Editorial

LEPROSY CONTROL IN 1978 AND BEYOND: WHO IS TO DO THE WORK?

In October 1969, on these very pages, Professor Michel Lechat wrote an Editorial which might have been entitled "The World Failure of Leprosy Control". He drew attention to the fact—now increasingly clear to everyone—that the hopes raised by the introduction of the sulphones 20 years previously were not being realized. In the years since his Editorial, the position has become generally worse, and a great deal more complicated. Since the original estimate by the World Health Organization in 1965 of a world total of 10,786,000 patients suffering from this disease, successive WHO Expert Committees have seen no reason to reduce the figure. It should, however, be stressed that it was arrived at with a number of important reservations, some of which are worth extracting here from WHO's 1966 "Guide to Leprosy Control":

(1) "The data collected or available in the literature on the prevalence of leprosy in most countries do not represent the real situation, because neither case finding nor reporting have reached the desired level".
(2) "Furthermore; in some countries the prevalence rate is apparently low because patients are discharged from case-lists as soon as the disease becomes inactive. In other countries cases of quiescent disease, especially tuberculoid, are maintained on the active list for many years. These observations as well as diff
patients from control make it difficult to obtain accurate data on the prevalence of leprosy."
(3) "It is stressed that these data are presented with many reservations and are intended to give an idea of the extent of the problem throughout the world".

The fact that several large countries have consistently failed to produce estimates of the numbers of their leprosy cases, together with the continued population increase, heavily affecting those countries where leprosy is endemic, has led to a figure of 15 million being quoted in recent years by a number of experienced observers, as a more realistic one for the world total of estimated cases, and WHO's most recent re-appraisal (Expert Committee on Leprosy, Fifth Report, 1977) states that "Revised estimates from a number of the larger countries indicate the total cases throughout the world may well exceed 12 million". Of these, it is estimated that only about 3 million are known to local authorities, and that only a fraction of them receive treatment with any degree of regularity. Equally disturbing is the fact that the continued

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Lepr. Rev. (1978) **49**, 187–198

Autoradiographic and Metabolic Studies of *Mycobacterium leprae*

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Highly purified suspensions of *Mycobacterium leprae* show a progressive increase in incorporation of [^{3}H]thymidine and [^{3}H]DOPA in short-term cultures as shown by scintillation counting. The intact bacilli are known to have a high permeability barrier. The experiments described suggest that [^{3}H]DOPA becomes trapped within this barrier and oxidized inside the bacilli. Tests by pre-treatment with diethyl dithiocarbamate (DDC inhibitor of DOPA), cold DOPA or hyaluronidase distinguish the uptake of [^{3}H]DOPA by bacilli from the effects of connective tissue contamination. Similar increases in labelling of bacilli by scintillation counting of cultures, have been observed by autoradiography of the organisms.

The scintillation method shows promise for rapidly identifying drug resistance in lepromatous patients relapsing while on treatment with dapsone (DDS), rifampicin, clofazimine or other anti-leprosy drugs.

Introduction

In the previous communication (Khanolkar, Antia and Ambrose, 1976) the application of short-term pulse labelling of suspensions of *Mycobacterium leprae* combined with high resolution autoradiography was described. In order to apply such methods to a systematic investigation of the biological and biochemical characteristics of *M. leprae*, it is necessary to know the dynamic aspects of uptake of various labelled metabolites during various periods of culture and also to know the extent to which bacterial and tissue contaminants play a role in the incorporation of metabolites. In this communication, the use of scintillation counting in place of autoradiography is described. This method makes possible the carrying out on a regular basis of systemic and quantitative investigations of metabolism and biosynthesis of leprosy bacilli. Therefore it has clinical applications, particularly for the study of dapsone (DDS) resistance and the development of alternative drug combinations for the treatment of new patients. Basic studies of the metabolic requirements of the organism and hence the improvement of the culture medium may also be investigated.

Received for publication 21 May 1978.

0305–7518/78/0901–0187$01.00/0

Materials and Methods

PREPARATION OF BACTERIAL SUSPENSIONS

The methods used for preparing bacterial suspensions have involved the minimum possible number of operations in order to avoid the risk of contamination by airborne organisms. After preparation, all suspensions are tested on nutrient agar for absence of contaminants. Each separate culture is also tested on nutrient agar and Lowenstein-Jensen medium at the end of the culture period. We have used the method for preparing suspensions from human nodules as described by Talwar, Krishanan and Gupta (1974). This was modified later during the present studies. The chopped up tissue was placed in ice-cold distilled water for 0.5 h and subsequently processed according to the method of Talwar *et al.* This we call the Modified Talwar method. After preparation of the suspension, microscopical checks were made on a standard sized drop coating a constant area of slide, to give the bacteriological and morphological (MI) index. The same slide was then observed through a microscope with a square eyepiece graticule, subdivided into 25 small squares. The average area covered by a single bacillus after acid-fast staining, as the average of a large number, was found to be 1/57th of the area of a small square. Twenty fields of the large square were counted for total number of bacilli. In addition, the areas of small squares covered by tissue fragments, mainly stained blue, by the methylene dye, were observed. Stopping down the condenser was sometimes helpful for determining these areas. The purity of a given suspension could then be assessed quantitatively as the percentage of total area covered by bacilli to the percentage covered by tissue.

MICRO-METHOD FOR AUTORADIOGRAPHY

The agar film technique, as previously described (Khanolkar *et al.,* 1976) has been modified as shown in Fig. 1. The coverslips are first dipped vertically in 1% agar and dried on filter paper to give a thin coating of transparent agar to assist adhesion. The reinforcement with a strip of cloth helps to preserve the agar film when culturing up to 12 days.

SCINTILLATION COUNTING

Microtubes, as shown in Fig. 1 (g) and (h), 40 mm × 8 mm (6 mm int. diam.) were used. They were convenient for processing 1×10^6 bacilli, and 100 or more replicate cultures could be set up from a single nodule. The cultures are pulse-labelled with 1 μCi/ml of [^{3}H]thymidine for 24 h, or for 6 h with DOPA. The tubes are then centrifuged for 10 min at 4000 rev/min. The visible pellet is washed 3 times with saline, treated with cold TCA and processed for scintillation counting according to standard methods.

CULTURE MEDIA

The problem of finding a medium suitable for the long-term culture of *M. leprae* is well known. Therefore, the absence of biochemical data concerning suitable sources of energy for *M. leprae,* alternative sources, such as citrate and pyruvate have been supplied as well as essential amino acids, particularly

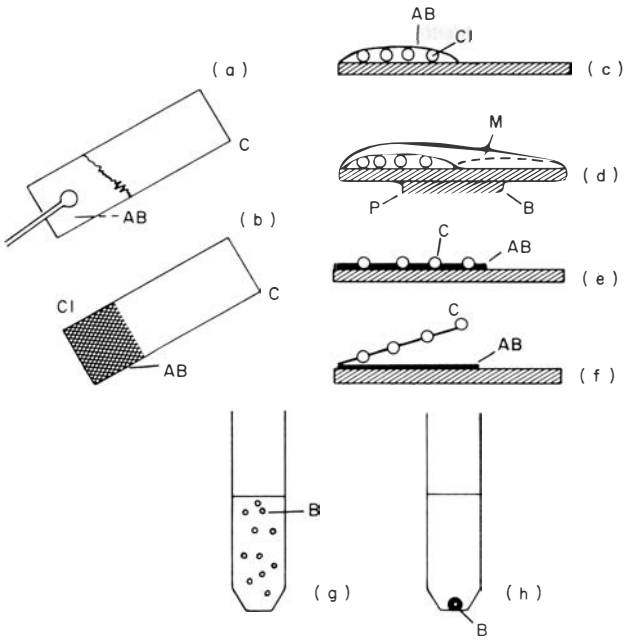

Fig. 1. *Culture methods for autoradiography and scintillation counting.* (a) Equal volumes of bacilliary suspension and 1% molten agar (maintained in water bath at 55°C) are mixed with wire loops on coverslips. (b) and (c) A small strip of loosely woven cloth is placed on molten agar to reinforce the gel. (d) The coverslip may be placed in a Leighton tube. For small volumes, to save labelled metabolites, it can be placed on a glass block (B) coated with a film of liquid paraffin (p) and incubated in a Petri dish in a humid atmosphere. After fixing it must then be washed in detergent to remove paraffin — (e) and (f). After processing and drying, the cloth can be peeled off leaving the dry agar film intact. (g) Microtube holding 0.5 ml. of culture medium and 1×10^6 bacilli for scintillation studies. (h) After pulse labelling and centrifuging a distinct pellet can be observed. This is washed and processed for counting.

asparagine and nucleotides. The new medium of Murohashi and Yoshida (1975) is simple and seems to satisfy most requirements for our short-term culture assay.

With this medium we find that, unless the MI is very low, the counts on pulse labelling after maintenance for 9 days show an increase as compared with controls labelled at day 0. On the basis of this finding we have used this medium for all the investigations supported here. However, the scintillation assay could be used to investigate modification of this medium or other possible media.

Experimental Results

PURITY OF BACTERIAL SUSPENSIONS

The micro-scanning method indicates that in the Talwar method of preparing suspensions, the tissue contamination is in the range of 30–40%. But with

TABLE 1
Analysis of bacterial content in suspensions prepared by the modified method

Patient OPD No.	Bacilli %	Tissue fragments %
99620	85	15
99722	84	16
61655	86	18
98571	94	6
30357	99	1

preparations made by the modified Talwar method as shown in Table 1, the tissue contamination is much less when expressed as the relative areas covered by tissue and bacilli. The relative dry masses will not be identical but are not expected to differ markedly from these figures. The extent of contamination with hyaluronic acid has also been assessed (Khadapkar *et al.,* 1977). A range of concentrations of a purified sample of human umbilical cord hyaluronic acid were used as standards. The test samples were supernatants obtained during centrifugation of the bacterial suspensions and the final purified suspension of *M. leprae,* obtained from the nodule. Drops of known volume were placed on standard areas of slides and allowed to dry slowly. These were then covered with a standard sized drop of toluidine blue solution as used in histochemistry. Metachromasia was observed to an extent depending on the concentration of acid mucopolysaccharide present. With the standard solutions, showing a colour range, the test samples were matched colorimetrically against a standard drop. The first washing from the bacilli was found to contain the equivalent of approximately 0.2 mg/ml of hyaluronic acid. The amount in the washed bacilli was below the detectable limit, at least 100 times less than that in the washings.

AUTORADIOGRAPHIC STUDIES OF CULTURED BACILLI

These studies were made as a further check on the localization of the labelling within the bacilli, prior to adopting the scintillation counting method.

With the Murohashi and Yoshida medium, the concentration of labelled metabolite can be reduced to 1 μCi/ml giving a low background count, not more than 18 background grains were observed per microscopic field. The average area covered by a bacillus being 1400 times less than the field area, the probability of a background grain lying over a bacillus will be 0.01. For 2 grains it will be 0.0001 and for 3 grains 0.000001. Photomicrographs taken both by transmitted light and with the polarizing vertical illuminator have been shown previously (Ambrose *et al.,* 1974; Khanolkar *et al.,* 1976). In Fig. 2 is shown a detailed analysis of a 12-day culture checked at high magnification for a group of granular bacilli and a group of labelled bacilli recorded from a camera lucida drawing. Due to slight variations in focal-plane it is difficult to record and present such detailed information by photography. Due to the porosity of the dry agar film, emulsion penetrates uniformly so that grains are developed immediately adjacent to bacilli and strictly in a straight line along the length of a bacillus. A comparison between labelling of bacilli and the morphological type of the acid-fast staining in the case of 5 untreated

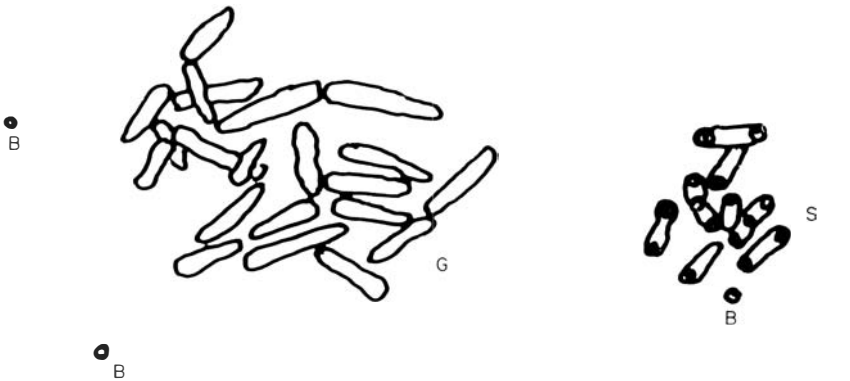

Fig. 2. Autoradiograph after maintaining for 12 days in culture (from Camera lucida drawing made at ×3000 mag.). G, Group of unlabelled and granular bacilli. S, Group of solid bacilli showing up to 3 grains per bacillus. B, Background grains. Labelled for 48 h with 1 μCi/ml of [^{3}H]DOPA.

lepromatous leprosy patients showed that labelling is almost entirely restricted to solid bacilli. In Fig. 3(a) is shown a histogram obtained in a culture maintained for 9 days before labelling with [^{3}H]DOPA. The frequency of clusters containing labelled bacilli is shown in Fig. 3(a), upper, and of

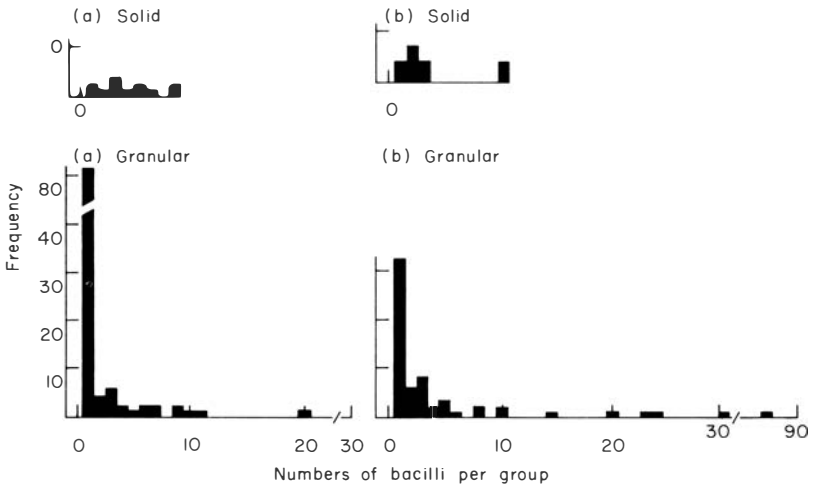

Fig. 3. Effect of added hyaluronic acid on the labelling of *M. leprae* with [^{3}H]DOPA as seen in autoradiographs (a) (upper, left). Solid bacilli. Histogram showing frequency of occurrence of clusters containing different numbers of bacilli (abscissa), after labelling for 48 h with [^{3}H]DOPA. These are the bacilli which show one or more silver grains along their length (mainly solid). (a) (lower, left). Granular bacilli. Similar histogram for the unlabelled bacilli on the same slide (mainly granular). (b) (upper and lower, right). Histograms for solid and granular groups similar to (a), but in this case 0.3 mg/ml of hyaluronic acid had been added to the medium before labelling. The general pattern of labelling is unchanged; adsorption of hyaluronic acid on previously unlabelled bacilli has not caused them to be labelled.

unlabelled granular bacilli, Fig. 3(a), lower. At day 0 labelling is restricted mainly to single bacilli, but at day 9 labelled clusters are also seen.

In Fig. 3(b) is shown a similar result when 0.3 mg/ml of hyaluronic acid had been added initially to the culture media. Although many grains were seen in the background due to oxidation catalysed by hyaluronic acid, there were mainly in different focal planes from bacillary clusters and it was possible to count this slide. The presence of hyaluronic acid does not affect the general pattern of labelling.

Due to high rate of incorporation, [^{3}H]DOPA is useful for autoradiography, although [^{3}H]thymidine also shows a small number of labelled bacilli.

THE ROLE OF TISSUE CONTAMINANTS IN THE INCORPORATION OF LABELLED METABOLITES

In Table 2 is shown the rate of labelling with [^{3}H]thymidine for fresh and heated bacilli. Twenty-four hours' labelling gives a good uptake, general absorption by tissue, as indicated in the heated specimens, being low. Absence of bacterial contaminants is checked on nutrient agar and on Lowenstein-Jensen medium.

In earlier studies with [^{3}H]DOPA, penicillin was not added to the medium. In autoradiographs non-acid-fast bacterial contaminants were sometimes seen, but none of these were labelled. A fungus, apparently *Aspergillus,* which is known to contain DOPA oxidase showed heavy labelling. Penicillin 100 i.u./ml is now introduced routinely into the culture medium. Cultures of *M. tuberculosis, M. phlei* and *M. smegmatis* in their respective growth media showed no grains in autoradiographs following [^{3}H]DOPA treatment. The role of connective tissue hyaluronic acid in the bacterial suspensions has been checked by 4 independent methods following the observations of Kato, Ishaque and Adopoe (1976) that this tissue can catalyse the oxidation of DOPA. The capacity of connective tissue to oxidase DOPA is not affected by heating (Kato *et al.,* 1976). In Table 3(a) the incorporation of [^{3}H]DOPA is

TABLE 2

Rate of labelling with [^{3}H]*thymidine as shown in scintillation counting*

Morphological Index	p mol of [^{3}H]thymidine incorporated per 10^7 bacilli		
	Time (h)		
	6	24	48
Fresh bacilli	$\times 10^{-3}$	$\times 10^{-3}$	$\times 10^{-3}$
4	9.78	15.3	16.6
4	76.4	67.6	102
5	80.8	72.0	106
Heated bacilli			
4	0.32	0.22	1.3
4	1.00	1.3	3.4

TABLE 3

(a) *Analysis of labelling of suspensions of* M. leprae *heated and pre-treated with DDC**

Patient	MI (%)	Ratio of bacilli/ tissue	Time of incu-bation (h)	pmol of [^{3}H]DOPA incorporated per 10^7 bacilli			
				Fresh bacilli		Heated bacilli	
				Column 1 control	Column 2 with DDC	Column 3 control	Column 4 with DDC
OPD No. 99355	5	Low	1	97.1	50.3	54.1	60.3
		(Talwar	6	291.1	98.7	157.4	148.7
		method)	8	162.9	132.0	117.0	90.0
L L Untreated	5	High	1	186.5	72.9	65.1	59.5
OPD No. 99620		(Modified	6	375.0	97.1	86.5	93.2
		Talwar					
LL Untreated		method)	8	223.2	70.0	61.8	61.5

* Control suspensions were incubated for 8 h in phosphate buffer, washed and incubated for a further 8 h before labelling with 2 μCi/ml [^{3}H]DOPA prior to counting at 1, 6 and 8 h. DDC-treated suspensions were also given similar washing after 8 h treatment.

(b) *Effect of pre-treatment with hyaluronidase on composition of bacterial suspensions*

Patient	Estimated by scanning of microdrop		Estimated by pre-treatment with hyaluronidase	
	Bacilli (%)	Tissue fragments (%)	Bacilli (%)	Tissue fragments (%)
OPD No. 99912	62	38	58	42
OPD No. 99620	85	15	75	25

*Control suspensions were incubated with [^{3}H]DOPA. Hyaluronidase-treated suspensions were incubated for 2 h and washed before incubation with [^{3}H]DOPA.

shown for a suspension prepared by the Talwar method (OPD No. 99355) and by the modified Talwar method (OPD No. 99620). In column 1 is shown the uptake by the fresh suspension which reached a peak at 6 h. In column 3 is shown the uptake by a heated preparation. In column 2 is shown the effect of treating fresh bacilli with diethyl dithiocarbamate (DDC), the copper chelator and inhibitor of diphenol-oxidase, for 8 h followed by washing, and further incubation for 8 h prior to labelling with [^{3}H]DOPA. The effect of DDC persists after washing, but in the case of the heated specimens it shows no inhibitory effect. The residual levels as seen in column 2 and 4 are similar, the amounts being reduced in the purified suspension prepared by the new method.

