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Editorial

THE TRAINING OF RESEARCH WORKERS IN LEPROSY

As a sub-title we might well have "Reflections of a leprologist on the discussions during a Round Table on 'The Training of Research Workers in the Medical Sciences' held at Geneva on 10 and 11 September, 1970, under the auspices of the Council of International Organizations of Medical Sciences".

A panel of distinguished speakers opened the topic, and participants from many countries, as well as representatives of many of the member-organizations of the Council, contributed to the discussions. Although specific subjects and specific medical problems were not considered, the relevance to leprosy of much of the debate was apparent. In point of fact, leprosy illustrates supremely well the need to prosecute research concurrently on many fronts, in the laboratory and in the field, and to apply new knowledge to old and intractable problems. The medical, social, and economic problems presented by leprosy bring into sharp focus the widely-felt concern to make medical research relevant to the vast populations of the Third World. The biomedical research worker of the future may well be a hyphenated hybrid, a doctor-sociologist or a geneticist-epidemiologist: all must have some concern with the social and community implications of research.

Although the main drawback to effective control of leprosy is the yawning gap, the unconscionably long time-lag, between new knowledge and its application, there is no gainsaying the need to discover more about *Mycobacterium leprae* and the fascinating range of tissue response it evokes. In other words, more research is needed. And more research means, in practical terms, more good work by more good people. Full-time research scientists must be attracted to leprosy in greater numbers, and the most likely means of achieving this goal is by the obvious and infectious enthusiasm of those already on the job. Once they have been attracted, what should happen to them then? How should they be prepared for their life-work? Some speakers at the Round Table drew up such a formidable programme of training in the basic sciences (with special importance attached to mathematics, biochemistry and biophysics) followed by wide experience in experimental techniques and in computerization, that by the time he arrived at the end of this protracted preparation the doctor-cum-would-be-research worker might well be too old and too highly trained to realize his creative potential to the full.

The individualistic approach typical of much clinical investigation in the past depended largely on first-class and well-equipped clinicians who combined in themselves several diverse attributes—a knowledge of medicine and of the scientific method, appreciation of the ethical aspects of research, etc. Flexibility and variability are surely necessary today. Undue rigidity may exclude the unconventional and unpatterned.

A large proportion of published work in leprosy comes, however, not from the full-time laboratory-confined research worker, but from busy practitioners who take the trouble to observe, record, evaluate and write. They pursue their research interests as an integral and necessary part of their service activities. In their immediate post-graduate years, they had no opportunity (or perhaps inclination) for a prolonged period of preparation. Perhaps they little imagined that they would one day feel impelled to ask questions and try to find the answers. Despite the sophistication of much research in leprosy today, and notwithstanding the continuing need to scrutinize every new advance in investigative technology for its possible application to leprosy, there is still a place—and perhaps a growing place—for those whose main interests lie in the wards, the operating theatre, or the rural clinic, rather than in the esoteric atmosphere of the laboratory and animal house.

Perhaps the time is ripe to try to rehabilitate clinical and epidemiological research in leprosy. After all, since "the proper study of mankind is man", the best-laid schemes of mice may not be entirely applicable to the human being. It is just conceivable that a sick bacillus that would despair and die in a hostile rodent micro-environment might regain its pristine powers of multiplication within the congenial confines of a human reticulo-endothelial cell. And no known animal can yet replace man in the wide range of response to leprosy infection. This is not to decry animal experimentation, which has recently been so obviously productive of results, but rather to emphasize the complementary necessity for accurate observation of the person exposed to or suffering from leprosy. Serious clinically-orientated research, in contra-distinction to purely laboratory investigations, needs to be reinstated in academic prestige.

One of the functions of this *Review*—and not the least important—is to encourage workers in all branches of leprosy to ask questions. Usually the answers are already available somewhere; hence the imperious necessity for keeping up to date and knowing where to consult the findings of co-workers. Sometimes the questions have not been asked, or have been only partially or imprecisely answered. As we proceed towards the frontiers of knowledge, the actual delimitations may be vague and ill-defined. If the right questions are now posed in the right way, valuable new knowledge and new insight may be forthcoming.

It is here that we recognize the wisdom of Bacon's well-known saying, "reading maketh a full man, conference a ready man, and writing an exact man". As he submits himself to the inexorable and inescapable discipline of the printed word, the part-time research worker in leprosy finds his acuteness of observation sharpened and his powers of expression increased.

Since ideas for profitable research, "hunches", and the like do not automatically continue to emerge as we become older, medical men and women coming with fresh and open minds to leprosy may well find themselve posing unanswered and perhaps unasked questions that may eventually prove of real significance. In such matters as cultivation of *Myco. leprae* in artificial media, healthy carriers, transmission, inoculation lesions, susceptibility to infection, and a host of other problems, new light may conceivably come from relatively simple investigations, with results that will be dazzlingly obvious in retrospect.

Last, but by no means least, are the psychological and moral qualities of the research worker. Motivation is important, but these go deeper than motivation. In leprosy, the strong social or humanitarian urge has always been predominant, and is still of overriding importance, not only in those faced daily with the grim spectre of human suffering but also in many who pursue their investigations in distant laboratory or research centres. Doctors are still called upon to care for patients rather than to treat diseases. And doctors, rather than research bio-scientists, must take the responsibility for clinical research. The combination of personal integrity and scientific competence is still needed. The research worker of today and tomorrow cannot help concerning himself with the flagrant disparities in the kinds of health care available in the affluent societies and in the developing countries where leprosy is most prevalent. Nor can he remain unmoved at the senseless squandering of irreplaceable natural resources in a context of undernutrition and preventable disease. He is responsible for the results of his successes.

The individual patient suffering from a slightly contagious mycobacterial infection, and from all the personal, social and economic accompaniments of that infection, is the object, the subject and the eventual beneficiary of leprosy research. May we have more of it.

News Items

LEPRA GOES INTERNATIONAL

The British Leprosy Relief Association, known since 1964 as LEPRA, has now become an international association. At an Extraordinary General Meeting of the Association held on 24 September, 1970, and presided over by Sir George Seel, K.C.M.G., Chairman of the Executive Committee, a resolution was unanimously passed that would have the effect of abolishing the clauses in LEPRA's Memorandum of Association restricting its activities to the countries of the British Commonwealth.

BELRA, the British Empire Leprosy Relief Association, was founded in 1924 by Sir Frank Carter, Sir Leonard Rogers, and the Rev. Frank Oldrieve, who became its first General Secretary. The world "Empire" was dropped from the title in 1957.

In establishing and sponsoring *Leprosy Review*, LEPRA has never subscribed to any artificial limitation of one of its principal duties, which is "to spread the knowledge that modern science could treat and finally prevent the spread of leprosy". Henceforth, however, LEPRA will be able to allocate funds to approved leprosy projects in all countries without distinction.

OPENING OF THE "COCHRANE ANNEXE" AT OXFORD

On 11 August, the veteran leprologist, Dr. Robert G. Cochrane, C.M.G., formally opened an Annexe—to be called after him—at the Slade Hospital, Oxford. For several years the need has been felt for a small unit where patients suffering from leprosy might be investigated and treated in close proximity to distinguished

Dr. and Mrs. Cochrane at the opening ceremony. (Reproduced by kind permission of *The Oxford Mail*)

medical specialists and research facilities. The inspiration for the creation of such a unit came from Dr. Cochrane himself (at the time Consultant Adviser in Leprosy to the Ministry of Health) with the wholehearted support of Dr. Renwick Vickers, dermatologist and Member of the Panel of Leprosy Opinion. Thanks to a generous grant from the Nuffield Foundation, which augmented the allocation from official sources, the Cochrane Annexe was erected.

The modest but adequate building comprises 6 single-bedded rooms for patients, a laboratory, a library, and offices, together with a larger room for group discussions, clinical meetings and scientific exhibitions. Selected patients will spend short periods in the wards for initial investigations, stabilization of treatment, research into nerve and orthopaedic complications, etc. Although the Annexe is primarily intended for patients suffering from leprosy, it will be no surprise to those acquainted with the eclectic dermatological interests of Dr. Vickers and his colleagues to learn that investigations in depth into various dermatoses are contemplated. The considerable clinical and pathological expertise of the Oxford School of Medicine will be at the call of the workers in the Cochrane Annexe.

As Dr. Vickers suggested in his opening speech at the well-attended ceremony, leprosy may not constitute in England a problem of vast dimensions or imposing threat, but—echoing the words of the Minister of Health at the Ninth International Leprosy Congress, London, 1968—he emphasized that British workers were under an obligation to utilize their considerable investigative resources in solving some of the outstanding problems posed by the disease.

