Editorial

REHABILITATION TODAY

The Report of the Director General of WHO to the 29th World Health Assembly in May 1976 included the following:

"Disease-orientated medicine needs to be complemented by disability-orientated medicine, and it should be realized in every country that the objectives of medicine are not only the prevention and cure of disease, but also the restoration of the individual to normal social function." (WHO, 1976*a*.)

This statement is of the highest relevance to leprosy workers today, concerned as they are with the most important bacterial cause of crippling disability in the world.

It is now 16 years since the WHO Expert Committee on leprosy defined what rehabilitation in leprosy really meant, in a statement most clear, concise and comprehensive:

"By rehabilitation is meant the physical and mental restoration, as far as possible, of all treated patients to normal activity, so that they may be able to resume their place in the home, society and industry. To achieve this, treatment of the physical disability is obviously necessary, but it must be accompanied by the education of the patient, his family and the public, so that not only can he take his normal place, but society will also be willing to accept him and assist in his complete rehabilitation." (WHO, 1960.)

This Committee was followed by an expert Scientific Meeting on Rehabilitation in Leprosy (WHO, 1961*a*) which went into the subject in greater detail. The co-ordinated use of medical, social, educational and vocational measures in rehabilitation was also advocated by the WHO Expert Committee on Medical Rehabilitation in 1969 (WHO, 1969).

While notable progress has been made, especially in the curative aspects of rehabilitation in leprosy, the underlying basic principles are still not universally recognized. This is well brought out in the proceedings of a recent 7 Nations Consultation on leprosy in S.E. Asia, organized by WHO at the highest level, and concerned with an area of the world in which there are estimated to be more than 4.5 million sufferers from leprosy. The Consultation honestly admitted that, "the prevention and treatment of deformities were regarded as rather neglected areas in the management of leprosy cases." (WHO, 1967*b*.)

Some of the reasons for this situation are not far to seek.

1. The average doctor is *disease*-orientated. If he has met leprosy at all in his medical education, it is likely to have been encountered briefly in the context of bacterial infections or dermatology, with emphasis on chemotherapy, and maybe some reference to remedial surgery. Face to face with the patient, he is likely to be at ease when prescribing dapsone, but feels out of his depth when confronted by the imponderable aspects of the patient's situation, especially if there is no

physiotherapist or social worker onto whose shoulders responsibility can be shifted. Too often disease-orientated medicine is not complemented by disability-orientated medicine, and this is a matter of great importance, because without it many of our efforts can become self-defeating. "Thus patients are deprived of aspects of *primary patient care* which might have saved them from progressive crippling deformity and social, economic, psychological and vocational disability" (10th International Leprosy Congress, 1973).

2. It is simpler to think of rehabilitation in terms of treating visible established deformity than it is to see our primary concern as the *prevention of disability*. The patient who looks physically normal and is mobile may be a wonderful testimony to the skill of surgeon and physiotherapist, but it should be more satisfying to contemplate those patients who have been saved from the need to consult a surgeon. This means that in rehabilitation we have to go back to root causes. The focal point of our activity is *the diagnosis and bringing into care of patients at the earliest possible point in their illness*, at a stage when physical disability is minimal and most amenable to treatment. On this basis rehabilitation must be seen as integral to the primary approach to the patient and his community, inseparable from the personal relationship between health worker and the people and the enlightened community attitude which promotes early diagnosis. "*Rehabilitation must start on the day of diagnosis*" (WHO, 1961*b*) is a worthy motto for leprosy workers at all levels, but even this is not sufficient. Effective rehabilitation demands that the day of diagnosis is pushed further and further back towards the onset of the first symptoms of leprosy. This will happen only when the stigma has been taken out of leprosy. The objectives of rehabilitation are thus inseparable from community enlightenment and health education.

As long as rehabilitation was thought of in terms of curing disability, its sphere was essentially the institution where the skills of surgeon and physiotherapist were available. These will always be needed, but the trend towards prevention rather than cure places the focal point of rehabilitation activity firmly at the periphery, with major responsibility on the shoulders of the local health worker and medical auxiliary. This is to be welcomed, because it is in line with current thought regarding medicine in developing countries, so clearly expressed in one WHO publication after another. National dignity requires that health planning must realistically relate the primary health needs of the greatest possible number of people to the limited economic resources usually available. In practice this imposes a pattern of medicine in which the front line of primary health care is held by a large corps of community health workers and auxiliaries of whom leprosy workers form a part. The training and orientation of these important members of the leprosy control team are thus extremely important. For them in particular an approach which sees the patient in his wholeness with interest and compassion is the starting point of rehabilitation. This orientation needs to be taught, because it is not the approach to leprosy sufferers which comes naturally to most of us. The Director General of WHO states that radical changes are required in education and training, with a new strong emphasis on the training and utilization of auxiliary and community health workers and their supervisors (WHO, 1976*c*). Where leprosy is concerned these changes need to relate to the responsible role of the local worker in relation to rehabilitation. We need more manuals in this respect like that of Kapoor (1975), but carrying the subject in even further detail.

Some interesting facets in rehabilitation are illumined by recent reports from India, where the concept of rehabilitation in leprosy first flowered. In a major report reviewed in this issue, and covering 7 years of research and observation of rehabilitation needs in a large leprosy control programme (Karat *et al.*, 1976), the authors found that out of 6038 patients in the area no less than 40% suffered from sensory loss or more advanced grades of disability due to leprosy. Bacilliferous types of leprosy were involved more severely than non-bacilliferous types, but regular administration of dapsone in such patients had a beneficial effect on nerve function, and a slow but steady decline in patients needing hospital care for trophic ulcers occurred as the project proceeded. The importance of rehabilitation at primary care level is thus clearly demonstrated. Ranjitkumar and Fritschi (1976) in this issue of *Leprosy Review* present a preliminary report concentrating on 88 of the most seriously involved patients in the same programme, those already rejected by family and community or in imminent danger of rejection. They have found that by personal assistance and training, domiciliary rehabilitation is in fact possible in a significant number of patients, and at a considerable saving as compared with the cost of sheltered industry. Another slant on the same subject comes from the study by Wright (1976) also in this issue, of 2 communities of rejected patients in India who have actually succeeded in establishing a more satisfactory economic level of living than prevails in the local population, while posing no medical hazard to surrounding villages. These villages illustrate the basic human need for security and affection in a community framework, something which we should never officiously try to alter.

The battle for the rehabilitation of leprosy patients has to be fought and won, not in the operating theatre, ulcer ward, physiotherapy department or protected workshop, but at the level of family and village. The rural leprosy worker or community health worker is in the forefront of this battle, and his wise training, supervision and support by those technically more highly qualified are immediate priorities.

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Autoradiographic Studies of *Mycobacterium leprae*

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A method is described for the radioactive labelling of fresh bacilli of *Mycobacterium leprae* using tritium labelled *o*-dihydroxyphenyl alanine as a metabolite. A reasonably good correlation has been obtained between the Morphological Index and the Labelling Index in the case of suspensions obtained from the nodules of a group of leprosy patients. A method for organ culture of the intact tissue of leprosy nodules has been developed. Thin sections have been prepared from these organ cultures for autoradiography using [^{3}H]-DOPA. With the aid of a polarizing vertical illuminator, the distribution of the metabolizing organisms within the tissues has been demonstrated.

Introduction

In view of the difficulty of maintaining the bacilli of *M. leprae* in culture for extended periods, the application of a short term assay system which offers possibilities for systematic laboratory investigation related to metabolism, immunological and chemotherapeutic studies has recently attracted attention (Druts and Cline, 1972; Ambrose *et al.*, 1974; Talwar *et al.*, 1974). By the use of high resolution autoradiography as described in the brief note by Ambrose *et al.* (1974), it is possible to observe the localization of silver grains immediately above individual bacilli. This method has now been extended to an investigation of the relationship of the Labelling Index to the Morphological Index and to a study of bacilli within living tissues obtained from the leprosy nodules.

Methods

AGAR FILM TECHNIQUE FOR SUSPENSIONS OF BACILLI OF *M. LEPRAE*

The bacterial suspension was prepared according to the method of Nishiura *et al.* (1969). A nodule from an untreated lepromatous leprosy patient was chopped

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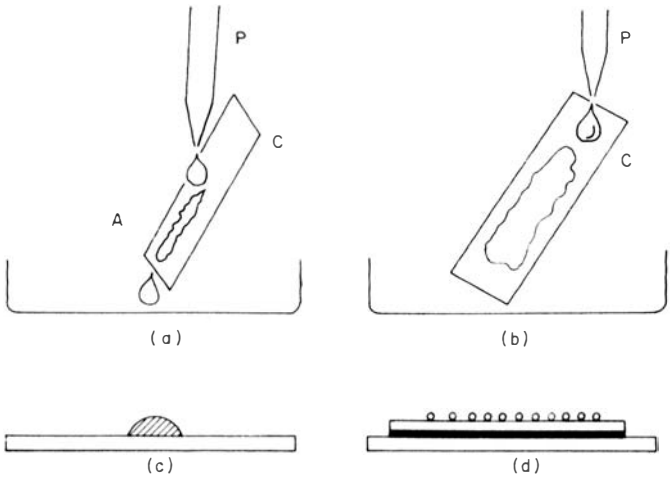

Fig. 1. Stages in the pouring of agar onto a glass coverslip to produce a thin layer. (a) The agar layer is prepared with first pipette. (b) Drop of suspension of *M. leprae* is placed on the agar, with a second pipette. P—pipette; C—coverslip. (c) Cross-section showing agar layer before drying. (d) Dried coverslip mounted with wax on a slide prior to dipping in emulsion. Bacilli lie on the upper surface.

up into small fragments with scissors, teased, and left overnight in a refrigerator in a cavity slide. The supernatant suspension containing bacilli was withdrawn the next morning, tissue debris having settled to the bottom of the slide. This procedure was performed under sterile conditions and a sample of the nodule plated on blood agar and Lowenstein-Jensen medium to rule out the presence of cultivable organisms. A 1% solution of agar was prepared and melted at 50° C in a water bath. A drop of the melted agar was poured on to a sloping coverslip followed immediately by a drop of bacillary suspension (Fig. 1). The coverslip was then placed horizontally in a Leighton tube to set. In this way a uniformly thin coat of agar was obtained containing embedded bacilli. One ml of culture medium (MEM + 10% human adult serum + 100 μ/ml pencillin) was added to the Leighton tubes, 2 of which were maintained as controls. To the other 2 tubes 5 μCi/ml of tritiated DOPA, [^{3}H]-DOPA, was added.

The Leighton tubes were incubated at 37° C for 48 h following which the coverslips were removed and washed 3 times with saline and then fixed in 10% formal saline overnight. They were then washed 6 times with distilled water and air dried. A melted drop of 50/50 paraffin and vaseline mixture was placed on a microscope slide and the coverslip mounted on it with the bacillary surface facing upwards.

K5 Ilford nuclear emulsion was diluted with an equal volume of water in a beaker placed in a water bath at 50° C. This was done in a dark room with a low intensity lamp. The slides with the mounted coverslips were dipped in the thin emulsion, following which they were dried with a fan and placed in a sealed box for 12-14 days in a refrigerator. They were subsequently developed for 5 min with Kodak D19 developer and fixed for 1 min in Amfix with hardener. After washing in running water for 20 min, the slides were air dried. The wax was removed with

xylol. The coverslips were stained in the usual way for acid-fast bacilli by the Ziehl-Neelsen technique and mounted, with the bacilli facing downwards on the slide, with D.P.X. mounting fluid.

ORGAN-CULTURE METHOD FOR INTACT TISSUE

Fragments not larger than 1 mm^3 were prepared from the biopsy and set up for organ culture on expanded stainless steel grids as shown in Fig. 2. The same medium incorporated with tritiated DOPA was employed for these experiments. Control cultures were maintained with the isotope.

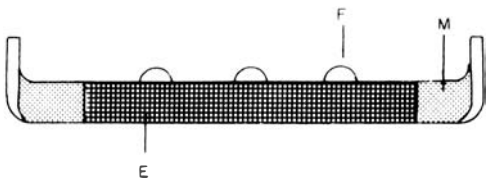

Fig. 2. Organ culture of small tissue fragments of a leprosy nodule. F—fragments; E—expanded metal grid; M—culture media.

The cultures were incubated for 48 h in a 5% CO_2 gassed incubator. They were subsequently washed with saline, fixed with 10% formol saline and embedded in paraffin blocks for histological sectioning. One μm thick sections were mounted on slides and autoradiographs were prepared by coating with K5 Ilford nuclear emulsion as before. The slides were stained by the Fite Faraco technique.

EXAMINATION OF THE AUTORADIOGRAPHS

For viewing by the usual method with transmitted light, light staining of the bacilli is desirable, and the agar film also needs to be comparatively thin. For clear identification of silver grains, particularly in well stained preparations and in the thin sections, the polarizing vertical illuminator M74 of Vicker Instruments, as developed by Rogers (1973) for autoradiography, is an added advantage as shown in Fig. 3.

With this system, the specimen can first be viewed with transmitted light and then with a dark field using polarized light and vertical illumination. Silver grains are clearly seen as bright spots on a dark field. Dye particles do not produce noticeable depolarizing reflections.

Experimental Results

There was a high degree of localization of silver grains immediately above the individual bacilli of *M. leprae* using the agar technique as already described (Ambrose *et al.*, 1974). In the earlier work the agar was poured on the horizontal surface of the coverslips. In some cases DOPA was difficult to wash out completely from the agar when the film was thick, resulting in a low background count. With the thinner agar preparation as shown in Fig. 1, the background count is extremely low. Some fragments of debris containing human cell melanin may get labelled but these can easily be distinguished from *M. leprae* by their morphology and staining. Compact bacilli are found to be labelled with 1 or 2

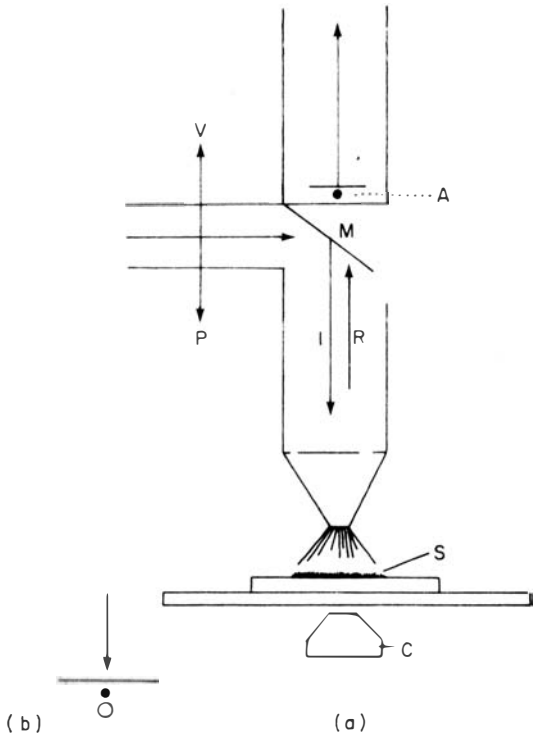

Fig. 3. Polarizing vertical illuminator used examine autoradiographs under dark field. V—vector of incident polarized light; M—partially reflecting mirror; I—polarized light incident on the specimen S; R—light back scattered and depolarized by silver grains; A—analyser with electric vector polarized perpendicular to the plane of the paper.

grains. A comparison of the Morphological Index, the Bacteriological Index and the Labelling Index for untreated patients is given in Table 1. In the third column data are given for 1000 bacilli counted in the usual transmission microscope. A comparison of this figure with that obtained using the combined transmission and vertical illuminator is given in the last column as a double check.

Within the thin sections of intact lepromatous tissue, extremely low background counts were obtained. Groups of the labelled bacilli against a completely dark field could readily by identified. It was found in general that the groups of labelled bacilli tended to appear in clusters in the tissue. Many areas containing stained bacilli were unlabelled. A typical field of labelled bacilli is shown in transmitted light field and in dark field with polarized light in Fig. 4 (a) and (b). Quantitative data obtained with a group of patients are summarized in Table 2.

Discussion

The work of Rees and Valentine (1962) has already indicated from statistical data, using the mouse foot-pad, that a relationship exists between the viability of

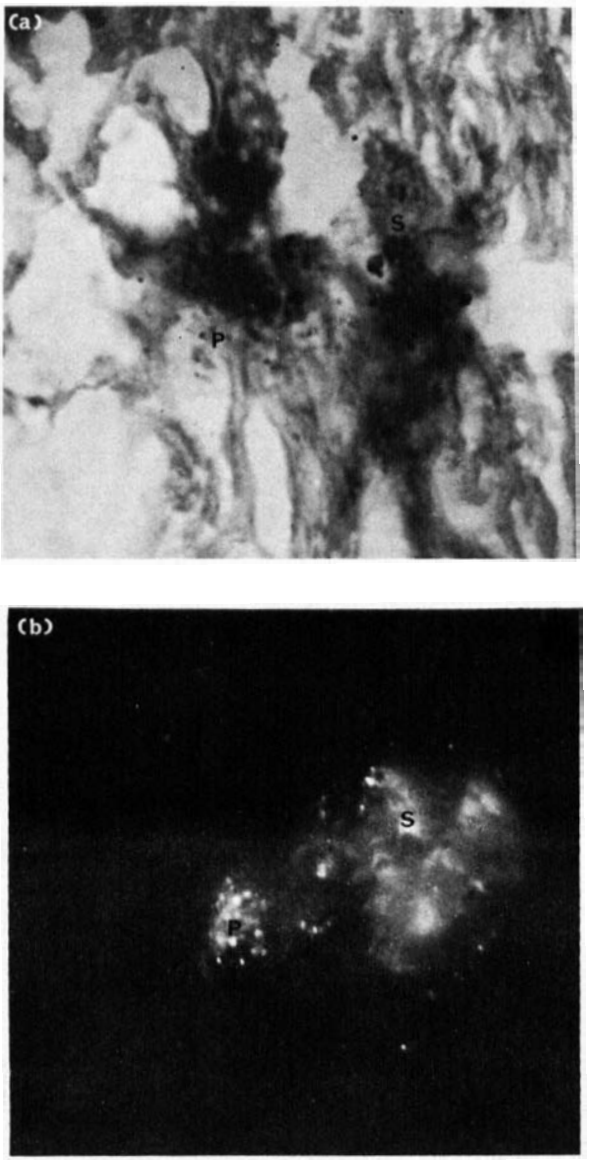

Fig. 4. Autoradiograph of the sections obtained from an organ culture of a leprosy nodule after incorporation of [^{3}H]-DOPA. (a) As seen in transmitted light. A packet of labelled bacilli is clearly visible at P. Many scattered bacilli at S. (b) The same as seen in the polarizing vertical illuminator. The silver grains adjacent to bacilli at P make the rod-shaped bacilli clearly visible. Some diffuse illumination of the tissue is produced by the dense grains at S. These were the only two labelled packets visible in the entire section. The extremely low background counts (dark field) can be seen surrounding the areas P and S.

TABLE 1

Comparison between Morphological Index and Labelling Index by incorporating [^{3}H]-DOPA into M. leprae

Specimen No.	Clinical diagnosis	BI	MI (%)	LI (%)	LI By polarizing microscope
1	LL	3+	4	3	5
2	LL	3+	4.5	5	8.5
3	LL	4+	5	6	3
4	LL	4+	0-1	1.5	0
5	LL	4+	4	4.5	4
6	LL	4+	2	3.1	2.2
7	LL	4+	4	N.D.	4[a]
8	LL	3+	1.4	3	3
9	LL	3+	3	4.7	6.2
10	LL	3+	4[a]	5	6

[a] Sample from early experiments with high background counts before agar pouring method (Fig. 1) was used, not suitable for counting in transmitted light.
BI = Bacteriological Index.
LI = Labelling Index.
MI = Morphological Index.
LL = Lepromatous Leprosy.
N.D. = Not done.
Heat-killed bacilli were used as a control. No silver grains were detected on the killed bacilli.

TABLE 2

Uptake of [^{3}H]-DOPA in thin sections of lepromatous nodules

Specimen no.	Clinical diagnosis	BI	MI (%)	Remarks
1	LL	4+	4	++
2	LL	4+	1	–
3	LL	2+	0	–
4	LL	4+	1.4	+
5	LL	4+	3	++
6	LL	5+	3.3	++
7	LL	3+	1	–
8	LL	5+	3	++

++ = Moderate level grains count.
+ = Low level grains count.
– = Grains count similar to the surrounding area.

M. leprae and the Morphological Index. For the present experiments, using the high level of labelling with [^{3}H]-DOPA of 5 μCi for 48 h, conditions are favourable for a saturation labelling of the organisms. Earlier studies (Ambrose *et al.*, 1974) have already shown that these conditions were optimal for a high level of the Labelling Index, as shown in Table 1. This data provides independent evidence that a relationship exists between the Morphological Index and the number of actively metabolizing bacilli.

The slightly higher values obtained before the use of thin agar techniques for some of the earlier cases may be due to the presence of the background labelling.

Evidence that the uptake of [^{3}H]-DOPA occurs with fresh bacilli of *M. leprae* has been recently obtained by Harris and Prabhakaran (1975). The uptake of [^{3}H]-DOPA by *M. leprae* and melanocytes which contain *o*-diphenoloxidase and by turtle heart cells which may be expected to exhibit catecholamine metabolism was demonstrated by scintillation counting of labelled cell suspensions. But *M. phlei* and armadillo fibroblasts, which do not contain *o*-diphenoloxidase, failed to incorporate DOPA. Treatment with diethyl dithio-carbamate, a copper chelating agent, prevented the incorporation of DOPA by *M. leprae*. DOPA is to be expected to become first attached at the specific receptor sites of *o*-diphenol-oxidase, which is a copper-containing enzyme. The eventual fate of the tritium atoms of DOPA cannot at present be decided, but that pigmented products are not the final product in the case of living bacilli is suggested by the complete absence of detectable pigmentation in suspensions obtained from patients. That DOPA is nevertheless metabolized is suggested by the autoradiographic studies described in the communication. The slides have by necessity to be subjected to extensive washing in water. To prepare slides for autoradiography which will retain water soluble compounds of low molecular weight such as DOPA, special techniques, as described by Rogers (1973) are likely to be required. It is distinctly possible that the tritium becomes incorporated, at least into large peptides, oligonucleotides or oligosaccharides, if not into cellular macromolecules.

The absence of grain counts over heat-killed bacilli strongly suggests that the active metabolism of the organisms is required for DOPA incorporation.

In the case of the bacilli located in the whole tissues, the Morphological Index is not easy to assess. The autoradiographic techniques may be of considerable value in assessing the relative viability of organisms located in intracellular spaces and within various tissues known to retain packets of viable bacilli.

Acknowledgements

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Studies of the Mouse Foot-Pad Technique for Cultivation of *Mycobacterium leprae*. 4. Statistical Analysis of Harvest Data

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An analysis of data generated by harvests of *Mycobacterium leprae* from the foot-pads of mice is presented. Acid-fast bacteria (AFB) were randomly distributed within the circles of a counting slide in fewer than half of the preparations; the AFB were more likely to be distributed randomly in those preparations containing fewer organisms. The mean coefficient of variation

$$100 \times \frac{\text{standard deviation}}{\text{mean}}$$

of the number of AFB was 29% for the 3 circles on a counting slide, 60% for the 4 foot-pads normally pooled for a harvest, and 48% for harvests from 4 replicate pools of 4 to 8 foot-pads. The doubling time of *M. leprae* during logarithmic multiplication in mice averaged 10.7 days, confirming an almost identical estimate made in an earlier study by a different technique. Finally, multiplication of *M. leprae* was found to be a little slower in mice inoculated in both hind foot-pads than in mice inoculated in only one.

This analysis confirms the precision of data generated by work with Shepard's foot-pad technique. Except for the case of foot-by-foot harvests, differences among measurements equivalent in time or numbers of AFB to 2 doublings of *M. leprae* appear certainly to be meaningful.

Introduction

Since its description by Shepard (1960), the mouse foot-pad technique for the cultivation of *Mycobacterium leprae* has been applied to the study of numerous experimental and clinical problems in leprosy. Although the technique is demanding, restricting its employment to a few laboratories, the results of its applications have been useful. The data generated in these laboratories and the conclusions and hypotheses based on them are widely used by investigators and clinicians unfamiliar with the technique. Knowledge of the limitations of the technique is required if the results of experiments are to be correctly interpreted and applied.

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In earlier papers in this series, we examined the fate of inoculated *M. leprae* (Levy *et al.*, 1974), studied the application of the mouse foot-pad technique to the measurement of the proportion of bacilli infective for mice in skin biopsy specimens obtained from leprosy patients (Levy and Murray, 1976), and measured the doubling time of *M. leprae* during logarithmic multiplication in the mouse foot-pad (Levy, 1976). In this report, we present a statistical analysis of the results of the mouse foot-pad harvest procedure.

Materials and Methods

All studies were performed on *M. leprae* harvested from the foot-pad tissues of locally-bred BALB/c mice according to the method described by Shepard (1960), and counted by means of a carefully-standardized technique (Shepard and McRae, 1968). The *M. leprae* employed in the studies were of a number of strains; however, the great majority of the studies, including all measurements of doubling time, were performed with the strain furnished by Shepard in 1967 and used subsequently as the standard strain for most of the work of this laboratory. Most of the studies to be reported were based on data generated in the course of other work. A few were carried out primarily for the purpose of this report.

Results

This study is divided into 6 sections: (1) Distribution of acid-fast bacteria (AFB) within the "circles" of the Reich counting slide (Bellco Glass Co., Vineland, N.J., U.S.A.); (2) variation of the number of AFB among the 3 circles of a counting slide; (3) variation of the number of AFB among replicate harvests, each from a single foot-pad; (4) variation of the number of AFB among replicate harvests, each from a pool of 4 to 8 foot-pads; (5) doubling time of *M. leprae* during logarithmic multiplication in mice; and (6) comparison of multiplication of *M. leprae* in mice inoculated in both hind foot-pads (BHF) with that in mice inoculated only in the right hind foot-pad (RHF).

DISTRIBUTION OF AFB WITHIN CIRCLES

If the AFB were distributed randomly—that is, if each organism were independent of every other organism—within the circles of a counting slide, the sampling distribution of the AFB in the microscopic fields examined would be a "Poisson distribution" with a mean of λ (Goldstein, 1964). To test whether the AFB were distributed according to a Poisson distribution, AFB were enumerated in each of 60 oil-immersion fields examined in the 3 circles on each of 20 slides, and the number was recorded field-by-field. A goodness-of-fit test using the χ^2 statistic (Goldstein, 1964) was used to determine whether the observed distribution of AFB/field corresponded with a Poisson distribution. An example of this calculation is shown in Table 1. In this case (specimen no. 8 of Table 2), 120 AFB were counted in 60 fields, yielding a mean (λ) of 2.00 AFB/field. The value of χ^2 calculated in this example is smaller than the critical value, indicating that the differences between observed and expected frequencies are not inconsistent with a Poisson distribution.