Kato *et al.* (1976) have shown that pre-treatment with hyaluronidase completely arrests the capacity of connective tissue to oxidase DOPA. In Table 3(b) is shown, in the case of a heavily contaminated sample (OPD No. 99620), the effect of pre-incubation with 0.2 mg/ml of Sigma hyaluronidase for 2 h followed by washing and labelling. Controls were similarly treated but without enzyme. Pre-treatment with cold DOPA followed by washing prior to labelling also inhibited uptake of [^{3}H]DOPA. Finally the effect of ascorbic

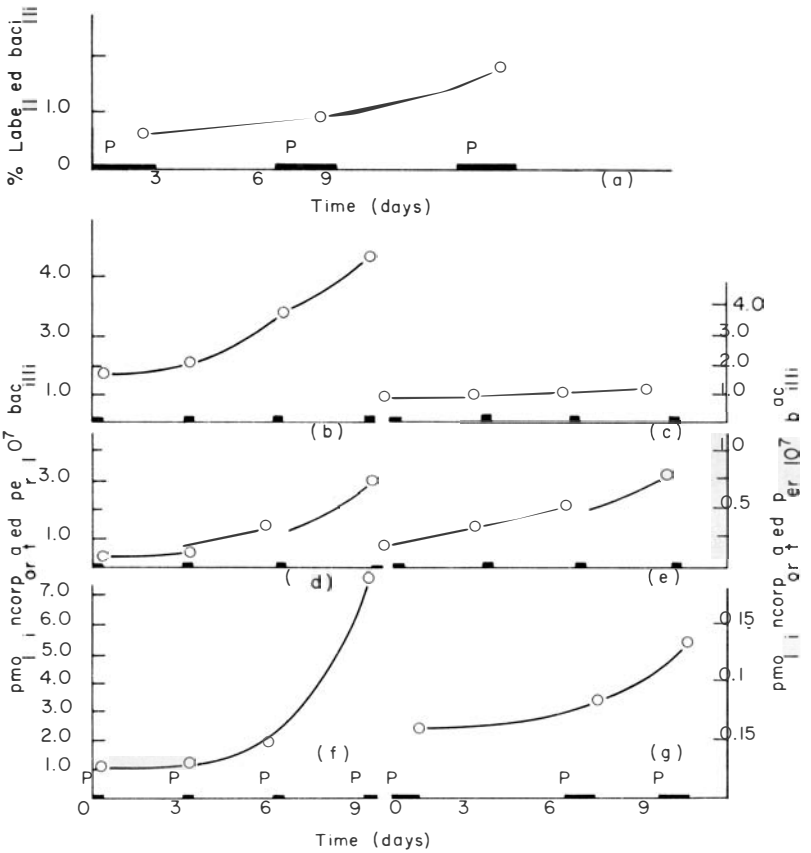

Fig. 4. Growth curves obtained by autoradiography and scintillation counting. (a) Increase in labelling of replicate autoradiographs pulse-labelled for 48 h with 1 μCi/ml of [^{3}H]DOPA. Untreated LL patient with MI 3%; P = pulse. (b) Similar growth curve obtained by scintillation counting for a highly purified suspension of *M. leprae* (only 1% tissue contamination, Case 30357 Table 1). Relapsed LL patient; MI 2%. (c) Heated suspension (60°C for 1 h). Supension prepared by Talwars method with considerable tissue contamination. (d) Growth curve for untreated LL patient; MI 4%; pulse-labelled with [^{3}H]DOPA. Rate of growth expressed as ratio of incorporation at day 9/incorporation at day 0 = 5.6. (e) Suspension of same nodule as (d). Including sediments removed at 500 rev/min prior to preparing suspension shown in (d), it is highly contaminated with tissue debris (38%). In this case 4 mM ascorbic acid was added immediately before pulse-labelling to inhibit all oxidation of [^{3}H]DOPA by tissue contaminants. Rate of growth = 5.8, is similar to (d). Repeated experiments have shown similar results with ascorbic acid. (f) Growth curve for untreated LL case, MI 6% — pulse-labelled with [^{3}H]DOPA. (g) The same suspension as shown in (f) but pulse-labelled with [^{3}H]thymidine.

acid was shown to give results similar to the previous 2 tests. Adding ascorbic acid even at 4 mM, only reduced the incorporation of [^{3}H]DOPA to the extent expected in proportion to the amount of tissue contamination [Fig. 4(e)]. Addition of ascorbic acid to reaction mixture of *M. leprae* reverses the reaction from purple pigment to pinkish indole 5:6 quinone (Prabhakaran,

1973). On the other hand it reverses completely in the case of non-enzymatic oxidation of DOPA to a colourless solution.

GROWTH CURVES

For the purpose of this communication growth is defined as an increase in the incorporation of [^{3}H]thymidine or [^{3}H]DOPA into the bacilli. Four growth curves were obtained by autoradiography, one being shown in Fig. 4(a). All showed a progressive increase in labelling at the end of 6 and 12 days, of replicate autoradiographs. In Fig. 4(b) is shown a similar curve obtained by scintillation counting in the case of a highly purified suspension of *M. leprae* (OPD No. 30357 of Table 1). In Fig. 4(c) is shown a result for a suspension containing tissue contamination heated to 60° C for 1 h. In Fig. 4(d) and 4(e) are shown results for the same suspension, but that shown in Fig. 4(c) was the preliminary sediment centrifuged at 500 rev/min, while 4(d) was from the usual supernatant centrifuged at 4000 rev/min according to the modified method. The sample 4(e) contained 38% of tissue and was treated with ascorbic acid to eliminate tissue oxidation of DOPA. Figure 4(f) and 4(g) show growth curves obtained with the same suspension using [^{3}H]DOPA and [^{3}H]thymidine. Figure 5 shows a reasonably good correlation between the morphological index and the growth rate as determined for the last 8 biopsies processed for culture.

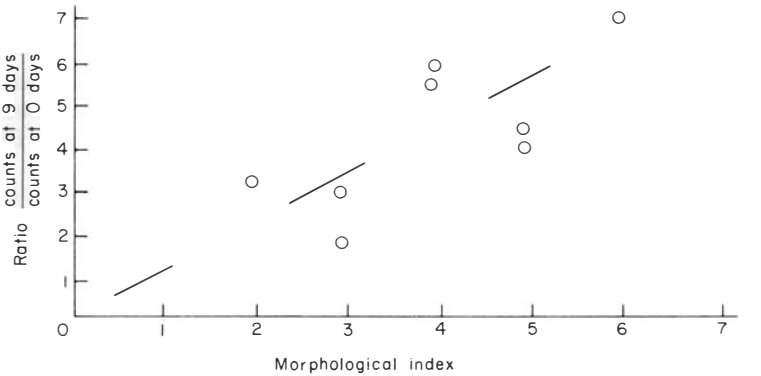

Fig. 5. Relationship between morphological index and the increase in counts at day 9 over counts at day 0, for a sequence of 8 cases tested recently.

In all cases the morphological index was checked independently at the A.L.H. (R.G.C.) and at FMR (S.R.K.).

Discussion

The use of labelled metabolites to assess metabolic activity and growth potential has already been shown to be of value with human tumours to measure drug response, effects of antisera etc., although most tumour cells do not survive for more than 1 or 2 weeks in the culture media at present

available. The approach adopted here has been basically similar for studies of *M. leprae*. Labelled thymidine is well recognized as a marker for DNA synthesis by living cells. The incorporation of this metabolite by *M. leprae* already ingested by human macrophages has been described by Drutz and Cline (1972), and by Talwar, Krishanan and Gupta (1974). The suspensions used for scintillation studies, as shown in Fig. 4(g), were pre-treated with distilled water which causes lysis of all mammalian cells. Tests for contaminating bacteria on nutrient agar and Lowenstein-Jensen medium were also negative, strongly suggesting that thymidine was also being incorporated into *M. leprae* in these cultures. Another widely used test for viability of mammalian cells has been the dye exclusion test. The high permeability barrier of the intact plasma membrane of living cells prevents the penetration of dyes such as lissamine green, but dead cells are rapidly stained. Such tests are not needed with bacteria which grow readily in culture, but are of value with *M. leprae*. The evidence presented in this paper supports other evidence that these bacilli also possess a high permeability barrier, which is only penetrated slowly by molecules such as DDC or cold DOPA. Once DOPA has entered the bacillus and been oxidized to quinone, this molecule cannot leak out before further stages of oxidation to insoluble products or incorporation into macromolecules has occurred. The radioactivity remaining will be due to those atoms which are not released during oxidation as $[^3H]_20$. Results obtained with ascorbic acid also indicate that special conditions exist within the leprosy bacilli which are not affected by the presence of this reducing agent in the surrounding medium. As shown in Fig. 4(b), a bacterial suspension containing a negligible amount of tissue contamination (OPD No. 30357 Table 1) still shows high incorporation of [^{3}H]DOPA at day 0 and subsequent increases up to day 9. Chatterjee (1977) has obtained some strains of acid-fast bacilli in long-term cultures obtained from leprosy nodules, which are also DOPA oxidase positive.

The various tests for the effect of tissue contamination on the initial counts i.e. scanning of microdrops pre-heating of suspensions, pre-treatment with DDC, pre-treatment with hyaluronidase (Tables 1 and 3) and the effect of ascorbic acid [Fig. 4(c)] give similar values for the effect of tissue contamination on the initial background count. This does not exceed 25–30% with the modified method of preparation and is generally much less than this.

The autoradiographic studies indicate that the solid bacilli incorporate sufficient labelled metabolites to produce grains in the autoradiographs. When embedded in the agar the penetration of the metabolite, and probably oxygen exchange, is further reduced so that 48 h is required for labelling. Earlier studies with the mouse footpad (Shepard and McRae, 1965) and clinical studies of decreases in the proportion of solid bacilli during the first 3 months of DDS treatment (Waters and Rees, 1962), favour the view that the solid bacilli are the viable and most actively growing fraction. The possibility that other morphological types are moribund, but recover their viability under favourable conditions cannot be excluded. The increase in the average number of grains per labelled bacillus, seen after 12 days in cultures, suggests that the increase in count with DOPA labelling is at least partly due to increased rate of metabolism, in which some previously moribund bacilli might be involved.

Nevertheless, the progressive increase in thymidine labelling, as shown by the curve of Fig. 4(g), suggests that bacterial replication may also be involved. There have been claims that other forms of *M. leprae* including coccal forms can multiply in the mouse footpad (Desikan, 1976). This problem has also been discussed by Dharmendra (1977). The acid-fast character of the bacilli obtained in clusters as seen in autoradiographs after 12 days, the bacterial specificity for DOPA labelling (Prabhakaran, 1973) and the absence of growth as double-checked, before and after culture, on nutrient agar and on Lowenstein-Jensen media, favour the view that this technique can be used for dynamic studies of the biological properties of *M. leprae*. This is borne out by data recently obtained on the response to DDS, rifampicin and clofazimine in these cultures (to be published). With untreated lepromatous leprosy cases, inhibition has been obtained in the range of concentration of DDS used in mouse footpad i.e. more than 100 times the sensitivity shown by any known contaminant. With relapsed cases DDS resistance has been observed up to concentrations corresponding to those observed in patients receiving between 100–200 mg daily dose of DDS, including cases already shown to be DDS resistant in the mouse footpad. Good response to rifampicin with the labelling test has been observed in 2 DDS-resistant cases who have now shown good clinical response to this drug.

Acknowledgement

We are grateful to Dr N. H. Antia and Dr K. K. Koticha for their help and encouragement. This work has been supported by grants from the Ministry of Overseas Development (U.K.) and the World Health Organization (Geneva). We are grateful to Mr Khadapkar S. V. for help with the scintillation counting and Mr Swamy for photographic assistance.

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Lepr. Rev. (1978) **49,** 199–202

Further Data on the Effect of Ethionamide and Prothionamide in Experimental Leprosy

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Experiments carried out in mice using intermittent administration of ethionamide and/or prothionamide indicate that the efficacy of these drugs are substantially impaired if they are given less frequently than 3 times a week. Irregular administration of these drugs could lead more rapidly to the emergence of resistance than is the case for dapsone. Results of the total minimal inhibitory test show that treatment of paucibacillary leprosy with prothionamide during 9 or 12 weeks can be envisaged.

Introduction

Ethionamide (α-ethyl thioisonicotinamide) and more recently prothionamide (α-propyl thioisonicotinamide) have been quite extensively used as second-line drugs in the treatment of tuberculosis. Using the proportional bactericidal test, Colston, Hilson and Banerjee (1978) have demonstrated that when either drug is fed to mice for 45 days at 0.1% in the diet, 98.6% of the inoculated leprosy bacilli were killed. This proportion is only surpassed by rifampicin.

The commercial production of ethionamide has now been discontinued, since prothionamide was found to be as active against *Mycobacterium tuberculosis* as ethionamide but better tolerated in man (Chambatte *et al.,* 1965; Martin-Lalande *et al.,* 1966; Lesorbre *et al.,* 1968; Mycskowska-Wilska and Pawelc, 1968; Glatthaar and Van Der Merwe, 1970). Against *Mycobacterium leprae* the 2 drugs have also an identical activity (Colston *et al.,* 1978*a*). In this paper the results of ethionamide-prothionamide in intermittent therapy and the total minimal inhibitory test are presented. Ethionamide was used at the start of the experiments, and it was replaced by prothionamide when ethionamide was no longer available.

Material and Methods

M. leprae strain 17547 isolated in our laboratory was used (Pattyn *et al.,* 1972) and mice inoculated with 5.10^3 bacilli. Treatment started 3 weeks post-infection. When ethionamide or prothionamide were given for periods of more

Received for publication 18 May 1978.
0305–7518/78/0901–0199$01.00/0

than 2 consecutive days, they were administered in the diet at a concentration of 0.1%. For regimens once, twice or thrice weekly drug administration, it was given by gastric cannula, 0.25 mg per mouse.

When multiplication in untreated control mice had reached 5.10^5 bacilli per footpad, the drug treated animals were killed and footpads examined for the presence of acid-fast bacilli.

Results

(A) INTERMITTENT ADMINISTRATION OF ETHIONAMIDE-PROTHIONAMIDE

As shown in Table 1 control treatment with continuous prothionamide inhibited completely the growth of *M. leprae* strain 17547. All intermittent regimens except the one of 3 administrations per week, were only partially active.

TABLE 1
Effect of intermittent administration of ethionamide prothionamide on the multiplication of M. leprae

Controls continuous treatment†	0/9*	A
3 days p. week‡	0/4	A
2 days p. week‡	5/10	P
1 day p. week‡	4/6	P
1 day every 2 weeks†	4/6	P
1 day every 3 weeks†	4/7	P
1 day every 4 weeks†	3/6	P
1 week every 3–4–6–8 and 12 weeks†	all positive	I

* Number of footpads positive/number of footpads harvested.
† Ethionamide given during initial 64 days, followed by prothionamide.
‡ Prothionamide.
A = active; P = partially active; I = inactive.

(B) TOTAL MINIMAL EFFECTIVE DOSE

Table 2 shows the results of treatment during 6–9 or 12 weeks. Clearly a 6-week treatment resulted only in a growth delay, whereas a 12-week treatment, was sufficiently bactericidal to result in absence of multiplication during the following 16 weeks of observation.

Discussion

Ethionamide-prothionamide are after rifampicin the most potent bactericidal antileprosy drugs. They are however rapidly excreted, and serum levels only exceed the MIC for about 24 h (Colston *et al.*, 1978*b*). We have previously shown that the efficacy of rifampicin in the mouse is still maintained when administered intermittently once every week, once every 2 weeks and even once every 4 weeks provided the dose administered is 2.5–10 times the

TABLE 2
Total minimal effective dose of ethionamide prothionamide

Duration of treatment	At plateau*	Plateau + 16 weeks†
Controls, untreated	12/12	
6 weeks	0/3	5/9
9 weeks	0/3	0/9
12 weeks	N.E.‡	0/9

* When bacterial multiplication reached 5.10^5 bacilli per footpad in the control mice.
† 16 weeks later.
‡ Not examined.

MED (Pattyn and Saerens, 1974). The efficacy of once weekly administration of rifampicin in man has also been studied (Pattyn *et al.*, 1975). In this experiment with prothionamide, the drug was administered at 10 to 3 times the MED, but intermittency could not be lowered beneath 3 administrations per week. This is unfortunate because there may be an advantage in supervised weekly intermittent treatment. The results of the intermittent experimental therapy with prothionamide and the studies of Colston *et al.* (1978*b*) suggest that irregular treatment with this drug could lead more rapidly to the emergence of resistance than with dapsone. In the total minimal inhibitory test, mice were followed for 16 weeks after the plateau phase of multiplication was reached in the control group. Although this length of follow-up could be too short to prove definitely complete sterilization of the infection, it is certainly reduced to such low levels that can be taken care of by the existing immunological defences in the paucibacillary human patient. It is generally accepted that in almost all infections chemotherapy does not kill absolutely all parasites, but that a minimal surviving fraction is eliminated by the host defence mechanisms. Only in the lepromatous patient with his immune deficiency should chemotherapy aim at a complete sterilization of the infection. It can therefore be concluded from the present results that 3–6 months prothionamide treatment courses (500 mg daily) of paucibacillary leprosy should be considered: previous experiments showed that a short course treatment of a paucibacillary infection with dapsone (Pattyn, 1977) was insufficiently bactericidal.

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Absence of β-Glucuronidase in *Mycobacterium leprae* and Elevation of the Enzyme in Infected Tissues

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β-Glucuronidase activity was determined in mouse footpads infected with *Mycobacterium leprae,* in the leprosy organisms separated from the liver and spleen of experimentally infected armadillos, and in the armadillo tissues. Enzyme assays in the mouse footpads were initiated 1 week after inoculation with *M. leprae* and continued at monthly intervals for 12 months. In the mouse footpads and in the armadillo tissues, *M. leprae* infection resulted in remarkable elevations of β-glucuronidase levels. The leprosy bacilli seemed to be devoid of the enzyme. In its properties like pH optimum, reaction velocity and effect of inhibitors, the activity detected in *M. leprae* resembled the host tissue enzyme rather than bacterial β-glucuronidase; and the activity was found to be superficially adsorbed on the bacilli. It is well established that phagocytes are rich in lysosomal enzymes. Evidently, the increased β-glucuronidase of the infected tissues is not derived from the invading organisms, but from the different types of phagocytic cells infiltrating the tissues.

Introduction

β-Glucuronidase is an important hydrolytic enzyme ubiquitously distributed in animal tissues and in tissue fluids. Phagocytic cells are especially rich in β-glucuronidase. In the mammalian liver, the enzyme is largely associated with lysosomes, and approximately one-third of the activity is distributed in the endoplasmic reticulum. The hydrolase is closely correlated with cellular proliferation and tissue repair; high levels of the enzyme are found in the reproductive and endocrine organs and in tumours. Skin cancers as well as normal skin contain β-glucuronidase. The enzyme has been reported in molluscs and in insects. Among bacteria, *Escherichia coli, Streptococcus pyogenes, Corynebacterium xerose, C. hoffmani* and rumen micro-organisms have been shown to produce β-glucuronidase (Fishman, 1955; Levy and Marsh, 1959). In bacterial infections characterized by intracellular parasitism of the invading organisms, marked elevation of hydrolytic enzymes has been observed (Allen, 1969).

β-Glucuronidase splits off D-glucuronic acid residues from oligosaccharides, thus degrading these complex molecules. In the body, glucuronic acid forms

Received for publication 9 February 1978.

0305–7518/78/0901–0203$01.00/0

conjugates of hormones, drugs and other substances, thus serving a detoxification function; also β-glucuronidase can hydrolyze such conjugates and release the organic compounds (Fishman, 1955). Because of their degradative activities, lysosomal enzymes, including β-glucuronidase, have a major role in the killing and digestion of ingested bacteria (Allen, 1969). In leprosy, as in certain other mycobacterial infections, a paradoxical situation occurs; the bacteria, instead of being destroyed by the phagocytes, seem to be protected in these cells (Goren, 1977; Brown *et al.,* 1969).

No significant elevation of β-glucuronidase was observed in peripheral blood leucocytes of leprosy patients (Avila and Convit, 1970; Garcia-Gonzalez *et al.,* 1977). Increase in acid phosphatase was demonstrated histochemically in histiocytes containing *Mycobacterium leprae* (Brieger and Allen, 1962). Based on histochemical evidence, it was concluded that *M. leprae* possesses β-glucuronidase (Matsuo and Skinsnes, 1974). In the course of our investigations over the past several years on lysosomal enzymes in leprosy, we studied the levels of acid phosphatase and β-glucuronidase in mouse footpads and in tissues of armadillos experimentally infected with *M. leprae.* We also determined β-glucuronidase activity in concentrates of the bacilli separated from infected armadillo tissues. Because acid phosphatase is extremely labile, its assay was found to be unreliable in tissues stored for varying periods of time. As such, acid phosphatase assays were not pursued further. However, β-glucuronidase gave consistent and reproducible results. The data presented in this report show that *M. leprae* purified from infected armadillo tissues apparently does not contain β-glucuronidase; in the mouse footpad, the enzyme activity continues to rise as the infection progresses; and in the infected armadillo tissues, the enzyme levels are elevated 2 to 3 times over that of the uninfected tissues.

Materials and Methods

ANIMALS AND MYCOBACTERIA

Approximately 8-week-old female Swiss mice of the NIH strain were used in the study. The experimental animals were inoculated in the left hind footpads with 1×10^4 *M. leprae.* The *M. leprae* used was a mouse-passage strain originally obtained from the skin biopsy of an untreated lepromatous patient. Control mice were of the same age and sex. Starting at 1 week after inoculation, a few mice (usually 5) were sacrificed at monthly intervals for 12 months. The soft tissues from the left hind footpads of both the experimental and the control animals were collected and stored at −80°C. At the time of assay, the material was thawed out, washed in cold saline, blotted and minced. The tissues were extracted in small amounts of water by grinding in a chilled agate mortar. The extract was centrifuged briefly, to remove the particulate matter. Enzyme determinations were made in the supernatant fraction. Protein was estimated by the method of Lowry *et al.* (1951). Enumeration of *M. leprae* in the mouse footpad was done at 6 months and 12 months, by the method of Hanks *et al.* (1964), modified by Kirchheimer.

