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XIII INTERNATIONAL LEPROSY CONGRESS, THE HAGUE, NETHERLANDS 11–17 SEPTEMBER 1988

Editorial

The XIII International Leprosy Congress, organized under the joint sponsorship of the *International Leprosy Association* and the *World Health Organization*, was held in the Congress Centre, The Hague, from 11–17 September 1988. It was attended by over 1200 delegates from more than 100 different countries, including all those where leprosy is endemic and, as on previous occasions, the original papers, poster presentations, exhibits and keynote speeches covered the entire subject of leprosy in all its aspects. The 5 plenary sessions, with keynote or 'state of the art' lectures, covered *Immunological tools for leprosy control* (P E M Fine, UK); *Recent developments in molecular biology* (B R Bloom, USA); *Operational aspects of multidrug therapy* (M Becx-Bleumink, Netherlands); *Nerve damage* (C K Job, USA); *Training and social aspects* (L B Valencia, Philippines). These early morning lectures were extremely well attended and generally much appreciated. Paul Fine reviewed the immunological tools which are under development and Barry Bloom the current work and advances in molecular biology, both in masterly fashion, but both drew attention, in their concluding remarks, to the gulf between research potential on the one hand, and actual *availability* for operational use on the other. Marijke Becx-Bleumink's review of operational aspects was warmly received by a packed house; her observations, largely based on experience in the Shoa Province of Ethiopia, were at the same time critical, stimulating and encouraging. She drew attention to the established effectiveness of multiple drug therapy as advised by the World Health Organization, and to the progress which has so far been made in many countries in implementation, whilst at the same time discussing the *lack* of progress, amounting in some cases almost to 'stagnation', in others. Indeed, the whole subject of the impediments at operational level to the faster and wider implementation of multiple drug therapy, soon became one of the outstanding topics for discussion at this Congress. Professor Job described the histopathological changes occurring in the nerves of patients with different forms of leprosy with a professionalism which can only come from life-time study, and emphasized our continuing ignorance about the basic mechanisms involved and the sad effect this has on our ability to predict and prevent nerve damage at clinical level. Lastly, Professor Valencia from the Philippines drew attention to the poor performance of social science to date, in the collection of reasonably objective data using proved methods in sociology, anthropology and psychology, to produce findings of *practical* importance in leprosy control.

From these absorbing and at times exciting sessions, the delegates (after pausing for a cup of coffee and the usual quick discussion or exchange of views on matters which sometimes turn out to have far-reaching importance at such international meetings), moved to the various floors and lecture rooms of the vast Congress Centre to oral or poster presentations on: clinical aspects; immunology; treatment; microbiology; surgery and rehabilitation; ophthalmology; nerve damage; experimental leprosy; epidemiology; experimental therapy; pathology; training and social aspects.

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It was clear from this Congress that mycobacterial diseases in general and leprosy in particular, are far from being left behind with regard to the application of recent advances in immunology, microbiology and molecular biology. Unfortunately, conflicting simultaneous sessions made it impossible for those interested in these aspects to hear much of what was presented. While such conflicts are inevitable at large international meetings of this type, perhaps more thought should be given in future to the scheduling of these sessions.

An area which has clearly been the focus of much attention over the last few years has been the use of our increasing knowledge of the detailed immunochemistry of mycobacteria to develop seroimmunological tools for diagnosis and epidemiology. Methods based on the detection of antibodies or antigens in body fluids or tissues were presented by many groups. While, as Paul Fine pointed out in his keynote address, the value of these techniques at the operational level is questionable, we have learned much about the immune response to *Mycobacterium leprae* from these studies, and new approaches presented at the Congress hold out much promise.

Leprosy is at the forefront of research in cellular immunology and this was reflected in the high quality of papers presented at the Congress. Convincing evidence for the presence of T suppressor cells (a subject of much heated debate among cellular immunologists) was presented, and new techniques for defining the antigens recognized by T cells (pioneered by leprosy research) are being used. In addition, much attention has centred on investigating T cell responses to those antigens which have been expressed by recombinant DNA technology, though there is little evidence, as yet, for their role in protective immunity. New evidence that *M. leprae* cell walls contain major immunogenic proteins provides a new lead to our understanding of the way in which *M. leprae* is recognized by the immune system.

The application of molecular biology to our understanding of leprosy and its causative organism has got underway during the last 5 years, with the cloning and expression of a number of *M. leprae* genes in *E. coli*, detailed studies of the nucleic acids of *M. leprae* and the development of mycobacterial genetics. This work was well represented during the Congress and holds out much promise for unravelling the complex interaction between host and pathogen. Preliminary evidence which was presented suggests that it might be possible to detect strain differences between *M. leprae* isolates, based on readily detectable differences between nucleic acid sequences, which could have important application in epidemiological studies. In addition, methods for studying the expression of mycobacterial genes and for transferring genes between mycobacteria could lead to important developments in the diagnosis of mycobacteriosis; further cloning of immunologically important molecules and enzyme targets for drugs; understanding the basic mechanisms involved in pathogenicity and for vaccine development.

Impressive progress in the area of experimental chemotherapy was presented. Although this field has taken something of a back seat in recent years, reports of new drugs, some belonging to totally new classes of compounds, and new methods for assessing drug activity were reported, which gave an air of unexpected excitement to these sessions.

With regard to the remaining subjects, including clinical and social aspects and the other less 'scientific' disciplines, the very number and diversity of them defies summary. But, in both oral and poster media, there were excellent contributions from all over the world, accompanied by lively discussion. It is, however, difficult to escape the comment that *presentation* could be improved; many of the transparencies projected during oral sessions were faded, illegible or crowded with indigestible detail. Several poster presentations had obviously been prepared at the last minute using rough sheets of paper and a felt pen, and the lighting in the poster area was generally substandard. It should not be beyond us to set minimum standards of presentation and to demand that they are respected.

Elsewhere in this Journal we publish accounts of the *Pre-Congress Workshops* which give some idea of current thinking on many aspects of leprosy. The *Abstracts*, thanks to a most generous and thoughtful initiative by the *Associazione Italiana 'Amici di R. Follereau'* were presented to each delegate on arrival. There were also teaching and training sessions on leprosy immunology;

epidemiology and control; the design and implementation of a leprosy information system; the pathophysiology of nerve damage; the assessment of management of neuropathic limbs; chemotherapy in leprosy; histopathological diagnosis of leprosy; how to diagnose and classify patients; leprosy control—educating community and patients; the eye in leprosy; recognition and management of reactive phenomena in leprosy. The organizers also provided excellent facilities for a number of stalls and exhibits, some of them for commercial companies, others for independent agencies dealing with teaching and learning materials in leprosy (TALMILEP), aids for the disabled (WLEREC and Modulan), and coordination (ILEP, International Federation of Anti-Leprosy Associations).

Can the general impact of such a momentous and wide-ranging Congress be summarized? Probably not. But one thing is clear—progress *is* being made. Multiple drug therapy, notably in the form recommended by the World Health Organization in 1982, is effective and available. The pace of implementation is improving, although there are grounds for believing that it should be faster and wider (many aspects of this vitally important subject were discussed at the *WHO Third Coordinating Meeting on Implementation of Multidrug Therapy in Leprosy Control* during the Congress and the conclusions will be circulated at a later date). Immunological tools and vaccines are not yet available and 'established', but remarkable progress is being made and in the next few years it is possible that they will be in use at operational level. There is an awareness that our knowledge of the mechanisms involved in nerve damage, notably in relation to reversal (upgrading) reactions is defective and that it calls, almost as a matter of urgency, for further study.

At the Opening Ceremony, a beautifully made film from WHO carried the title 'Multidrug Therapy for Leprosy: an End in Sight'. There is much to be done and we should not underestimate the magnitude of the task ahead, but the main message from this historic Congress in The Hague is that we can now control and perhaps eventually eradicate this disease.

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Pre-congress workshops

From 7–10 September 1988, workshops were held on the following subjects: Immunology; Chemotherapy; Epidemiology; Leprosy control, evaluation and integration; Information systems; Diagnosis and clinical aspects; The role of the ILA in training; Prevention and management of impairment; Vaccine trials; Social aspects; Health and education; Recommendations on rehabilitation; and Microbiology. As on the occasion of previous congresses, the reports were prepared by the chairmen and rapporteurs and distributed to all delegates.

Immunology

In the last 5 years it is clear that advances in basic immunology are rapidly expanding our understanding of the immunology of leprosy. The workshop arbitrarily divided the field into five general areas which we have attempted to summarize:

IMMUNOGENETICS

Additional evidence has accumulated that HLA-genes control the type of leprosy that develops in infected, susceptible individuals. Different HLA-alleles are associated with the respective leprosy types but susceptibility to leprosy *per se* is not under HLA control. No association has been found between HLA and reversal reactions and no studies have been done with respect to HLA and ENL. The mechanisms of the HLA influence in leprosy may be via differential binding of processed antigenic peptides by the polymorphic domains of HLA molecules. As a possible example, helper-T cell clones recognize different epitopes on the ML 65 KD protein that are segregated according to the class II restrictor element used (DR1, 2, 3 and 5 but not DR 4, 6, 7 or 8). It is not clear yet how antigen epitope specificity is related to protective or pathological responses. Future studies should include a search for a human counterpart to the murine Bcg R/S phenotype/gene and understanding the mechanism of the association between epitope specificity and MHC restriction element specificity and their relationship with protection, immunopathology and vaccine efficacy.

MYCOBACTERIUM LEPRAE (ML) ANTIGENS AND MOLECULAR BIOLOGY

In recent years, 7 protein (10, 65, 36, 35, 28, 18 and 12 kD) and 2 glycolipid (PGL-1, LAM-B) antigens have been identified from ML. Much detail is now available. In general a large number of ML-specific and cross-reactive epitopes have been identified, many down to the molecular level. One of the most studied, for example, is the 65 kD protein. It has now been shown to contain 1 specific and 10 cross-reactive antibody epitopes and at least 1 specific and 10 cross-reactive T cell epitopes. Virtually all of these proteins are being expressed from recombinant DNA libraries. The gene (and amino acid) sequences are now complete for the 65 kD protein as well as the 18 kD protein and major portions of the 10 kD protein. In addition to the ML antigenic epitopes which have been identified by these techniques, there is evidence that many more T cell epitopes exist on other ML proteins. Goals in the study of molecular biology of ML continue to be production and

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identification of ML antigens for: 1, the development of immunodiagnostic tests (serology and skin tests); 2, dissection of the immunoresponse to ML (*e.g.* identify antigens important in protective cell-mediated immunity and hypersensitivity, pathologic immune responses, reactions or autoimmunity); 3, understanding the structure and function of ML which may shed light on how the organism resists killing by the immune system by some individuals; and 4, development of a subunit antileprosy vaccine.

MACROPHAGES (M)

Evidence is lacking that failure in macrophage (M) function is the basis for host susceptibility to leprosy. Examples in which activated M successfully cope with ML were discussed and contrasted with experimental models where ML-infected M became defective in afferent and efferent function. The anatomical source of M being studied was emphasized. Caution was expressed about solely studying readily obtainable (blood, peritoneal cavity) M. Interest should be focused more on M from the leprosy lesions themselves. Collectively *in vivo* and *in vitro* mouse studies and clinical trials of local immunotherapy in LL patients suggest that killing and clearance of ML from lepromatous lesions likely depends on the influx of new M into the lesions rather than activation of resident ML-burdened M. Future studies should address: 1, the importance of antibody in the phagocytosis of ML by M; 2, clarification of early events in phagocytosis (phagosome acidification, fusion with lysosomes); 3, whether ML *do* escape from the phagosome into the cytoplasm; 4, kinetics of M traffic into the lepromatous lesion; and 5, importance of infected M as target cells for cytotoxic T cell lysis or destruction by new M. Finally the mechanisms of ML entry into nonphagocytic cells should be studied and the importance of these infected host cells in pathogenesis explored.

CELL MEDIATED IMMUNITY (CMI)

Lymphocytes can be divided functionally into helper, cytolytic, and suppressor subclasses and by phenotype and genetic (MHC) restriction into CD4+, Class II restricted and CD8+, Class I restricted subgroups. Generally CD4+ T cells are helpers and CD8+ are cytotoxic. Exogenous antigens preferentially induce CD4+ T cells while newly synthesized or endogenous antigens induce CD8+ T cells. Killed ML should induce CD4+ helper T cells. Intracellular bacteria, including ML, can activate CD8+ T cells to lyse antigen primed M. CD4+ T cells as well as CD8+ T cells may express cytolytic activity that could result in the release of ML from host cells of low microbicidal potential (ineffective M, Schwann cells, somatic cells) and thus could function in protection. Reversal reaction-type phenomena occur locally after PPD, interferon gamma or IL-2 are infected into the skin lesions of LL patients. ML-specific suppressor T cell clones have been described. In TT skin lesions, CD8+ cells appear to be cytolytic and in LL lesions, suppressive. The role of distinct suppressor T cells in the pathogenesis of unresponsiveness in LL is not clear. Different T cells both produce and respond to different interleukins. The types, quantities and interactions of these different interleukins may play a role in the development of an individual's type of leprosy. Future studies should continue to explore: 1, the mechanisms of ineffective CMI in LL; 2, which immunomechanisms contribute to protection and which to disease; 3, the traffic of mononuclear cells into leprosy lesions; and 4, the characteristics of the cellular infiltrate in leprosy lesions including (a) functional studies on cells isolated from these lesions, (b) studies using CD4+ cell markers of maturity and antigen exposure (CD45R and CD45), and (c) studies using CD8+ cell markers for cytolytic capability (CD28].

SEROLOGY

Over the past 5 years, four types of antigens have been evaluated in leprosy serology: 1, PGL-1; 2, ML-specific epitope monoclonal antibody inhibition assays; 3, antibody assays to synthetic

peptides of specific and cross-reactive epitopes on ML proteins; and 4, the cross-reactive LAM-B of ML. Assays utilizing PGL-1 and its synthetic analogues have had the most widespread application. With these assays virtually 100% of LL patients but only approximately 30% of paucibacillary (PB) patients are positive. Antibody levels are positively correlated with BI in untreated multibacillary (MB) patients and fall (together with BI) in treated MB patients. Anti-LAM-B and monoclonal antibody inhibition assays fall more sharply. Antibody assays are not helpful in predicting reactions. Several prospective studies of contacts of MB patients have identified an increased relative risk of developing clinical leprosy in seropositive individuals. Future studies should include: 1, further exploration of synthetic peptides; 2, refinement of techniques for monitoring patients on chemotherapy; and 3, further evaluation of antigen detection systems in clinical specimens using both immunological and DNA probe techniques such as the polymerase chain reaction.

THE FUTURE

In addition to a number of specific areas requiring attention which have been mentioned, there are several broad recommendations for the next 5 years. At present there does not seem to be enough basic knowledge to suggest new field trials of new potential vaccines. Effort should continue to integrate leprosy into general scientific research to increase the number of researchers and the variety of skills working on a leprosy vaccine. In the shorter term the immunopathology of possible autoimmunity in leprosy, and the pathogenesis and possible immunomodulation of ENL and reversal reactions, particularly neural reactions, deserve high priorities.

R HASTINGS, *Chairman*

Chemotherapy

The 5 years since the Delhi Congress have seen a number of important advances in the chemotherapy of leprosy. Studies have continued to demonstrate a high frequency of relapse with secondary dapsone resistance during dapsone monotherapy, and a high prevalence of primary dapsone resistance, further emphasizing the need for multidrug therapy (MDT). On the other hand, primary resistance to rifampicin has not yet been recognized, even in those areas in which secondary resistance to rifampicin has occurred as a consequence of rifampicin monotherapy. Most importantly, implementation of MDT has expanded, so that, by this time, more than 2 million patients have completed MDT. MDT has been implemented in most endemic countries, although, in the majority of countries, only a proportion of patients has been covered. MDT has been widely accepted, both by patients and by leprosy control personnel; the three components—rifampicin, dapsone and clofazimine—have been extremely well tolerated, and patient compliance has been at least as good as in the days of dapsone monotherapy. The main difficulties have been in the reliable delivery of the drugs to the patients by the leprosy control infrastructure; however, MDT has proved to be operationally feasible where the infrastructure is adequate. Relapses have been remarkably few in the short term—fewer than 1% per year, despite the persistence of viable *Mycobacterium leprae* in a significant proportion of patients after MDT for 1 or more years, and caseloads have been substantially reduced in many areas.

Drugs of 2 additional classes exhibited bactericidal activity against *M. leprae*. Two fluoroquinolones—pefloxacin and ofloxacin—are fully active against *M. leprae* in mice, pefloxacin is rapidly bactericidal in patients with previously untreated lepromatous leprosy, and ofloxacin is currently in clinical trial. Minocycline, a lipid soluble tetracycline, is also fully active against *M. leprae* in mice, and is soon to be tested in men.

At this time, a number of important problems await resolution. A few patient paucibacillary leprosy appear to be more appropriately treated by the regimen for multibacillary leprosy, but we

are as yet unable to recognize these patients before treatment. The slow resolution of some paucibacillary lesions makes patients and staff unwilling to stop therapy. And it has been difficult to distinguish some late reversal reactions from relapse. The most effective way of using the new fluoroquinolones and minocycline to strengthen MDT had not yet been established, and the potential role of immunotherapy remains unclear. The potential role in leprosy control of rifampicin as chemoprophylaxis has not yet been assessed, and measurement of the impact of MDT upon transmission of *M. leprae* in the community is made much more difficult by the lack of a reliable test for latent infection. There is a continuing need for new bactericidal drugs that are well tolerated and not prohibitively expensive, especially if they are suitable for intermittent, supervisable administration. Sensitive and reliable *in vitro* methods of detecting viable *M. leprae* and measuring their susceptibility to drugs are needed to aid the assessment of treatment—outcome, and to facilitate the screening of new compounds. It is hoped that application of the techniques of molecular biology will assist in the achievement of these goals, and suggest new, potentially useful approaches to the cultivation of *M. leprae*. During the next 5 years, MDT should be implemented in all countries for all patients. Simultaneously the effectiveness of MDT over the long term must be assessed as precisely as possible. Special efforts should be undertaken to facilitate the distinction between reversal reaction and relapse, and relapse of multibacillary leprosy must be documented whenever possible by inoculation of mice and drug-susceptibility testing. Trials ofloxacin and minocycline should be mounted to measure the potential of these drugs for strengthening MDT. Work should be continued in the areas of the biology of *M. leprae*, to understand better the action of the presently available drugs, and to discover new targets of drug action. The search for additional new drugs should continue. The development of immunomodulating agents, including vaccines, should be pursued, both to strengthen chemotherapy and for immunoprophylaxis.

Efforts to cultivate *M. leprae* to assess the potential role for chemoprophylaxis and to develop means of detecting subclinical infection with *M. leprae* should be encouraged. And operational studies should be undertaken, particularly to find the most effective methods of delivering MDT in a variety of geographical and socioeconomic environments.

L LEVY, *Chairman*

Epidemiology

MAGNITUDE OF THE PROBLEM

The estimate of the global magnitude of leprosy has changed little for more than 20 years. Moreover such estimates are often based on unsystematic criteria and reports. Therefore in order to make a realistic projection of the global problem, the workshop recommends:

1 Acceptance of the *definition* of a *case* of *leprosy* as recommended by the WHO Expert Committee, *i.e.* 'A case of leprosy is a person showing clinical signs with or without bacteriological confirmation and requiring chemotherapy.'
2 That prevalence rates should be based on *registered* cases as per the above definition.

As per present data, the total number of registered cases world-wide is approximately 5 million. Eighty-two percent of the registered cases are from 6 countries. Since 1985, the number of patients who completed treatment appears to be higher than the newly detected cases. It must however be noted that even in countries of low or medium prevalence, there can be pockets of high prevalence.

TRENDS OF LEPROSY

Some countries have reported well-documented declining trends of leprosy; some areas and some countries still show increasing trends. Thus in general there is a great need to carefully evaluate the work done during the last decades. This calls for the collection of reliable data on the patients and on the populations to which they belong. These data should be analysed separately by age, sex and type of disease. Trends in relation to factors such as BCG coverage must also be analysed.

While criteria for classification can differ to some extent country-wise, there is need for *standard criteria* of classification, taking into account clinical, bacteriological and immunological status of the patient.

URBAN LEPROSY

Trends in urban leprosy have not been sufficiently analysed so far. This is partly due to the operational difficulties in the control programmes and the fact that most of the urban programmes are of recent origin. It is recommended to have studies to evaluate the pattern and trend of leprosy in urban areas, in order to investigate whether epidemiological factors and trends differ in urban areas compared to rural areas and to know whether there are unique epidemiological features in urban areas.

SOCIOECONOMIC DETERMINANTS

Though the relationship between leprosy and poor socioeconomic conditions is widely recognized, the factors that contribute to the transmission of infection and/or the development of the disease under such conditions are not established. Ideally there is a need to collectively study factors such as overcrowding, migration, hygiene, nutritional state, intercurrent infection, ethnic variations, BCG coverage, housing etc., and to separate them in the analysis.

SEROEPIDEMIOLOGICAL TOOLS FOR LEPROSY

Almost all data and reports on leprosy are based on clinical disease. There is very little indication of subclinical infection. None of the available tests to detect subclinical infection is sufficiently specific or reliable for use under field conditions. Therefore there is need for development of a reliable test appropriate for field use. Skin tests used during immunoprophylactic trials have demonstrated the usefulness of detecting individual susceptability and to study the immunological conversion induced by potential vaccines. Such studies need to be encouraged.

RECENT PROGRESS IN EPIDEMIOLOGICAL WORK AND RESEARCH

1 Epidemiological applications of serological tests in the context of prevalence and incidence studies.
2 Confirmation of HLA-linked determinants for lepromatous as well as tuberculoid leprosy.
3 Discovery of natural *M. leprae* (or *M. leprae*-like) infections in armadillos and monkeys.
4 Institution of large-scale vaccine trials providing population laboratories for leprosy research.
5 Application of case control study methodology to identify risk factors, *e.g.* armadillo contact and absence of BCG vaccination.
6 Shift to MDT regimens and initial studies aimed at measuring reaction and relapse rates after different regimens.

PRIORITY ISSUES FOR THE FUTURE

1 Clarification and application of rigorous case definition.

2 Studies of the implications of HIV infections for leprosy risk and type.
3 Application of new genetic tools (*e.g.* restriction fragment length polymorphisms) in family segregation analysis studies.
4 Application of new serological tools to study sources, modes of *M. leprae* transmission and risk factors.
5 Cohort and case control studies of resistance, and relapse rates with new drug regimens.
6 Descriptive and analytical studies of the epidemiology of reactions and disabilities.
7 Longitudinal and cohort analyses to describe trends in leprosy incidence, and to identify the determinants of any changes.

M ZUNIGA, *Chairman*

Leprosy control, evaluation and integration

The strategy for leprosy control continues to be based on secondary prevention, that is early detection and chemotherapy for all cases of leprosy. Today the most effective chemotherapy is multidrug therapy (MDT) and already 40% of the Worlds 5 million registered cases have benefited. There is urgent need to speed up MDT implementation, to make it accessible to at least 80% of the estimated cases, in a phased, time bound, target-oriented programme as part of national health plans.

To achieve this end, in many circumstances, the advantages of integration within a well functioning health service should be recognized. Primary health care can provide a comprehensive, continuous and adequate leprosy service, with specialized training, technical support, referral services, treatment delivery, supervision and evaluation.

Many issues pertinent to leprosy control have been discussed in other workshops, and hence are not included in this report (Epidemiology, Health Education, etc.) The recommendations of the 6th WHO expert committee were generally endorsed, taking special note of the definition of the case of leprosy and of the prevalence rate in operational terms. There was a full discussion of a number of important operational points:

1 It is recommended that MDT be implemented as a country-wide, community-based service. Reports received suggest that, with proper implementation, MDT cannot only reduce the caseload, but also constitute an important factor in the decline of leprosy.
2 The effectiveness of MDT suggest that no deviation from the present recommended PB and MB regimes (adapted as necessary for operational reasons) are needed. Reports indicate that the acceptance and compliance are good, therapeutic problems during and after therapy, including reactive episodes, relapse and persistence of active lesions do not hamper the programme. On the other hand, injunctions use of MDT, without proper supervision or not in the correct combinations, must be avoided, because of the risk of drug resistance. The use of thiomides is not recommended under field conditions.
3 The suggestion by the 6th WHO expert committee is that all smear positive patients be treated as MB was endorsed. The need for greatly improved quality for skin smears was recognized. It was recommended in MB cases smears should be taken at least once at the start of MDT and again on completion of treatment. Although in some circumstances it may be necessary to start MDT based on clinical judgement alone, efforts must be to carry out bacteriological examination as soon as possible.
4 The operational problems of continuing surveillance after stopping chemotherapy, and continuing treatment in MB cases after 2 years if they are still positive were recognized. At the moment, however, there is insufficient evidence to deviate from the original WHO guidelines.
5 Skills in the prevention of management of disability should be strengthened. The training of appropriate staff in the recognition and treatment of reactive episodes, involving nerves (and eyes)

with steroids in the field is essential. Adequate referral facilities for each case, as well as the correction and care of established deformity needs the urgent attention it deserves.

6 The cost-effectiveness of different types of surveys for case detection need further evaluation. It is suggested that contact examinations be done systematically at least once at registration of a new case. The importance of health education in case detection was emphasized.

7 Greater attention has to be paid to monitoring and evaluation. There is an urgent need to apply the established indicators and to develop new ones to help programme managers in decision making. Such monitoring and evaluation is not only needed at every level of programme implementation but should include all the components of leprosy control. Management information systems should be designed with this in view in order to improve the effectiveness of the programme implementation.

8 The rapidly increasing urban population, slums, shanty towns and their related leprosy problems, threatens all the achievements of the leprosy control today. Though in both urban and rural areas the methodology is similar, urban leprosy control has to be recognized as a specialized area, varied and complex calling for an imaginative and an unconventional approach, within the framework of urban health care system. All programme managers should recognize that involvement of all medical personnel, the community, the use of health education, selective surveys and emphasis on rehabilitation are needed.

9 Multidisciplinary action oriented health systems research will go a long way to clarifying operational problems. Research at selected regional centres is needed to evaluate the impact of MDT. Operational research in key result areas needs to be carried out to improve efficiency of the programme.

10 Tests for the detection of the subclinical infection, when applicable in the field will greatly assist in the control of leprosy. The results of vaccine trials in progress are not yet available. Till the availability of the field-tested vaccine, the strategy for leprosy control will have to rely on secondary prevention.

11 The rapidly increasing prevalence of human immunodeficiency virus infection and increasing concern of governments should not result in lowering of the priority given to leprosy control. Relationship between HIV infection and leprosy needs investigation. Suitable precautions should be taken by leprosy control staff dealing with patients possibly infected with HIV, Hepatitis B and other such infections.

12 The assistance already given by nongovernmental organizations and contributing agencies was fully recognized. In view of the need for the governments of endemic countries to increase MDT coverage, even greater efforts are called for to mobilize resources. Governments should strengthen their cooperation with the NGOs and coordinate their activities, within the national health plans, so that no leprosy patient is denied MDT for want of resources. In conclusion there is renewed optimism that with increased MDT coverage, integration, mobilization of resources, human resources development, training, increase participation by the NGOs, enhanced monitoring, evaluation and health systems research leprosy control will become a reality.

H SANSARRICQ, *Chairman*

Information systems

The terms of reference were:

1 To deliberate on major issues confronting information systems, including the lack of reliable indicators and possible solutions.

2 To identify serious gaps in present knowledge that affect information systems so as to suggest priorities for research.

1 The meeting, having reviewed various information systems as reported by participants and considering difficulties experienced in implementation, reaffirmed that OMSLEP forms the base for developing systems in all countries with appropriate modifications to suit the requirements of the country. It recommended that: 1.1, Clarification should be made on WHO regimen and on definitions used, specifically with regard to case, adequacy of MDT, and application of reporting system in urban areas. 1.2, The system should give due priority to the needs of field workers; only appropriate data should be collected for analysis, interpretation and programme assessment at the district/state and national level. 1.3, Any system should provide adequate and timely feedback to field workers.

2 The meeting recognizing that the indicators suggested in OMSLEP do not cover the needs of promotional activities and that proxi indicators give only an indirect assessment, recommended that: 1, Promotional programme should specify the exact aims and objectives of various activities, so as to build in evaluation. 2, Research be conducted into developing appropriate indicators for such purposes. The meeting appreciated that appropriate indicators are needed to meet not only scientific concern but also to satisfy political needs.

3 The meeting recognized that there was insufficient information not specific to leprosy, which is important in assessing programme effectiveness, e.g. coverage of the population by the programme or health services, equal accessibility to health care services by all individuals in a country and therefore, recommended: That such indicators for health service accessibility and equity be developed through health systems research.

4 The meeting highlighted various other problems that affect information systems and recommended that: a, a centralized register be maintained in each country to prevent duplication of recording of case; b, each country ensure that patients under the care of private doctors are also reported to the national system; c, NGO's (Non Governmental Organization) maintain information systems in line with national systems; d, the workshop on chemotherapy considers developing clearer definitions for the terms regularity, irregularity and defaulters which do not require such complex calculations; e, research be carried out to ascertain the cost in terms of time and human resources for information systems so as to provide the most appropriate approach for maintaining and developing this system; and f, any information system should report on all activities within a unified system.

5 The meeting considered the suitability of computerization for information systems; but it recognized that computers may not be the appropriate solution in all circumstances, and reaffirmed the need for each country to consider all the pros and cons of such computerization as detailed in the document.