In Table 2, the values of λ and χ^2 are listed for each of 20 specimens, together with the corresponding degrees of freedom. Eleven of the 20 showed differences between observed and expected frequencies of the numbers of AFB/field greater

TABLE 1

Expected and observed distributions of AFB for specimen No. 8 yielding a total of 120 AFB/60 fields ($\lambda = 2.00$ AFB/field)

No. AFB/ field	Frequency		
	Expected		Observed
0	$60e^{-2.0}$	= 8.12	11
1	$60(2.0e^{-2.0})$	= 16.24	14
2	$\frac{60(4.0e^{-2.0})}{2}$	= 16.24	14
3	$\frac{60(8.0e^{-2.0})}{3!}$	= 10.83	11
⩾ 3	60 − 51.43	= 8.57	10
Total		60	60

$$\chi^2 = \frac{(8.12-11)^2}{8.12} + \frac{2(16.24-14)^2}{16.24} + \frac{(10.83-11)^2}{10.83} + \frac{(8.57-10)^2}{8.57} = 1.88^*$$

*The critical value of χ^2 for $P=0.01$ with 3 degrees of freedom=11.3; therefore, the observations are consistent with random distribution of the AFB.

TABLE 2

χ^2 as a function of λ for 20 specimens*

Specimen no.	λ	DF†	χ^2	Specimen no.	λ	DF†	χ^2
1	0.12	1	0.48	11	2.38	3	47.69‡
2	0.45	1	9.96‡	12	2.42	4	1.12
3	0.60	1	0.33	13	3.48	3	10.35§
4	0.82	1	0.89	14	4.43	4	7.83
5	1.27	2	26.62‡	15	5.72	3	42.63‡
6	1.37	1	49.28‡	16	6.75	5	22.07‡
7	1.85	3	15.49‡	17	7.47	5	36.78‡
8	2.00	3	1.88	18	8.20	1	55.92‡
9	2.02	3	2.26	19	9.88	4	1211‡
10	2.05	3	11.53§	20	60.67	1	4095‡

* λ = mean no. AFB/oil immersion field.
† Degrees of freedom.
‡ $P < 0.01$.
§ $0.01 < P < 0.05$. $P > 0.05$ for all values of χ^2 not footnoted.

than would be consistent with a Poisson distribution. Two additional specimens yielded χ^2 values larger than the critical values for $P = 0.05$ but smaller than those for $P = 0.01$, suggesting that these were borderline cases.

The frequency distributions of the number of AFB per field of 3 representative specimens are shown in Fig. 1. The expected and observed frequencies for

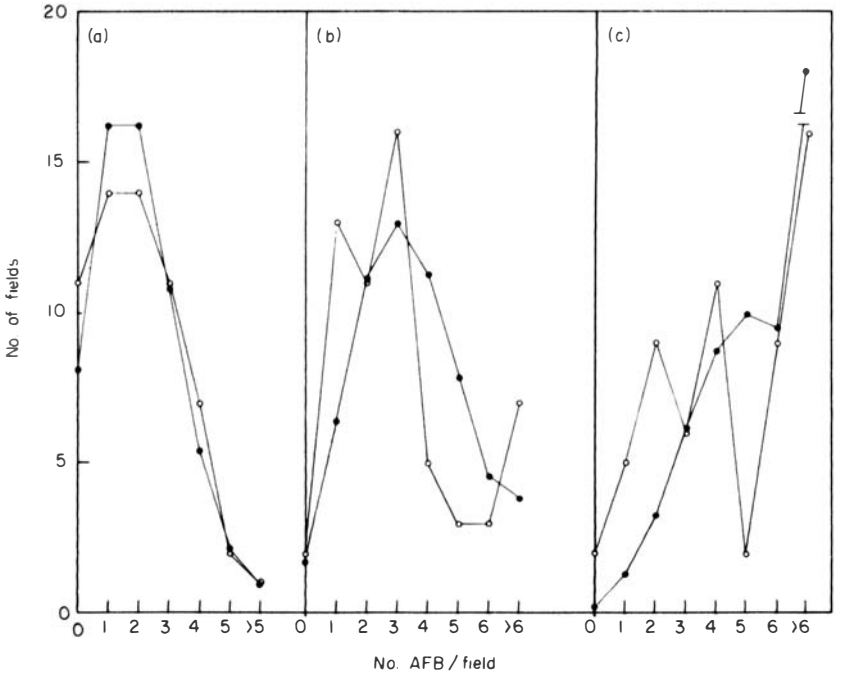

Fig. 1. Frequency distribution of the number of AFB/field of three representative specimens. (a) Specimen no. 8; $\lambda = 2.00$, $\chi^2 = 1.88$, $P > 0.05$. (b) Specimen no. 13; $\lambda = 3.48$, $\chi^2 = 10.35$, $0.05 > P > 0.01$. (c) Specimen no. 15, $\lambda = 5.72$, $\chi^2 = 38.73$, $P < 0.01$. The expected frequency (●) and (○) the observed frequency.

specimen no. 8, shown in the left-hand panel, are quite similar. The expected and observed frequencies are plotted in the center panel for specimen no. 13, which yielded a χ^2 value of borderline significance. In this case, more than the expected numbers of fields contained 1 AFB and more than 6 AFB, whereas the numbers of fields containing 4 or 5 AFB were smaller than expected. The excessive number of fields observed to contain small numbers of AFB is even more apparent in the distribution of AFB shown in the right-hand panel from specimen no. 15, which yielded a significantly large value of χ^2. In this case also, the number of fields containing intermediate numbers of AFB is smaller than expected.

Inspection of the data of Table 2 suggests that the AFB are less likely to be distributed according to a Poisson distribution in preparations yielding larger values of λ. This impression may be tested by plotting $\log_{10}\chi^2$ as a function of $\log_{10}\lambda$ for these 20 samples. The regression of $\log_{10}\chi^2$ on $\log_{10}\lambda$, shown in Fig. 2, has a slope significantly greater than 0, confirming that AFB from specimens with smaller values of λ are more likely to be randomly distributed in the circles of a counting slide than are AFB from specimens yielding larger values of λ.

VARIATION OF THE NUMBER OF AFB FROM CIRCLE TO CIRCLE

The variation of the number of AFB among the 3 circles of a counting slide was determined for the 233 slides prepared during 1975 that yielded a total of at least

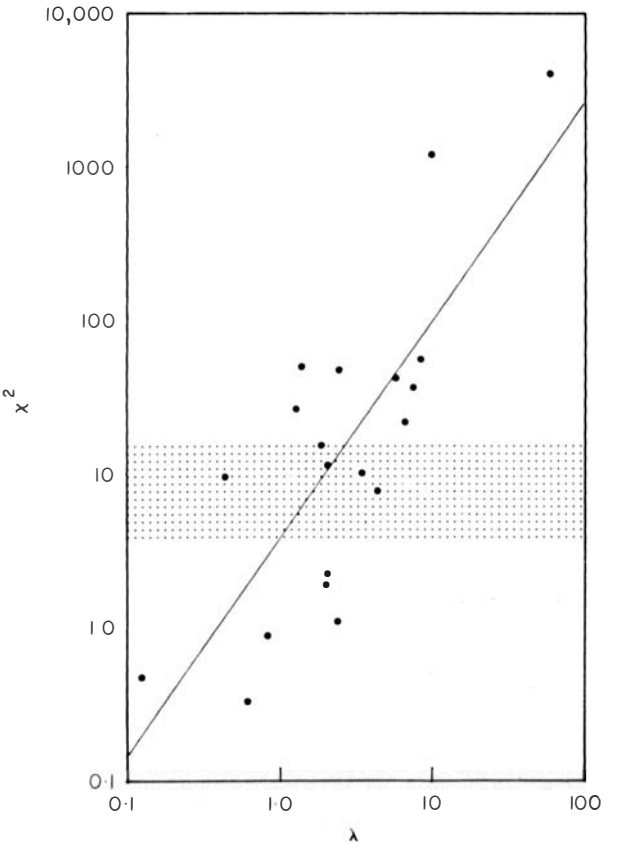

Fig. 2. $\text{Log}_{10}\chi^2$ as a function of $\log_{10}\lambda$ (λ = mean no. ABF/field) for 20 preparations in which the number of AFB counted in each microscopic field was compared to that expected from a Poisson distribution. The shaded area represents the region of critical values of χ^2 for $0.05 > P > 0.01$ and 1 to 5 degrees of freedom. The equation of the regression line is: $\log_{10}\chi^2 = 1.14 + (1.41 \pm 0.55)(\log_{10}\lambda - 0.40)$; the correlation coefficient, $r = 0.78$.

50 AFB in the 60 fields examined. For each slide, the mean and standard deviation of the number of AFB in each of the 3 circles were calculated. The variation from the mean of the 3 circles of the number of AFB counted in each of the circles is expressed as the coefficient of variation (C.V.), which is 100 times the standard deviation divided by the mean (Goldstein, 1964). Use of this statistic permits one to consider the variation among the 3 counts independently of an effect of the magnitude of the counts, as shown by the regression of the C.V. on the total number of AFB:

$$\text{C.V.}/100 = 0.35 - 0.00012\,(\text{no. AFB} - 210).$$

The 95% confidence limits around the estimate of the slope of the regression line include 0 and the correlation coefficient, $r = 0.12$, a value not different from 0 at

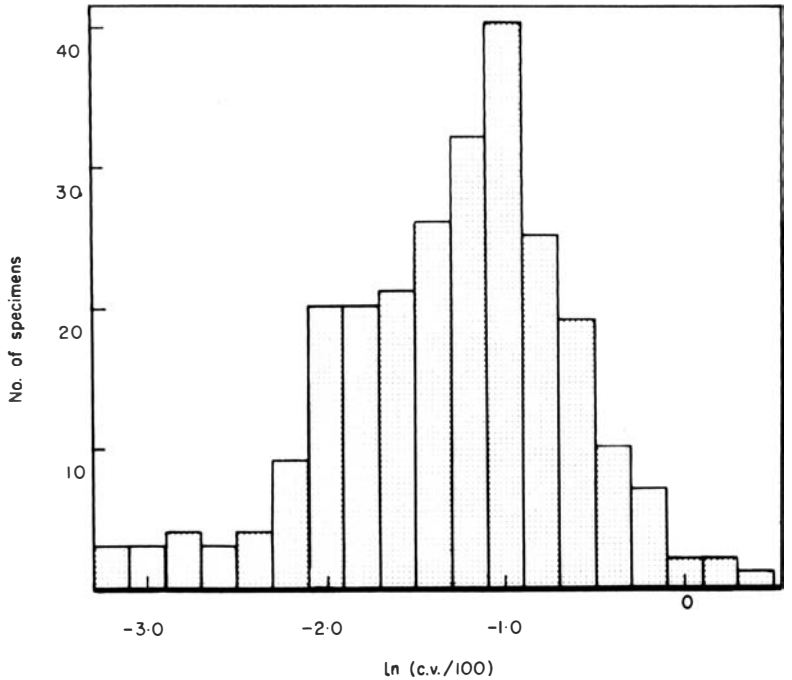

Fig. 3. Frequency distribution of ln (C.V./100) of the number of AFB/circle for 233 specimens.

$P = 0.05$. Thus, the C.V. did not depend on the total number of AFB counted in the 3 circles of each slide.

The C.V. was not normally distributed, so that the dispersion of the individual values of the C.V. about the mean value could not easily be described. The natural logarithm (ln) of the C.V. was distributed as shown in Fig. 3; the χ^2 goodness-of-fit test showed that this distribution was consistent with a normal distribution ($P > 0.1$). The mean C.V. was 29%, with 95% confidence limits of 26 and 31%.

In 25 of the 233 slides (10.7%), the number of AFB in 1 of of the 3 circles fell outside the range −50% to +100% of the mean for the 3 circles. These slides yielded larger values of the C.V. Moreover, 14 of 77 slides yielding 50 to 99 AFB/60 fields were included in this group, whereas only 11 of the 156 slides with 100 or more AFB were included; these proportions are significantly different ($P < 0.01$). Thus, a disproportionately large fraction of these 25 slides was composed of slides with smaller numbers of AFB.

VARIATION OF THE NUMBER OF AFB FROM FOOT TO FOOT

On 13 occasions, harvests of *M. leprae* were performed from 4 individual foot-pads of mice of the same group. These results are summarized in Table 3. The ratio of the largest to the smallest number of AFB harvested from the 4 foot-pads of a group ranged from 1.67 to 20.0, with a mean of 6.66. The mean C.V. for the 13 sets of 4 foot-by-foot harvests, calculated by averaging the values of ln C.V., was 60%, with 95% confidence limits 45 and 79%.

TABLE 3

Variation of the number of AFB from foot to foot

Experiment no.	Number of AFB/foot-pad (x 10^5)				Ratio*	C.V. %
1	1.38	3.60	8.89	13.1	8.49	78
2	8.95	14.4	19.4	59.3	6.63	90
3	1.90	7.27	11.5	12.4	6.53	58
4	1.01	1.30	1.56	2.04	2.02	30
5	3.77	4.63	5.69	10.6	2.81	49
6	1.35	2.22	6.48	17.0	12.6	106
7	0.85	1.02	3.92	6.00	7.06	84
8	3.92	5.85	8.36	13.3	3.39	52
9	0.70	0.84	0.84	1.55	2.21	39
10	27.6	30.3	39.9	46.2	1.67	24
11	2.21	2.88	5.11	13.3	6.02	87
12	1.39	3.77	8.45	8.46	6.09	64
13	0.28	1.70	2.79	5.61	20.0	87

* Ratio of largest to smallest number in set of 4.

TABLE 4

Variation of the number of AFB/foot-pad among replicate harvests from pools of 4 to 8 foot-pads

Experiment no.	Number of AFB/foot-pad (x 10^5)				Ratio*	C.V. (%)
1	0.85	4.22	4.70	6.34	7.43	57
2	6.30	8.03	9.76	13.4	2.13	32
3	2.04	2.07	2.24	4.80	2.35	48
4	4.52	7.94	18.5	35.0	7.74	83
5	2.38	3.32	5.07	6.94	2.92	45
6	4.45	4.53	11.0	13.1	2.94	54
7	0.17	0.50	0.50	0.68	4.00	46
8	0.73	0.87	0.96	1.09	1.50	17
9	4.47	5.17	6.26	7.31	1.64	21
10	0.82	1.50	2.10	2.82	3.44	47
11	13.00	15.0	19.0	32.0	2.46	43
12	0.29	0.41	1.30	3.00	10.3	100
13	0.88	0.91	2.10	2.39	2.72	50
14	8.65	20.0	31.0	39.0	2.31	54
15	0.12	0.19	0.60	0.88	7.33	80
16	3.34	5.90	8.50	9.54	2.86	41
17	1.05	1.35	1.56	1.87	1.78	24
18	0.19	0.41	0.42	1.41	7.42	90
19	3.20	5.92	11.2	11.3	3.53	51
20	3.62	3.98	6.69	11.1	3.07	54
21	5.62	15.6	21.7	22.5	4.00	48
22	9.00	20.1	30.4	30.9	3.43	46
23	0.85	1.50	2.53	3.74	4.38	59

* Ratio of largest to smallest number in set of 4.

VARIATION OF THE NUMBER OF AFB FROM HARVEST TO HARVEST

On 23 occasions, 4 harvests of *M. leprae* were performed on the same day, each from the pooled tissues of 4 to 8 foot-pads of mice inoculated in the same experiment. The results of these harvests are presented in Table 4. The ratio of the largest to the smallest yield of the replicate harvests ranged from 1.50 to 10.3, with a mean of 4.08. The mean C.V. for the 23 sets of 4 replicate harvests, calculated by averaging the values of ln C.V., was 48%, with 95% confidence limits 40 and 57%. Thus, the variation among replicate harvests of *M. leprae* from pools of 4 to 8 foot-pads is smaller than that among harvests from individual foot-pads, but greater than that found among circles prepared from the same bacterial suspension.

DOUBLING TIME OF *M. LEPRAE*

On many occasions during the past 8 years, 2 harvests of *M. leprae* were made during logarithmic multiplication of the same strain of organisms. On 129 occasions, the earlier of the 2 harvests yielded $> 5 \times 10^4$ but $< 5 \times 10^5$ AFB/foot-pad. The slope of the line joining the 2 harvests was calculated for each of the 129 experiments, in order to derive an estimate of the doubling time of this strain of *M. leprae* during logarithmic multiplication. The mean slope was 0.094 $\log^2$ units (0.094 doublings)/day; the reciprocal of this slope represents a mean doubling time of 10.7 days. The 95% confidence limits around this estimate of the mean doubling time are 9.89 and 11.6 days per doubling.

MULTIPLICATION IN BOTH HIND FEET COMPARED TO THAT IN ONE HIND FOOT

In 8 experiments, mice of one group were inoculated RHF and mice of a second group were inoculated BHF with equal portions of the same bacterial suspension. Harvests of *M. leprae* from the pooled tissues of 4 foot-pads were performed at intervals, growth curves were constructed, and the number of days fron inoculation to multiplication to the level of 10^6 AFB/foot-pad was calculated from each growth curve. On the average, the time required for multiplication to 10^6 AFB/foot-pad of mice inoculated BHF was 6.5% longer than that of mice inoculated RHF; however, this value is not significantly different from 0. The regression of the time to 10^6 AFB/foot-pad of mice inoculated BHF on that of mice inoculated RHF is shown in Fig. 4. The slope of the regression line, 1.41, is significantly larger than 1.0, indicating that multiplication of *M. leprae* is somewhat slower in mice inoculated BHF than in those inoculated RHF.

In order to interpret this finding, it is necessary to consider the possibility that fewer AFB were systematically inoculated LHF than RHF. In 16 experiments, mice that had been inoculated BHF were killed, and harvests of *M. leprae* were performed from separate pools of 4 RHF and 4 LHF. In 10 of these experiments, the numbers of AFB harvested from both pools were $> 10^5$ and $< 10^6$/foot-pad. These data are presented in Table 5. The mean difference between the yields of *M. leprae* from pools of RHF and LHF was not significantly different from 0. If the organisms may be assumed to have multiplied at the same rate in either hind foot-pad of mice inoculated in both, then there is no evidence that the number of *M. leprae* inoculated LHF was systematically different from that inoculated RHF.

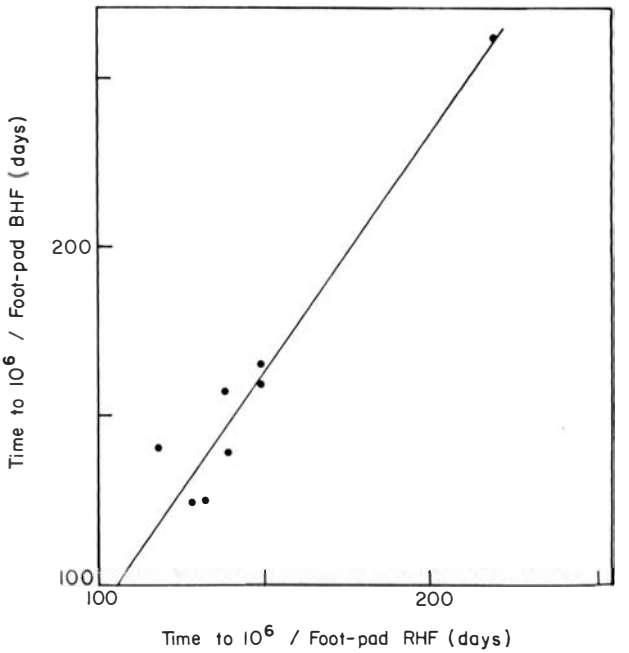

Fig. 4. Time to 10^6 AFB/foot-pad in mice inoculated BHF as a function of the time to 10^6 AFB/foot-pad in mice inoculated RHF. The equation of the regression line is: No. days BHF = 158 + (1.41 ± 0.29) (No. days RHF − 146); $r = 0.98$.

TABLE 5

Variation of the number of AFB/foot-pad from LHF to RHF

Experiment no.	Number of AFB/foot-pad ($\times 10^5$) RHF	LHF
1	5.79	4.12
2	1.94	4.10
3	3.93	2.27
4	1.36	3.70
5	9.16	6.84
6	3.74	3.03
7	9.70	5.27
8	1.99	1.51
9	4.62	2.86
10	2.56	2.16

Discussion

Application of Shepard's technique for cultivation of *M. leprae* in the mouse foot-pad (Shepard, 1960) have produced a considerable body of data that have subsequently been employed in making clinical decisions and in the selection of the drugs for trials and for use in therapy, to name but a few important uses. But because the technique is carried out in only a few laboratories, many workers who use its results do not understand clearly its strengths and limitations. The performance of the technique during the 10 years since this laboratory was established has generated much data, an analysis of which may be informative to some of the "consumers" of these and similar data. The purpose of this report is to present such an analysis.

The presentation is divided into 6 sections. The first 4 sections represent a progressive increase of scale, beginning with the distribution of AFB among the microscopic fields examined in a circle of a counting slide, and ending with the reproducibility of the harvest procedure performed on the pooled tissues of a number of mouse foot-pads. The last 2 sections represent "by-products" of this analysis—a second look at the doubling time of *M. leprae,* and a comparison of the rate of multiplication in mice inoculated in both hind foot-pads to that in animals inoculated in one.

In the performance of Shepard's technique, AFB are enumerated in only a minute fraction of the bacterial suspension that results from a harvest of *M. leprae.* A 10-μl aliquot of a suspension 2 ml or greater in volume is pipetted onto each of the surfaces bounded by the 3 circles of a counting slide, and AFB are enumerated in 20 1250× oil-immersion fields along the equator of each circle. The diameter of a 1250× field of one of our microscopes is 0.18 mm, and that of a circle of a slide from the batch currently in use is 1.13 cm. Therefore, one field represents 0.00025 of the area of a circle, or 0.0025 μl, and the 60 fields examined represent 0.15 μl, or 0.00075 of a 2-ml suspension. Had we established that the AFB were independent and randomly distributed, in the sense that their distribution fit a Poisson distribution, then accurate estimates of the number of AFB in the mouse foot-pad could be readily obtained, the only source of variation being sampling (experimental) error. However, the results indicate that one cannot assume the AFB to be randomly distributed on the circles of a counting slide, especially when the numbers of AFB are large.

Such a situation could have resulted from "clumping" of the *M. leprae,* both as closely-grouped organisms and as groups of well-separated organisms contained within tissue cells that had escaped disruption. These phenomena, encountered not infrequently in work with mouse foot-pad technique as described by Shepard (1960), would produce more fields containing both larger and smaller numbers of AFB than would be predicted from the mean number of AFB per field. The demonstration that a non-random distribution was associated with larger numbers of organisms is difficult to interpret. Even a small value of λ, the mean number of organisms/oil-immersion field, is consistent with multiplication of *M. leprae.* Specimen no. 8 ($\lambda = 2.0$), for example, represented a yield of 5.62×10^5 AFB/foot-pad, more than 100 times the number of *M. leprae* inoculated. Thus, the association of non-randomness with larger numbers is not simply a result of multiplication.

Because it is not possible to quantitate the increased variation that results from the non-random distribution of the AFB, other methods of assessing the precision

of the mouse foot-pad technique must be employed. The C.V. offers another means of assessing variation in a population. Although it may lack a degree of precision, it possesses the advantages of independence of the size of the population—in this case, the number of AFB counted. And we have shown that the ln C.V. is approximately normally distributed, permitting the application of normal statistics for testing the hypotheses and determination of confidence intervals. The usefulness of the C.V. resides in its relationship to the standard deviation. If the mean number of AFB counted in the 3 circles of a counting slide is 100, then the "true mean" lies in the range 100 ± 29 (mean $\pm$ (C.V./100) $\times$ mean), and the 95% confidence limits around the number of AFB/foot-pad calculated from the mean or total number of AFB counted in 3 circles would be 29% larger and 29% smaller than the number calculated.

One might expect that the C.V. for replicate slides would be smaller than that for replicate circles—i.e. that the variation of the number of AFB between duplicate slides would be smaller than that among triplicate circles, and that the C.V. for 2 repeated counts on the same slide would be smaller yet. In the course of another study (Levy *et al.*, 1976), 2 observers performed duplicate counts on 20 slides. The mean values of the C.V. calculated for each observer were 9 and 14%; the mean ratios of the larger to the smaller number of *M. leprae* per slide were 1.33 and 1.31, respectively. These values are significantly smaller than those for replicate circles.

Although *M. leprae* are most often harvested from pools of mouse foot-pad tissues rather than from the tissues of individual foot-pads, the variation of the number of AFB from foot to foot was of some interest. Harvests from sets of 4 foot-pads yielded a C.V. of 60% and a mean ratio of the largest to the smallest number of AFB/foot-pad of 6.66. This situation is quite different from that representing more common application of foot-by-foot harvesting, in which one seeks evidence of variation among animals or evidence that a marginally adequate inoculum has infected some animals but not others. In these latter applications, one would expect a much larger value of the C.V., with a much broader range between the smallest and largest number of AFB/foot-pad.

One would also expect that the variation among harvests of *M. leprae* from replicate pools of mouse foot-pad tissues would be smaller than that among individual foot-pads. This was indeed the case; the C.V. is smaller for harvests from replicate pools of from 4 to 8 foot-pads than for harvests from individual foot-pads. A most important observation is that pooling the tissues does not, under ordinary circumstances, obscure really unacceptable variation from foot to foot.

One by-product of this study was the measurement of the doubling time of a single strain of *M. leprae* during logarithmic multiplication in mice. In another study (Levy, 1976), we found the doubling time, measured by a different technique, to be 11.1 ± 1.9 days. The measurement made in this study—10.7 ± 0.8 days—is virtually identical.

A second by-product was the comparison of rates of multiplication of *M. leprae* in mice inoculated BHF to that in mice inoculated only RHF. Multiplication in animals inoculated BHF was significantly slower, but the difference of rates was so small that it was not meaningful biologically. The difference did not reflect a systematically smaller inoculum LHF. This observation is of some practical importance. In many experiments we inoculate mice BHF rather than RHF in order to economize on mice, cages and the demands on the staff of our animal

house. We had been reassured that this was an acceptable procedure by the results of an earlier study (Levy, 1975), in which we showed that mice infected with *M. leprae* in one hind foot-pad 15 to 60 days earlier were not protected against *M. leprae* challenge in the second hind foot-pad. At every challenge interval, multiplication of *M. leprae* in the challenge inoculum was compared to that in control mice not previously infected. The control mice were inoculated BHF. Perhaps had the control mice been inoculated RHF, we might have observed a small effect of the primary infection at the earlier intervals similar to the differences observed in the current study between mice inoculated BHF and those inoculated RHF.

The data presented in this analysis suggest that the mouse foot-pad technique as described by Shepard exhibits a considerable degree of precision. Except in the case of foot-by-foot harvests, differences among measurements equivalent in time or in numbers of *M. leprae* to at least one doubling (or halving) may be accepted as real; differences equivalent to two doublings appear certainly to be meaningful.