Liver and spleen of "normal" and infected armadillos were collected

aseptically at autopsy and were held at 0°C or at −80°C before processing. Ten percent homogenates of these organs were made in water. Suspensions of the leprosy bacilli were prepared as reported before (Prabhakaran *et al.,* 1976) from the liver or spleen of 9-banded armadillos experimentally infected with *M. leprae* (Kirchheimer and Storrs, 1971; Kirchheimer *et al.,* 1972). The bacilli were disrupted by ultrasonic oscillation (Prabhakaran *et al.,* 1973). In an effort to remove superficially adsorbed host-tissue materials from the bacterial concentrate, it was treated with trypsin (final concentration 0.5%), NaOH (0.1 N), sodium dodecyl sulfate (1%), or sodium deoxycholate (0.5%) for 30 min at 0°C, except for trypsin, which was done at 37°C.

Mycobacterium phlei was grown in Proskauer-Beck medium (Youman's modification) at 37°C for 2 weeks. The bacilli were harvested by centrifugation and washed twice with cold saline and once with deionized glass-distilled water.

ENZYMES AND CHEMICALS

Purified *E. coli* β-glucuronidase (Type VII), bovine liver β-glucuronidase (Type B-10), phenolphthalein glucuronic acid (sodium salt) and glycine buffer were purchased from the Sigma Chemical Co., St Louis, MO, USA. Other chemicals used were of the highest purity commercially available.

ENZYME ASSAY

The enzyme assay was done essentially by the standard procedure of Talalay *et al.* (1946). The reaction mixture consisted of the following constituents: 0.1 M acetate buffer (pH 4.7), 0.4 ml; 0.01 M phenolphthalein glucuronic acid, 0.1 ml; sample, 0.5 ml. The sample blank contained 0.5 ml buffer and 0.5 ml sample, and the reagent blank 0.9 ml buffer and 0.1 ml substrate. For *E. coli* β-glucuronidase, 0.075 M phosphate buffer (pH 6.8) was substituted for acetate buffer; 40 units of the enzyme were added. The mycobacterial suspensions used contained $2–5 \times 10^9$ organisms/ml. The final concentration of the inhibitors tested was 0.005 M. After the reaction mixture was incubated at 37°C for 60 min, 1 ml of 5% TCA was added, followed by 2.5 ml of alkaline glycine reagent. Volume was made up to 6 ml with 1.5 ml of water. The reaction mixture was centrifuged and the purple colour of the supernatant fraction was read at 540 nm in a Beckman DU-2 spectrophotometer. The readings were corrected for any absorbance due to the sample or reagent blanks. The amount of phenolphthalein liberated from the substrate by the enzyme was calculated from a standard curve. All reactions were carried out in duplicate and each experiment was done at least 3 times. The results reported are mean values of representative experiments.

Results

MOUSE FOOTPADS

M. leprae inoculated in the mouse footpads multiplied normally; enumeration of the bacilli showed $2.4 \pm 0.18 \times 10^6$ organisms per footpad in 6

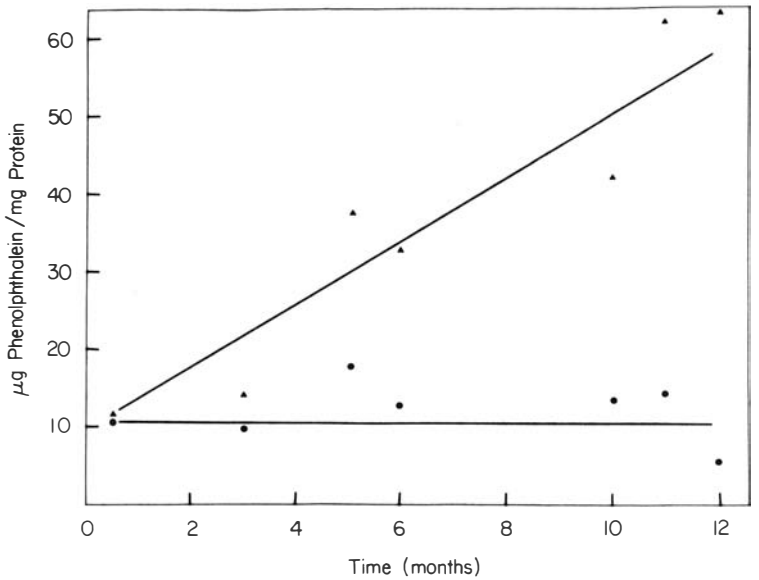

Fig. 1. β-glucuronidase of mouse footpads infected with *M. leprae*. (▲—▲) Experimental; (●—●) control.

months, and $1.8 \pm 0.15 \times 10^7$ in 12 months. β-Glucuronidase activity was assayed in the footpad tissues at monthly intervals for 12 months. To correct for variations in individual assays, the values obtained for both the control and the experimental samples were subjected to regression analysis. The results are presented in Fig. 1. In the uninfected mice, the enzyme activity remained fairly stable throughout the experiment. However, β-glucuronidase continued to rise in the footpads of mice where *M. leprae* multiplied progressively. These results do not indicate whether the higher activity is derived from the bacilli or from the cells that infiltrate the infected footpads.

ARMADILLO TISSUES

β-Glucuronidase was determined in the liver and the spleen tissues of both the infected and the uninfected armadillos. (On the average, the infected organs

TABLE 1

β-Glucuronidase in "normal" and infected spleen tissue of armadillos: μg *of phenolphthalein released/*mg *protein*

Number of animals	"Normal"	Infected
1	32.2	92.1
2	37.7	173.5
3	44.4	110.5
Mean	38.1	125.4

TABLE 2
β-Glucuronidase in "normal" and infected liver tissue of armadillos: µg *of phenolphthalein released/*mg *protein*

Number of animals	"Normal"	Infected
1	77.4	188.9
2	162.0	200.0
3	118.4	286.3
4	130.8	234.4
Mean	122.2	227.4

contained over 10^9 *M. leprae* per g of tissue.) Compared to the controls, the activity in the infected spleen was elevated by over 3 times, and by almost 2 times in the liver (Tables 1 and 2).

M. LEPRAE

It was not clear whether the source of the elevated enzyme activity observed was the invading organisms or the host tissues. To ascertain this, *M. leprae* was separated from both the spleen and the liver tissues and β-glucuronidase activities determined in the bacterial preparations. The preparative procedure involved differential and density-gradient centrifugations in solutions of sucrose and KCl (Prabhakaran *et al.,* 1976). When assayed without further purification, these bacterial suspensions contained β-glucuronidase. When the bacilli were disrupted by ultrasonic oscillation, more enzyme acitivity was not released. Both intact and disrupted organisms gave similar results. When the disrupted suspension was fractionated, the supernatant contained a greater amount of β-glucuronidase than the particulate fraction. Additional washing resulted in further loss of enzyme activity from the bacterial particles (Table 3). Evidently the enzyme is not firmly attached to the bacterial membranes.

TABLE 3
Distribution of β-glucuronidase in M. leprae *disrupted by ultrasonic oscillation:* µg *of phenolphthalein released*

Sample	Unheated	Heated
Unfractionated suspension	47.0	0
Particulate fraction	15.0	0
Supernatant fraction	20.0	0
I wash of particulate fraction	5.0	0

To determine whether the activity was due to enzyme molecules superficially adsorbed on the bacilli, intact *M. leprae* preparations were treated with trypsin, NaOH and 2 detergents. Trypsin-treatment removed over 50% of the activity from the bacilli. NaOH and sodium dodecylsulfate produced total inactivation, and only a residual activity was left on treatment with deoxycholate (Table 4). Probably, β-glucuronidase is not an inherent property of the leprosy bacilli, but is only superficially attached to the organisms.

TABLE 4
Effect of different treatments on β-glucuronidase of M. leprae: μg *of phenolphthalein released*

Treatment	Untreated bacilli	Treated bacilli
Ultrasonic oscillation	29.0	28.0
Trypsin	66.0	37.0
Deoxycholate	46.0	7.0
Sodium dodecyl sulfate	46.0	0
NaOH	65.0	0

TABLE 5
β-Glucuronidase in M. phlei: μg *of phenolphthalein released*

Sample	Unheated	Heated
Intact bacilli	0	0
Disrupted bacilli	0	0

Another mycobacterium tested, *M. phlei,* both intact and disrupted had no β-glucuronidase (Table 5).

pH OPTIMA

In order to characterize the enzyme activity detected in the untreated *M. leprae* suspensions, properties of the enzyme were compared with those of β-glucuronidase of *E. coli* and of mammalian liver. Bacterial and mammalian β-glucuronidases are distinguished from one another by their pH optima. With phenolphthalein glucuronide as substrate, optimal hydrolysis by the mammalian enzyme takes place at pH 4.5–5.2, and by the bacterial enzyme at pH 6–7 (Levy and Marsh, 1959). The pH-activity curve of the enzyme in armadillo liver is shown in Fig. 2. The pH optima for the β-glucuronidase of *E. coli* and of the *M. leprae* suspension are given in Fig. 3. It may be seen that the activity detected in *M. leprae* resembles closely that of the host tissue, rather than the bacterial enzyme.

REACTION VELOCITY AT HIGH SUBSTRATE LEVELS

Increasing substrate concentrations resulted in a pronounced inhibitory effect on *E. coli* β-glucuronidase. No such effect was detected with mammalian β-glucuronidase or with *M. leprae*; the results obtained with armadillo liver and with the leprosy bacilli gave hyperbolic curves. The reaction velocity of the liver enzyme is shown in Fig. 4. The results obtained with *E. coli* and *M. leprae* are presented in Fig. 5. It is evident that the activity in the leprosy bacilli is similar to that of the host tissue enzyme.

Figure 6 represents the double-reciprocal Lineweaver-Burk plot of the reaction velocity of *E. coli* β-glucuronidase. K_m value of the enzyme was found to be 8.0×10^{-5} M. This compares favourably with the K_m of 7.5×10^{-5}M reported for sheep rumen bacteria (Levy and Marsh, 1959),

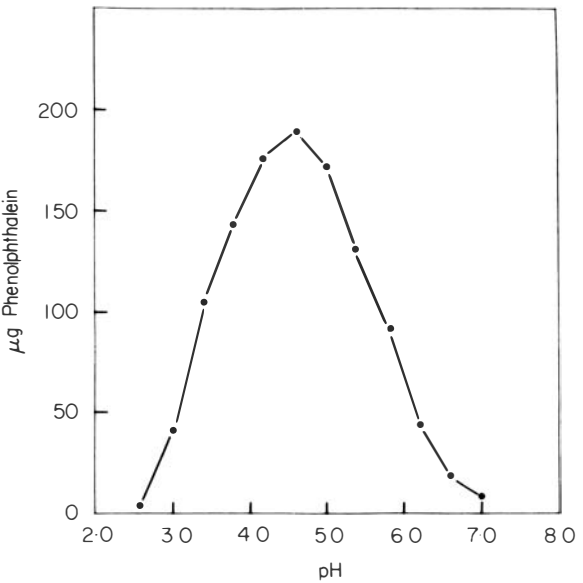

Fig. 2. pH-Activity curve: armadillo liver.

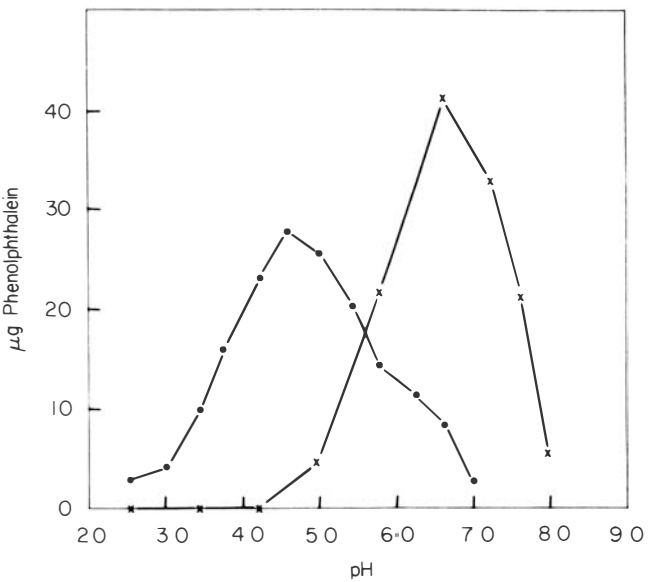

Fig. 3. pH-Activity curve: *M. leprae* and *E. coli*. (●—●) *M. leprae*; (×—×) *E. coli*

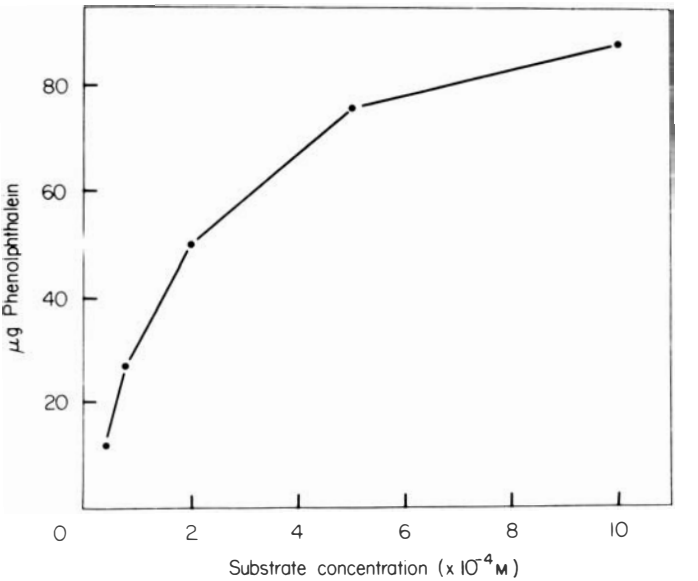

Fig. 4. Reaction velocity at high substrate levels: armadillo liver.

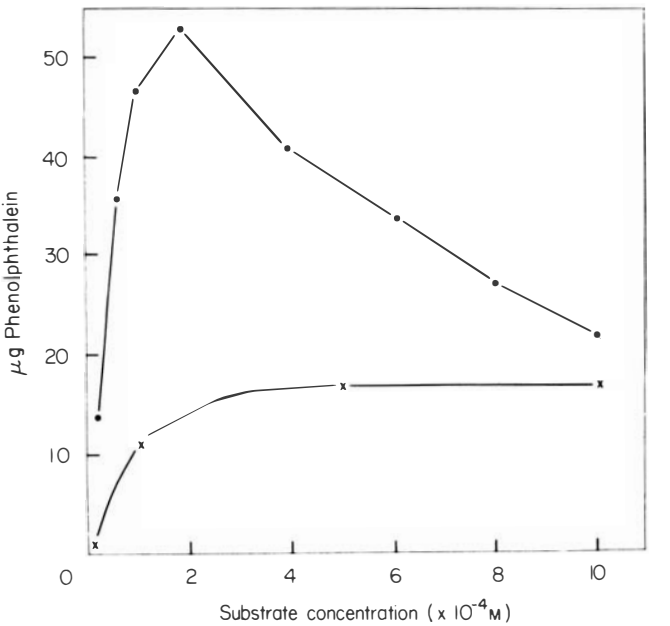

Fig. 5. Reaction velocity at high substrate levels: *M. leprae* and *E. coli*. (✖—✖) *M. leprae*; (●—●) *E. coli*.

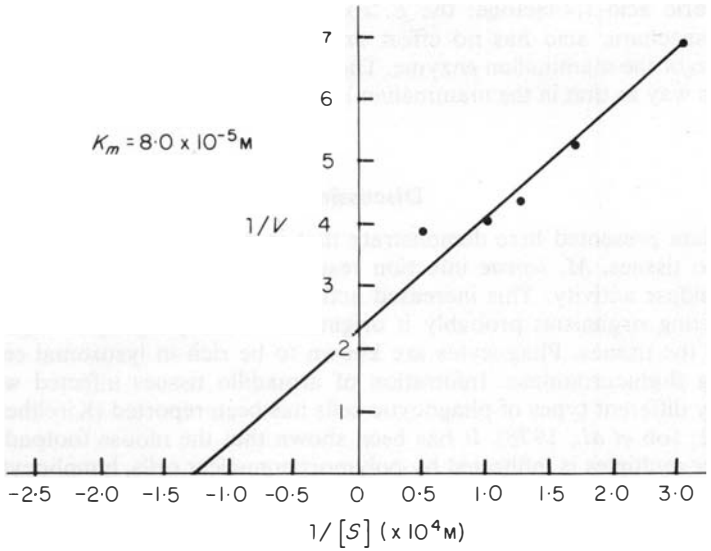

Fig. 6. *E. coli* β-glucuronidase: double-reciprocal plot of substrate concentration *versus* reaction velocity.

confirming the reliability of the assay procedure we adopted for β-glucuronidase. However, it may not be valid to derive such kinetic data with crude preparations like tissue homogenates and the *M. leprae* suspensions.

INHIBITORS

In addition to the above criteria, bacterial and mammalian β-glucuronidases are differentiated by the effects of inhibitors on the enzymes. Contrary to what has been reported recently (Matsuo *et al.*, 1975), ascorbic acid by itself has no inhibitory effect on β-glucoronidase (Levy and Marsh, 1959). We tested 2 specific inhibitors of the enzyme *M. leprae* suspensions, armadillo liver homogenates, and purified bovine liver and *E. coli* β-glucuronidases. The results are given in Table 6. The mammalian enzyme is completely inhibited by

TABLE 6
Effect of inhibitors on β-glucuronidase: μg *of phenolphthalein released*

Sample	D-Saccharic acid-1,4-lactone			Galactosaccharic acid		
	−Inhibitor	+Inhibitor	% Inhibition	−Inhibitor	+Inhibitor	% Inhibition
Armadillo liver	115	0	100	99	26	74
M. leprae	32	0	100	37	5	87
β--glucuronidase (beef liver)	192	0	100	110	11	90
β--glucuronidase (*E. coli*)	56	14	75	59	59	0

D-saccharic acid-1,4-lactone; the *E. coli* enzyme is inhibited only partially. Galactosaccharic acid has no effect on *E. coli,* whereas it shows 70–90% inhibition of the mammalian enzyme. The activity present in *M. leprae* behaves the same way as that in the mammalian liver.

Discussion

The data presented here demonstrate that in mouse footpads as well as in armadillo tissues, *M. leprae* infection results in a significant elevation of β-glucuronidase activity. This increased activity is apparently not derived from the infecting organism; probably it originates in the phagocytic cells which infiltrate the tissues. Phagocytes are known to be rich in lysosomal enzymes including β-glucuronidase. Infiltration of armadillo tissues infected with *M. leprae* by different types of phagocytic cells has been reported (Kirchheimer *et al.,* 1972; Job *et al.,* 1978). It has been shown that the mouse footpad where *M. leprae* multiples is infiltrated by polymorphonuclear cells, lymphocytes and monocytes (Evans *et al.,* 1973).

The activity detected in the *M. leprae* separated from the armadillo tissues seems to be of host-tissue origin. In its properties like pH optima, reaction velocity and effect of inhibitors, the activity in the bacilli resembled β-glucuronidase derived from mammalian tissues rather than the bacterial enzyme. Moreover, the enzyme seemed to be superficially adsorbed on the organisms, and could be easily removed by various treatments. Apparently, β-glucuronidase is not an intrinsic property of *M. leprae.* This conclusion is supported by the finding that another lysosomal enzyme, acid phosphatase, detected in *in vivo*-grown tubercle bacilli is derived from the host cells (Kanai, 1969). The precise role of the lysosomal acid hydrolases in leprosy infection remains to be elucidated.

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Lepr. Rev. (1978) **49,** 215–222

The Effects of Tilorone on Mycobacterial Infections of Mice

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Tilorone (2,7-bis[2-diethylaminoethoxy]fluoren-9-one dihydrochloride), administered in a concentration of 0.05 g per 100 g in the mouse chow, was found to inhibit multiplication of *Mycobacterium leprae* in the mouse footpad. Infection was enhanced in mice inoculated with *M. marinum* or *M. lepraemurium* to which the drug was administered in the same dosage. Tilorone appears to have exerted an antimicrobial effect on *M. leprae* that outweighed the immunosuppressive effect of the drug on the mouse host.

Introduction

We have recently demonstrated that intensive treatment of mice infected with *Mycobacterium leprae* with the interferon inducer polyinosinic:polycytidylic acid (polyI:C) inhibits multiplication of the organisms in the mouse footpad (Levy and Merigan, 1977). These results led us to study the effects on *M. leprae* infection of treatment of mice with another synthetic interferon inducer, tilorone (2,7-bis[2-diethylaminoethoxy]fluoren-9-one dihydrochloride). Treatment with tilorone was accompanied by inhibition of multiplication of *M. leprae* in the mouse footpad, but the character of the inhibition appeared more consistent with that produced by an antimicrobial agent than with an effect that could be attributed to enhancement of host resistance. Therefore, we also studied the effects of tilorone treatment of mice infected with *M. marinum* and *M. lepraemurium.* The results of our studies suggest that tilorone inhibited multiplication of *M. leprae* in the mouse footpad by a direct antimicrobial action rather than by non-specific enhancement of host resistance.