INTERNATIONAL LEPROSY COLLOQUIUM, BORSTEL (GERMANY), 26-27 AUGUST, 1970

Under the joint auspices of the Borstel Forschungsinstitut, the Skin Department of the University Clinic of Hamburg and the German Leprosy Relief Association (Deutsches Aussätzigenhilfswerk of Würzburg), a highly successful Colloquium on leprosy was held on 26 and 27 August, 1970, at the Borstel Institute, 25 miles north of Hamburg. There were 105 registered and invited participants from 23 countries, not counting several score visitors who helped to fill the auditorium, where 73 papers were presented in English, French and German (with simultaneous translation provided).

Such meetings of workers and research scientists interested in leprosy, held between the quinquennial Congresses of the International Leprosy Association, bring together in fruitful contact not only well-known figures seen from time to time in both hemispheres, but also isolated leprologists and microbiologists who rarely have the opportunity of discussing their problems with others working in related fields. For this reason, this Colloquium is to be welcomed. Although many of the papers provided nothing new, and presented merely a rehash of published work on hackneyed themes, other contributers gave up-to-the-minute results of original work.

Problems of cultivation of the fastidious *Mycobacterium leprae* were discussed with new insights and with the aid of simple laboratory apparatus. The mouse footpad inoculation technique is being routinely utilized for screening selected groups of anti-mycobacterial drugs. More work is being done on the globulin fractions of the serum in lepromatous leprosy. The sophisticated techniques of electron microscopy, immunofluorescence and experimental microbiology are being pressed into the service of leprosy investigation.

The application of recent advances in knowledge to the field control of leprosy regrettably lags far behind. Few of the participants in the Colloquium were concerned with the frustrating and disappointing organization of practical leprosy control projects. The epidemiologists elaborated their theories, and the reconstructive surgeons reported the results of their operative procedures. Shoemakers brought us down to earth with a pedestrian account of the problems of the ulcerated and anaesthetic extremities of millions of our fellowmen.

Seventy-three papers in 2 full days represented a great deal of reading and listening, and perhaps too little time for thinking and appraising and discussing. However, the closing session came alive as some of the participants took the opportunity for getting to grips with the practical problems confronting the specialist workers.

The excellent arrangements made by the Borstel Institute, and particularly the genial supervision of Dr. E. Freerksen and Dr. J. Kimmig, merit nothing but praise. Hotel reservations, transport, culinary arrangements (including a superb banquet at Trembüttel) were very much appreciated.

The papers presented at the Colloquium will be published in a special number of the *International Journal of Leprosy*.

C.I.O.M.S.

These initial letters stand for the Council of International Organizations of Medical Sciences. The Council unites specialist bodies—such as the International Leprosy Association—in a non-governmental and representative agency that can concern itself with broader matters of medical policy and direction, research and training, ethics and responsibility. Loosely-knit but strong, informal yet influential, working in close association with the World Health Organization and UNESCO yet remaining vigorously independent of these bodies, the Council provides a forum for serious discussion and debate. It encourages the holding of international meetings where these may serve some useful medical purpose. It addresses itself from time to time to the larger areas where medicine and ethics meet, and has organized useful Round Table discussions on such themes as "Biomedical science and the dilemma of human experimentation", "Heart transplantation", "Evaluation of drugs—whose responsibility?", which have resulted in publications that should be taken seriously by governments and medical research councils.

The International Leprosy Association is a member of the Council of International Organizations of Medical Sciences, and has profited practically from its adherence, having received a grant from that body towards expenses of translation and publication incurred at the London (1968) Congress.

The Council is at present conducting a comprehensive study on the nomenclature of diseases: it is to be hoped that the classification of leprosy and the meanings to be attached to the terms used in this specialty will be clarified and delimited—to the benefit of those who read (in English, French, Russian or Spanish) as well as those who write.

Another matter of common concern to both the C.I.O.M.S. and leprosy is the rapid and accurate dissemination of advances in the biomedical sciences. It is not enough to discover and to record: despite the enormous and inescapable difficulties consequent on the accumulation of knowledge and the fragmentation of science, the really important advances must be made available, in understandable language, to wider audiences. The C.I.O.M.S. encourages member organizations to forge links with similar bodies and stimulates the developing awareness of mutual dependence and collective concern. In the whole matter of medical education (of auxiliaries as well as doctors), now as never before subject to change and flux and experimentation, the C.I.O.M.S. could undertake an invaluable role in correlating and coordinating the various national and international groups currently studying some aspects of this important topic.

FONTILLES (SPAIN)—PLANS FOR EXPANSION

The dynamic Medical Director of the Fontilles Leprosy Sanatorium, Alicante, Spain, Dr. Terencio de las Aguas, has prepared plans to augment the facilities already available at the Sanatorium and to increase both the teaching and research aspects of the programme.

At present, about 300 leprosy patients are under treatment at Fontilles as in-patients, and there are beds for another 200. A total of 3000 patients have been treated since the institution began admitting patients. The

medical team comprises (in addition to the Medical Director), 3 resident doctors, 5 qualified (male) nurses, as well as 24 nursing sisters of the Order of the "Terciarias de la Immaculada", and 25 volunteer helpers of both sexes. Visiting specialists in the main branches of medicine make their services available to the patients.

The well-known journal *Fontilles* publishes original scientific articles on leprosy—some 281 so far—as well as news and abstracts of interest to Spanish medical readers.

The teaching activities of the staff have had a wide influence on the standards of leprosy care beyond Spain itself. Nineteen courses of instruction have been given, 10 of them for qualified doctors and the rest for paramedical staff, and altogether about 500 students have profited from these courses. The Order of Malta has generously borne the main financial burden.

The plans for the future are largely based on the stimulation and encouragement afforded by ELEP (the Co-ordinating Committee of the European Leprosy Organizations) and include 2 courses annually for doctors with a maximum of 25 doctors for each course. It is intended that the courses should last for 15 to 20 days. The media of instruction would be English, French, German and Spanish. The lecturers would be drawn not only from the staff of Fontilles, but also from Spanish Medical Colleges and abroad. It may be possible later to inaugurate bursaries for students wishing to devote longer periods of, say, 3 to 6 months, to leprosy studies.

The clinical and laboratory facilities at present available would, in the opinion of Dr. Terencio, make Fontilles an ideal European Research Centre for leprosy. If this suggestion meets with acceptance, some new buildings will be required and additions will have to be made to the medical and technical staff.

BRITISH COUNCIL FOR REHABILITATION OF THE DISABLED, FOURTH INTERNATIONAL SEMINAR, EDINBURGH, 27 JUNE TO 3 JULY, 1971

The British Council for Rehabilitation of the Disabled is organizing its Fourth International Seminar and Exhibition in Edinburgh, Scotland, from 27 June to 3 July, 1971, on the theme "Rehabilitation—a Unified Concept" (international, governmental, and local government hospital services). Official participants will include: Her Majesty's Government, the United Nations Organization, the International Labour Office, and the World Health Organization.

While no papers on leprosy are to be invited, it is not unlikely that reference will be made to leprosy in the reports of the official participants at the plenary sessions. A most fruitful session on leprosy, including papers and discussion, was included in the programme of the Eleventh World Congress of the International Society for Rehabilitation of the Disabled (I.S.R.D.), which was held in Dublin from 14 to 19 September, 1969 (see *Leprosy Review*, 1970 **41**, 5 and 57).

Enquiries are invited from the Secretary General of the British Council for Rehabilitation of the Disabled (Commander Ian R. Henderson), Tavistock House (South), Tavistock Square, London, WC1H 9LB.

AMERICAN CONGRESS OF REHABILITATION MEDICINE

The 49th Annual Meeting of the American Congress of Rehabilitation Medicine will take place in San Juan, Puerto Rico, from 7 to 12 November, 1971. Preceding the meeting, there will be a Prosthetics Course arranged by the San Juan Veterans Administration Hospital from 1 to 5 November, the subject being "Recent advances of prosthetics and orthotics". After the meeting, members will be free to take a post-Congress tour to Caracas, Venezuela (13 to 16 November), to join the Second Caribbean Congress on Rehabilitation Medicine.

Those interested are invited to get in touch with Mr. Creston C. Herold, Executive Director, American Congress of Rehabilitation Medicine, 30 North Michigan Avenue, Chicago, Illinois 60602, or Dr. Herman J. Flax, President, American Congress of Rehabilitation Medicine, 310 De Diego Street, Suite 301, Santurce, P.R., U.S.A. 00909.