Acknowledgements

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Serum Iron and Total Iron Binding Capacity in Burmese Patients with Leprosy

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Serum iron and total iron binding capacity were estimated in the sera collected from 96 male leprosy patients (age 20-40 years) belonging to Leprosy Hospital, Htaukkyant. The sera from 22 healthy subjects were included in the study as controls. A low level of serum iron (below 50 μg%) with normal iron binding capacity was recorded in 36 out of 60 patients (60% with lepromatous leprosy, 14 out of 36 patients (39%) with tuberculoid type and only 4 out of 22 controls (18%). There was no difference in the prevalence of low serum iron and percentage saturation between those leprosy patients who had been on DDS and ferrous sulphate pills for at least 2 years and untreated new leprosy patients.

Bone marrow from 7 patients with anaemia and low serum iron, as well as the liver at autopsy from 3 male patients with advanced lepromatous leprosy and low socio-economic status showed normal iron content. Injection of parenteral iron (10 daily injections of Imferon) to 6 patients with anaemia and low serum iron also showed no increase in haemoglobin level.

Thus the anaemia in leprosy is not due to true lack of iron in the body (tissue iron deficiency). However, reduction in serum iron level is probably due in part to the toxic process of the disease upon the body.

Introduction

Anaemia is one of the common complications in patients with lepromatous leprosy. Low levels of serum iron with normal total iron binding capacity (TIBC) and transferrin in British patients with leprosy were found by Tin Shwe and his co-workers in 1972, but their study was based on a limited number of treated leprosy patients belonging to a developed country where the socio-economic status of the patients is entirely different from that of our country.

Burmese leprosy patients have been supplied with ferrous sulphate pills together with antileprosy drugs as a routine to prevent anaemia, but the usefulness of iron pills is not known. This study was carried out in order to establish the effect of ferrous sulphate pills on the iron status of leprosy patients.

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Materials and Methods

Estimation of serum iron and total iron binding capacity was carried out on the sera collected from a random sampling of 96 male leprosy patients (ages between 20-40 years) belonging to Htaukkyant Leprosy Hospital, Rangoon, Burma. Of these, 60 patients had lepromatous leprosy while the remaining 36 belonged to the non-lepromatous tuberculoid group. Half of the patients were untreated and newly diagnosed cases at the hospital while the remainder had been treated as in-patients of the hospital with antileprosy drugs (DDS 50-100 mg) and ferrous sulphate (5 grains or 325 mg) daily for not less than 2 years. All the patients were clinically free from renal and hepatic disorders. The blood samples were taken in the morning or afternoon and not in the evening.

The sera from 22 healthy males belonging to the same age group and who came to the hospital for skin infestations other than leprosy were included in the study as controls. The serum iron and total iron binding capacity were estimated by the method of Shade *et al.* (1954).

The haemosiderin content of the bone marrow from 7 patients with anaemia and low serum iron, and the liver non-Hb iron from 3 male autopsied patients with advanced lepromatous leprosy and low socio-economic status were also estimated.

Ten injections of parenteral iron (2 ml Imferon each containing 100 mg of iron as ferric hydroxide were given daily to 6 patients with anaemia and low serum iron level and their haemoglobin level was determined at weekly intervals for 4 to 6 weeks.

Results

Out of 60 patients with lepromatous leprosy, 36 patients (60%) showed a reduction in iron level (< 50 μg%) with normal or reduced level of total iron binding capacity. There was no significant difference in the level of mean serum iron or TIBC between those patients who had not taken ferrous sulphate pills and those who had taken ferrous sulphate and DDS for not less than 2 years.

Out of 36 patients with tuberculoid leprosy, 14 patients (39%) showed a reduction in serum iron level with normal or reduced total iron binding capacity. In this group also there was no significant difference in the level of serum iron or TIBC between those patients who had not taken ferrous sulphate pills and those who had taken ferrous sulphate and DDS for not less than 2 years.

Out of 22 controls, 4 subjects (18.2%) showed reduction in serum iron level with normal level of total iron binding capacity. The details are given in Table 1.

The prevalence of patients with reduced serum iron level is significantly higher in patients with lepromatous leprosy when compared to those with tuberculoid leprosy and controls. The mean level of serum iron is also significantly reduced in patients with leprosy when compared to controls with the exception of the untreated lepromatous group. A similar significant difference in the level of total iron binding capacity between leprosy patients and controls is observed, with the exception of the untreated lepromatous group. However, the mean level of percentage saturation is not significantly reduced in patients with leprosy, the only exception being the treated lepromatous group.

Taking all untreated leprosy patients as a whole, 24 out of 50 (48%) had serum iron level below 50 μg%, while the treated group had 26 out of 46 (56%)

TABLE 1

Serum iron (SI) total binding capacity (TİBC) and percentage saturation (% sat.) of healthy controls and leprosy patients

Subjects	No.	Mean ± S.D.			Number with	
		SI	TIBC	% Sat.	SI < 50 μg %	% Sat. < 15
Control	22	106 ± 48	338 ± 71	31 ± 15	4 (18)	5 (23)
Tuberculoid						
Untreated	19	74 ± 47	268 ± 68	28 ± 15	7 (37)	5 (26)
Treated	17	69 ± 39	275 ± 61	26 ± 14	7 (41)	6 (35)
Tuberculoid (all)	36				14 (39)	11 (33)
Lepromatous						
Untreated	31	74 ± 56	297 ± 88	25 ± 15	17 (55)	12 (26)
Treated	29	52 ± 42	266 ± 85	20 ± 14	19 (66)	14 (20)
Lepromatous (all)	60				36 (60)	26 (20)
Total leprosy	96				50 (52)	37 (21)

Figures in parentheses represent percentage of respective total.

with low serum iron. Similarly 17 out of 50 (34%) untreated leprosy patients and 20 out of 46 (43%) treated patients had percentage saturation below 15. Thus the prevalence of low serum iron and reduced percent saturation in leprosy patients is significantly higher when compared to those in the control group, i.e. 18 and 23% respectively).

Bone marrow taken from 7 patients with anaemia and low serum iron showed stainable iron (haemosiderin). The liver autopsy specimens taken from 3 male patients with advanced leprosy and low socio-economic status also showed normal iron content.

The 6 patients with anaemia and low serum iron who had been given parenteral iron (10 injections of Imferon) showed no increase in haemoglobin levels.

Discussion

Reduction in cell size and haemoglobin content are characteristic of the fully developed picture of iron deficiency anaemia in which the synthesis of haemoglobin is reduced. In the early evolution of this type of anaemia either of these morphological changes may be seen, but biochemical investigations reveal the underlying disorder that leads eventually to its production. The levels of plasma iron and its carrier protein transferrin most frequently measured as the total iron binding capacity (TIBC) of plasma and the percentage of saturation often provide a clue to the cause of defective haemoglobin synthesis (Turnbull, 1971).

In our investigation a random sample of male patients with ages between 20-40 years was chosen for study because of the lesser risk of iron deficiency in males.

Although serum iron and total iron binding capacity in patients with lepromatous leprosy did not differ significantly from those with non-lepromatous leprosy they were significantly lower than those of controls. This finding, however, is not in full agreement with the earlier study where Tin Shwe *et al.* (1972) using the spectrophotometric method found no reduction in total iron binding capacity, and serum transferrin level in patients with lepromatous leprosy, but the mean level of serum iron was significantly reduced in patients with lepromatous leprosy, though their method of serum iron estimation was different from that of the present study.

The lowered serum iron levels may be due to a reduction in iron supply, which again either may be due to a true lack of iron in the body (body iron deficiency) or to a failure in the delivery of iron from iron stores in bone marrow leading to what has been called "iron deficient erythropoisis". The true lack of iron in the body (body iron deficiency) would be due to an iron deficient diet, poor absorption of iron from the intestine, or excessive loss of iron following acute or chronic haemorrhage. The failure in delivery of iron from stores to bone marrow may occur if transporting protein transferrin is deficient, or if associated with chronic disorders (Cartwright, 1966; Cartwright and Lee, 1971).

In this present study the patients were free from acute and chronic haemorrhages, and defective iron absorption has not been reported in leprosy. The bone marrow taken from 7 patients with anaemia and leprosy, as well as the liver of 3 patients who had died of leprosy showed that there is no deficiency of iron in the stores. Therefore the reduction in serum iron level is probably due to impaired delivery of iron from the stores to the circulation as a result of the toxic process of the disease upon the body.

More than half of the patients who are on regular ferrous sulphate pills also showed reduced serum iron levels. Since those patients are provided with such pills daily to be taken in the presence of the nurses there is no possibility that they may not be taking the pills. Therefore the value of routine iron pills in leprosy remains to be judged from the cost effectiveness point of view. Instead, multivitamin and other measures may be needed to improve the general nutritional status of the patients.

Acknowledgements

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A Preliminary Report on a Study of Serum Alpha-1-Antitrypsin and Immunoglobulin Levels in Lepromatous Leprosy

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Introduction

While it is known that alpha-1-antitrypsin deficiency is associated with hereditary pulmonary emphysema (Laurell, 1963), respiratory distress syndrome in premature infants (Evans *et al.*, 1970), and juvenile liver cirrhosis (Sharp, 1971), its concentration in serum is considerably increased under various other physiologic and pathologic conditions (Ganrot and Bjerre, 1967; Kueppers, 1968, 1972; Schultze and Heremans, 1966; Cleve and Behrend, 1966).

Young *et al.* (1973) reported that alpha-1-antitrypsin levels, measured by the trypsin inhibitory capacity method, were increased in patients with untreated active sarcoidosis when compared with patients with inactive sarcoidosis and with age-sex matched healthy controls. Since the etiology of sarcoidosis is, at present, unknown, it would be of interest to assay these globulins in a chronic granulomatous disease of known etiology.

Human leprosy provides such an example. This disease is caused by a very slowly developing bacterial infection in which the antigenic load of the bacterial population cannot be rapidly decreased even by vigorous chemotherapy and antibiotic treatment. Therefore, human lepromatous leprosy affords an opportunity for the study of the effect of continuous antigenic stimulation on alpha-1-antitrypsin levels. In addition, immunoglobulin levels can be examined.

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Materials and Methods

Sera from 34 hospitalized patients under treatment for lepromatous leprosy were obtained. These patients had been examined clinically and radiographically and smears of nasal or skin scrapings were positive for acid-fast bacilli in all subjects. Thirty normal controls donated sera for study. The patient and control groups were comparable with respect to age (mean ages are 46 for cases, 48 for controls) and sex (approximately 10% of each group are female). All patients and controls were Caucasians of Turkish origin. No patient was in a "reactional" state at the time of blood sampling.

Sera were separated and frozen within 24 h and stored at -20°C until assay. Quantitation of IgG, IgA, IgM and alpha-1-antitrypsin globulins was performed with Behringwerke* agar diffusion plates containing specific antisera (Mancini *et al.*, 1963) and antigen reference standards. Precipitin ring diameters were measured to the nearest 0.1 mm using an immunodiffusion reader.

Results

The mean levels of IgG, IgM, IgA and alpha-1-antitrypsin were all elevated among the leprosy patients compared with the normal controls (see Table 1). An examination of the correlation coefficients among these immunoglobulins and alpha-1-antitrypsin in the 2 groups showed higher correlations in the patient group.

These differences in correlation structure in the patients and controls suggested the possibility of some heterogeneity among leprosy cases. Three patients identified from plots of the data appeared to differ from the remaining patients.†

When these 3 patients were removed, the variables were uncorrelated among both the cases and controls. Therefore, these variables could be treated as independent variables.

Comparison of the mean levels of these variables, normals and the cases with the outlets removed indicated that the levels of the 3 immunoglobulins and alpha-1-antitrypsin were significantly elevated among the cases. The t values‡ for

* Farbwerke Hoechst AG, 6230 Frankfurt (Main) 80, Behring Department.

† These leprosy patients and their levels are:

Patient	IgG	IgM	IgA	Alpha-1-antitrypsin
Nr. 4-M.S.	5760	226	570	1000
Nr. 11-B.L.	3600	586	570	1400
Nr. 28-K.E.	1220	120	266	240

‡ While the variances are significantly larger (except IgM) among the leprosy cases, the more conservative statistical approaches did not affect the results (cf. G. W. Snedecor and W. G. Cochran (1967), *Statistical Methods*, 6th ed. Iowa State University Press, Ames, Iowa, pp. 114-118).

the differences between the leprosy patients and controls are:

	t	d.f.	P-level
IgG	5.92	59	<0.001
IgM	4.11	59	<0.001
IgA	4.89	59	<0.001
Alpha-1-antitrypsin	6.05	59	<0.001

TABLE 1

Serum immunoglobulins and alpha-1-antitrypsin levels in Turkish patients with lepromatous leprosy and normal controls

Group	IgG	IgM	IgA	Alpha-1-antitrypsin
A. Normal controls (n = 30)				
Mean (mg %)	1671.3	164.0	248.7	400.3
S.D.	477.6	82.8	81.6	82.5
Correlation with alpha-1-antitrypsin	0.156	0.151	−0.003	1.000
B. Leprosy patients (n = 34)				
Mean (mg %)	2982.9	270.8	395.0	610.3
S.D.	1184.2	122.6	137.7	219.0
Correlation with alpha-1-antitrypsin	0.304	0.270	0.439	1.000
C. Leprosy patients (n = 31) outliers removed				
Mean (mg %)	2930.3	267.0	387.9	584.2
S.D.	1080.0	111.2	134.9	147.1
Correlation with alpha-1-antitrypsin	0.087	−0.030	0.223	1.000

Discussion and Summary

The quantitative assay of alpha-1-antitrypsin and immunoglobulins in this study demonstrates that levels of IgG, IgA, IgM and alpha-1-antitrypsin are all elevated in the sera of patients with leprosy when compared with normal controls. In addition, these immunoglobulins are uncorrelated with alpha-1-antitrypsin in either leprosy patients or in normal controls.

Thus, the independence of immunoglobulin classes which has been observed in normal individuals (Allan Smith *et al.*) appears to be unchanged in leprosy even though chronic antigenic stimulation by *M. leprae* results in marked changes in concentration in all of the immunoglobulin classes.

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Domiciliary Rehabilitation (Preliminary Report on an Experiment in Self Employment of Disabled Ex-Leprosy Patients)

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An experiment in the rehabilitation of patients on the verge of displacement from their families due to economic stringency, is described. The principle is that the patients are enabled to remain in their homes and, as far as possible, resume their previous trades, or take up new trades.

The early results of this experiment are presented. On the whole domiciliary rehabilitation justifies its continuation while greater accumulation of experience will further reduce the failure rate.

"Restoration of the handicapped to the fullest physical mental, social, vocational and economic usefulness of which they are capable", is one of the widely used definitions for Rehabilitation (definition issued by the National Council on Rehabilitation, 1942).

Rehabilitation can be effected in a number of ways, depending on the type of disability, availability of funds and specialized personnel and the cultural background of the locality. There could be no hard and fast rule to say a particular type of disability should be handled in a particular way. The whole effort should be directed towards a dehabilitated man being helped to return to self sufficiency.

Nobody ever becomes a beggar from preference. In the first instance he is always driven to it by hunger and need. Leprosy is not a disease of beggars. It is a disease which results in beggars because the community rejects a patient after he has lost his job and hence ceases to be a useful member of it. Domiciliary Rehabilitation aims to assist the patient to remain a useful member of his family and thus avoid the danger of displacement both from his home and from his community. It is well known that, having once become a beggar, it is psychologically extremely difficult for the individual to return to the shackles and bonds of organized society, from the freedom and irresponsibility of the vagrant. To live in society, to be a breadwinner in one's family and to move harmoniously with one's neighbour involves a certain discipline. The beggar has lost the desire for discipline and hence his rehabilitation is very difficult indeed.

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Domiciliary Rehabilitation aims at the prevention of vagrancy and begging by discovering a person's needs before he is in fact displaced from his own society.

This paper aims to present the early results of what we have termed Domiciliary Rehabilitation where we take not only treatment but also rehabilitation to the homes of our patients.

This project is based on the following assumptions:

The handicapped person will be self-employed and remains in his family who retain responsibility for his care.

The captial cost per work place is limited to the provision of the means of production only, without buildings and other overheads and only with the minimum supervision and assistance if required.

In the case of leprosy patients colonization of the patients in group rehabilitation may have a tendency to perpetuate stigma. This is avoided in a Domiciliary Rehabilitation programme.

Description of the Project

The Gudiyatham Taluk (total population 418,000–1971 census), has been assigned by the Government to S.L.R. & T.C. for leprosy control work since 1964. The total number of registered leprosy patients as on 31 December, 1974, was 8921. The prevalence rate of leprosy is 21.34/1000 population. The estimated number of patients having advanced (WHO grade III) (WHO Expert Committee on Leprosy, Fourth Report, 1970) deformity is approximately 450 persons, i.e. 5%. Most of them have satisfactory home conditions. Others are in danger of being rejected from their homes or are a serious burden because they are unproductive. The project concentrated on the latter group and those who have just been dehabilitated. There are two exceptions, who were drawn from the nearby Government Leprosy Beggars Rehabilitation Home.

The aim of the project was both investigation and service.

SURVEY

It was sought to determine the number of disabled leprosy patients in the Gudiyatham Taluk of N.A. District, Tamil Nadu, who are at present at risk of being displaced from their homes and having to resort to socio-economic dependency, and to assess their employment status and work potential.

REHABILITATION

The aim was to provide a means of production to the patient and/or his immediate family thus restoring him to gainful and socially acceptable employment and to follow up and evaluate the cost effectiveness of different types of aid.

Method of Operation

One of us went out with the mobile clinic team accompanied by a senior Paramedical Supervisor, to each of the 44 clinics operating in this area. We talked with the patients and the paramedical workers, and sought out any patients who were in an unsatisfactory financial and social position. They were then visited and interviewed in their homes in the village. Their neighbours were also interviewed to get their support, advice and co-operation.

If no other means such as job placement, general advice or counselling could be applied, then, if a suitable aid within the budget was available, the patient was selected for rehabilitation.

Depending on environmental factors, such as the location of his house, patient's disability and his aptitude, it was decided whether the patient could be helped and if so what kind of assistance was most useful. The available alternatives were:

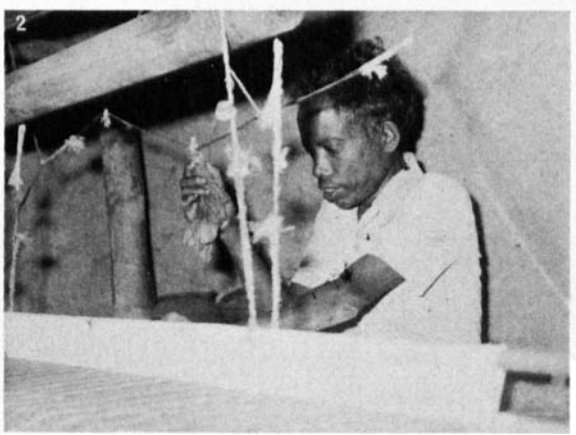
Training in handicraft in the centre and then providing the patient with an appropriate means of production.

If the patient was found to have a good practical knowledge of any trade, he could be offered a return to the same trade if possible.

Sometimes livestock or agricultural assistance could be rendered.

A detailed social and occupational history was taken, and was presented to the committee in the centre. The committee consisted of an Epidemiologist, a Non-medical Supervisor, an Occupational Therapist and the two authors of this paper. After presentation of the patient's case and discussion, a plan was drawn up based on the discussion which the social worker had had with the patient and his family. If the patient needed training he was admitted for this; care was taken to limit this training period to not more than a month at a time. The patient was then sent home with his loom. He was visited by the Social Worker, where possible, frequently in the beginning and later once a week or month.

To a few, additional assistance has been given by way of provision of raw materials and help in marketing; and to some others (Fig. 1) (the two who had come out of the Government Rehabilitation Home) even a hut had to be provided near the local village for their shelter.



Figs 1 to 3.

It was felt undesirable to give assistance away without a contract for return. Hence in each case a bond was executed wherein the beneficiary contracted to return the capital in some form or another. In the case of livestock, the patient agreed to return the original animal or its progeny after weaning. In other forms of aid usually they agreed to reimburse the capital by instalments. This was done mainly to make patients feel that they were responsible for their production unit, and also to have a better control over them. A separate file is maintained for each patient with their social and economic history, the committee's decision, the expenditure involved, the bond and notes made during the visits after issue of the units. A special ledger folio is also opened to account for any reimbursements that are made towards the original capital investment.

Results

NUMBER OF PATIENTS

The project was planned to investigate and cater to the need of the people resident in the local taluk. However, inevitably as the first patients were being helped, a large number of requests, both by post as well as in person came from patients far and near. In many cases these requests for help were recommended by the persons responsible for their treatment and care. Two patients (Fig. 1) came from the nearby Government Leprosy Beggars Rehabilitation Home. They had earlier been our patients and had been trained as weavers before they became beggars. They had no home, and hence, as an experiment, they were included in the project, and were provided with a one-room hut each with a loom installed.

TABLE 1

Total number of patients investigated	88
No. of patients assisted:	
Domiciliary rehabilitation	16
Job placement	4
No. of patients who are in the process of being rehabilitated	4
No. of patients rejected	49
No. of cases pending decision	15

Analysis of reasons for rejection:

TABLE 2(a)

Within Gudiyatham Taluk

S. No.	Reasons	No.
1.	Investigated and found to be well off	9
2.	Not interested	6
3.	Died while under consideration (T.B.)	1
4.	Only wanted salaried job	2
	Total	18

TABLE 2(b)

Outside area

S. No.	Reasons	No.
1.	Too far away for investigation or visits	10
2.	In homes, no shelter outside	5
3.	Already rehabilitated by other centres	5
4.	No suitable programme within our limits	2
5.	Irresponsible and no genuine interest shown	4
6.	Only wanted salaried job	5
	Total	31

Discussion

REJECTED PATIENTS

From Tables 2(a) and (b) it is clear that Domiciliary Rehabilitation is possible only in selected cases. The indications have yet to be worked out on the basis of the experience derived in the course of time from the assisted patient, but preliminary factors to be considered are:

The patient's desire to work. Patients who were not interested in what we had to offer could obviously not be included.

The patient's initiative to know in what direction he wanted assistance. The best successes were in patients who had a definite request that was within our scope to render.

The patient's courage to face the insecurity of self employment, against the security of a salaried appointment.

Some of the less successful of our selected cases indicate that there are other factors to be considered which have yet to be established.

TABLE 3

Period of follow-up of patients assisted

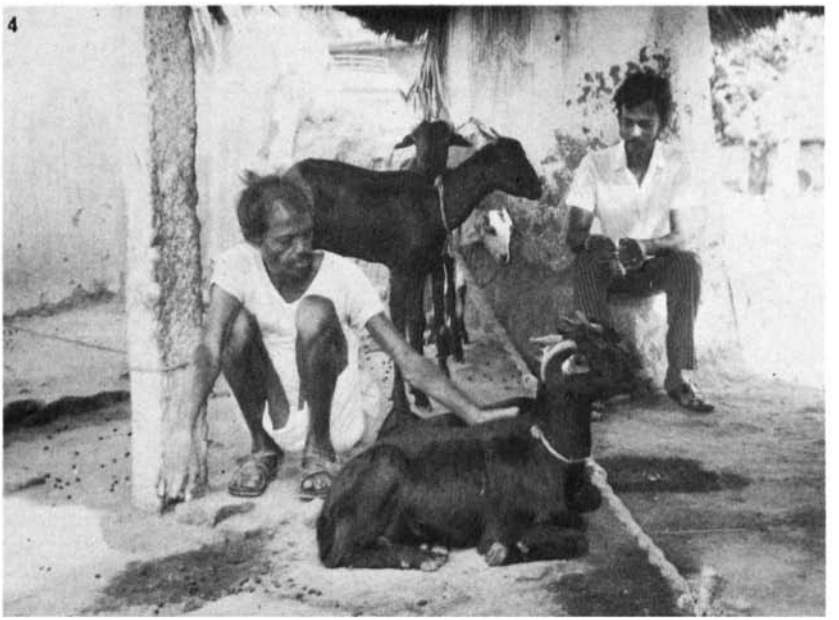
12 months	4
9-12 months	4
6-9 months	6
3-6 months	—
3 months	2
Total	16

Average 8 months

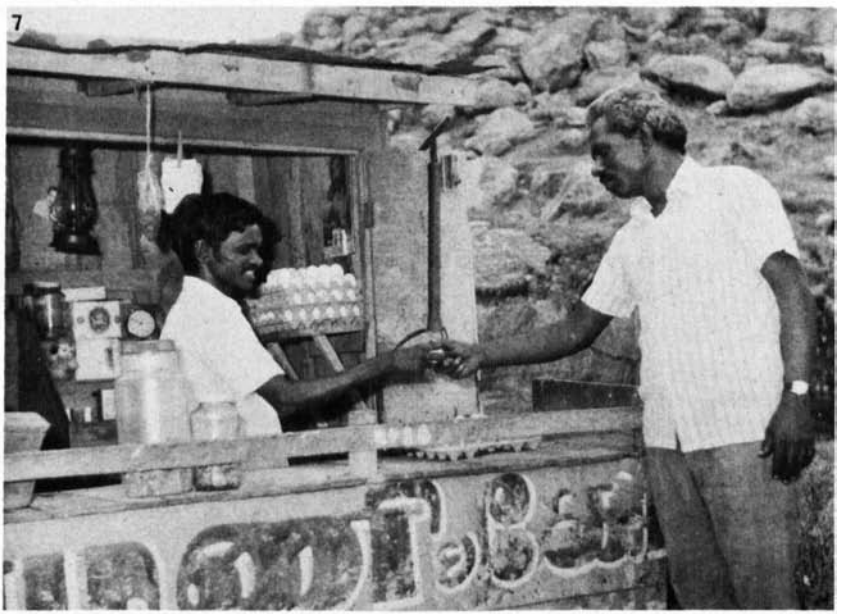
TABLE 4

Results

Satisfactory	6
Fair	3
Failure	3
Reserved	4



Figs 4 and 5.



Figs 6 and 7.