6 Training prior to the implementation of any information system is a fundamental requirement.

LIM KUAN JOO, *Chairman*

Diagnosis and clinical aspects

DIAGNOSIS

In a great number of highly endemic countries, case-finding is undertaken by health workers, who in integrated programmes and in primary health care should be able to make the diagnosis, treatment and follow-up not only of leprosy patients but also of cases of other diseases. Consequently, diagnostic methods should be simple and easily applied in the field. When necessary and wherever possible more elaborative methods should be employed.

Leprous neuropathy (new approaches)

The diagnosis of early neural involvement (especially as for indeterminate or tuberculoid cases) may be achieved in skin biopsies by identifying the antigen in the nerve, with the use of monoclonal antibodies and the presence of the Schwann cell using S-100 protein (immunoperoxidase techniques).

Neurophysiological examinations (electromyogram, nerve conduction velocity, Hoffmann reflex, F wave, and cerebral evoked potentials) may be useful for early leprosy diagnosis.

However, these examinations can only be performed by trained specialists and cannot be undertaken in the field.

Subclinical infection diagnosis

FLA abs, ELISA test, and SACT (serum antibody competition test) may detect subclinical infection. Household contacts were found to have positive reactions and a proportion of them became seronegative.

Further investigations in areas of different endemicity are required to throw light on this subject. Dot-ELISA methods (micromethod) easily applied in villages, and inexpensive, should be preferentially employed.

Household contacts with positive reactions should be tested with lepromin, and the nonreactors (more prone to acquire leprosy and develop the L type) kept under strict surveillance. Although it is not mandatory, for many lepromin negative contacts to develop clinical manifestations, an attempt could be made to prevent their appearance by appropriate intervention.

Probe for M. leprae *identification—recombinant DNA technology*

Cloning of mycobacterial proteins by recombinant DNA technology might offer interesting possibilities for identifying *M. leprae* in suspected specimens (viable and non-viable ones) in addition to the study of the mechanisms of drug-resistance, screening drugs, etc.

CLINICAL ASPECTS

Early manifestations of leprosy

The first signs are cutaneous and occasionally neurological ones. The earliest skin lesion (indeterminate form) appears as one or as a few hypopigmented and sometimes erythematous macules. They are flat, without infiltration, with rather ill-defined margins, and some sensory loss. Most often they are distributed on the extremities. Sometimes there may be a hair loss on the lesions. These are often transient and self-healing. Leprosy bacilli are not found or are extremely scanty, by the routine methods.

The lepromin response is positive in a very high proportion of indeterminate cases. Most of the cases evolve towards the tuberculoid type and only in a small proportion towards the lepromatous type.

Occasionally, neurological symptoms and signs precede the onset of skin lesions. Tuberculoid lesions are very often an early manifestation.

Reactional episodes

Reactional episodes represent acute or subacute phenomena, with local and/or general involvement and occurring in the chronic course of leprosy. Following studies on cellular and humoral immunity, the reactional states have been divided into 2 groups:

Reaction type 1: associated to cell-mediated immunity, may result in improvement (up-grading or reversal reaction) or worsening (down-grading) of the disease.

Reaction type 2: is an immunocomplex syndrome characteristic of the lepromatous type. Cutaneous manifestations may consist of: 'Erythema Nodosum' (EN), and less frequently 'Erythema Multiforme' and 'Erythema Necrosans', often accompanied by constitutional symptoms and systemic involvement.

The correct diagnosis of reactional episodes is important for the appropriate treatment. Differentiation of reversal reaction and relapse is also important in cases who have completed MDT.

The study of the reactional states is recommended with regard to: lysosomal activity, immune-complexes, autoantibodies, use of immunoperoxidase techniques, cell-mediated immunity and ultra structure of the nerve particularly to identify the sites of involvement.

The silent nerve paralysis

While in reactional states painful neuritis is easily recognized in a large number of cases, worsening of the disease process in the nerve is not heralded by pain or paresthesia. Sensory and motor deficit occur insidiously. This may occur in the early stages of the disease or later. Diagnosis of this condition in the early stages, by routine sensory and motor assessments, is necessary to institute treatment and avoid irreversible deformities.

Lucio's leprosy—Lucio's Phenomenon

Lucio's leprosy is a variety of lepromatous type, characterized by a diffuse and generalized skin infiltration which never presents nodules and with a special kind of lepra reaction: 'Erythema Necrosans' (Lucio's Phenomenon). It is mainly seen in Mexico. A few cases have been reported from some other countries.

J C GATTI, *Chairman*

The role of the ILA in training

INTRODUCTION

There are two principal roles for professional associations in training. The first is to provide opportunities for continuing education for members and other professionals and the second, to recruit and train additional professionals. It is generally agreed that there is a great unmet need for training in leprosy, particularly amongst people responsible for the clinical care of leprosy patients in countries where the disease is endemic, especially where integration has been adopted as a national policy. With these presuppositions in mind the members of the workshop considered the topic in 5 aspects: 1, The *International Journal of Leprosy* and other journals; 2, Associations of leprosy professionals; 3, Teaching and learning materials; 4, Undergraduate medical education; and 5, Possibilities for a resource information network.

THE *INTERNATIONAL JOURNAL OF LEPROSY* AND OTHER JOURNALS

Initially the very existence of journals devoted to leprosy must be justified. As long as there is a need for a specialized body of knowledge which makes up leprology there will need to be an International Leprosy Association and its Journal. This need will exist as long as leprosy remains a health problem, *i.e.* for as long as the disease is not 'adequately' controlled.

Given that leprosy journals are still needed, to whom should these journals be directed? Certainly the relatively small group of full-time leprosy researchers and leprosy physicians (2000?) should be served. As well as the significant numbers of physicians and surgeons, who must care for leprosy patients in specialist or general medical practice. Many paraprofessionals working in leprosy should find at least some parts of leprosy journals of interest. As a practical matter it is most unlikely that leprosy journals can be of value to much larger but nonprofessional groups such as community health workers. A leprosy journal should provide leprosy professionals with a focus for their discipline, a means of exchanging information, and a constant motivation to perform better. A focused, well-informed, and well-motivated professional leprosy worker—in a laboratory, at the bedside or teaching—is our greatest asset in caring for today's leprosy patients and in preventing tomorrow's. For the membership of the association the journal should foster a sense of pride in belonging to a group with a high professional standard as well as being a convenient, reliable and readable source of accurate, timely and stimulating information through original articles, editorials, review articles and current literature summaries. For writers including those who fill the correspondence columns and especially for the younger professionals the Journal provides not only motivation and an opportunity to share their work with others, but with exposure to the disciplines that publication in a reputable Journal entails and with exposure to free, critical and kindly advice from experienced reviewers.

ASSOCIATIONS OF LEPROSY PROFESSIONALS

Leprosy associations are defined as being those composed primarily of professional workers with a serious interest in this disease. Such professionals need not be leprosy workers exclusively, but will include any recognized medically oriented discipline. In providing training opportunities to those actively or potentially engaged in leprosy work control activities, it is important that the private sector is not overlooked, as in many parts of the world private practitioners are becoming more involved in such activities. It is essential for the ILA to identify local or national associations (or appropriate institutions) with actual or potential ability and willingness to provide training for those with a need, and to design mechanisms by which whatever support required is provided to these associations so as to enable them to conduct effective training programmes.

Although not defined at present, there needs to be a focus on specific targeted actions which will enhance these efforts, but it is deemed important to at least first identify ILA members who can and will collaborate in training activities; determining the mechanisms of their support, including financial support, is of prime importance.

Finally, the ILA and its individual members should strongly consider making serious efforts to convince governments of various nations that leprosy continues to be a serious global problem, and that support of leprosy control activities remains extremely important.

TEACHING AND LEARNING MATERIALS

It is recognized that there is a very great unmet need for teaching and learning materials especially for general medical workers at all levels involved in integrated leprosy programmes. However apart from once again drawing attention to the need it was agreed that no specific role for the ILA could be identified in this area. The possibilities for the provision of material specifically designed for self-assessment and self-instruction were also captured—again it was emphasized that the greatest needs are to be found amongst those working in general health services who have limited numbers of patients to care for. However it was considered questionable whether self-instructional material would be of greater utility to this group than basic handbooks or manuals and the matter was referred for further study.

UNDERGRADUATE MEDICAL EDUCATION

Leprosy is either not included at all or is allocated insufficient time in the curriculum of many medical schools. The ILA should emphasize the importance and value of teaching leprosy patient management and disease control in medical schools especially in leprosy endemic countries. The following approaches were discussed: Interdisciplinary teaching in conjunction with dermatology, infectious diseases, neurology, epidemiology, ophthalmology and rehabilitation medicine. The involvement of disciplines outside the clinical medical field including sociology and psychology in order to increase the understanding of and eventually reduce the stigma associated with the disease. The preparation and distribution of leprosy training material. The identification and encouragement of individual teaching staff who are interested in leprosy.

RESOURCE INFORMATION NETWORKS

There is a need for a resource information network to identify leprosy information materials and leprosy specialists worldwide, particularly in the field of training. Such a resource would be especially helpful to persons working in remote areas without access to good libraries and computer-search facilities. Because of the practical difficulties and costs inherent in the development and maintenance of these networks it was proposed to proceed with caution and possibly begin with a relatively small network linking individuals working in training institutions.

W F ROSS, *Chairman*

Prevention and management of impairment in leprosy

Great strides have been made in the chemotherapy of leprosy, but despite adequate treatment, including MDT, patients continue to develop incapacities. Limited and unreliable information is available about the disability pattern in this new group of patients either active or discharged from treatment.

Where leprosy is common, it is identified as deformity and disability by the public. The failure of control programmes to master the problem of deformity is seen by the public as failure to cure the disease. Discharge of patients from the register of MDT removes the stigma of deformity only from statistics. It remains and accumulates in the sight of the public and of new patients who need treatment. Only if disability is controlled will leprosy control programmes win and maintain the confidence that is essential to success.

Treating and preventing disability should be an integral part of any control programme. We also strongly support the long-term follow-up of patients released from MDT programmes with regard to appearance or worsening of disabilities in spite of 'bacterial cure'. Regular testing should include accurate measurement of nerve function in the eyes, hands and feet and recording of other disabling or stigmatizing physical signs in the face like nose collapse and loss of eyebrows. Every effort should be made at this stage to ensure that treatment and preventive measures are available to the disabled patients that are no longer on the active register.

Training of personnel working in leprosy, but specifically in the areas of prevention and rehabilitation is of utmost importance. Too big a gap continues to exist between available knowledge and implementation. For this we need support and funding. Education in rehabilitation and prevention should start at the top with health care and government officials, administrators and also reach the heart of medical schools and allied health training centres. The well-known training centres should be better supported and probably a few secondary ones developed. Cooperation and communication among the major training centres should be stimulated.

More time devoted to presentations on disability, rehabilitation and prevention should be made available at leprosy congresses.

In spite of past efforts in education and training there is still stigma in the disease, even among health care personnel and we should continue on all fronts in our education programme. No special status or financial benefits (like automatic pensions) should be given to the patient solely on the basis of the diagnosis of leprosy. Doing so only adds to the problems already present in trying to rehabilitate these patients especially in the social and vocational areas.

There is a need for a more workable disability grading system for control programmes. We recommend that the modified grading system presented at the March 1987 WHO Consultation on Disability Prevention and Rehabilitation in Geneva be implemented. This grading system only uses grades 0, 1 and 2, eliminating the existing confusion about grades 2 and 3. We would like to suggest that the term anaesthesia should be replaced by an objective measurement representing protective sensation. However, specifically for control, prevention and management of disability we need more detailed testing systems. Control programme records should have provision for initial and follow-up nerve function examination. It is recommended that a range of filaments be used to identify levels of normal, diminished and lost protective sensation. Successful treatment of peripheral nerve impairment requires the early recognition of changes in nerve function. This will allow treatment for the nerve before the damage becomes irreversible. Only regular and accurate testing of nerve function will alert us in cases of nerve impairment without symptoms. The role that physical and occupational therapists could have in all these areas needs to be stressed.

A programme, treating and preventing disability successfully, as a back-up to the control team, will give credibility to that control programme. Foot care in particular is an aspect of treatment that patients notice and can help to promote compliance and confidence in the programme.

The loss of sight is the most devastating disability in leprosy because it most often is associated with loss of sensation in hands and feet. Basic screening of eye function and status is easy and quickly done. Every leprosy worker, at all levels, should be trained in the basic examination of the eye. Every patient has to have his eyes examined initially and all multibacillary patients at regular intervals. Routine prevention and treatment is possible in the vast majority of cases by specially trained paramedical workers. Medical officers can be adequately trained to deal with the more complex problems and supervise the eye programme by training in special centres.

We would like to emphasize again that leprosy is not a disease of just the skin, but also of nerves and that it produces social, emotional and physical disabilities. These can be prevented in many cases by appropriate measures at the appropriate time. When already established, rehabilitation is more difficult, but these cases must also be treated. No patient should be denied his right to these modalities of treatment, but he also must be made an active and responsible participant in his treatment and prevention programme. Many patients can be taught to recognize 'reportable events' like changes in eye, sensory or motor function and nerve pain and look for help as indicated, but monitoring is still necessary in most cases because many patients are not aware of ongoing changes as in the 'silent neuritis'.

F DUERKSEN, *Chairman*

Vaccine trials

The participants for the workshop came from a wide range of disciplines which included epidemiologists, statisticians, and immunologists. The participants had rich experience in large-scale vaccine trials. The group discussed the results from the 4 major BCG vaccine trials to generate information that could be useful to the ongoing and future leprosy vaccine trials.

1 BCG TRIALS

1.1 Burma

Some 26,000 healthy children were included in the trial and one half of them received BCG, the other half served as controls. Two batches of Glaxo freeze-dried vaccine were used. Very little protection (10%) was observed with the first batch, and as much as 30% with the second batch. Although the secondary batch was a little more potent, both batches had acceptable potency. Combining the results from both batches, it was concluded that the protection conferred by BCG was of a very modest level.

1.2 Uganda

A trial of BCG vaccine against leprosy among 19,000 children in Uganda, all contacts or relations of known cases, began in 1960, using the Glaxo strain of BCG. The efficacy of BCG against early forms of tuberculoid leprosy during the first 8 years was 80%, with evidence of continued protection up to 23 years. The degree of protection was independent of age, sex, and the child's exposure to infection.

1.3 South India

BCG prophylaxis study against tuberculosis in South India included the leprosy component, 5 years after the large-scale study (intake 1968–71) began, involving 200,000 persons. An overall 25% protection against all forms of leprosy has been recorded in 5–12·5 year period. Two different strains of BCG (French and Danish) gave similar results. With a lower dose of BCG (0·01 mg) there was a lower level of protection, and with the higher dose (0·1 mg), there was a higher level of protection seen against different types of leprosy in all age and sex groups.

1.4 Papua New Guinea

The intake period for the trial was about 1 year in 1963–64 and involved about 5000 persons. At the end of 9 years of follow-up, the efficacy rate of BCG was 46%. This trial was carried out in an area virtually free of tuberculosis and environmental mycobacteria.

1.5 Reasons for the differences

The differing results of the trials of BCG vaccine in leprosy were similar to the experiences with BCG against tuberculosis. Possible explanations include, (a) differential exposure of the population to *M. leprae* and other mycobacteria, (b) differences in immunogenetic characteristics of the population, (c) different strains of *M. leprae*, and (d) BCG strains could vary with respect to their protective effect.

2 ANIMAL MODELS

The limitations of the mouse model in experimental situations to judge vaccine efficacy were highlighted. Conflicting results have been reported by different investigators. The participants suggested that additional animal models should be developed.

3 ONGOING TRIALS

3.1 Venezuela

A large-scale trial was started in Venezuela in 1985, to test the ability of a vaccine based on a mixture

of killed *M. leprae* and live BCG to protect against leprosy. Participants in the trial were selected from among the household contacts and other close contacts of prevalent leprosy patients in three states of Venezuela. After initial skin testing with PPD and leprosy soluble antigens (LSA), about 30,000 contacts, aged 6–64 years were randomized in a double-blind fashion to receive either BCG or BCG plus *M. leprae*. The trial population is being followed through annual surveys for the occurrence of leprosy, each year a sample of participants are skin tested with PPD and LSA. To date, the incidence of leprosy in the trial population has been about 0·75 cases per 1000 per year, and no side-effects to vaccination have been observed other than those normally associated with BCG. The skin test studies show no differences in response to PPD between the 2 randomized groups, but large and significant differences in responses to LSA up to 3 years postvaccination (the maximum follow-up time to date). It is expected that sufficient cases will have occurred within the next 2 years to evaluate the initial protective effect against leprosy.

3.2 Malawi

The Karonga Prevention Trial is a randomized controlled trial of BCG and BCG plus killed *M. leprae* vaccine against leprosy (and tuberculosis). Among individuals without a BCG scar prior to entry into the trial, the protective efficacy of BCG plus killed *M. leprae* will be assessed against vaccination with BCG alone. In individuals with a BCG scar, the protective efficacy of repeat vaccination with either BCG or BCG plus killed *M. leprae* vaccine will be assessed. The initial vaccination with prior BCG, was mostly given several years ago. The intake phase started in December, 1985 and is expected to be completed at the end of 1989. The first follow-up survey will take place from 1991 to 1994. First results can be anticipated by 1995.

3.3 ICRC Vaccine

ICRC bacilli, a group of leprosy-derived cultivable mycobacteria exhibit cross-reactivity with *M. leprae* with reference to both B and T cell antigens, this forms the basis of their use in the vaccine preparation. It has been demonstrated that the ICRC vaccine brings about lepromin conversion in a proportion of lepromatous leprosy patients and in 95% of contacts, as well it possesses immunotherapeutic potential. The vaccine is currently undergoing a large scale immunoprophylactic trial in Maharashtra, India, from February 1987. It is a randomized, double-blind and controlled trial. The target population consists of 40,000 healthy household contacts of leprosy patients (all forms) between 1 and 65 years old and both sexes. The trial has 2 arms, receiving, (a) 1×10^9 radiation attenuated ICRC bacilli, and (b) one fifth the standard dose of BCG which is the control arm. Lowering the incidence of the disease (all forms of leprosy) will be used as the criterion of the vaccine efficacy. The trial is expected to last 10 years. To date, about 20,000 contacts have been vaccinated. In addition, a separate large-scale study on the immunotherapeutic efficacy is underway. Simultaneously, a vaccine containing a very high molecular weight (approx. 10^6) cell wall component of ICRC bacilli is now undergoing phase I and phase II clinical studies.

3.4 M.welchii *(L.) Vaccine*

M.w is a non-pathogenic, fast-growing soil mycobacterium, similar, but not identical, to mycobacteria listed in Runyon's group IV. It shares several antigens with *M. leprae*, but has also additional CMI-reactive antigens not present in *M. leprae*. An immunotherapeutic trial with this vaccine was initiated in December 1986 in 2 hospitals in Delhi. The patients belong to LL, BL and BB type of leprosy, and are bacillary positive, lepromin negative. All 89 patients reported to date received MDT, with 52 of them given, in addition, immunotherapy with *M.w* vaccine. The first dose consisted of 10^9 autoclaved *M.w.* bacilli. Repeat doses of 5×10^9 bacilli are to be given at 3-month intervals, up to 8 injections. Preliminary results are promising.

4 PROPOSED TRIAL

South India

CJIL Field Unit, ICMR, Madras proposes to carry out a vaccine trial against leprosy to test the efficacy of 3 candidate vaccines: (a) BCG plus armadillo-derived killed *M. leprae*, (b) ICRG vaccine, and (c) *M.w* vaccine. This trial would include 260,000 individuals from the Chingleput district, an area known to be hyperendemic for leprosy.

5 LABORATORY SUPPORT

Presently there are no proxy indicators available to judge the efficacy of a vaccine. The only method available would be measure the protective efficacy in terms of reduction in the incidence of clinical disease. Recently several *M. leprae* specific antigens/epitopes have been studied as candidates for tools for the detection of infection with *M. leprae*. The ongoing large-scale leprosy vaccine trials provide ideal, well characterized 'population laboratories' for the evaluation of candidate assays. The initial observations suggest that several assays may have epidemiological value.

5.1 PGL-1 Antibody

Findings from Venezuela indicate the high relative risk of developing clinical disease in individuals with high levels of antibodies for the phenolic glycolipid. However this type of survey would not be useful as a screening test to identify high risk individuals, as a large proportion of cases came from individuals with low levels of antibody response. Findings from Malaŵi, which are of cross-sectional nature, do not indicate any predictive value for the PGL antibodies. The levels are similar in contacts as well as noncontacts.

6 Skin test antigens

M. leprae derived soluble antigens, particularly the Rees and the Convit antigens, are being used for research purposes. Results from Venezuela show that the Convit antigen indurations are positively and strongly correlated to the Mitsuda antigen late reactions. However, results from Malaŵi and India bring out the deficiencies with these antigens in terms of reproducibility, sensitivity and specificity.

7 Development of second generation subunit vaccines

The recent application of recombinant DNA technologies, together with the availability of *M. leprae*-specific monoclonal antibodies and the ability to derive *M. leprae*-specific T cell clones, has led to the identification and characterization of at least 6 major protein antigens from *M. leprae*. Based on this approach, the identification of those antigens which are capable of evoking an appropriate cellular immune response in man should contribute to the development of a new generation of leprosy vaccines. Several groups are actively engaged in attempts to introduce genes coding for mycobacterial antigens into various potential vaccine vehicles, including vaccinia virus, BCG, and salmonella. In addition, several recent reports suggest that *M. leprae* cell wall-associated antigens may be candidates for a subunit vaccine.

8 Methodological and design issues

Like most vaccine trials, those for leprosy should in general include, (a) prior evidence of protection as for example from animal studies, sensitization studies, etc., (b) selection of trial area, trial groups

and determination of sample size, (c) choice and definition of 'control' and vaccine groups and dose of vaccine, (d) revaccination criteria, when indicated, (e) procedures for avoiding bias such as randomization, coding, ('blinding' etc.), (f) exclusion criteria, (g) standardization of leprosy diagnosis, (h) quality control of vaccines and field procedures, (i) resurveys and provision to monitor adverse effects, (j) information system including data processing, and (k) duration of trial and rules for stopping.

9 Additional epidemiological information from vaccine trials

While the trial should, in principle, concentrate on its specific objectives, the population base and the resources deployed permit, in general, the gathering of critical epidemiological information, such as trends in incidence rates, with very little additional effort. Such information is generally of value in interpreting the trial results. Care should be taken that this extra effort is kept separate and does not interfere with the conduct of the vaccine trial.

10 Conclusions

(a) Vaccine trials should be carried out simultaneously in different areas of the world with uniform methodology which includes procedures, vaccines and doses.

(b) At least one large-scale trial should compare the 3 candidate vaccines currently available: BCG + killed *M. leprae*, ICRC bacillus, and *M.w* bacillus.

(c) Serious consideration should be given to immunotherapeutic and immunoprophylactic trials in view of their importance to control programmes.

M D GUPTE, *Chairman*

Social aspects

INTRODUCTION

The group composed of social scientists, clinicians and leprosy programmers deliberated on the comprehensive meaning of the social aspects of leprosy. After reviewing reports of earlier workshops on the social aspects of leprosy and taking into consideration the concurrent workshops on Health Education Rehabilitation. At this Congress the group identified the role of social sciences in leprosy as the focus on the workshop.

The contribution of the social sciences extends to all aspects of leprosy control including transmission, treatment, training and health services delivery. The group reaffirms that leprosy control also includes the prevention of associated deformities (WHO TRS 675).

For the purpose of leprosy control, the social sciences include disciplines of anthropology, economics, psychology, history, sociology, philosophy, linguistics, political science and law.

The workshop reviewed the social science publications related to leprosy and noted the theoretical and methodological limitations of many of these studies. Therefore the research results have been of little practical significance. Furthermore, it was also noted that majority of passed research on the social aspects of leprosy had not been conducted by trained social scientists.

The group strongly advocated a need for scientific rigour in terms of research design, methodology, data collection and analysis of research data. This would result in increased scientific reliability and validity, thus facilitating effective use of research results in leprosy control.

The role of a multidisciplinary research team was emphasized. This team should include social science researchers and leprosy programmers at all levels from field leprosy workers to policy planners.

The dissemination of research results was identified as a critical issue. These results need to be (a), clear; (b), easily accessible; and (c), translatable to be of value in leprosy control.

Research in other areas of health, disease and illness is a valuable source from which social scientists in the field of leprosy could benefit. For example, extensive research on compliance improving strategies to hypertension and diabetic regimens would be of importance. The group agreed for the need of detailed background papers from sources. These 'state of art' papers would include an annotated bibliography and would form the basis of further leprosy related scientific enquiries.

The group recommended that a centralized documentation centre of social science and leprosy be established as part of the WHO Leprosy Archives in Geneva.

RESEARCH

The priorities of social science research on leprosy include the following:

1 Those having an immediate relevance to better leprosy control, e.g. improved patient treatment compliance.
2 The differential impact of monotherapy and multidrug therapy on patients, community and health workers concept of cure.
3 The study of social epidemiological factors related to the transmission of leprosy should be conducted by multidisciplinary teams of social scientists, epidemiologists and other researchers.
4 The study of social environment which creates fear on account of prevalence of ulcers and deformity resulting in nonparticipation of community in leprosy control programmes.

RESEARCH PROGRAMMES

1 Studies in health beliefs, behaviour and practices of the community, health providers and patients.
2 Impact of multidrug therapy on the community and health services.
3 Impact of existing health systems (vertical and integrated) on the health functionaries and their relative efficiency.
4 The diagnosis, treatment and rehabilitation problems of women.
5 Studies on patients' and families' self-stigmatization and low self-esteem.
6 The status of leprosy health personnel in the entire health care system.
7 Effective communication between health providers and patients.
8 Legislation regarding leprosy.
9 Cost-effectiveness of various approaches in leprosy control programmes.
10 Concept of 'cure' amongst patients, community and health providers under monotherapy and multidrug therapy.
11 Semantic problems associated with various aspects of leprosy.

TRAINING

The training for social scientists needs to include an orientation to the various problems of leprosy control. The group also emphasized the need for special training in social science research methodology for other leprosy workers who may form part of the multidisciplinary research team. Special funding should be made available for training to attract social scientists to the field.

CONCLUSION

Social science insights aim to provide scientific understanding of the process of change that is brought about in the behaviour, attitudes and practices of various sectors of society towards leprosy.

R K MUTATKAR, *Chairperson*

Health education

RECOMMENDATIONS ON PUBLIC AWARENESS ACTIVITIES IN LEPROSY

Members of the workshop recommend that:

1 There be someone in the leprosy programme with the task of liason with media personnel, who can provide access to media expertise, channels etc. The liason person should have experience and skills or benefit from extra training in communication methods.
2 Awareness activities be continuous throughout the year, not only on World Leprosy Day.
3 Simple studies be conducted locally (including information from experienced workers) to provide background information about current beliefs, practice and attitudes towards leprosy among different groups. Information from these studies should be used in public awareness activities.

RECOMMENDATIONS FOR PATIENT EDUCATION

1 That health workers try to understand the reasons for noncompliance, rather than labelling a patient 'uncooperative'.
2 The major emphasis in leprosy care be on educating the patients to want to be treated. Effective patient education should remove the need for defaulter tracing.
3 All health workers should receive training in patient education skills. Having acquired these skills, they need time to talk with patients. This may require adjustment in the programming of clinics and workload.
4 Guidelines be agreed upon, whereby patient education is carried out in small steps and in a progressive manner according to the patients needs. Written records to monitor progress in patient education and to obtain feedback could be used.

RECOMMENDATIONS ON TRAINING HEALTH WORKERS

1 A course or module on health education in leprosy be included in the training programme of all leprosy training centres, i.e. regional, national, local. Where courses do not exist they should be developed; where there are courses but their content is not adequate, they should be revised.
2 The following strategy be adopted for in-service training; a, an initial workshop on health education/communication skills to demonstrate the approach and to identify potential trainers; b, a second workshop to train the trainers; and c, a third visit to assist the trainees in implementing in their own area what they have learned. This strategy had budgetary implications, and should cover a minimum period of 3 years.
3 The main learning objectives for the leprosy workers will be: a, to learn to look at leprosy through the eyes of patients, their families and public; b, to acquire skill in translating ideas into language which the patient, family and public can easily understand; and c, to learn interpersonal skills to ensure that their communication is effective; and, d, to apply what they have learned, using guidelines for specific situations.

4 In the evaluation of training, the emphasis should be on assessing through follow-up and supervision, how trainees use the health education guidelines in patient and community care.
5 Training of teams of leprosy workers in an area is preferable to training just one level of health worker.
6 A list be drawn up of available resource persons to provide such training. The list should be made available to countries through ILA member associations and the ILA coordinating bureau.

RECOMMENDATIONS ON HEALTH EDUCATION IN LEPROSY

1 Development of materials be at local level, so as to be consistent with local language and culture, but production could be done centrally.
2 Priority should be on pictorial materials used by field workers for patient and community education. Every field worker should have a set of flash cards and be trained to use them.
3 Separate materials should be designed for different target groups.
4 Materials should be pre-tested before production, evaluated and then revised. When possible, advice from a communication specialist should be obtained.
5 ILA takes initiative in identifying agencies to help with material production in various regions, and provides funds to implement the above recommendations.

RECOMMENDATIONS ON IMPLICATIONS FOR HEALTH EDUCATION RELATED TO THE INTRODUCTION OF MULTIDRUG THERAPY (MDT)

1 Prior to the implementation of MDT: a, all health workers involved in leprosy care be trained in appropriate managerial, clinical and health educational aspects of the MDT programme; and b, all patients receive adequate education concerning MDT. The initial preparatory training of staff and education of patients should be reinforced periodically.
2 The objective of leprosy control includes the prevention of disability as well as interrupting transmission of infection. Therefore, health education in all aspects of self care remains a high priority.
3 Where appropriate, the general population should be informed about MDT prior to its implementation.