ELEP MEDICAL COMMISSION

Under the Chairmanship of Dr. L. P. Aujoulat, the Medical Commission of ELEP (the European Federation of Anti-leprosy Associations) met in Paris on 23 and 24 October, 1970, to examine numerous projects for financial assistance submitted to member-organizations and to advise on matters of policy.

Voluntary agencies are no longer concerned only with fund-raising and propaganda, but are becoming more and more involved—in co-operation with each other and with Governments—in the larger matters of epidemiology and research. The advice of the Medical Commission was sought on new proposals for co-operative projects, on desirable new emphases in national leprosy campaigns, and on new teaching facilities available. Since the bodies represented in ELEP are raising annually considerable funds for leprosy work all over the world, it is more than ever necessary that they should seek and heed expert advice on the spending of these funds.

IN VITRO CULTIVATION OF *MYCOBACTERIUM LEPRAE*

At the International Leprosy Colloquium held at the Forschungsinstitut, Borstel (Hamburg, Germany) on 26 and 27 August, 1970, Dr. R. Bönicke presented a paper that may in future years be seen to be of fundamental importance in the history of the continuing attempts to induce *Myco. leprae* to remain viable and to multiply in artificial culture media.

Dr. Bönicke uses specially made U-shaped tubes, separated in the middle of the U by a sintered glass filter plate (Schott G 5). Liquid culture medium is delivered to each limb of the tube. Into one limb the inoculum containing living *Myco. leprae* is introduced; the other limb is utilized for periodical replacement of the medium. The rationale of the procedure is that an organism with a long generation time would be subject to the accumulated toxic effects of products of metabolism and inherent biochemical degradation; if these could be removed, as formed, before they reached toxic concentrations, the organisms would perhaps continue to multiply.

Dr. Bönicke has demonstrated such multiplication over a period of 9 to 12 months, giving a generation time of between 20.8 and 41.3 days, with an average of 28 days.

The happy choice of some such simple physical principle has long been awaited by microbiologists, and it is possible that other workers will now employ the same or similar methods for cultivating an organism that has so far resisted the acumen and resource of generations of bacteriologists.

Letter to the Editor

Dr. T. F. Davey's article "Rural Leprosy Control Problems in Biafra and Central India: a Comparison", in *Leprosy Review* (1969) **40**, 197, struck a responsive note in the minds of those of us who are struggling with the problem in South India. The discrepancy between the apparent ease with which the problem could be tackled by those writing of their successes in Africa, and the difficulties we experience here in India is most striking.

In this Centre, we are trying to combine the domiciliary approach to the control of both leprosy and tuberculosis, using the same administrative set-up and the same paramedical field workers for both. Our paramedical workers were given the standard approved short course in leprosy. All that they know about tuberculosis we have taught them here while they are on the job. In their house-to-house surveys and informal contacts in the villages, they give equal stress to detection of, and education regarding, leprosy and tuberculosis. Both groups of patients come to the same roadside clinics for their medicines and laboratory tests.

In theory, the combination appears very logical and should represent a step forward. However, I have been concerned about the lack of convincing evidence that the efforts of the paramedical workers in the villages have actually made a difference in the regularity of attendance of the patients or the results of treatment in either disease. The present programme has not yet been in operation long enough for valid statistical analysis to be possible. However, a review of 15 years' experience in a programme confined to leprosy in another district showed to my dismay that the attendance and results were no better in the control area covered by the paramedical workers than in an outside area with the same kind of roadside clinics but no paramedical workers. My impression here, so far, is that the differences between patients in the control area and those in the other district, both as regards leprosy and tuberculosis, are equally small.

The problem appears to be one of *motivation*—the urge that makes patients come for treatment, and continue treatment. This motivation seems to be easily achieved in Africa, but is achieved only with the utmost difficulty in the villages of South India. The seriousness of the problem is not apparent to those practitioners who sit in their offices in traditional hospitals and wait for patients to come to them, because the only patients they see are the more intelligent and educated, that is, those who are already motivated, or they would not have come in the first place. A study in depth of the psychological and social factors that influence the motivation of these people would seem to be indicated.

The findings of the international team studying the related problems of motivation to accept family planning in the villages of India and Pakistan might well prove helpful to those working in the field of leprosy.

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Cell-Mediated Immunological Processes in Leprosy*

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A large number of organisms such as viruses, protozoa, helminths, fungi and bacteria, especially mycobacteria, need cell-mediated immunological processes for their elimination. As well as being involved in protection, cell-mediated immunological processes are also involved in a number of allergic reactions to products derived from mycobacteria. Cell-mediated immunological processes can be demonstrated by a number of *in vitro* reactions. Leprosy can present with a wide range of different clinical patterns. The clinical spectrum of leprosy can be shown to depend on the degree of the cell-mediated immune response of the host against *Mycobacterium leprae*. Thus in tuberculoid leprosy there is a high degree of cell-mediated immune response whereas in lepromatous leprosy such a response is virtually absent. There appears to be a constitutional predisposition to lepromatous leprosy. In addition to a specific loss of cell-mediated immune response against *Myco. leprae*, there is also a non-specific drop in the ability of patients with lepromatous leprosy to show other aspects of cell-mediated immune response, e.g. contact sensitivity and skin homograft rejection. There is also a relative impairment of the ability of lymphocytes to react *in vitro*. Lymph nodes from patients with lepromatous leprosy show a deficiency in those areas associated with the development of cell-mediated immune responses. The article includes a discussion on the possible causes of deficiencies in cell-mediated immune responses in lepromatous leprosy.

THE NATURE OF THE IMMUNOLOGICAL RESPONSE

The body can respond to an antigenic stimulus in 2 fundamentally different ways. The end result of each is the production of a factor which will interact specifically with the antigen, setting off a train of events leading either to the elimination of the antigen from the body or the induction of an inflammatory reaction. In one case, the specific antigen-reacting factor is a serum protein, an immunoglobulin molecule, which we can refer to as a "humoral antibody". In the other, there is the production of lymphocytes which have a similar property of reacting specifically with the antigen. The production of these specifically sensitized lymphocytes is known as the "cell-mediated immune response" Although both humoral antibody and sensitized lymphocytes react specifically with the antigen, the subsequent train of events, which each leads to, is different. Thus, humoral antibodies may react with antigen on cell surfaces to produce anaphylactic phenomena (asthma, hay-fever, urticaria, etc.) or react with complement and be deposited in the walls of blood vessles, causing vasculitis, glomerulonephritis, arthritis, uveitis, etc. The interaction between humoral antibodies and the antigens of certain bacteria, such as pneumococci or *Salmonella*, may increase the phagocytosis and elimination of these organisms from the body. However, a large number of micro-organisms cannot be eliminated by the action of humoral antibodies alone. These organisms require the interaction of sensitized lymphocytes and macrophages for their elimination. It is becoming more apparent that cell-mediated immune processes are necessary for the protection of the body from viruses, protozoa, helminths, fungi and many bacteria, including organisms as different as the treponemata, *Brucella* and the mycobacteria.

*Reprinted from *Bull. Wld Hlth Org.*, 1969, **41**, 779-792, by kind permission of Chief, Technical Publications, WHO.

The need of cell-mediated immune processes for the elimination of mycobacteria has been known for many years. Much of the early work on this subject was performed on *Mycobacterium tuberculosis*, and started with the observation of Koch (1891), who demonstrated that if virulent tubercle bacilli were injected subcutaneously into a guinea-pig which was already infected, the animal reacted against the organisms differently from an uninfected animal. The rejection of the organisms was associated with a massive inflammation which developed at the site of injection. A similar type of inflammation could be induced if the guinea-pig, or a similarly infected human subject, was inoculated intradermally with a culture filtrate of the microorganisms (the tuberculin reaction). Subsequently, it has been shown that both the elimination of the organisms from the body and the inflammatory reaction produced by a sensitized person against the organisms or culture fluid are caused by the reaction with sensitized lymphocytes and macrophages, cell-mediated immunological reactions.

Both the cell mediated immune response and the humoral-antibody response are functions of the central lymphoid tissues of the body. An immunological response may be considered to have a number of phases. There is first contact with antigen and this is followed by a phase of cellular proliferation, lymphocytes in the case of cell-mediated immune responses, and plasma cells for humoral-antibody production. Cellular proliferation takes place mainly in the lymph nodes, spleen or the lymphoid tissue of the gut. As a result of this, either sensitized lymphocytes or humoral antibody are present in a sufficient concentration to react with the foreign substances (antigens) on subsequent contact.