TABLE 5

Case No.	Sex	Age	Unit supplied	Date	Cost/unit Rs.	Previous employment
1	M	33	Standard handloom (Fig. 2)	July 1975	430	Weaver
2	M	42	Standard handloom (Fig. 3)	October 1975	430	Weaver
3	M	37	Bullock cart (second-hand)	December 1975	420	Bullock cart driver in Rehabilitation Centre
4	M	28	Bunk shop deposit and capital for 1500 eggs (Fig. 7)	January 1976	750	Started egg business by himself
5	M	55	Four goats (Fig. 4)	July 1975	360	Was caring for 3 goats belonging to somebody else for 1 meal a day
6	M	60	Silk screen printing unit	September 1975	400	Silk screen artist and later admin. asst. in a leprosy institution
7	M	42	Provision of ploughing and seeds for his own land (1 acre)	July 1976	100	Farmer (owned 2 acres)
8	M	60	Pregnant cow (Fig. 5)	July 1975	675	Had cows earlier. Recently wage labourer
9	M	40	Pregnant cow	November 1975	565	Original wage labourer in oil mill. Recently wage labourer
10	M	35	Pregnant buffalo (Fig. 6)	October 1975	412.50	Wage labourer. Lived alone. Domestic servant
11	M	49	Pregnant(?) buffalo	May 1975	387.50	Unmarried, living with brother and wife. Father had had some livestock
12	M	38	Purchase of 4 cents of land	January 1976	540	Wage labourer
13	M	38	Mat loom and training and house (Fig. 1)	January 1976	900	Beggar admitted in Beggar Home (learnt weaving here)
14	M	30	Mat loom and training and house (Fig. 1)	January 1976	900	Beggar admitted in Beggar Home
15	M	41	Working. Capital for fire-wood and kerosine oil trade	June 1976	50	Illicit drug and liquor trade
16	M	23	Cost of cultivation of 2 acres land	June 1976	250	Had 2 acres land. Joint ownership with brother

Detailed case by case analysis

Cause of social deterioration	Result	Reimbursement
Fire in hut	Very satisfactory	Regular
Patient was a hired weaver, lost job because too slow of disabilities	Very satisfactory	Slow
Discharged from Rehabilitation Centre	Failure (after 6 months bull died– neglect due to patient leaving home for medical treatment)	Nil
Stoppage of local supply. Inadequate capital for advance payment	Very satisfactory	Regular
Poverty	Satisfactory	Only possible when goats proliferate
Lost job because of bad vision	Failure (patient absconded leaving equipment behind)	Nil
1. Repeated hospitalization due to very severe deformities; 2. land became dry because of reduction in water level	Satisfactory (patient has now ploughed both acres without assistance)	Not yet. Will pay after present harvest (3 months)
Daughter's marriage necessitated sale of cows	Reserved (delivered a calf but refused to permit milking, so no returns. Cow now pregnant again)	Not yet
Lost job due to reactions. Progressive deterioration due to deformities	Fair (cow gave calf, only slight milk. Patient now has two more cows to care for. Patient now married and happy)	Not yet
Poverty	Reserved (buffalo delivered dead calf. Now buffalo is pregnant again)	Not yet
Brother started drinking after prohibition lifted	Failure (buffalo proved not pregnant hence animal returned to us)	–
Threatened eviction from home if he did not buy the land	Very satisfactory	Regular
Wage labourer drifted into vagrancy	Fair (frequently off sick, intermittent work. Wife helps him, just been trained)	Not yet
Wage labourer drifted into vagrancy	Fair (frequently off sick. Remarried, wife being trained)	Not yet
Eviction from family by father	Reserved (no follow-up yet)	Not yet
Land leased and patient institutionalized 6 years	Reserved (land is now cultivated)	Not yet

ASSISTED PATIENTS

Satisfactory, indicates that the expectations of the team have been fulfilled. Fair, is used when the patient's condition and morale have improved but the result is short of our full expectations. Failure, is the term applied when neither the patient nor the team were satisfied. The reserved category is when no conclusion can be reached yet.

Without any question the best assistance to give is the emergency assistance when the patient has just lost his work. Examples of this are the first two weavers (Case No. 1 and 2—Table 5—Figs 2 and 3).

Those who were living at a bare subsistence level and who could be assisted by increasing capital or facilities also registered as satisfactory. These include the person (Fig. 4) who had the care of someone else's goats and who was given 4 goats for himself (Case No. 5) and the person (Fig. 7) who had an itinerant egg delivery service and to whom a bunk shop and capital was given (Case No. 4) and the patient whose fields were ploughed (Case No. 7).

The failures are more difficult to analyse because they are due sometimes to misadventure and sometimes to our own inexperience. These include the pregnant buffalo who turned out not to be pregnant (Case No. 11) and the bull which died (Case No. 4). This latter was partly due to the irresponsible behaviour of the patient in leaving his wife alone for more than a month to take care of the bull, while he was admitted in a hospital 100 miles away for ulcer treatment. The case of the silk screen printing equipment is difficult to analyse. The patient had rather poor vision and had produced some very indifferent results in an order we ourselves had given him. Then he absconded and his whereabouts are not known.

COST EFFECTIVENESS

The most commendable thing about this form of rehabilitation is its cheapness and the fact that the patient is no worse off in the event of failure than he was before. Whereas with other forms of rehabilitation the patient may be still more displaced by virtue of having been removed from his home environment. The average capital investment per work place in our series was Rs.536/-. To this must be added at least a portion depending on the number of beneficiaries, of the salary of a highly skilled social worker, and transport expenses for supervision, interviews, etc. On the whole, it could be said that rehabilitation is achieved at a cost in the order of Rs.1000/- per work place. Compared with the cost of sheltered industry this is relatively insignificant.

Conclusion

On the whole it would seem that the success of the experiment lies in careful selection of the cases to be assisted.

Rehabilitation has no single solution. For each case the choice has to be made as to which type of rehabilitation is most likely to succeed. The choices now available include:

Job placement where the patient is assisted to find a salaried post. For the educated or skilled with minimum deformity.

Vocational training and sheltered workshop. Employment or subsequent job placement for the younger and more educated group.

Domiciliary rehabilitation for the older and usually rural patient who has not yet been displaced from home.

After these three possibilities are considered there still remains a group of patients for whom no direct assistance towards self sufficiency is possible and for whom some sort of pension scheme must be considered. Such a scheme will often enable the patient to remain with his relatives and contribute his pension towards his keep. The pension must however be disbursed in small amounts at not greater than weekly intervals and at the most peripheral level of the administration, namely, the block headquarters. This is in order to ensure that the patient actually remains with his family and does not go to the city to beg.

Probably the most important contribution that this experiment is making is to demonstrate the need for attention to be directed to the prevention of dehabilitation. The patient is now allowed to become community dependent but is assisted at the stage when he may only have become family or neighbour dependent. As in the case of treatment of disease, so in rehabilitation, the earlier the diagnosis, the more effective and the cheaper the treatment.

Acknowledgements.

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Leprosy Villages—Are They All Outdated? A Survey of two Leprosy Communities

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A medical and social survey is reported on 2 villages in India composed originally of rejected leprosy patients and developed over the past 30 years, near to a large leprosy hospital. The results are surprising. These communities pose no leprosy problem to the adjacent population, thanks to the concern of the hospital authorities. The general economic standard is low, with begging an important factor, but the level of child health and nutrition in these communities was found to be superior to that among the local population, while the residents have developed a sense of community responsibility which replaces that lost from their homes and has created a reasonable degree of stability.

Introduction

One of the major problems of leprosy hospitals has been that of patients who no longer need in-patient care but appear to have nowhere to go. Over the years, almost on the doorstep of the Victoria Hospital, Dichpalli, Central India, 2 villages have sprung up where a number of leprosy patients discharged from the hospital live. Most of them settled there because relationships with their families had broken down, some because of permanent deformities and disability which made them afraid to move away from a sympathetic community and medical facilities. The records show that the first house in Devanagar was built in 1948 by a patient discharged from the hospital. By 1954, with the population steadily increasing, the inhabitants decided to split the community, and some residents moved about half a mile away where they built the village of Devapalli.

Over the years both these villages continued to grow and many healthy children were born there. Various attempts were made to put these children on prophylactic dapsone and their parents on regular leprosy treatment, but it appeared that the weekly clinics in Devanagar and Devapalli were totally inadequate. In the first place relationships between these people and the hospital were not ideal. Patients from these communities were constantly coming to the hospital for treatment of complaints often of a trivial nature. Having been rejected by their own families, what they sought more than anything else was attention and affection. That the busy hospital outpatient department was not always in a

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position to meet this need resulted in misunderstandings and poor co-operation. Secondly, it appeared that many patients were not permanently resident in these villages, and this caused problems with registration and regular treatment. Finally, there appeared to be considerable general health problems which could not be dealt with during weekly visits. It was therefore decided in 1974 to hold daily clinics, to attend to general complaints as well as leprosy, and to establish an Under-Fives Clinic in each village.

Ten months later when relationships between the hospital and the leprosy villages had much improved, a survey was undertaken to assess the medical situation and the social problems which these patients are facing, using for comparison where nutrition was concerned, an Under-Fives Clinic at a neighbouring village of local people.

Materials and Methods

The registered population of Devapalli and Devanagar consisted of 432 and 494 respectively. Only a few of these were permanently resident, the majority leading an itinerant existence. A team of 2 doctors, 2 nurses and a paramedical worker visited every house and each member of the family was examined by a doctor. All patients were classified according to the Ridley and Jopling scale, deformities were noted, and records were made of other chronic diseases, especially tuberculosis. At the same time the nursing staff did a social survey amongst the adults to determine the size of each family, length of residence in the community, occupation and recent contacts with the home village.

On the side of the community there was much interest and co-operation in this survey. Three hundred and six adults and 112 children were examined out of a registered population of 926. The remainder were absent (see Discussion).

Results

LEPROSY SURVEY

Of the *adults* examined, almost half had lepromatous (LL) leprosy but most of it not very active. When skin smears were done only 6 patients (2%) had a positive Morphological Index. About 30% were within the BL, BB, BT range. Less than 10% had tuberculoid leprosy and the remainder, mainly healthy adults born in Devanagar or Devapalli, showed no signs of leprosy. Among the *children* only one case of leprosy (TT) was discovered, a boy who had never had any prophylactic

TABLE 1

Classification of leprosy amongst the surveyed population

Type of leprosy	Adults	%	Children	%
Lepromatous leprosy LL	150	49	—	—
Borderline lepromatous BL	31	10	—	—
Borderline BB	37	11	—	—
Borderline tuberculoid BT	32	10	—	—
Tuberculoid TT	28	10	1	1
Observation	—	—	5	4
No signs of leprosy	28	10	106	95

dapsone. Five children were placed on observation. The remaining 106 had no signs of the disease, though many were children both of whose parents had lepromatous leprosy. Twenty-eight patients (9%) were currently receiving treatment for tuberculosis.

DISABILITY SURVEY

Of the 306 adults examined 172 (56%) had noticeable thinning or complete loss of eyebrows and 114 (37%) had appreciable deformity of the nose. Those with contracted hands numbered 101 (33%), but many of these were very slight. Seventy-eight (25%) showed bone absorption of the hand and 71 (23%) bone absorption of the feet. Only 27 (9%) had trophic ulcers. There were 4 cases (1%) of Lagophathalmos and 4 (1%) of foot drop.

TABLE 2

Frequency of deformities amongst the adult population

Deformity		Number	Percentage %
Face	Eyebrows almost or completely lost	172	56
	Lagophthalmos	4	1
	Nose collapsed	114	37
Hands	Mobile contractures	69	22
	Absorption	78	25
	Stiff joints	33	11
Feet	Trophic ulcers	27	9
	Footdrop	4	1
	Absorption	71	23

NUTRITION SURVEY

In Table 3 the level of protein/calorie malnutrition among the 112 children in these leprosy villages is compared with that obtaining among a similar group among the local population attending an Under-Fives Clinic at the village of Mentrajpalli.

TABLE 3

Frequency of protein calorie malnutrition in Devanagar, Devapalli and in a nearby village, Mentrajpalli, estimated according to Morley's Road to Health Chart

Degree of nutrition	Devapalli and Devanagar		Mentrajpalli	
Normal "Road to Health"	29%	65%	16%	36%
1st degree protein calorie malnutrition	36%		20%	
2nd degree protein calorie malnutrition	29%	35%	39%	64%
3rd degree protein calorie malnutrition	6%		25%	

The general condition of the children in Devanagar and Devapalli compared favourably with that of the children in the neighbouring village who attend a similar Under-Five Clinic.

SOCIAL SURVEY

Almost half of the residents are totally or partially dependent on begging. The remainder work as coolies, farm their own land, follow village occupations or are involved in irregular practices, e.g. smuggling. Only 4 families claim to be receiving support from relatives.

Among the 166 women interviewed 71 (43%) have no children, 52 (31%) have one child, 22 (13%) have 2 children, whilst only 10 (6%) have more than 3 children.

Discussion

Experience over the past 10 months suggests that the 45% of the total population who were interviewed are representative of the number of people actually resident in Devanagar and Devapalli at any one time. We have described as "temporary residents" all those who were away at the time of the survey, plus 125 who stated that they spent more than 3 months of the year elsewhere. This leaves us with a permanent adult resident population of 181 (less than 20% of the total registered population).

Many of the *temporary* residents, who move between the cities of the north and Devanagar or Devapalli are involved in begging and appear to be quite content with their life. All they need from us is encouragement to continue regularly with their treatment. We make provision for them to take up to 6 months supply of DDS with them when they leave us. However, some expressed concern about the lack of regular education for their children.

The large number of patients with lepromatous leprosy (LL) and facial deformities, and the relatively small number of deformed hands incapable of work, suggest that social inacceptability rather than economic dependence is the reason for settling in a leprosy village. In this part of India the stigma attached to leprosy is very great and this has left many patients deeply resentful and suspicious. Fear of further rejection often suppresses all initiative to seek employment. This attitude even applies to projects for rehabilitation and the general attitude of the *permanent* residents is that of preferring to be left alone. Up to a point, they may be right: the majority have come to a compromise with their environment and have more or less rehabilitated themselves within the community of fellow patients. The number of rejected and disabled patients who really need what we usually call "rehabilitation" may well be much smaller than we are inclined to think. Among themselves the permanent residents form a happy community and appear to act as one family, forgetting or ignoring the barriers normally created by caste and religious differences. This is one of the most striking features of life in these leprosy villages.

The fact that of the 112 children examined, only one was diagnosed as having leprosy is most gratifying, as is the general level of nutrition among them. Nearly all children have had some prophylactic DDS in the past but this has certainly been very irregular. At the Under-Fives Clinic, started in 1974, there is 100% registration of the under-five population. These children are regularly examined and supplied with prophylactic dapsone. In addition, the following services are provided: immunization (BCG, Smallpox, DPT and polio), nutritional advice and the treatment of illnesses. This clinic has formed a valuable link with the adult population who are enthusiastic in bringing the children for examination and treatment.

The three main reasons for this rather surprising picture are:

The smaller families due to the high proportions of parents with lepromatous leprosy, which often causes sterility.

The fact that so many families are dependent on begging for their livelihood. Much of the "earnings" of a beggar are in the form of cooked food which is not resaleable but which will be eaten by the family.

The absence of the extended family where one or two wage earners may be required to feed and educate nephews and nieces. In Devapalli and Devanagar the normal family unit consists of the parents and their own children. However, the benefits of the extended family system (an abundance of affectionate and attentive relatives which gives such security to Indian children) are here provided by the whole village community.

Following the establishment of the Under-Five Clinic the most pressing need for the children is education. There is a well-established nursery school in Devapalli run by two devoted Christian patients. From there the children are able to attend the Government Primary School in a nearby village. In Devanagar a qualified teacher has been appointed by the Government. Following their education to junior level most of the children move away and go to live elsewhere with relatives who arrange for their marriage, while some remain behind to marry other children who were born in the community. These form the largest proportion of the adults examined who have no signs of the disease.

Conclusions

Living conditions in two communities of rejected or disabled leprosy patients appear to be much better than is usually presumed. Village hygiene and the health of the children compare favourably with those of the neighbourhood. Obviously there is no need to segregate the children from their parents. Another striking feature is the relative happiness of the community, possibly due to a "fellowship of suffering"—an experience shared by all of the residents.

Among the deformities caused by leprosy collapse of the nose appears to be one main reason for rejection. Regular nose care is therefore an essential part in the treatment of all lepromatous patients to prevent this disaster occurring.

The greatest needs of the two communities seem to be:

Regular leprosy treatment and general health care.
Educational facilities for the children.

Rehabilitation of the adult population is on the whole not a felt need, though there may be individual cases where this form of help is required. The community itself is the main factor in psychological rehabilitation and therefore such patient villages should not be regarded as undesirable.

Acknowledgements

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Report of the Second IMMLEP Task Force Meeting 1–5 December, 1975

Leprosy workers everywhere are aware of the great importance of the World Health Organisation Special Programme for Research and Training in Tropical Diseases, with the establishment of IMMLEP, the Task Force dedicated to the discovery of a specific vaccine against Mycobacterium leprae. *A summary report of the first meeting of IMMLEP was reprinted with the permission of the World Health Organisation in* Leprosy Review, *Vol. 47, No. 3. We are happy to print here, also with permission, the Report of the Second IMMLEP Task Force Meeting.*

Three specific goals were established for the IMMLEP Task Force at its initial meeting in November 1974. They were briefly: (a) To develop *M. leprae* specific skin test antigens suitable for carrying out epidemiological studies of leprosy; (b) to develop a vaccine for leprosy which would induce sensitization for cell-mediated immunity to *M. leprae* and engender resistance to the disease, and (c) to understand the nature of the pathological complications of leprosy so that they can be prevented or better treated. While the great hope of the programme is to be able to immunize against leprosy, the Task Force remains aware that the induction of sensitivity to antigens of *M. leprae* does not lead automatically to production of protective immunity. Only with appropriate testing and clinical assessment of protective effect, will it be possible to know if the vaccine can be developed.

The present second IMMLEP Task Force meeting, held December 1975, has as its focus the epidemiological problems of leprosy. A summary of the achievement of one year of research by the Task Force members present included: (a) An improved method of purification of bacilli containing less than 1% of contaminating material from armadillo tissue; (b) development of three different soluble skin test antigen preparations, all of which demonstrated in preliminary trials considerable *M. leprae* skin-test reactivity in tuberculoid leprosy patients and low levels of skin-test reactivity with lepromatous or non-exposed individuals; (c) three small-scale field studies were carried out on two of the soluble antigens, and one large epidemiological survey (2300 tests) was carried out with one of the products; (d) a mycobacterial infection in some wild armadillos in one district was discovered and the organism involved, in preliminary tests, showed several similarities to *M. leprae*; (e) immunochemical analysis of isolated mycobacteria demonstrated 45 definable components and indicated both that a standardized reference system for antigens of *M. leprae* is feasible, and that individual antigenic components could be purified and monospecific antisera to them produced, and (f) comparison of antigenic properties, both by serological techniques and skin testing, indicated a group of readily cultivated fast growing species with striking antigenic similarities to *M. leprae*.

On the basis of these findings protocols were prepared for: (a) Calibration and comparison of the available skin test antigens in terms of dose response, and (b) frequency of reactivity in leprosy patients, contacts and healthy individuals in non-endemic areas. First formulations were prepared of protocols for testing three epidemiological hypotheses relating skin test reactivity to: (a) Susceptibility to

disease; (b) exposure to bacilliferous leprosy patients; (c) subclinical infection; (d) exposure to environmental mycobacteria, and (e) responsiveness to a future vaccine when developed, and the order of priorities suggested. Ethical considerations are a permanent feature of all IMMLEP studies.

In pursuit of the specific goals of IMMLEP, future plans include: (a) Continued follow-up of skin-test antigen purification and testing, (b) analysis of the epidemiological data obtained so far, and (c) initiation of vaccination studies including optimal use of adjuvants in laboratory animals, with progress in those areas to be reviewed at a third IMMLEP Task Force meeting to be held in a year's time.

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Summary Report

INTRODUCTION

At the direction of the Steering Committee, the second Task Force of IMMLEP met from 1 to 5 December, 1975, in Geneva to consider the results obtained so far, and to formulate further investigations of the new skin test reagents related to leprosy. The Task Force comprised the

members of the Steering Committee, 5 members of the 1974 Task Force meeting, 5 new members, and 5 members of the WHO secretariat.

The present Task Force activities related to the development of the new skin test reagent's may be summarized as follows.

STATUS OF *M. LEPRAE*-DERIVED SKIN TEST ANTIGENS

Three kinds of skin test reagents have been prepared from leprosy bacilli by Task Force members, and two of them have been subjected to preliminary field testing.

Skin test reagent LC

This reagent was elaborated by Dr J. Convit from supernatants of lepromins prepared from heavily infected human and armadillo tissues. (Lepromins are suspensions of whole killed leprosy bacilli, crudely separated from tissue.) Whole lepromins evoke 2 types of reaction in sensitized persons. Firstly, the Fernandez reaction producing an erythematous phase, oedema and induration 48 h after injection, and in some ways analogous to the tuberculin reaction. Secondly, the Mitsuda reaction, producing a tuberculoid granuloma at the site of injection 3 weeks to 1 month later. This latter reaction is a response to whole bacilli in the reagent, and the former reaction is to soluble antigens. Thus, Dr Convit's reagent (LC) is designed to evoke the Fernandez and not the Mitsuda reaction.

Results (*Bull. Wld Hlth Org.* (1975), **52**, 193-197) were presented of skin tests carried out with the entire supernatant and with ammonium sulphate precipitates of this material, reconstituted to the original volume. These reagents produced equivalent results and their activity was removable by trypsinization. Using the reagents, small groups of patients with lepromatous and tuberculoid disease were found negative and positive respectively as defined in Dr Convit's publications. Villagers in 3 villages, 1 in Chile where leprosy is not known to occur, and 2 in Venezuela with different levels of leprosy prevalence, were tested with these reagents, with whole lepromin, and with the tuberculin PPD. There were less than 4% of persons producing positive reactions in the Chilean village, and 45% of adults were positive in the Venezuelan villages. However, in the Venezuelan village of lowest endemicity, 29% of children were positive, and in the village of greatest endemicity the percentage of positive children was 45% and did not differ from that of the adults.

Skin test reagent LR

This is prepared by Dr Rees and Dr Draper from leprosy bacilli killed by irradiation and extracted from infected armadillo tissues. The bacilli are exhaustively freed of tissue antigen by methods outlined in Protocol No. 2/75 of this summary, and then broken by ultrasound. The solution is freed from particles by centrifugation and filtration through membrane filters with a pore size of 0.22 μm. This reagent is diluted for use according to protein concentration. Several batches have been prepared and tested in 6 countries.

The earliest batch, LRA4, was tested firstly in Malaysia and Ethopia on very small numbers of well documented lepromatous and tuberculoid leprosy patients. From these tests a dose of 2 μg protein in 0.1 ml was selected for testing on secondary schoolchildren and tuberculosis patients in Libya. One hundred and fifty persons were tested in Libya and the reagent was found to be too concentrated. In view of this, a reaction size of 12 mm was selected as positive, and by this criterion 8% of the schoolchildren and 16% of the adult tuberculosis patients produced positive reactions. (Leprosy is considered to have a prevalence of 3/1000 in Libya.) An improved batch, LRA6, was tested on volunteer university students in England and Norway where leprosy is non-endemic. Two doses, 2 μg and 0.2 μg of protein of the reagent were tested simultaneously with tuberculin. One out of 70 Oxford students and 11 out of 60 Oslo students reacted positively to LRA6. There was a relationship between LRA6 positively and strong positivity to 5 TU of PPD (RT23) in the Norwegian data of Dr Harboe. In other areas (Oxford and Libya) such a relationship has not been found. The exploration of this apparent inconsistency is unknown and the specificity of LRA6 will be further investigated in non-endemic areas for leprosy (see Protocol No. 5/75).

LRA6 was assessed in 1500 persons as part of a larger study in Burma, using a dose of 0.2 μg protein. It was found that 3% of patients with lepromatous leprosy and 39% of those with tuberculoid leprosy produced positive reactions as compared with 18% among the general adult population. An interesting sex difference was noted in the general population, amongst tuberculoid patients and amongst contacts of lepromatous disease; in each case, women were markedly less reactive than men, and this was especially so amongst the close contacts. The findings amongst villagers from villages with differing prevalences of leprosy were interesting. It was found that reactivity to LRA6 reached a peak at a prevalence of 3% where up to 70% of persons from some villages produced positive reactions. At higher prevalences the percentage of positive reactors was 25% (+ or −10%). (The nature of this phenomenon will be further pursued as outlined in Protocols Nos. 7-8/75.)

Skin test reagents prepared by Dr Stanford and Dr Shield from 21 other species, subspecies or serotypes of mycobacteria were also used in the Burma study, and 13 of them were compared with LRA6 (see working paper, Annex 1).

As found previously a relationship between certain of these and *M. leprae* was detected. Three reagents in particular (vaccin B, nonchromogenicin and lactin) were found to produce results similar in many ways to those obtained with LRA6. The remaining reagents fell into two groups, those with some relationship to the leprosy bacilli (chelonin, vaccin, vaccin A, diernhoferin, smegmatin and gordonin), and those apparently unrelated (flavescin, xenopin and marianin); one reagent (marinin) was considered unreliable. Many results of the study carried out in Burma still await assessment.

Skin test reagent LK

This reagent was prepared by Dr Kirchheimer (*Leprosy in India* (1975), **47**, No. 3, 142-150) in small quantities from leprosy bacilli carefully separated from frozen armadillo livers. The organisms were broken by mechanical means and by ultrasound, and the soluble part was separated. Protein was precipitated with trichloracetic acid and redissolved in buffer. A small group of lepromatous and tuberculoid leprosy patients were tested with 116 μg doses of this material. The very few results available look favourable.

EPIDEMIOLOGICAL STUDIES WITH LYMPHOCYTE TRANSFORMATION TESTS

Dr Bergquist presented an interim report of the results of the, as yet, incomplete study of LTTs carried out in an area of Ethopia on contacts of lepromatous or tuberculoid leprosy patients and control persons not known to have contact with any form of the disease. The antigens employed were *M. leprae* (whole organisms, a filtered sonicate, and dharmendra antigen), BCG, *M. avium,* and *M. gordonae* (whole organisms). Although some differences were found, the study taken as a whole did not show a significant variation of responses between the 3 groups tested. The results will require further analyses as more information is added to the study.

PREPARATIONS FOR NEW EPIDEMIOLOGICAL STUDIES

(a) The epidemiology of leprosy in selected areas was reviewed. See working papers Annexes Nos. 2, 3 and 4.

(b) New studies in non-endemic areas. Consideration was given to comparative studies on the presently available skin test antigens (LRA6 and LC) to establish their specificity versus tuberculin. The study will be carried out as set out in Protocol No. 5/75.

(c) The potential application of *M. leprae* derived skin test antigens as outlined at the last Task Force meeting (see IMM/73.3 Protocols Nos. 8 and 9) were reassessed (see Protocols Nos. 7-11/75).

The priority for these studies recommended by the Task Force to the Steering Committee was that the first study to be undertaken should be TDR/IMMLEP/75.8, Protocol No. 5/75. Protocol No. 6/75 will be carried out subsequently to Protocol No. 5/75. This protocol may

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* Accepted by the Steering Committee at its meeting on 5-6 December, 1975.

have to be modified depending on the outcome of Protocol No. 5/75 study. Further epidemiological studies, Protocols Nos. 7, 8, 9/75 should, if possible, be carried out as a joint prospective study after completion of Protocol No. 6/75.

Supply of M. leprae

Infected armadillo tissue will be submitted to the IMMLEP programme as agreed at the last Task Force meeting (IMM/74.3 Protocol No. 1). However, the yields from sacrificed animals have been lower than that found in those dying from advanced disease (see Protocol No. 2/75, Annex). Thus, the Task Force recommended strongly that the Steering Committee should make plans immediately for expanding the supply of *M. leprae* to meet the needs of the programme in the future.

Mycobacterial infection of wild armadillos from certain areas was reported by one of the centres. Preliminary observations indicate that the mycobacterium in question may be *M. leprae*. A collaborative investigation with public health authorities has been started to define the possible public-health importance of this animal reservoir. The precautions necessary to ensure a non-contaminated supply of *M. leprae* were discussed in detail and set out in Protocol No. 1/75.