Materials and Methods

Tilorone was generously supplied by Merrell-National Laboratories, Cincinnati, Ohio. The *M. leprae* were of a strain that had been originally isolated from a lesion of a patient with previously untreated lepromatous

Received for publication 25 May 1978.

leprosy by C. C. Shepard, Center for Disease Control, Atlanta, Georgia, and subsequently carried in mouse passage. Inocula of *M. leprae* were prepared from organisms recovered from the footpad tissues of mice inoculated earlier in the hind footpads. The *M. lepraemurium,* of the Hawaiian strain, were supplied by B. H. Tepper, Leonard Wood Memorial Leprosy Research Laboratory, the Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland, and subsequently carried in mouse passage. Inocula of *M. lepraemurium* were prepared from organisms recovered from the omental fat of mice inoculated intraperitoneally (i.p.) earlier. The culture of *M. marinum* was a gift of A. Back, City-County Health Department, San Francisco, California. The mice were locally bred BALB/c mice. Drug was incorporated into the mouse chow by means of a liquid-solid twin-shell blender (Patterson-Kelly Co., East Stroudsburg, Pennsylvania).

Inoculation of mice in the footpad with *M. leprae* or *M. marinum* and harvest of acid-fast bacteria (AFB) from the infected tissues were performed by published methods (Ng *et al.,* 1973; Shepard, 1960; Shepard and McRae, 1968). In the case of *M. leprae*-infected mice, the harvested AFB were only enumerated. In the case of mice inoculated in the footpad with *M. marinum,* the harvested AFB were enumerated by microscopic examination, and the numbers of colony-forming units (CFU) were counted in aliquots of the same suspensions. Footpad swelling was measured by means of a "Schnelltaster" caliper ("Quick-test", Dyer Co., Inc., Lancaster, Pennsylvania). Mice were also inoculated intravenously (i.v.) in a tail vein with *M. marinum* or i.p. with *M. lepraemurium.* These mice were observed at short intervals, deaths were recorded, and the "survival index" of Smith and Westgarth (1957) was calculated.

Results

M. LEPRAE INFECTION

Groups of 15 mice each were inoculated with $10^{3.7}$ *M. leprae* in each hind footpad. Several groups served as untreated controls. To one group of mice, tilorone was administered incorporated in the mouse chow in a concentration of 0.05 g per 100 g, the maximal tolerated dosage, for a period of 30 days, beginning 70 days after inoculation. Tilorone was administered in the same dosage to the mice of another group for 150 days, beginning on the day of inoculation. The results of treatment of *M. leprae*-infected mice with tilorone are shown in Fig. 1, in which each point represents the results of harvest of *M. leprae* from the pooled tissues of 4 footpads. The 95% confidence limits around the results of such a harvest are -50% and +100% when the yield of AFB per footpad is near 10^6 (Krushat *et al.,* 1976). The broken line represents the linear regression of the $\log_{10}$ number of *M. leprae* per footpad on the number of days after inoculation of untreated control mice during logarithmic multiplication. The slope of the regression line yields an estimated doubling time of 12.4 days. The harvest of *M. leprae* performed 147 days after inoculation yielded $10^{5.39}$AFB per footpad of mice treated between days 70

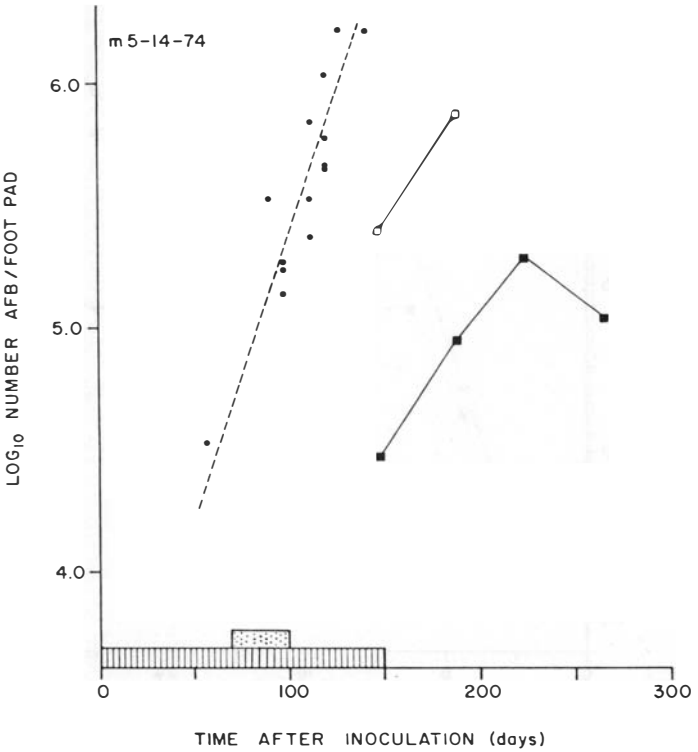

Fig. 1. Multiplication of *M. leprae* in the footpads of mice as a function of time after inoculation. The broken line is the regression of the $\log_{10}$ number of AFB per footpad in untreated mice on the number of days after inoculation. (●) Results of harvests of *M. leprae* from the footpads of untreated mice; (■) results of harvests from mice treated with tilorone for 150 days from day of inoculation; (□) results of harvests from mice treated with tilorone for 30 days from day 70 after inoculation. The shaded bars along the abscissa represent the periods of drug administration.

and 100 after inoculation, and $10^{4 \cdot 48}$ AFB per footpad of mice treated from the day of inoculation. Subsequent harvests demonstrated that, after termination of treatment, the organisms multiplied in both groups of mice. After the mice had been treated with tilorone for 30 days, the *M. leprae* multiplied almost to the maximal level of $10^{6 \cdot 0}-10^{6 \cdot 3}$ at a rate not greatly different from that in the untreated mice. After the 150-day period of treatment with tilorone, the organisms multiplied at the same rate but to a somewhat lower maximum, plateauing at the level of $10^{5 \cdot 03}-10^{5 \cdot 28}$. These results demonstrate that multiplication of *M. leprae* was modestly inhibited in mice treated with tilorone administered for 30 days during logarithmic multiplication of the organisms. Multiplication of *M. leprae* was inhibited more strongly in mice treated with tilorone for 150 days, beginning on the day of inoculation.

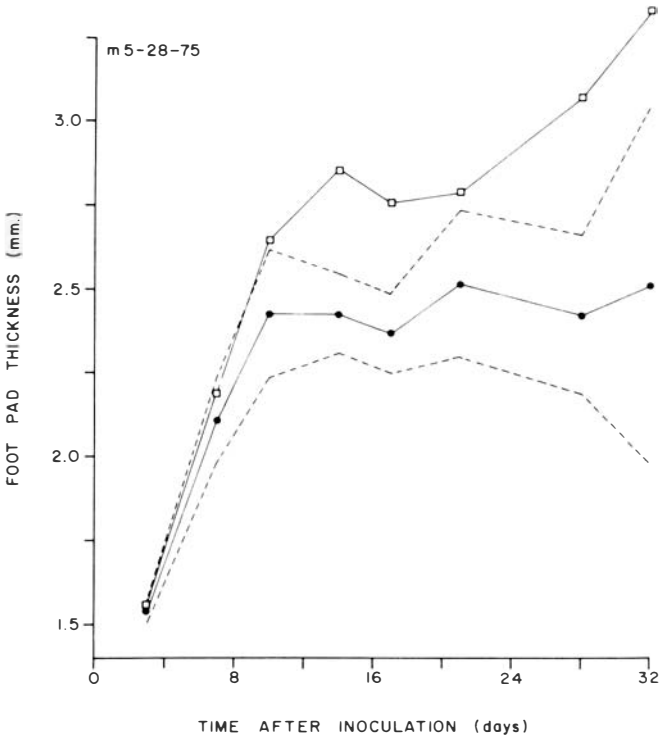

Fig. 2. Footpad thickness of mice as a function of time after inoculation with *M. marinum*. (●) Results of measurements in untreated mice; (□) results of measurements in tilorone-treated mice. (— — —) 95% confidence limits around measurements of footpad thickness in untreated mice. The points at 28 and 32 days represent measurements made on 6 feet (3 mice); all other points were based on measurements of 10 feet (5 mice).

INFECTION WITH *M. MARINUM* AND *M. LEPRAEMURIUM*

The effects of tilorone treatment of mice infected with *M. marinum* and *M. lepraemurium* were also studied. In one experiment, tilorone was administered continuously in a concentration of 0.05 g per 100 g of diet to 10 mice inoculated with $10^{3 \cdot 7}$ *M. marinum* in both hind footpads, beginning one week before inoculation. Ten untreated mice similarly inoculated were observed as controls. As shown in Fig. 2, footpad swelling was greater in the tilorone-treated mice than in the untreated control mice. The results of harvests of *M. marinum* from the infected footpad tissues of some of the same control and tilorone-treated mice are summarized in Table 1. In the control mice, the numbers of AFB and of CFU appeared to reach maximal values 14–21 days after inoculation, after which the numbers diminished. Maximal numbers of AFB and CFU were achieved in tilorone-treated mice at about the same time as in control mice; however, the maxima were at least one order of magnitude

TABLE 1

Results of harvests of M. marinum *from untreated and tilorone-treated mice after footpad inoculation*

Treatment	Time after inoculation (days)	AFB No. per footpad ($\times 10^5$)	CFU No. per footpad ($\times 10^5$)
None	7	8.42(4.21–16.8)*	1.43(1.32–1.54)
	14	11.4(5.70–22.8)	1.25(1.03–1.47)
	21	10.2(5.10–20.4)	4.24(2.95–5.53)
	49	1.47(0.74–2.94)†	0.10(0.07–0.13)†
Tilorone 0.05 g/100 g	7	7.88(3.94–15.8)	1.34(1.24–1.44)
	14	64.2(32.1–128)	80.2(79.7–80.7)
	21	82.0(41.0–164)	94.0(93.0–95.0)
	49	114(57.0–288)†	112(104–120)†

*Mean (95% confidence limits).
†Each value for the number of AFB or CFU per footpad was derived from the pooled footpad tissues of 2 mice (4 footpads), except for the 49-day values, which were derived from harvests from the pooled tissues of 4 mice (8 footpads).

greater in the treated mice, and the numbers did not decrease during the period of observation. In two subsequent experiments, mice were challenged i.v. with $10^{6 \cdot 6}$ or $10^{7 \cdot 6}$ *M. marinum* suspended in Hanks' balanced salt solution (BSS); control mice were left untreated, whereas 0.05 g per 100 g tilorone was administered to other groups of mice continuously, beginning 1 week before challenge. As shown in Table 2, the survival of tilorone-treated mice was much shortened compared to that of untreated mice.

In a final experiment, untreated mice and mice to which the administration of 0.05 g per 100 g tilorone was begun on the day of inoculation were challenged i.p. with 10^7 or 10^8 *M. lepraemurium* suspended in BSS. As shown in the lower portion of Table 2, the survival of tilorone-treated mice after challenge with *M. lepraemurium* was much shortened compared to that of control mice. Although the intake of tilorone by *M. marinum*- and *M. lepraemurium*-infected mice was not measured, it undoubtedly decreased as the animals became ill. Nevertheless, it appears certain that the mice died of infection and not of drug toxicity. The *M. leprae*-infected mice administered tilorone in the same dosage for 150 days showed no evidence of toxicity; as demonstrated by the data of Table 2, no *M. marinum*- or *M. lepraemurium*-infected mouse treated with tilorone survived longer than 118 days.

Discussion

Poly I:C has been demonstrated to protect mice against infection with a wide variety of bacterial, fungal, and protozoal pathogens (Merigan, 1973), and we have recently demonstrated that intensive treatment of mice with poly I:C for a short time during logarithmic growth inhibits multiplication of *M.*

TABLE 2
Survival of untreated and tilorone-treated mice after intravenous challenge with M. marinum *or intraperitoneal challenge with* M. lepraemurium

Experiment no.	Challenge	Survival	
		Control	Tilorone-treated
1	*M. marinum* $10^{7.6}$	25.4* [20, 26, 30, 33(7), 41(9),† 44, 48(3), 56(4), 57, 59(2)]	51.3 [12, 16, 20(27), 23]
2	*M. marinum* $10^{7.6}$	18.7 [30, 35(2), 38(2), 41, 46(5), 59, 83,(4), 84, 93, 132, 135]	41.7 [18(5), 21(2), 23(4), 25(5), 46(4)]
	$10^{6.6}$	<7.37 [41, 46, 65, 83(2), 155, 187, 260(13)‡]	29.0 [21(2), 23, 28(4), 30(3), 46(10)]
3	*M. lepraemurium* 10^{8}	7.02 [106, 113(3), 125(3), 128(3), 149(2), 160, 167, 222, 229, 238(2)]	11.0 [83, 86, 90(4), 93(2), 97(2)]
	10^{7}	5.27 [149(2), 167, 170(2), 173(2), 183, 187, 194, 201, 204(2), 208, 211, 222(4), 225]	9.25 [97(2), 100, 111(2), 113(2), 118(2)]

*Survival index of Smith and Westgarth (1957).
† [Number of days from inoculation to death (number dead on day indicated)]. If the number dead is not specified, only one mouse was found dead.
‡The number of mice indicated within the parenthesis survived on the day indicated.

leprae by a mechanism that does not depend upon interferon induction (Levy and Merigan, 1977). The purpose of this study was to examine the possibility that tilorone, another synthetic inducer of interferon, might also non-specifically enhance the resistance of mice to infection with *M. leprae*.

The distinction between a direct antimicrobial effect of a drug and action of a drug to enhance host resistance may not be easily made. In the case of poly I:C, the inference that the effect was exerted on the host and not on *M. leprae* rested primarily on the extremely broad spectrum of drug action (Levy and Merigan, 1977; Merigan, 1973). When tilorone was administered in an oral dosage of 0.05 g per 100 g (50–100 mg per kg body weight) to *M. leprae*-infected mice, multiplication of the organisms was inhibited. Consistent with a direct antimicrobial effect was the apparent resumption of multiplication of *M. leprae* virtually immediately after cessation of drug administration at a rate not very different from that in untreated mice. Failure of the organisms to multiply to the level of 10^6 per footpad after the end of the 150-day period of tilorone administration may be taken as evidence of enhanced resistance of the mice. However, it appears equally likely that the *M. leprae* infection was eradicated

in the footpads of some of the treated mice, whereas organisms survived treatment and were able to multiply to the normal maximum in the footpads of other mice once treatment had stopped; because the harvests were made from the pooled tissues of 4 footpads, tissues devoid of *M. leprae* diluted the organisms present in the other footpad tissues in the pool, yielding mean numbers of *M. leprae* per footpad significantly smaller than the normal maximum.

The results of the studies in *M. marinum*- and *M. lepraemurium*-infected mice appear clearly to demonstrate that the resistance of the mice to these pathogens is decreased as a consequence of tilorone treatment.

This dual effect of tilorone on mycobacterial infections of mice should, perhaps, have been anticipated. Before this work was begun, it had already been reported that, although tilorone demonstrated antiviral effects both *in vivo* and *in vitro,* including some effects possibly not mediated by the mechanism of interferon induction, the drug prolonged survival of skin allografts in mice (Mobraaten *et al.,* 1973), both stimulated and inhibited the resistance of mice to challenge with allogeneic leukemia cells (Friedlander *et al.,* 1974), and selectively depleted the lymphocytes of thymus-dependent areas of the lymphoid tissues of mice and rats (Levine *et al.,* 1974). More recently, the drug has been shown to exhibit anti-inflammatory properties in rats (Megel *et al.,* 1975), the survival of allografts in rats has been shown to be prolonged by tilorone (Wildstein *et al.,* 1976), and inhibition of T-lymphocyte function has been confirmed (Gibson *et al.,* 1976; Megel *et al.,* 1974, 1976).

Particularly pertinent are the results of studies by Collins (1975) and by Gruenewald and Levine (1976). Collins found that oral administration of tilorone at dosages of 10 or 100 mg per kg body weight on alternate days to mice inoculated with *Listeria monocytogenes, M. bovis* (BCG), *M. tuberculosis* H37Rv, or *Salmonella enteritidis* was accompanied by enhanced multiplication of the organisms, and reduction of the response to tuberculin in the case of the mycobacterial infections. The minimal inhibitory concentrations of tilorone were 60 μg per ml for BCG, 250 μg per ml for *L. monocytogenes,* and 500 μg per ml for *S. enteritidis.* Grunewald and Levine found that tilorone, administered in a single subcutaneous dose of 50 mg per kg, increased susceptibility of mice to intravenous challenge with *L. monocytogenes.*

Thus, it appears likely that, in the case of *M. leprae*-infected mice, tilorone exerted the same immunosuppressive effect demonstrated by our studies of *M. marinum*- and *M. lepraemurium*-infected mice, and by the studies of Collins (1975) and Gruenewald and Levine (1976) of mice infected with *L. monocytogenes, M. tuberculosis,* and BCG. That multiplication of *M. leprae* was inhibited during the period of tilorone treatment of the mice suggests, therefore, that *M. leprae* are much more susceptible to the antimicrobial effect of tilorone than either of the other two mycobacterial species studied.

Acknowledgement

This work was partially supported by the U.S. Leprosy Panel of the U.S.-Japan Cooperative Medical Science Program administered by the Geographic

Medicine Branch of the National Institute of Allergy and Infectious Dieseases, National Institutes of Health, Bethesda, Maryland, U.S.A. (Interagency Agreement IAA 2-YO1-AI-10004).

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Lepr. Rev. (1978) **49**, 223–229

Concentration and Persistence of Bacilli in the Fingers and Toes of Patients with Lepromatous Leprosy

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In 41 patients with lepromatous leprosy, the fingers and toes were found to be the site with the highest bacillary load, the fingers being more productive than the earlobe or buttock and the toes being more productive than the buttock. Neither was the bacillary index at the finger significantly different from that at the toe, nor was the bacillary index at the terminal phalanx significantly different from that at the middle phalanx in either the finger or the toe. However, the terminal phalanx of the finger harboured more solid bacilli than the middle phalanx. In 14 long-treated low index cases where BI had registered a fall, and was not more than 2+ at any of the routine smear sites, the fingers and toes harboured more bacilli than the earlobe. In one long treated smear negative case, the terminal phalanges of the fingers and toes proved to be the only skin sites positive for bacilli, all other routine sites, being acid-fast bacilli negative.

Introduction

Ridley and Jopling have reported that the fingers were the skin sites with the greatest bacterial load and the greatest number of solid staining bacilli following a study on 30 patients with lepromatous leprosy (Ridley *et al.*, 1976). It was decided to see whether this concentration of bacilli held good in tropical India as well. They had studied toes only in 8 cases. Since closed footwear is hardly used in India, it was decided to study toes as well, hoping to compare the bacillary load at fingers to that at toes in barefoot patients.

Material and Methods

Forty-one patients with lepromatous leprosy from the Schieffelin Leprosy Research & Training Centre, Karigiri, were studied at random. All of them had treatment for periods ranging from 6 months to 20 years. Surprisingly we

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Received for publication 11 May, 1978.

0305–7518/78/0901–0223$01.00/0

found no barefoot patients. All patients studied wore the open type of footwear, i.e. sandals or slippers that are closed at the sides but leave the toe-tips exposed. Smears were taken from routine sites such as right ear-lobe, left forehead, right chin, and left buttock. Nasal mucosa was also studied, but as part of another project. It has not been included as a routine site in statistical calculations in this study. The special sites studied were:

(a) dorsum of the middle and terminal phalanges of the middle finger of both hands;
(b) dorsum of the middle and terminal phalanges of the second toe of both feet.

In either case (1) if there was a clinical lesion on the digit or (2) if the digit was missing, the adjacent digit was studied (3rd toe in the case of feet). The modified Ziehl-Neelsen method was used for staining; BI was estimated according to Ridley's logarithmic scale (Ridley, 1964) and MI according to the criteria of Waters and Rees (Waters and Rees, 1962).

On finding that more bacilli were found at the fingers and toes as compared to accepted sites such as earlobe and buttock, it was decided to see whether the fingers and toes harboured bacilli in greater numbers in long-treated cases where the BI at routine sites had registered a fall. We arbitrarily classified those with a maximal bacillary load not exceeding 2+ at any of the routine sites as low index cases, and compared the bacterial load at fingers and toes to that at routine sites in those cases. Fourteen such cases were studied, all having had treatment for 3 years or more. We also studied 8 long-treated smear negative cases at routine and special sites to check for persistence of bacilli, if any, at the fingers or toes.

Results

The results of the initial random study are summarized in Table 1.

BACTERIAL INDEX RESULTS

(a) The maximal BI at the fingers was significantly greater than the maximal BI at the earlobes, the BI at the middle phalanx being greater than that at the earlobe ($P < 0.02$), and the BI at the terminal phalanx also being greater than that at the earlobe ($P < 0.05$).
(b) The maximal BI at the fingers was significantly greater than the maximal BI at the buttock; the BI at middle phalanx being greater than that at the buttock ($P < 0.001$), and the BI at the terminal phalanx also being greater than that at the buttock ($P < 0.01$).
(c) In 35 out of 39 cases, the finger was more productive than the nasal mucosa (in 2 cases nasal mucosa could not be studied).
(d) In 26 out of 41 cases the finger was more productive than the forehead and in 29 out of 41 cases, more productive than the chin.
(e) The maximal BI at toe was significantly greater than the maximal BI at buttock, the BI at middle phalanx being greater than that at the buttock ($P < 0.01$), and the BI at terminal phalanx being greater than that at the buttock ($P < 0.02$).