Production of humoral antibody takes place in the plasma cells which are found at the cortico-medullary junction or in medullary cords of lymph nodes or in the red pulp of the spleen, and is usually associated with the formation of germinal centres in the cortex of the lymph nodes or the splenic white pulp. The proliferation of lymphocytes associated with cell-mediated immune reponses takes place in what is often referred to as the paracortical area of lymph nodes or in the area of the white pulp of the spleen around the central arteriole. The paracortical area of the lymph node is found situated between the true cortex and the medulla and between the lymph follicles and germinal centres (Oort and Turk, 1965).

Both this area and the areas of lymphocytes in the white pulp of the spleen round the central arteriole have been found to be dependent on thymus integrity in late foetal and neonatal life (Parrott, de Sousa and East, 1966). The differentiation of lymphocytes, probably sensitized by contact with antigen in the periphery (Medawar, 1958), into large pyroninophilic cells (immunoblasts), which can be shown to divide into other small lymphocytes takes place in the paracortical area of lymph nodes as part of a cell-mediated immune response (Oort and Turk, 1965). Removal of the thymus in neonatal life depletes these areas of lymphocytes and is associated with an inability to manifest such a response (Miller, 1961).

Babies born with congenital thymic hypoplasia are found to be unable to produce a cell-mediated immunological response and the paracortical areas of their lymph nodes and the area of the white pulp of the spleen round the central arteriole are deficient in lymphocytes and replaced by reticulo-histiocytes. A similar appearance is found in the lymphoid tissue of animals treated with anti-lymphocyte serum (Turk and Willoughby, 1967) which also prevents the development of cell-mediated immune responses. Neonatally thymectomized animals, babies with congenital thymic aplasia and animals treated with anti-lymphocyte sera are able to produce humoral antibodies, have normal levels of immunoglobulins in their serum and have normal numbers of plasma cells in their lymphoid tissue.

As well as being involved in the protection of the body against a wide range of microorganisms, cell-mediated immune responses are involved in a number of allergic responses to substances produced by a number of microorganisms. These reactions, like the tuberculin reaction mentioned above, take 24 to 48 hr to

develop and include the "reaction of immunity" to vaccinia virus, the mumps skin test, skin reactions to streptococcal antigens such as those found in mixtures of streptokinase and streptodornase, to fungal antigens such as coccidiomycin and histoplasmin, and to protozoal antigens such as the Montenegro test for leishmaniasis. The reaction of rejection of a skin homograft is another example of a cell-mediated immune response, so also is contact sensitivity to simple chemical agents such as 2,4-dinitrochlorobenzene (DNCB) which attach readily to skin proteins.

Following contact between sensitized lymphocytes and antigen in the periphery, a soluble substance is released which has a direct effect on macrophages. The nature of the substances on or within the lymphocytes, with which the antigen reacts specifically, is not known. However, many people feel that, because the reaction resembles humoral antibody reactions so closely, the reacting molecule must be related in some way to an immunoglobulin molecule. Thus it has been suggested that it might be a polypeptide chain such as the Fd fragment of the immunoglobulin molecule, or some similar peptide chain carrying an amino-acid sequence specific to the particular antigen for which it is coded to react.

The soluble substances released by the incubation of specifically sensitized lymphocytes with specific antigen are in the molecular-weight range of approximately 70,000. These substances can be shown to have a direct effect on the cell surface of macrophages, decreasing the surface electrostatic charge, increasing their adhesibility both to each other and to similarly charged surfaces, and decreasing their permeability to certain organic chemicals with molecular weights from 600 to 900, such as diphosphopyridine nucleotide and triphosphopyridine nucleotide. The relationship of these changes to the development of an inflammation in the skin with erythema, induration and a mononuclear cell infiltrate is not yet completely clear. However, the soluble mediator can be isolated after culture of specifically sensitized lymphocytes with specific antigen and, when this is injected intradermally, it will produce an inflammatory reaction which has many of the characteristics of a tuberculin reaction (Bennett and Bloom, 1968; Pick *et al.*, 1969).

The effect of the interaction between specifically sensitized lymphocytes and antigen on the ability of macrophages to eliminate mycobacteria has also not been completely elucidated. However, Berthrong and Hamilton (1959) have observed the intracellular growth of these organisms in macrophages. Normally, tubercle bacilli multiply intracellularly and form cords which engorge the cells so that they eventually burst. Macrophages from immune animals do not allow the bacilli to grow intracellularly in this manner, and they survive longer than non-immune macrophages although they may have still ingested the organisms. At the same time it has been shown that macrophages from immune animals have a high rate of dehydrogenase enzyme activity (Allison, Zapposodi and Lurie, 1962), and an increase in lysosomal enzymes (acid phosphatase, *b*-glucuronidase and cathepsin). Lysosomes of macrophages from animals immune to mycobacteria are also probably more fragile than normal, as indicated by the release of considerable amounts of lysosomal enzymes, especially *b*-glucuronidase, into the plasma following the intravenous injection of an endotoxin (Saito and Suter, 1965*a*, *b*). The little work that has been published on the phagocytosis of *Myco. leprae* by macrophages suggests, as will be discussed later, that in tuberculoid leprosy, where there is a high degree of cell-mediated immunity, macrophages can readily lyse phagocytosed *Myco. leprae*, whereas in lepromatous leprosy, where cell-mediated immunity is deficient, the organisms are still phagocytosed but not lysed. Moreover, the lysis of *Myco. leprae* by macrophages from patients with lepromatous leprosy is immunologically specific (Beiguelman, 1967).

A few *in vitro* reactions have been studied extensively over the last few years in relation to cell-mediated immunity. One of these is related to the increased adhesiveness of macrophages following the release of a soluble substance after the interaction of specifically sensitized lympho-

cytes with specific antigen. The macrophages are grown in tissue culture in small capillary tubes. Under normal conditions, they migrate from these tubes in a fan-like manner. However, if they are in close contact with antigen and specifically sensitized lymphocytes, or incubated together with the soluble substances released from specifically sensitized lymphocytes incubated with antigen, they fail to migrate from the tubes. For this reason, the soluble substance released by incubation of specifically sensitized cells with antigen is referred to as the "migration inhibition factor" and the reaction is referred to as "inhibition of macrophage migration *in vitro*".

The other reaction performed in tissue culture is more related to the changes which occur to lymphocytes in lymph nodes following first contact with antigen, during the development of a cell-mediated immunological response. It will be remembered that after first contact with antigen, probably in the periphery, lymphocytes differentiate into large cells in the paracortical area of lymph nodes, prior to division into other lymphocytes. These large cells which are synthesizing DNA, and which can thus incorporate radioactively labelled thymidine, are referred to as "immunoblasts". Similar transformation of small lymphocytes into "blast" cells can be produced in tissue culture *in vitro*. However, this occurs most frequently if the person or animal has had a previous exposure to the antigen. Under these conditions, no more than 10% of the small lymphocytes are transformed. There are a number of substances which will regularly transform 80 to 90% of the small lymphocytes, without any known, previous exposure of the individual to the substance. The substances, or non-specific mitogens, include phytohaemagglutinin (PHA), a similar mitogen from pokeweed (*Phytolacca americana*), streptolysin S and the filtrate of *Staphylococcus aureus* cultures. Similar transformation can be obtained by treating the lymphocytes with an anti-immunoglobulin allotype serum. Although the relationship between lymphocyte transformation with specific antigen and cell-mediated immune response or humoral antibody production is highly controversial, there is a direct relationship between the ability of lymphocytes to be transformed by PHA and the ability of individuals to show cell-mediated immune phenomena. Thus an inability both to be sensitized to show contact sensitivity to DNCB and to have their lymphocytes transformed by PHA occurs in patients with congenital thymic aplasia, Hodgkin's disease and sarcoidosis.

THE SPECTRUM OF CLINICAL LEPROSY

It has been recognized for a considerable time that leprosy may present clinically in a wide variety of forms. The pleomorphism of the presentation of the disease was first rationalized by the separation of those forms of the disease where the presentation was one of peripheral nerve damage from the form where the lesions mainly took the form of nodular deposits in the skin. The association of anaesthetic patches in the skin with a surrounding raised erythematous edge or with a large erythematous plaque led to the description of these lesions as "tuberculoid" owing to their occasionally resembling the lesions of lupus vulgaris. The nodular lesions are generally referred to as "lepromatous" as they are typical of this form of disease. Thus leprosy has become divided into 2 polar forms, "tuberculoid" leprosy and "lepromatous" leprosy. However, more recently many descriptions have been made of patients who may show, at one and the same time, manifestations of both polar forms of the disease. This form of leprosy has been referred to as "dimorphous" or "borderline" leprosy. Occasionally, patients may present with just one or more hypopigmented macules. These cases may be difficult to classify into one or other of the more florid forms of the disease, and are referred to as "indeterminate" leprosy. However, the disease rarely stays in this form and may develop with the production of more typical tuberculoid or lepromatous lesions. It is thought that the indeterminate state is probably the earliest form of the disease and that many patients pass through it before they develop the typical manifestions of tuberculoid or lepromatous leprosy.