RECOMMENDATIONS FOR THE EDUCATIONAL TASKS OF THE PERIPHERAL HEALTH WORKER IN LEPROSY CONTROL

1 A variety of types of peripheral health worker be recognized in different places. They range from the informal contact person in the community to the trained health worker. They all influence community opinion about leprosy, and are able to help patients emotionally and socially.
2 The informal contact person or villagers should be identified and listened to. They will devise their own activities, and the health worker gives support when requested.
3 The trained peripheral health worker encourages members of the community to carry out activities like drama, puppet shows, small group discussions to inform the community about leprosy. Other tasks can include: a, explaining to the patient and his family about treatment of leprosy; b, how to prevent deformity; c, about reactions; and d, possible side-effects of the drugs.

J NEVILLE, *Chairwoman*

Recommendations on rehabilitation

1 INTRODUCTION

Any leprosy programme which fails to address the patients disability and dislocation from society, is, especially from his perspective, a failure.

2 DEFINITION

Rehabilitation is the process of maintaining in, or restoring the individual to, his/her rightful place in society.

3 OBJECTIVES

Rehabilitation may be implemented by the achievement of the following objectives:

1 Encourage early treatment of the disease, and perseverance until cure.
2 Recognize early signs of damage to eyes and nerves, and apply appropriate preventive measures and treatment.
3 Develop reconstructive surgery and ophthalmology programmes, including the providing of specialist training in these fields.
4 Find (or create) for the individual job opportunities appropriate to his/her ability. This may involve vocational/other training, and encouragement towards achieving independence, and the ability to compete for employment in the community.
5 For the severely disabled, make life possible with dignity and fellowship, regardless of their physical condition.

4 SPECIFIC RECOMMENDATIONS

While many aspects of rehabilitation have been discussed before, at earlier congresses and elsewhere, and by other groups at this Congress, our group would like to emphasize the following points:

1 Disability control. It is the responsibility of staff at all levels, peripheral, regional, and central, to: a, set measurable disability control and rehabilitation objectives, and evaluate progress towards them; and b, enable patients to minimize their own disability problem. Patients and families are often even more motivated than paramedical workers, and maybe quick to learn simple techniques testing for levels of risk, and following up by preventive care. They must be used.
2 Disability recording. The WHO system for grading of disability is for statistical purposes, and not for recording the progress in individual patients. Detailed records are necessary to monitor changes in eye and nerve status. However these will be too complicated for routine assessments. Hence it is essential that there be, in addition, a *simple* disability record for field use, from which changes in eye status and sensation, strength, and secondary problems can be immediately identified, and appropriate action taken on a priority basis.
3 Implications of multidrug therapy (MDT). Careful record keeping has particular significance now, with the implementation of MDT, where there is an increased threat of nerve damage, especially in borderline patients.

Field personnel must be trained in early recognition and active treatment of eye/nerve damage at the peripheral level. It is imperative that they be able to prevent damage/deterioration, otherwise the compliance and confidence of patients and others will be adversely affected. This intensive surveillance must be continued even after the completion of MDT in the patient. Care must also be taken to be aware of nerve damage in patients with silent neuritis.

4 SURGERY

a Nerve involvement. In the management of neuritis, when medical treatment alone has been shown to be ineffective, the addition of a nerve decompression procedure will relieve pain, and may give good functional recovery. This has special significance with regard to decompression of the posterior tibial nerve, which may restore plantar sensation, and prevent ulceration. To prevent permanent paralysis, the procedure must be done before the nerve is irreversibly damaged, and may be done by any surgeon with appropriate training.

b Reconstructive surgery. Surgery in patients with established paralysis is not of an emergency nature. As long as proper care is taken to prevent the development of secondary deformity, such surgery may be delayed, until a competent surgeon is available.

c Eyelid surgery. Patients who have *lagophthalmus*, especially if corneal sensation too is impaired, are at risk of corneal breakdown. Some type of tarsorraphy should be done as soon as possible.

In those patients with corneal sensation, other sophisticated procedures (such as temporalis transfer) may be done, after proper evaluation. If ulceration is already present, a temporary tarsorraphy using a 'matrass' suture to close the lid should be done as an emergency procedure. Facilities and personnel with appropriate training should be available, at field level, in all control programmes.

d Intraocular surgery. Intraocular procedures may be essential for controlling glaucoma, or for restoring visual function, as in cataract extraction. Some of these patients are at risk for serious post-operative complications. They include those with impaired corneal sensitivity, and those with a recent history of iritis. They should be referred for surgery to a skilled ophthalmologist experienced in ocular leprosy.

e Ulcer and foot care. In anaesthetic feet, ulcers can often be prevented by daily care, careful examination, and the use of appropriate footwear. The patient should be trained and encouraged to take responsibility for this.

Ulcers, once they have occurred, need rest. In uncomplicated cases this need not be neccessarily in bed: some simple ulcers may be managed by wearing protective footwear and minimizing walking; others will need the use of established procedures for ulcer healing. We emphasize that the provision of footwear, and other disability preventing activities, must be an integral part of any leprosy control programme.

5 CONCLUSION

Rehabilitation is not an additional service to leprosy control, that can be left out if funds are limited. It is *fundamental* to the success of control, which may be a waste of money without it.

M BRAND, *Chairman*

Microbiology

SOURCES

The nine-banded armadillo still provides bulk quantities of *M. leprae* of acceptible microbiological quality, so long as protocols for controlling contamination are followed. Smaller quantities of *M. leprae* of high quality (high viability, low chance of contamination) are available from nu/nu mice. In some regions of the world, adequate supplies for research are not available.

PURIFICATION

The 'IMMLEP 1/79' procedure yields suspensions comparable in viability with unpurified

suspensions. Other satisfactory methods involving density-gradient centrifugation exist; one appears to fractionate *M. leprae* cells according to density (possibly related to intactness). A small-scale method for purifying *M. leprae* from human biopsies needs to be developed.

STRUCTURE AND COMPOSITION

Evidence is emerging for the presence in *M. leprae* of a 30 kDa protein like that secreted by *M. bovis*. Capsular material can be observed electron-microscopically and may form the electron-transparent zone of intracellular mycobacteria. 'Buried' proteins linked to the peptidoglycan of *M. leprae* apparently exist. Most components of the envelope (some unique to *M. leprae*) have been identified and MoABs, potentially useful in studying ultrastructure, raised to most. *M. leprae* (like *M. tuberculosis*) inhibits phagosome-lysosome fusion in phagocytic cells.

MOLECULAR BIOLOGY

Five immunodominant antigens were cloned and 2 have been sequenced. As SDS-denatured proteins they are recognized by antibodies and by some T cells. A repeated sequence of DNA has been discovered in *M. leprae*; probes for this may be useful for detection and identification. Expression of *M. leprae* genes has been obtained in *E. coli* and *Streptomyces*. There are encouraging prospects of cloning *M. leprae* genes in cultivable mycobacteria. An immunomodulatory fusion protein has been reported. Genes cloning for identifiable enzymes have been expressed: citrate synthetase and biotinylated proteins (probably acyl-CoA carboxylases, involved in lipid synthesis). Analysis of the sequence of 16S rNA placed *M. leprae* phylogenetically in the group of slow-growing mycobacteria; analysis of the sequence of the ribosomal RNA gene gave preliminary indications of strain differences in *M. leprae*.

BIOCHEMISTRY

M. leprae apparently depends on the host for purines, but is able to synthesize its own pyrimidines. It can synthesize fatty acids de novo and can use the glycoxylate shunt. However, phosphotransacetylase seems deficient; acetate is incorporated into lipids only in intracellular *M. leprae*. Palmitate is incorporated into PGL-I and is readily oxidized. Iron-chelating molecules have not been detected but those from *M. neoaurum* can be utilized. Cytochrome B1, but no others, has been identified. Catalase levels are very low but can be detected by immunoprecipitation methods.

VIABILITY TESTING AND DRUG SCREENS

Systems were described for assessing viability and drug susceptibility *in vitro*. Different systems might be optimal for each purpose. It was noted that compounds have different relative activities in different systems. It is essential to use good quality bacteria for drug screening. Assays for viability varied greatly in sensitivity. Palmitate catabolism appeared to be a particularly promising assay for drug screening; assays based on synthesis of macromolecules will probably be useful. No results of comparisons by double-blind trial of existing *in vitro* drug screens are available.

CULTIVATION

Two new tissue culture systems seem promising; *M. leprae* multiplied in mouse fibroblasts (but lost infectivity) and also in Schwann cell cultures. Axenic multiplication has been described: in the presence of 'adjuvant' mycobacteria; in conditioned medium; in a microaerophilic medium (coccoid forms); in normal media. Identification criteria (see below) should be applied to all these cultures with precautions to ensure that the initial inoculum is diluted out.

IDENTIFICATION

Use of several or all of the following are recommended for identification of cultures claimed to be *M. leprae*: mycolic acids (types by high-resolution chromatography after alkaline methanolysis; species by GC–MS); PGL-1 (using MoABs); G + C content of DNA (56%); DNA hybridization. Restriction fragment analysis will be valuable when fully developed. Reaction with specific MoABs to the 'famous five' antigens is suggested with the caveat that 3 of them appear to be stress proteins and may not be expressed in cultured *M. leprae*. Immunodiffusion analysis can rule out known, cultivable mycobacteria. Growth in normal mouse footpads, and nerve involvement, are characteristic (though not technically taxonomic tests).

P DRAPER, *Chairman*

Lepr Rev (1988) **59,** 309–316

Mucosal immunity to mycobacteria in leprosy patients and their contacts

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Accepted for publication 24 June 1988

Summary Since the development of leprosy may follow the formation of an initial lesion in the nose, mucosal immune responses might be important in the protective immune response to *Mycobacterium leprae*. Salivary antibody responses to *M. leprae* and other mycobacteria were therefore investigated in leprosy patients and healthy contacts using ELISAs against whole mycobacteria and an *M. leprae*-specific glycolipid constituent (PGL1) of the external surface of *M. leprae*. Lower levels of salivary IgA directed against *M. leprae* were found in household contacts (at high risk of developing leprosy) than in hospital contacts (low risk of leprosy). Samples from the local indigenous population with no known leprosy contact showed an intermediate number of positive salivary IgA responses against *M. leprae* and untreated patients were less likely to be positive than treated patients.

Correlation was found between salivary antibody responses to *M. leprae*, *M. scrofulaceum* and *M. tuberculosis*, suggesting the presence of some cross-reacting antibody. Few patients and no healthy subjects had detectable antibody responses against an epitope of PGL1, suggesting that this important serum antibody response is not a major component of the mucosal immune response to *M. leprae*. Since there appears to be a secretory IgA response to *M. leprae* which is least likely to be found amongst those with the disease and in those individuals with increased risk of developing leprosy, we suggest that the mucosal immune system might be of importance in a putative protective response to infection by the leprosy bacillus.

Introduction

Leprosy bacilli are excreted in large numbers from the nose in lepromatous leprosy[1] and it is widely believed that the nose may also be the site of initial infection, although other methods of transmission cannot be excluded at present.[2] By analogy with tuberculosis, it seems feasible that local cell-mediated immunity (CMI) might be important in producing resolution of the initial

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infection.[3] However, organisms entering the upper respiratory tract must penetrate epithelial barriers before establishing an infection and so mucosal immunity appears to offer an alternative explanation for the successful defence against *Mycobacterium leprae* mounted by the vast majority of people coming into contact with the disease.[4] This might involve interference with the adherence of inhaled bacilli to mucosal surfaces. Although the mechanism(s) involved in adherence of *M. leprae* to cells are not fully understood, adherence of other bacteria to mucosal surfaces may involve specific receptors,[5] or may depend upon the hydrophobicity of the organism.[6,7] Prevention of adherence depends upon physical factors such as a constant flow of mucus which traps organisms and on the secretion of humoral factors such as lysozyme and immunoglobulins (predominantly IgA) directed against bacterial antigens.[8,9]

Until recently, the few published studies of mucosal immunity in leprosy have concentrated on patients[10,11] who by getting the disease have demonstrably failed immunologically. Abe *et al.*[11] showed that most lepromatous patients do not produce a salivary IgA response to *M. leprae* and that treated patients are more likely to show a response than active cases. The same authors[12] have recently shown that a high proportion of contacts of leprosy patients have detectable IgA against *M. leprae* in the saliva, which they hope might prove useful for the early diagnosis of leprosy.

Salivary IgA responses to *M. leprae* might well be linked to mucosal and systemic CMI responses, since both may result from mucosal presentation of antigen.[8,13] Healthy contacts of untreated leprosy patients are the most likely subjects to be challenged continuously by *M. leprae* in an endemic population and a high proportion of these individuals are likely to be producing a protective immune response. In this investigation we sought salivary antibodies to mycobacteria in two contact groups: household contacts of untreated leprosy patients (who have an increased risk of leprosy), and hospital workers who are also exposed daily to live leprosy bacilli, but who have a low risk of developing leprosy. For comparison, we have included groups of untreated patients, treated patients and unselected subjects from the indigenous population in the study.

The ELISA methods were chosen because they detected antibody to surface antigens on *M. leprae* and other species of mycobacteria. Salivary IgA antibody cross-reacting with other mycobacteria might also be effective in preventing adherence of *M. leprae*, and consequently the lack of strict immunological specificity of the whole cell mycobacterial ELISA used was considered advantageous. Since the saliva samples for the first study in Bangladesh were chemically preserved for transport back to Scotland for analysis (a procedure which might have altered the levels of IgA present), a second study was performed in Fiji using an ELISA developed for use in the field. This also allowed comparison not only of the methodological factor but also of the salivary antibody responses of two geographically distinct populations in which leprosy is endemic.

Materials and methods

SUBJECTS

The saliva samples used for the initial study were obtained by IAC during a field study in northern Bangladesh in 1986.[14] A total of 253 subjects were included in the investigation. These were divided into five groups: 52 untreated leprosy patients, 53 treated leprosy patients, 78 household contacts of untreated patients, 20 hospital contacts, and 50 indigenous control subjects. The Ridley–Jopling classification[15,16] was confirmed by skin biopsy in 41 of the untreated patients and in 11 of the treated patients. The age and sex distribution in each group is given in Table 1. The untreated patients were classified on the Ridley–Jopling scale as 4 TT, 23 BT,1 BB, 9 BL, 8 LL and 7 Idt, while there were 29 BT, 1 BB, 9 BL, 14 LL patients in the treated group.

Following this study, one of the ELISA methods used was adapted for use in the field and a further survey was performed amongst leprosy patients and their contacts in Fiji. A total of 163 subjects were examined, including 56 treated leprosy patients, 24 household contacts of treated patients, 23 hospital workers, 39 indigenous subjects (mainly medical students), and 21 treated

Table 1. The age and sex distribution of Bangladeshi and Fijian subjects

Group	No.	Male:female ratio	Age (years) Mean	SD	% < 15 years
Bangladeshi series					
New patients	52	1:0·86	33·1	11·9	5·8
Treated patients	53	1:0·20	34·1	10·5	0
Household contacts	78	1:0·95	23·4	15·0	35·9
Hospital contacts	20	1:0·82	35·2	11·1	0
Control group	50	1:0·79	24·9	8·1	0
Total	253	1:0·68	28·9	12·9	12·2
Fiji series					
Treated patients	56	1:0·72	49·6	19·8	3·6
Hospital contacts	23	1:1·88	39·7	10·9	0
Household contacts	24	1:1·67	26·8	13·2	16·7
Indigenous population	39	1:1·86	22·7	9·9	0
Treated TB patients	21	1:0·91	31·2	14·4	4·8
Total	163	1:0·90	35·9	18·7	4·3

tuberculosis patients. The age and sex distribution of the subjects is given in Table 1. The treated patient group were classified clinically on the Ridley–Jopling scale as 9 TT, 5 BT, 2 BB, 14 BL, and 26 LL.

SALIVA COLLECTION AND PRESERVATION

Saliva was collected by asking subjects to suck orange-flavoured multivitamin tablets (Haliborange, Evans Medical, Beaconsfield, England) and expel saliva over a period of 5 minutes. Large particles of food were removed and the saliva samples were preserved for transport by the addition of the following chemicals (final concentrations in parentheses) to inhibit bacterial growth and enzymatic proteolysis: sodium azide (0·5 g/l), iodoacetamide (2·00 mM), phenylmethylsulphonylfluoride (0·34 mM), and ethylenediamine tetra-acetic acid (2·00 mM). Prior to the field project, measurement of total IgA levels by nephelometry in saliva samples kept at room temperature for 3 months showed no appreciable loss of IgA. Following transport to Scotland, the saliva samples were centrifuged at 11,000 *G* for 15 min in a MSE Microcentaur centrifuge and divided into 3 equal aliquots, 2 of which were frozen for future study. Three of the saliva samples were lost in transit. In the Fijian study, vitamin C tablets (Vit Valu Laboratories, Sterling Pharmaceuticals Ltd, Sydney, Australia) were used to stimulate salivation and the samples were refrigerated until the ELISA could be performed (maximum delay: 12 hr).

ELISAS

ELISA studies using whole y-irradiated mycobacteria (*M. leprae*, *M. tuberculosis* and *M. scrofulaceum*) provided by Dr R J W Rees (Division of Communicable Diseases, Clinical Research Centre, Harrow) were performed on all of the saliva samples. The saliva samples from Bangladesh were screened for IgA, IgM and IgG against *M. leprae*, and for antibody of any subclass against whole y-irradiated *M. scrofulaceum* and *M. tuberculosis* (strain H37Rv). In addition, the Bangladeshi samples were screened in an ELISA using a BSA glycoconjugate (also provided by Dr R J W Rees) which mimics a major epitope of the phenolic glycolipid (PGL1) of *M. leprae*.[17] This glycoconjugate consists of the intact terminal disaccharide of the natural antigen coupled to BSA by reductive amination and corresponds to conjugate 11 as used by Brett *et al.*[17] The Fijian samples were screened for anti-*M. leprae* IgA only.

For the ELISA using whole mycobacteria, plastic ELISA plates (Nunclon, Gibco Ltd, Paisley, Scotland) were treated with 0·1% gelatine and dried at 60°C for 3 h before adding whole mycobacteria to the wells at a concentration of 10^7/ml in a volatile ammonium carbonate buffer (pH 8·2) and drying overnight at 37°C. The wells were blocked with 0·1% bovine serum albumin (BSA) in phosphate buffered saline (pH 7·1) (PBS) for 1 h at 37°C, and washed 4 times over 15 min with 0·1% BSA in PBS before the saliva samples were added. Saliva samples from Bangladesh were diluted 1:10 in 0·1% BSA in PBS and incubated in prepared plates at 37°C for 90 min. The plates were washed 4 times before horseradish peroxidase (HRP) conjugated antihuman immunoglobulin (against IgA, IgM, IgG or polyvalent) was added (1 : 1000 dilution) and incubated at 37°C for 90 min. Following a further 4 washes, the HRP was developed by incubation for 30 min at 37°C with freshly prepared 0·4 mg/ml o-phenylenediamine (OPD) containing 0·05% hydrogen peroxide in a citrate-phosphate buffer (pH 5·0). The reaction was stopped with 2·5 N sulphuric acid and the results read at 490 nm using an automatic Dynatech MR 580 microELISA reader.

For use in Fiji this technique was modified by: 1, using undiluted saliva; and 2, by the use of 2 mM 2,2′-azino bis(3-ethylbenzthiazoline sulphonic acid) (ABTS) with 5 mM hydrogen peroxidase as the final substrate.[18] All incubations following coating of the bacilli onto the wells were performed at 24°C, necessitating a longer incubation time for the saliva (2·5 h). The HRP–ABTS reaction was stopped with 0·1% sodium dodecyl sulphate (SDS) and read visually against a white background as positive or negative by comparing wells with *M. leprae* and wells without *M. leprae*. Since the ABTS reaction was being used under field conditions for the first time, the opportunity was taken to compare the effect of ABTS incubation at 24°C and 32°C. The results differed in 15 out of the 163 cases, in all of which the 24°C assay was positive and the 32°C assay negative.

DATA ANALYSIS

The results of the ELISAs are expressed as the difference in absorbance between the mean of triplicate wells coated with the whole mycobacteria or the glycoconjugate and a single well without mycobacteria. Since background absorbances in both the whole mycobacterial and BSA glycoconjugate ELISAs varied between 0 and 0·030, an insignificant level in most positive specimens, a single control well was used. This allowed 3 wells to be coated with antigen to reduce errors between positive salivas (maximum coefficient of variation = 20%) and as a result it was possible for all of the saliva samples to be screened at one time.

Since the whole mycobacterial assay does not distinguish between specific and cross-reacting antibody, no unequivocally negative control group could be tested to determine a cut-off point for the Bangladeshi series. Therefore the absorbance differences were compared directly for each group using nonparametric statistical methods.

The results from the Fijian study were subjectively determined as positive or negative, and it therefore was necessary to define an arbitrary cut-off point for comparison of the Bangladeshi data with the Fijian results. Since 82% of the Fijian treated patient group were positive and this group was the only one common to both studies, the cut-off point was defined as the absorbance value at which 82% of the Bangladeshi treated patients would be regarded as positive.

The results were analysed using Statgraphics (STSC, California, USA) software. The results of the Bangladeshi assays were compared using Spearman Rank Correlation and the Mann–Whitney U test.

Results

Salivary IgA against whole *M. leprae* was detectable in some individuals in all of the Bangladeshi subject groups (Figure 1). Occasional saliva samples produced high background values in wells coated with gelatine, but not *M. leprae*. These patients may have antibody to gelatine in their saliva and were therefore excluded from statistical analysis. In the leprosy patients, comparison of the

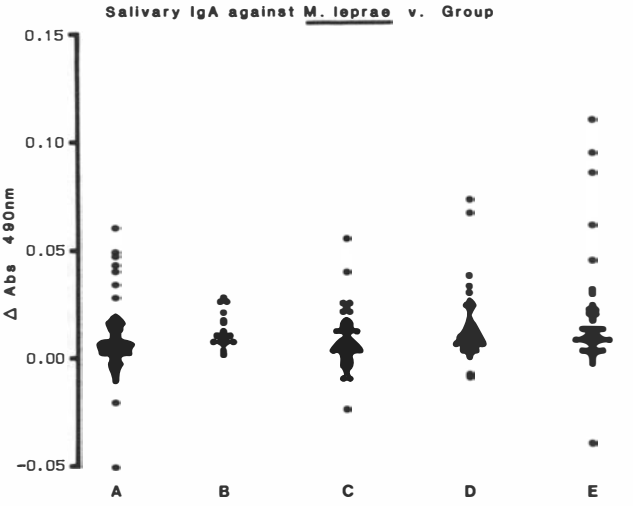

Figure 1. Salivary IgA antibody response to whole y-irradiated *M. leprae* for each subject group. A, household contacts; B, hospital contacts; C, untreated patients; D, treated patients; and E, indigenous subjects.

salivary IgA titres to *M. leprae* with the Ridley–Jopling classification showed no discernible pattern of response. The ELISA for IgM anti-*M. leprae* antibodies showed lower absorbance differences than the IgA ELISA, but the general distribution of the results was a similar pattern in both Ig subclasses and there is weak rank correlation between the results of these ELISAs for all subjects ($r = 0{\cdot}29$, $P < 0{\cdot}001$). This relationship appears to derive from a stronger correlation between the IgA and IgM results in the treated patient group ($r = 0{\cdot}55$, $P < 0{\cdot}001$) and in the indigenous control group ($r = 0{\cdot}49$, $P < 0{\cdot}001$). The ELISA for salivary IgG against *M. leprae* was uniformly negative in all subject groups.

The percentage of positive IgA responses against *M. leprae* in each subject group from both Bangladesh and Fiji are shown in Table 2. The low percentage of positive results amongst the household contacts compared with the other groups is particularly striking, and is apparent in both study populations. When the results from the study groups in Bangladesh (Figure 1) are compared using the Mann–Whitney U Test, significantly lower absorbance differences are obtained in the household contacts than hospital contacts ($P < 0{\cdot}01$), although both these groups have daily exposure to *M. leprae*. The household contacts also have a significantly lower IgA anti-*M. leprae* response than the treated patient group ($P < 0{\cdot}005$) and the indigenous subjects ($P < 0{\cdot}003$). The larger proportion of positive responses amongst treated patients compared with untreated patients is also statistically significant ($P < 0{\cdot}05$).

The results of the ELISAs for polyvalent antibody against *M. tuberculosis* and *M. scrofulaceum* (Figure 2(a) and (b), show significant correlation with the IgA response to *M. leprae* ($r = 0{\cdot}29$, $P < 0{\cdot}001$ and $r = 0{\cdot}21$, $P < 0{\cdot}001$ respectively) and with each other ($r = 0{\cdot}33$, $P < 0{\cdot}001$). This suggests that there is a salivary antibody response to closely related epitopes on external surface antigens of *M. leprae* and the other mycobacteria studied. However, the results from treated tuberculosis patients in Fiji shows that these subjects have similar positivity for salivary IgA against *M. leprae* as the indigenous healthy population. If the whole *M. leprae* ELISA was detecting antibody against *M. tuberculosis* as well as *M. leprae*, one might expect a considerably higher number of positive responses in tuberculosis patients. Nevertheless, the rates of positivity in each of the subject groups from Bangladesh in the ELISA for antibody against whole *M. tuberculosis* are similar to those seen in the IgA anti-*M. leprae* assay. The household contact group have lower responses in the *M. tuberculosis* ELISA than the hospital contacts ($P < 0{\cdot}001$), the treated patients ($P < 0{\cdot}001$), and the indigenous subjects ($P < 0{\cdot}001$). There is also a lower mucosal antibody

Table 2. Percentage of positive responders (numbers in brackets) in ELISA for salivary IgA against *M. leprae* for Bangladesh and Fiji populations. Cut-off value for Bangladesh ELISA calculated by regarding 82% of treated leprosy patients (D) as positive

Subject group	Bangladesh % positive	Fiji % positive	Total % positive
A	64·0 (48/75)	41·7 (10/24)	58·6
B	90·0 (18/20)	87·0 (20/23)	88·4
C	62·7 (32/51)	None tested	62·7
D	82·7 (43/52)	82·1 (46/56)	82·4
E	78·7 (37/47)	69·2 (27/39)	74·4
F	None tested	71·4 (15/21)	71·4
Missing values:	8	0	8
All	72·7 (178/245)	72·4 (118/163)	72·5

A, household contacts; B, hospital contacts; C, untreated patients; D, treated patients; E, Indigenous subjects; F, treated TB patients.

response to *M. tuberculosis* amongst the untreated patients compared with the treated patients ($P < 0.001$).

Salivary antibody against the BSA glycoconjugate[17] which mimics an epitope of PGL1 was only detected in 3 lepromatous patients, all of whom exhibited high absorbance levels.

Discussion

Both of the ELISA methods used in this study detect antibody against surface antigens of *M. leprae*. Although the whole mycobacterial assay is relatively crude compared with the specific assay developed by Brett *et al.*,[17] it has proved sufficiently robust to give reproducible results under field conditions and it has the advantage of detecting antibody directed against any external antigens, whether these are specific to *M. leprae* or not. ABTS is a peroxidase substrate which has optimal activity at 25°C[18] and its use in the final stage of the ELISA has proved eminently suitable for assays under field conditions. In Fiji, better results were obtained by performing the HRP–ABTS incubation at 24°C than at 32°C.

The proportion of saliva samples with positive ELISA results for IgA against *M. leprae* in household contacts of both Fijian and Bangladeshi patients is significantly lower than that found in hospital workers who have a similar (and possibly even greater or longer) exposure to *M. leprae*. Indigenous subjects, with a lower degree of exposure to leprosy bacilli have an intermediate number of positive responses. The increased proportion of treated patients with demonstrable IgA responses against *M. leprae* compared with untreated leprosy patients appears to parallel increases in CMI against *M. leprae* which may occur as a result of treatment[14] and confirms the previous observation made by Abe *et al.*[11]

Thus there appears to be a lack of a salivary IgA response to *M. leprae* amongst many apparently healthy contacts of untreated leprosy patients and amongst the patients themselves. Mucosal antibody responses to *M. tuberculosis* and *M. scrofulaceum* are also weaker in these individuals, suggesting that there may be some general suppression of mucosal immunity in both groups affecting mucosal responses to mycobacteria. Various factors have been found to abrogate mucosal immune responses, notably malnutrition and intercurrent infection.[8,19]

Very few salivary antibody responses were detected against the BSA glycoconjugate which mimics an epitope of PGL1, a finding consistent with the low number of positive results obtained in saliva by Abe *et al.*[12] using purified PGL1 in an ELISA for salivary antibody. This suggests that PGL1 is not important as an antigen in the mucosal immune response to *M. leprae*. Comparison of

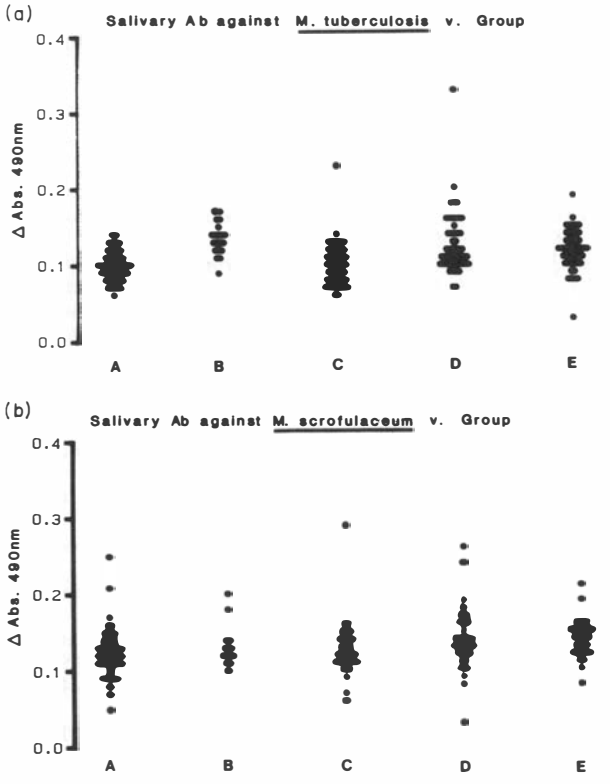

Figure 2. Salivary antibody response to whole y-irradiated: (a) *M. tuberculosis* H37Rv; and (b) *M. scrofulaceum.* A, household contacts; B, hospital contacts; C, untreated patients; D, treated patients and; E, indigenous subjects.

the results of salivary and serum ELISAs for antibody against *M. leprae* in the Bangladeshi patients[20,21] showed little correlation, suggesting that the basis for these responses may be substantially different.