TABLE 1

Bacterial and morphological indices of patients by routine sites, finger and toe

Patient	Earlobe		Forehead		Chin		Buttock		Finger		Toe		Nasal mucosa		Duration of treatment (years)
	BI	MI	BI	MI	BI	MI	BI	MI	BI	MI	BI	MI	BI	MI	
1	6	0.5	5	0.5	4	0	4	0	5	1	5	0	2	0	2.5
2	4	0	4	0	3	0	3	0.5	4	0.5	4	0.5	1	0	10
3	3	0	3	0	5	0	3	0	4	0	4	0	1	0	10
4	3	0	3	0	2	0	2	0	4	0	3	0	4	0	1
5	4	1	4	0	4	0	4	0	6	1	4	0.5	5	1.5	8
6	3	0.5	3	0.5	3	0.5	3	0.5	4	1.5	5	2	2	0	13
7	6	0	5	1	4	1	4	1	6	1.5	5	1	1	1	7
8	5	0	4	0	3	0.5	4	0	6	0	5	0.5	0	0	2.5
9	3	0	2	0	1	0	3	0	5	0	3	0	0	0	7
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15
11	0	0	1	0	0	0	0	0	0	0	0	0	0	0	20
12	2	0	3	0	5	0	4	0	4	0	4	0.5	0	0	9
13	4	0	4	0	4	0	4	0	4	0	4	0	2	0	1
14	0	0	0	0	0	0	0	0	2	0	2	0	0	0	20
15	1	0	0	0	0	0	1	0	2	0	2	0	2	0	3
16	2	0	1	0	0	0	0	0	3	0	3	0	0	0	4
17	0	0	0	0	0	0	2	0	1	0	1	0	0	0	12
18	1	0	0	0	1	0	1	0	1	0	0	0	0	0	3
19	1	0	1	0	2	0	1	0	2	0	3	0	1	0	20
20	3	0	3	0	2	0	3	0	3	0	4	0	0	0	1.5
21	4	0	4	0	3	1	3	1	4	0.5	5	3	3	0	4
22	1	0	0	0	0	0	1	0	2	0	1	0	0	0	5
23	6	0	6	1.5	3	0	4	0	4	0	3	1	3	0	12
24	4	0	4	1.5	4	0	3	0	3	0	3	1	0	0	0.5
25	3	0	2	0	2	0	2	0	5	0.5	3	0	1	0	4
26	4	0	4	0	5	0	3	0	5	0	4	0	2	0	4
27	5	0	5	0	5	0	4	0	4	0	5	0.5	2	0	5
28	4	0	3	0	3	0	1	0	4	0	2	0	2	0	2
29	2	0	3	0	2	0	0	0	3	0	2	0	0	0	3
30	0	0	1	0	1	0	1	0	2	0	1	0	0	0	15
31	6	1	4	0	4	0	3	0	4	0	6	1	3	1.5	2
32	2	0	2	0	2	0	1	0	4	0	5	0	0	0	16
33	2	0	3	0	2	0	4	0	3	0	3	0	0	0	5
34	1	0	1	0	1	0	2	0	2	0	2	0	0	0	6
35	2	0	2	0	1	0	2	0	0	0	1	0	—	—	4
36	2	0	2	0	1	0	2	0	3	0	3	0	—	—	5
37	3	0	3	0	2	0	4	0	4	0	2	0	0	0	1
38	5	0	4	0	4	0	4	0	5	1	3	0	0	0	3
39	3	1	4	0	4	0	4	0	5	0.5	4	0.5	2	0	12
40	3	0	3	0	2	0	3	0	3	0	3	0	1	0	5
41	4	0	3	0	2	0	3	0	4	0	3	0	0	0	4

(f) The toes were more productive than the forehead in 18 out of 41 cases, and as highly productive as the forehead in 15 out of 41 cases.

(g) The toes were more productive than the chin in 23 out of 41 cases, and more productive than the nasal mucosa in 31 out of 39 cases.

(h) Five per cent of the patients who were acid-fast bacilli negative at the earlobe and buttock showed bacilli at the fingers and toes.

When BI at earlobe and buttock were low, the fingers and toes showed a greater bacillary load.

No statistically significant difference was observed between the average or maximal BI at fingers or toes as compared to the same at routine sites. However, while the routine site with maximal BI varied from patient to patient the fingers and toes proved to be sites with bacillary loads as high as that at the best routine site in 32 out of 41 cases. The BI at the fingers was not significantly different from that at the toes. The BI at the terminal phalanx was also not significantly greater than that at the middle phalanx in either the finger or toe.

MORPHOLOGICAL INDEX RESULTS

(a) The maximal MI at fingers was significantly greater than that at the buttock ($P < 0.05$). However, it was not significantly greater than that at the earlobe.
(b) The maximal MI at the terminal phalanx of the finger was significantly greater than the maximal MI at the middle phalanx, of the finger ($P < 0.05$). However such a difference did not exist in the case of toes.
(c) The maximal MI at the toes was significantly greater than the maximal MI at the earlobe ($P < 0.05$).
(d) The maximal MI at the toes was also significantly greater than the maximal MI at the buttock ($P < 0.01$).
(e) However, no significant difference was seen between the average or maximal MI at finger or toe when compared to the same at routine sites. The MI at finger was not significantly different from that at the toe.

RESULTS FOR THE 14 LOW INDEX CASES

(a) In 10 out of 14 cases the finger was more productive than the earlobe.The maximal BI obtained at the finger was significantly greater than that at the earlobe ($P < 0.05$).

TABLE 2

Special site	BI	MI
Right middle finger, middle phalanx	0	0
Left middle finger, middle phalanx	0	0
Right second toe, middle phalanx	0	0
Left second toe, middle phalanx	0	0
Right middle finger, terminal phalanx	2+	0
Left middle finger, terminal phalanx	1+	0
Right second toe, terminal phalanx	2+	0
Left second toe, terminal phalanx	0	0
Nasal mucosa	0	0

Routine sites were all acid-fast bacilli negative.

The 8 smear negative cases studied for persistence of bacilli at routine sites, fingers and toes were AFB-negative at all sites.

(b) In 11 out of 14 cases, the finger was more productive than the forehead.
(c) In 9 out of 14 cases the finger was more productive than the chin.
(d) In 9 out of 12 cases the finger was more productive than the nasal mucosa.
(e) The toe was more productive than the earlobe in 9 out of 14 cases. The maximal BI obtained at the toe was significantly greater than the maximal BI at the earlobe ($P < 0.05$).
(f) The toe was more productive than the forehead in 9 out of 14 cases and more productive than the chin in 9 out of 14 cases.
(g) The toe was more productive than the nasal mucosa in 8 out of 12 cases.
(h) However no statistically significant difference was got between the BI's at toe and buttock. Also the average or maximal BI at finger or toe was not significantly different from that at the routine sites. But while the routine site with maximal BI varied from person to person, the finger and toe were sites consistently as highly productive for bacilli as the best routine site in 11 out of 14 cases.

CASE NO. 14

This case merits special mention because here the fingers and toes were the only skin sites positive for acid-fast bacilli, all other routine skin sites being acid-fast bacilli negative. The patient is a 27-year-old male with a 15-year history of disease on regular treatment for 10 years. His BI had registered a progressive fall with treatment. In 1967 his average BI had been 3.62 and it fell to 0.75 in 1974. No smears were done after that until 1977 when on routine smear examination he was found to be AFB-negative. However, on routine and special site smear studies, we got the results given in Table 2.

Discussion

Our findings are similar to those of Ridley and Jopling. Comparing the bacillary load at each routine skin-smear site including nasal mucosa to that at the finger, it was found that the finger harboured significantly greater numbers of bacilli than all these sites. Similarly, the toes proved more productive than the chin, buttock and nasal mucosa. Besides this, in long-treated cases, bacilli were found to persist in significantly greater numbers at the fingers and toes as compared to the earlobe, forehead, chin and nasal mucosa. These findings argue for the inclusion of finger skin smears on a routine basis for the bacteriological diagnosis of leprosy, assessment of progress following treatment, and finally before declaring a patient AFB-negative. The middle phalanx of the finger gave a BI as good as the terminal phalanx, but the MI at the terminal phalanx was significantly greater than that at the middle phalanx. This further suggests that the dorsum of the terminal phalanx of the finger be the site for skin smears.

The epidemiological significance of these findings is not known. Pedley, following his "contact smear" study (Pedley, 1970) has shown that bacilli probably do not emerge through intact skin. Even so, the high incidence of ulceration due to the vulnerability of the constantly used anaesthetic finger,

promises a fair amount of infective potential to the lepromatous finger, so rich in solid-staining bacilli.

Just why the bacilli should concentrate in the fingers is not known. Thermographic studies by Hastings (1968), and Anish (1971), have shown that bacilli are present in greater numbers in the cooler areas of the body. Anish reports a relatively higher bacillary load in the cool forearm as compared to the warm axilla and scalp in patients with lepromatous leprosy. That the MI at the terminal phalanx was greater than that at the middle phalanx in the finger suggests that there may be a temperature gradient across the phalanges. A lower drug concentration at the fingertips consequent to a lesser perfusion as a result of disuse atrophy, and probably ulceration and fibrosis could be another reason for a high and persisting bacillary load in these areas. Such a decreased perfusion would make the finger a cooler site also. However, only thermographic studies can give a final answer. The rich nerve supply of the finger may be another reason why the neurotropic bacilli are present there in such large numbers.

But why the toes, which have an equally rich nerve supply and suffer similar trophic changes should register a relatively low BI is another poser. But then, none of the patients studied were barefoot. Even with the open type of footwear, air circulation is restricted and constant contact with the same surface is bound to generate a certain amount of frictional heat, all of which would go to make the toes not-so-cool a site. Still it is noteworthy that there were more solid bacilli in the toes than at the earlobe or buttock.

An important finding was that in at least one patient, who was AFB-negative at routine sites, we found bacilli persisting in the fingers and toes. It is striking that all the bacilli detected were at the terminal phalanges, the middle phalanges being AFB-negative. Perhaps like the nerve and Dartos muscle, which are proven sites of bacillary persistence, the terminal phalanx harbours persisters as well. Such persisters might play an important role in relapse in lepromatous patients.

Our not finding AFB in the fingers or toes of the 8 smear-negative cases does not necessarily go against this hypothesis since this study has all the disadvantages of a cross-sectional study. Perhaps the persisting bacilli if any had also been eradicated. The ideal way of studying persistence would be to do skin smears from routine sites and fingers and toes at intervals following initiation of therapy in patients with lepromatous leprosy and to check for persistence of bacilli in large numbers at the fingers and toes while the routine sites registered a fall in BI. We intend following up this patient to see whether he is heading for a relapse. If so, at least in his case, the terminal phalanges of the fingers and toes were the skin sites at which the bacilli persisted.

Acknowledgements

We are indebted to Mr Muthuratnam of the Department of Biostatistics, Christian Medical College and Hospital, Vellore, for analysis of the data and to Mr J. Ramamurthy for his secretarial help. We are also grateful to Mr K. Vilwanathan for his assistance in carrying out the study.

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Ocular Leprosy in Iran: Findings of a Random Survey at the Baba Baghi Leprosarium, Tabriz*

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Ocular manifestations in 100 cases of leprosy, mostly lepromatous, in a racial group susceptible to serious leprosy are presented. The pathogenesis of the eye involvement is discussed. Its prevention and management are recounted solely with a view to emphasize the fact that the institution of simple procedures will go a long way in calling a halt to the ocular tragedy in leprosy.

"To lose one's eye sight when the sense of touch has already gone is a double disaster and it requires only a little imagination to appreciate the magnitude of such calamity."

Walter Fancott

Introduction

Visual impairment, sometimes culminating in total loss of vision is a major tragedy in the life of a patient with leprosy. Deformities, disabilities and neuropathic ulceration in the peripheral parts of the extremities sometimes lead to dehabilitation of the patients, thereby entailing loss of self-support, man power and sometimes even self respect. Such a situation can, however, be stemmed and even retrieved by instituting appropriate preventive and corrective measures, and the patient returned to society as a self-reliant and useful citizen. In sharp contrast, involvement of the eyes with the consequent impairment or loss of vision in the course of leprosy infection renders the patient utterly helpless and absolutely dependent. The fact that this visual disability or loss of vision is to a large extent preventable, if only certain simple and appropriate measures had been taken in time, adds poignancy to the situation.

When we started work at the Baba Baghi Leprosarium in 1975, one of the things that arrested our attention was the unusually large number of patients with facial nerve damage manifesting itself often as a total facial paralysis. The frequency and severity of damage to the unprotected eye consequent on the

Received for publication 3 March 1978.

* Based on a paper presented at the Seminar on "Evaluation of leprosy", held in Teheran, Iran, in June 1976.

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0305–7518/78/0901–0231$01.00/0

nerve paralysis resulting in various grades of visual loss was also striking. Damage to the facial nerve does occur in leprosy, but the frequency of such damage encountered here was out of all proportion to that seen in India. It was the experience of one of us (K. R.) that in India the involvement of the facial nerve in leprosy was very infrequent and that this more often manifested itself as an isolated paralysis of its zygomatic branch rather than that of the entire nerve. In the Baba Baghi Leprosarium the visual damage sustained by some of the patients in all types of leprosy consequent on the loss of the protective mechanism of the eye and its subsequent neglect was supplemented by the occurrence of chronic plastic iridicyclitis in the lepromatous cases. There were a few instances where both these factors were in operation in the same subject. In view of the magnitude and severity of the the ocular involvement it was felt worthwhile to undertake a random survey of the ocular damage caused by leprosy in this institution with a view to determine its frequency and nature.

Material and Methods

Our random survey of the ocular involvement was confined to the cases admitted into the wards of the Baba Baghi Leprosarium for the treatment of the complications of leprosy or intercurrent illnesses and also to the patients attending the outpatient department for advice. The patients were first subjected to a clinical examination supplemented with the examination of skin smears in order to classify the cases. This was followed by examination of the face in general, and of the eyes and their adnexa in particular. The findings were recorded on a suitably drawn proforma which included the biodata of the patient, the clinical classification of the case, the presence of paralysis or paresis of the muscles of the face, sensory status of the face and the cornea, presence of madarosis and the condition of the eyes in respect of the sclera, cornea, iris and the pupil. The elicitation of corneal sensation in some of the subjects was found to be very difficult because of the total paralysis of the eyelid muscles. The examination of the eye was carried out with a hand-torch and binocular loupe.

None of us was a qualified ophthalmologist, but owing to long association with the speciality (leprosy) and by force of circumstances, a working knowledge of the recognition and management of the ocular involvement in leprosy had been acquired. This is our apology for any shortcomings or mis-interpretation of findings in this paper.

The survey under report covers the findings in 100 patients, comprising 80 lepromatous, 8 borderline and 12 indeterminate cases examined at random. In some of these subjects who appeared to have been quiescent for years ('burnt-out' cases) the classification of the disease was made from the residual signs or stigmata of the disease when present and occasionally by surmise.

Findings

The findings of the study are briefly as follows:

(1) The involvement of the seventh cranial nerve manifesting as total facial paralysis or isolated paralysis of the zygomatic branch was detected in 49.0%

of the cases examined, the former accounting for 26.0% and the latter for 23.0%. These patients exhibited varying grades of visual defect as a result of the damage sustained by the cornea in the unprotected eye. This manifested itself as anything varying from nebula and leucoma to complete replacement of the cornea by dense scar tissue (Figs 1 and 2). This type of ocular involvement occured in all types of leprosy. The taste sensation on the anterior two-thirds of the tongue could not be tested in these subjects owing to the difficulty in communicating with each other. This is mentioned particularly because one of

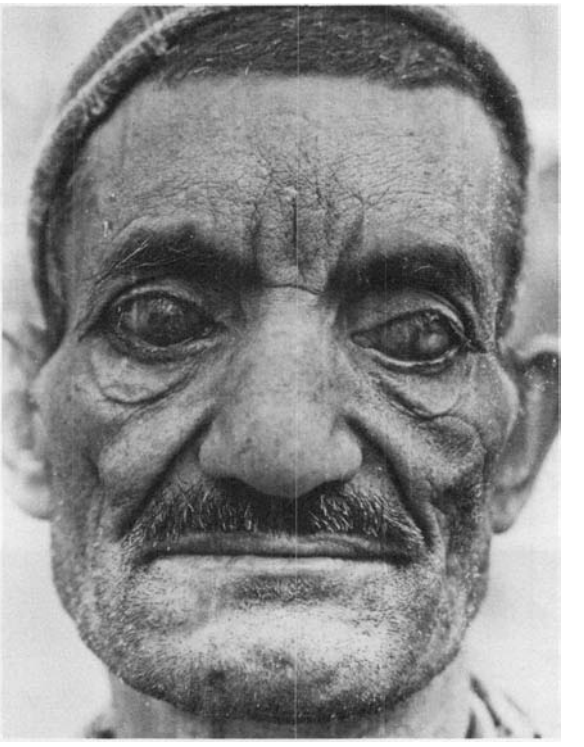

Fig. 1. Bilateral lagophthalmos with exposure keratitis.

us (K. R.) while working at the Central Leprosy Teaching Research Institute, Chengalpattu, India, detected the loss of taste over the anterior two-thirds of the tongue in 2 patients who manifested isolated paralysis of the zygomatic branch.

(2) The involvement of the elements in the anterior segment of the eye with its protean manifestations consequent on bacillary invasion was observed in the multibacillary types of leprosy, especially lepromatous cases. One of the surprising findings was the presence of signs of chronic plastic iridocyclitis in 2 cases clinically labelled as indeterminate leprosy. The manifestations in the

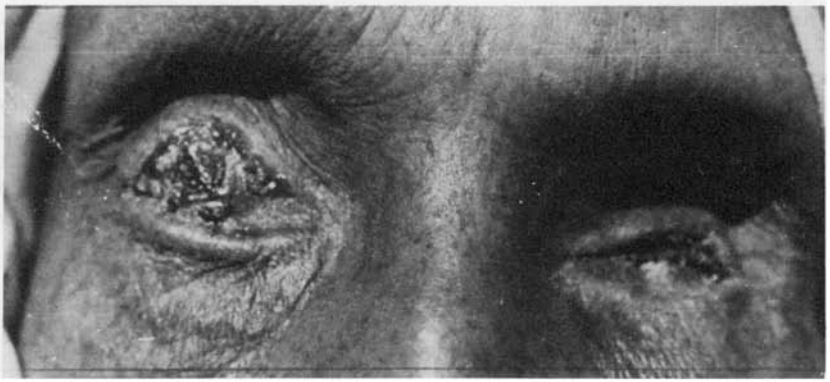

Fig. 2. Replacement of the cornea by dense scar tissue.

order of their frequency were: chronic plastic iridocyclitis with its sequelae, sclerosing keratitis, subscleral nodule, corneal and ciliary staphyloma. Iris pearls or lepromata were not detected in any of the cases by the procedure adopted. Cataract, not an infrequent complication in lepromatous cases in India, was seen in 4 subjects. What appeared to be a widely dilated pupil, but in reality the result of iris atrophy, was seen in 2 cases, either on one or both sides. Anaesthesia of the cornea was detected in 5 cases. There were 2 cases each of dacryocystitis (Fig. 3) and phthisis bulbi.

Chronic plastic iridocyclitis of varying grades of severity was observed in 76.0% of lepromatous cases, 2 of the 8 cases of regressed borderline leprosy and 2 cases of indeterminate leprosy. In most of the instances, the iris with a

Fig. 3. Dacryocystitis.

small irregular pupil was firmly bound down by posterior synechiae. In one case the pupillary aperture was plugged with exudate (Fig. 4). Atrophic changes in the iris varied from localized areas of loss of pigment leaving behind the stroma of the iris to total loss of iris substance leading to a "moth-eaten" appearance and coloboma of the iris (Figs 5 and 6). In one case this led to a condition of polycoria (Fig. 7).

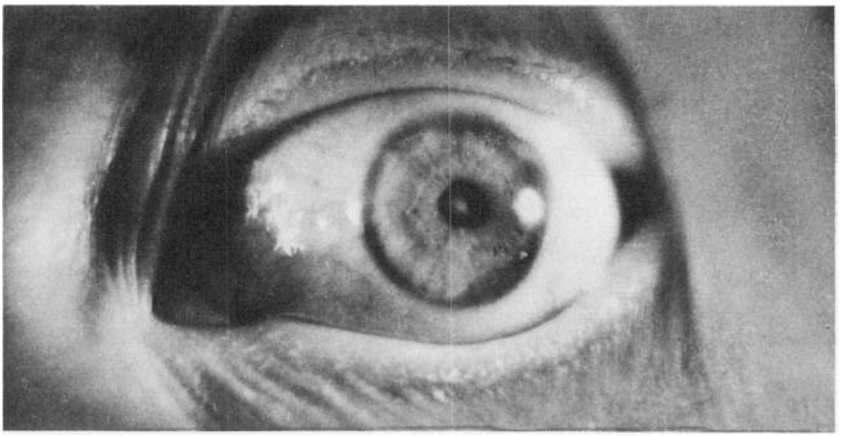

Fig. 4. Exudate in the pupillary aperture.