Thus, clinically, leprosy forms a spectrum between the 2 polar forms—tuberculoid and lepromatous leprosy—and patients may present with varying lesions depending on the degree to which tuberculoid or the lepromatous elements contribute to the disease. Apart from those with the 2 polar forms, patients may vary from time to time in the degree to which the 2 elements contribute to the disease.

Thus the disease may often be considered to be "mobile". A patient with leprosy at the tuberculoid end of the spectrum may move to a more borderline condition, or a patient with borderline leprosy may move to the lepromatous end of the spectrum as a result of an exacerbation of the disease. Conversely, on sulfone therapy a patient may move from the lepromatous end of the leprosy scale towards a more borderline position, or a patient with borderline leprosy may move towards a tuberculoid position.

The lesions in the 2 polar forms of leprosy show marked histological differences. In tuberculoid leprosy, the granuloma is invariably infiltrated with a dense collection of small lymphocytes and, despite well-developed collections of histiocytic cells (macrophages), *Myco. leprae* are not found on standard methods of examination. This is in marked contrast to the appearance in lepromatous leprosy where small lymphocytes are absent or scanty and the macrophages are packed with leprosy bacilli which may form globi. The tissues throughout the body may be infiltrated with macrophages containing proliferating bacilli which, it appears, cannot be eliminated. As with the clinical state, the histological appearance shows a broad spectrum between the 2 polar forms, the main variable being the proportion of small lymphocytes present in the lesion, and the intensity of parasitism of the macrophages by mycobacteria. Also, in parallel with the trend towards the lepromatous end of the scale as the disease worsens, lesions may show a decrease in the number of small lymphocytes and an increase in the number of bacilli present. Similarly with treatment, as the clinical condition swings back towards the tuberculoid end of the spectrum there will be an increased infiltration with small lymphocytes as the bacterial load gradually decreases.

In an attempt to provide a scheme whereby the patient can be assigned at any time to the correct place on the spectrum, Ridley and Jopling (1966) attempted to characterize a number of points on the leprosy spectrum, understanding, however, that the disease in a number of patients was mobile and that the patients could only be assigned to one place on the scale at a particular point in time. By closely correlating the clinical state of the patient with the histological appearance of the lesions, Ridley and Jopling were able to define 5 points on the spectrum. The 2 polar forms of the disease at the tuberculoid and lepromatous ends of the spectrum are referred to as TT and LL, respectively, while the central position, dimorphous or borderline leprosy, is referred to as BB. They added 2 other points, BT between TT and BB, and BL between BB and LL. It was recognized that patients with single-lesion TT rarely, if ever, swing forward across the spectrum, and patients with true polar LL also rarely swing back across the spectrum. However, within the range BT–BL there could be considerable variation from year to year. In south-east Asia, lepromatous leprosy appears to be more labile than it is in other parts of the world and patients have a form of lepromatous leprosy which is far less stable than that found in LL patients in other parts of the world, presenting histologically with a slightly different picture in which the granuloma consists of undifferentiated histocytes, often with a fibrocytic appearance. These patients, intermediate between BL and LL with a potentiality to revert to BL on treatment, are sometimes referred to as LI (Ridley and Waters, personal communication). It must, however, be emphasized that the points TT, BT, BB, BL, LI and LL are arbitary points on what is a spectrum of disease. Patients can be at any particular time at points intermediate between the 5 marker points, i.e. TT/BT, BT/BB, BB/BL, BL/LI or LI/LL, depending on the state of their disease at that time.

THE IMPORTANCE OF THE SPECTRUM CONCEPT IN ASSESSING THE STATE OF RESISTANCE OF THE PATIENT TO HIS DISEASE

The correlation between the lepromin test and the state of clinical leprosy has been accepted for many years. Thus patients with tuberculoid leprosy are well known to be strongly lepromin-positive, while those at the lepromatous end of the scale are lepromin-negative. The presence of a positive lepromin test in a patient with indeterminate leprosy would indicate that he is more likely to develop tuberculoid leprosy, whereas a negative lepromin test would indicate a considerable risk of lepromatous leprosy developing.

At this stage it is profitable to digress slightly and discuss the nature and importance of the lepromin test. The antigen used is an extract of lepromatous tissue containing particulate material, especially particulate mycobacteria, as well as antigens derived from the human tissue from which the extract is derived. The reaction appears in 2 phases: a typical, delayed hypersensitivity reaction—the Fernandez reaction—maximal 48 to 72 hr after intradermal inoculation, and a papular granulomatous reaction—the Mitsuda reaction—which develops 3 weeks after inoculation. It is the Mitsuda reaction which is used by most leprologists to indicate whether a patient with leprosy is at the tuberculoid end of the scale. Although this reaction takes place as long as 3 weeks after inoculation, and is not a typical delayed hypersensitivity reaction, it is probably evidence of a high degree of cell-mediated immunity directed against the mycobacterial antigens. As the inoculated material is particulate, it is retained in the tissues for a long period and does not diffuse always as rapidly as does tuberculin. However, it may be that in the form in which it is injected the antigen is not readily available to react with the sensitized lymphocytes of a cell-mediated immune process and possibly the 3-week latent period is the time taken for the mycobacterial antigens to be exposed and made available, perhaps by tissue enzymes.

The lepromin test is not in itself an indication of infection with *Myco. leprae*, but indicates that the patient has had previous contact and has been immunized with these antigens which are probably common to a number of mycobacteria. Normal subjects can be immunized to produce positive lepromin tests by repeated intradermal injections of the killed antigen. However, it does indicate that the patients are capable of mounting a strong cell-mediated immune reaction against *Myco. leprae* associated with marked lymphocytic infiltration.

A positive lepromin test and the extensive lymphocytic infiltration in the lesions of patients with tuberculoid leprosy of the TT type indicate that these patients have a high degree of cell-mediated immunity directed against *Myco. leprae*. This means that they have in their circulation sensitized lymphocytes capable of activating macrophages and interacting with *Myco. leprae* antigens. This would account for their lesions having the appearance of typical cell-mediated allergic reactions (i.e. prolonged erythema, induration and infiltration with small lymphocytes). It would also account for the fact that mycobacteria are absent from the lesions. Thus they also have a high degree of cell-mediated *immunity* and are capable of eliminating *Myco. leprae* from the body, although they over-react with an intense allergic reaction to the antigens at the same time.

In tuberculoid leprosy of the BT type lymphocytic infiltration is of the same degree as that in TT leprosy and the extent of the allergic reaction in the skin is as intense. However, more than one lesion is present and mycobacteria can be found in the lesion, indicating that the degree of cell-mediated immunity is less, so that not all organisms are immediately eliminated. The lepromin test is also not so intensely positive as in the polar form (TT). Throughout the BB and BL forms, cell-mediated immunity appears to be increasingly less as indicated by the negative lepromin test, decreasing lymphocytic infiltration in the lesions and increasing numbers of mycobacteria that can be found.

This loss of cell-mediated immunity increases

so that in the LI or polar LL forms of leprosy there may be no lymphocytic infiltration and the mycobacteria proliferate throughout the body as though the body possessed no defence mechanisms towards the organisms at all. Mycobacteria are found growing in colonies and forming globi within the macrophages in an apparently undisturbed manner.

Thus the spectrum of clinical leprosy from TT to LL appears to reflect a massive cell-mediated immune response at the one extreme to a complete absence of cell-mediated immunity at the other. The main problems to be discussed in understanding the various manifestations of leprosy are why in some patients there is this high degree of cell-mediated immunity and in others cell-mediated immunity is absent, why some patients starting with lesions indicating a high degree of immunity can develop lesions indicating a progressive loss of immunity, and why some patients with little or no immunity can, on chemotherapeutic treatment, develop lesions of a different type, indicating that they are regaining a certain degree of immunity against the organisms. This latter state may be particularly distressing since, at the same time as they begin to eliminate organisms from their body, they will start to develop allergic reactions, affecting not only the skin but also the nerves, of a cell-mediated immune type directed against residual antigen. The resulting disease may not only be temporarily more severe than their original lepromatous state, but also may result in permanent scarring or nerve damage.