PURIFICATION OF *M. LEPRAE* FROM TISSUES

An improved system for preparing electron-microscopically clean suspensions of *M. leprae* from infected armadillo tissues was discussed (Protocol No. 2/75). The method resembled in part that of Kirchheimer (see above) and that described previously (IMM/74.3, Protocol No. 2, Appendix 1), but separation in an aqueous two-polymer phase system replaced sucrose density gradients.

The importance of determining the effect of the enzymes used on the bacterial antigens was emphasized, and also the need to measure recoveries of bacteria. The method of IMM/74.3, Protocol No. 2, had been used successfully in several laboratories. It was agreed that the high-speed supernatant fraction from the preparation described in Protocol No. 2/75 should be examined for presence of antigen of the Convit type. Consistent yields of bacteria from tissues were obtained, but were lower than described in IMM/74.3, Protocol No. 2, probably because the tissue now came from killed armadillos rather than animals dying of leprosy (see Protocol No. 2/75, Annex).

CHARACTERIZATION OF *M. LEPRAE* ANTIGENS

Dr Harboe described the potentialities of the crossed immunoelectrophoresis system for study, purification and fractionation of antigens of mycobacteria, and urged the necessity for reference antigen/antibody systems to be developed. The method had been used to study antigens of *M. lepraemurium* and *Mycobacterium BCG* and should now be applied to *M. leprae* as purified bacteria became available. Reference systems for *M. leprae* antigens based on both precipitation in gel methods and cell mediated immune systems are highly desirable.

GENERAL APPRAISAL

From the documents presented at the meeting and from the discussions, the progress made since the first Task Force meeting can be summarized as follows.

(1) The supply of infected armadillo tissues has been met as committed in the first Task Force meeting.

(2) A procedure for purification of *M. leprae* has been established and further improved to the extent that the antigenic material produced contained less than 1% of host tissues.

(3) A bank of armadillo infected tissues has been established and materials distributed to the various investigators.

(4) The methods for an antigen reference system have been established.

(5) Three skin test preparations have been developed.

(6) The results of preliminary field studies undertaken with two available antigens can be summarized as follows:

(a) A supernatant from Mitsuda type lepromin (LCH) prepared from armadillo or human

heavily infected tissues (autoclaved preparation) showed a difference in reactivity of populations in endemic and in non-endemic areas.

(b) A protein extract of purified bacilli from armadillo infected tissues (LRA6) showed a high reactivity in polar tuberculoid leprosy patients, and very little reactivity in lepromatous patients. The results obtained in non-endemic areas are at present conflicting.

Extensive studies in a highly endemic area have been carried out and the results are being assessed.

(7) New studies using the LTT have been reported.

(8) Protocols on methods for new studies making use of existing antigenic preparations as well as of others which could be developed, have been designed during this meeting.

(9) Administrative procedures for the Task Force have been established (see Interim Report of ST Chairman, 1975, Annex 5).

The cost involved in these achievements, which only represent a part of the total IMMLEP activities, is only in the order of US$75,000, all administrative costs for 1975 inclusive.

List of Protocols

No.	*Title*
1/75	Supply of *M. leprae* for IMMLEP programme.
2/75	Proposed system for preparing purified suspensions of *M. leprae* from tissues of infected armadillos.
3/75	Purification and characterization of *M. leprae* antigens.
4/75	General considerations for skin test studies to be undertaken in endemic areas.
5/75	Comparative testing in non-endemic areas.
6/75	Calibration and comparison of available antigens.
7/75	Possible desensitization as a consequence of heavy exposure to bacilliferous lepromatous patients.
8/75	Relation of skin-test unresponsiveness of contacts to their susceptibility to disease.
9/75	Subclinical infection.
10/75	Environmental mycobacteria.
11/75	Skin tests in relation to trials of potential vaccines.

PROTOCOL NO. 1/75. SUPPLY OF *M. LEPRAE* FOR IMMLEP PROGRAMME

The Task Force reviewed the procedures to be followed in the light of a recent report by Dr Walsh from the Gulf South Research Institute, that a mycobacterial infection had been found in wild armadillos captured in sout-west Louisiana. While it was agreed that there was insufficient evidence at present to establish beyond doubt that any of these were *M. leprae* infections, most of the available data are not inconsistent with such a conclusion.

The importance and implication of this observation was considered as it related to the IMMLEP programme and commitment. Fortunately, the tissues from the infected wild animals have been distributed rapidly to some IMMLEP members and others. Attempts to identify these mycobacteria are under way. In addition, a detailed sampling programme of wild armadillos in Louisiana and neighbouring states has been undertaken by the Center for Disease Control (United States of America) (60 animals from 10 areas). It is anticipated that more definitive data will be available within 3 to 4 months. This information will be made available to IMMLEP.

In the meantime the following procedures were accepted:

1. *Screening of eight- and nine-banded armadillos prior to inoculation with* M. leprae

(a) *Clinical examination.* Full clinical examination at the time of capture, and all animals with skin nodules or ulcerations, or enlarged lymph nodes, would be killed. Smears from the above sites would be stained for AFB, and tissues from animals with positive smears will be cultured and subjected to histopathological examination. (From the centres in the United States of America data will be reported to CDC, and the information obtained from all centres is to be reported to IMMLEP.)

(b) *Full bacteriological examinations of clinically normal animals.* (1) *Ear skin*: Scrape or tissue clip to be examined for AFB; ear clip tissue also to be examined histologically. (2) *Blood*: Examination of buffy coat for presence of AFB. (3) *Nasal smear*: Examination for AFB.

Cultures for mycobacteria will be set up on all AFB positive material.

All AFB positive animals are to be killed and tissues taken for histopathological examination. CDC and IMMLEP are to be notified as in 1(a). All tests currently available and applicable to the identification of acid-fast organisms as *M. leprae* should be carried out on representative animals on a continuing basis.

2. *Quarantine of armadillos before inoculation with* M. leprae

Animals that are AFB negative will be held for:

(1) Four months in a holding area isolated from contact with wild armadillos and as protected as possible from rodents and arthropods. They will then be re-examined as in 1(b) just before inoculation (procedure for GSRI and Venezuela); or

(2) For 2 weeks and then they may be inoculated with *M. leprae* as long as ear clips are taken 3 and 6 months later for bacteriological and histological examination (procedure for Carville).

(In future all animals failing to acclimatize to captivity should be killed and autopsied.)

3. *Inoculation with* M. leprae *and subsequent isolation*

All animals will be inoculated by intravenous route as previously agreed. However, in future only *M. leprae* from leprosy patients or from first passage armadillos currently held at Carville and Venezuela will be used. Until further notice no armadillo passaged *M. leprae* from GSRI will be used for inoculation.

All *M. leprae* inoculated armadillos will be kept separately from animals awaiting inoculation.

In order to produce heavy infection in a high proportion of animals with *M. leprae* obtained from patients, IMMLEP will provide the centres at GSRI, Carville and Caracas with fresh suspensions containing large numbers of AFB on wet ice or liquid nitrogen. It is hoped to be able to provide sufficient *M. leprae* for each animal to receive 1.0×10^8 AFB intravenously.

Various procedures are being explored to predict the optimal time for killing infected animals.

4. *Source of wild armadillos*

Until further information is available from the present studies, only armadillos obtained from areas in which mycobacterial infection has not been found will be used for IMMLEP.

5. *Investigations at time of harvesting from infected armadillos for passage*

From each animal, skin and peripheral nerves will be submitted for histological examination for the presence of AFB in dermal or peripheral nerves.

From each animal a fresh sample of bacilli will be prepared for:

(a) Pyridine test.

(b) (D)-Dopa test.

(c) Fluorescent antibody test (air-dried smears to be prepared on thin glass slides; test will probably be undertaken by Dr Abe).

(d) Mouse foot pad inoculation.

Other tests may be proposed depending on the results from further studies on bacilli from the freshly trapped and mycobacterially infected animals.

6. *Investigations at time of harvesting tissues from armadillos for distribution through IMMLEP.*

(a) Animals due to be harvested prior to 1 July, 1976, are subjected to all steps listed in section 5 above.

(b) After this time, animals inoculated with human material or first passage armadillo

material will be subjected to cultural procedures for mycobacteria, to skin and peripheral nerve histopathology, and whichever of the tests in section 5 are found most specific.

ANNEX TO PROTOCOL NO. 1/75. SUPPLY OF *M. LEPRAE* 1976-1978

Supply centres

The following numbers of heavily infected animals will be available in 1976:
Gulf South Research Institute, New Iberia, Louisiana, United States of America–up to 15 armadillos;
USPHS Hospital, Carville, Louisiana, United States of America–up to 3 armadillos.
The following numbers will be available in 1977:
Gulf South Research Institute–up to 15 armadillos;
USPHS Hospital–up to 10 armadillos;
Instituto Nacional de Dermatologia, Caracas, Venezuela–up to 5 armadillos (of the smaller 8-banded species).
The following number will be available in 1978:
Gulf South Research Institute–up to 15 armadillos;
USPHS Hospital–up to 15 armadillos;
Instituto Nacional de Dermatologia–up to 5 animals.

PROTOCOL NO. 2/75. PROPOSED SYSTEM FOR PREPARING PURIFIED SUSPENSIONS OF *M. LEPRAE* FROM TISSUES OF INFECTED ARMADILLOS

Tissues used so far: liver, spleen, subcutaneous leproma.

All were frozen at -20°C or (usually) at -70°C. All were sterilized by 2.5 Mrad [^{60}Co] gamma radiation.

(There seems no reason why: (a) Fresh tissues might not be used, or (b) why unirradiated tissue might not be processed. However, the latter alternative involves the hazards of: (i) infection with *M. leprae* of the operator, and (ii) contamination of the digests with other organisms.)

Materials needed

(1) SE buffer (0.3 M-sucrose, 0.25 mM-dipotassium EDTA adjusted to pH 7.1 to 7.2 with KOH.
(2) Triton X100 (conveniently handled as 20% w/v solution).
(3) Tris buffer (0.1 M-Tris adjusted to pH 7.2 with HCl).
(4) Tween (0.05% Tween 80 in water).
(5) Sodium azide.
(6) Collagenase (from *Clostridium histolyticum*; we used "type II" from Koch-Light with low proteinase activity; a cruder type might do).
(7) Trypsin (crystalline).
(8) Chymotrypsin (crystalline).
(9) Pronase (type B from Calbiochem; could be replaced by *Streptomyces grisius* proteinase from other sources).
(10) PEG (polyethylene glycol 6000 from BDH; 130-170 ethylene oxide units per molecule).
(11) Dextran (Dextran T500 from Pharmacia; mol. wt about 500,000).
(12) Phosphate buffer (conveniently 0.5 M-potassium phosphate, pH 6.9).
(13) Sodium chloride (conveniently 1 M- or 2 M- NaCl).
(14) Calcium chloride (M-$CaCl_2$).

Apparatus needed

(1) Homogenizer (Sorvall Omnimix has especially fast motor and is easy to keep cool; others would probably do).
(2) Centrifuge (capable of spinning samples at 10,000 x g; Sorvall RC-2, RC-2B, RC-5 suitable).

(3) Separating funnel.
(4) Magnetic stirrer.

Homogenize tissue in SE buffer, 30 ml buffer/10 g tissue, 3 min in Omnimix at top speed, cooled in water with ice.

Centrifuge 500 x g, 5 min. Preserve supernatant. Homogenize sediment as before, centrifuge as before, combine supernatants, discard sediment.

Centrifuge 10,000 x g, 10 min. Discard supernatant. Resuspend in SE, centrifuge as before. Pour off supernatant and as much as possible of fluffy top layer of sediment. Keep cold up to this point.

Suspend sediment in 1% Triton x 100 in SE buffer, stand at room temperature (20°C) for 1 h to lyse mitochondria.

Centrifuge 10,000 x g, 10 min, discard supernatant. Wash sediment twice with SE buffer, centrifuging as before. Pour off as much as possible of fluffy upper layer of sediment during washing. There is usually a sharp demarcation between the fluffy uppper layer and the firm lower layer. (There is usually a small whitish (collagen) pellet at the base of the lower layer.)

Suspend in Tris buffer, add a little sodium azide, one drop of calcium chloride, and collagenase at 100 μg/ml. Incubate at 37°C, 24 h. (If *viable* bacilli were needed, an alternative to axide should be sought.)

Add trypsin and chymotrypsin at 100 μg/ml. Incubate 24 h, 37°C. (This step has so far only been necessary with subcutaneous leproma, which gave a suspension with more non-bacterial particles at this stage than did liver.)

Add pronase at 100 μg/ml. Incubate 24 h, 37°C. (May be prolonged up to 4 days to cope with large amounts of extraneous protein, but the addition of the trypsin step is probably a more satisfactory alternative.)

Centrifuge 8000 x g, 10 min. Wash sediment twice with Tween, centrifuge as before. Suspend in small amount of Tween.

Prepare aqueous two-phase polymer-system. For 100 g batch use 7.0 g Dextran, 5.0 g PEG; water to 75 g (weigh it!). Stir, or stand and then stir, until polymers are dissolved. (This can conveniently be done by brief stirring after standing overnight.) Add phosphate buffer and sodium chloride to achieve final concentrations of 0.01 M each, add bacterial suspension, add water to 100 g.

Stir system until uniform, then stand in separating funnel until phases separate. Run off lower phase and interface (usually coloured). Dilute top phase with equal volume of water, adding Tween 80 (10% w/v solution) to give 0.1% final concentration. (A 100 g system gives about 50 ml of upper phase.)

Centrifuge diluted upper phase 8000 x g, 30 min. Wash sediment in Tween several times, centrifuging at 8000 x g for 10 min. (If there is a significant translucent brownish layer above the whitish bacterial layer it may be removed by suspending it gently without disturbing the bacteria.)

Suspend bacteria in water and freeze-dry *or* preserve as a suspension in Tween 0.05%.

ANNEX TO PROTOCOL NO. 2/75. PROGRESS REPORT ON *M. LEPRAE* AND SKIN TEST ANTIGEN PREPARED FROM INFECTED ARMADILLOS AT THE IMMLEP *M. LEPRAE* BANK (DR REES)

Yield and reproducibility

Using the definitive system for preparing suspension of *M. leprae* from tissues of infected armadillos developed by P. Draper since the first meeting of the IMMLEP Project Group, held in Geneva in November 1974, highly reproducible yields of purified *M. leprae* have been obtained. All this data presented in the following table is based on infected tissues from killed armadillos:

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* But see Protocol 3/75.

Tissue			*M. leprae* Yield		
Armadillo No.	Organ	Wet weight (g)	Batch No.	Dry weight (mg)	%
A81	Spleen	41	A.7	56	0.137
	Liver	40	A.8	77	0.193
	Liver	82	A.9	166.7	0.203
A29	Liver	80	A.10	102.3	0.128
	Liver	80	A.11	89.5	0.112

Thus, to date 323 g wet weight infected liver and spleen have yielded 492 mg dry weight *M. leprae*, giving a mean yield of 1.5 mg dry weight *M. leprae*/1.0 g wet weight infected tissue. These yields are less than we reported (2.7 mg *M. leprae*/1.0 g tissue) in November 1974 (IMM/74.3, Appendix II, page 12) probably because then our methods were: (1) Less precise and (2) were based on limited information from tissues of infected armadillos which were allowed to progress to death.

To date approximately 300 g dry weight *M. leprae* have been distributed to the IMMLEP programme.

Purity

Electron microscopical examination of each of these preparations of bacilli showed them to be free from contamination, including the presence of collagen (see section below on purity of skin test antigen derived from these preparations of *M. leprae*).

Distribution and handling of freeze-dried preparations of M. leprae

To date, our preparations of purified *M. leprae* have been freeze-dried from water suspensions and like all other purified mycobacterial preparations in water are difficult to resuspend without aggregation. Present studies show that the problem of aggregation can be overcome by initial resuspension in the presence of surfactants and that with these surfactants the maximum concentrations of *M. leprae* in suspension can be obtained under the following conditions:

MAXIMUM CONCENTRATION OF *M. LEPRAE* RETAINED IN SUSPENSIONS FROM FREEZE-DRIED STATE

Concentration of freeze-dried *M. leprae*/ml in suspending fluid	
0.5 mg	0.1% Tween 80/saline
0.25 mg	0.05% Tween 80/saline
1.0 mg	0.05% Tween 85/saline

(To obtain a homogeneous suspension the freeze-dried *M. leprae* should be placed in a dry glass homogenizer and the suspending fluid added slowly during grinding.)

From any of the above combinations *M. leprae* will remain homogeneously in suspension at two-fold dilutions adding saline without any further Tween.

SKIN TEST ANTIGEN

In order to expedite studies on a soluble skin test antigen derived from *M. leprae* obtained from infected armadillo tissues, a large-scale batch of *M. leprae* was prepared from infected armadillo tissues using the earlier method described by P. Draper (Appendix I, IMM/74.3). This preparation has been used throughout for the major skin tests carried out in Burma, Norway and the United Kingdom. There is a stock of 110,600 skin test doses (0.2 μg protein) of LRA6.

Reproducibility of antigen

As agreed by IMMLEP small samples of antigen have been prepared from each new batch of purified *M. leprae* for comparing with LRA6 by skin tests in leprosy patients.

The yield of soluble protein antigen is approximately 25% of the dry weight of *M. leprae*.

Test for purity

Quantitative skin tests with protein from uninfected armadillo tissues in groups of guinea pigs sensitized with the same protein or *M. leprae* skin test antigen, incorporated in complete Freund adjuvant, show that LRA6, LRA7 and LRA8 contain 1%, $< 0.68\%$ and $< 0.13\%$ armadillo protein, respectively.

Standardization of skin test antigen in guinea-pigs

Pilot studies using animals sensitized with purified preparations of *M. leprae* incorporated in incomplete Freund adjuvant indicate that this method is very sensitive. Preliminary comparative results using LRA6, LRA7 and LRA8 give similar delayed type hypersensitivity reactions related to their protein content. It is planned to use this method to study cross reactions between *M. leprae* and other species of mycobacteria.

Problems

(1) The possible effect of four different proteolytic enzymes on antigens of bacteria. Experiments are currently under way using *M. lepraemurium* as a model.

(2) Supernatant from high speed (10,000 x g) centrifuging probably contains Convit's type of antigen. This should be recovered if suitable techniques become available.

(3) Difficulty of resuspending freeze-dried bacteria.

PROTOCOL NO. 3/75. PURIFICATION AND CHARACTERIZATION OF *M. LEPRAE* ANTIGENS

1. *Purpose*

The goals are:

(a) To provide a soluble, stable preparation from *M. leprae* which is as specific for *M. leprae* as possible, cross reacts minimally with other mycobacteria, and is non-sensitizing by skin testing. The product should contain as few immunologically irrelevant, non-reactive components as possible.

The potential uses of this type of *M. leprae* preparation are described in Protocols Nos. 7, 8, 9 and 11/75.

(b) To prepare individual antigenic components from *M. leprae*. Experiments with different preparations of this kind are necessary to obtain information on the relationship between antibody formation and cellular immune reactions against the different components of *M. leprae* in patients with various forms of clinical disease and in individuals exposed to *M. leprae* who remain healthy.

Sensitive assays for antibody formation against selected *M. leprae* antigens may provide positive findings in infected individuals who develop lepromatous disease with minimal clinical symptoms and who have negative skin tests.

Antibody formation and cell mediated immune reactions against particular antigenic components may be related to important pathological processes such as nerve damage, reversal reactions and erythema nodosum leprosum (ENL).

Work with purified antigen would facilitate investigations of the taxonomic relationship between *M. leprae* and other mycobacteria.

(c) To establish a reference system for characterization of *M. leprae* by crossed immunoelectrophoresis.

Anti-*M. leprae* antibody should be produced in one laboratory so that antibody and polyvalent *M. leprae* antigen can be sent to other laboratories to serve as a control and reference for their immunodiffusion systems.

Tests with crossed immunoelectrophoresis including direct comparison with the reference

system are valuable for examination and comparison of various *M. leprae* antigen preparations made for skin testing and other purposes.

(d) The possible use of animal models based on skin tests and *in vitro* tests of cell mediated immunity for evaluation and comparison of various *M. leprae* antigen preparations should be explored.

(e) To find whether antigens that are important in immune reactions in leprosy have corresponding components with similar structure in readily cultivable mycobacteria.

2. *Materials suitable for fractionation*

(a) Antigens will mainly be isolated from purified leprosy bacilli obtained from infected armadillos.

(b) Some important antigens of *M. leprae* may be present in the tissue fluid of infected armadillo tissue. After the initial homogenization and centrifugation procedures involved in purification of bacilli (see Protocol No. 2/75), the supernatant fluid should be saved and stored at -20°C, to be used for antigen fractionation when adequate methods have been developed. Different techniques should be explored for this purpose. Immunoadsorbent procedures based on the use of antisera against *M. leprae* are expected to be particularly useful since they may permit specific recovery of *M. leprae* antigens from the quantitatively dominating animal proteins in the fluid.

(c) Purification of antigens from circulating immune complexes in lepromatous leprosy patients with and without ENL may provide materials that are particularly valuable for establishing the relationship between certain antigenic components of *M. leprae* and clinical course of the disease.

3. *Fractionation procedures*

Development of fractionation procedures using some other mycobacteria is important, to establish the most valuable techniques and to save precious antigenic material, but the work should be applied to *M. leprae* itself as soon as possible.

Several approaches should be explored since it cannot be predicted which methods will lead to purification of antigens most important in protective immunity or clinical complications. Differences in "know how" in different laboratories are also important in this respect.

The separation may be based on conventional biochemical procedures such as salt fractionation, electrophoresis, ion-exchange chromatography, gel filtration, phase partition and lectin-affinity chromatography.

The possibility of using immunological techniques for fractionation should be explored, particularly immunoadsorbent procedures. These are based on the use of antibodies as reagents of high specificity, and make it possible to concentrate the work on fractions that appear to be particularly important from other sources of immunological information. They are particularly suited for work with small amounts of antigen.

Monospecific antisera against individual components of BCG have been made by immunization of animals with precipitates from crossed immunoelectrophoresis, and similar antibodies may be very useful for isolation of individual antigenic components of *M. leprae*.

4. *Testing of the preparations obtained*

Tests for specificity of antigenic preparation described in section 1(a). The preparation should be characterized by:

(i) Strong reactivity with antisera against *M. leprae*; (ii) minimal reactivity with antisera against other mycobacteria; (iii) minimal reactivity with antisera prepared against normal armadillo and human tissue; (iv) cell mediated immune reactions as measured by delayed hypersensitivity skin reactions, lymphocyte transformation tests, and migration inhibition tests in animals, and patients with tuberculoid leprosy should show similar specificity and lack of cross reactivity. Skin testing in healthy individuals in leprosy non-endemic areas is described in Protocol No. 5/75.

The stability of the preparation should be ascertained and should include information on the effect of storage at -20°C and 4°C, heating and dilution.

Tests for purity of preparation described in section 1(b):

(a) Precipitable components should give a single band by crossed immunoelectrophoresis or a system of similar sensitivity using a potent, polyvalent anti-*M. leprae* antibody.

(b) Non-precipitating components with immunological reactivity should be proved homogeneous by adequate biochemical techniques.

PROTOCOL NO. 4/75. GENERAL CONSIDERATIONS FOR SKIN TEST STUDIES TO BE UNDERTAKEN IN ENDEMIC AREAS

1. *Antigen(s) to be tested*

(1) The antigens to be considered must be as specific as possible for *M. leprae*, stable under the conditions to be tested, and non-sensitizing (cf. Protocols Nos. 7/75 and 3/75).

(2) The optimal dosage for discriminating positive reactors from non-reactors must be known (to be derived from Protocol No. 6/75).

(3) The maximal potency available is required, i.e. a high frequency of reactivity in known tuberculoid leprosy patients (cf. Protocol No. 6/75).

(4) Antigens should be coded.

(5) Additional antigens could be added to this as seems appropriate.

2. *Methodology*

(1) Skin tests should be carried out intradermally in 0.1 ml volume on the volar surface of the forearm.

(2) For multiple tests on single individuals, randomization of test sites is preferable.

(3) Tests must be accurately read by an experienced reader for diameter of induration, diameter of erythema and/or oedema, and subjectively for intensity of erythema (cf. Protocol No. 8/75).

(4) Accurate and detailed records of the study population group must be kept. Some parameters to be included are given in Protocols Nos. 7/75 and 8/75.

3. *Population studied*

In order that the most useful information can be obtained from skin test results, it is important that any population to be tested be characterized with regard to frequency and prevalence of leprosy and tuberculosis, BCG vaccination status, and exposure to environmental mycobacteria. The study group should be group by age, e.g. 6-11, 12-18 and 19-40 years. Sociological parameters which may affect contact with leprosy cases should also be considered (Protocols Nos. 7/75 and 8/75).

In testing populations of leprosy patients, their disease status, determined clinically and histopathologically, should be recorded as follows:

TT, BT, BB, BL, LL and (if possible) I.

Duration of disease, nature of disease (e.g. quiescent, active or reactive), treatment (type, duration and regularity) must be recorded.

4. *Ethical considerations*

(1) It must be appreciated that none of these protocols can be carried out without the approval of the governments concerned, and/or the groups being studied, which will be sought in advance.

(2) All patients with leprosy discovered in the course of any trial should receive treatment.

PROTOCOL NO. 5/75. COMPARATIVE TESTING IN NON-ENDEMIC AREAS

The purpose is to compare different antigenic preparations from *M. leprae* by skin testing in non-endemic leprosy areas and to delineate whether there is a correlation with tuberculin sensitivity.

(1) The LRA6 preparation, 2.0 μg/skin test (0.1 ml intradermally).

(2) The LC preparation as prepared from infected armadillos (LCA) (0.1 ml intradermally, standardized on the basis of original count of acid-fast bacilli = 1.6×10^8/ml).

(3) A control preparation made in the same way from lymph nodes from uninfected armadillos standardized to the same total protein concentration.

(4) PPD, RT23, 2 TU/intradermal injection in 0.1 ml volume (obtained from Copenhagen).

Note. Arrangements will be made for the LRA6, LCA and control preparations to be tested for endotoxin (Limulus assay) as a sensitive indicator of the presence of non-*M. leprae* bacterial products.

Test subjects

(1) Healthy volunteers, male or female, aged 18-25, who have given informed consent and who have not been exposed previously to any test procedure with *M. leprae* antigens, but who may or may not have been BCG vaccinated. It should be recorded whether they have been vaccinated once or more, and at which time.

(2) Patients with active pulmonary tuberculosis, sputum positive at time of diagnosis and within 18 months of commencement of treatment. The drug regimen should be recorded and should not include immunosuppressive agents. No adverse effects or more intense skin reactions have been recorded using 1.5 TU RT23 in cases of pulmonary tuberculosis (J. Guld *et al., Bull. Wld Hlth Org.* (1958) **19**, 845-951).

For both groups, individuals from leprosy endemic areas must be excluded.

Areas selected

(1) Chile (southern part). In this area healthy individuals from 2 additional age-groups (children of 10 years or less, and adults 50 years and over) will be studied.

(2) Norway.

(3) United Kingdom.