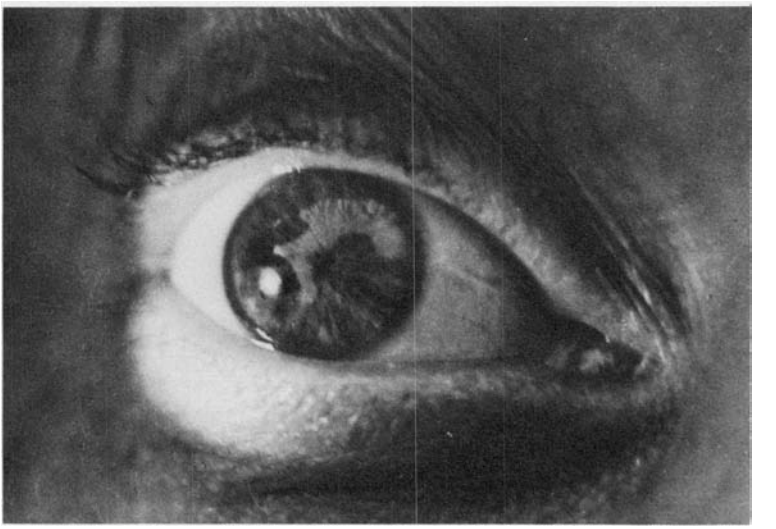

Fig. 5. Atrophy of the iris—"moth-eaten" appearance of the iris.

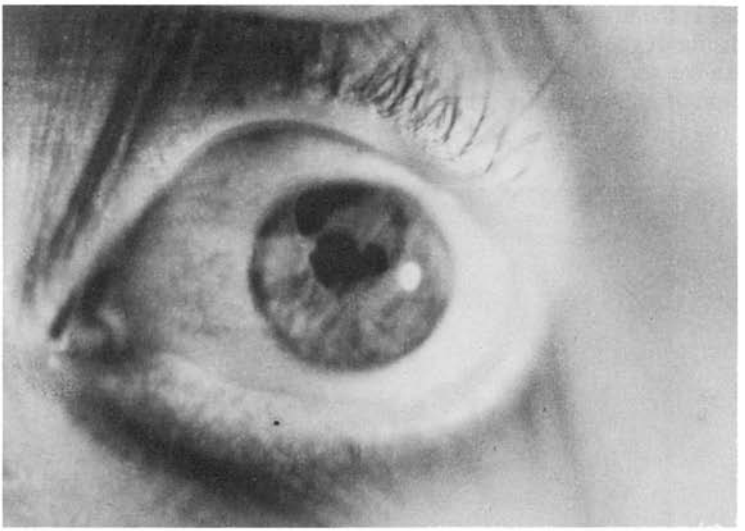

Fig. 6. Atrophy of the iris, leading to loss of iris stroma—coloboma of the iris.

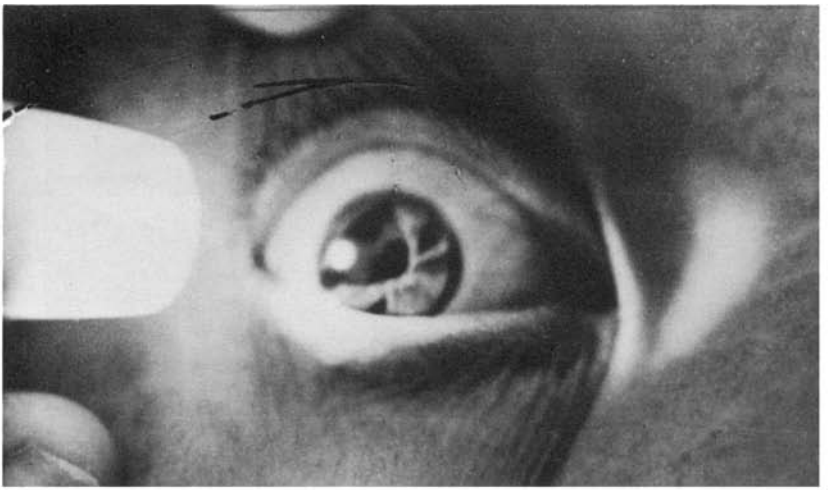

Fig. 7. Atrophy of the iris leading to "polycoria".

Discussion

Ocular involvement in leprosy occurs in one of 2 ways: (i) from mechanical causes and here the discussion will be limited to the damage arising from the loss of "blink reflex", the protective mechanism of the eye, following damage to the zygomatic branch of the facial nerve, and (ii) actual invasion of the elements in the anterior segment of the eye by *Mycobacterium leprae* in the lepromatous and near lepromatous cases producing, among other things, an insidious, painless and relentlessly progressive destruction of the iris and the ciliary body.

Facial nerve involvement in leprosy is encountered in all types of the disease with perhaps greater frequency in the borderline and tuberculoid cases. One of the peculiarities of this nerve involvement in this disease is the great tendency for the occurrence of an isolated paralysis of one of the branches rather than of the nerve trunk itself. The branch of predilection appears to be the zygomatic branch. In India, Dewan (quoted by Antia, 1966) observed facial nerve involvement in 3.0% of cases and that this often manifested itself as lagophthalmos. Abraham (1976) observed lagophthalmos in 2.0% of cases. The lower branches of the facial nerve are said to be more involved in the yellow and white skinned races (Antia *et al.*, 1966*a*). Attempts have been made to investigate the cause of facial nerve damage in leprosy. Brand's hypothesis (1959) that *M. leprae* prefers the colder parts of the body and the nerves for its localization and destructive work would perhaps explain the damage to the zygomatic branch with greater frequency. Antia *et al.* (1966*b*) by extensive operative exploration of the facial nerve in the side of the face confirmed the clinical impression that the zygomatic branch was the one most frequently affected. They also observed that the greater frequency of involvement of the distal part of the nerve to the orbicularis oculi muscle "possibly arose from the anastomosing sensory branches of the maxillary nerve". Secondary factors such as "the apposition of this nerve against the unyielding bony arch of the zygoma and its possible compression there, increased fibrosis and fibrous tunnels sleeving these branches", together with exposure to cold and trauma, all may play a part in the picking out of this branch with greater frequency. From the observations made on the Baba Baghi subjects in Tabriz where during the winter months there is very heavy snowfall and the temperature goes well below zero, it would appear that by far the more significant factor operating in the causation of paralysis of the facial nerve could be the effect of cold temperature on the superficially located parts of the nerve which have been conditioned for damage by the invasion and inflammation set up by *M. leprae*. If this were so, the frequency of such involvement should be more in the winter months. This needs elucidation. Another factor operating in these subjects is the unchecked progress of the disease in the absence of specific treatment. In addition to the above, inflammatory infiltration into the nerve and spasm of the vasa nervora leading to ischaemia of the nerve and thereby interference with its function may be the other factors operating in these cases.

The manner and nature of involvement of the elements in the anterior segment of the eye by bacillary invasion in the multibacillary types of leprosy

are too well known to merit repetition here. It may however be mentioned that the frequency and severity of ocular involvement in these cases is directly proportional to the duration and degree of advancement of the disease, the ethnic background and treatment status of the patient. Harrell (1977) in his survey of "ocular leprosy in the Canal zone" observed a high prevalence of leprotic ocular disease (96.0%) amongst 48 patients, mostly lepromatous, and that 64.0% gave evidence of present or past iritis". He believes that this high prevalence "is most probably due to the advanced age of the patients, the lengthy duration of their illness and the high percentage of persons affected by the lepromatous form of the disease". Gupta (1976) in a report based on the examination of 300 cases of leprosy, mostly lepromatous, at the Central Leprosy Teaching and Research Institute, Chengalpattu, found that: (i) the incidence of ocular complications was 69.0% (nature not mentioned), (ii) 50.0% of the patients without ocular involvement were young subjects (less than 20 years old) suggesting that the ocular complications were most frequent with the increasing duration of the disease and (iii) the incidence of blindness was low, 3.0% resulting from cataract, 2.0% from corneal opacities and *0.66%* due to iridocyclitis. This extremely low prevalence of chronic plastic iridocyclitis leading to loss of vision is presumably due to access of the Sanatorium population to specific treatment and also to their ethnic background. In sharp contrast, the involvement of the iris and the ciliary body in the Baba Baghi cases is very high and the destructive effect of this involvement is also striking. This, we believe, is largely due to the neglect of specific treatment and also to their racial extraction. The atrophic changes observed in the iris in some of the cases and which Brand (1976) calls "progressive iris atrophy" are said to be the consequence of "prolonged perhaps low grade iris inflammation". She believes that this condition contributes to the development of a low grade glaucoma "arising from the silting of the pigment lost from the iris against the trabeculae at the anterior angle, thereby obstructing the outflow of aqueous".

Management

"Foresight prevents blindness" was the theme with which the World Health Day April 1976, "put the spot light on the darkness which envelopes so many" (World Health, 1976). In leprosy the institution of certain simple preventive and therapeutic measures will go a long way in averting a calamitous situation in both the paralytic and Mycobacterial involvement of the eye. These methods are well known but merit repetition here, by way of emphasis.

In the paralytic type of eye involvement the eye should be protected from glare and dust during the waking hours by the use of an eye shade. Under rural conditions this can be achieved by teaching the patient to tie his turban cloth or any piece of clean cloth across the affected eye. Dark glasses with side-protectors will achieve the same purpose and perhaps look more elegant, if the patient can afford it. During the night, the patient is advised to instil a drop of castor oil or liquid paraffin into the affected eye followed by the application of a pad of cotton wool (or a pad soft cloth) and bandage. These simple measures

will help in preventing the occurrence of exposure keratitis and its disastrous sequelae. By way of medical treatment, in those cases detected soon after the occurrence of paralysis, administration of corticosteriods and anti-inflammatory drugs is worthy of trial. In addition, vasodilator drugs such as nicotinic acid in a dose of 100 mg twice a day after food, can be of benefit when given along with injections of vitamin B1, B6 and B12. Physiotherapeutic exercises comprising active forced closure of the lids, massage of the paralysed muscles and electrical stimulation of the viable muscles will considerably aid in the recovery of the protective function in the eye. Recovery of paralytic deformities in leprosy is unpredictable. Medical and physiotherapeutic measures are to be vigorously pursued. When these measures fail surgical procedures will have to be undertaken, depending upon the needs of the patient and the availability of facilities.

In the potentially serious form of eye involvement arising from bacillary invasion of the elements of the eye, institution and uninterrupted continuation of specific treatment in appropriate doses, supplemented by a periodical examination of the eye and protection of the eye from external elements with dark glasses will serve to save the eyes from damage.

Over and above these measures, health education of the patients with regard to the care of the eyes is vital. Many of them are not only ignorant of the potentially serious damage the disease may cause the eyes, but also believe that visual damage sometimes culminating in total loss of vision is a necessary accompaniment of the disease.

Acknowledgements

Grateful thanks are expressed to the patients who willingly submitted to the examination. Thanks are also due to Sri R. Gopal for secretarial assistance.

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Lepr. Rev. (1978) **49,** 241–248

Leprosy and the Community

LEPRA CHILDREN'S FUND

Ever since 1936, Lepra has appreciated that leprosy treatment can be most effective when given regularly to those unfortunate children who suffer from the disease. Today, it is still our policy to encourage the early diagnosis and regular treatment of children and this we do by giving small per capita grants in respect of all those children who are receiving treatment for their leprosy. In 1977, £42,000 was spent in this way and 30,000 children living in 12 different countries benefited as a result of grants from this fund.

Lepra now recognizes the need to give combined therapy, at least in the initial stages, to multi-bacilliferous cases and in such cases, the first year's per capita grant is increased by £5.

Currently the per capita grant for the first 100 children in any African control scheme is £5 and for India £4. In order to obtain a grant, it is necessary for those in charge of leprosy control work to send a list of the children's names up to the age of 18 years, with particulars of their sex, age, type of leprosy and progress, certified by the Medical Officer in Charge to the effect that the children listed are receiving treatment for active leprosy and are likely to require a further year's treatment.

A brief statement is also required to show how the previous year's grant has been spent. The money can be used for anything which will facilitate the early diagnosis and regular treatment of child sufferers. It can include fuel for vehicles, staff salaries, extra drugs and even school books or clothes in certain cases.

Copies of the instructions and the necessary application forms can be obtained from the Director of Lepra at Fairfax House, Causton Road, Colchester, Essex CO1 1PU, England. It is earnestly hoped that all those working in the field who are treating children and need a little more financial help will ask for it. Money is available but unless it is asked for, it cannot be used for this most important aspect of leprosy work, the early diagnosis and regular treatment of children suffering from leprosy.

G. F. HARRIS

LEPROSY RELIEF ASSOCIATION "EMMAUS-SWITZERLAND"

In February 1956 following the very cold weather experienced in Switzerland and inspired by the events which marked the founding of the movement Emmaus in France, several circles were formed in Switzerland to aid the isolated and the families affected by the hard winter and lack of means to heat their homes. From that time on, organized associations were founded in

Geneva, Berne, Zurich, Basle and the Jura with the motto "Serve first those who suffer most". These associations are on the one hand groups of *"Friends of Emmaus"* and on the other *"Communities of rag-pickers"*.

The "Friends of Emmaus" are volunteers who hold normal jobs. They undertake to help the more unfortunate, according to the local needs. At the same time, they try to eliminate the causes of hardship and to make the public authorities as well as the population aware of existing problems.

In 1959 a few members of the "Friends of Emmaus-Berne" were moved by the fate of leprosy victims, who, to their way of thinking, belonged to "those who suffer most" in the sense of the Emmaus motto. They therefore decided, in virtue of the same principle, to obtain for these leprosy victims the means of overcoming their physical and moral suffering. On the other hand, they decided to try and awaken the conscience of both the Swiss public and the public of the endemic countries to the painful problems of these victims. The same year a National committee for relief to leprosy victims was founded within the Swiss Emmaus Federation and in 1967 a group, which remained attached to this Federation but at the same time autonomous, was founded. This group was created because of the ever increasing and important tasks which arise from the immeasurable needs of some 15 million leprosy victims.

The first operational steps of "Aid to Leprosy Victims" were already taken in 1960 thanks to the first campaign organized in January in favour of the leprosy victims and which brought in 350,000 Swiss francs. A ward for leprosy patients was equipped at the new hospital in Vellore (India). A young Swiss volunteer went to Cameroon to determine what type of aid might be given and at the same time to work in improving the leprosarium in Nden.

Every year since 1960 the campaign in favour of leprosy victims goes out by means of a propaganda leaflet to every family in 15 Swiss cantons (about 5 million people). This leaflet informs the public not only of the ways of fighting against leprosy, but even gives particulars and exact figures about the assistance granted. The Swiss Emmaus Leprosy Relief Work in fact estimates that only knowledge of the technical assistance and human problems together with maximum efficiency can bring a real solution to those very often too numerous sufferers in our world who sometimes find themselves living beside our overbearing wealth. It is in this sense that Swiss Emmaus Leprosy Relief Work publishes a little pamphlet entitled "More happiness for leprosy victims" and even consults the public on certain problems (aid to disabled leprosy victims and to ex-patients).

The amount taken from collections has increased (more than 3 million francs in 1977); influence has strengthened; there are now over 100 beneficiary centres and some 500,000 patients received some form of aid last year. The *Swiss Emmaus Leprosy Relief Work* thinks however that money, although indispensable, should not enter into the matter when confronted with the human aspects of the problem. Its aims, in spite of being based on a mass campaign to reach out to the greatest possible number of sick, consist in caring for each leprosy victim according to his particular worries and difficulties, in giving him work and offering him a normal place in society. Furthermore the facts about leprosy as a disease should be taught in the endemic countries. Leprosy should be the object of controlled research and be integrated little by little into the

national health infrastructure. To attain these goals, the Swiss Emmaus Leprosy Relief Work without distinction of race, creed, caste or colour, helps all those who are willing to apply these principles i.e. private institutions, missions, governments and international organizations.

WHO SPECIAL PROGRAMME FOR RESEARCH AND TRAINING IN TROPICAL DISEASES: NEWSLETTER 10 JANUARY 1978, GENEVA, TDR/NL/78.1

This Newsletter records the following developments in the field of leprosy:

27 NEW RESEARCH PROJECTS APPROVED

The Steering Committee of the Scientific Working Group on the Immunology of Leprosy (IMMLEP) approved 27 new research proposals of a total 40 applications received, at its fifth meeting in Geneva, 9–11 June 1977. Eight proposals were not approved and five deferred until the next meeting which is to be held 1–3 February 1978.

The committee noted the continuing increase in supply of *M. leprae* with expansion of the armadillo farms in London and Caracas and the establishment of new facilities in Paraguay. Other possibilities for increase of production are also being explored. The IMMLEP farm, which had about 70 armadillos in 1975, will probably increase its stock to approximately 250 nine-banded armadillos by 1978. The *M. leprae* bank (London) reported that as of 1 June 1977 their stock of infected *M. leprae* material was about 5 kg of infected tissues, 300 mg of purified bacteria and 30 mg of skin test antigens. Protocols have been prepared for storage of *M. leprae* on liquid nitrogen and transportation on wet ice.

The Steering Committee agreed that there should be further study of a method for *M. leprae* purification and fractionation to evaluate the effect of using proteolytic enzymes in the purification procedures. The finding of disseminated mycobacterial infection in wild armadillos has been confirmed, but further research is required to establish its significance. Some areas, as for example Florida, appear to be infection-free.

It was agreed that serological methods in leprosy should be reviewed at the next SWG meeting, 19–22 June 1978, and considered for incorporation in the IMMLEP programme.

The Committee defined the following steps as prerequisite to beginning sensitization studies in humans:

—measurment of the sensitivity to irradiation of *M. leprae* and *M. lepraemurium;*
—sterility tests;
—acute toxicity tests;
—limulos endotoxin assay;
—toxicity studies in primates;
—potency test for sensitization (this is being established in guinea pigs).

All these studies are to be carried out with *M. leprae* purified from armadillo tissues. Production of *M. leprae* to be used in humans should be undertaken in licensed premises.

Participants in the Steering Committee meeting were:

Dr. B. Bloom, Albert Einstein College of Medicine, *New York,* N.Y., United States of America.
Dr J. Convit, Instituto Nacional de Dermatologia, *Caracas,* Venezuela.
Dr T. Godal, Radium Hospital, *Oslo,* Norway (Chairman).
Dr R. J. W. Rees, National Institute for Medical Research, *London,* United Kingdom.
Dr H. Sansarricq, World Health Organization, *Geneva,* Switzerland (Secretary).
Dr G. Torrigiani, World Health Organization, *Geneva,* Switzerland.
Dr J. Walter, World Health Organization, *Geneva,* Switzerland.

SEVEN APPLICATIONS APPROVED BY THELEP SC

The Steering Committee of the Scientific Working Group (SWG) on the Chemotherapy of Leprosy (THELEP) held its second meeting in Geneva, 7–9 November 1977. The proposed strategic plan for THELEP is now in the final stage of preparation.

Of thirteen grant applications reviewed by the Committee, two were approved in the area of drug development (total U.S. $ 30,000), two for field studies of dapsone resistance (total U.S. $ 12,300) and three for laboratory studies (total U.S. $ 44,500).

The Committee reviewed the status of clinical trials of drugs with a view to resolving a number of issues related to the Standard Protocol which had been left open at the last SWG meeting. The THELEP Coordinator reported on his site visit to Chingleput, including the state of readiness of the clinical trials there. Several pharmaceutical companies have offered to donate supplies of rifampicin for use in the first two years of these trials.

Three members of the Committee will meet with two outside experts for three days in Geneva in early February 1978 to draft protocols for surveys of dapsone resistance. These protocols will be distributed to Steering Committee members for review before the next Committee meeting, at which they will be discussed and perhaps finalized.

In regard to testing chemotherapeutic regimens on a mass scale the Committee decided it should follow closely the results of the Burma rifampicin trial (a WHO/SEARO sponsored activity), but for the present should take no action to initiate new trials of mass chemotherapy elsewhere.

The Committee decided to defer further activity on chemotherapy trials in non-lepromatous leprosy.

Discussion of the area of drug development produced agreement on the following points, amongst others:

(1) the data base was too small to permit development of a set of criteria based on structure-activity relationships, with which to select existing compounds for screening against *M. leprae*;

(2) fewer than 100 compounds per year could be screened against *M. leprae* with the mouse foot pad system, considering the available capacity of existing laboratories;

(3) no non-*M. leprae* screen appears to predict activity against *M. leprae;*

(4) THELEP should consider promoting the development of improved screening procedures based on *M. leprae* or components of *M. leprae* (enzymes);

(5) THELEP should consider the support of its own armadillos to provide a supply of *M. leprae* and components for the new screening procedures.

It was agreed that a sub-committee should meet with a few experts in drug development to plan and schedule new THELEP-sponsored efforts in drug development in time to report to the next meeting of the Steering Committee, which is scheduled to be held in Geneva, 24 – 26 April 1978.

A broad programme of training and institutional strengthening activities of both IMMLEP and THELEP is envisaged. A committee charged with planning this programme will meet in Geneva for two days at the end of January 1978. A Standardization Workshop will also be held in September 1978 which will be open to participants from outside THELEP.

Steering Committee members participating in the meeting were:

Dr J. Levy, Department of Comparative Medicine, Hebrew University-Hadassah Medical School, *Jerusalem,* Israel (Chairman).

Dr E. de Maar, WHO, *Geneva,* Switzerland.