The association between lower levels of secretory IgA against *M. leprae* in household contacts of leprosy patients and their greater risk of developing leprosy, together with the apparent rise in salivary IgA levels following chemotherapy in leprosy patients, leads us to suggest that a mucosal immune response to *M. leprae* may be one of the major protective factors preventing leprosy in those who come into contact with the disease.

Further studies will be required to determine the specificity and possible significance of the IgA response to *M. leprae*, although an *M. leprae*-specific response may not be essential for protection. We hope to investigate which antigens are involved in the IgA response against *M. leprae* which should make it possible to produce more sensitive ELISAs for the detection of mucosal immune responses to *M. leprae*. It will be necessary to follow up a large population of leprosy contacts over several years to determine whether a positive IgA anti-*M. leprae* antibody response correlates with protection.

Acknowledgments

We wish to thank Dr M S Islam, Dr T Ali, Mr S Halda, Dr J Ivanyi, Dr R J W Rees, Professor C du Florey, Dr E C Dawlako, Professor H Lander, and Dr T E Isles for their assistance. The visit by IAC to Bangladesh was supported by the award of the Becton–Dickinson Travelling Scholarship by the

Royal College of Pathologists and the laboratory work was funded by LEPRA. The visit by NDCS to Fiji was funded by the St Francis Leprosy Guild, Roussel Laboratories Ltd, and LEPRA. We are grateful to Mr R Fawkes and Mr S McPherson for preparation of the figures.

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Lepr Rev (1988) **59,** 317–327

Serum antibody responses to mycobacteria in leprosy patients and their contacts

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Accepted for publication 27 July 1988

Summary The aim of this descriptive study was to investigate the relationships between serum antibody responses to different mycobacteria in leprosy patients and contacts. The results of ELISAs for serum antibody against whole mycobacteria (*Mycobacterium leprae*, *M. tuberculosis*, and *M. scrofulaceum*) were compared with the results of an *M. leprae*-specific ELISA for antibody against an epitope of PGL1. The IgG response was found to be predominant in ELISAs for antibody directed against whole *M. leprae*, while the IgM response was greatest in the assay for antibody against PGL1. Some healthy hospital workers were found to have appreciable levels of IgM anti-PGL1. Since infection in this group is unlikely, chronic exposure may result in humoral responses to PGL1 in addition to subclinical leprosy. None of the ELISAs studied were able to give greater than a 55% sensitivity at 95% specificity and none were considered suitable for serodiagnostic use.

Significant correlation was found between the results from the whole mycobacterial ELISAs, which could be explained on the basis of cross-reaction between antibodies directed against common antigens. However, similar correlations were found between the results of the *M. leprae*-specific ELISA and the assay for antibody against whole *M. tuberculosis* and *M. scrofulaceum* which were greater than those for antibody against whole *M. leprae*. Infection with *M. leprae* may produce general stimulation of immunological memory for common mycobacterial antigens resulting in responses to antigens belonging to other mycobacteria to which the host has been exposed previously.

Introduction

Humoral responses in leprosy were first studied by complement fixation,[1,2] haemagglutination[3,4] and immunodiffusion.[5,6] In recent years, a number of other techniques for the detection of antibody directed against whole *M. leprae* or antigens derived from leprosy bacilli have shown some promise as serodiagnostic tests for leprosy. These include a qualitative fluorescent antibody (FLA–ABS) test,[7] and ELISA or RIA methods[8–10] which use whole *M. leprae* as antigen. Tests using whole mycobacteria rely upon absorption of the sera for their specificity and are not as reliable for

0305-7518/88/059317+08 $01.00

serodiagnosis as ELISAs or RIAs using antigens specific to *M. leprae*,[11,12] but whole mycobacterial assays have proved useful in descriptive studies of humoral immunity in leprosy patients.

The highest levels of antibody to antigens of *M. leprae* are generally found in lepromatous patients, while few tuberculoid patients or contacts have positive titres, although there is considerable variation in the levels within each group on the Ridley–Jopling scale. Antibody is produced against a wide variety of antigens in the serum of leprosy patients.[13–16] The majority of these antigens cross-react with antibody to other mycobacteria, but have nevertheless proved useful in studies of immunity in leprosy using radioimmunoassay (RIA).[6,11,17] Species-specific antigens such as the phenolic glycolipid PGL1 which coats the surface of the leprosy bacillus have been described more recently.[18] The highest titres of antibody to PGL1 occur in lepromatous patients and the response is predominantly of the IgM subclass.[19] Since IgM responses are thought to occur at an early stage in the development of leprosy,[20] serodiagnosis of leprosy seemed to be eminently feasible. A number of newer ELISAs based on synthetic glycoconjugates which mimic the immunogenic epitope of PGL1[21,22] or monoclonal antibody inhibition[23–25] are now available. The more recent assay methods are said to provide good separation of healthy individuals from patients and are not confused by the presence of immunity to other species of mycobacteria, although their ability to detect subclinical or indeterminate leprosy under field conditions has yet to be proven.[16]

The aim of most recent studies of antibody levels in the serum of leprosy patients and their contacts has been the production of a diagnostic test for leprosy at a subclinical stage, before nerve damage and transmission of the disease have occurred.[6,26] In some studies, an additional objective has been to identify those members of a population who are at risk of leprosy. The object of the present investigation was to compare serum antibody levels against several different mycobacteria in leprosy patients and their contacts with an *M. leprae*-specific assay for antibody against PGL1, to clarify the role of the humoral response in the pathogenesis of the disease. Since cross-reacting antibodies against antigens shared by several species of mycobacteria are part of the humoral immune response to *M. leprae* and may be of pathological significance, the lack of strict serological specificity of the methods used for their detection was not a major consideration.

Materials and methods

SUBJECTS

During a field study in the North-east of Bangladesh in 1986, a total of 253 subjects were examined, as described previously.[27] The subjects were divided into 5 groups: 52 untreated leprosy patients, 53 treated leprosy patients, 78 household contacts of untreated patients, 20 hospital contacts, and 50 indigenous 'control' subjects. Since the control group was drawn from a population with a high prevalence of leprosy, it should be noted that many will have been exposed to leprosy and that a few might even have subclinical leprosy. Serum samples were obtained by venepuncture and preserved for transport back to Scotland by the addition of sodium azide to a final concentration of 0·5 mg/ml serum. Only 10 of the serum samples were lost in transit. Skin biopsies were taken from 41 of the untreated leprosy patients and from 11 of the treated leprosy patients to confirm the diagnosis and their Ridley–Jopling classification. Of the 52 new patients, 4 were classified as TT, 23 BT, 1 BB, 9BL, 8 LL, and 7 Idt. The treated group consisted of 29 BT, 1 BB, 9 BL, and 14 LL patients. The serum samples brought back from Bangladesh were centrifuged and divided into aliquots which were frozen at $-20°C$ if they were not required immediately.

ELISA METHODS

Two assays were used during this study: the first was intended to detect all antibody directed against the external surface of whole mycobacteria, while the second was an *M. leprae*-specific assay which has been shown to detect antibody directed against a single epitope of PGL1.[21]

A serum standard composed of equal quantities of serum from 5 multibacillary patients was used as a positive control in screening experiments in which up to 264 samples were assayed simultaneously. Twelve sera from healthy Scottish volunteers were used as leprosy-negative controls. All of the sera were assayed for IgA; IgG and IgM antibodies against whole *M. leprae* and against the BSA glycoconjugate. In further experiments, whole *y*-irradiated *M. scrofulaceum* and *M. tuberculosis* were substituted for *M. leprae* and all of the sera were assayed for polyvalent antibody activity against these mycobacteria.

ELISA with whole, y-irradiated mycobacteria

An ELISA method for detecting antibody against whole *M. leprae* was developed from published methods.[9,19,28] Polystyrene ELISA plates were treated with 0·1% gelatine prior to coating on the bacilli to reduce the background absorbance and improve bacterial adhesion to the plates. Whole *y*-irradiated mycobacteria of 3 species (armadillo-derived *M. leprae* batch CD67, *M. tuberculosis*, strain H37Rv and *M. scrofulaceum*) were donated for this study by DR R J W Rees (Division of Communicable Diseases, Clinical Research Centre, Harrow). The whole bacilli at a concentration of 2×10^7/ml were suspended in a volatile ammonium carbonate buffer (pH 8·2) and dried onto the plates overnight at 37°C.[9]

Following blocking with 5% normal goat serum (NGS) in 0·01 M phosphate buffered saline (PBS), pH 7·1, the serum samples were added at a dilution of 1:200 in 5%NGS and the plates were incubated at 37°C for 60 min. Tween and other detergents were avoided as both blocking and washing agents in order to conserve lipids on the external surface of the bacilli.[29] The plates were washed with 1% BSA in PBS and antihuman immunoglobulin—horseradish peroxidase (HRP) conjugate (Sigma, Poole, Dorset, UK) was added at a 1:1000 dilution in PBS. In some assays class-specific second antibody was used: in others, polyvalent second antibody was employed. The peroxidase substrate, o-phenylene-diamine (O-PD), was prepared freshly for each experiment as a 0·4 mg/ml solution in citrate phosphate buffer (pH 5·0).[21] Hydrogen peroxide was added immediately before use at a concentration of 0·05%. The reaction was stopped after 30-min incubation with 2·5 N sulphuric acid.

ELISA using BSA glycoconjugate

The ELISA method developed by Brett *et al.*[21] (Batch No. 11, provided by Dr R J W Rees) uses a glycoconjugate of BSA which mimics the *M. leprae*-specific epitope of PGL1 and consists of the intact terminal disaccharide of the natural antigen linked to BSA by reductive amination. Since no lipid is present, coating of the antigen onto the plates is easier and there is no need to avoid the use of detergents such as Tween in the assay method. To reduce levels of nonspecific IgM binding in control wells without the glycoconjugate, the method was modified slightly by changing the diluent used with test samples from the BSA/Tween/PBS wash to 5% normal goat serum + 0·5% Tween 20 in PBS, pH 7·2.

Analysis of Results. Since O-PD was used as the substrate in both ELISAs, the results were expressed as the absorbance at 490 nm, measured using a Dynatech MR580 MicroELISA autoreader. The results from individual patients are expressed as the mean absorbance of triplicate wells coated with *M. leprae* minus the absorbance of a single well which did not contain *M. leprae*.

Triplicate antigen-coated wells were used in preference to duplicate wells to produce data from each experiment which could be used to assess the variability of the results. The background absorbance in control wells without bacteria was $< 30\%$ of the serum standard and the coefficient of variation (CoV) in the results from uncoated or coated wells was $< 15\%$. There was less variation of the results in the BSA glycoconjugate ELISA (CoV $< 5\%$), probably attributable to the more reliable coating obtained with a soluble antigen. Since it was desirable to perform simultaneous assays on all of the sera at once and only 11 plates could be physically handled in one ELISA, single uncoated wells were used to estimate the background absorbance with each sample.

Comparison of the ability of the various ELISAs to discriminate between patients and nonpatients was performed by defining an arbitrary cut-off point as the absorbance at which 95% of the healthy subjects would be regarded as negative. Approximately 5% of the healthy contacts of leprosy patients are likely to be developing the disease at any one time,[30] although the exact figure will vary between different geographical areas. Alternative methods for the assessment of ELISA positivity rely upon data from a disease-free control population from the same area as the test population, a requirement which was not available in this study.[31]

The results were analysed using SPSS, version H, release 9·1, on a DEC10 mainframe computer and Statgraphics, version 2·0 (STSC, California, USA). Since results from the ELISAs did not conform to a normal distribution, the relationship between individual ELISAs was examined using the Spearman rank correlation coefficient.

Results

The results of ELISAs for antibody of IgG, IgM, and IgA subclasses directed against *M. leprae* in each group of subjects are shown in Figure 1(a), (b) and (c) respectively. The highest absorbances at 490 nm occurred in ELISAs for IgG antibody against *M. leprae* in sera from patients, with few positive results in the other groups. The absorbances for IgM and IgA antibodies were considerably lower and did not show good separation of patients from healthy subjects. ELISAs to detect polyvalent antibody against whole γ-irradiated *M. scrofulaceum* and *M. tuberculosis* in serum were also performed (Figure 2(a) and (b)). These show some separation of the groups with high levels of antibody in many of the patients and relatively few positive results in the healthy subjects. The difference is most marked in the assay with *M. tuberculosis* as antigen.

The results of the ELISA for IgM against the BSA glycoconjugate are shown in Figure 2(c): there are many patient sera with high absorbances and there are also occasional positive results among the healthy contacts. High levels of IgG and IgA against this epitope of PGL1 were found in the patient groups, but none in the healthy subjects (results not shown). However, the absorbances in the IgM ELISA with BSA glycoconjugate were much higher at the same serum dilution than the IgG and IgA assays.

Comparison of the whole cell ELISA results against grade on the Ridley–Jopling scale[32,33] for sera from the treated and untreated patients shows no convincing pattern in the ELISAs for IgG against *M. leprae* (Figure 3(a)) or in the other immunoglobulin subclasses, but there is a graded antibody response across the spectrum with *M. tuberculosis* (Figure 3(b)) and *M. scrofulaceum*, with the highest levels in the lepromatous patients. There is also a graded response in the ELISA for serum IgM against BSA-glycoconjugate (Figure 3(c)), which is less marked in the IgG assay and least strong in the IgA assay. Furthermore, when the results for the treated lepromatous patients are compared with the untreated patients, the absorbances in the treated group appear to be lower, although this apparent diminution in the IgM response to BSA-glycoconjugate is not statistically significant.

Correlation of the results from each individual for the different assays performed using the Spearman rank correlation coefficient reveals the existence of some relationships between the assay results, although in many cases these are not particularly strong. The results from different immunoglobulin subclasses for whole *M. leprae* correlate poorly within the study population and within the subject groups. There is a small correlation between the results from the BSA-glycoconjugate ELISAs for different immunoglobulin subgroups, suggesting that the results with whole *M. leprae* are complicated by the different degree of response within each immunoglobulin subgroup to the many antigens present in the ELISA using whole mycobacteria. Good correlation ($r = 0{\cdot}59$, $p < 0{\cdot}0001$) was observed between the responses to whole *M. tuberculosis* and whole *M. scrofulaceum* in the study population. This is presumably due to extensive similarity between the surface antigens of these two species.

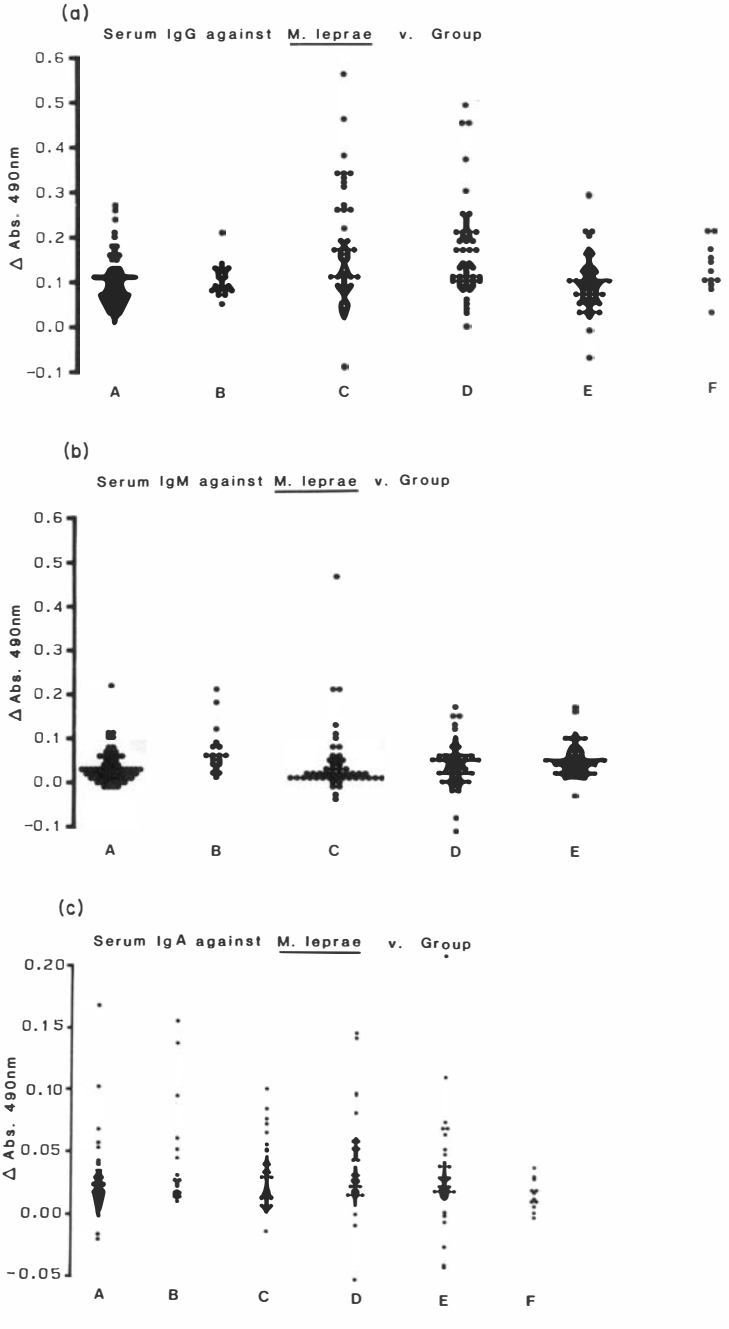

Figure 1. Serum antibody responses to whole *y*-irradiated *M. leprae* for each subject group: (a) IgG, (b) IgM, and (c) IgA subclasses. A, household contacts; B, hospital contacts; C, untreated patients; D, treated patients; E, 'control' group; F, Dundee controls.

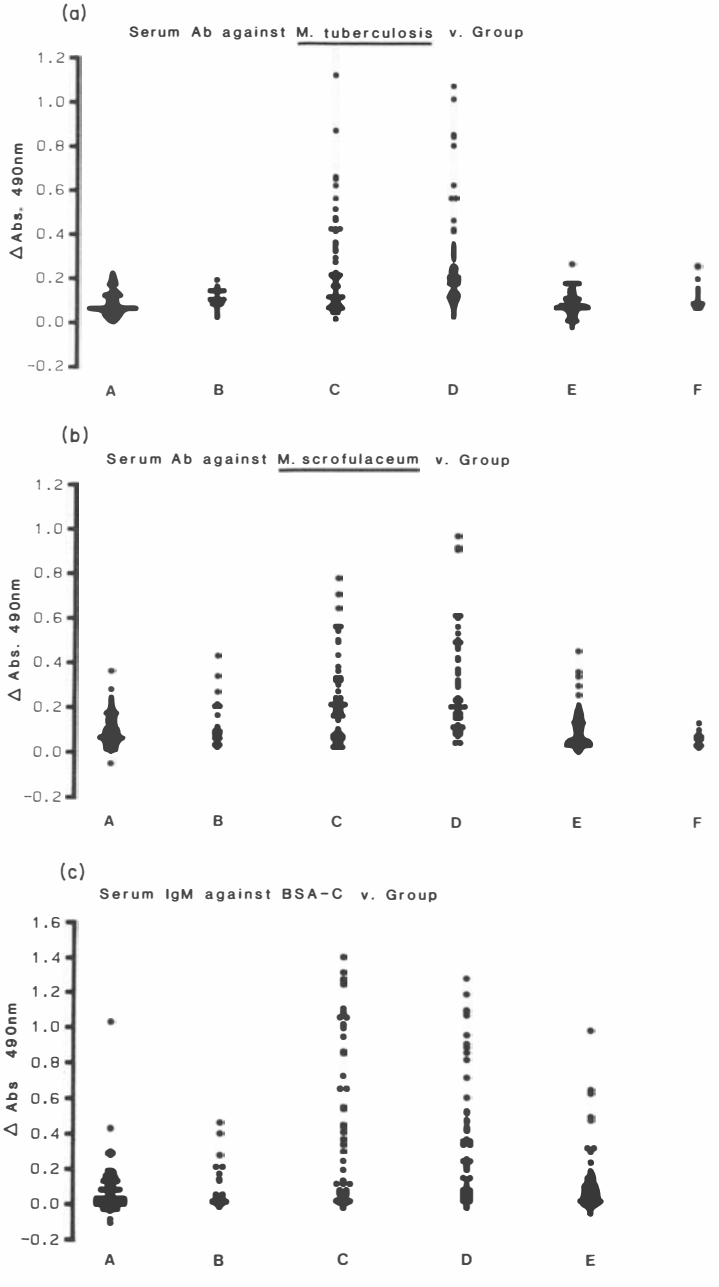

Figure 2. Serum antibody response to (a) whole *y*-irradiated *M. tuberculosis* H37Rv, (b) whole *y*-irradiated *M. scrofulaceum*, and (c) BSA glycoconjugate 11^{21} (IgM subclass) in each subject group. A, household contacts; b, hospital contacts; C, untreated patients; D, treated patients; E, 'control' group; F, Dundee controls.

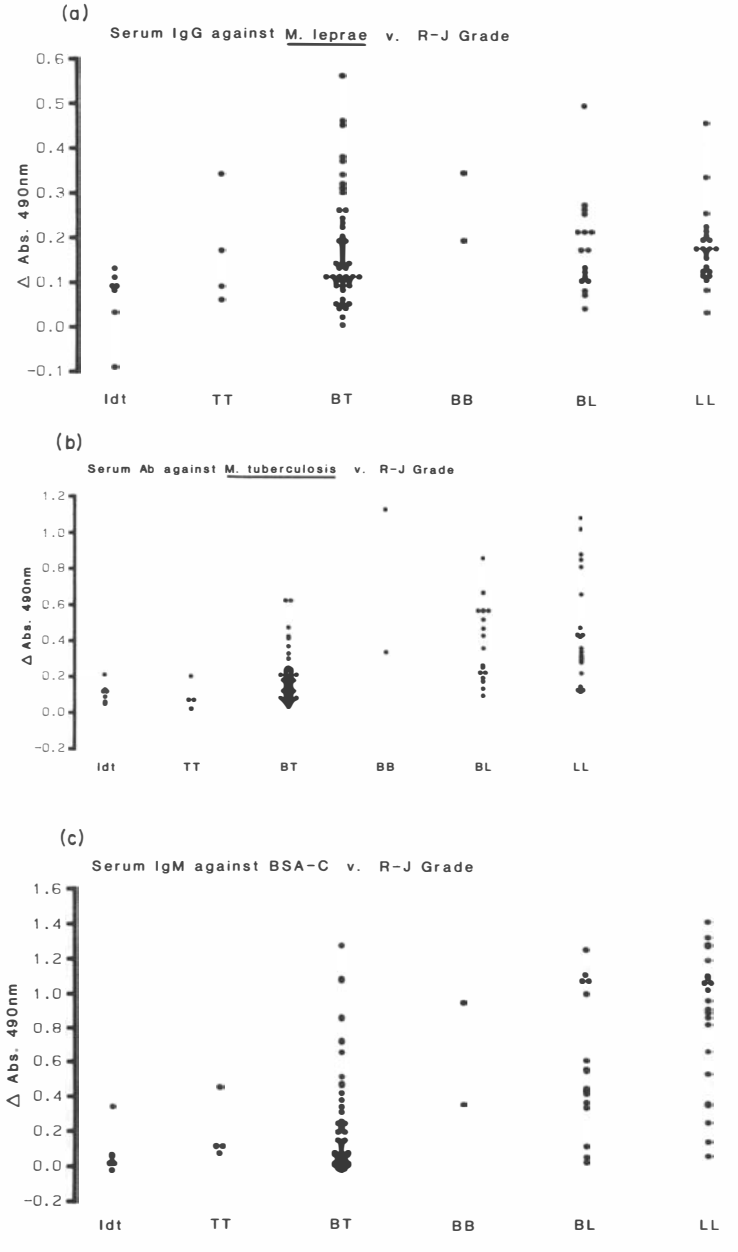

Figure 3. Serum antibody responses to (a) whole *y*-irradiated *M. leprae* (IgG subclass), (b) whole *y*-irradiated *M. tuberculosis* H37Rv (all subclasses) and (c) BSA glycoconjugate 11^{21} (IgM subclass) for treated and untreated patients classified on the Ridley–Jopling scale.

Table 1. The percentages of positive responders to each serum ELISA in each subject group

ELISA	Cut-off value	Subject group A	B	C	D	E
IgG to ML	0·207	5	5	28	29	6
IgM to ML	0·112	4	17	8	10	6
IgA to ML	0·075	3	15	6	10	6
IgG to BSA-C	0·017	4	10	34	26	6
IgM to BSA-C	0·429	3	5	46	34	10
IgA to BSA-C	0·016	6	0	38	30	8
Ab to TB	0·173	8	5	52	60	2
Ab to SC	0·276	3	10	32	46	8
n	—	71	20	50	50	50

A, Household contacts; B, Hospital contacts; C, Untreated patients; D, Treated patients; E, Indigenous 'control' group.

ML, *M. leprae*; BSA-C, BSA glycoconjugate; TB, *M. tuberculosis*; SC, *M. scrofulaceum*; *n*, Number of subjects (missing values excluded).

NB. The 95% limit was calculated by taking the 8th highest value of the results from the healthy subject groups (A, B and E) as the cut-off value.

When all of the subjects are considered together, the IgG response to *M. leprae* shows significant correlation with the serum antibody response to both *M. tuberculosis* ($r = 0{\cdot}46$, $p < 0{\cdot}0001$) and *M. scrofulaceum* ($r = 0{\cdot}38$, $p < 0{\cdot}0001$). The correlations are of similar strength in the untreated patient group ($r = 0{\cdot}47$, $p < 0{\cdot}001$ and $r = 0{\cdot}36$, $p < 0{\cdot}01$), but only the antibody response to *M. tuberculosis* correlates significantly in the treated patient group ($r = 0{\cdot}31$, $p < 0{\cdot}05$) and there are no significant differences in the healthy subject groups. These observations may be attributable to cross-reaction between different species in the whole microbial cell ELISA, but there is also a significant correlation between the serum antibody response to *M. tuberculosis* or *M. scrofulaceum* and the IgM response to BSA-glycoconjugate from all the subjects ($r = 0{\cdot}47$, $p < 0{\cdot}0001$ and $r = 0{\cdot}41$, $p < 0{\cdot}0001$ respectively). Stronger correlations between the results of these ELISAs also occur in the untreated patients ($r = 0{\cdot}68$, $p < 0{\cdot}0001$ and $r = 0{\cdot}52$, $p = 0{\cdot}0003$ respectively), but not in the other subject groups. There was no correlation between the serum IgM response to BSA-glycoconjugate and the serum IgM response to whole *M. leprae*, although there was some correlation with the IgG and IgA responses to *M. leprae* ($r = 0{\cdot}34$, $p < 0{\cdot}001$ and $r = 0{\cdot}17$, $p < 0{\cdot}008$ respectively).

The percentage of positive antibody responses for each group was estimated using a cut-off point calculated by regarding 95% of the individuals in the normal subject groups as negative. The results are shown in Table 1. The assays offering the best discrimination between patients and healthy subjects were: 1, IgG to whole *M. leprae*; 2, IgM to BSA glycoconjugate; and 3, antibody of any subclass to whole *M. tuberculosis*. However none were able to distinguish reliably between healthy subjects and paucibacillary patients.

Discussion

The two ELISAs employed in this study allowed the detection of antibody against 1 *M. leprae*-

specific glycolipid epitope on the surface of the organism and against a wide variety of antigens expressed on the surface of 3 mycobacterial species. This contrast was designed to give an overview of the humoral responses to *M. leprae* and other mycobacteria in each subject group.

The results of the present ELISA studies to whole γ-irradiated mycobacteria are similar to those found by other workers using whole bacilli as antigen.[8,10,29] More of the subjects produce an IgG response than either IgM or IgA and the absorbances in the IgG ELISA are higher than they are for the other classes of immunoglobulin. Moreover, the subjects show a similar response to other species of mycobacteria (in this case, *M. tuberculosis* and *M. scrofulaceum*), confirming the findings of Douglas *et al.*[10] In patients, the lack of a graded antibody response to *M. leprae* across the spectrum of disease from TT to LL (Figure 3(a)) is probably in part due to the inclusion of treated patients in the results, although there is certainly great variation in the antibody response within each grade and some untreated lepromatous patients show low levels of antibody activity in all of the ELISAs (Figures 3(a), (b) & (c)).