Reading and recording of skin tests

All reactions are to be read at 48 and 72 h. The primary recording should be of both the transverse and vertical diameters of induration in mm. Diameters and intensity of erythema and oedema are also to be recorded. An experienced skin tester will be allocated for each area. It may be possible to arrange for injections and readings to be made by one person for tests in Norway and the United Kingdom. The reader should not have access to the 48 h results when reading the tests at 72 h.

Distribution and coding of antigens

The same antigen preparations should be used in the different areas. To this end, the various preparations will be sent to Dr Rees, NIMR, London, whence they will be distributed in coded, identical bottles.

Group size

For healthy subjects in the 18-25 year age-groups, 60 in all three areas, and 60 in the 2 additional age-groups in Chile.

In each of the 3 areas 20 patients with pulmonary tuberculosis will be tested.

Suggestions for future follow-up studies depending on the results of the above

(1) Testing with antigen preparations from environmental mycobacteria. The reaction to LRA6 should be compared with the reaction to similarly prepared antigens from mycobacteria selected from those used in the LRA6 skin test trial in Burma. In addition, studies of the *in vitro* correlates of delayed hypersensitivity may be performed.

* Intensity to be recorded as +, ++ and +++ (weak, medium and strong).

(2) It is planned to investigate, in Chile, the influence of previous testing on reaction size and the possibility of sensitization with regard to the LRA6 as well as the LCA antigen, and the antigen from uninfected armadillo tissue. With this end in view, information enabling accurate identification will be obtained from all subjects at the time of first testing.

The interval between the first and one or two subsequent tests should not be less than 6 months.

PROTOCOL NO. 6/75. CALIBRATION AND COMPARISON OF AVAILABLE ANTIGENS*

Purpose

(a) To determine the optimum dose of presently available skin test antigens in healthy persons in a highly leprosy endemic area and in patients with tuberculoid leprosy.

(b) To compare the reactivity of these antigens, using optimum doses, in patients with leprosy.

1. *Calibration in schoolchildren in Chingleput District, India*

1.1. In an ongoing programme of tuberculin testing and BCG vaccination, each antigen will be given as a second test in a few hundred children, preferably in a secondary school. These children shall not be tested with similar antigens subsequently.

1.2. Preliminary studies will start in the first instance with LRA6 in a dose indicated by the detailed study of data from Burma. Readings will be made after 72 h.

1.3. LRA6 will be received in 1 ml vials (2 μg/0.1 ml) for further dilution in the field.

1.4. During the first week a starting dose, probably of 0.25 μg, will be tested. The dosage will be increased from week to week until a maximum permissible dose is reached (producing the strongest acceptable reaction). The maximum dose available from the preparation is 3 μg/0.1 ml. Around the optimum level, 2 doses will be used alternately to establish the dose-response function.

1.5. Similar studies will be made of the LCA antigen and possibly of an "environmental" antigen. For the LCA antigen, readings will be made at 48 h.

2. *Calibration in tuberculoid patients*

Concurrently or subsequently, suitable dose levels will be established in tuberculoid patients not previously tested with lepromin. In selecting the starting dose, the possibility must be taken into account that reactions in these patients may be stronger than in schoolchildren. About 100 patients may be needed, assuming they are willing to receive multiple tests (which would be preferable). For each antigen, a dose will be chosen with suitable right-hand mode, if possible identical with a dose suitable for use in the general population. In particular for LRA6 and LCA antigen, equivalent doses in terms of the right-hand mode should be chosen.

3. *Comparison of antigens in patients*

3.1. A comparative study in leprosy patients will include LRA6 and LCA antigen, each in a dose thus chosen. RT23, 2 TU is given as a third test, and an "environmental" antigen expected to be antigenically close to *M. leprae* might be given as a fourth test.

3.2. The tests are allocated randomly to skin sites in the forearms in each individual.

3.3. For the duration of the study, all patients will be tested except for authentic tuberculoid cases, identified by clinical examination. A random third of this latter group will be selected for testing. A total of 200 patients tested is probably sufficient.

3.4. The reading should be done by a technician with no knowledge of leprosy immunology. All reactions will be read both after 48 and 72 h.

3.5. Independently of the skin reactions (before the testing) each case will be described classified clinically. For all patients not excluded from testing, material will be collected for histological examination (preferably in duplicate, in India and abroad).

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* This protocol may have to be modified dependent on the results of the study outlined in Protocol No. 5/75.

4. *Replication outside India*

The last study (the comparison in patients) should preferably be carried out according to the same protocol if taken up outside India. Preliminary calibration studies need not be identical and might be limited or omitted if the studies in India are thought sufficient.

PROTOCOL NO. 7/75. POSSIBLE DESENSITIZATION AS A CONSEQUENCE OF HEAVY EXPOSURE TO BACILLIFEROUS LEPROMATOUS PATIENTS

1. *Hypothesis to be tested*

Is the frequency and intensity of skin test reactions diminished in contacts of active untreated lepromatous patients as compared with contacts of tuberculoid patients and treated (one year) lepromatous patients?

2. *Methodology*

(1) Skin test with a selected antigen.
(2) Appropriate tests of cell mediated immunity if possible, on a sampling basis.

3. *Selection of appropriate area for study*

Requirements. The study should be carried out in a context in which known and predictable household contact with the patient population can most clearly be defined.

Parameters to be considered include: (a) Prevalence and incidence of disease in the area; (b) age; (c) duration of contact; (d) sex; (e) relationship to patient; (f) social habits of population: these must be assessed to establish that the contact of different sexes, age-groups, etc., with lepromatous cases is the same. If there is a difference, it is necessary to define exactly what this difference in degree of contact may be; (g) duration and regularity of treatment of patients; (h) bacteriological examination of nasal smears is to be carried out.

The study should be carried out in an area where the majority of existing cases are known and registered, and can be appropriately classified according to the stage of their disease. The clinical status of the contacts should be established at the beginning and at the conclusion of the study. The population must be relatively stable.

4. *Requirements for the antigen preparation to be used in such a study*

(1) It must show a high frequency of positive reactivity in tuberculoid (TT) leprosy patients (preferably $> 80\%$).
(2) It should give low reactivity in lepromatous (LL) patients ($< 5\%$).
(3) It should give low reactivity in non-exposed healthy individuals ($< 10\%$).
(4) The antigen must be non-sensitizing so that changes in reactivity of the control population can be examined at later times.
(5) Appropriate dosages of the antigen, stability and storage conditions must be established.

5. *Anticipated problems*

(1) Contacts may, in fact, have been leprosy patients in the past or may be incubating the disease.
(2) The higher the endemicity of the area, the less reliable will be the predictability of the exposure and non-exposure of the test group to active lepromatous patients. The incidence in the villages selected should not be so high that a plausible low contact control group cannot be identified.
(3) The lower the endemicity, the more difficulty is experienced in finding the required number of contacts.
(4) That of obtaining precise data on the amount and duration of contact.

PROTOCOL NO. 8/75. RELATION OF SKIN TEST UNRESPONSIVENESS OF CONTACTS TO THEIR SUSCEPTABILITY TO DISEASE

1. *Hypothesis to be tested*

Is consistent failure of contacts to react immunologically to skin tests with *M. leprae*

antigens related to:

(a) Increased frequency of clinical disease.

(b) Increased frequency of acquiring the lepromatous form?

2. *Methodology*

(1) Skin tests will be made with antigen meeting requirements specified in Protocol No. 7/75.

(2) A large population will be tested twice with an interval of 2 years. The population group to be studied will consist of the contracts negative to both of the tests. (The group consisting of those who were negative at the first testing but positive at the second one might form part of the population studied according to Protocol No. 9/75.)

(3) The susceptibility to disease of these individuals can be evaluated in 2 ways: (a) Prospectively, i.e. the entire study group would be examined for disease 5 years after the second negative test reading. (More frequent examination is desirable if practicable.) (b) Case control method, i.e. cases of leprosy reporting to health services in the indexed study group would be recorded and linked to original skin test status.

3. *Selection of area*

(1) It should be hyperendemic for leprosy.

(2) Careful follow-up must be possible.

(3) The population must be relatively stable.

(4) Since the Burma trial area has high endemicity, and 1500 people there have already received one test with LRA6, it should be considered for such a study, subject to government agreement. A proportion of the general population who subsequently develop leprosy may be subjected to diagnostic lepromin testing in conjunction with the format of the WHO Leprosy BCG trial. In the context of this protocol, therefore, this problem is unavoidable.

(5) There should be a good record keeping system whereby previous skin test results can be traced.

PROTOCOL NO. 9/75. SUBCLINICAL INFECTION

1. *Hypothesis one*

Only a fraction of the total population exposed to *M. leprae* in a given endemic area will sooner or later acquire the disease. A further fraction of the exposed population may develop subclinical infection and this may be recognizable by testing with an *M. leprae* skin test reagent.

2. *Methodology*

Total populations in low endemic, moderate endemic and high endemic areas should be skin tested once or more to establish incidence of infection in different situations. This could be done by evaluating a proportion of positive reactors according to different variables such as age, sex, contact state, familial aggregation, etc.

Requirements for antigen preparation to be used in such a study would be the same as in Protocol No. 7/75. If tests were to be carried out on a single occasion a sensitizing skin test antigen could be accepted.

3. *Problems*

3.1 Negative skin test reaction, single or persistent, could indicate either: (i) That there has been no exposure to *M. leprae,* or (ii) that there has been exposure to *M. leprae* but no sensitization to the organism for one of several reasons, i.e. (a) low dose, (b) early stages of infection in those that ultimately develop clinical disease (see Protocol No. 8/75).

3.2. If only one test were undertaken, the study would be inconclusive unless a considerably larger proportion of the population as compared to the proportion of leprosy cases to be expected, were found to respond.

3.3. Such a study could be carried out in a more refined way, as outlined in Protocol No. 8/75.

4. *Hypothesis two*

Individuals in a preclinical stage of the disease, but who are destined to develop lepromatous disease, may already show immunological aberrations such as high levels of antibodies to certain *M. leprae* antigens.

5. *Methodology*

In any prospective epidemological study to be undertaken, one may consider the desirability of collecting serum samples from the population. These samples could be kept frozen until lepromatous patients appear and then compared (see Protocol No. 5/75) with samples from those who developed tuberculoid leprosy or remained free of clinical disease.

6. *Problems*

(1) Blood collection may be resented by the population and interfere with their co-operation.

(2) The cost involved in the collection of a large number of serum samples must be considered before this project is undertaken.

PROTOCOL NO. 10/75. ENVIRONMENTAL MYCOBACTERIA

The principal reason for investigation of environmental species of mycobacteria is to find a possible alternative to *M. leprae* for production of a vaccine for leprosy.

There also exists the possibility that delayed hypersensitivity to certain environmental mycobacteria might have an important influence on the epidemiology of leprosy, and affect the outcome of skin test and vaccine trials. Mycobacterial species used in this project will be selected as a result of taxonomic studies relating this organism to *M. leprae.*

Existing knowledge

1. Experience in the field and the laboratory indicates a close relationship between the leprosy bacillus and environmental mycobacteria (e.g. skin tests, lymphocyte transformation tests, and immunodiffusion).

2. Preliminary studies have attempted to identify which species should be further investigated. (a) Investigations using skin test reagents have shown that attention might be directed to *M. vaccae, M. nonchromogenicum,* and just possibly *M. marinum* among the 22 mycobacterial species investigated. (b) Investigations using laboratory animals and employing both skin tests and lymphocyte transformation have indicated a relationship between *M. leprae* and *M. vaccae* in particular.

Present investigation

1. Seven mycobacterial strains (4 of *M. vaccae* and 3 of *M. nonchromogenicum*) have been selected for special study. Stable rough variants of each strain are being isolated for use and will be preserved by freeze drying for later reference. The exact cultural biochemical and antigenic characteristics of each organism are to be determined, and each organism will be used to immunize guinea pigs, mice and rabbits in parallel with animals immunized with *M. leprae* from human material. Specific reagents will be prepared from each strain and used for:

(1) Skin testing the guinea pigs and mice 6 weeks after immunization.

(2) Lymphocyte transformation on lymph nodes of mice killed at the same time that other mice are skin tested.

(3) Immunodiffusion analysis, to be carried out using antisera from the immunized rabbits. This material will also be made available for crossed immunoelectrophoresis.

It is hoped that this experiment will further select the best strains of *M. vaccae* and *M. nonchromogenicum* for field investigations of leprosy patients and their contacts (provisionally Malawi might be considered as a suitable country for this study).

2. Studies of vaccines prepared from selected mycobacterial strains are being carried out in mice infected with *M. leprae* via the foot pad route.

3. An investigation has just begun into the acquisition of delayed hypersensitivity to those

environmental bacteria encountered by experimental animals. This will be followed by attempts to suppress peripheral immunological responses by overloading with specific mycobacteria. The principal purpose of this experiment would be to produce an animal model for the apparent suppression of reactivity to leprosy bacilli seen in close contacts of the disease.

Future studies

1. Antigens prepared from organisms selected in the present studies will be included, together with those currently used, in the lymphocyte transformation studies on contacts of leprosy patients in Ethopia.

2. Dependent upon the completed analysis of the Burmese results, very carefully planned studies should be performed on the populations of selected villages in Burma. These villages would be chosen on the basis of different prevalences, and as far as possible incidences, of leprosy (high, middle and low). The reagents to be used would include a leprosy antigen (as selected from Protocol No. 7/75) and antigens prepared from the 2 or 3 most promising environmental species. Lymphocyte transformation as well as skin testing should be considered for this study.

Problems arising

1. Some degree of protection from tuberculosis is afforded by contact with certain other mycobacterial species, and a similar situation may complicate the epidemiology of leprosy.

2. The particular species selected as being related to the leprosy bacillus are themselves difficult to classify precisely, and much confusion exists as to their precise identity.

PROTOCOL NO. 11/75. SKIN TESTS IN RELATION TO TRIALS OF POTENTIAL VACCINES

Introduction

One of the primary objectives of the IMMLEP programme is the development of an effective antileprosy vaccine (IMM/74.3). Important applications of skin test antigens will be to help assess the effectiveness of the vaccine.

1. *Assessment before vaccination*

The protective effect of an antileprosy vaccine is likely to be affected by the epidemiological characteristics of the areas to be tested. Pre-vaccination skin testing may be important in several respects. For example:

1.1. Skin testing should be used to establish the age at which natural conversion occurs, to allow the test population to be vaccinated before extensive conversion due to exposure to *M. leprae* has taken place.

1.2. The proportion of positive reactors to skin tests amongst contacts of lepromatous and tuberculoid patients may vary from one area to another and affect the outcome of vaccination. If this is found to be the case, several areas should be tested, to allow vaccination to be undertaken in 2 areas with contrasting patterns.

Requirements. In order to allow a skin test to be used in this respect, it is necessary that it is not sensitizing and has a high specificity for *M. leprae.*

1.3. A strong reaction to the skin test may prove usefully prognostic of an adverse reaction to vaccination.

2. *Assessment of efficacy of vaccination*

The skin test antigen would be used to compare the ability of potential vaccines to produce sensitivity to antigens of *M. leprae* both in non-endemic and endemic areas.

Requirements. (1) The skin test must be non-sensitizing. A sensitizing skin test would be unsatisfactory, unless it was possible to use *in vitro* tests such as LTT before vaccination; (2) the reaction to the antigen must be correlated with protective immunity, (a) by ensuring that the degree of sensitivity to the antigen corresponds to the position of a leprosy patient on the

Ridley-Jopling scale, (b) prospective studies in high risk groups comparing sensitivity to antigen with development of leprosy may also be considered.

3. *Post-vaccination assessment*

3.1. Non-converters and converters to be compared in a vaccine trial for subsequent development of leprosy. Detailed measurement of reaction sizes will be needed to draw maximum information from the trial.

3.2. Measurements of conversion rates and distribution of reaction sizes among various categories of contacts (it will be necessary also to measure natural conversion rates) should be compared with incidence rates of leprosy.

3.3. Lepromin testing (Mitsuda reaction) of at least a proportion of the population should also follow vaccination as part of a prospective trial to determine whether a negative reaction is associated with development of lepromatous leprosy.

4. *Problems*

4.1. The protocol is concerned with skin testing in relationship to vaccination. It will have to be modified as protocols for vaccination trials are developed.

4.2. It is possible that no skin test can be developed that will show complete correlation between reactivity and protective immunity. Nevertheless, skin testings may be useful to check potency of a vaccine and coverage of the population in a vaccination programme.

It should be borne in mind that lepromin (3.3. above) may interact with the vaccine to alter the level of protective immunity achieved. Lepromin testing may have to be confined to a proportion of the population.

Lepr. Rev. (1976) **47**, 333–340

Leprosy and the Community

REPORT ON AN INTER-COUNTRY CONSULTATIVE MEETING ON LEPROSY

New Delhi, India, 18-20 December, 1975. WHO Project SE ICP MBD 002

This Consultation, organized by the World Health Organization, brought together health authorities and research workers from 7 countries in S.E. Asia, India, Bangla Desh, Nepal, Burma, Sri Lanka, Thailand and Indonesia, for a discussion of matters of mutual interest in leprosy control. The report, by Dr K. S. Seal contains numerous items of general concern, and demonstrates the value of such Regional Consultations, in this case concerned with an area in which there are believed to be over 4.5 million sufferers from leprosy. The objectives of the meeting were:

(1) To review and analyse the magnitude of the leprosy problem in member countries;
(2) to develop strategies for the control of the disease;
(3) to develop guidelines relating to the necessary steps needed to implement the strategies;
(4) to review the methods of control, treatment and rehabilitation in the programme, and
(5) to review the present research activities and to identify areas for further research in order to strengthen leprosy control programmes.

The following are some of the important conclusions of the Consultation.

CASE FINDING

(a) Intensive health education of the community at all stages of case-finding is of fundamental importance.

(b) It is necessary to define in broad terms the nature and extent of the problem. This can often be best done by means of a random sampling survey to provide data on age, sex, form and specific prevalence in addition to overall prevalence.

(c) The case-finding programme should be systematic by defining operational areas so that epidemiological and operational evaluation can be made periodically.

(d) The actual choice of case-finding methods should be related to the level of endemicity in the country or region in which leprosy control is to be carried out.

(e) It was accepted that a country, region or area with a prevalence of more than 10/1000 should be considered hyperendemic.

(f) Even in areas with a prevalence of less 1/1000 active case-finding methods are necessary, and the lepromatous rates should always be taken into consideration.

(g) The most practical methods of case-finding are the examination of

contacts, especially child household contacts of infectious cases and of persons reported to be suffering from leprosy.

(h) In areas where the rate approaches that of hyperendemicity, school or child surveys provide a good indication of the prevalence in the adult population.

(i) In highly endemic areas, mass surveys may be justified. They should be conducted by teams and should aim at examining not less than 95% of the target community.

(j) Mass surveys are no substitute for the effective case-finding potentialities of the conscientious, well-motivated worker at village level.

(k) The support of the general health staff, doctors and paramedical and auxiliary workers is important as an adjunct to active case-finding.

EFFECTIVE TREATMENT

The increasing evidence of sulphone resistance among lepromatous and borderline cases on ambulatory treatment is due, it is believed, to inadequate and/or irregular treatment and necessitates the review of drug regimens and treatment arrangements. Bacilliferous cases should be rendered non-infective as early as possible.

In general the treatment intervals for patients to receive tablets should, whenever possible, be reduced in order to obtain better clinical control.

Where it is possible, lepromatous patients should have part of the treatment by a supervised dose.

During the first 3 months of chemotherapy, careful supervision should be exercised over the lepromatous patients to ensure adequate therapy in anticipation of a significant reduction in infectivity.

It was recommended that in order to shorten the period of closely supervised therapy, collaborative field studies should be conducted on the value of rifampicin as a single initial dose and in daily doses during the first month in bringing the Morphological Index below 1. In addition, trials should be conducted with DDS and clofazimine.

CASE MANAGEMENT–REACTIONS AND PREVENTION AND TREATMENT OF DEFORMITIES

The management of reactions in the field and the prevention and treatment of deformities were regarded as rather neglected areas in the management of leprosy cases. It was recommended that the staff of all leprosy units should be skilled and equipped to undertake the prevention and treatment of deformities.

TRAINING

A review was given by each delegate of training facilities in their respective countries for all types of health personnel. In general, the area where such training was the weakest and yet the most necessary in endemic countries was that of undergraduate medical education. It was considered that greater efforts should be made to provide short courses for private medical practitioners, and that each country should have a working manual for the guidance of workers engaged in leprosy control.

INTERNATIONAL VOLUNTARY AGENCIES

Dr Sansarricq highlighted the assistance provided by the international voluntary agencies to the leprosy control programmes in the countries of the South-East

Asia Region. The meeting emphasized the importance of greater involvement of these agencies in the leprosy control activities.

RESEARCH

Dr Sansarricq provided a succinct but interesting survey of current problems and recent advances in research on leprosy and outlined WHO's special programme. Since the Central Training and Research Institute, Chingleput, India, is collaborating with WHO in research on the chemotherapy of leprosy in India, a brief review of recent work was given by Dr Iyer, Director of the Institute.

Conclusions and Recommendations

OBJECTIVE

The aim is to bring about sufficient reduction in the amount of infection in the community to interrupt transmission of the disease, so that it is controlled at a level where it ceases to be a serious public health problem.

TARGETS

(1) In addition to preventing indeterminate cases from progressing into lepromatous leprosy, at least 75% of the estimated lepromatous and borderline cases should become bacteriologically negative and should remain so. The meeting felt that this should be possible in the Region within a period of 10-15 years.

(2) To achieve this target in the highly endemic areas at least 95% of the population should be examined in the expectation of detecting 95% of the infectious cases.

(3) All patients detected should be brought under regular supervised treatment immediately on detection, priority being given to the infectious or potentially infectious forms. This operation, in the opinion of the meeting, should be completed within a period of 3-5 years, in the highly endemic areas, after the launching of the programme.

(4) The existing national control programmes in each country should be maintained and further strengthened so that the intensified programme can be built upon them.

Strategies for implementation and needs were also discussed. This was clearly a very valuable Consultation.

THE INTERNATIONAL CONFERENCE OF THE LEPROSY MISSION

Singapore, 1976

S. G. BROWNE

The Conference held in Singapore from 3 to 15 May, 1976 was something of an event. The last such Conference was held in Lucknow in 1953, which was well reported in an article by William Bailey that appeared in *Leprosy in India* (1954) **26**, 53-62. The sulphones were just coming into practical therapeutics, and reconstructive surgery for the deformities of leprosy was beginning. The leaders of The Leprosy Mission, the oldest and the biggest voluntary agency concerned with leprosy, thought the time opportune in 1976 to take a hard look at present policies and future prospects, so as to utilize the resources of the Mission in the best possible ways for the benefit of the individual leprosy sufferer and the community of which he should form an integral part.

Gone are the days when custodial care for the favoured few in a context of Christian compassion was all that could be offered to the "lepers in India". And impossibly distant seems the Second International Leprosy Conference at Bergen (in 1908 it was) when the learned participants listened spell-bound as a layman, Wellesley Bailey, the founder of the Mission, spoke out of the breadth of his experience and encouraged the medical scientists and the government physicians to tackle realistically the problem of leprosy. When the Congress agreed—and this was the only matter apparently on which they were agreed—that established leprosy was quite incurable, it was Bailey who detected a faint glimmer of hope. They did not know at the time that the year before, two German chemists, Fromm and Wittmann, had synthesized diamino-diphenyl sulphone.

The 1976 Conference drew over 90 invited participants from some 35 countries, representing the "Western" world (including Australia and New Zealand) in which most of the million and a quarter pounds sterling is now raised annually, and also a gratifying number of representatives from those countries—particularly India—where the Mission expends the bulk of its money. Although doctors accounted for only a quarter of the invited participants, other members of the medical team—nurses, physiotherapists, occupational therapists, hospital administrators—were well to the fore, and regional secretaries and selected members of the Councils of various auxiliaries made up a knowledgeable and interested group.

As befits a Christian body concerned with compassionate caring as well as medical competence, the Conference studied in depth the changing role of the Mission as world medical opinion moved away from concentration on a single disease to community health and prevention. It was generally appreciated that although the Mission during the 102 years of its existence may have sought to isolate in thought and action the neglected victims of leprosy, and has stimulated governments and other voluntary agencies to take cognizance of the importance

of leprosy in the community, yet its future role may change as it strives to ensure that the leprosy sufferer is accorded his rights as a citizen to social and medical justice. Thus, far from becoming redundant, the Mission will have a significant and continuing role to play in community health programmes. Its medically serious contribution is being increasingly recognized by governments faced not only with the backlog of deformity, but also with an intractable medico-social problem whose solution cannot be sought in purely therapeutic or surgical terms. It is in this respect that the dedicated and highly motivated team-member—from research scientists like Professor C. K. Job to the humblest paramedical worker—will be able for many years to come to contribute by precept and example to a government countrywide programme of leprosy treatment and control.

The Conference paid tribute to the pioneering endeavours of those associated with the Mission whose influence continues to be apparent in the world of leprosy, especially in the realms of compassionate caring for individuals hopelessly deformed and hence rejected by society; modern medicinal treatment with sulphones, clofazimine, rifampicin and the rest; reconstructive surgery; rehabilitation; integration of leprosy into community health programmes; insistence of the importance of the individual in the mass; the relevance of social, cultural and psychological factors in any scheme for leprosy control and treatment.

Far from resting upon the laurels of the pioneers, the Conference considered the challenge of the possibility of the emergence of sulphone-resistance on a wide scale and the financial implications of multi-drug therapy, the significance of persister leprosy bacilli, measures to counteract the transmission of leprosy in overcrowded communities, the place of reconstructive surgery and rehabilitation in a total programme of medical care where financial constraints are paramount, the necessity for overcoming social stigma and community cultural pressures, the need to conform to government directives while somehow retaining the initiative and flexibility characteristic of voluntary agencies, the opportunities for field and epidemiological research, etc.

In addition to these medical concerns within The Leprosy Mission purview and responsibilities, the Conference discussed the promotional and educational aspects of its world-wide task. Far from being a mere fund-raising organization, The Leprosy Mission looks forward to increasing its impact for good on the problem of leprosy in many countries, bringing together the accumulated wisdom of the past, a youthful zest and enthusiasm, and a motivated service to the victims of leprosy.

The Conference expressed its disappointment that leprosy is a neglected subject in the curricula of many medical schools, even in countries where the disease is prevalent, and discussed ways in which the challenge of leprosy could be presented to students and interns, to nurses and physiotherapists: visits of roving lecturers (especially leprologists and reconstructive surgeons), the provision of transparencies and taped commentaries, the production of brochures and illustrated booklets, the encouragement of visits of medical students during their elective periods to leprosy centres, the utilization of the experience of consultants (particularly dermatologists and orthopaedic surgeons) who would after early retirement become available as lecturers and teachers, the granting of scholarships for impecunious medical students showing an interest in leprosy, etc. The Conference stressed the need to make medical folk in general aware of the sheer interest of leprosy today in the light of immunological and microbiological

research, and the need for epidemiological investigations of comparable excellence and coverage. It is not only the challenge of case-finding, but the even more demanding task of case-holding that accounts for the failure of many leprosy control programmes.