A CONSTITUTIONAL PREDISPOSITION TO LEPROMATOUS LEPROSY

Lepromatous leprosy seems not to occur in a fixed proportion of leprosy infections. In a population in which the rate of leprosy infection is decreasing there is not a parallel fall in the incidence of lepromatous leprosy. It has therefore been suggested by Newell (1966) that the development of lepromatous leprosy in an infected person is a host-determined characteristic that is present in a fixed proportion of all people everywhere.

Evidence that leprosy in its various forms occurred more frequently in certain families in endemic areas has been cited by a number of workers since it was first demonstrated by Danielsen and Boeck in 1848. Jamison and Vollum (1968) have recently cited evidence which suggests that leprosy occurs in families in which there is a naturally occurring weakness in their ability to develop a cell-mediated immune response. When tuberculin-negative children with a family history of leprosy were vaccinated with an avirulent strain of mycobacterium containing antigens which cross-reacted with *Myco. tuberculosis* (vole tuberculosis vaccine) only 18% were converted to tuberculin positivity, whereas 90% of children who came from families where there was no history of leprosy were converted to tuberculin positivity by the vaccine. This would indicate that children from families in which leprosy occurs are less able to develop a strong cell-mediated immune response, than children from families in which leprosy does not occur.

A similar relation between weakness of an ability to show a cell-mediated immune response and the development of leprosy can be shown in experimental animals. Under normal conditions it is only possible for *Myco. leprae* to proliferate locally and for a limited number of generations when inoculated into mice and other rodents, and after a period of 8 months, the organisms are rejected by the body. However, if adolescent mice are thymectomized and irradiated with 900 R, a process known to suppress the ability of the body to develop a cell-mediated immune response, the mycobacteria are able to spread throughout the body and produce a disease resembling leprosy in man (Rees *et al.*, 1967). The signs include nodular swellings of the skin similar to those seen in patients with lepromatous leprosy. If the mice received lymphoid tissue from animals with a normal state of cell-mediated immunity, the infection was far less severe than in those animals which had been thymectomized and irradiated but had not received lymphoid cell replacement. If animals were allowed to develop an infection like lepromatous leprosy and were then given an

inoculation of lymphocytes the nodular swellings became inflamed and subsided, after which the lesions developed a condition resembling that seen in borderline leprosy with nerve damage (Rees and Weddell, 1968). This would indicate that in experimental animals, a condition resembling lepromatous leprosy can be produced by eliminating cell-mediated immunity, and that the lepromatous state can swing towards the borderline state if cell-mediated immunity is restored partially by a transfusion of viable lymphocytes capable of mounting an immune reaction against *Myco. leprae.*

This suggests also that the constitutional defects predisposing to the development of lepromatous leprosy might be a weakness in thymus function in the young person, or an equivalent defect in the adult.

THE ABILITY TO SHOW OTHER ASPECTS OF CELL-MEDIATED IMMUNITY IN LEPROMATOUS LEPROSY

A generalized depression of the ability to mount a cell-mediated immune response has been demonstrated recently, by a number of authors, in patients with lepromatous leprosy (Waldorf *et al.*, 1966; Bullock, 1968). The general disability to show delayed hypersensitivity reactions was demonstrated by an inability of patients with lepromatous leprosy to become sensitized with DNCB (Waldorf *et al.*, 1966). Altogether, 75% of patients with lepromatous leprosy without erythema nodosum leprosum failed to be sensitized to DNCB whereas only 1 out of 5 patients with dimorphous (borderline) leprosy failed to be sensitized in this way; the technique used to assess whether sensitivity had developed was to skin-test the patients with 100 and 50 μg of DNCB in acetone. The result is curious since it has been found that the application of 100 μg of DNCB in acetone can produce a non-specific inflammatory reaction in unsensitized individuals (Turk and Waters, 1969). Moreover, 6 out of the 17 patients who could not be sensitized with DNCB were shown at the same time to be able to mount a cell-mediated immune reaction to tuberculin. As no record is given of what proportion of patients reacted to the 100-μg dose only and what proportion to both the 100- and 50-μg doses, this report shows some unsatisfactory features. The authors also found it difficult to explain why a similar state of anergy or inability to be sensitized with DNCB did not occur in patients in the same part of the lepromatous spectrum but who were having attacks of erythema nodosum leprosum. Also, it is difficult to interpret these results because the patients were only classified broadly into lepromatous or dimorphous leprosy without any assessment as to whether they were moving in the clinical spectrum of leprosy. We do not know whether the patients classified as lepromatous had the polar LL form of the disease or a form between LL and BB.

In another report by Bullock (1968), the Ridley-Jopling scale (TT-LL) was used to place the patients more accurately according to the clinical and histological state of their disease. As well as pre-existing sensitivity to lepromin, tuberculin, *Candida* and trichophytin, the ability of patients to become actively sensitized was assessed by their response to picryl chloride. Picryl chloride is a much weaker sensitizer than DNCB in experimental animals. It is known that DNCB will sensitize some 90% of normal individuals, and Bullock (1968) was able to sensitize 28 out of 30 normal people with this agent. Although patients in this series were originally classified according to the Ridley-Jopling scale, patients with TT and BT leprosy are referred to as tuberculoid and patients with BL and LL as lepromatous. In the patients with lepromatous leprosy who had been under treatment for less than 18 months, 70% could not be sensitized with picryl chloride; however a discrepancy again exists because only 50% of these patients were tuberculin-negative. Moreover, 10% of the patients were lepromin-positive, suggesting that they were closer to the borderline or tuberculoid end of the spectrum than the other patients in this group.

In a second group of lepromatous patients who had been treated for longer than 18 months, only 47% could not be sensitized with picryl

chloride. In this group, 40% were lepromin positive. A further problem in interpreting this result is that as many as 55% of the patients with tuberculoid leprosy failed to be sensitized with the picryl chloride. There appears to be no logical explanation why patients with tuberculoid leprosy fail to be sensitized to picryl chloride when 90% of controls can be sensitized easily. One possible thought is that the 2 populations are not identical and that the controls come from a population which is more readily sensitized to picryl chloride than the group from which the patients with tuberculoid leprosy are derived. One explanation that Bullock (1968) himself has suggested is that in tuberculoid leprosy cell-mediated immunity is deviated so strongly to produce the reaction against *Myco. leprae* that no reserves are available to react with other antigens. The report of increased immunological activity following therapy in patients with lepromatous leprosy is interesting and has been observed in other groups of patients (Waters, personal communication). However, difficulty is obtained in interpreting these results as BL and LL patients are grouped together and it would be important to know whether increased ability to be sensitized to picryl chloride was associated with clinical evidence of an increase in the immune state, such as a movement across the immunological spectrum of the disease from LL to BL.

The finding of patients who were tuberculin-sensitive but unable to be sensitized to DNCB, poses an interesting problem. These patients could have a degree of cell-mediated immunity high enough to respond to antigens to which they were exposed before they developed leprosy, but insufficient to respond while they had their leprosy. Another possibility is that contact-sensitizing agents provide a weaker antigenic stimulus than bacterial antigens. In recent experiments (Turk and Waters, 1969) it has been found that patients with leprosy who could not be sensitized to DNCB could, however, be sensitized to develop delayed hypersensitivity to keyhole-limpet haemocyanin (KLH). KLH provides a more powerful antigenic stimulus than DNCB which acts by modifying the body's own proteins. The inability to respond to DNCB does not therefore indicate a complete anergy in respect to cell-mediated immunity, but suggests that this property is only somewhat weakened.

IN VITRO REACTIVITY OF LYMPHOCYTES FROM LEPROMATOUS PATIENTS

The failure of lymphocytes from patients with impaired cell-mediated immunity, by non-specific mitogens such as PHA, has been described in a number of diseases. These include primary thymic dysplasia, Hodgkin's disease, sarcoidosis, Sjögren's disease and primary biliary cirrhosis. It is possible that in these conditions the lymphocytes which are capable of being stimulated are those that are under the control of the thymus in neonatal life and are thus the same class of lymphocytes that can be stimulated to proliferate into "sensitized lymphocytes" in a cell-mediated immune response. As has been discussed above, considerable evidence is accumulating that there is an impaired ability of patients with lepromatous leprosy to manifest a wide range of cell-mediated immune reactions. These include contact sensitivity to simple chemical sensitizers and delayed hypersensitivity to antigens from *Candida* and trichophytin. Moreover, experiments by Job and Karat (personal communication, cited by Hart and Rees, 1967) in 4 patients with lepromatous leprosy indicate that these patients show prolonged survival of allogeneic skin grafts. In normal cultures of lymphocytes, approximately 80% of the cells can be transformed into lymphoblastoid type cells by the addition of PHA. Dierks and Shepard (1968) found that the cells from 8 active lepromatous patients had a low response to PHA (only 10% of the cells being transformed by this mitogen). Of these patients, 4 had erythema nodosum leprosum and this did not affect the response of the patients. In another series, 3 patients with active lepromatous leprosy were found to have a markedly diminished response. One surprising result in this series

was that 3 patients with tuberculoid leprosy also had a diminished response to PHA. The reason why patients with lepromatous leprosy fail to be stimulated with PHA and show a diminished ability to manifest delayed hypersensitivity reactions, could be explained as being due to a general failure to manifest cell-mediated immune reactions. This might be genetic as suggested by Jamison and Vollum (1968). However, other causes should be considered, such as the changes which are seen in the lymphoid organs of such patients which will be discussed in another section. Whether these changes themselves are also hereditary and due to thymus dysfunction will also be discussed.