The serum IgM response to BSA glycoconjugate 11[21] is considerably greater than the IgG or IgA response. This is in keeping with the results of other studies using PGL1 or synthetic glycoconjugates,[21,22] all of which have shown that the IgM response to this determinant is immunodominant.[19] However, it appears that several of the hospital contacts have a significant titre of IgM antibody to the BSA conjugate in their serum (Figure 2(c)) which may reflect chronic exposure to *M. leprae*. Thus chronic exposure as well as infection may be important in producing serological antibody responses to this particular antigen and this may complicate attempts to use this ELISA as a serological tool for diagnosis. Many of the treated patients in this study had only been treated for a few months and it is therefore not surprising that few differences between treated and untreated patients are apparent in the ELISA results.

Correlation of the various ELISA results for individual subjects shows that there is linkage between the antibody responses to *M. leprae* and *M. tuberculosis*. The same is true of *M. leprae* and *M. scrofulaceum*, but to a lesser extent. Since there is considerable difference between the surface antigens of *M. leprae* and *M. tuberculosis*, the explanation may lie in the host response to common external antigens. However, the smallest correlation between the whole mycobacterial assays and the BSA-glycoconjugate assay occurred with *M. leprae* and the strongest with *M. tuberculosis*. Induction of cross-reactive antibodies might explain this, but infection with *M. leprae* could also produce general stimulation of immunological memory for mycobacterial antigens resulting in co-responses to antigens belonging to other mycobacteria. Such a link might well have survival value, since exposure to different, antigenically related organisms is an everyday occurrence. Further evidence for 'co-responsiveness' may come from specific assays for antibody responses to individual antigenic determinants specific to different species of related mycobacteria.[34] A similar phenomenon, known as 'original antigenic sin,' has been described by Ivanyi.[35]

The serum antibody response to external antigens of *M. leprae* is a complex one, with many antigens involved, some of which cross-react with other mycobacterial species. Responses to different antigens may be dominant in the different immunoglobulin subclasses. These differences have already proved useful in the development of tests for subclinical leprosy and may be exploited further. In this study, the response to *M. tuberculosis* offered the best discrimination between patients and healthy subjects with a 55% sensitivity at 95% specificity.[36] Addition of other responses to form an index does not significantly improve the sensitivity, due to their correlation with the *M. tuberculosis* response and their low intrinsic sensitivity. Antigen capture assays using monoclonal antibodies may provide a better alternative for early diagnosis of leprosy, since these do not depend upon the host response to the infection.[37-39]

Acknowledgments

We wish to thank Dr I H Cochrane, Mr S Halda, Dr T Ali and Dr M S Islam for their assistance in

Bangladesh and Mr R Fawkes for preparing the figures. We are grateful to Professor C du Florey for help in analysing the data. IAC was supported by the award of the Becton–Dickinson Travelling Scholarship by the Royal College of Pathologists and by LEPRA.

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Use of soluble antigens in leprosy epidemiology

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Accepted for publication 16 May 1988

Summary Rees and Convit antigens prepared from armadillo-derived *Mycobacterium leprae* are presently available. This study was undertaken to understand the skin test reactions produced by these antigens in comparison to tuberculin. A standard tester skin-tested about 250 individuals. The indurations were read at the end of 48 h for Rees and Convit and at the end of 72 h for tuberculin by a standard reader who read these reactions following blind procedures again after 2 h. The values of standard deviations for the mean differences were 1·0, 2·6 and 2·4 mm for tuberculin, Rees and Convit antigens respectively. Standard deviations for the mean differences for two different tests using the same antigen on the same individual twice, were 3·0, 6·0 and 5·3 mm respectively. Two batches of Rees antigen gave reasonably consistent results, but the skin-test readings with 2 batches of Convit antigen differed substantially. The available antigens need further improvement.

Introduction

The need for an antigen like tuberculin has been felt for a long time for the study of epidemiology of leprosy.[1] Lepromin is a minimal vaccine and it cannot serve this purpose.[2] Drs Convit and Rees have prepared soluble antigens from *M. leprae* of armadillo origin.[3] These antigens are known after their names. Two batches of Convit and Rees antigens were supplied to our Unit by the IMMLEP programme of the World Health Organization. Several studies have been undertaken using these antigens. The present paper deals with the nature of the skin reactions produced by these antigens, reproducibility of the skin-test readings, and comparison of two different batches of these antigens with respect to skin-test reactions. It was necessary to undertake these studies as there is no reported research work on these aspects of skin-test reactions in leprosy. For the sake of comparison, tuberculin skin testing was also undertaken.

Material and methods

STUDY POPULATION

The skin-test antigen studies were conducted in 2 villages from Sriperumbudhur Taluk, Chingleput District, Tamil Nadu. Prevalence of leprosy in these 2 villages was over 50 per 1000 population examined.

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ANTIGENS

In most of the studies mentioned below, we used Rees antigen (batch CD19) and Convit antigen (batch SA-IND, 1-16-86). For studying batch variations, batch Wel-3-CD73 of Rees antigen and batch IB-Lote-2 4-6-87 of Convit antigen were also used. These antigens were received from Drs Rees and Convit, through the courtesy of IMMLEP, TDR, WHO. Tuberculin PPD (RT 23, 1 TU per dose) was used for comparisons and was procured from the BCG Laboratory, Madras, India.

Rees antigen is a soluble antigen preparation of gamma irradiated *M. leprae*, purified according to IMMLEP protocol, with particulate material removed.[3] The Rees antigen preparations contained 10 μgms protein/ml for both the batches used.

Convit antigen is also a soluble antigen and is prepared from 'live' *M. leprae*. Suspensions of *M. leprae* are passed through a standard French press. The extract is centrifuged and filtered following standard procedures. Preparations after necessary dilutions are autoclaved.[3]

SKIN TESTING AND READING

The skin-test antigens were given by superficial intracutaneous injections on the dorsum and the volar side of the forearm. The specified quantity, 0·1 ml, was measured according to the markings on the barrel of a tuberculin syringe. We used Omega tuberculin syringes, and 26 G, 3/8″ platinum needles, supplied by IMMLEP, TDR, WHO. Each skin test was read at specified intervals, the reaction was felt, and if the induration was present its transverse diameter was measured. All measurements were made with a ruler calibrated in millimetres. Each palpable reaction was also examined for the density of induration. We adopted these standard procedures as they are followed for the tuberculin testing and reading.[4]

The following studies were undertaken to standardize various procedures in Rees and Convit skin tests. A standard tuberculin tester and a standard tuberculin reader carried out all the testings and readings reported below. For all of these studies the reader was unaware of the identity of the skin-test antigen while reading a reaction. This blinding was achieved by providing him with a secretary at the time of readings.

Study 1 *Optimum interval for skin-test readings*

Tuberculin skin tests were read at the end of 72 h. Reactions following Rees and Convit antigen skin tests were measured at 24-, 48- and 72-h intervals in a set of 767 individuals. It was possible to make all 3 readings for both Rees and Convit antigens in 471 individuals. These findings are given in

Table 1. Skin-test readings for Rees and Convit antigens at 24-, 48- and 72-hr interval in 471 individuals.

	Reactions	
Interval (h)	Rees antigen	Convit antigen
	Mean ± SD (mm)	
24	11·47 ± 5·71	9·52 ± 4·98
48	11·00 ± 6·17	10·04 ± 5·44
72	9·41 ± 5·84	8·61 ± 5·30
	Paired 't' values	
24 *vs* 48	1·95	2·73
48 *vs* 72	7·54	7·49

Table 1. There was a marginal difference in 24- and 48-h skin-test readings, 72-h readings were significantly lower when compared with the 48-h readings. The findings were similar when all the available skin-test readings were considered. It was decided to use the 48-h readings, in preference to 24 h readings, to avoid the early nonspecific response.

Study 2 *Intrareader variations*

Rees and Convit antigens (0·1 ml of each) were injected in the dorsum of the forearms, allocation of the antigens to right or left forearms was done randomly. The reader read all the tests twice at an interval of approximately 2 h. The initial part of the study (April 1987) involved approximately 250 individuals.

It was felt desirable to document the intrareader variations in reading tuberculin tests for the sake of comparison with Rees and Convit reactions. This part of the study was also done with approximately 250 individuals.

Following these initial exercises, along with the interbatch variation studies, the standard reader was asked to repeat the same exercise for Rees and Convit antigens in a limited number of individuals. Eighty-six and 61 paired observations for Rees and Convit skin-test readings were made. This work was done in July 1987, 3 months after the initial intrareader variation study.

Findings for the intrareader variations are given in Table 2.

Study 3 *Intertest variations*

Two skin tests of 0·1 ml of Rees and Convit antigens were performed on the selected individuals on the same day. Readings were made after 48 h. Approximately 200 individuals were involved in Rees and Convit antigen studies. Tuberculin exercise was essentially for comparison and was done in about 300 individuals. The selected individuals were retested with the same antigen previously used in them after a gap of approximately 1 month. Findings of this study are given in Table 3.

Study 4 *Interbatch variations*

This study was done in two parts. In the first part, 2 batches each of Rees and Convit antigens were skin tested in two different groups of individuals. This part of the study brought out distinct batch variations for Convit antigen. It was felt desirable to repeat this study in one group of individuals who received all the 4 skin tests for 2 batches each of Rees and Convit antigens. Injection sites for the 4 skin tests were selected on the basis of randomization. The findings were confirmed (Table 4, Figure 1).

Table 2. Intrareader variations—tuberculin, Rees and Convit antigens.

	Tuberculin	Rees		Convit	
	June 87	April 87	July 87	April 87	July 87
Number	252	250	86	251	61
Mean reactions (mm)					
I reading	13·27	14·83	15·17	13·37	10·26
II reading	13·18	15·13	14·94	13·90	10·31
Mean difference ($\bar{d}$) (mm)	0·09	0·30	0·23	0·53	0·05
Standard deviation ($\bar{d}$) (mm)	1·00	2·60	1·21	2·40	1·30

Table 3. Intertest variations.

	Tuberculin	Rees	Convit
Number	314	191	204
Mean reactions (mm)			
Test I	12·24	10·93	9·82
Test II	13·00	11·08	10·80
Mean difference ($\bar{d}$) (mm) (II–I)	+0·76	+0·15	+0·98
Standard deviation ($\bar{d}$) (mm)	2·98	5·96	5·32
Probability	<0·05	>0·05	<0·05

Table 4. Interbatch variations for two* batches of Rees and Convit antigens (1987).

Antigens		No. tested	Mean reactions (mm)		$\bar{d}$ (mm)	SD for $\bar{d}$	Correlation coefficient for I and II
			Batch I	Batch II	[I–II]		
Convit	Expt 1	74	10·64	7·88	2·76	5·47	0·53
	Expt 2	95	12·33	9·38	2·95	4·73	0·66
Rees	Expt 1	94	15·50	14·97	0·53	2·82	0·81
	Expt 2	95	14·94	14·63	0·31	3·65	0·77

* Rees antigen batches: I. CD19; II. Wel-3 CD73. Convit antigen batches: I. SA-IND, 1-16-86; II. IB-Lote-4-6-87.

Observations and discussion

INTRAREADER VARIATIONS

It could be seen from Table I that the readings for the tuberculin indurations were very close to each other, the mean difference being 0·09 mm, with a standard deviation of 1·00 mm. The difference in the readings of the same reaction twice was within ±2 mm on 95% of the observations.

With respect to Rees and Convit antigens, the initial intrareader variation was fairly wide with standard deviations of 2·60 and 2·40 mm respectively. It is worth noting that this amount of variation was seen in a standard tuberculin reader. As could be seen from the small number of paired observations, 3 months later, the standard deviations came down to 1·21 and 1·30 mm for Rees and Convit antigen readings.

INTERTEST VARIATIONS

Table 3 brings out the marked differences between tuberculin and the leprosy soluble antigens. With tuberculin, the value of standard deviation for mean difference between two tests was 2·98 mm. Ninety-five per cent of the observations were within ±6 mm of the mean difference. These observations are similar to those quoted by Rajnarain in standard tuberculin readers.[5] With respect to the soluble antigens, the standard deviations were 5·96 and 5·32 mm. It is expected that this difference also would come down with experience.

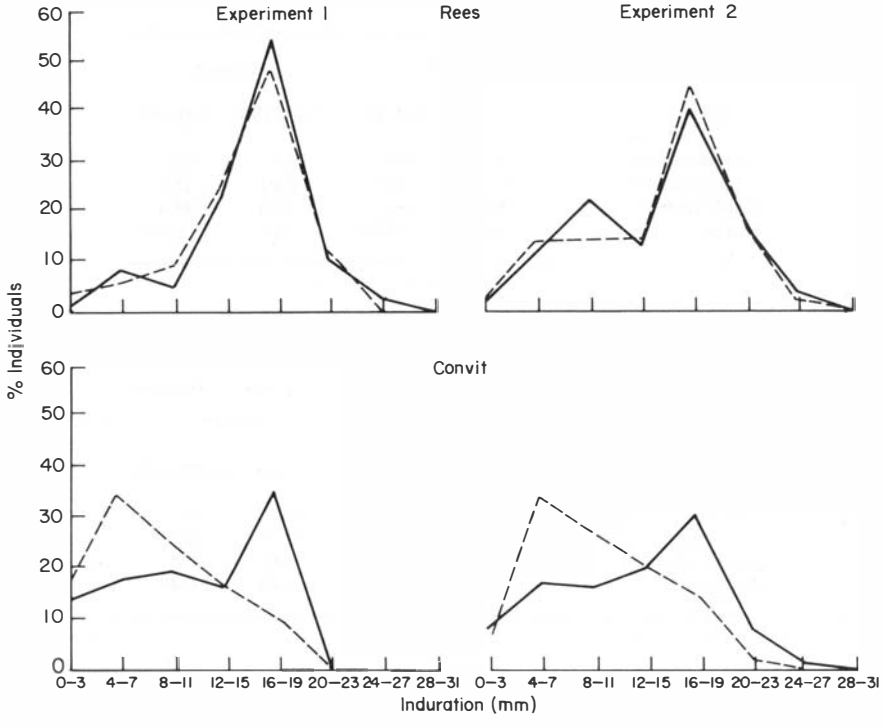

Figure 1. Interbatch variations: Rees and Convit skin-test reactions. ———, Batch I*; – – – –, Batch II.
* Rees—Batch I: CD19; Batch II: WEL-3-CD73. Convit—Batch I: SA-IND 1-16-86; Batch II: IB-Lote 4-6-87.

INTERBATCH VARIATION

Two batches each of Rees and Convit antigens were tested in two different experiments. Information from Table 4 and the frequency distributions from Figure 1, clearly bring out the differences between 2 batches of Convit antigen. Both these batches were prepared following similar procedures and were standardized in Venezuela by Dr Convit (personal communication). The reason for the difference observed by us is difficult to understand.

SKIN TEST REACTIONS TO THE SOLUBLE LEPROSY ANTIGENS

In the process of the various studies mentioned above, the density of the indurations to the soluble antigens were also recorded. The scale adopted for this was arbitrary. Soft reactions meant reactions merging almost imperceptibly with the normal skin around. Hard reactions were quite distinct in the margins. When we looked for the density of indurations due to Rees and Convit antigens in the initial stages of the present work (1239 indurations), about 30% of them were 'soft', 30% hard and the remaining 40% were in between and did not pose difficulty in reading them to the standard tuberculin reader. Reactions to tuberculin were almost invariably hard or firm in consistency. The reason for this soft consistency is not known. This soft consistency of the

Table 5. Implications on positivity due to intrareader variations.

	Rees		Convit	
Antigens	April 1987	July 1987	April 1987	July 1987
Number of individuals	250	86	251	61
% positives by both	69·2	83·7	64·1	45·9
Concordance	90·4	96·5	92·0	98·4
Kappa	0·75	0·86	0·82	0·97

Table 6. Implications on positivity due to intertest and interbatch variations.

	Rees		Convit	
Antigens	Intertest	Interbatch	Intertest	Interbatch
Number of individuals	191	95	204	95
% positives by both	30·9	63·2	31·4	30·5
Concordance	73·3	85·3	75·0	69·5
Kappa	0·46	0·65	0·49	0·43

indurations, makes them extremely difficult for the correct and consistent reading even for an experienced skin-test reader.

The experience of Ponnighaus and Fine in Malawi using various batches of Rees antigen and a smaller number of Convit-type antigens has been reported[3] (TDR, IMMLEP). They found changes in the skin reactivity to these antigens in a time span of 3 months. Part of these 'changes' could be on account of the inherent nature of these antigens, which produce comparatively softer reactions.

IMPLICATIONS OF VARIATIONS IN REES AND CONVIT SKIN TEST READINGS

Observed variations in the skin test readings are expected to lead to variable interpretation of the skin tests in terms of positives and negatives. In the initial exercise for the intrareader variations, the magnitude of this kind of error was not very high. It was further possible to reduce this difference substantially with experience (Table 5). Efforts to read positive and negative reactions in the intertest and interbatch experiments were almost frustrating (Table 6).

Conclusions

Comparatively recent availability of soluble antigens in leprosy have raised the possibility of promising research in leprosy epidemiology. However very little published work is available documenting the methods needed for skin testing and reading these antigens. The studies reported here bring out certain problems in reading these reactions consistently. This difficulty could be partly on account of the softness of indurations produced by these antigens. It is possible to reduce the intrareader variation with experience. However, there is a continued requirement for research on standardizing and improving the presently available soluble skin test antigens in leprosy. It is also necessary to produce better skin test antigens than the ones presently available.

Acknowledgments

The authors are grateful to Drs Rees and Convit for the supply of Rees and Convit antigens and also to IMMLEP, TDR, World Health Organization, for arranging the supply of antigens to us. The authors appreciate the statistical assistance by Mr S Kannan and the secretarial assistance by Mr N K S Brahaspathy.

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A concentration method for detection and quantitation of bacillaemia in leprosy and its comparison with other techniques

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Accepted for publication 11 May 1988

Summary Detection and quantitation of bacillaemia in 50 untreated cases of leprosy were evaluated by the buffy coat method, the haemolysis method and the present Petroff's method. Bacillaemia was detected in 29 (58%) out of 50 cases and in 32 LL–BL cases it was detected in 28 patients with a success rate of 87·5%. Both the haemolysis method and Petroff's method were found useful in estimating the bacillary load per millilitre of blood. Importantly, the smears of concentrated deposit obtained by Petroff's method revealed only AFB free from any artefacts and also yielded high bacterial counts. In conclusion, Petroff's method of concentration was found superior over other methods for detection and quantitation of bacillaemia in the lepromatous spectrum (LL–BL) of the disease.

Introduction

Lowe[1] appears to be the first worker to observe bacillaemia in leprosy. Subsequently many workers[2–6] have demonstrated bacillaemia in various types of leprosy, especially in the lepromatous spectrum of the disease. Fite[7] has found leprosy lesions of blood vessels and suggested a continuous shedding of bacilli into circulation. The bacilli have been found in the endothelial cells of blood vessels in the skin and subcutaneous veins of leprosy patients.[8–10] Bacillaemia has been detected by various concentration methods.[2–4]

In the present communication, we have used Petroff's method of concentration for detection as quantitation of bacillaemia in different clinical types of leprosy and the results are compared with those obtained by other concentration methods to evaluate the usefulness of our technique in determining the bacillary load in the blood of a leprosy patient.

Material and methods

Fifty untreated cases of leprosy consisting of 22 LL, 10 BL, 10 BT and 8 TT patients were investigated. Skin smears from 5 different sites were taken for BI and skin tests using DNCB and

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lepromin were done for cell-mediated immunity (CMI) to select only such cases which were definite and unequivocally classifiable according to the Ridley–Jopling clinical scale.[11]

Specimens of venous blood were drawn twice (double syringe technique[2]) at the antecubital fossa where the lepra bacilli are sparse in the skin with negligible contamination of specimens. The first 8 ml of blood was collected in a plain sterile bottle and the serum separated was used for the estimation of serum proteins and immunoglobulins. The second 13 ml specimen was obtained in a fresh syringe and collected in a heparinized container (10 IU/ml blood) for detection and quantitation of bacillaemia. Alliquots of 3 ml, 5 ml and 5 ml of heparinized blood were used for the concentration of AFB by buffy coat[2] haemolysis[4] and the present modified Petroff's technique respectively.

Since Petroff's method of concentration for AFB in the sputum and other exudates using 4% NaOH is a well known technique, we have used this method with slight modification. In the modified Petroff's method of concentration, the sediment obtained from 5 ml of blood by haemolysis technique was homogenized with 0·5 ml 1% NaOH for 5 min, mixed with 10 ml distilled water and centrifuged at 3000 rpm for 30 min. The concentrated deposit thus obtained was suspended in 0·1 ml (100 μl) saline. In each specimen, 2 smears of 10 μl were prepared from saline suspension obtained by the haemolysis method and modified Petroff's method separately. Bacilli counted in 2 ZN stained smears (20 μl) would indicate the number of bacilli/ml of blood as calculated by $5 \times B/5$, where numerator 5 represents 100 μl (ie 5×20 μl) saline suspension of the deposit, B total number of bacilli in 2 smears (2×10 μl) and denominator 5 represents 5 ml of blood used for concentration.

Results

The data of this study are presented in Tables 1 and 2. Table 1 shows the comparison of 3 concentration methods for detection of bacillaemia in different clinical types of leprosy revealing positive skin smears. Bacillaemia was frequently detected in the lepromatous spectrum of the disease, especially in clinically LL cases. Out of 22 LL cases with positive skin smears, bacillaemia was demonstrated in all patients by Petroff's method, in 20 patients by the haemolysis method and in 17 patients by the buffy coat method achieving a success rate of 100%, 91% and 77·3% respectively. In 10 BL cases with positive skin smears, bacillaemia was detected in 6 cases, 4 cases and 3 cases by the respective methods thus revealing detection rate of 60%, 40% and 30% in that order. All the 3 concentration methods did not help in the detection of bacillaemia across the tuberculoid spectrum of the disease and only in 1 out of 10 BT cases was bacillaemia demonstrated by Petroff's method.

Out of 32 cases of leprosy in the lepromatous spectrum of the disease, bacillaemia was seen in 28 cases (87·5%) by Petroff's method, 24 cases (75%) by the haemolysis method and 20 cases (62·5%)

Table 1. Detection of bacillaemia by different concentration methods in various clinical types of leprosy

Type of leprosy	No. of cases	No. of cases showing positive skin smears	Detection of bacillaemia by: Buffy coat No. (%)	Haemolysis No. (%)	Petroff's No. (%)
LL	22	22	17 (77·3)	20 (91)	22 (100)
BL	10	10	3 (30)	4 (40)	6 (60)
BT	10	2	0	0	1 (10)
TT	8	0	0	0	0

Table 2. Bacillaemia detected by concentration methods in the lepromatous and tuberculoid spectrums of the disease

Disease spectrum	No. of cases	No. of cases with positive skin smears	Detection of bacillaemia by		
			Buffy coat No. (%)	Haemolysis No. (%)	Petroff's No. (%)
Lepromatous (22 LL + 10 BL)	32	32	20 (62·5)	24 (75)	28 (87·5)
Tuberculoid (10 BT + 8 TT)	18	2	0	0	1 (5·5)

Table 3. Bacillary load by the haemolysis method and Petroff's method

Type of leprosy	No. of cases	No. of cases with bacillaemia	Bacillary load/ml blood by	
			Haemolysis method Mean ± SD	Petroff's method Mean ± SD
LL	22	22	1955 ± 150	2550 ± 585
BL	10	6	1308 ± 75	1415 ± 95
BT	10	1	0	560
TT	8	0	0	0

by the buffy coat method (Table 2). Bacillaemia was detected in 1 (5·5%) out of 18 cases in the tuberculoid spectrum of the disease only by the Petroff's method.

Table 3 shows the bacillary load/ml blood as revealed by the haemolysis method and Petroff's method, which were useful to make bacterial counts in the smears of concentrated deposits. Bacterial counts of 2550 ± 585 by Petroff's method as against counts of 1955 ± 150 by the haemolysis method per ml blood were observed in 22 LL cases with bacillaemia. Out of 6 BL cases with bacillaemia, bacillary load of 1415 ± 95 and 1308 ± 75/ml blood was observed by Petroff's and haemolysis methods respectively, whereas bacterial counts of 560 and 0/ml blood were recorded by the respective methods in 1 BT case associated with bacillaemia.

Discussion

In the present study bacillaemia was observed in 29 out of 50 cases of leprosy with a detection rate of 58%. On the other hand the positivity rate of bacillaemia was 85·5% (28 cases) in the lepromatous spectrum of 32 LL–BL cases. Bacillaemia in LL cases by the buffy coat method has been reported by different workers[2,4,9] with success rates of 81·8%, 100%, and 16%, respectively as against our results of 77·3%. Various studies[4-6] with the haemolysis method have shown a positivity rate of 52%, 85·7% and 100% in that order as compared to 91% success achieved in the present study. Importantly, the present work has shown bacillaemia in 100% of LL cases by Petroff's method.

A success rate of 42·8% and 55·5% by the haemolysis method was observed by some workers[4,6] in demonstrating bacillaemia in BL to BT spectrum of the disease. A positivity rate of 60% by Petroff's method was observed in BL cases in our hands. These findings have clearly revealed the superiority of Petroff's method over other methods in the detection of bacillaemia in the lepromatous (LL & BL) spectrum. None of the 3 concentration methods were helpful in demonstrating AFB in the blood of TT cases.

Both the haemolysis method and Petroff's method could be used to assess the bacillary load per millilitre of blood. The smears of concentrated deposit obtained by Petroff's technique revealed only AFB free from artefacts as against the AFB smears of deposit by the haemolysis method. On the other hand, Petroff's method yielded high bacterial counts when compared with the haemolysis method.

In the present study Petroff's methods proved superior to other methods for demonstration and quantitation of bacillaemia in lepromatous spectrum (LL & BL) of the disease, especially in BL cases.

Acknowledgments

The authors are thankful to Dr K V Desikan, Directory, Central Jalma Insitute of Leprosy for kindly supplying lepromin.

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Histopathologic observations on the persistence of *Mycobacterium leprae* in the skin of multibacillary leprosy patients under chemotherapy

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Accepted for publication 11 May 1988

Summary In the study of 782 biopsy specimens from 195 patients during and after chemotherapy we compared the numbers of *Mycobacterium leprae* stained by the Fite–Faraco (FF) and the Gomori methenamine–silver (GMS) techniques. In many patients large amounts of non-acid-fast *M. leprae* or remnants thereof remained 66 months after starting effective multidrug therapy. The GMS stain is a useful method for assessing the efficacy of methods for enhancing bacillary clearance in multibacillary leprosy patients.

Introduction

The Fite–Faraco (FF) stain, or modifications thereof, is used routinely to demonstrate *Mycobacterium leprae* in tissue sections.[1] As the *M. leprae* degenerate in the host, either naturally, or in response to therapy, acid-fastness diminishes and is eventually lost even though whole carcasses of the bacilli and/or bacillary remnants persist.[2] The silvering of leprosy bacilli in tissues was first described in 1948.[3] The Gomori methenamine–silver (GMS) method,[4] as employed by Grocott[5] for fungi, appears to stain all *M. leprae* in tissues, whether or not they have lost their acid-fastness.[2,6] We present here the results of observations on multiple biopsy specimens from 195 treated patients followed over a period of up to 66 months.

Methods

The data reported here are from multibacillary patients in a prospective multicentre multidrug chemotherapy trial in Pakistan (Karachi), India (Bombay, Chetput, Madras) and Sierra Leone (Freetown). Histopathologic classification was according to Ridley–Jopling,[7] and was the criterion used in dividing the patients into borderline–lepromatous (BL), subpolar lepromatous (LL_s), and polar lepromatous (LL_p). Three hundred and seven patients entered the study, but data for this report are from only 195 patients who had multiple skin biopsies stained by both the FF and GMS methods. All patients were treated for 3 years with one of the following drug regimens: DDS (100 mg/da); DDS (100 mg/da) + rifampicin (600 mg/da); Isoprodian (2 tabs) + rifampicin (100 mg/da).

0305-7518/88/059341+06 $01.00

Table 1. Semiquantitative grading scale for *M. leprae* in skin biopsies, for FF and GMS staining

Grade	Number of bacilli	Comparative BIG*
1	none	—
2	rare/occasional	1+
3	few	2+
4	moderate	3+ to 4+
5	large	5+ to 5·5+
6	massive	6+ to 6·5+

* This is an estimation. These values of the Bacterial Index of Granuloma (BIG) are as described in Ridley, DS, *Skin Biopsy in Leprosy* (Documenta Geigy), Ciba-Geigy, 2nd ed. Basle, Switzerland, 1985, pp. 59–60.

One tablet Isoprodian contains 50 mg DDS, 175 mg INH and 175 mg prothionamide. We report on findings in 1504 biopsy specimens taken at approximately 6 months to 1-year intervals over a 66-month period.

Sections from all specimens were stained by a routine FF[1] method and 782 of the same specimens were stained by the GMS[8] technique. Sections were stained by GMS usually only after 18–24 months of therapy. All sections were processed in the same laboratory at the Armed Forces Institute of Pathology (AFIP). The numbers of bacilli in all FF and GMS sections were graded semiquantitatively by the same observer (WMM) (Table 1). Using the assigned values of 1 to 6 designated in Table 1, means were calculated for the bacillary density in both FF and GMS stained sections. Each mean represents evaluations of specimens of 10 or more patients. Regression analyses were performed and correlation coefficients calculated. Testing for significance between differences of means was by the Signed Rank Test.