As a Founder-Member of ELEP (now ILEP), The Leprosy Mission is conscious of the need to work together with other bodies pursuing similar objectives, and plays a very active part in joint activities. In the realm of publications, too, the Mission is becoming increasingly active, particularly in the production of material intended for medical auxiliaries and doctors.

If Lucknow 1953 indicated the way forward into effective chemotherapy and reconstructive surgery, Singapore 1976 may well mark the beginning of an era of sober consolidation of activities and a continuing mobilization of medical and non-material resources to face the common foe.

LEPRA REPORT 1976

The Annual Report of Lepra for 1975, the 52nd in a long series, tells in a modest way a continuing story of distinguished service and dedication which has been characteristic of Lepra from the beginning. It involves not only leprosy workers overseas, but many people in Britain whose offering of time, energy, skill and concern has enabled the work to continue.

In a year of economic stress the income of Lepra at £432,738 fell only slightly as compared with 1974, and in spite of a great increase in costs it was possible to maintain to the full the services rendered overseas. Twenty-one nations benefited from Lepra aid, with Malawi, India and Sierra Leone heading the list. The establishment by the World Health Organization of IMMLEP is an international development of great challenge and hope. Lepra has been associated with IMMLEP in 1975 both through Dr Rees, Chairman of Lepra's Medical Advisory Board, who is a permanent member, and Dr Molesworth, Director of the Lepra eradication project in Malawi. At the same time Lepra gave financial help to enable Dr Celia Moss to work in Burma in the interest of IMMLEP.

Leprosy Review has had a prosperous year, a notable feature being the large Supplement covering the Second International Leprosy Colloquium on the Chemotherapy of Leprosy, Today and Tomorrow, held at the Forschungsinstitut, W. Germany. After 10 years of operation the Lepra pilot leprosy control project based on Blantyre has been handed over to the Government of Malawi. The Lepra Child Adoption Scheme has been brought to a conclusion and replaced by the very successful Children's Fund which facilitates the work among child out-patients. In these and in other ways, notably in training, the work of Lepra continues, and we offer all concerned our best wishes for an outstandingly successful year in 1976.

HEALTH PERSONNEL: WHO PRESS RELEASE WHO/24 OF 23 APRIL, 1976

RADICAL CHANGES REQUIRED IN EDUCATION AND TRAINING

Radical changes in health manpower development are required in the coming years to make it relevant to present and forseeable future community health requirements.

The need for "non-traditional" solutions to health personnel problems plaguing almost every country in the world today is stressed by the Director-General of the World Health Organization in his report on health manpower development prepared for the 29th World Health Assembly, meeting in Geneva on 3 May, 1976.

Changes can only be carried out in the countries, and by the countries themselves, WHO playing its role as directing and coordinating authority for international health work. The task of WHO is to function as a change agent, stimulating thought and action, and promoting innovations—"often in the face of conventional wisdom", says the report.

The need for non-traditional solutions stems directly from the realization that simply to train more doctors and nurses who are mainly orientated towards diseases and hospitals will not solve health problems in the foreseeable future.

This means that in addition to the emphasis laid on "classical" categories of health workers—such as physicians and nurses—a new strong emphasis should be laid on the training and utilization of auxiliary and community health workers and their supervisors.

Far from being diminished, the number, role and importance of "classical" health workers will have to be increased. Their education—properly geared to the progress of science and to the needs of society—must be made relevant to community health needs and demands without reducing its basic quality.

Health professionals, says the report, should be prepared for their leadership role in the health team and in community development so that, through the use of auxiliaries, a much broader part of the community will benefit from their knowledge and skill.

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News and Notes

11TH INTERNATIONAL LEPROSY CONGRESS, MEXICO CITY, 1978

The next International Leprosy Congress will be held in Mexico City from Monday, 13 November, 1978 till Saturday, 18 November. The local Honorary President will be Professor F. Latapi, and the Chairman of the local Organizing Committee is Dr Amado Saul.

Dr J. Convit, the President of the International Leprosy Association has appointed a representative Committee to advise him on the form and contents of the Scientific Programme of the Congress. This Committee will meet in January 1977, and will consider the numerous representations made from various individuals and interested bodies, so that the needs of all kinds of leprosy workers may be catered for as far as possible.

As in London (1968) and Bergen (1973), the Secretary-General of the Congress is Dr S. G. Browne, C.M.G., O.B.E.

Preliminary notices of the Congress, including indications of the themes to be considered, and all information concerning the submission of abstracted full papers, will be published as soon as possible after the President's Advisory Committee in January 1977.

LEPROSY SEMINAR IN IRAN

A successful international seminar under the title "Evaluation of Leprosy" was held in Teheran, Iran, from 21 to 23 June, 1976. The seminar was under the distinguished patronage of Her Imperial Majesty Farah Pahlavi, Empress of Iran, and was organized by the Leprosy Assistance Association of Iran, in particular its Secretary, Dr Siyadat. Well-known participants came from many countries—England, France, Switzerland, U.S.A., Brazil, Argentina, Senegal, India, Indonesia and Korea. Many doctors and research workers from Iran itself, as well as expatriates (from France, Switzerland, India and Korea) working in the leprosy service in Iran at Mashad, Tabriz and Behkadeh, took an active part in the proceedings. Many aspects of leprosy came under review, from the latest research work on immunotherapy by the lymphocyte transfusion, to a critical evaluation of the late results of reconstructive surgery.

The problem of leprosy in Iran is not huge or unmanageable, the prevalence rates ranging from about 0.5 to 2.47/mille, and an estimated total number of sufferers about 30,000 in a population of 30 million. However, difficulties of access, extremes of climate, the scattered nature of the population, impermeable social prejudices, and the absence of an adequate medical service in outlying parts of the country—all tend to make case-finding and case-holding matters of real concern.

The Empress, who is well known for her practical interest in leprosy and all other social ills affecting the citizens of Iran, gave an audience to visitors from abroad and some of the Iranian doctors. She urged a greater collaboration between the Health Ministry and the voluntary agency that has played so notable a part in bringing the problem of leprosy to the attention of the Iranian people.

"PARTNERS"—A NEW MAGAZINE FOR PARAMEDICAL WORKERS IN LEPROSY

The Leprosy Mission is to be congratulated on producing a journal specially designed for paramedical workers in leprosy, and aiming to give up to date information on the scientific and technical aspects of their work while providing a forum for the sharing of experience. Written in a style which should have wide appeal, this Journal, which is under the editorial direction of Dr Browne, can do nothing but good, and we wish it every success. It is intended to publish *Partners* every 6 months, and copies are available gratis from The Leprosy Mission, 50 Portland Place, London W.1, England.

PERSONAL

Dr Stanley Browne, CMG, OBE, has been elected a Fellow of King's College, London. The Fellowship is the highest honour in the gift of the College. Dr Browne was a student at the College from 1927 to 1930.

At a recent meeting of the Council of the Royal Society of Tropical Medicine and Hygiene, Dr Browne was elected as President-elect. He will assume the office of President of the Society for a period of 2 years from June 1977.

Dr W. Felton Ross, late Director of the All Africa Leprosy and Rehabilitation Training Centre (ALERT) at Addis Ababa, Ethopia, has been appointed Medical Director of American Leprosy Missions. During his 11 years as Director of Training, ALERT has become one of the largest and most forward looking leprosy training institutions in the world. We offer him our most cordial good wishes as he takes up his new appointment.

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Letters to the Editor

From The Director, Lepra, Fairfax House, Causton Road, Colchester CO1 1PU

The Medical Advisory Board of Lepra, at its meeting held on the 10 June, 1976, expressed its deep concern for the action taken by the President of the American Leprosy Mission in using the recent claim that the leprosy bacillus has been cultivated *in vitro*—as a basis for a special fund raising drive. Members of the Board consider that any attempt, by organizations dedicated to the control of leprosy and the welfare of the leprosy patients, to exploit a discovery of this magnitude before it has been documented and proved, can only harm the work of anti-leprosy organizations generally and more especially so far as their fund raising activities are concerned. The public will soon lose confidence and become disillusioned if claims of this nature are exploited without adequate explanation and before the claim can be substantiated.

Members of the Lepra Medical Advisory Board strongly condemn such publicity and trust that those in responsible positions in anti-leprosy organizations throughout the world, will exercise great care to ensure that their fund raising literature and publicity is not in any way misleading.

G. F. HARRIS

From Dr G. Warren, The Leprosy Mission, c/o Christian Hospital, Manoram, Thailand

The Editorial of *Leprosy Review* of March 1976 (Vol. 47, No. 1) on the complications of treatment with clofazimine provides interesting reading for those who are familiar with this drug. But it has caused real concern amongst the less clinically experienced, some of whom have become fearful that its use will often produce a high incidence of undesirable toxic effects and may even cause death.

All who have worked for any length of time with clofazimine realize that only a small proportion of patients on the drug do develop gastro-intestinal symptoms (the figures to support this are quoted in the Editorial), and that in an even smaller proportion are the symptoms severe enough to warrant a change in drug therapy. Well supervised trials with clofazimine were carried out at widely separated centres and as in any drug trial, deaths did occur which were usually well investigated. In most cases some other cause of death was easily found, but the possibility of the use of clofazimine, or any other drug being given at or immediately before death, contributing to the cause of death would be difficult to rule out.

These drug trials showed clofazimine to be an effective means of controlling E.N.L. and that its dangers are so much less than those of prednisolone that it has

become the drug of choice in many centres for treating patients with E.N.L. Although the usual dosage is 100 mg daily this may need to be increased to 200, 300 or even more per day adequately to control the E.N.L. in severe cases. Once the E.N.L. is controlled we do try to reduce the dosage of clofazimine to the lowest level that will control the E.N.L., but many patients do need 900-2100 mg/week for long periods of time. Even on these higher doses the incidence of gastro-intestinal symptoms is low.

In many clinics the control of E.N.L. must be achieved by clofazimine or prednisolone, and especially where close monitoring is not possible clofazimine is the drug of choice. It is hoped that an Article such as this one does not lead to discarding the use of clofazimine and so to less efficient E.N.L. control and irregular anti-leprotic drug therapy.

The two cases are interesting and it would be informative to read the full case histories including the results of the tests done and to know the final medical state of the second patient. From the details given it is obvious that clofazimine cannot be assumed as the undoubted cause of the gastro-intestinal symptoms in either case—especially as in the second patient the first symptoms reported occurred more than 3½ years after cessation of treatment with clofazimine. Such cases should of course be properly investigated and reported to the Dunlop Committee, but until definite proof is obtained incriminating a specific drug, it is hoped that any printed reports, while informing workers of the possible complications will not be such that will deter the field workers from using the drug in adequate dosage to achieve the desired therapeutic effect.

Personally I have treated many more than 100 patients with clofazimine—some on 300 and 400 mg daily for many months, and can only remember 2 patients with significant gastro-intestinal symptoms. In one of these the symptoms settled when we realized he had been taking the drug on an empty stomach, and he took it with his food thereafter. In the other a very detailed investigation was carried out—including laparotomy which revealed heavily pigmented lymph nodes in the mesentery—and revealed no obvious cause of the symptoms. Once he was reassured the symptoms subsided without stopping the clofazimine which was only being given at 100 mg at the time, and had been at that dose for many months. Fortunately, when these complications occur they rarely if ever cause an acute crisis, so that awareness of their possibility should help the field worker in the management of patients. In the areas where clofazimine is frequently used there are also many other causes of gastro-intestinal symptoms; these must be ruled out before clofazimine is definitely incriminated. If we do a proper work-up and treat the patient for any intercurrent diseases we should not be caught blaming a drug which has solved far more problems than it has caused.

GRACE WARREN

COMMENTS ON DR WARREN'S LETTER BY DR W. H. JOPLING

The purpose of the Editorial in question was not to condemn the use of clofazimine in the treatment of leprosy but to alert doctors using the drug to be aware of possible gastro-intestinal side effects. Like Dr Warren I have used the drug extensively and have had no cause to change my policy; in fact the only side effect seriously mitigating against its use is skin discoloration in light-skinned patients. Regarding the last case recorded in the Editorial, the chief interest lies in the finding of dense deposits of clofazimine crystals in mesenteric glands 4 years after stopping the drug.

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Book Reviews

Leprosy in Children, by F. M. Noussitou, in collaboration with H. Sansarricq and J. Walter, with an introduction by S. G. Browne. World Health Organisation, Geneva, 1976. (ISBN 92 154053 2), 22 pp. Price Sw. fr. 9.00, U.S. $3.60.

The official description of this booklet is as follows.

"This book is concerned with the epidemiology, clinical features, diagnosis, classification, treatment and prognosis, as well as psychosociological aspects of leprosy in children. The main points stressed are:

(1) A significant proportion of all leprosy cases start in childhood;
(2) practically always, lesions in children are initially benign and bacteriologically negative;
(3) complete regression with minimal or no sequelae takes place under treatment in a very high proportion of cases. Spontaneous cure is a common feature in infantile leprosy but a significant number of untreated cases evolve to adult forms of the disease with high risk of infectivity and serious disabilities;
(4) the methodical examination of children, mostly by means of school surveys in leprosy control programmes, has considerable advantages. The book deals with practical problems encountered in the diagnosis of leprosy in children, and the clinical features are illustrated in 13 colour plates."

This is a most valuable booklet, authoritative, attractively presented and illustrated with a series of excellent colour plates. The text, directed to the general practitioner, gives in clear and concise style a balanced and comprehensive account of leprosy in childhood as likely to be seen in general practice and rural survey work. The presentation is up to date, giving commendable space to psycho-social aspects, health education and school surveys. The authors mention the variation in the appearances of leprosy between one part of the world and another. Here there is some orientation towards S.E. Asia, but this if anything is an advantage. Dr Browne has written a valuable Introduction which picks up some points deserving extra emphasis.

The statement that lepromatous leprosy is uncommon before the age of 15 years (p. 14) is matched by only 2 photographs being devoted to this most important type of leprosy, both of them illustrating relatively advanced disease. In the reviewer's experience in Central India, lepromatous leprosy, especially a degeneration from indeterminate and borderline leprosy, was by no means rare in childhood, indeed never less than 40 cases of this, some of them in young children, were in need of close hospital supervision. Photographs of multimacular pre-lepromatous lesions and the earliest stages of lepromatous infiltration and nodulation, e.g. on the earlobe, would have enriched the booklet, with if possible a photograph of juvenile leprosy as described in the Introduction.

The dictum on page 20 suggesting that if in doubt patients should be registered and treated as leprosy cases needs careful reservation. With really careful examination, including close observation of loss of sweating, competent smears, and where very early lepromatous leprosy is in question, careful clinical and bacteriological examination of the nose, the area of doubt becomes very small indeed. This is the type of case in which the judgment of an experienced leprologist is indicated. One does not condemn any child to a long course of therapy without careful judgement, and more emphasis could have been placed on this with advantage.

These are specialized aspects which in no way detract from the value of this excellent booklet to the general practitioner for whom it is intended, and to whom it can be recommended very strongly.

T. F. DAVEY

Rehabilitation of Leprosy Patients in a Comprehensive Control Programme in the Gudiyattam Taluk, S. India, by Mrs S. Karat, D. A. Ranney and P. V. Kurian, 132 pp.

This report in book form of 7 years of co-ordinated study of rehabilitation problems in a large comprehensive leprosy control programme contains many points of interest. Chapter headings include: Base Line Data on Disability, Aspects of Nerve Status, The Development of Disability, Restoration of Muscle Balance, Preservation of Anaesthetic Limbs and Vocational Training. Important observations include the high prevalence of neurological deficits, the heavy involvement of patients with lepromatous leprosy and the beneficial effect on these of dapsone therapy, the assessment of surgical procedures, and the exploration of domiciliary care and prevention of trophic ulcers. Inevitably in a study which is breaking new ground more questions are raised than are resolved, and some subjects are dealt with in greater detail than others. There is for instance the poor outlook even under dapsone therapy for so many disabilities in people with non-lepromatous leprosy. This is not in accord with the review's experience, but the terms of reference need defining. With the mass of information available the classification of patients according to the Ridley-Jopling scale would have been very helpful and probably resolved the rather surprising finding that only 11.6% of patients had Borderline leprosy, and also that only 21% of tuberculoid cases suffered from neurological disability. This figure suggests that large numbers of patients with tuberculoid leprosy had the disease in an exceedingly mild form and therefore the question of progress under dapsone was largely irrelevant. Some details of the grades of disability would have been helpful, especially as the details in Table 1.3 do not lend themselves to summation from this angle. A pertinent question is also how assiduously massage and hand and foot exercises were carried out at outpatient clinics. No details of this are given. The general impression is nevertheless one of admiration at the enormous work involved in summarizing so many statistics and, very important indeed, the domiciliary orientated approach of the whole subject.

The Report is not offered for sale. It is available from Dr E. Fritschi, Superintendent, S.L.R. Sanatorium, Karigiri, via Katpadi, N. Arcot, S. India, provided that the cost of postage is covered.

T. F. DAVEY

Lepr. Rev. (1976) **47**, 347–358

Abstracts

1. MICROBIOLOGY

1. SKINSNES, O. K., MATSUO, E., CHANG, P. H. C. & ANDERSSON, B. ***In vitro* cultivation of leprosy bacilli on hyaluronic acid based medium. 1. Preliminary report.** *Int. J. Lepr.*, 1975, v. 43, No. 3, 193-203.

Recent studies by the authors have indicated that hyaluronic acid might be an essential metabolite for *Mycobacterium leprae,* and one which enhances the multiplication of the organism in the mouse abdomen*. They now apply these findings to the cultivation of *M. leprae in vitro* using a liquid medium (LA-3) based on hyaluronic acid with the addition of yeast extract, bovine albumin, glycerine and phosphate buffer; and also a solid medium (LA-3p) with similar ingredients plus agar. These media were inoculated with organisms from 4 patients with untreated lepromatous leprosy (one in relapse) and from 2 mouse abdominal walls. All strains of bacilli from the above sources grew well on the LA-3 medium except that one was lost by contamination. Initial growth was observed after 2 to 6 weeks. The authors identify the cultured organism as *M. leprae* for the following reasons:

(1) Pathologic and experimentally determined rationale for the essential *M. leprae* nutrient requirement.

(2) Several cultures having the same characteristics have been isolated from LL patients widely separate in time and by geography.

(3) Failure of culture isolates to subculture on the usual media employed in the cultivation of mycobacteria at both 37° C and room temperature.

(4) 1° cultures in liquid medium successfully transferred to 2° liquid medium and to 2° agar medium plates.

(5) Bacillary isolates and bacilli of 1° and 2° liquid medium cultures, as well as LA-3P cultures, all stain with pooled LL serum, FITC coupled, *M. leprae* specific antibody with which a broad range of other mycobacteria do not react.

(6) *M. lepraemurium* also presents good growth on this medium.

This is a very important claim, and the authors are clearly convinced of its veracity. However, there are a number of surprising facts to be taken into account which indicate that, if the new organism is *M. leprae,* then the orthodox view of *M. leprae* is almost wholly at fault. The organism whose growth in mice is promoted by hyaluronic has a partly extracellular habitat. It does not require a cool tissue site and its *in vitro* culture temperature is 37° C. Its growth is faster than would be expected from experience of leprosy in mice. On first isolation it is non-acid-fast and shows suggestions of branching, and only after about six weeks is it predominantly acid-fast. It gives specific immunofluorescence only during the early non-acid-fast phase; by 3 weeks it is negative. Results with the electron microscope tellurite viability test indicate that it is the non-acid-fast phase that is viable, whereas well-stained acid-fast forms may not be viable. These rather revolutionary results deserve some caution pending their confirmation, which it is hoped will be forthcoming.

D. S. Ridley

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* Matsuo *et al. Int. J. Lepr.,* 74, 42, 399 and 75, 43, 1.

2. STORRS, E. E., WALSH, G. P. & BURCHFIELD, H. P. **Development of leprosy in another species of armadillo *Dasypus hybridus* (L.); genetic and immunologic implications.** *J. Trop. Med. Hyg.,* 1975, v. 78, Nos 10/11, 216-218.

The development of severe disseminated leprosy in one of a pair of seven-banded armadillos (*Dasypus hybridus*) after inoculation with *Mycobacterium leprae* is reported. This is a smaller animal than the nine-banded armadillo, but is of interest in that it regularly produces 8-16 monozygous young, compared with 4 in the case of its larger relative. This feature gives *Dasypus hybridus* a potential importance in studying the relationship of susceptibility in leprosy to genetic factors.

T. F. Davey

3. CLOSS, O. **Experimental murine leprosy: growth of *Mycobacterium lepraemurium* in C2H and C57/BL mice after foot-pad inoculation.** *Infection & Immunity,* 1975, v. 12, No. 3, 480-489.

Forty-three female C57/BL and C3H mice were inoculated with 2.7×10^6 *Mycobacterium lepraemurium* into each hind footpad. The foot thickness and the number of acid-fast bacilli in the footpad and popliteal and inguinal lymph nodes were recorded. In addition the morphological index and the mean bacillary length were determined in the footpad and in the popliteal lymph node. The bacilli multiplied in both strains during the first 4 weeks after inoculation. After that time no further increase in acid-fast bacilli was observed in the C57/BL strain; the bacilli became elongated and the morphological index decreased. These changes were preceded by a local swelling of the footpad due to the onset of an immune reaction. Thus, under the present conditions, C57/BL mice were able to resist experimental infection with *M. lepraemurium* by developing an immune response. In C3H mice no indication of an immune reaction was detected, and the bacilli continued to multiply throughout the observation period. The mouse footpad model seems to provide an excellent basis for the use of experimental murine leprosy to study immunity to mycobacterial infections. Certain aspects of the present model are discussed in relation to the mouse footpad model as used in the study of *M. leprae* infection in mice.

4. STANFORD, J. L *et al.* **Preliminary taxonomic studies on the leprosy bacillus.** *Br. J. Exp. Path.,* 1975, v. 56, No. 6, 579-585.

Antigens extracted from leprosy bacilli obtained from infected human and armadillo tissues have been examined by immunodiffusion analysis with serum samples from lepromatous patients and with immune sera raised in rabbits. Using the best combinations of serum and antigen extracts, 12 antigenic constituents were found in the leprosy bacilli. Six of these were antigens common to all mycobacteria and nocardiae, 4 were specific to the leprosy bacillus and the position of 2 could not be determined. Groups ii and iii antigens (i.e. those associated with the slow growing and fast growing subgenera of mycobacteria) were not found in the leprosy bacillus, suggesting some relationship with *M. vaccae* and similar strains, in which these antigens are also missing. Lymphocyte transformation tests performed on lymph node cells of mice infected or immunized with leprosy bacilli also showed the leprosy bacillus to have a closer relationship with *M. vaccae* than with other mycobacteria.

5. PRABHAKARAN, K. **A survey of attempts at cultivation of *Mycobacterium leprae in vitro* and experimental transmission of leprosy to animals.** *Lepr. India,* 1975, v. 47, No. 4, 325-336.

A selected survey is made of repeated efforts over the past 100 years at cultivation of *Myco. leprae* in chemically defined media and in tissue cultures. It is pointed out that none of the

claims of success on continued cultivation of the bacilli *in vitro* has been corroborated by other workers. Attempts to transmit human leprosy to experimental animals are reviewed. Except for the nine-banded armadillos (which have a limited geographic distribution and which have so far not been bred in captivity), no other species of animals has been found to be naturally susceptible to the systemic form of the disease.

[There are 74 references, but these do not include those to some of the early work mentioned in the text.]

6. GANAPATI, R. & CHULAWALA, R. G. **Bacteremia in leprosy and its relation to distribution of *M. leprae* in skin.** *Lepr. India,* 1976, v. 48, No. 1, 42-47.

Evidence of bacillaemia through examination of heparinized blood smears was obtained in 17 of the 20 cases (85%) of untreated leprosy cases belonging to the spectrum ranging from BT to LL. Among 17 cases whose blood smears were positive for AFB, the endothelial cells of blood vessels in skin lesions showed AFB in 11 instances (64.7%) and in 7 (41.2%) of these cases biopsies obtained from apparently normal skin also showed bacilli in the blood vessels. The fact that blood smears may show AFB even in patients belonging to types classifiable as BT-BB in the Ridley Jopling scale (a child aged 3½ years showed this feature) emphasizes the importance of investigations to assess thoroughly the extent of bacillation in leprosy patients.

[The technique for the preparation of smears from heparinized blood is described.]

7. PATTYN, S. R., DOCKX, P., JACOB, W., ROLLIER, R. & ROLLIER, M. T. ***Mycobacterium leprae* in human skeletal muscle.** *Ann. Soc. Belg. Méd. Trop.,* 1975, v. 55, No. 6, 643-646.

Leprosy bacilli were found in 24 out of 31 muscle biopsies from treated lepromatous patients. Bacilli were localized within macrophages and endothelial cells of the interstitial connective tissue, none were found inside muscle cells. This may be the result of treatment. Morphological intact bacilli were not observed. The widespread occurrence of leprosy bacilli in the human body is in accordance with the concept that leprosy is a generalized infectious disease.

8. PADMA, M. N. & DESIKAN, K. V. **Bacillaemia in leprosy.** *Indian J. Med. Res.,* 1975, v. 63, No. 6, 888-892.

In this careful and well-documented study, the authors found alcohol-acid-fast bacilli in the venous blood of 77 out of 114 patients with lepromatous leprosy. Stringent precautions were taken to avoid contamination of the blood sample by bacilli present in the dermis overlying the punctured vein from which blood was taken. The buffy coat (obtained after centrifugation at 1500 rev/min) was spread on a clean new microscope slide and stained by Ziehl-Neelsen's technique. Of the 77 cases in which bacilli were found, in 67 they were present within leucocytes, 62 of them being in monocytes; in 29 of these, they were also present extracellularly. Solid rods (morphologically normal, and presumably viable bacilli) were found in 19 cases.

Although bacilli were present on average in only 1 field in every 500 examined, the total bacillary load per ml of plasma might be as high as over 40,000.

These findings [which incidentally confirm the routine examinations in some laboratories 40 years ago] are discussed in the light of the well-known observation that endothelial cells engorged with viable leprosy bacilli are often found lining the blood vessels in sections of skin from patients with lepromatous leprosy. Further studies are necessary to determine whether the bacteraemia is intermittent or constant, whether it is to be correlated with clinical activity or acute exacerbation, and whether with more delicate staining techniques bacilli could be demonstrated with greater ease and in patients with non-lepromatous leprosy.

S. G. Browne

9. BALENTINE, J. D., CHANG, S. C. & ISSAR, S. L. **Infection of armadillos with *Mycobacterium leprae*. Ultrastructural studies of peripheral nerve.** *Arch. Path. Lab. Med.*, 1976, v. 100, No. 4, 175-181.