Dr N. E. Morrison, Department of Pathobiology, Johns Hopkins University, *Baltimore,* Maryland, United States of America.

Dr R. J. W. Rees, National Institute for Medical Research, *London,* United Kingdom.

Dr H. Sansarricq, WHO, *Geneva,* Switzerland (Secretary).

Dr C. C. Shepard, Center for Disease Control, *Atlanta,* Georgia, United States of America.

Dr J. Walter, WHO, *Geneva,* Switzerland.

Dr M. F. R. Waters, National Institute for Medical Research, *London,* United Kingdom.

ILEP

At the recent (13–16 April 1978) meetings of the Medical Commission and General Assembly of ILEP, held in Würzburg, W. Germany, the representatives of the Member-Organizations studied with interest and a certain satisfaction the Reports of Professor M. F. Lechat (currently chairman of the Medical Commission) and Monsieur Pierre van den Wijngaert, the Secretary General.

ILEP is now well established as the co-ordinating body for a score of national and international voluntary agencies concerned with leprosy. Its members are responsible for disbursing the considerable sum of nearly 17 million $U.S., which they raised in 1977 and using this money — on the advice of the guidelines laid down by the Medical Commission — to the greatest benefit of leprosy sufferers throughout the world. Over 6% of the total grants made is spent on various research projects.

Some healthy disquiet was expressed at these meetings that in spite of tremendous efforts, ILEP members are still reaching only 1 in every 10 leprosy sufferers in the world, and that the virtually static situation is now complicated by the twin problems of dapsone-resistance and persister leprosy organisms.

The participants, representing the various Member-Organizations of ILEP, were urged to study the pros and cons of integrating their leprosy activities into government programmes for comprehensive primary health care, and to encourage joint schemes wherever possible — co-operative efforts for tackling other diseases than leprosy, and joint efforts between various voluntary agencies.

The membership of the Medical Commission for the next 3 years was determined by the election of 12 doctors. Four former members of the Commission were with acclamation accorded the title of Honorary Members in recognition of their outstanding service over the years; these are: Drs Gilbert and Wegener, Professor Janssens and General Richet.

Professor M. F. Lechat emphasized in his Annual Report the increasing importance that should be given to the medical components of leprosy programmes sponsored by voluntary agencies. He stressed in particular the training of medical auxiliaries, the need to take advantage of the best advice, and to combine medical competence with compassionate care. The Medical Commission has shown itself very active in encouraging and participating in joint meetings with other bodies in various countries, and in striving to raise the standards of patient care in the diverse projects aided by Member-Organizations of ILEP.

The following is the Press Report of the XII General assembly of ILEP:

XIIth GENERAL ASSEMBLY OF ILEP

Würzburg. 22 voluntary agencies from 20 countries, Members of the International Federation of Anti-Leprosy Associations met in Würzburg 13–16 April for their 12th General Assembly. This international conference started with the meetings of the Medical Commission and of panels who dealt with the social aspects and training in leprosy, International Year of the Child, proclaimed by the United Nations for the year 1979, and health education. The General Assembly, during the meetings held on Saturday and Sunday, invested as President for the period 1978–1980 Mr Askew, International General Secretary of the Leprosy Mission (London) and choose as his successor for the period 1980–1982 Mr Thomassen, President of the Nederlandse Stichting Voor Leprabestrijding (Amsterdam).

On proposal of the Medical Commission, whose Members were renewed, the General Assembly nominated as Honorary Members of the Medical Commission: Dr Gilbert (Geneva), Dr Wegener (Würzburg), Général Richet (Dakar) and Prof. Janssens (Antwerpen).

It was decided to transfer the Co-ordinating Bureau of ILEP to Paris.

Following the reports of the General Secretary, of the Medical Commission and of the panels, the budget for 1978–1980 was fixed. A working Group was created for implementing combined leprosy-tuberculosis campaigns, and it was decided to continue the enquiry undertaken by one of the Member-Organizations "Les Amis du Père Damien" in cooperation with Prof. Lechat, on the leprosy situation in the world in order to devise a global strategy.

The next Working Sessions will be held: the first one in Oslo next June and the second one in Carville (U.S.A.) in November on the occasion of the

International Congress at Mexico. The next General Assembly will be held in 1980 — the General Assemblies are held every two years — in London.

The report of the General Secretary pointed out that last year the Member-Organizations of ILEP have supported up to an amount of 24.5 million U.S. dollars 700 projects in 74 countries. In the report of the Medical Commission it was recommended that, due to the increasing emergence of sulfone resistance, priority be given to combined treatment in some cases and that new drugs be made available.

A delegate of the Ministry of Health from Togo, together with a representative of the World Health Organization attended the meetings.

During a reception offered by the Mayor of Würzburg and the Minister-President of Bavaria, full appreciation of the work undertaken by the Member-Organizations in the field of leprosy was expressed.

The delegates of the Member-Organizations attended an oecumenical service on the occasion of the conference.

(Press Release approved by the General Assembly of Würzburg.)

FIFTH WORKSHOP ON LEPROSY; ACWORTH LEPROSY HOSPITAL SOCIETY FOR RESEARCH, REHABILITATION AND EDUCATION IN LEPROSY, WADALA, BOMBAY –31, HELD ON 23 NOVEMBER 1977

The Proceedings summarize 5 papers:

(1) R. Ganapati and R. G. Chulawala. "Diagnosis of Early Leprosy with Reference to Histopathological Features".
(2) D. K. Dastur, G. L. Porwal and J. S. Shah, "Very Early Non-lepromatous Leprosy — Histochemistry and Electron Microscopy of Nerves".
(3) D. K. Dastur, S. M. Daver, G. L. Porwal and C. R. Revankar. "Long Treated Lepromatous Leprosy — Histochemistry and Electron Microscopy of Muscles and Histochemistry of Nerves".
(4) C. V. Bapat. "Evolution of Culture Media for Cultivation of *M. leprae 'in vitro'*".
(5) K. S. Pradhan, M. B. Bhide and C. V. Bapat. "Use of ICRC Bacilli as Vaccine against *M. leprae* in Mouse Foot Pad".

Paper 1 deals with a subject which is still of practical importance to those who see large numbers of patients in an endemic area, namely the extent to which skin biopsy is helpful in the diagnosis of leprosy (or other diseases), where the only manifestation of disease is a skin macule, often small in size. Dr D. J. Harman at the Leprosy Study Centre in London, has during the past few years reviewed a considerable number of biopsies from patients in various parts of the world labelled clinically as "indeterminate", and concluded that a histopathological diagnosis can be made in most of them (leprosy or not leprosy, and if the former, a classification), provided that the material is well-taken and examined in detail. It would be interesting to receive the comments of Drs Ganapati and Chulawala on their own material from India in view of this finding. The summary of *Paper 2* says that "the study showed turnover of

Schwann cells", another subject of continuing interest in view of the fact that most authorities regard their turnover in the normal state as nil, or exceedingly low once myelination has taken place. In the discussion of Papers 2 and 3, Dr S. S. Pandya referred provocatively to our inability to do something which may seem basic, if not simple, in the study of nerves, namely to distinguish motor and sensory fibres in a fascicle. (In fact, it cannot be done, and the lysosomal studies in myelinated and unmyelinated fibres, reported in these papers, did not shed new light.)

A. C. McDOUGALL

Lepr. Rev. (1978) **49,** 249–253

News and Notes

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

The attention of all our readers is drawn to this important Congress. There is still time to register for it. Registrations, hotel reservations, social events, tours and in general all administrative matters concerning this Congress will be handled by the Local National Committee. Please address to: *XI International Congress of Leprosy, Asociacion Mexicana de Accion Contra La Lepra A.C., Dr Vertiz 464 Mexico 7. D.F. Mexico.*

THE EAST AFRICAN LEPROSY BULLETIN, MARCH 1978, v. 6, No 1

It is encouraging to see another issue of this Bulletin, thanks to financial support from the Netherlands Leprosy Relief Association. The Editorial Board comprises Drs Anderson, Chum and Broekman (Kenya) and Dr Nsibambi (Uganda). This issue is mainly concerned with (1) Abstracts from the WHO Fifth Report, Expert Committee on Leprosy, Technical Report Series, 607, (2) a summary of the recommendations of the 1st International Workshop on Chemotherapy of Leprosy in Asia, Philippines. January, 1977, (3) a summary of the WHO Special Programme for Research and Training in Tropical Diseases (this is an excellent short account of the Programme, for those not already familiar with it), and (4) a circular letter from the All Africa Leprosy and Rehabilitation Training Centre, (ALERT), P.O. Box 165, Addis Ababa, Ethiopia. The latter summarizes some of the research findings at ALERT and their consequences for field work, and it is unfortunate that the third page of this potentially important letter (page 22) is a printing/typing error, reproducing page 21. Perhaps this could be remedied in the next issue. The Editorial Board of Leprosy Review wishes to record its interest and good wishes for the future production of this Bulletin, from an area of Africa where leprosy is obviously still a major problem.

A. C. McDOUGALL

INTERNATIONAL YEAR FOR DISABLED PERSONS — 1981

Just as they are all more or less directly concerned with the International Year of the Child (1979), in the same way governments and voluntary agencies connected with leprosy programmes throughout the world will wish to share in the activities that will be organized during the year 1981 — designated by the

United Nations as the International Year for Disabled Persons. While the statutory bodies of the United Nations will naturally assume the major role in the central organization of action to be taken at government level, much of the success of the local activities in the various countries will depend upon the initiative and enthusiasm of members of voluntary agencies. It is here that the anti-leprosy associations, especially those working together under the aegis of ILEP, will be able to offer their expert knowledge of the local situation concerning those whose handicap is due to leprosy, and to help organize national activities in favour of the handicapped. The social discrimination and disabilities under which leprosy victims still suffer should not be lost sight of in any action organized for this purpose.

The Council of World Organizations Interested in the Handicapped has been asked, through its Executive Committee, to prepare a protocol for the voluntary agencies whose activities it represents. Leprosy has a voice on this Council, through the International Leprosy Association, which is one of its founder-members; its Secretary-Treasurer (Dr S. G. Browne) represents the Association both on the Council and on the Executive Committee. At its meeting in Paris on 3 April 1978, the Executive Committee made suggestions for a draft protocol to be submitted to the United Nations, stressing the following points:

—the need to organize activities appropriate to the local situation in different countries;
—the prevention of deforming conditions and diseases (leprosy is obviously an example of these);
—the provision of services in rural areas;
—the importance of reducing the stigma still attaching to physical handicaps (especially, we might add, those due to neglected leprosy);
—the need for suitable teaching materials for medical students, doctors, auxiliary medical workers and all engaged in any way with the care of those suffering from some kind of physical or mental disability.

CONGRESS IN CAIRO

The "Cairo Second International Leprosy and Tropical Dermatology Congress" which was held in Cairo on 20 and 21 March 1978 attracted about 300 participants from 8 near-east countries and 4 from outside the Arab world. The joint organizers were Dr S. G. Browne (London) and Professor M. El Zawahry (Cairo).

Most of the papers on leprosy were presented by dermatologists working in one or other of the University hospitals in Egypt. They were of high order and gave evidence of the continuing interest of dermatologists of all degrees of seniority in one of the intractable problems of this part of the world.

Among the papers on tropical dermatology, those on cutaneous leishmaniasis and cutaneous bilharzia attracted considerable attention.

The Congress followed a Memorial Congress held to commemorate the 150th anniversary of the founding of the Kasr el Aini School of Medicine, the oldest and most prestigious of Egypt's Medical Schools.

S. G. BROWNE

WHO SETS NEW SERVICE FOR READERS OF ITS "*BULLETIN*": WHO PRESS RELEASE WHO/19 OF 25 APRIL 1978

Publication to stress "Information of Immediate Use" for Health Workers

The World Health Organization has redesigned its oldest scientific journal, the internationally esteemed *Bulletin,* around a new service for readers.

The change centres on a section called "Update", created by the editors to carry current, "state of the art", reports on scientific aspects of public health problems written by leading experts around the world.

The publication's change in style stems from a policy shift in editorial emphasis and begins with the current issue.*

In a statement explaining the changes, Dr Halfdan Mahler, WHO Director-General, says that the 30-year-old *Bulletin's* "sole function" will no longer be the publishing of original articles of research.

Instead, it will be edited to carry "information of immediate use in health development", and to appeal to a wider audience. Aimed at particularly are the generally over-burdened scientist and "health manager" who are hard-pressed for time but who need accurate information speedily.

As much as better health relies on antibiotics, vaccines and other medical developments, so too is it equally dependent on the spread and application of technical knowledge. Thus, as put by Dr Mahler: "Each 'Update' article will provide a concise and authoritative account of the current state of the art being written about, with particular emphasis on information of immediate use in health developments.

"For this reason, unlike conventional review articles, they do not contain a detailed historical survey of all aspects of the subject, and a long list of biographical references."

Instituted to meet new worldwide health priorities, the editorial shift, he says, will "ensure that the best current knowledge is available to and applied by those in a position to make use of it".

These 'Update' articles launch the new readers' service:

—"The Three Types of Human Viral Hepatitis", by Professor A. J. Zuckermann, University of London, U.K.

—"Advantages and Disadvantages of Killed and Live Poliomyelitis Vaccines", by Professor Joseph L. Melnick, Baylor College of Medicine, Houston, Texas, U.S.A.

—"Le Rôle du Laboratoire de Virologie dans un Pays en Développement", by Professor R. Sohier, Université de Lyon, and Professor O. G. Gaudin, Université de Saint-Etienne,

—"Species Complexes in the Simuliidae", a summary of what is known about species of the African blackfly, the transmitter of river blindness. It is by six experts.

—"Ocular Onchocerciasis", or river blindness, by Dr B. Thylefors, a WHO consultant. The disease affects some 20 million throughout the world, blinding up to half a million.

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* Bulletin of the World Health Organization, Vol. 56, No. 1, 1978.

Despite the editorial shift, the *Bulletin* still publishes articles on research. Eight are carried in the current issue, including a combined Spanish-Scottish study on illness that affects travellers taking package tours.

Bulletin articles are in either English or French. An article in one language is accompanied by a detailed summary in the other. There is also a Russian edition of the *Bulletin* published in Moscow.

OLD ISSUES OF *LEPROSY REVIEW*

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

NOTICE TO AUTHORS

With the completion of Volume 49, Dr Davey will be retiring as Editor of this Journal. Prospective authors are advised that original papers and other material offered for publication in Volume 50 No. 1 should be sent to Dr Colin McDougall, The Slade Hospital, Headington, Oxford OX3 7JH. This change of address becomes operative from 1st September 1978.

SURGICAL CONGRESS, NAIROBI 5–6 DECEMBER 1978

We have been asked to draw the attention of our readers to this Congress, held in conjunction with the Association of Surgeons of East Africa, the International Federation of Surgical Colleges, and the International Society for Burns Injuries. In addition to Symposia there will be opportunity for free papers including burn injuries. The Secretary of the Congress is Mr I. J. P. Loefler, PO Box 47934, Nairobi, Kenya.

PERSONAL

Dr Ruth Pfau Honoured

Dr Ruth Pfau's work for leprosy sufferers in Pakistan, based on the Marie Adelaide Leprosy Centre in Karachi, and reaching out to the whole country has recently received well-merited recognition. She has been invested with the Commanders Cross Order of Merit — the highest award granted to civilians — by the Federal Republic of Germany. The Government of Pakistan has also decorated her with its highest civilian award — "HILAL-i-IMTIAZ" (the Crescent of Achievements).

We add our own congratulations to those that Dr Pfau has already received.

S. G. BROWNE

Dr S. G. Browne

During a recent visit to India (27 January to 11 February), Dr S. G. Browne delivered an address entitled "India's future role in the fight against leprosy" at a meeting in New Delhi organized around the launching of a book "A Window on Leprosy" edited by Dr B. R. Chatterjee. In the presence of the Prime Minister of India and other notabilities, the Vice-President of India officially launched the volume, which is published to mark the Silver Jubilee of the Gandhi Memorial Leprosy Foundation. The occasion coincided with the observance of the 30th anniversary of the assassination of Mahatma Gandhi and of World Leprosy Day. The Hind Kusht Nivaran Sangh, offshoot of BELRA (now LEPRA), also celebrates at this time its 50th anniversay.

In addition to lecturing in various centres in India (notably New Delhi, Calcutta, Madras, Karigiri, Bombay and Wardha), Dr Browne conducted a seminar in Calcutta for the Sisters and Brothers of Mother Teresa's Missionaries of Charity who are engaged in caring for leprosy sufferers, and recommended that their socially exemplary activities should be medically improved and integrated into the Greater Calcutta Leprosy Control Programme and in other towns where they are at work.

While in Calcutta, Dr Browne was presented with the J. N. Chowdhury Medal for his contributions to tropical medicine, and asked to deliver the J. N. Chowdhury Memorial Oration on "Some Growing Points of Leprosy Research" at the School of Tropical Medicine.

Book Reviews

Leprosy for Medical Students and Practitioners by K. K. Koticha, Acworth Leprosy Hospital, Bombay 400 031. Price Rs. 12/-; U.S. $2; U.K. £1.
First Edition, 1978.

This is a 32-page booklet on pages rather larger than A4, intended mainly to supplement the lectures given at the Acworth Leprosy Hospital. One of the early sections contains interesting information on the distribution of leprosy in India, and in Greater Bombay and the City of Bombay itself. Of the currently estimated world total of 11-12 million cases of leprosy, India is estimated to have 3 millions, with a prevalence of 10 or more per thousand in Tamil Nadu, Andra Pradesh, Orissa, West Bengal, Bihar, Karnataka and Maharashtra. Bombay alone has over 80,000 cases. The main body of the text gives a standard account of the subject including aetiology, pathogenesis, clinical findings, histopathology, diagnosis, treatment and control. The dosage of dapsone is detailed on page 21, starting with 25 mg daily, increasing at 3-monthly intervals to 100 mg daily after 9 months. In the management of reaction on page 24, it is advised that dapsone should be stopped or "the dose reduced and substituted by Diphenyl thiourea (DPT)". The author advises that the latter should be continued for 6–12 months, after which "... DDS should be re-introduced though now in very small doses, e.g. 10 mg twice a week and gradually increased".

Although this first edition is marked 1978, some of the advice and information seems a little out of date (for instance, *DPT* is no longer in production by the CIBA Company; *ethyl mercaptans* for skin inunction have a short section on page 23, but *combined drugs for lepromatous leprosy* (same page) are not described in adequate detail; *research on the armadillo* is not mentioned). This booklet should be of value in the Bombay area and for the continued teaching of leprosy at the Acworth Hospital, but it may have some difficulty in competing with others of similar length and price, produced in India and elsewhere.

A. C. McDOUGALL

The Book of Outlines, by S. Hasan, 1977. Published by Hind Kusht Nivaran Sangh, Andhra Pradesh Branch, 3-4-760 Barkatpura, Hyderabad 500 027. Price Rs. 16/-.

This is a 126-page paperback, A4 size, written for "Para-medical workers and readers, who are interested in elementary anatomy, physiology, pathology, bacteriology, food, communicable diseases; and leprosy with its variable problems". The author is himself a para-medical worker, who joined the Leprosy Training Centre, Hyderabad, as a physiotherapist in 1968. The book is divided into two sections of approximately equal length, the first dealing with the list of general topics noted above, and the second with leprosy as a special subject. There is an enormous amount of information in these pages, and on the whole it is well set out and easy to read, but the quality of the hand-drawn illustrations is not always good and some of those illustrating basic anatomy are misleading. There are a large number of spelling mistakes which should be corrected in the next issue, and the title of the book could well be changed to something more descriptive of the actual contents. Mr Hasan has put a great deal of work into this publication which, although composed largely of standard, accepted knowledge, may nevertheless be of value to para-medical workers in India coming to the study of leprosy for the first time.

A. C. McDOUGALL

Lepr. Rev. (1978) **49**, 255–261

Abstracts

47. HARBOE, M., CLOSS, O., BJORVATN, G., KRONVALL, G. & AXELSEN, N. H. **Antibody response in rabbits to immunisation with *Mycobacterium leprae*.** *Infect. Immun.*, 1977, v. 18, No. 3, 792–805.

The authors' summary reads as follows:

"*Mycobacterium leprae* purified from liver tissue of an infected armadillo (the A/10 preparation) was tested for antigenic composition by immunization of rabbits and characterization of the antibody response by crossed immunoelectrophoresis. The rabbit antisera detected 7 distinct components in the *M. leprae* preparation. This number is far lower than in similar experiments with other mycobacteria. The *M. leprae* sonic extract gave far fewer lines after polyacrylamide gel electrophoresis and staining with Coomassie brilliant blue than sonic extracts prepared from BCG, *M. smegmatis,* and *M. phlei* adjusted to the same protein concentration based on the Folin assay. The 7 components detected in *M. leprae* cross-reacted extensively with *M. avium,* BCG, *M. lepraemurium, M. smegmatis,* and *Nocardia asteroides*. The 7 components are involved in immune reactions in leprosy; antibodies against all of them were demonstrated in sera from patients with lepromatous leprosy, but the specificity of the antibodies varied from patient to patient. The reason for the demonstration of so few antigenic components and some of the implications of these findings for the use of armadillo-grown *M. leprae* to develop specific skin test reagents and in other aspects of leprosy research are discussed".