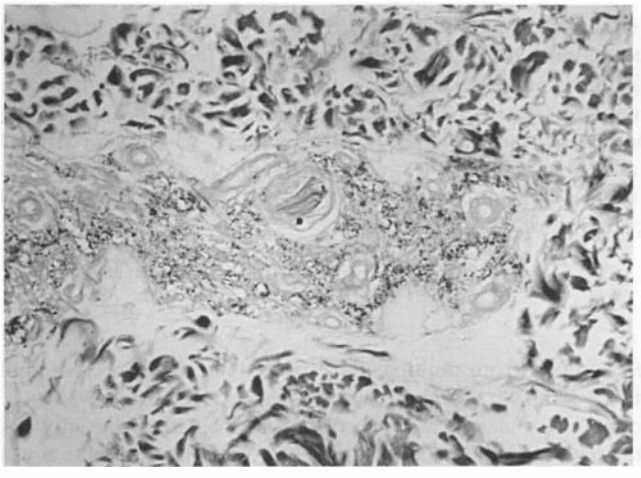

Figure 1. Skin of a subpolar lepromatous patient treated with DDS plus rifampicin for 36 months. This specimen was taken 13 months after cessation of therapy. Note the large amounts of silvered (black deposits) bacillary material in the histiocytes, and smaller amounts in the nerve. Fite–Faraco stained parallel sections did not reveal acid-fast bacilli. GMS stain, × 250. (AFIP Neg. 87–6570).

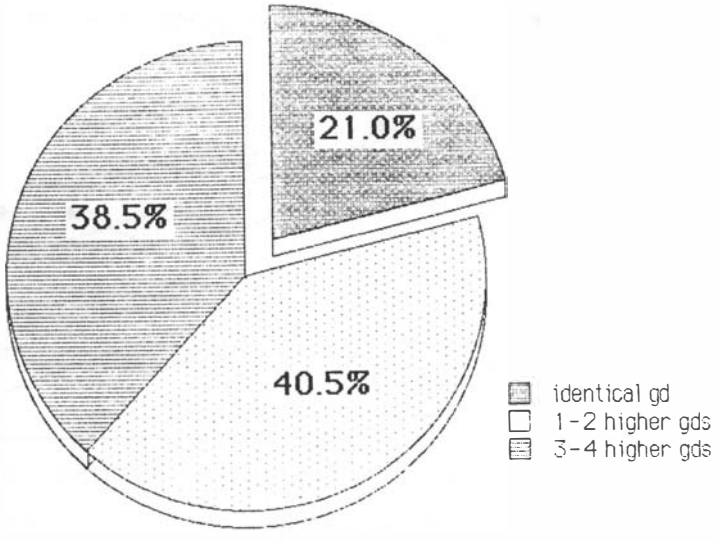

Figure 2. GMS grading in 533 AFB positive specimens.

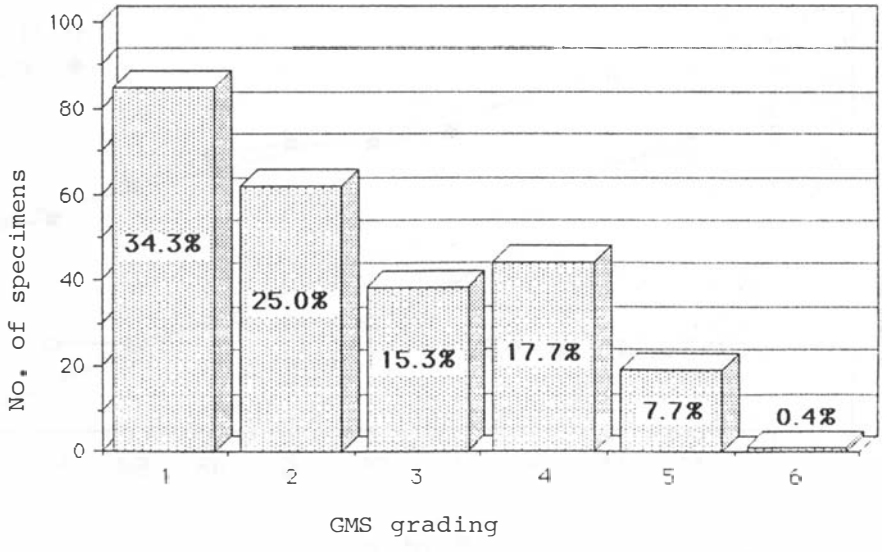

Figure 3. GMS grading in 248 AFB negative specimens (for details of grading see Table 1).

Results

Figure 1 illustrates silvered *M. Leprae* in a GMS stained section of an LL_s patient 13 months after completing 3 years therapy with DDS + rifampicin.

Of the 782 specimens studied by both the FF and GMS stain, 533 were positive for acid-fast bacilli (AFB): 112 (21%) had identical numbers of organisms in the FF and GMS stained sections, and in 421 (79%), the GMS gave higher yields of bacilli (Figure 2). In the 248 sections that revealed

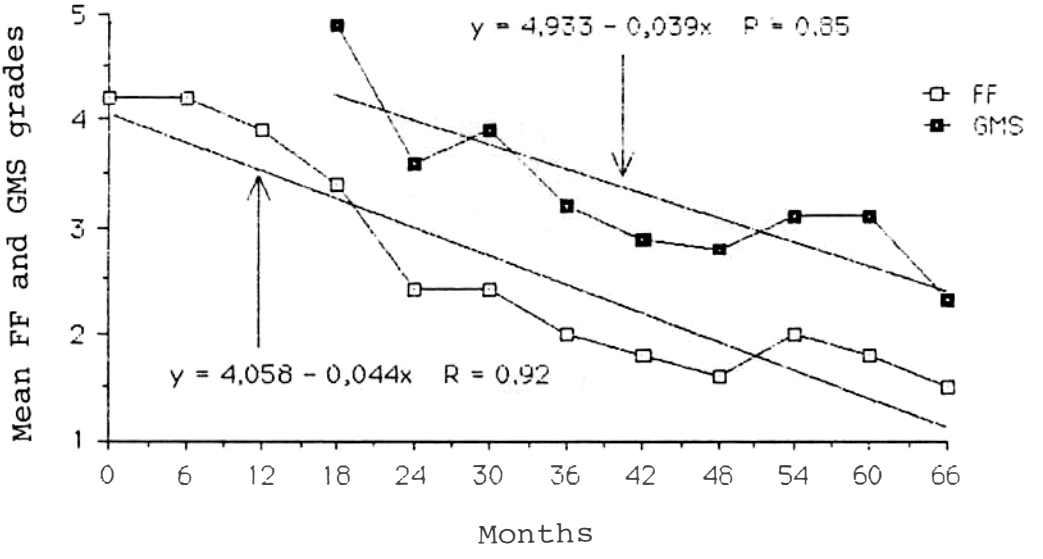

Figure 4. Mean FF and GMS grades during and after treatment in LL_p patients.

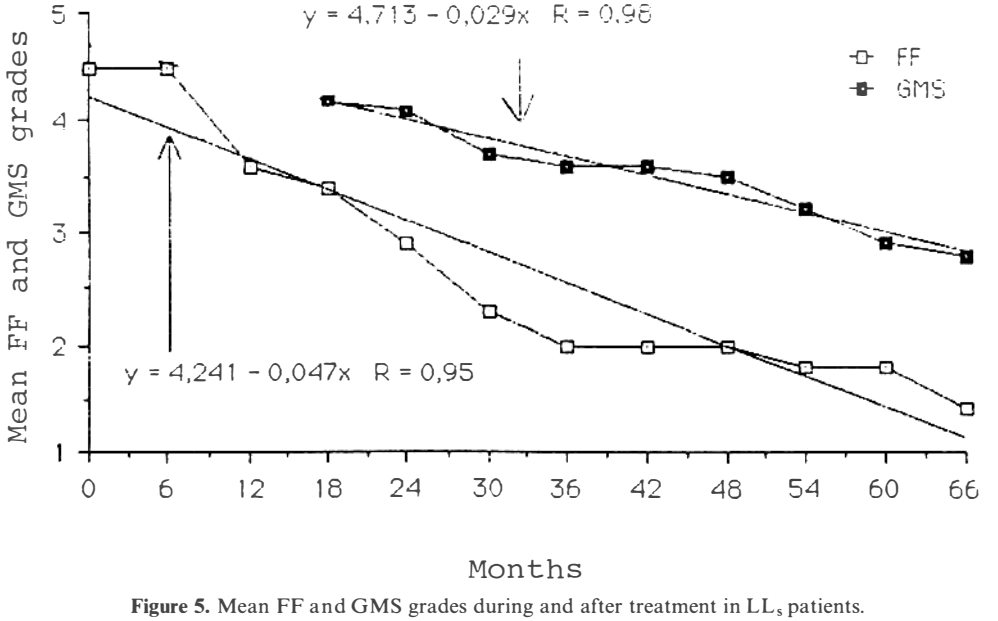

Figure 5. Mean FF and GMS grades during and after treatment in LL_s patients.

no AFB in FF sections, 84 (33·9%) were GMS-negative, and 164 (66·1%) were GMS-positive, with differences ranging from 1 to 5 grades (Figure 3).

Changes in the numbers of *M. leprae* in the tissue sections over the 66-month period of observations in the LL_p, LL_s, and BL patients were evaluated. Regression analyses show linear downward regressions for both staining techniques in all three groups of patients (Figures 4–6). The closest fit ($r = 0{\cdot}98$) was in the specimens from LL_s patients stained by the GMS (Figure 5). The

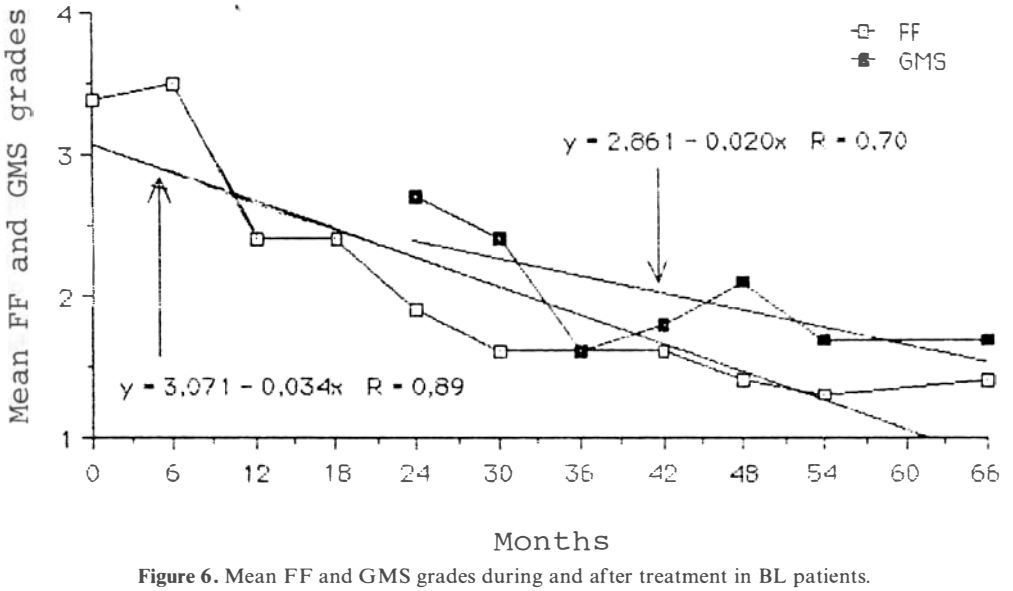

Figure 6. Mean FF and GMS grades during and after treatment in BL patients.

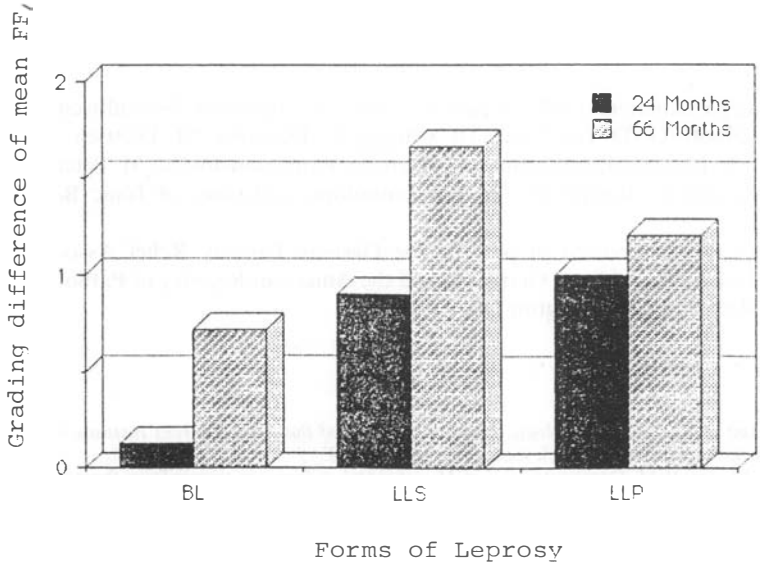

Figure 7. Difference of mean FF and GMS grades at 24 and 66 months for LL_p, LL_s, and BL patients.

GMS sections consistently show larger numbers of organisms than the FF stains. These differences are statistically significant ($p < 0{\cdot}05$) for LL_p, LL_s, and BL patients.

The negative slope of the FF line as compared to the GMS line is always more pronounced, leading to an increase in the difference of the FF and GMS grade means over time (Figure 7). The greater divergence of the regression in LL_s and BL patients (Figures 5 and 6) suggests that LL_s and BL patients are slightly more efficient than LL_p patients in diminishing the acid-fastness of *M. leprae*.

There was no difference in the degree or duration of persistence of carcasses of *M. leprae* in the three different treatment regimens used in this chemotherapy trial.

Discussion

This large-scale study demonstrates the persistence of carcasses and bacillary debris of *M. leprae* long after they have lost their acid-fastness. The persisting carcasses and debris are continuing sources of antigen which contribute to the morbidity, e.g. erythema nodosum leprosum.

The acid-fastness of *M. leprae* is weaker than for other mycobacteria, but as in the other mycobacteria the acid-fastness is related to mycolic acid in the cell wall.[9] The mode of action of methenamine–silver staining is not completely understood, but, in part, depends on the hydrolysis of saccharides to yield aldehyde groups. The silver of the stain is then reduced by the aldehyde of the hydrolysed carbohydrate.[10] Reducing lipids could produce a similar result. Thus, the differential staining of degenerating *M. leprae* suggests that the integrity of the mycolic acids is lost long before the carbohydrate (or lipid components) of the cell wall lose their reducing properties. The identity of the persisting cell wall components is unknown. Immunohistochemical studies with specific antibodies may be useful in the *in situ* identification of the persisting cell wall material, and may provide information on the intracellular digestion of *M. leprae*.

Enhancement of the removal of persisting bacillary material in multibacillary patients (e.g. immunotherapy) is an objective of more effective therapy of leprosy. The GMS stain provides a simple technique for following the efficacy of such treatment modalities as may become available.

Acknowledgments

The following participated in other clinical or laboratory aspects of the multicentre chemotherapy trial: M. Aschhoff, G. D. Burchard, Th. Chiang, V. Devanbu, M. Dietrich, S. Engelhorn, R. Ganapati, P. Irudayaraj, J. Jayakumar, P. Kern, U. Laukamm-Josten, M. Peters, R. Pfau, J. and M. Rangaraj, and L. Revankar. The histopathologic assistance of Isaac Banks is gratefully acknowledged.

This study was supported in part by the German Leprosy Relief Association (DAHW), Würzburg, Federal Republic of Germany, and the American Registry of Pathology at the Armed Institute of Pathology, Washington DC.

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Lep. Rev (1988) **59,** 347–354

Protective sensation in the foot in leprosy

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Accepted for publication 22 June 1988

Summary Plantar ulceration is a significant problem in leprosy patients and accounts for a large proportion of hospital admissions. Three methods of sensory testing were employed to see if a high-risk group of people with loss of protective sensation might be selected, so that the serious first ulcer might be prevented. Three groups of patients were studied: 41 leprosy patients with ulcers, 41 without ulcers and 48 control subjects without leprosy. The results show that either or both Semmes–Weinstein nylon monofilaments and biosthesiometer may prove a more reliable method of sensory testing than the standard WHO pencil stimulus.

Introduction

Plantar ulceration resulting from damage to the anaesthetic foot is a major problem in the management of leprosy patients. Patients with ulcers form a large proportion of those admitted to leprosy hospitals, and these admissions can cause loss of income as well as further physical disability and social isolation for the patients. Loss of 'protective sensation' is a major factor in ulcer formation, which will in turn lead to a scarred foot, even more prone to further ulceration. Therefore, prevention of the first ulcer must be a priority in any leprosy programme.

The strategy for prevention is primarily education of the patient about care of insensitive feet, and the issue of protective footwear such as microcellular rubber (MCR) sandals. With limited resources, the clinician needs to define a high-risk group quickly and easily, in order to concentrate attention on these individuals.

The purpose of this study was to assess two techniques of defining such a group at risk of plantar ulceration. A vibration meter or 'biothesiometer' (Figure 1) has been used in detecting early loss of vibration sensation in diabetic patients in the UK[1] but there is only one report of its use in the leprosy field.[2] The second method was evaluation of pressure sensation using a graded range of nylon monofilaments (Semmes–Weinstein filaments, Figure 2) which have been useful in assessment of mild nerve damage.[3] These monofilaments have been used in research on plantar sensation in patients with leprosy in America[4] but have not been assessed in barefoot populations in the Third World.

The aim has been to compare these two methods of assessment, together with the standard WHO test, and to define a point at which loss of 'protective sensation' may be said to occur.

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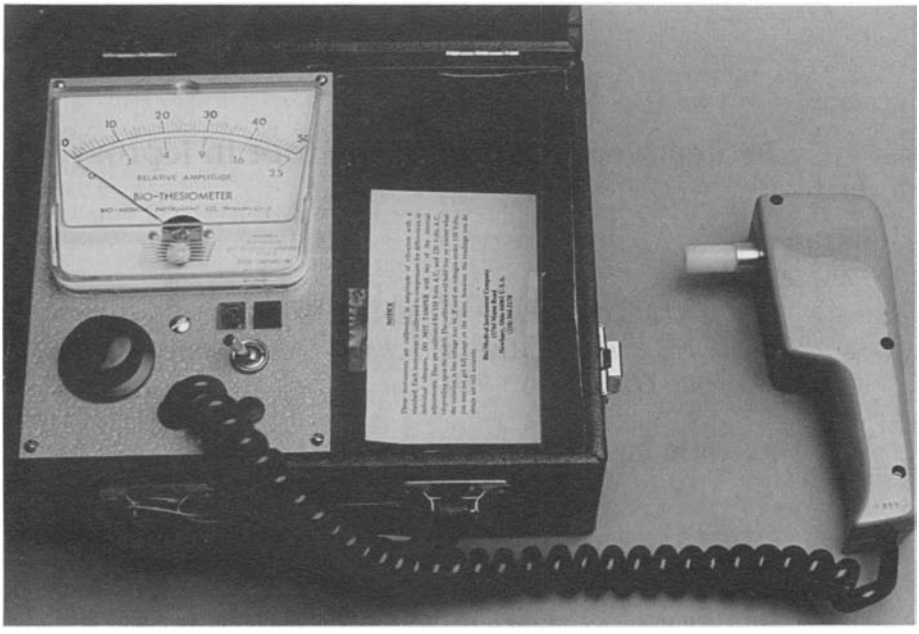

Figure 1. Biothesiometer.

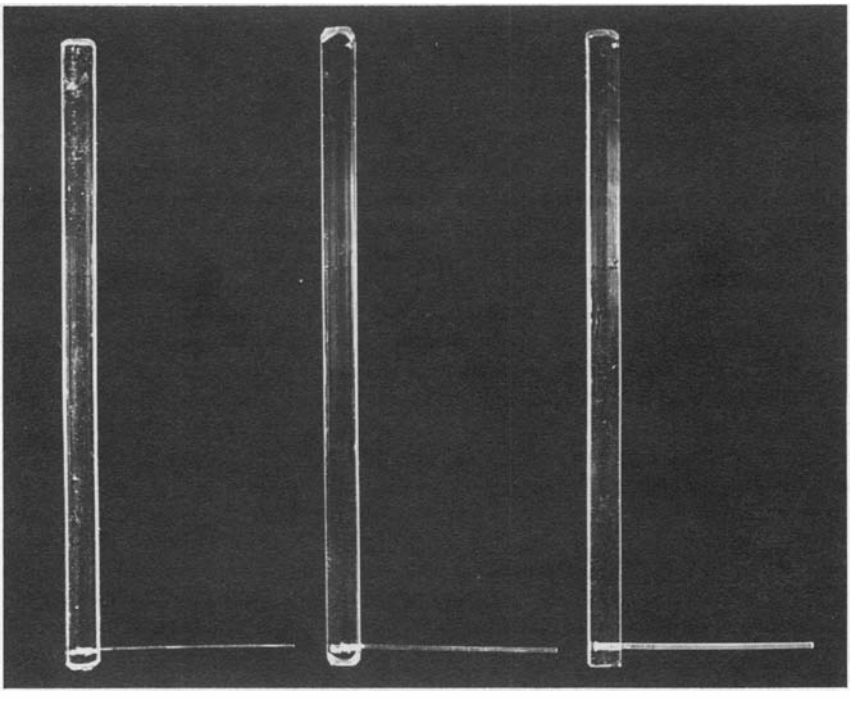

Figure 2. Semmes–Weinstein monofilament.

Materials and methods

Three groups of patients were considered: leprosy patients with plantar ulcers, leprosy patients without ulcers, and a control group without leprosy, all matched for age and sex (see Table 1). The leprosy patients were drawn from in-patients and out-patients at the Dr Bandorawalla Leprosy Hospital, near Pune, India, and the controls were local agricultural workers and non-leprosy patients at the Dermatology Clinic at Sassoon Hospital in Pune. Excluded from all 3 groups were those patients with known diabetes mellitus, any other foot pathology, and those unable to understand the test (of whom there were 4). The group with ulcers included all those with a past history of or present plantar ulceration, but no clinical signs of tarsal disintegration (included in a separate study). The non-ulcered group excluded any leprosy patient with a past history or suspicion of ulcer, and also any patient in lepra reaction.

Materials used were: 1 set of 20 Semmes–Weinstein graded nylon monofilaments (Research Designs Inc.), consisting of monofilaments or 'hairs' range numbers 2·84 to 6·65 (these numbers relate to the logarithm of the force required to bend the hair); 1 biosthesiometer delivering a vibrational stimulus at a fixed frequency of 120 Hz and of varying amplitude from 0 to 25 μm (Bio-Medical Instrument Co., Ohio, USA); a 2B pencil used for WHO standard sensory testing.

Method

Patients were tested in a quiet room, with the aid of a translator. Details of the patient's age, sex, history of past and present ulceration and any history of operations were noted. Disease type, duration and treatment were also recorded.

After adequate explanation and demonstration, the patient's sensory threshold using the nylon filaments was determined at 3 sites; great toe pulp, first and fifth metatarsal heads. The method is described elsewhere,[5] and the threshold was taken as that filament at which the subject could accurately and reproducibly detect the site of pressure stimulus. The highest threshold from the 3 sites was taken as the threshold for that foot.

Standard sensory testing as recommended by WHO,[6] using pressure from a pencil point at the 3 sites was assessed. A 'positive' WHO test was taken as the ability to point accurately to the site of dimpling at all 3 positions, a 'negative' test implying inability to recognize one or more positions.

Vibration sensation was tested at 2 sites, the great toe pulp and the medial malleolus, as described.[7] The vibrating rod was applied at each site with constant light pressure and the amplitude of vibration slowly increased until the subject first noticed the sensation. This was repeated 3 times at each site and the mean amplitude calculated. The sum for each foot was recorded from the means at the 2 sites, with those subjects unable to feel a maximum vibration being given an arbitrary value of 25 μ.

Table 1. Comparison of groups.

	Controls	Patients without ulcers	Patients with ulcers
Number of patients	48	41	41
Number of feet measured	96	82	59*
Age range	13–55	15–54	12–55
Mean age (years)	32·7	31·7	32·4
Sex ratio	32:16	31:10	31:10

* 18 subjects had bilateral ulcers

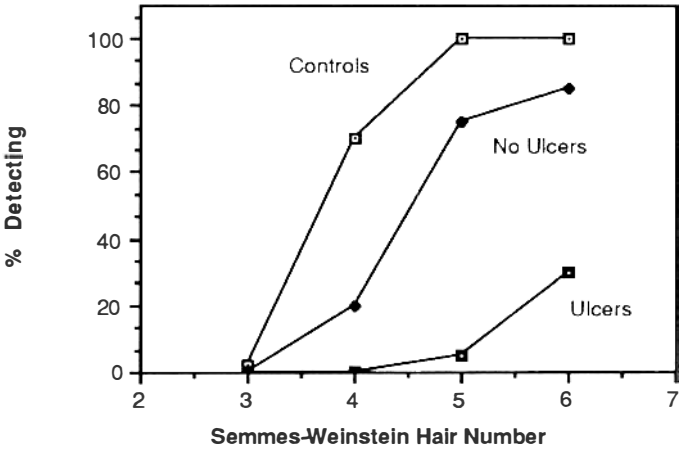

Figure 3. Distribution of pressure sensory thresholds in feet of patients and controls.

Results

The composition of the 3 groups (patients with ulcers, patients without ulcers and controls) are shown in Table 1. 130 subjects were measured, 41 in the ulcered group, 41 in the non-ulcered group, and 48 controls. Fifty-nine feet with ulcers were examined.

Figure 3 shows the cumulative frequency curves for these 3 groups, of sensation threshold when tested with Semmes–Weinstein monofilaments, i.e. the proportion of patients able to feel each filament. Considering the threshold value represented by the 5·07 filament, this could be detected by 99% of the control feet, by 70% of feet of leprosy patients without ulcers, and only 5% of feet of patients with ulcers. (Differences significant at $p < 0{\cdot}01$ using chi-squared test).

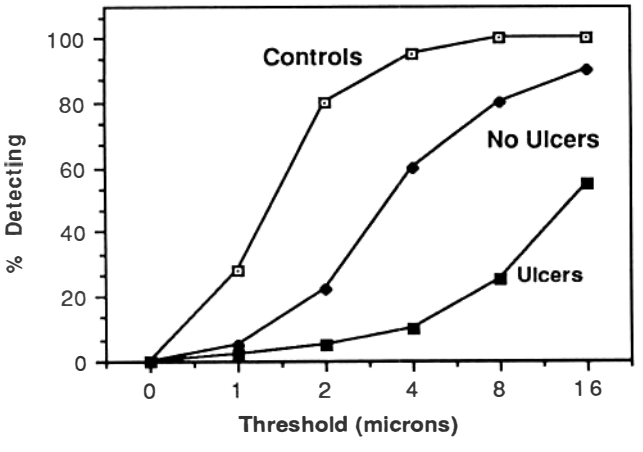

Figure 4. Distribution of vibration sensory thresholds in feet of patients and controls.

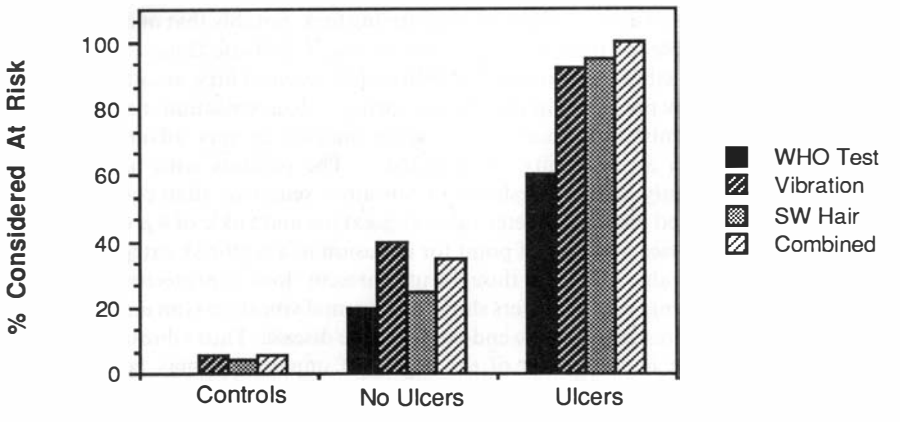

Figure 5. Percentages of patients considered to have lost protective sensation by four criteria.

The cumulative frequencies of vibration threshold are demonstrated in Figure 4 for the 3 groups. Considering a threshold value for a foot of 4 μ this could be detected by 99% of normals. 60% of non-ulcered patients, and 10% of patients with ulcers. (Differences significant at $p < 0{\cdot}01$ using the chi-squared test.)

Sixty-one per cent of patients with ulcers were unable to detect the pencil stimulus as described, and these results are considered in comparison to the other methods of testing in figure 5.

Discussion

The concept that loss of a certain level of 'protective sensation' in anaesthetic feet puts them at risk of plantar ulceration is widely used.[4] The aim of this study was to define this level in patients with leprosy, and to do so using simple clinical tools which might be applicable in a First or Third World hospital. In the past a battery of tests has been proposed in order to evaluate peripheral neuropathy in leprosy patients,[8] including the use of graded nylon filaments and optional low frequency (60 Hz) and high frequency (250 Hz) tuning forks. It was thus of further interest to compare different modalities of sensory testing in the same patients.