More difficult to understand is the diminished responsiveness of the lymphocytes of patients with tuberculoid leprosy to respond to PHA. This may be associated with the diminished ability of these patients to develop delayed hypersensitivity to picryl chloride. One suggestion that has been put forward (Bullock, personal communication) is that the lymphoid tissue of such patients is responding so strongly to antigens from *Myco. leprae* that there is no residual activity to respond to other antigens. This would explain an inability to respond to an antigen, but it is difficult to see how this effect could cause an inability of the lymphocytes to respond to non-specific mitogenic stimulation with PHA.

Extremely interesting experiments have been described by Bullock and Fasal (1968). In these experiments, a depression of DNA synthesis, indicative of the transformation of lymphocytes into blast cells was found in lymphocytes from patients with lepromatous leprosy when exposed to PHA and streptolysin O (SLO) which also acts as a mitogen probably through its antigenic properties. A similar depression in the transformation of lymphocytes of patients with tuberculoid leprosy was found, as was described by Dierks and Shepard (1968). However, one of the most fascinating aspects of this investigation was that the depression of response to SLO was found only when the lymphocytes from lepromatous patients were suspended in autologous plasma. When the cells were suspended in homologous plasma from normal subjects no depression of activity was found. When the cells of normal subjects were suspended in the plasma of some lepromatous subjects depression in the ability of these cells to be transformed by SLO was observed. However, plasma from lepromatous patients did not suppress the response of lymphocytes from normal subjects to PHA. Thus the plasma from some patients with lepromatous leprosy contains a factor which can inhibit the response of normal lymphocytes to SLO. Much might be learned about the depression of cell-mediated immunity in leprosy if the nature of this factor could be discovered.

A similar phenomenon has been described by Melli *et al.* (1968) in patients treated with heterologous anti-lymphocyte serum. After 14 to 21 days of treatment, there was a severe depression of the response of the patients' lymphocytes to PHA. However, this only occurred if the cells were cultured in autologous plasma. In the presence of homologous plasma the response of the cells was normal. Correspondingly, the plasma of patients treated in this way would inhibit the ability of PHA to transform lymphocytes from normal people. Melli *et al.* (1968) considered, reasonably, that the factor present in the plasma of patients treated with anti-lymphocytic serum, which inhibited the transformation of lymphocytes by PHA, was in fact an anti-lymphocyte antibody. It would thus appear that a factor is present in the plasma of both patients with lepromatous leprosy and those treated with anti-lymphocytic serum which could inhibit the response of normal lymphocytes by non-specific mitogens. In both these cases it should be easy to find out whether the factor involved is an immunoglobulin, and thus an antibody. If an anti-lymphocytic antibody is present in the circulation of patients with lepromatous leprosy, this could have considerable implications. It is now well known that anti-lymphocytic antibodies act specifically by suppressing the circulating pool of long-lived small lymphocytes, influenced by the thymus in neonatal life, which are necessary for the

normal manifestation of a cell-mediated immunological reaction. Treatment of animals with anti-lymphocytic antibody has an effect very similar to thymectomy in neonatal life, in depleting the paracortical area of lymph nodes and the corresponding area of the spleen of these small lymphocytes, without affecting the lymphocytes in the lymph follicles, thought to be of direct bone-marrow origin, the germinal centres and the plasma cells in the medulla, involved in humoral antibodies production. Thus the presence of an anti-lymphocytic antibody in the circulation selectively inhibits cell-mediated immune processes without affecting the production of most types of circulating antibody. If an anti-lymphocytic antibody was present in the circulation, this could account for the anergy in respect of cell-mediated immunity in certain patients with lepromatous leprosy. How could such an antibody develop? Such an antibody would be an "autoantibody". Autoantibodies of a wide variety have been found in patients with lepromatous leprosy; they include the rheumatoid factor, thyroglobulin autoantibodies, antinuclear factor and the Wassermann antibody which is an autoantibody directed against heart muscle (Bonomo and Dammacco, 1968). The presence of these different autoantibodies may be due to the exposure of hidden antigenic groups as a result of tissue damage caused by the presence of such large amounts of microorganisms throughout the body. A similar wide spectrum of autoantibodies is found in a number of other chronic infective diseases such as tuberculosis and syphilis. In experimental animals the production of autoantibodies can be stimulated by the injection of an emulsion of homologous tissues in oil containing mycobacteria, thus reproducing the antigenic stimulus which the body receives from its own tissues in certain chronic infective diseases.

Thus an important step forward in understanding the mechanism of the loss of cell-mediated immunity in patients with lepromatous leprosy will be the characterization of the factor which inhibits the transformation of lymphocytes by SLO in the plasma of some of these patients.

IN VITRO EXAMINATION OF MACROPHAGE ACTIVITY IN LEPROSY

Although much work has been done on the behaviour of macrophages in experimental animals with tuberculosis, little study has been made of macrophage activity in patients with leprosy. Beiguelman (1967) has developed a technique for testing the lysogenic ability of macrophages for *Myco. leprae*. He observed the behaviour of macrophages from leprosy patients maintained in a tissue-culture medium after the addition of the dead leprosy bacilli. The macrophages from both lepromatous and tuberculoid patients actively phagocytosed the dead leprosy bacilli; the macrophages from the tuberculoid patients completely lysed the ingested bacilli, so that they became free of lipids. However, the macrophages from the lepromatous patients were unable to lyse the organisms and became transformed into typical lepra cells. Their cytoplasm was found to contain numerous bacilli and droplets of lipid material which stained readily with Sudan Black. A similar study was reported by Hanks (1947, pp. 21, 31, 38) who cultured phagocytic cells morphologically resembling fibroblasts from patients with leprosy. Those from patients with tuberculoid leprosy were able to lyse the organisms, whereas the cells from patients with lepromatous leprosy degenerated and released the organisms free into the medium. Although unable to lyse *Myco. leprae*, cultured macrophages from patients with lepromatous leprosy have been reported to be able to lyse *Myco. lepraemurium* and *Myco. tuberculosis* (Beiguelman, 1967).

The inhibition of migration of macrophages *in vitro* in the presence of sensitized lymphocytes and antigen is now a technique regularly used in the study of cell-mediated immunity. This technique, developed originally in experimental animals by Rich and Lewis in 1932 using tissue explants, has been adapted to free-cell suspensions by George and Vaughan (1962). More recently, methods have been described where this technique can be adapted for use with human tissues. In one of these methods (Thor, 1967; Thor and Dray, 1967) human lymph-node cells are cultured as a free suspension for 72 hr

prior to being placed in the capillary tubes. The lymph-node cells then grow out in a fan-like manner, similar to that seen in cultures of guinea-pig macrophages. In the presence of specific antigen they are inhibited from migrating.

Another modification is that described by Søberg and Bendixen (1967). In this modification, peripheral blood leucocytes are used in the capillary tubes and inhibition of the migration by specific antigen. Both adaptations of the leucocyte migration technique appear to correlate well with the presence of delayed hypersensitivity. The first technique has the disadvantage that it uses lymph-node cells and needs biopsy, whereas the second technique uses peripheral leucocytes. Moreover, the second technique has been performed with particulate organisms, especially *Brucella abortus*. The development of the cells' ability to be inhibited from migrating in the presence of specific antigen correlates well with the known course in time of the development of cellular, rather than humoral, immunity (Søberg, 1968).

The technique of Søberg and Bendixen (1967) lends itself as an *in vitro* approach which could be used to investigate cellular immunity against *Myco. leprae* in humans. The antigen could be killed *Myco. leprae*, similar to that used by Beiguelman (1967) to investigate macrophage activity. The technique of Thor (1967) is more difficult in that it uses lymph-node cells which are more difficult to obtain than peripheral blood leucocytes and also entails a 72 hr culture of the cells as a free-cell suspension, with the associated risk of any long-term tissue culture.