Peripheral nerves of armadillos were studied 16 to 30 months after intradermal or intravenous inoculation with *Mycobacterium leprae*. Numerous bacilli were found within macrophages, Schwann cells, and perineural cells; endothelial cells, pericytes and fibroblasts were involved as well. The bacilli were characteristically contained in membrane-limited vacuoles that were interpreted as being phagosomes. Some of the phagosomes contained granular, membranous, and vesicular debris considered to be bacillary degradation products, suggesting that lysosomal activity was present within the phagosomes. Multivesicular bodies, a few of which contained bacilli, were abundant in macrophages and perineural cells. An unusual proliferation of irregular tubulovesicular profiles was noted, especially in Schwann and perineural cell cytoplasm, surrounding and within phagosomes containing bacilli. The pattern of cellular involvement of neural structures with *M. leprae* was similar to that observed in lepromatous leprous neuritis in humans.

10. MATSUO, E. & SKINSNES, O. K. **Specific direct fluorescent antibody identification of *Mycobacterium leprae*.** *Int. J. Lepr.*, 1975, v. 43, No. 3, 204-209.

In vitro cultivation of *M. leprae* requires a rapid, specific identification procedure for monitoring the cultures. A method utilizing direct FITC-coupled lepromatous, specific serum globulin is described in detail with suggestions for improvement. After various purification and adsorption procedures, notably against human liver powder and *M. tuberculosis*, a fluorescent serum preparation is obtained which specifically reacts with *M. leprae* and not with other mycobacteria.

2. BIOCHEMISTRY, PATHOLOGY, IMMUNOLOGY

11. CONVIT, J., PINARDI, M. E., AVILA, J. L. & ARANZAZU, N. **Specificity of the 48-hour reaction to Mitsuda antigen. Use of a soluble antigen from human and armadillo lepromin.** *Bull. Wld Hlth Org.*, 1975, v. 52, No. 2, 187-191.

Two antigens were tested and compared in relation to the 48-h Fernandez reaction. They were obtained from standard human and from standard armadillo lepromin. All the tests were negative in patients with lepromatous leprosy and highly positive in those with tuberculoid leprosy and in lepromin-positive contacts. There was total agreement in all tests done with the two types of antigen. The antigen component has the following basic properties: it precipitates with 80% saturated ammonium sulfate; it is not destroyed by autoclaving or by treatment with 0.4% phenol; it is non-dialysable; and it is destroyed by treatment with trypsin.

12. CONVIT, J. *et al.* **Tests with three antigens in leprosy-endemic and non-endemic areas.** *Bull. Wld Hlth Org.*, 1975, v. 52, No. 2, 193-198.

A study comparing the 48-h and 30-day reactions produced by three antigens was made in areas of low and high leprosy endemicity in Venezuela and in areas of Chile, a non-endemic country. The antigens used for the intradermal tests were standard Mitsuda antigen, supernatant from standard Mitsuda antigen, and PPD. The results indicate that there is no difference in the Mitsuda reaction of persons living in areas of high or low endemicity, but they show a statistically significant difference between the reactions in persons who live in endemic areas and those of persons living in a country where the disease has not been described. The

difference in the Fernandez reaction obtained with the supernatant was not statistically significant between the two population groups in the endemic country, but was highly significant when comparing the endemic and the non-endemic countries.

13. LIM, S. D., JACOBSON, R. R., PARK, B. H. & GOOD, R. A. **Leprosy XII. Quantitative analysis of thymus-derived lymphocyte response to phytohemagglutinin in leprosy.** *Int. J. Lepr.*, 1975, v. 43, No. 2, 95-100.

The immune status of various leprosy patients was evaluated by using a micromethod to evaluate lymphocyte responses to phytohemagglutinin (PHA). In our study, whole blood was used and the degree of response to PHA stimulation was expressed in terms of unit volume of blood. A markedly decreased response to PHA stimulation was noted in patients with active lepromatous leprosy. Patients with active lepromatous leprosy who have been proved drug (DDS) resistant showed less response than did those of drug sensitive patients with active lepromatous disease, while the patients with active lepromatous leprosy complicated by *erythema nodosum leprosum* (ENL) showed higher response than did those of patients with no complicated ENL.

Comparing the results obtained to those obtained using other methods for T cell analysis indicates that these results reflect the number of T lymphocytes in the leprosy patient. Thus, this simple method is of value in assaying the presence and responses of T lymphocytes in the leprosy patient.

[For Pt XI, see *Trop. Dis. Bull.*, 1975, v. 72, abstr. 1663.]

14. SHER, R., HOLM, G., KOK, S. H., KOORNHOF, H. J. & GLOVER, A. **T and CR^+ lymphocyte profile in leprosy and the effect of treatment.** *Infection & Immunity*, 1976, v. 13, No. 1, 31-35.

Thymus-derived lymphocytes (T lymphocytes) and complement receptor-bearing lymphocytes (CR^+ lymphocytes) were estimated by using erythrocyte rosettes and erythrocyte-antibody-complement rosettes as markers in untreated lepromatous and untreated tuberculoid patients and in healthy controls. Treated lepromatous cases were also investigated. Ten cases of untreated lepromatous patients were reassessed 6 months or more after therapy commenced. A significant decrease in both percentages and absolute numbers of CR^+ cells in the untreated lepromatous leprosy subjects was observed. This decrease showed a return to normal levels after treatment. The percentage of T cells in the untreated lepromatous cases was normal; however, the absolute numbers of T cells and the total lymphocyte count showed a significant decrease. After therapy, the T cell population was unchanged but the total number of lymphocytes increased significantly with treatment. The absolute number of T and CR^+ cells was significantly less in the untreated than in the treated lepromatous patients.

15. REA, T. H., GOTTLIEB, B. & LEVAN, N. E. **Apparently normal skin in lepromatous leprosy. Histopathological findings.** *Arch. Derm.*, 1975, v. 111, No. 12, 1571-1574.

Biopsy specimens of apparently uninvolved skin from 34 patients with lepromatous leprosy were studied histologically. Bacilli were found in 30 of 31 specimens from clinically polar or near-polar lepromatous patients but not in the 3 from nonpolar patients. A predominantly perivascular distribution of infiltrate and bacilli is consistent with the hematogenous spread of infection. Subclinical, diffuse lepromatous leprosy is found in patients with nodular lesions and may precede the development of nodules. Study of apparently uninvolved skin may be helpful in classifying patients, in interpreting immunologic responses, and in elucidating the natural history of the illness.

16. HALDAR, B. & DUTTA, A. K. **Cutaneous vascular reactivity in tuberculoid leprosy lesion.** *Lepr. India,* 1975, v. 47, No. 4, 307-315.

In order to study the nervous and non-nervous cutaneous vascular responses in tuberculoid lesions of leprosy under chemical, mechanical and thermal stimuli, the following experimental studies were performed in 38 cases with control study in each:

(i) Adrenaline induced blanch reaction and periblanch erythema.
(ii) Focal bleeding time.
(iii) Surface temperature variations and adjustments following local application of cold.

In the lesions the results showed less prolonged blanch reaction and ill-developed periblanch-erythema, relatively prolonged focal bleeding time; slightly lesser degree of initial surface temperature, higher degree of declination in surface temperature following cold application and higher level of rise following heat application. Hunting type of reaction after both heat and cold applications was observed in control and test sites almost equally.

The collective results testify the phenomenon of inhibited nerve tonus in leprosy lesions consequent to organic affection of sympathetic fibres resulting in a vascular atonia.

17. DÍAZ ALMEIDA, J. G., TORRES, G. & ABREU, A. Deficiencia de glucosa 6-fosfato-deshidrogenasa en el enfermo de lepra. Informe preliminar. [**Glucose-6-phosphate dehydrogenase deficiency in leprosy. A preliminary report.**] *Revta Cub. Med.,* 1975, v. 14, No. 5, 687-691.

The English summary appended to the paper is as follows:

For the first time in Cuba a preliminary study on glucose 6-phosphate dehydrogenase (G6PD) deficiency using a modification of the Beutler's method (Kapa's system) in patients from Havana Leprosarium is made for determining the relation between G6PD deficiency and a lower defensive capacity of the organism against *M. leprae.* An enzyme deficiency only in patients with the lepromatous form and in those with backgrounds of repeated acute reactions was found.

18. MEHTA, L., SHETTY, V. & ANTIA, N. H. **Study of early nerve lesions in mice infected with *M. Leprae.*** *Lepr. India,* 1976, v. 48, No. 1, 31-35.

The present study is of quantitative histology in immunologically intact mice inoculated with *M. leprae.* Total 12 sciatic nerves are studied. The fibres are grouped as large, medium and small sized fibres. Initially there is loss of small sized fibres. At later stages there is involvement of all sized fibres and ultimately Wallerian type of degeneration sets in. The process of regeneration is more active than that of hyman leprosy of tuberculoid type. This study adds a new dimension in understanding the pathogenesis of leprosy.

[This paper is illustrated with 5 electron micrographs on plates.]

19. BEDI, B. M. S., HARRIS, E. B., NARAYANAN, E. & KIRCHHEIMER, W. F. **Delayed hypersensitivity tests with *Mycobacterium leprae* purified protein derivative.** *Lepr. India,* 1976, v. 48, No. 1, 8-18.

Skin tests were conducted on 3 lepromatous, 3 dimorphous and 6 tuberculoid leprosy patients and 3 others not suffering from leprosy with lepromin, purified protein derivative from *M. leprae* of armadillo origin and tuberculin. Results show that a delayed hypersensitivity reaction could be produced with PPD in 72 hours on all Mitsuda positive cases, with one anomalous exception, without cross reaction to tuberculin. The results were promising from the point of view of substituting lepromin with PPD in usual tests.

20. CHEN, T. S. N., DRUTZ, D. J. & WHELAN, G. E. **Hepatic granulomas in leprosy: their relation to bacteremia.** *Arch. Path. Lab. Med.*, 1976, v. 100, No. 4, 182-185.

A clinicopathologic study of liver disease was conducted on 28 patients with leprosy who lived in Taiwan. None of the patients exhibited symptoms or signs of liver disease. Hepatic granulomas were found in 21 patients. Histologically, the infiltrates were epithelioid, foam cell, and histiocytic in type. Hepatic dysfunction was absent, except for mild sulfobromophthalein elevations in the severely infected cases. Hepatic granulomas correlated with the cutaneous reactions in lepromatous leprosy, but the association was poor for other stages of disease. Hepatic involvement varied with the severity of cutaneous infection and with the frequency and intensity of bacteremia. An estimated 1000 to 10,000 acid-fast bacilli/ml of blood was required to generate the hepatic infiltrates.

21. SAHA, K., DUTTA, R. N. & MITTAL, M. M. **Immunologic aspects of leprosy as related to leucocytic isoantibodies and platelet aggregating factors.** *Int. J. Lepr.*, 1975, v. 43, No. 3, 239-248.

The incidences of various iso- and autoantibodies in a random population of 112 unselected leprosy patients is presented. Low titers of leucocytic isoantibodies and platelet aggregating factor were detected in the sera of a variable number of such patients. The leucoisoagglutinins were found in 8% of the sera of tuberculoid as well as lepromatous leprosy patients, whereas the leucoisocytotoxins were detected in a larger percentage of the lepromatous (40%) as well as tuberculoid (28%) cases. The platelet aggregating factors (PAF) were positive in 51.2% and 45% of lepromatous and tuberculoid cases respectively. Of the 21 positive sera for PAF, the antiplatelet factor by antihuman globulin consumption test could be demonstrated only in 66.6% and 50% of lepromatous and tuberculoid sera respectively. To study the frequencies of these newly detected antibodies or antibody-like factor and to compare their occurrences with other well-documented autoantibodies present in the sera of leprosy patients: cryoglobulins, antinucleoprotein antibody and thyroglobulin autoprecipitin were also studied in the sera of the same population of leprosy patients. It has been observed that the simultaneous occurrence of all these auto- and isoantibodies in the serum of one patient is a rare phenomenon. Leucocytic and platelet counts of these patients having antibodies against leucocytes and platelets were found to be within normal limits. Accordingly, it is suggested that the low levels of antileucocyte antibody and antiplatelet factor are probably harmless to the hosts. On the other hand, it is postulated that these antibodies may act as enhancing factors by being specifically adsorbed on the lymphoid cells, thus rendering them unresponsive to mitogenic stimulus *in vitro*. From these studies it seems that leprosy, especially the lepromatous type, is associated with some of the serological features suggestive of an autoimmune aberration.

22. LOUIE, J. S. & GLOVSKY, M. **Complement determinations in the synovial fluid and serum of a patient with erythema nodosum leprosum.** *Int. J. Lepr.*, 1975, v. 43, No. 3, 252-255.

Simultaneous serum and synovial fluid CH50, C1, C4, C2, C1 esterase inhibitor and C3 protein were determined in a patient with acute erythema nodosum leprosum. The pattern of synovial fluid complement activity coupled with the demonstration of multiple lepra bacilli free and within histocytes is more comsistent with an infectious than an immune complex induced synovitis.

23. SAMUEL, I., SAMUEL, D. R. & GODAL, T. **Hepatitis associated antigen (HAA) in leprosy.** *Ethiop. Med. J.,* 1974, v. 12, No. 4, 175-178.

One hundred and forty leprosy patients selected from the outpatient department of ALERT, Addis Ababa, were tested for hepatitis-associated antigen in their blood. Eleven or 8% of these patients were positive for HAA. The prevalence of HAA in leprosy patients was not significantly higher than in the normal population. Our studies did not show any increase in the prevalence of HAA in lepromatous cases as compared to tuberculoid cases.

3. CLINICAL

24. REA, T. H. & LEVAN, N. E. **Erythema nodosum leprosum in a general hospital.** *Arch. Derm.,* 1975, v. 111, No. 12, 1575-1580.

This is a study of 32 patients in Los Angeles, California, suffering from lepromatous leprosy complicated by erythema nodosum leprosum (ENL) reaction. There were 17 men and 15 women in the series, their ages ranging from 18 to 72 years, and 22 of these patients first presented with ENL reaction although they had never received any anti-leprosy treatment. In 5 women the initial attack of ENL occurred during pregnancy.

The authors describe the clinical, laboratory and histological findings, and discuss the possible mechanisms involved. They stress that their experience is contrary to the generally held opinion that this type of reaction usually complicates anti-leprosy therapy, and suggest that it should be considered to be a manifestation of leprosy rather than a complication of its treatment.

W. H. Jopling

25. QUAGLIATO, R., BECHELLI, L. M., ALMEIDA, J. O. & ARANTES, M. A. A. **Bacteriological status (point prevalence) of lepromatous outpatients under sulphone treatment.** *Bull. Wld Hlth Org.,* 1975, v. 52, No. 1, 57-62.

This is a study of 337 patients with lepromatous leprosy who had been under dapsone therapy as outpatients for 1-26 years in São Paulo, Brazil. 167 (50%) showed bacteriological positivity of skin smears when the slides were examined by a paramedical technician, who spent 10 min on each slide, but the rate was 99% when each slide was examined for 30-60 min by a bacteriologist. The authors conclude that, in mass campaigns, bacteriological negativity should be checked by a well-trained bacteriologist and the smears should be made by an experienced leprologist, before lepromatous patients are released from supervision.

[A more practical conclusion would be that lepromatous patients should be treated for life.]

W. H. Jopling

26. TIN SHWE, MYA THEIN & SOE MINT. **Prevalence of pulmonary tuberculosis in patients with leprosy.** *Burma Med. J.,* 1975, v. 21, 39-44.

The authors quote figures showing the high incidence of pulmonary tuberculosis among leprosy patients being treated in leprosaria in various parts of the world, figures which suggest that persons suffering from leprosy have an increased susceptibility to tuberculosis. This investigation, designed to establish if there is any justification for such a hypothesis, was confined to leprosy outpatients attending clinics at Rangoon General Hospital, and 603 patients aged between 20 and 40 years were studied (301 tuberculoid and 302 lepromatous). Seven patients with radiological evidence of pulmonary tuberculosis were found in the tuberculoid

group (2.3%) and 8 in the lepromatous group (2.6%), figures which compare favourably with those from Burma as a whole among adults of similar age-group (2.6-3.6%). Two important conclusions can be drawn from this study: firstly, that figures giving the incidence of pulmonary tuberculosis among in-patients in leprosaria bear no relation to the situation in that particular region or the country as a whole; secondly, that patients suffering from the lepromatous type of leprosy are no more susceptible to tuberculosis than are those suffering from the tuberculoid type.

W. H. Jopling

4. THERAPY

27. GELBER, R. H. & REES, R. J. W. **Dapsone metabolism in patients with dapsone-resistant leprosy**. *Am. J. Trop. Med. Hyg.*, 1975, v. 24, No. 6, 963-967.

Acetylation of dapsone (DDS) and sulfamethazine (SMZ), and plasma clearance of DDS were studied in Malaysian Chinese with lepromatous leprosy including 40 DDS-resistant and 44 non-resistant patients. Neither a patient's acetylation characteristics (DDS or SMZ), nor his plasma clearance rate, appeared to have predisposed him to the development of DDS resistance. A potentially important drug interaction between rifampin and DDS was discovered. After ingestion of rifampin for a minimum of 2 weeks, the plasma clearance of DDS was increased and the relative amount of the acetylated DDS was decreased. The implications of these results for the treatment of lepromatous leprosy are discussed.

28. CHAUDHRY, S. B. R. & DESIKAN, K. V. **Sulphone-resistance in leprosy: a report of three cases**. *Lepr. India*, 1975, v. 47, No. 4, 283-290.

The experimental transmission of leprosy to the foot-pads of mice has provided a very useful technique to demonstrate the occurrence of sulphone-resistant strains of *M. leprae*. Although this method is at present the most satisfactory laboratory confirmatory test, it is not practicable to apply it to screen a very large number of cases of leprosy under sulphone therapy today. A clinical detection of sulphone-resistance is therefore very essential. Three cases of lepromatous leprosy detected clinically to be sulphone-resistant and proved subsequently by the mouse foot-pad model are reported.

[A report from Chingleput, Tamil Nadu, India.]

29. LEVY, L. & PETERS, J. H. **Susceptibility of *Mycobacterium leprae* to dapsone as a determinant of patient response to acedapsone**. *Antimicrob. Agents Chemother.*, 1976, v. 9, No. 1, 102-112.

In the course of a clinical trial of acedapsone therapy in 17 patients with lepromatous leprosy, the rate of response to therapy was measured by inoculation of mice with *Mycobacterium leprae* recovered from biopsy specimens of skin lesions obtained before treatment and at intervals of 4, 12, and 24 weeks after institution of treatment. The susceptibility of each isolate of *M. leprae* to dapsone (4,4′-diaminodiphenylsulfone [DDS]) was measured by passaging organisms that had multiplied in mice to new groups of untreated mice and to mice treated with DDS incorporated in the mouse chow in concentrations of 10^{-5}, 3×10^{-5}, and 10^{-4} g/100 ml. The rate of response to acedapsone therapy and the susceptibility of patient strains of *M. leprae* to DDS varied widely among patients. All isolates were inhibited from multiplication by treatment of mice with 10^{-4} g of DDS per 100 ml; all but two isolates were susceptible to 3×10^{-5} g of DDS per 100 ml; and 17 of 36 isolates, representing nine patient strains, were susceptible to 10^{-5} g of DDS per 100 ml. Plasma levels of DDS measured in the

mice administered these diets show that the minimal inhibitory concentration of DDS for *M. leprae* isolated from untreated patients is about 3 ng/ml. No relationship could be demonstrated between DDS susceptibility of pretreatment isolates of *M. leprae* and the rate at which patients responded to acedapsone therapy. Neither acedapsone treatment of patients nor DDS treatment of mice appeared to select genotypically more resistant *M. leprae*.

5. EPIDEMIOLOGY AND CONTROL

30. KYAW LWIN & ZUIDERHOEK, B. **Case detection rates for Central Burma (1962-1972).** *Int. J. Lepr.*, 1975, v. 43, No. 2, 125-128.

A short description of the leprosy control program in Burma is given and the decrease of the case detection rates during the period 1962-1972 are presented to show the effects of control measures in the program emphasizing the importance of early case detection through annual examination of household contacts and school children, regular treatment and health education.

31. JARAMILLO, A. O. & DE LA CRUZ, M. R. **Le lepra en Costa Rica.** *Acta Méd. Costarric.*, 1975, v. 18, No. 3, 151-207.

The first person with leprosy recorded in Costa Rica was the child of a Spanish family in about 1734. By 1883 there were 32 leprosy patients in a lazarette. Sulphonotherapy was introduced in 1945 and the first cure was recorded in 1947. The Department for the Control of Leprosy was created in 1948. Active prophylaxis was initiated in 1952 with the compilation of a register of contacts. From 1954, cutaneous lymph was collected for diagnosis from persons living with patients. In 1962 a specialist was appointed to conduct a dermato-neurological examination of all contacts. A "new programme for the control of leprosy in Costa Rica" was created in 1974 which prohibited the internment of patients in the leprosy sanatorium and decreed that they should be treated in general hospitals. A rehabilitation programme was also initiated. By 1974 there were 518 registered cases, and their geographical distribution is recorded, but it is estimated that there are probably some 1300 cases. The spread of the disease is to be controlled by dermatological examination of all suspects and groups of persons at risk. Persons living with a patient must be examined dermato-neurologically once a year for 5 years; at present, 3282 contacts are under observation. Chemoprophylaxis is appropriate for juveniles living in close contact with a patient in the lepromatous or dimorphic stage. Leprosy patients should generally be treated in outpatient clinics and should be examined dermato-neurologically and bacteriologically twice a year in the case of those with lepromatous or dimorphic lesions and once a year in tuberculoid and indeterminate cases.

Ann Grant

32. LECHAT, M. F., MISSON, J. Y., VELLUT, C. M., MISSON, C. B. & BOUCKAERT, A. Un modèle épidémionétrique de la lèpre. **[An epidemiometric model of leprosy.]** *Bull. Wld Hlth Org.*, 1974, v. 51, No. 4, 361-373. English summary.

This valuable paper, which breaks new ground, should be consulted in the original by all concerned with the epidemiology and control of leprosy. Taking as their bases a mass of

observations collected over 16 years in the Belgian Leprosy Centre at Polambakkam, India, concerning 35,262 leprosy patients, the authors have elaborated a tentative epidemiometric model that should be tried out in other situations.

The model takes cognizance of 10 different states of leprosy infection, which correspond to the different stages through which any individual is presumed to pass, from "susceptible" and "latent" to "inactive" and "no longer taking treatment". Certain assumptions are made, some of which are open to question and need clarification, e.g. every individual is born susceptible to leprosy infection and may contract the disease if exposed to effective contact; any person with active leprosy may serve as a source of infection; tuberculoid leprosy may pass into the lepromatous form, through an intermediate stage; transition from active to inactive is a clinically based decision, although infectivity is assumed; the risk of relapse is identical, whether patients whose leprosy is quiescent take treatment or not; the latent form always develops into active leprosy, which does not regress in the absence of treatment.

A detailed analysis of the Polambakkam statistics allows the authors to suggest a series of mathematically expressed equations for several parameters, which have been used in a computerized simulation of leprosy prevalence and incidence rates in a variety of hypothetical and actual situations.

Preliminary results run closely parallel in certain respects to accepted epidemiological findings dating back some years, such as the observations that patients with lepromatous leprosy are 4 times as contagious within the household as those with tuberculoid leprosy, that infectivity diminishes rapidly with anti-leprosy treatment, that (because of their numbers) patients with tuberculoid leprosy play a major part in the persistence of the leprosy endemic. The model indicates that, whereas the latent period of tuberculoid leprosy is about 4 years, that of lepromatous leprosy is but 2.2 years. After 10 years of treatment, the chances of a patient with lepromatous leprosy becoming "cured" are practically nil.

Some tentative conclusions are of immediate applicability. A reduction in the rate of default would show a greater effect on the endemic than segregation of patients with lepromatous leprosy. A vaccine that would prevent the development of lepromatous leprosy would show the greatest beneficial results. Intensification of case-finding activities and the placing under effective treatment as soon as diagnosed of all persons suffering from leprosy, and especially those with lepromatous leprosy, are desirable goals, if not immediately achievable throughout the world.

S. G. Browne

33. RAO, P. S. S., KARAT, A. B. A., KALIAPERUMAL, V. G. & KARAT, S. **Transmission of leprosy within households.** *Int. J. Lepr.*, 1975, v. 43, No. 1, 45-54.

The wealth of statistical data available in the records accumulated in the Gudiyatham Taluk (Tamil Nadu, India) leprosy programme is exemplified by this analysis. During the years 1962 to 1970, in a population of about 400,000, over 97% of the 23,285 contacts in 5088 families having a leprosy patient were examined clinically. Against an annual incidence of leprosy of the order of 0.8 per 1000 patient-years in the general population, the incidence among household contacts was 6.8 per 1000 patient-years. The rates for males and females, when analysed for each of the major types of leprosy and expressed as total sex incidence, showed no appreciable differences. Where the index case in the household suffered from a multibacillary form of leprosy, the secondary attack rate was significantly higher.

The most vulnerable age-groups were found to be 5-14 years for boys, and 5-9 years for girls, the actual clinical manifestations appearing after a variably long latent period. When there was more than one index case in the family, the attack rate doubled. When the index case was of a bacilliferous type of leprosy, there was a higher proportion of such types among the secondary cases [a possibly fortuitous finding that does not imply strain differences].

S. G. Browne

34. RASI, E., CASTELLAZZI, Z., GARCIA, L., QUEVEDO, L. & CONVIT, J. **Evaluation of "chemical isolation" in 1168 leprosy patients' homes.** *Int. J. Lepr.*, 1975, v. 43, No. 2, 101-105.

"Chemical isolation" (treatment of open cases as a measure of control for transmission between contacts) is evaluated by a retrospective study of 7232 household contacts of 1168 leprosy patient homes. Contacts comparable in age and type of exposure were arranged in subgroups according to whether they were born before (Group A) or born after (Group B) beginning treatment of the index cases had begun. Additionally, the whole group of contacts, both of open (LL and BB) and close (TT and I) cases were evaluated.

Among comparable contacts of LL and BB cases, the infection rate in the contacts before initiation of treatment is higher than in that of contacts after initiation of treatment. The protection afforded by the treatment to the exposed group (Group B) is on the order of 66%.

The morbidity occurring in the group born after the initiation of index case treatment apparently results from partial persistence of infectiousness of the case under treatment.

6. SOCIOLOGY AND REHABILITATION

34. ROTBERG, A. **The ten enemies of prevention of hanseniasis are related to "Leprosy". A "Psycho-social-somatic phenomenon".** Reprinted from *Leprologia*, 1974, v. 19, No. 2, 281-285.

Leprosy is not a *disease like any other*, but a chain of phantasies, superstitions, stigma, sensationalism, ignorance, fear, rejection and infamous terminology—around a core of signs and symptoms of the somatic disease. One of the consequences is that we are still *case finding* instead of seeing patients and contacts *finding us*. The ten enemies of prevention are: (1) Latent segregationism; (2) hesitant integrationism; (3) the infamous pejorative *leprosy*; (4) sensationalism; (5) misguided charity; (6) psycho-social amateurism; (7) non-attentive education; (8) low salaries and lack of personnel; (9) obsolete legislation; (10) deficient teaching. . . .

Thanks are due to the Director, Bureau of Hygiene and Tropical Medicine for permission to reprint Abstracts from *Tropical Diseases Bulletin* May, June and July 1976.

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