Anaesthesia is not the only important factor in the aetiology of plantar ulceration in leprosy. Nerve damage leads to loss of autonomic supply and hence drying of skin, and motor damage can result in undue pressures on certain parts of the sole, e.g. through drop foot.[9] Another extremely important factor is the patient's health education and subsequent personal foot care and use of protective shoes. Thus in the group without ulcers there is a proportion of patients who have lost protective sensation and so are still at high risk of ulceration. Indeed it is exactly these patients whom one wishes to identify, before the first ulcer has developed.[10]

BIOSTHESIOMETER

Although the biosthesiometer has been in use for over 40 years, it received little attention in the UK before the early 1980s. Since then, it has been used in the study of peripheral neuropathy in diabetic patients (another group susceptible to plantar ulceration) in whom the problems of anaesthesia are compounded by the vascular complications of that disease.[11] The vibrometer or biosthesiometer has

a number of advantages over clinical testing using a tuning fork, notably that of quantification[12] and normal values are available for the UK population for use in diabetic clinics.[7]

In contrast, testing of vibration sensation in leprosy has received little attention and it has been thought that it is relatively preserved in this disease, being a 'deep sensation' namely 'position and vibration sensation are entirely normal except in the unusual or very advanced case in which multiple major nerves in an extremity are affected.'[13] The patients with ulcers in this study demonstrated a significantly higher threshold for vibration sensation than controls and patients without ulcers. A combined biosthesiometer value at great toe and ankle of 4 μ would identify 90% of patients with ulcers if used as a cut-off point for inclusion in a high-risk category (see Figure 5), and readings above this value would be thought to represent 'loss of protective sensation' in this disease. That 40% of patients without ulcers showed abnormal vibration is an indication that loss of vibration sensation is not restricted to the end-stages of the disease. Thus vibration sense in leprosy is not only impaired, but measurement of the degree of impairment may be of some value in distinguishing patients at risk of ulceration.

Advantages of the use of a biosthesiometer in this situation are that it is an easily reproducible test, and relatively easy for the patients to understand. In practical terms it is expensive and requires an electrical supply which is not always available in the Third World clinic. A further disadvantage is the increase in normal thresholds, both mean and variance, with age,[7] thus limiting its usefulness over the age of 60. However, out of a potential population of over 80 patients with ulcers, only 2 were over 60 year old, and this would not appear to be a serious practical limitation in leprosy work. Other potential limitations have been pointed out by Williams *et al.* in a recent paper.[14]

SEMMES–WEINSTEIN MONOFILAMENTS

Nylon filaments have been previously recommended in the identification of sensory neuropathy in leprosy[8] and have been used in the United States to define a minimum sensory level in patients with ulcers.[4] It was suggested that similar experiments should be performed in the Third World, where barefoot walking is common and normal values may be different. Our results provide further evidence that such testing is of value in identifying loss of 'protective sensation'. Use of a threshold value of 5·07 (11·8 g) would identify 95% of patients with ulcers if used as a minimum cut-off point foridentification of a high-risk group, and by this criterion 24% of patients without ulcers would be in such a category. These results agree with those of Birke & Sims,[4] who also proposed a 5·07 filament for this purpose. It is interesting to note that the average value of our control population (4·11) does not differ significantly from their normal quoted value (4·17), despite the fact that the majority of controls were accustomed to barefoot walking. Loss of vibration sensation accompanies loss of pressure sensation, a correlation first suggested by Von Frey in his seminal work on the subject.[14]

Although use of a set of monofilaments is a slow and expensive method of testing, use of a single filament such as the 5·07 hair as a proposed discriminatory test is quick, and it is possible to make such individual filaments.[8] One disadvantage of this method of testing is that the patients find it relatively difficult to understand, and care must be taken to avoid 'cheating'. The force exerted by a filament is thought to change with a variety of factors including temperature and possibly wear, so regular recalibration may be required. It has been pointed out that the monofilaments are 'simple to use but easy to misinterpret.'[16]

WHO STANDARD METHOD

The WHO-recommended method of testing has the advantage of being quick, simple and cheap, but lacks the reproducibility of either of the above methods. Although a pencil was used in this study, many centres use a ballpoint pen which has a more constant tip, therefore minimizing variation in pressure with a constant force.

In this series only 61% of patients with ulcers would be said to have lost protective sensation by this criterion, and so a significant proportion of such patients would not have been included in a high-risk group. In many ways, use of a single hair may be regarded as a more refined version of this test, with greater sensitivity and reproducibility.

COMPARISON OF METHODS

Four methods of identifying high-risk patients are compared in Figure 5—5·07 monofilament alone, biosthesiometer alone, WHO test alone and filament and biosthesiometer combined. Use of the last method would identify 100% of patients with ulcers as belonging to the high-risk category, while use of a 5·07 filament alone would identify 95% of patients and could thus provide a useful screening test in a Third World clinic. An additional 24% of patients without ulceration would have lost protective sensation by this criterion.

Since this is a retrospective study it is not possible to give predictions of risk on the basis of this data, but as such it might form the basis of a prospective investigation.

Conclusion

It has been proposed that measurement of vibration sensation may be a useful clinical tool in the assessment of the insensitive foot in leprosy, even in the relatively early stages of the disease. The use of a single monofilament either alone or in combination with biosthesiometry might prove a reliable method for identifying patients at high risk of plantar ulceration in a leprosy clinic. For this purpose, a 5·07 monofilament would appear to be the most appropriate.

It should, however, be emphasized that simply identifying a patient as 'high-risk' is only a small part of ulcer prevention. Much work needs to be done before this stage in early detection and treatment of leprosy, and much remains to be done afterwards, for effective patient education in foot care and lifelong footwear is an all too often neglected part of leprosy management.

Acknowledgments

We would like to thank all the staff at the Dr Bandorawalla Leprosy Hospital, Pune, its Hon. President Dr Jal Mehta, and Dr Sunil Tolut, Dermatology Dept., Sassoon Hospital, Pune, for their help and support with this project. We are indebted to Mr Anjan Dei for his help with translation. We would also like to thank Dr Colin McDougall and Professor L Klenerman for providing us with ideas and equipment.

We are grateful for grants from LEPRA, *Journal of Bone and Joint Surgery,* St Francis Leprosy Guild, Medical Defence Union, Anglo-American Corporation (Zimbabwe) Ltd, and Oxford University Medical School.

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Letters to the Editor

THE ACTUAL LEPROSY SITUATION WITHIN KATSINA STATE, NIGERIA

Sir,

Katsina State, one of the 21 states of Nigeria, is situated in the central upper north of the country bordering Kano State in the east, Kaduna State in the south, Sokoto State in the west and Niger Republic in the north. It covers an area of 23,400 sq km and has a population of 3·5 to 4 million people.

As calculated from the only data available to us (C M Ross 1951, WHO–LAT survey 1960, NSL survey 1977 and our own findings 1983–88), the actual leprosy situation at the present time seems to be the following:

With a prevalence of 1·5–2 per thousand, there are in total some 6500 leprosy patients with signs of either active or inactive infection, of whom 1200 patients have multibacillary and 5300 paucibacillary leprosy.

Of these 6500 leprosy patients, a minimum of 5500 patients have been registered at least once in their life and as such have received or are still receiving antileprosy drug therapy, so far only monotherapy with DDS. At the moment there are still some 3300 leprosy patients on the treatment registers.

We regard any patient who is still active after 5 years of treatment for multibacillary leprosy, or 2 years of treatment for paucibacillary leprosy, as being dapsone resistant. On this basis, there are at present a maximum of 120 patients with probable dapsone resistance between Babbar Ruga Hospital Clinic and the other clinics in this area.

There are a maximum of 250 new leprosy patients a year, giving an annual incidence rate of 0·0625 per thousand; this reflects the transmission of some 2–5 years ago. But it is important to record that we have in this State over 60% disability grade 2 or more (involving hands in 50%, feet in 30% and eyes in 15% of all 6500 patients). There are therefore at least 4000 leprosy patients with gross disability of hands and/or feet and/or eyes.

Conclusion and prognosis

Reviewing the trend over the last 35 years, the leprosy 'epidemic' in Katsina State is almost under control, and with an intensified control programme involving better organization/documentation/recording/supervision, active case finding, introduction of MDT etc., it should be possible to almost completely interrupt transmission by the year 1995, so that by the year 2000 there will be no more than 200–300 leprosy patients in need of drug treatment.

However, in view of the enormous disability rate, care (rehabilitation, footwear, ulcer care, reconstructive surgery, etc.) for some thousands of patients has to be continued at least until the year 2010—if not longer.

K WAALDIJK

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CARCINOMA IN PLANTAR ULCERS OF LEPROSY PATIENTS: A REPORT OF 4 CASES FROM TURKEY

Sir,

Plantar ulcers are a commonly observed complication in leprosy. Malignant development may be associated with chronicity, continuous trauma, neglect of skin care or osteomylitis.

Publications on the subject are few and generally in the form of case reports. In this report we present 4 cases observed during the period 1984–87 at Istanbul Leprosy Hospital.

Case Reports

Case 1. A 56-year-old male farmer from Kars. Formerly classified as having lepromatous leprosy (LL). The patient was hospitalized due to a chronic plantar ulcer of 3 years duration on his right foot. The biopsy results of the ulcer showed it to be 'epidermoid carcinoma'.

Results of a previous biopsy taken at Diyabakir were reported as 'epidermoid carcinoma in primary stage'. The report of further lymph node and lesion biopsies taken on 5 July 1984 was 'reactive hyperplasia in the lymph node and epidermoid carcinoma in the lesion'.

On 19 July 1984 a below-knee (BK) amputation was performed on the patient and a prosthesis was made following the healing of the wound. The patient is presently still alive with no evidence of metastasis.

Case 2. A 45-year-old female from Tunceli, classified as having LL. She had a plantar ulcer of 20 years duration from which a biopsy was taken and reported as 'Grade 1 epidermoid carcinoma'. A BK amputation was performed, a prosthesis fitted and the patient was walking well prior to discharge. One year after discharge, she was re-admitted with a new lesion on the same leg which on biopsy showed 'epidermoid carcinoma'. The patient, who was told that she couldn't benefit from chemo- or radiotherapy, died at home.

Case 3. A 50-year-old female from Samsun, classified as borderline tuberculoid (BT) leprosy. In December 1985, this lady was examined in her home and a plantar ulcer on her left foot of 20-years duration was discovered. The basic steps in ulcer care were explained to her. In March 1986, she was admitted to hospital with a 'cauliflower-like' growth on the plantar ulcer site and enlarged groin lymph glands. Lesion and nobe biopsies revealed epidermoid carcinoma. Her condition gradually deteriorated and a few months later she died.

Case 4. A 53-year-old male, farmer from K. Maras, classified as BT. In May 1986, this patient was seen in his home with a chronic plantar ulcer. In April 1987, he was admitted to hospital with a 'cauliflower-like' growth on the ulcer. Biopsy results showed epidermoid carcinoma. On 7 May 1987, a BK amputation was performed, later a prosthesis was fitted. This patient is doing well to date following the BK amputation.

Discussion

The time factor has a significant influence on plantar ulcers becoming malignant.[2,4] Malignant degeneration of plantar ulcers of between 3- and 20-years duration was seen in the 4 cases in Turkey and this correlates with findings in other reports.

Generally, the incidence of malignant plantar ulcers is not very high.[1,2] Of the patients examined in this hospital over the past 10 years only 4 cases of carcinomas were detected.

Carcinoma is generally seen in leprosy patients over 30 years old.[2–4] Our cases however were in the 45–56 age group. In other studies the incidence of carcinoma was higher in men[2 4] but in our cases the male : female ratio was equal.

According to Fleury,[2] TT and BT types of leprosy are more prone to malignancy. Out of our 4 cases, 2 were BT and 2 were LL. The reason for malignant change occurring more often in TT and

BT groups is presumably due to the greater frequency of peripheral nerve damage and consequent chronic ulcers.

For 3 of the cases reported here, BK amputations were performed.

Chronic ulcers that have degenerated into malignancy, can easily escape our notice if we are not careful. The diagnosis may be delayed for years and thus the possiblities for treatment are greatly reduced. For this reason it is important to check suspicious chronic ulcers for the present of malignant degeneration, at frequent intervals. It would be interesting to know, perhaps from your readers, if there is any evidence that malignant degeneration is commoner (or perhaps less common) in chronic ulcers due to leprosy, as compared with similar ulcers due to other conditions?

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LEPROSY IN BANGLADESH

Sir,

In January–February 1988 I had the opportunity to visit Bangladesh and to work in a leprosy hospital about 150 miles east of Dhaka. I thought it might be of interest to record some of my impressions for your readers.

Bangladesh is a delta region formed by the rivers Ganges and Brahmaputra. It is bounded by India on 3 sides and has a small boundary with Burma (Figure 1). The land is largely flat and fertile, the main crop being rice. Other crops include jute, tea and cotton. Eighty per cent of the population live in the rural areas and depend on agriculture for their livelihood. Most have a hand-to-mouth existence.

I was very apprehensive about heading off to Bangladesh on my own and standing in the crowded customs department of Zia International Airport, with no sign of the person who was to meet me, I thought my worse fears had been realized. But I soon got through the customs, saw the board held-up with my name on it and I was on my way through the crowds of beggars and taxi drivers, to a jeep bound for Dhaka. I had several days in Dhaka at the beginning of my elective, as it was not considered safe for me to travel the 150 miles by train to Kamalganj alone. There I visited the centre for rehabilitation of the paralysed which is run by an expatriate, Valerie Taylor, she set up the centre several years ago when she came to Bangladesh as a physiotherapist, and they now have around 100 patients in their care.

The centre is run by volunteers and they concentrate on making patients self-sufficient. They deal with medical problems such as bed sores, then using physiotherapy, occupational therapy and a lot of encouragement, set about the slow progress of rehabilitation, I found all the volunteer workers to be very dedicated and I was surprised to see how cheerful all of the patients were and how well they coped with their disabilities. Most of the patients are involved with handicrafts or painting, not just as a mode of occupational therapy, but as a way to earn a little money.

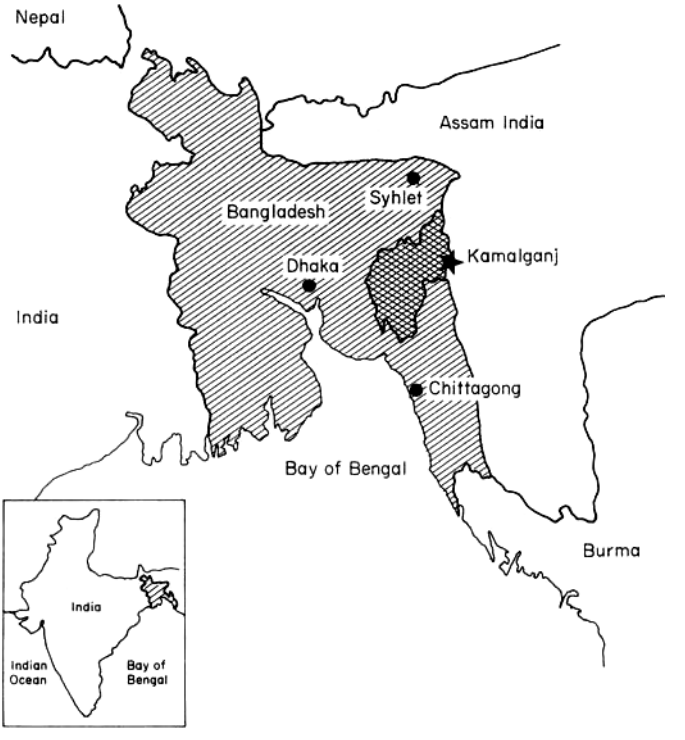

Figure 1.

I travelled to Kalmalganj on the Joyantika Express and we arrived in Kamalganj at 8.30 pm after a 40-minute jeep ride along a 6 mile dirt-track, which is the main road. While there I stayed in the HEED (Health, Education and Environmental Development) Guest House, situated to the rear of the hospital, a beautiful, peaceful place, surrounded by pineapple groves and Bamboo forests. I paid 145 Taka (£2.50) per day for full board.

Bangladesh is the most densely populated rural nation in the world with a population of approximately 100 million which is increasing rapidly. Bengali is the official language; English is spoken only among the educated. I found the language barrier at times very frustrating and often had to rely on paramedical workers to act as interpretors, but by the end of my elective I could carry out a short conversation and had acquired a limited vocabulary.

The life expectancy of the average Bangladeshi is 47 years, with only 1 doctor for every 8500 people. In Dhaka there is only 1 hospital bed for every 1500 patients. The infant mortality rate is 135 per 1000 live births.

Bangladesh is generally said to have a typical tropical monsoon climate. There is a wide variation in temperature. I was there during its winter months where the temperature ranged from 6°C at night to 27°C during the day. During the summer (April–September) the temperature can reach 40°C and the relative humidity is high 80–95%. The weather is very changeable with cyclones and monsoons resulting in flooding and extensive damage.

The HEED Leprosy Control Project and Hospital are located near Kamalganj, a sub-district or Upazula of Moulvibazar District. It is about 150 miles east of Dhaka on the main railway line to Sylhet. In 1976 the Government of Bangladesh gave HEED a mandate to conduct a Leprosy Control Programme in Kamalganj Upazula, using the existing facilities which had been established in 1968 as a leprosy sanatorium. A base hospital was built in 1979 and the leprosy work in the Tea

Gardens commenced in 1981. There are now 49 out-patient clinics which are visited on a monthly basis, most of them are in the tea gardens where leprosy remains a major problem.

In 1988 Teleopara out-station was opened, so allowing paramedical workers to stay permanently outside Kamalganj and provide a local service to the leprosy patients to the South of Moulvibazar District. The Leprosy Hospital is a modern building with 38 beds, most of which are reserved for leprosy patients, a small number being available to tuberculosis, and patients requiring minor surgery. The hospital has its own operating theatre, physiotherapy department, pharmacy, laboratory and out-patient area. There are also facilities for making shoes, wheelchairs and walking aids. The hospital staff consists of 2 medical officers, Dr Ian Cochrane and Dr Francoise Luthie. The remaining staff are Bangladeshis—12 paramedical workers, 5 nurses, 2 physiotherapists, shoemaker and orderly. Most of the staff are Christians and religion plays an important part in everyday life.

The prevalence of leprosy in Bangladesh is approximately 9 per 1000 population. The HEED Leprosy project is responsible for the care of 1400 patients. The vast majority of these being situated outside Kamalganj Upazula.

The main workload for the staff at Kamalganj centres on the use of multiple drug therapy, using dapsone, clofazimine and rifampicin, as recommended by WHO in 1982 (Technical Report Series 675). Finding defaulters and maintaining a review system after completion of treatment adds to the already stretched resources of the leprosy programme.

During my elective, I saw mainly leprosy patients and became familiar with their examination and charting of findings. I saw many disabilities associated with late detection and treatment. I also learned the procedure for taking skin smears, skin biopsies and how to stain them with the Ziehl–Neelsen method.

Health education is very important for leprosy patients and I watched paramedical workers teach the patients how to care for their feet, which are often involved with trophic ulcers.

The staff at Kamalganj said that attitudes to leprosy are improving. Patients coming earlier for treatment and that they are accepting that leprosy is not a curse from Allah. I was impressed by their dedication and their determination to keep the programme going.

There was an out-patient clinic every Monday where general medical patients would attend. The conditions included pulmonary and skin tuberculosis which are relatively common, as are skin disorders such as leucoderma, scabies and fungal infections. Pneumonia, asthma and bronchitis were frequently seen. Buerger's disease and peripheral vascular disease are common because of the high cigarette consumption.

I found all the signs to be much grosser and the diseases more advanced on presentation. Infectious diseases, especially the diarrhoeal diseases, were very common, including dysentry, cholera and typhoid. Another problem disease is malaria, especially chloroquine-resistant disease due to *P. falciparum*.

In Bangladesh the effects of a poor diet are constantly present, although I found it ironic that in Dhaka, which has become relatively Westernized, ischaemic heart disease, diabetes mellitus and obesity are becoming such a problem that dieting is fashionable amongst the affluent.

I feel my elective in Bangladesh was a very worthwhile experience—I met so many different people and learned at first hand about the problems they face in a poor developing country. I also saw leprosy and many other conditions which are rare in Britain and began to appreciate the extent to which socio-economic and cultural factors affect peoples lives and their patterns of illness.

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SUSAN M RANCE

SLIT-SKIN SMEARS FROM THE FINGERS IN LEPROSY

Sir,

In previously published studies[1–4] attention has been drawn to the interesting and unexpected finding of positive slit-skin smears in the fingers of leprosy patients. In view of this we decided to investigate the value of including this site in a study of 278 smears taken from 220 patients in the LEPRA Control Project, based in Lilongwe, Malawi, Central Africa, during the period July 1986 to May 1987.

Our trained leprosy control assistants were instructed to take a slit-skin smear from the finger of all multibacillary patients on active antileprosy chemotherapy as well as any new ones who presented themselves during the course of the study. The finger site was to be included as an extra site whenever a smear was being taken. We routinely take slit-skin smears from multibacillary patients approximately every 6 months. Our routine sites are both earlobes plus at least 2 other sites corresponding with active or old, previously smear-positive, skin lesions.

We chose the dorsum of the proximal phalanx of the 3rd digit (middle finger) of the left hand. The skin is loose enough to pinch and smear properly. The site bleeds easily after releasing the skin, but the bleeding is also easily stopped. We had no reported complications of any kind.

As can be seen from the Tables 1 and 2, the finger site was mostly not useful. In only 3·2% (9/278) of the smears was the Bacteriological Index (BI) of the finger site higher than any of the routine sites. In 71·2% (198/278) of the smears the BI of the finger site was actually the lowest or equal to the lowest routine site. Furthermore, the average BI of the finger sites was much lower than the average BI of the highest routine sites and in fact compared almost equally to the average BI of the lowest routine sites.

The value of the finger smear might have been twofold: 1, in a suspected new patient a positive finger smear in the absence of any other positive site would certainly influence the treatment to be given and maybe even clinch the diagnosis in an otherwise clinically doubtful case. This did not occur in our study which included 32 new multibacillary patients; 2, in a multibacillary patient on antileprosy chemotherapy a positive finger site in the absence of other positive sites would influence

Table 1. The BI of the finger site compared to the BI of the highest routine site (earlobe or lesion) from 278 smears taken from 220 patients

BI finger site \ BI highest routine site	0	1	2	3	4	5	6
0		22	39	22	5	5	1
1	2	4	16	9	7	6	3
2	1		11	12	14	8	2
3	1		3	12	23	10	9
4					5	5	10
5					1	4	4
6						1	1

Notes: 1 Average BI of finger site: 1·63.
2 Average BI of highest routine site: 3·32.
3 Finger site is highest: 9/278 = 3·2%.
4 Finger site is the only positive site: 4/278 = 1·4%.

Table 2. The BI of the finger site compared to the BI of the lowest routine site (earlobe or lesion) from 278 smears taken from 220 patients

BI finger site	BI lowest routine site 0	1	2	3	4	5	6
0	63	16	10	5			
1	10	15	13	5	4		
2	13	5	19	9	2		
3	3	6	23	17	8	1	
4	1		2	8	6	2	1
5			1	3	3	2	
6				1		1	

Notes: 1 Average BI of finger site: 1·63.
2 Average BI of lowest routine site: 1·62.
3 Finger site is lowest: 76/278 = 27·3%.
4 Finger site equals lower routine site: 122/278 = 43·9%.

the length of treatment before discharge. This indeed occurred in 4 of our patients as can be seen in Table 1. In these 4 patients the treatment was extended because of their positive finger sites. All 4 subsequently produced a BI of zero at their finger sites and were then discharged from treatment. (Our current policy is to treat until smear negative.) All 4 patients had their treatment extended by about 1 year.

Because of the very small percentage of finger smears which would influence a clinical decision we have in our project decided not to use the finger site routinely.

These observations from Malaŵi are clearly at variance with those already reported in the literature. Furthermore, Jopling has recently confirmed the value of examining the fingers in a report of his investigations of the Malta Leprosy Eradication Project.[5] We consider that our selection of patients and laboratory techniques were comparable to those of the other investigators, and we are at a loss to explain the markedly different results obtained. It would be of interest to learn of the experience of clinicians from other parts of the world.

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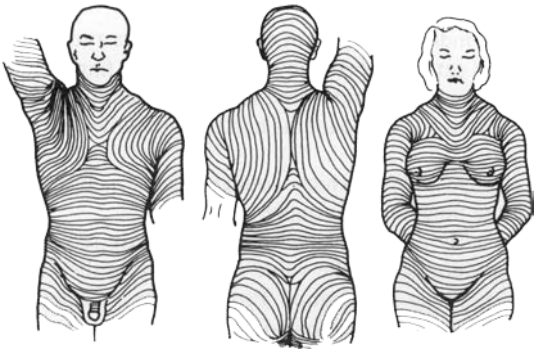
Teaching Materials and Services

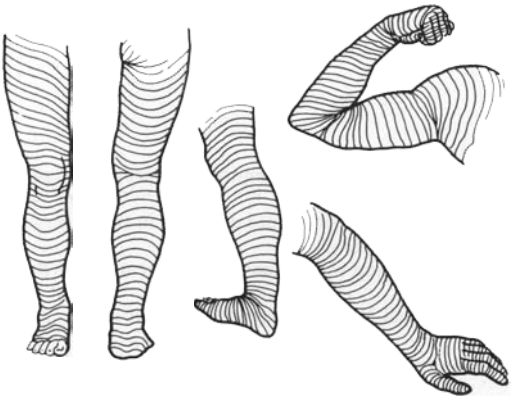
Skin biopsy; 'ABC of Dermatology'; British Medical Journal

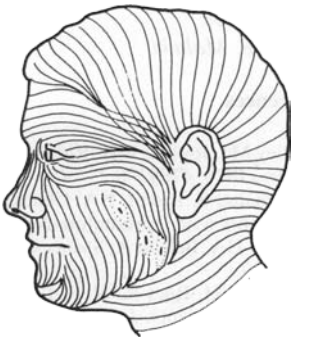
We are most grateful to the author, Dr D W S Harris, Department of Dermatology, The Royal Infirmary, Edinburgh, EH3 9YW, Scotland and to Dr Stephen Lock, Editor of the *British Medical Journal*, for permission to publish this section from a recently published article: *BMJ*, **296,** 12 March 1988. Although intended for dermatologists, there are several important points of technique which will repay attention.

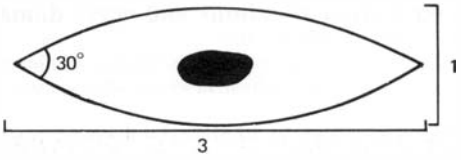
Many common conditions can successfully be dealt with by simple techniques which, once acquired, can easily be used in general practice. In this article several of the most useful are discussed.

Skin biopsy is used to establish a diagnosis (incisional) or to remove a lesion (excisional). In both cases the incision should be elliptical and should run parallel to the skin wrinkle lines.









As a rule of thumb the long axis of the wound should be about three times as long as its short axis. The apical angle should be no more than 30°.

Procedure

(1) Explain the procedure to the patient, warning about the scar that will result from it. This may be slight on the face but more prominent in areas susceptible to keloid formation, such as the sternum, shoulders, and upper outer arms.

(2) Establish that the patient is not allergic to local anaesthetics.

(3) Obtain consent.

(4) Mark out the planned incision with sterile gentian violet before injecting the local anaesthetic.

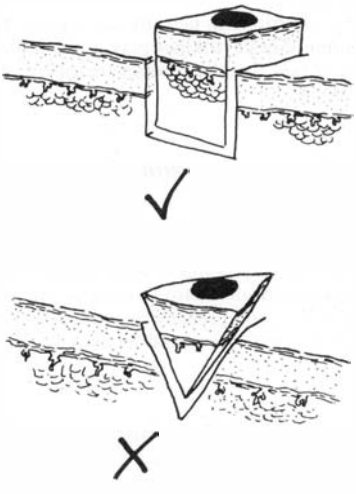
(5) Anaesthetize with 1–2% plain lignocaine. Lignocaine–adrenaline combinations, though they help to reduce bleeding, should not be used on the extremities. The mild discomfort of the injection may be lessened by injecting very slowly and avoiding lignocaine–adrenaline mixtures.

(6) A number 15 blade is used, cutting at 90° to the skin surface. Wedge shaped incisions heal poorly, as illustrated.

(7) A skin hook may be used to lift one end of the specimen to release its underside. Forceps crush specimens and cause pathological artefacts.

(8) Removal of sutures—from the face at 4–5 days, from the trunk at 7 days, and from the leg at 10 days.

(9) Elevation and compression bandaging are advisable when removing lesions from the lower leg.



Guidelines for the development of a national AIDS prevention and control programme

'The epidemic of Aids (acquired immunodeficiency syndrome) is a world health problem of extraordinary scale and extreme urgency. It represents an unprecedented challenge to the public health services of the world. This book is the first in a series of publications to be produced by WHO with the aim of helping national authorities to meet this challenge. It provides information on the establishment and organization of a national programme for the prevention and control of AIDS, covering definition of programme objectives, development of strategies, identification of appropriate activities, and evaluation of achievements and disease trends.'

Apply: Office of Publications, WHO, 1211 Geneva 27, Switzerland. Price US $4.80.

Promotion of research on leprosy reactions and nerve damage

From the *TDR Newsletter* No. 25, Winter/Spring 1988:

'During the last decade, advances in research on the chemotherapy and immunology of leprosy have led to significant improvements in leprosy control tools, which, in some endemic areas, have dramatically changed the pattern of leprosy.

Little or no progress has been made, however, in research on leprosy reactions and nerve damage—two important areas of prevention and treatment. The involvement and later destruction of the peripheral nerves is a specific universal characteristic of leprosy, and the consequences, particularly deformities and disabilities, are of great significance both to the patient and to the community.

The onset of nerve damage in leprosy can be either rapid or insidious. However, recent information indicates that insidious "quiet nerve paralysis" (silent neuritis) is far more common than previously suspected.

Leprosy reactions, apart from producing considerable physical and mental discomfort, are a major cause of nerve damage and consequent disability. Leprosy reactions can sometimes occur even after the cessation of multidrug therapy, potentially undermining patient confidence in the efficacy of modern treatment. Furthermore, some of the advantages expected of fixed-duration multidrug therapy—diminished workload and lowered costs of leprosy control—may be offset by requirements for the treatment of leprosy reactions, which requires care that is often more demanding than chemotherapy itself.