[This article, which has 38 references, describes findings of major importance (for instance, to WHO's IMMLEP programme) and should be read in the original by anyone interested in this field of leprosy research.]

A. C. McDougall

48. KOLLER, W. C., GEHLMANN, L. K., MALKINSON, D. & DAVIS, F. A. **Dapsone-induced peripheral neuropathy.** *Arch. Neurol.,* 1977, v. 34, No. 10, 644–646.

The author's summary reads as follows:

"Peripheral neuropathy is a rare complication of dapsone therapy. This neuropathy appears primarily to be of the motor type, and recovery occurs on discontinuation of the drug therapy. The patient in this report developed a marked motor deficit as well as a selective marked loss of vibration sense shortly after the initiation of a relatively low dose of dapsone. Recovery was rapid on cessation of the therapy. This patient was found to be a slow acetylator of isoniazid, and therefore is probably a slow acetylator of dapsone. The possible mechanisms of the neurotoxicity of dapsone and the role of altered metabolism are discussed."

[Unlike some of the other 9 cases in the world literature, this is an excellently documented and referenced case, highly convincing for dapsone as the cause of the neuropathy reported. The total of 9 world cases is obviously very small indeed for a drug which has been used — in fact since 1950 — for the treatment of many hundreds, perhaps even a few thousand cases of dermatitis herpetiformis, and more recently for the treatment of various dermatological disorders of obscure aetiology. High dosage has often been used over a period of many years. The authors discuss possible mechanisms involved in dapsone neuropathy, drawing attention to the division of patients into fast and slow acetylators for dapsone, similar to that known to exist for isoniazid, adding that "the mechanism of the neurotoxicity of dapsone could be due to impaired metabolism with slow acetylation." They suggest that if slow acetylation is found to correlate

with the development of peripheral neuropathy in other cases, "it would be justifiable to determine the acetylation phenotype prior to initiating dapsone therapy". Apart from the impracticability of doing this, the fact that 50% of people are slow acetylators of dapsone and that the half-life of dapsone is not related to acetylator phenotype casts some doubt on the points made in the final paragraphs of this interesting report.]

A. C. McDougall

49. **Research activities of the National Institute for Leprosy Research, Higashi-murayama-shi, Tokyo, Japan.** Special issue for the 20th Anniversary, July, 1975.

This 257-page document in fact appeared in November 1977, and contains a detailed description of research work carried out at this centre since its foundation in 1955. The main section headings are: Cultivation and Metabolism, Transmission, Host–Parasite Relationships in Leprosy and Anti-leprosy Drugs. Several hundred experiments are described and pages 252–256 list those which have been published in the medical literature. Some of the projects are now of little more than documentary interest, but there is nevertheless a great deal of useful information, particularly under the headings of metabolism, culture and electron microscopy, which should be invaluable to those working in experimental leprosy.

A. C. McDougall

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

I. MICROBIOLOGY

50. DESAI, A. C., APTE, S. N. & BHIDE, M. B. **The infectivity of drug resistant cases.** *Lepr. India,* 1977, v. 49, No. 1, 54–58.

Growth curves in the mouse footpad of *Mycobacterium leprae* derived from untreated patients with lepromatous leprosy are compared with those of proved dapsone-resistant *M. leprae* derived from patients after more than 5 years of dapsone therapy. The close similarity between the respective growth curves suggests to the authors that dapsone-resistant bacilli are as infective as their dapsone-sensitive counterparts.

T. F. Davey

51. DHOPLE, A. M. & HANKS, J. H. ***In vitro* growth of *Mycobacterium lepraemurium*, an obligate intracellular microbe.** *Science.* Washington, 1977, July 22, v. 197, 379–381.

This study of the limited *in vitro* growth of *Mycobacterium lepraemurium* was undertaken in the hope that the results would be of value to the culture of *M. leprae*. Firefly luminescence was used to measure the percentage of adenosine triphosphate (ATP) in a suspension or culture. Results with this ultrasensitive method confirmed that *M. lepraemurium* is capable of extra-cellular growth in diffusion chambers implanted in the peritoneal cavities of mice, and that growth is obtained in Nakamura's system. A 17-fold increase in mass was obtained after adaptation of the organism to growth *in vitro* in an improved modification of the latter method, though growth ceased after 6 weeks. A merit of ATP estimation as an index of metabolic activity for organisms such as *M. leprae* would be that data can be obtained immediately from unwashed host-grown organisms.

D. S. Ridley

52. KOHSAKA, K., MORI, T. & ITO, T. **Lepromatoid lesion developed in nude mouse inoculated with *Mycobacterium leprae*. Animal transmission of leprosy.** *Lepro,* 1976, v. 45, No. 3, 177–187.

Eight nude mice (BALB/c-*nu/nu*) were inoculated when 5 weeks old in the right hind footpad with 1×10^4 *Mycobacterium leprae* obtained from a relapsed lepromatous patient. The mice were kept under specific pathogen free (SPF) conditions in a Vinylplastic-isolator. The generation time of *M. leprae* was similar to that in control mice (in which Shepard-type limited multiplication occurred). However, spread outside the footpad was detected in a mouse killed at 13 months after inoculation. The 3 mice which survived to be killed at 17 to 22 months, all showed swelling of their inoculated footpads. Histological examination of the latter revealed lepromatous lesions with foamy histiocytes full of acid-fast bacilli; dermal nerves were involved and the histological figures published are compatible with *M. leprae* (compared with *M. lepraemurium*) infection. Lepromatous lesions were also detected in the eyelids, ears, nose and tail. Further proof that the lesions were due to infection with *M. leprae* was obtained in a number of ways, including failure of growth on artificial medium, and the results of the reaction in tuberculoid and lepromatous patients to lepromin prepared from a swollen footpad. Passage experiments are in progress in both *nu/nu* and normal mice.

[This is an important paper. Although the claim by Rees *et al.* to obtain lepromatous leprosy in thymectomized-irradiated mice (*Nature*, 1967, v. 215, 599) was confirmed by Job *et al.* (*Trop. Dis. Bull.,* 1975, v. 72, abstr. 1391), many workers have experienced great difficulty in keeping such mice alive for a sufficient length of time. Immunologically-deficient animals are required to study persisting viable *M. leprae* in treated leprosy patients, and the nude mouse kept under SPF conditions could prove to be an acceptable alternative.]

M. F. R. Waters

53. NAKAMURA, M., ITOH, T. & WAKI, C. **[Isolation of a cultivable mycobacterium from an armadillo subcutaneous tissue infected with *M. leprae* and characterization of this isolated strain.]** *Lepro,* 1976, v. 45, No. 4, 217–222. [In Japanese.]

The English summary appended to the paper is as follows:

"A strain of acid fast bacillus was isolated from a leproma of armadillo infected with *M. leprae* during the cultivation trial. Colonies were easily formed on Ogawa egg medium 1–2 weeks after inoculation, and were yellow.

"This isolated mycobacterium was identified as a type of Scotochromogen, which belonged to Group II atypical mycobacterium, by biological and biochemical characterizations."

54. ELLISTON, E. P. & TAYLOR, C. E. **Separation of *M. leprae* from human leproma and the development of a cytoplasmic skin test antigen from purified bacilli.** *Int. J. Lepr.,* 1976, v. 44, No. 3, 319–331.

With the object of producing a purified leprolin antigen that could be used for the epidemiological study of leprosy, leprosy bacilli were isolated without heating from a homogenized lesion by a flotation technique, digested enzymatically and sonicated. After repeated processing a dialysate (leprolin) was obtained that was free of cell wall material, and also a separate particulate fraction. These 2 antigens elicited positive skin test reactions in people with tuberculoid leprosy and negative reactions in lepromatous leprosy. The positive reactions were enhanced by the purification procedure. In a group of children not exposed to leprosy, the particulate antigens gave stronger reactions after BCG vaccination than in tuberculin negative children. Leprolin gave a low level response in both groups. Full evaluation was handicapped by limitation of supplies of antigen.

D. S. Ridley

55. MATSUO, E. & SKINSNES, O. K. **Immunologic identification of *M. leprae*. Immunofluorescence and complement fixation.** *Int. J. Lepr.,* 1976, v. 44, No. 3, 301–314.

An attempt was made to identify *Mycobacterium leprae* specifically by indirect immunofluorescent techniques. The antibody was the purified IgG fraction of serum of patients with lepromatous leprosy, adsorbed against *M. tuberculosis* and conjugated with fluorescein isothiocyanate. The organism, cultured by the authors in a hyaluronic acid based medium (LA-3) and thought to be *M. leprae,* gave strong fluorescence, especially about the periphery of the bacilli, but not all bacilli fluoresced. A similar result was obtained with bacilli in a cryostat section of a nodular lesion of lepromatous leprosy, but not with *M. lepraemurium, M. tuberculosis* or 12 other species of mycobacteria grown in LA-3 medium. *M. avium* produced faint fluorescence.

With complement fixation, a method based on antigen dilution gave more promising results than methods using serum dilution. The results seemed to correspond to those with immunofluorescence.

It was concluded that these methods were a means of specific identification of *M. leprae.* The specific antigen was considered to be a surface antigen with a lecithin-phospholipid component. The results strongly reinforced the claim that *M. leprae* is readily cultivated in LA-3 medium (*Trop. Dis. Bull.,* 1976, v. 73, abstr. 2053). Many technical details are given in the paper.

D. S. Ridley

56. OGAWA, T. **[Attempt at growth of *M. leprae* in mice.]** *Lepro,* 1976, v. 45, No. 4, 223—229. [In Japanese.]

The English summary appended to the paper is as follows:

"The technical procedures of experiment were explained briefly in Table 1. Bacterial suspension prepared from lepromas was injected into mice once or several times at weekly intervals by subcutaneous or intravenous route, or by both routes. Animals were killed at various intervals 2–16 months after injection. At necropsy, lesions were sought by gross inspection. Portions of various organs were removed and ground in mortar to make the homogenates. Smears made from the homogenate were stained by Ziehl-Neelsen's method and examined microscopically. The homogenate treated with 1% sodium hydroxide solution and then inoculated onto the egg yolk medium (for *M. lepraemurium*; also for *M. leprae* (?)) and Ogawa 1% egg medium (for cultivable mycobacteria). The tubes were incubated at 37°C for over 3 months. The details of single inoculation experiments and multiple inoculation experiments were shown in Tables 2 and 3.

"Ten experiments containing four with single inoculation and the other six with multiple inoculation were carried out. But one experiment, Expt. (4), exhibited a probable contamination and its results will be described in the following paper separately.

"In nine experiments, gross findings were all negative. Cultivation trials showed a few, smooth, and buff colonies, supposedly atypical mycobacteria, from two specimens only, but no colonies of mycobacteria, especially suspected of *M. leprae,* have been isolated.

"On the other hand, microscopic examination revealed the presence of acid-fast bacilli in the tissues of various organs. Among the two experiments, Expts. (3)–1 & –2, showed remarkable microscopical findings summarized in Table 6. As shown, in the subcutaneous experiment Expt. (3)–1, acid-fast bacilli found were in the injection site and superficial lymph nodes, and none of the tissue of viscera. In the intravenous experiment, Expt. (3)–2, acid-fast bacilli were detected in the spleen, liver and lungs, but smaller in number, comparing with those of the injection site just mentioned. No bacilli were found in the superficial lymph nodes. In both of the experiments the acid-fast bacilli had a tendency to decrease in number steadily. Where numbers of bacilli were present, globi were often seen, but these were usually small in size and loose in arrangement. And, indeed, it was uncertain whether the bacilli had multiplied within the tissue or not. The microscopic findings obtained in all the experiments were summarized in Table 7.

"As the materials of leproma used differed from experiment to experiment, it was impossible to compare directly the values of percentage for smear-positive specimens. As a whole, however, it seemed fairly justified in concluding that the microscopic findings were superior in the multiple

inoculation than in the single inoculation. This fact was accorded with the observations reported by previous workers."

[The tables are in English.]

57. NAKAMURA, M. **Multiplication of *Mycobacterium lepraemurium* in cell-free liquid medium. 10. Factors involved in the starting material of *M. lepraemurium* for the growth *in vitro* and *in vivo*.** *Lepro,* 1976, v. 45, No. 4, 203–210. **11. Establishment of the ND-5 medium.** *Ibid.,* 211–216. [In Japanese.]

The English summaries appended to the papers are as follows:

10. "Factors involved in the inoculum of *M. lepraemurium* for the growth in NC-5 medium as well as in mice were studied and the results obtained are as follows:

"1. Significant multiplications of *M. lepraemurium* obtained from infected subcutaneous tissue, liver, and spleen in NC-5 medium were observed. Therefore, it is obvious that the growth of bacilli in NC-5 medium is independent from the sources of the materials used. The multiplication ability of the bacilli in NC-5 medium is kept for 2 months at −20°C (in a freezer).

"2. No effects of treatments with 0.1% trypsin, 0.2% pronase, and 0.1% desoxycholate at 37°C for 60 min on the potentiality of the growth in NC-5 medium were recognized. The treatment with petroleum ether somewhat destroyed the ability.

"3. The growth rate of purified bacilli was superior than that of crude material.

"4. The potentialities of the growth of *M. lepraemurium* in NC-5 medium as much as in mice were completely destroyed by the treatment with below pH 6 at 37°C for 60 min and by heating at 50°C for 30 min. On the other hand, a complete destruction of the growth potentiality of bacilli in NC-5 medium was resulted by UV irradiation for 2.5 min, whereas the leproma producing ability in mice was maintained even by irradiation for 60 min."

11. "In order to improve the NC-5 medium, the Dubos medium (pH 7.3) was used as a basal medium, instead of Kirchner medium. The complete medium thus prepared is referred to as ND-5 medium. In this medium, *Mycobacterium lepraemurium* quickly multiplies in the form of binary fission without an extraordinary elongation. Possible generation times could be calculated by repeated experiments as 1.4–2.6 days. A slightly degenerative change in the cells during prolonged cultivation was observed by electron microscopy. This medium has some advantages for inhibiting other bacterial contaminations. Serial subcultivation is not tested yet."

[For earlier parts see *Trop. Dis. Bull.,* 1976, v. 73, abstr. 897.]

2. IMMUNOLOGY, PATHOLOGY

58. NIRMALA, V., CHACKO, C. J. G. & JOB, C. K. **Tuberculoid leprosy and tuberculosis skin—a comparative histopathological study.** *Lepr. India,* 1977, v. 49, No. 1, 65–69.

"Since it has been found hard to differentiate histopathologically tuberculoid leprosy from tuberculosis of the skin, a study of 20 biopsies from each of those conditions was undertaken to identify if possible some of their characteristic features.

"In tuberculoid leprosy along with tuberculoid granulomata there is always selective involvement and destruction of nerves, lack of fibrosis, absence of caseous necrosis and often epidermal atrophy. In cutaneous tuberculosis, on the other hand, in addition to tuberculous granuloma, there is often a proliferative reaction of the epidermis, areas of ulceration, absence of nerve destruction, marked increase in the reticulin, significant fibrosis and occasionally caseous necrosis."

59. WAHAL, P. E. *et al.* **Nephrotic syndrome complicating erythema nodosum leprosum (E.N.L.).** *J. Ass. Physns India,* 1977, v. 25, No. 6, 423–426.

"A case of lepromatous leprosy who developed renal amyloidosis with nephrotic syndrome as a complication of Erythema nodosum leprosum (ENL) reaction has been described. The remission

and exacerbation of clinical and biochemical picture of nephrotic syndrome coincided with subsidence and recurrence of lepra reaction. The case report emphasises the importance of early detection and treatment of E.N.L. episodes in lepromatous leprosy in an attempt to possibly prevent the development of this irreversible grave complication in these cases."

60. SINGH, T., KAUR, S., KUMAR, B., SAWHNEY, B. B. & CHOPRA, J. S. **A study of motor and sensory nerve conduction in leprosy.** *Indian J. Med. Res.,* 1977, v. 65, No. 5, 632–639.

"Motor and sensory nerve conduction velocities were studied in ulnar, median, lateral popliteal and posterior tibial nerves in 40 patients with leprosy and compared with 50 age-matched controls. The conduction velocity was found to be decreased in all varieties of leprosy and in all segments of the nerves. Lateral popliteal nerve was found to be the most frequently involved nerve. A clinico-electrophysiological correlation was found between nerve involvement clinically in the form of thickening of nerve weakness and wasting of muscles supplied by the nerve and the degree of conduction abnormality. Motor and sensory nerve conduction velocities were found to be equally affected in the neuropathy of leprosy. The study did not substantiate the presumption that sensory nerve conduction is more affected than motor conduction. It is suggested that for an evaluation of the severity of leprosy polyneuritis, nerve conduction velocity and distal delay especially for the motor nerves should both be tested."

61. KARAT, A. B. A. & RAO, P. S. S. **Haematological profile in leprosy. Part I—General findings.** *Lepr. India,* 1977, v. 49, No. 2, 187–196.

"Haematological studies in 904 adult leprosy patients with different types of leprosy, in various stages of the disease and treatment are described. Haemoglobin, packed cell volume, serum albumin and serum iron are significantly lower among lepromatous leprosy patients as compared with non-lepromatous patients. The serum B12 levels were significantly higher among the lepromatous group. Acid fast bacilli have been demonstrated in skin smear negative leprosy patients with indeterminate and tuberculoid leprosy, suggesting occurrence of bacillaemia in these groups of patients."

The discovery of acid-fast bacilli in bone marrow in 5% of men with indeterminate leprosy and in 4.3% of men with tuberculoid leprosy is of particular interest.

T. F. Davey

62. EL SHIEMY, S., EL HEFNAWY, H., FATTAH, A. A., EL HAWARY, M. F. & FARES, R. **Muscle invovlement in leprosy and its correlation with serum aldolase activity.** *Int. J. Derm.,* 1977, v. 16, No. 7, 587–593.

"Thirty-six leprosy patients underwent muscle biopsy; the specimens were studied for serum aldolase activity. The authors concluded that muscle degeneration occurs only in lepromatous leprosy due to direct invasion by leprosy bacilli increasing serum aldolase activity during the active degenerative phase of the muscle fibers."

3. CLINICAL

63. VACHHARAJANI, S. D., RASTOG, D. S., ARORA, P. N. & SOHI, A. K. **Leprosy in tuberculosis.** *Indian J. Tuberc.,* 1977, v. 24, No. 3, 135–136.

"The present article reports four cases of leprosy one lepromatous and three tuberculoid types. In all these cases, the leprosy was detected in confirmed cases of pulmonary tuberculosis who were on antituberculous drugs for varying intervals of 8–20 weeks.

"It is presumed that leprosy became active and manifest while pulmonary tuberculosis was active and being treated. This perhaps casts doubt about the antigenic similarity between the

tubercle and leprosy bacilli. It is emphasised that in a TB Hospital, careful search for detecting leprosy among its patients should be made periodically, even though the association of the two diseases is not very frequent."

4. THERAPY

64. HAMILTON, J. M. **The place of electrical stimulation in the physiotherapy of leprosy.** *Lepr. India*, 1977, v. 49, No. 2, 197–206.

"The production of nerve and muscle impulses by faradic and interrupted direct current, and the 'strength-duration curves' plotted for normal, denervated, and partially denervated muscles, are described. The advantages and disadvantages of such electrical stimulation in the testing of recent paralysis, the treatment of recent paralysis, and following tendon transfer surgery, in leprosy patients, are discussed. In the light of these, electrical stimulation is concluded to have only a minor role in the physiotherapy of leprosy."

65. KERHARO, J. La pharmacopée sénégalaise: note sur quelques traitements antilépreux traditionnels pratiqués dans le Baouar (préfecture de Kebemer). **[Senegalese pharmacopoeia: notes concerning certain traditional antileprous treatments practised in Baouar (prefecture of Kebemer).]** *Bull. Soc. Méd. Afr. Noire Lang. Fr.,* 1977, v. 22, No. 3, 321–329. English summary.

5. EPIDEMIOLOGY, PREVENTION, CONTROL

66. NAIK, S. S. & GANAPATI, R. **Regularity of dapsone intake by leprosy patients attending urban treatment centre.** *Lepr. India,* 1977, v. 49, No. 2, 207–215.

"Dapsone/Creatinine in urine ratios were determined in statistically randomised samples of 965 leprosy patients attending out-patient department of Acworth Leprosy Hospital and 44 inmates of the Hospital. The percentage of irregularity in DDS treatment was found in 43 and 22.6 respectively in out-patients and inmates of the Hospital. The need to assess the possible response for irregularity in treatment is stressed and the hazard of infectious cases remaining without treatment or with incomplete treatment is pointed out."

[The authors followed the methods described by Low and Pearson, *Trop. Dis. Bull.,* 1974, v. 71, abstr. 2797.]

6. REHABILITATION AND SOCIAL ASPECTS

67. MUTATKAR, R. K. **Health education in leprosy. An evaluation.** *Lepr. India,* 1977, v. 49, No. 2, 234–239.

This is an evaluation by the University of Poona of health education programmes related to leprosy pioneered in that city by the Gandhi Memorial Leprosy Foundation, and undertaken on behalf of the Foundation. Two areas are compared, and random sampling methods employed. The study indicates that the methods employed have effectively changed both attitudes and behaviour of the people towards leprosy, but the process is slow and needs repeated contact between educator and people in a continuous programme.

T. F. Davey

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