The apparent lag in the development of new methods for the prevention and treatment of nerve damage and leprosy reactions is due largely to the fact that the pathogenesis of these processes is not fully understood. In addition, to date no suitable animal model has been developed for the study of nerve damage or leprosy reactions. Consequently, fundamental research is urgently needed to develop a better understanding of the mechanisms involved in both disease processes. Animal models should be established, and new methods of preventing and controlling nerve damage and leprosy reactions, including systems for screening such methods, should be developed. These research activities represent a challenge to both clinicians and basic scientists.

The TDR Steering Committees on the Chemotherapy (THELEP) and the Immunology (IMMLEP) of Leprosy have singled out these activities in their strategic plans for future research. Interested scientists are invited to submit research proposals related to nerve damage and leprosy reactions. Specific research topics include:

- development of suitable animal models;
- elucidation of effector mechanisms in peripheral neuritis and leprosy reactions, including definition of the antigens involved and of the role lymphokines/cytokines play in the inflammatory process;
- development and/or identification of new drugs for better treatment of neuritis and leprosy reactions.

H. Engers and Ji Baohong

Leprosy for medical practitioners and paramedical workers; R H Thangaraj and S J Yawalkar

The third (revised) edition of this excellent booklet (1988), published by Ciba-Geigy Ltd, Basle, Switzerland, and available free of charge, has now appeared and will be distributed at the *XIIIth International Leprosy Congress in the Hague.* There are no fewer than 135 colour pictures of high quality, together with a considerable number of diagrams and charts of great practical value. Both authors have wide experience of leprosy, particularly in India, and the text is at the same time informative, comprehensive and readable. This booklet is almost certainly the most up-to-date and useful of its kind in existence, and as with the previous editions, will surely have an enormous circulation.

OXFAM–LEPRA pack of teaching–learning materials on leprosy

The 'mini-pack' of 10 documents was started in 1983 and sold about 670 packs by the time the service was stopped in mid-1988, with distribution to virtually all leprosy-endemic countries in many different parts of the world. A few 'archives' copies have been retained for reference. Including the contribution of the original large pack of 25 documents, started early 1982, OXFAM has distributed over 10,000 separate items of teaching–learning material in this way. There is a possibility, still under discussion, that a similar pack will now be assembled for tuberculosis.

Medical Education Newsletter, Dundee, UK

We have just received the latest issue of the excellent Medical Education Newsletter from Dundee; Centre for Medical Education, The University, Dundee DD1 4HN, Scotland, UK, which, as usual, contains dozens of interesting items of information. Headings include: International Encyclopedia of Teaching and Teacher Education; Diploma Course in Medical Education; Distance Learning; BLAT Centre for Health and Medical Education, London; Computers in Medical Education.

Technical guide for sputum examination in tuberculosis

This excellent booklet on sputum examination in tuberculosis is available from the International Union Against Tuberculosis, 3, rue Georges Ville, 75116, Paris, France. It describes collection, storage, the laboratory, registration of specimens, preparation of smears, staining, microscope examination and recording systems. This extremely clear and practical guide should be in the hands of all who run tuberculosis control programmes (and translated if necessary).

Teaching Aids at Low Cost (TALC), UK

TALC, PO Box 49, St Albans, Herts, AL1 4AX, England, produce books, slides and 'accessories' on; Mother and Child Care; Nutrition and Child Growth; Disability and Appropriate Technology; Health Care Services; Education and Communication; Medicine (the latter including 'common medical problems in the tropics', etc).

The slide sets have 24 colour transparencies, available either as strips, or ready-mounted, or mounted and in a plastic folder, together with full explanatory, self-instructional written texts. The prices for the full range of items are remarkably low; and lower for applicants from developing countries. The sets on leprosy are:

1 Lp. Leprosy. A description of the disease with particular reference to childhood. This is currently (late 1988) under revision, but sets of the first issue are still available.
2 LpCn. The classification of leprosy. Immunology leads to improved classification.
3 LpD. Leprosy lesions in skins of different colours. Diagnosis in Asian patients.
4 LpN. Care of the nerve damaged limb. How to teach patients to care for their limbs in leprosy and other neurological conditions to preserve residual function.

OXFAM; Questions and Answers on MDT for Leprosy

The third edition has now almost sold out, and a revised fourth edition is with the printers, including some information on AIDS and leprosy, and incorporating positive proposals received from readers in many leprosy-endemic countries. Health Unit, OXFAM, 274 Banbury Road, Oxford OX2 7DZ, England.

Health workers for the Third World, BOMS, London

The Bureau for Overseas Medical Service and the Appropriate Health Resources and Technologies Action Group have developed a new course for health workers who are interested in working in the Third World. The five-day course from 18–22 April will cover topics such as education, nutrition, maternal and child health, water and sanitation, essential drugs, and disease prevention. Further information from Catherine Gibb, Training Officer, BOMS, Africa Centre, 38 King Street, London WC2E 8JT.

Heiser Program for Research in Leprosy

Leprosy research today

Current research in leprosy falls under three main headings: bacteriology, immunology, and chemotherapy.

Bacteriological research revolves around the fact that *Mycobacterium leprae* has not been cultivated *in vitro* (on bacteriological media). Dr Armauer Hansen in Norway first observed the organism microscopically in 1873, but the organism was not grown outside the human body until 1960 when Charles C Shepard found that it would grow in the footpads of mice. More recently, the armadillo (*Dasypus novemcinctus*) has been found to be highly susceptible and to grow large numbers of *M. leprae* in its tissues. While attempts to grow *M. leprae* on bacteriological media continue, the armadillo material has made possible a wide variety of new studies of the organism. Specific antigens have been isolated and characterized, and studies of DNA homology have been applied to a better classification of *M. leprae* and its relationship to other bacteria.

On the immunological front, both cellular and humoral immunity are under intensive investigation. The role of the various subsets of lymphocytes is being studied *in situ* in biopsies of leprotic skin, and the macrophage, in which the organisms appear to grow, is also a focus of attention. A number of monoclonal antibodies to surface antigens of *M. leprae* are being used in attempts to identify the antigens that are most significant in pathogenesis for possible vaccine development.

Chemotherapeutic work involves efforts to develop new and more effective drugs. The organism appears to be developing resistance to dapsone, the drug most widely used in therapy of leprosy. The other effective drugs are too costly for use in the areas of high incidence of the disease. At best, treatment requires long periods of time, and better, more rapidly effective drugs are sorely needed.

The research on leprosy is thus directed on the one hand at a better understanding of the microorganism and the pathogenetic mechanisms by which it causes disease and on the other at developing methods of treatment and control. The approaches are interrelated, since new information on the pathogen, and the host response to it, is required for the development of rational measures of control.

The Heiser Program for Research in Leprosy, 450 East 63rd Street, New York, New York 10021, USA.

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News and Notes

Application for research grants, LEPRA, UK

Purpose of LEPRA research grants

The eradication of leprosy is LEPRA's ultimate goal. Towards that end, LEPRA's main policy is to extend the WHO recommended multidrug therapy to as many leprosy patients as possible by means of domicillary control programmes and to encourage and support research which is directly relevant to the understanding, prevention, and cure of leprosy.

LEPRA is therefore prepared to make grants for single projects which are designed to answer a single question or a small group of related questions in these areas. Such support will usually be limited to a maximum period of 3 years. Continuation of a grant within that period will be subject to annual review after receipt of a progress report, required at the end of each calendar year. Proposals for the support for a programme of research, rather than a finite project, will also be considered but not normally for an initial period of more than 3 years. In both cases an application for an extension of the grant beyond the normal 3-year period will be considered on its merits.

Personal direction of projects

It is expected that the grant holder will be actively engaged in his/her own project.

Applications

Applications may be submitted at any time, and will be considered at the earliest possible meeting of LEPRA's Research Grants Committee, which normally meets in late January, May, and early October. Applications are required 6 weeks in advance of each meeting.

For full application protocol and forms please write to: Projects and Research Officer, LEPRA, Fairfax House, Causton Road, Colchester, Essex CO1 1PU, and *not* to ILEP.

TDR: a new type of grant for scientists

The UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases (TDR) announces the establishment of a TDR Project Development Grant which will be used to enhance the involvement of scientists from developing countries in research targeted at the development of new and better tools to control major tropical diseases.

This grant is open only to national scientists of developing countries who are interested in pursuing research on one or more of the TDR target diseases—malaria, schistosomiasis, filariasis (including onchocerciasis), African trypanosomiases, Chagas' disease, the leishmaniases and leprosy. It is designed to assist scientists in formulating technically sound proposals suitable for consideration for financial support by the various TDR Steering Committees.

The maximum amount allowable per investigator under this non-renewable grant is US $10,000. These funds may be used to seek the advice of recognized experts in the preparation of a research proposal on a subject area of interest to TDR; to gather baseline or other preparatory data; and/or to initiate preliminary research.

How to apply: Interested scientists are invited to submit a proposal on the official TDR 'Director's Initiative Fund' Application Form,* Completed application forms should be sent to the Office of the Director, TDR, World Health Organization, 1211 Geneva 27, Switzerland.

All requests for further information should be addressed directly to the Secretary of the Steering Committee that corresponds to the applicant's proposed topic of research, as listed below.

Chemotherapy of Malaria: Dr E B Doberstyn
Immunology of Malaria: Dr L Martinez
Applied Field Research in Malaria: Dr S Goriup
Schistosomiasis: Dr N R Bergquist
Filariasis: Dr C P Ramachandran
African Trypanosomiases: Mr F A S Kuzoe
Leishmaniases: Dr F Modabber
Chemotherapy of Leprosy: Dr Ji Baohong
Immunology of Leprosy: Dr H Engers
Biological Control of Vectors: Dr B Dobrokotov
Epidemiology: Dr R H Morrow Jr.
Social and Economic Research: Dr C Vlassoff

* Available from the Communications Officer, TDR, at the address given below.

Send all requests, addressed as specified above to: World Health Organization, 1211 Geneva 27, Switzerland.

Medical electives bursaries: Association of Commonwealth Universities, UK

The Commonwealth Foundation invites applications for Medical Electives Bursaries from senior medical students of approved medical schools in the Commonwealth, who are nationals of a Commonwealth country and are planning to spend an elective period elsewhere within the Commonwealth between 1 June 1988 and 31 May 1989 (UK students: 1 May).

The Lennox-Boyd Memorial Trust is offering Medical Electives Bursaries for senior medical students in Commonwealth medical faculties/schools, to assist them to spend their elective period in a Commonwealth (normally a Third World) country other than their own. These are separate from the bursaries offered by the Commonwealth Foundation.

Five bursaries, each of up to £1000, are offered with tenure at any time between 1 June 1988 and 31 May 1989. Their purpose is to enable Commonwealth medical students to gain, during their elective period, practical experience elsewhere within the Commonwealth—normally, although not exclusively, in a tropical Commonwealth country.

For further details contact: The Association of Commonwealth Universities, John Foster House, 36 Gordon Square, London WC1H OPF, UK. Tel: 01-387 8572.

(Our understanding is that the offers will be renewed every year. These bursaries are by no means well known and could obviously be valuable to students in the Commonwealth, including the UK. *Editor*).

Wellesley Bailey Scholarship

The Leprosy Mission (International) has funded a training and research scholarship named after the Mission's founder, Wellesley Bailey. The Scholarship(s) will be awarded annually up to a maximum value of £5000 to enable a leprosy worker to engage in an approved research project or in training in one of The Leprosy Mission's centres. Application forms and further details are available from the International Director, The Leprosy Mission (International), 80 Windmill Road, Brentford, Middlesex TW8 0QH.

Robert Cochrane Fund for Leprosy

The Fund, in memory of the great leprologist Robert Cochrane, is administered by the Royal Society of Tropical Medicine and Hygiene. It is to be used to finance up to 3 travel fellowships each year to a maximum value of £1200 each.

The Fund will support travel for:

1 Leprosy workers who need to obtain practical training in field work or in research.

2 Experienced leprologists to provide practical clinical training in a developing country.

There is no restriction on the country of origin or destination providing the above requirements are fulfilled.

Application forms are available from the Society and completed forms must be received by the Society at least 6 months ahead of the proposed trip. All applications must be sponsored by a suitable representative of the applicant's employer or study centre, and agreed by the host organization. A two-page report on the travel/study should be submitted to the Society within 1 month of the recipient's return.

Apply: Robert Cochrane Fund for Leprosy, Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London W1N 4EY.

Brazilian Annals of Dermatology

This medical journal is the official organ of the Brazilian Society of Dermatology, Caixa Postal 389, 20000, Rio de Janeiro, RJ, Brazil. Partly because of the relatively few medical publications in Portuguese which are circulated, but also because it frequently carries articles on leprosy, it is worth watching. The latest issue has a particularly interesting contribution on the geographic distribution of dermatologists in Brazil, of which there are currently 2109. In the main areas of the country (Norte, Nordeste, Sudeste, Centro-oeste and Sul) the number of inhabitants per dermatologist varies between 75,051 and an amazing 166,487. It would be interesting to have information on their actual or potential role in the recognition, treatment and management of patients with leprosy.

AMREF: East Africa

From *Africa Today*, Spring 1988:

AMREF, the African Medical and Research Foundation works for better health for people in East Africa; mainly in Kenya, Somalia, Southern Sudan, Tanzania and Uganda.

For the last 30 years AMREF has created and organized health care projects that are relevant and useful in the rural communities. Its Flying Doctor Service takes health care to isolated regions; the immunization programmes protect the young against preventable diseases; the training of Community Health Workers means practical health care is a part of everyday life; and the medical research work saves lives and develops techniques of fighting ill-health that are cheap and appropriate in a rural situation.

AMREF is a charity. We make no profits and depend on funds from government and non-government aid agencies in Africa, Europe and North America, as well as from private donors. AMREF has 8 international offices in Canada, Denmark, France, Germany, the Netherlands, North America, Sweden and the United Kingdom, the headquarters are in Nairobi, Kenya.

For further information please contact: African Medical and Research Foundation, London House, 68 Upper Richmond Road, London SW15 2RP.
Tel: 01-874 0098.

You can also contact AMREF at its Nairobi Headquarters: AMREF, Wilson Airport, PO Box 30125, Nairobi, Kenya. Tel: Nairobi 501301/2/3. Telex: 23254 AMREF.

People's Republic of China: role of doctors in medical colleges and general hospitals in the continuing fight against leprosy

The latest edition of the *China Leprosy Journal*, Volume 4, Number 2, June 1988, has an editorial entitled *All the Doctors in Medical Colleges and General Hospitals must be Fresh Activists in the Struggle for Eradicating Leprosy*. The author is Qin Shide, from the Dermatology Department of the Hospital attached to Qingdao Medical College. The article opens as follows:

'Qingdao is located in the half island of Shandong province where leprosy is a frequently-occurring disease. For several decades doctors in the Dermatological Department of the hospital attached to Qingdao Medical College have been treating patients from the coastal areas of Shandong province and also some Shandong patients who now live in the Northeast, Northwest and Jiangsu province. In recent years, the number of patients has sharply dropped. This is a good omen for eradicating leprosy in our country in a short period of time. However, we must not lower our guard in preventing leprosy in practice. In our Out-patient Department, we still meet some misdiagnosed leprous patients.

'According to our statistics, the majority of patients go to their local general hospital for the first treatment. Most of them have experience of being misdiagnosed. Since most of the doctors at this level have never seen a case of leprosy.' In order to improve this situation, the article goes on to explain: 'We have closely cooperated with the Qingdao Dermatological Association and Hospital to organize one academic activity every 2 weeks. Seminars are often held to exchange developments on the prevention and treatment of Leprosy. We also arrange for visiting doctors, medical students and doctors in other general hospitals to visit the leprosy hospital with the aim of increasing awareness of diagnosis and treatment among health workers.'

It was said in 'The Strategies of War Kingdoms' that 'Ninety Li is only half of a hundred Li journey'. This means that the going is the toughest towards the end of a journey'. (One must sustain one's effort when a task is nearing completion.) 'When a country enters a phase in which there are fewer and fewer patients with leprosy, more and more effort is required to maintain vigilance, so that the last remaining patients are detected early and treated properly. In this task, medical students and all doctors must be the principal force contributing to the eradication of leprosy in this country.'

[See also final paragraph of '*Problems of tuberculosis in decline*'; by N W Horne; editorial in *British Medical Journal*, Volume 288, 28 April 1984; '. . . the first country to eliminate tuberculosis will be that country which regards it as a serious problem right to the end.' *Editor*].

TDR: support for telefax equipment

In an effort to support and encourage better communication among TDR-supported institutions, TDR will consider requests for telefax equipment in research project proposals submitted to TDR in future.

This support is available only to investigators and institutions in developing countries.

TDR will approve up to US$4000 for purchase of a telefax machine. Institutions will be encouraged to purchase this equipment locally.

In most countries, telefax equipment is regulated by national postal authorities. Principal Investigators should ensure that the make and model of the telefax equipment requested has received the necessary local authorizations. The availability of maintenance and repair services should also be verified.

Apply: TDR, WHO, 1211 Geneva 27, Switzerland.

Erwin Stindl Memorial Orations, India: 1984–88

We are grateful to Dr D S Chaudhury, Director, GRECALTES, 35/1/A, Old Ballygunge, 1st Lane, Calcutta 700 019, India, for sending copies of orations delivered in India under the above title from 1984 to 1988. The subjects were: 'An overview of immunological aspects of human leprosy'; 'Recent ideas and progress in the treatment of leprosy'; 'Biochemical aspects of leprosy'; 'Immunodiagnosis in leprosy'; 'Nerves and veins in leprosy'.

Action in International Medicine (AIM), UK

We have received the following information on this newly-formed charity from The Assistant Librarian, The Royal College of Physicians, 11 St Andrews Place, London NW1 4LE.

1 What is AIM?
AIM is established by Colleges and Academies (and equivalent institutions and associations) of medicine, nursing and allied health professions as a private, non-profit, wholly professional organization with the objective of cooperating in measures to reduce, prevent and alleviate diseases and disabilities in the poor and deprived regions of the world.
2 What is the purpose of AIM?
In general, any activity which effectively reduces the burden of ill health in the South or Third World would justify AIM's existence.

In legal words, the objectives have to be widely defined in terms of education and relief of suffering.

In practice, AIM must seize the first opportunity to demonstrate its usefulness. WHO, which is strongly supportive of the potential value of AIM, proposes that the best immediate task for AIM would be to collaborate with WHO in developing systems of intermediate health care, which coordinate hospital and primary health care.
3 Why is AIM needed?
Despite great efforts by WHO and other private and intergovernmental agencies concerned with health, treatable and preventable diseases and disabilities continue to cripple many millions of the world's poorest people. WHO identifies 33 countries where, to give just one example, the deaths of women in childbirth are between 50 and 400 times the maternal mortality in the developed countries. Fourteen million children under 5 years die each year from diarrhoea, malaria, measles, acute respiratory infections, tetanus and other conditions of which many could be avoided by pre-natal care, breast-feeding and education in nutrition. Mass diseases and parasitic infestations drain the economic viability of large populations, from generation to generation.
4 What is the attitude of AIM to the health problems of the Third World?
AIM's hope is to promote among all relevant professions an international climate favourable to the rapid improvement of health in the least developed countries.

The overall goal is to raise health status; to increase knowledge of how the health of communities and populations can be improved.

AIM fully recognizes that countries are responsible for their own development, and whatever support AIM can provide will be based on this principle.

All who serve AIM in any capacity will have to respect the sensitivities, cultural characteristics and psychosocial background of the people among and with whom they work.

AIM is confident that active cooperation between members of the health professions of the developed and less developed countries will be of benefit to both sides—and conceivably will help to create a network of greater mutual respect and understanding.

AIM will supplement and in no way attempt to supersede the many efforts being made to improve Third World health.
5 What can members of the medical, nursing and allied health professions in the developed world do for the Third World?
Most medical professionals have difficulty in meeting the needs of people in their own countries and communities. They may deplore the avoidable miseries of unknown millions but have no time, few opportunities and little relevant training to intervene.

They can, however, commit themselves to a mass expression of concern and a determination that an intolerable human situation cannot be allowed to continue.

AIM is attempting to mobilize this concern and determination. It is practical to start by enrolling those colleges, academies and similar institutions which are primarily involved in the standards of medical education and training. Their involvement in the health problems of the Third World may be expanded through AIM. Their leadership is essential.

(This almost astonishingly wide-ranging and ambitious project is headed by a group of eminent medical figures in the UK. Its relationship to somewhat similar objective and activities within WHO and other agencies is as yet unclear, but its potential contribution to the improvement of Third World health appears to be considerable. *Editor*.)

The Leprosy Mission International, London: change of address

TLMS have moved from Portland Place and their new address is: The Leprosy Mission (International), 80 Windmill Road, Brentford, Middlesex TW8 0QH, UK.

Essays on Leprosy by Oxford Medical Students

This book of 184 pages has just been published for the St Francis Leprosy Guild (21 The Boltons, London SW10 9SU) by the Department of Dermatology, The Slade Hospital, Headington, Oxford OX3 7JH, England. Copies are available from Dr T J Ryan at this address: price £10.00 each, including postage.

The Preface reads as follows:

'One of the rewards of being associated with leprosy research in Oxford, including the better strategies for

control programmes, has been the interest of successive generations of students in the Oxford Medical School. Several have chosen the subject for dissertations in their Final Honours School or for participation in competitions, such as LEPRA's Annual Essay Prize or the Renwick Vickers Prize for Dermatology. Often such essays have been rich sources of information and ideas as well as usefully comprehensive reviews. For that reason, we have selected some recent essays for publication in association with the Conference of 'Dermatology in the developing World' sponsored by the International Society of Dermatology and the International Society of Dermatopathology, September 1988, to be held in Oxford. We are grateful to the students themselves, to the journals which have already published a selection of their essays and to the St Francis Leprosy Guild and Squibb–ConvaTec for their support.'

The essays selected are the following:

The mode of transmission of human leprosy. Mark Machin; The *in vitro* cultivation of *Mycobacterium leprae*. James Hutchinson; Aetiological factors in delayed-type hypersensitivity reactions in leprosy. Paul Klenerman; The influence of immunosuppression and immunodeficiency on infections with leprosy and tuberculosis. Nicola H. Strickland; The mechanism of nerve damage in leprosy. Miles Parkes; Hypopigmentation in leprosy: its mechanism and significance. Melanie Parker; Are lymphatics important in the pathogenesis of leprosy? Tania Mathias; The mode of action of dapsone in leprosy and other disorders. Benjamin Mancey-Jones; A study of the efficacy of Duoderm dressings applied to chronic ulcers on patients at a rural south Indian hospital. James Mumford and Susan Mumford; and 'Naaman's dilemma'—factors influencing the compliance of patients to prescribed drugs in chronic diseases, with particular reference to leprosy. Rodney Macrorie.

Leprosy and women: seminar in India, February 1988

Dr K V Desikan, Leprosy Histopathology Centre, Mahatma Gandhi Institute of Medical Sciences, Sevagram 442 102, via Wardha (Maharashtra), India, has kindly supplied the following information of a seminar in India:

A seminar on 'Women and Leprosy' was held at Manohardham Dattapur, Wardha on 15 February 1988 to coincide with the anniversary of the death of its founder Shri Manoharji Diwan. It was inaugurated by Mr Neil Winship, Director of LEPRA and presided over by Shri S P Tare, Director, Gandhi Memorial Leprosy Foundation, Wardha. Mrs Kamala Desikan, Secretary of Tsubosaka Dera Kushtha Sewa Pratishthan described the problems of women leprosy patients and explained the objectives of a seminar on an important problem demanding a high priority in socio-economic rehabilitation. The papers included the special scientific problems of leprosy in women, sociological aspects of leprosy in women, special rehabilitative measures for women, and the important role of women in community participation for antileprosy work.

Leprosy control in Spain

The Third National Meeting on Hansen's Disease (Leprosy) was held in Tortosa, Catalunya, Spain 5–7 May 1988. This meeting, convened by Ciba-Geigy, Barcelona, had the main object of discussing multiple drug therapy on a national scale in Spain, possibly using blister calendar packs for both pauci- and multi-bacillary patients. It was attended by about 60 people including representatives from the Ministry of Health and Central Pharmacy in Madrid, the Department of Health in Barcelona and all the provinces with a significant leprosy problem. It is important to note that responsibility for leprosy control in Spain has now been devolved to communities ('communidades'), of which there are 17; Catalunya, Valencia, Murcia, Andalucia, Canary Islands, Balearic Islands, Estramadura, Galicia, Navarra, Madrid, Pais Vasco, Castillo La Mancha, Castilla Leon, Aragon, Asturias, Cantabria and Rioja. There is no 'central' leprosy 'department' in Madrid; the work of individual patient management and control must now be organized at 'communidad' level.

Invited speakers at the two-day meeting covered; 1, the present situation with regard to leprosy in Spain; 2, multiple drug therapy for leprosy; 3, the use of blister calendar packs, with specific reference to the controlled trial which is currently under way in Thailand; and 4, the possibility of using the OMSLEP system for the recording of basic data on individual patients and epidemiological trends.

Spain has approximately 5000 patients registered, but there is doubt about this figure; it is thought that a detailed and systemic analysis would reveal that the true total (prevalence) is in the order of 3000. The yearly incidence of new cases is about 80. The most seriously affected areas are those where socio-economic conditions are poor. The lepromatous rate was recorded as 66% by WHO in 1979. Many cases presenting for the first time have considerable disability. BCG was stopped some years ago (on the grounds that it was of no practical value and confused the interpretation of tuberculin testing in infants suspected of clinical TB). Multiple drug therapy is used in some areas, but patchily and on a small scale. The incidence of dapsone resistance is not well documented, but many cases in Spain have been on dapsone monotherapy for 10–20 years; irregularity and inadequate dosage are both more than likely.

Conclusions are still to be drafted and circulated for discussion, but it can be recorded that the consensus view of the participants, with backing from Madrid and Barcelona, was that MDT according to WHO recommendations should be used on a national scale in the form of blister-calendar packs, with implementation as soon as reasonably possible. All active multibacillary cases will receive a minimum of 2 year's treatment.

A manual of procedures is to be written and circulated in the near future.

Leprosy Review Index

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®Lamprene Geigy

(= clofazimine)

The highly effective antileprosy drug with anti-inflammatory[1] properties

For the prevention[2] and treatment[3] of lepra reactions (ENL)

Suitable for use in combined regimens for the prevention and treatment of dapsone-resistance in lepromatous and dimorphous forms of leprosy[4]

1. Browne, S. G.: Lepr. Rev. *37,* 141 (1966)
2. Azulay et al.: Lepr. Rev. *46* (Suppl.), 99 (1975)
3. Schulz, E. J.: Lepr. Rev. *42,* 178 (1972)
4. Yawalkar, S. J., Vischer, W. A.: Lepr. Rev. *50,* 135 (1979)

Composition: Clofazimine. *Capsules* of 50 mg and 100 mg. Indications: Lamprene, employed in combination with dapsone and rifampicin (®Rimactane), serves as treatment for multibacillary forms of leprosy, such as lepromatous (LL), borderline lepromatous (BL), and mid-borderline (BB) leprosy, as well as erythema nodosum leprosum (ENL). Combined chemotherapy is necessary in order to prevent the emergence of resistant strains of *M. leprae.* Dosage: *Adults* (of approx. 60 kg body weight): for the treatment of multibacillary leprosy (LL, BL, BB) the WHO (World Health Organisation) recommends the following dosage schedule: Lamprene: 300 mg once a month under surveillance + 50 mg once a day as self-medication. Rifampicin: 600 mg once a month under surveillance. Dapsone: 100 mg once a day as self-medication. This threefold combination should be administered for at least 2 years and, whenever possible, until such time as the skin smears become negative. If the patient develops ENL, the treatment with dapsone and rifampicin should be continued as before, whereas the dosage of Lamprene should be raised to at the most 300 mg per day. These high daily doses must not be given for longer than 3 months. *Children:* Children should receive lower doses adapted to their body weight. Administration: The capsules should be taken at mealtimes or together with milk. Contra-indication: Known hypersensitivity to clofazimine. Precautions: Leprosy patients suffering repeatedly from abdominal pains and diarrhoea, as well as those with liver or kidney damage, should if possible not be treated with Lamprene. Treatment with daily doses of Lamprene exceeding 100 mg should not be continued for longer than 3 months, and during this time the patient should be kept under medical supervision. If gastro-intestinal symptoms develop during the treatment, the dosage should be reduced or the interval between doses prolonged. In the event of persistent diarrhoea or vomiting, the patient should be hospitalised. Pregnancy and lactation: As in the case of any form of drug therapy, Lamprene should be employed with caution during pregnancy, especially in the first 3 months. Clofazimine crosses the placental barrier and causes temporary discoloration of newborn infants. The active substance also passes into the breast milk. Unwanted effects: The following side effects have been observed: Reddish to dark-brown discoloration of the skin and of the leprous lesions, particularly in pale-skinned patients at sites exposed to light. Discoloration of the hair, conjunctiva, cornea, and lacrimal fluid, as well as of sweat, sputum, urine, and faeces. This discoloration is reversible, although in the case of the skin it often does not disappear completely until some months after the cessation of treatment. Dryness of the skin, ichthyosis, pruritus, photosensitivity, acneform eruptions, and non-specific skin rashes. Nausea, vomiting, abdominal pains, diarrhoea, anorexia, loss of weight, and eosinophilic enteropathy. Storage: Protect from heat and moisture. Packs: 100 capsules of 50 mg or 100 mg.

Further information is available on request.