In further experiments, Thor *et al.* (1968) incubated peripheral blood lymphocytes with soluble antigen for 24 to 48 hr, then added the supernatant to a suspension of guinea-pig peritoneal macrophages in capillary tubes. The incubation of specifically sensitized human lymphocytes with the specific antigen causes the release of a migration inhibition factor similar to that described by Bennett and Bloom (1968) which inhibits the migration of the guinea-pig macrophages. This technique, although also using peripheral blood cells, is difficult and involves cultivation of the lymphocytes prior to the release of the factor; being a 2-stage cultivation procedure, it is not an easy technique but could be adapted to leprosy research under carefully controlled experimental conditions.

Extensive research on cell-mediated immunity *in vitro* is thus recommended along 2 lines. The first, using the leucocyte-inhibition technique of Søberg and Bendixen (1967) or Thor *et al.* (1968), should give information on the presence of sensitized lymphocytes capable of secreting a migration inhibition factor after reaction with *Myco. leprae* or soluble antigens derived from the organisms. This substance can be presumed to act on leucocyte cell surfaces causing increased stickiness of these cells. The second approach should be an extension of the work of Beiguelman (1967) to investigate what it is that causes the macrophages of tuberculoid leprosy patients to lyse *Myco. leprae* after phagocytosis, whereas the macrophages from lepromatous patients cannot lyse the organisms. It could perhaps be shown that there was inhibition of leucocyte migration by *Myco. leprae* using the blood from tuberculoid leprosy patients and that *Myco. leprae* fails to inhibit the migration of leucocytes from lepromatous leprosy patients. Moreover, a soluble substance could be obtained from the culture supernatants of leucocytes from tuberculoid leprosy patients with *Myco. leprae*, which might be able to stimulate the macrophages from patients with lepromatous leprosy to lyse *Myco. leprae*.

THE HISTOLOGICAL APPEARANCE OF LYMPHOID TISSUE FROM PATIENTS WITH LEPROSY

As has been mentioned above, the lymph nodes from experimental animals treated in various ways to suppress cell-mediated immunity show a particular histological appearance. Neonatal thymectomy or treatment with antilymphocyte serum causes a marked depletion of lymphocytes from the paracortical areas of the lymph nodes and these cells are replaced by pale-staining reticulo-histiocytes. The germinal centres and

their marginal cuff of small lymphocytes are unaffected by this process, as also are the plasma cells at the cortico-medullary junction and in the medullary cords. A similar picture is found in the lymphoid tissue of children with congenital thymic aplasia or with the Wiskott-Aldrich syndrome which is also associated with a failure on the part of the patient to show cell-mediated immune reponses. However, lymph follicles, germinal centre formation and plasma cell proliferation in the medulla, which are associated with humoral antibody formation are unaffected by this process.

In a recent study, a similar histological pattern has been found in the lymph nodes of patients with lepromatous leprosy (Turk and Waters, 1968). The lymphocytes in the paracortical area of the lymph nodes were almost completely replaced by reticulo-histiocytes. These cells were morphologically similar to the cells seen replacing the lymphocytes in the paracortical area of lymph nodes from the guinea-pigs treated with anti-lymphocyte serum (Turk and Willoughby, 1967). These cells also had striking phagocytic activity and in advanced cases they were seen to have ingested large numbers of *Myco. leprae*. However, this appears to be a secondary phenomenon as in cases that were nearer the borderline part of the spectrum, often there were no bacilli within these cells. The infiltration was confined exclusively to the paracortical areas of the lymph nodes and can be considered as due to drainage of these cells from areas of histiocytic infiltration in the periphery. The germinal centres were normal and their marginal zone of small lymphocytes was not decreased. The medullary cords of the lymph nodes were unaffected and contained large numbers of plasma cells. In borderline leprosy there was some infiltration of the paracortical areas with these histiocytic cells but there were considerable numbers of small lymphocytes still present. In one case, where the biopsy specimen had been taken after treatment for 7 months (during which the patient had moved clinically from the lepromatous end of the spectrum to borderline leprosy, indicating that he had regained a certain degree of cell-mediated immunity) there was evidence of the beginning of repopulation of the paracortical areas with small lymphocytes. Some of the capillaries in the paracortical area were dilated and were surrounded by a cuff of small lymphocytes. At the tuberculoid end of the spectrum a completely different picture is seen. The paracortical areas are well developed and packed full of small lymphocytes; an occasional immunoblast can also be found. Germinal centres are not seen in the epitrochlear nodes and only an occasional plasma cell is seen. This is consistent with the observation of Hanks (1961) that no significant levels of antibody are found in those forms of the disease (tuberculoid) that are characterized by very small numbers of bacilli, strong lepromin reactions and a frank tendency to self-healing. On the other hand, patients with lepromatous leprosy, who have no immunity to infection, possess large amounts of circulating antibody to mycobacterial antigens. A similar finding was made by Rees *et al.* (1965) who found precipitating antibody in the sera of all patients with lepromatous leprosy directed against a number of different mycobacterial antigens and crude homogenates of leprosy tissue. In contrast, antibodies directed against mycobacterial antigens were only rarely found in the sera of patients at the tuberculoid end of the leprosy spectrum.

ERYTHEMA NODOSUM LEPROSUM

Although the antibodies against mycobacterial antigens do not contribute to the elimination of the mycobacteria, they are in a position to react with mycobacterial antigens *in vivo*. It is probable that the interactions between these antibodies and mycobacterial antigens exposed as a result of chemotherapy underlie the condition known as erythema nodosum leprosum. This condition takes the form of an acute vasculitis in the skin associated with polymorphonuclear leucocytic infiltration. It may be associated with iridocyclitis, arthritis and even glomerulonephritis. This symptom complex is reminiscent of that found in serum sickness and is then associated with the circulation of antigen-antibody complexes formed in slight

antigen excess which circulate and become deposited in small blood vessels, producing in the skin lesions which resemble the Arthus phenomenon. There is no doubt that the skin lesions of erythema nodosum leprosum have many features in common with the Arthus reaction as seen in experimental animals and may even have a haemorrhagic centre.

Erythema nodosum leprosum always occurs in that part of the leprosy spectrum where cell-mediated immune processes against the mycobacteria are absent or minimal and under conditions where there are large amounts of circulating antibodies against mycobacterial antigens, so high that they can be detected by techniques as crude as precipitation in agar (Rees *et al.*, 1965).

POSSIBLE CAUSES OF DEFICIENCES OF CELL-MEDIATED IMMUNITY IN LEPROSY

Depression of cell-mediated immunity in lepromatous leprosy is probably due to an interaction of a number of different factors and cannot be attributed to a single mechanism.

In the first place, there is no doubt that in patients with lepromatous leprosy a fundamental constitutional defect contributes considerably to the eventual deficiency. This is indicated by the observation that lepromatous disease is a host-determined characteristic that is possessed by a fixed proportion of all people everywhere (Newell, 1966). This observation is supported by the finding that the children of parents with leprosy showed a marked reduction in their ability to become converted to tuberculin positivity when vaccinated with vole tuberculosis vaccine, as compared with normal children (Jamison and Vollum, 1968).

The appearance of lymph nodes from patients with lepromatous leprosy is very similar to that seen in children with congenital thymic aplasia (Turk and Waters, 1968) and suggests that the defect might be due to an inherent deficiency in thymus control of cell-mediated immunity. The suggestion is supported by the observation of Rees *et al.* (1967) that a disease resembling lepromatous leprosy can be produced by infection in mice following a combination of adolescent thymectomy and deep X-irradiation.

However, it is more likely that this is due to drainage of histiocytes from peripheral lesions. Such cells passing down the afferent lymphatics will tend to accumulate in the paracortical areas. Although the appearance of lymph nodes resembles those seen under conditions where there is thymus deficiency, there is no evidence that this plays any role in these appearances in leprosy. There is a suggestion from the work of Bullock and Fasal (1968) that an anti-lymphocytic antibody could be present in the plasma of such patients. Moreover, Gaugas (1968) has found that a combination of thymectomy at 6 weeks of age and treatment with antilymphocytic antibody will allow *Myco. leprae* to proliferate to the extent of reproducing many of the features of lepromatous leprosy. As has been discussed above, such an antilymphocytic antibody could be developed as an autoimmune phenomenon as a result of extensive tissue damage during infection with *Myco. leprae.*

Another non-specific factor that could be relevant to the depression of cell-mediated immunity in leprosy is that the ability to develop delayed hypersensitivity is suppressed in guinea-pigs pretreated with Freund's adjuvant (Jankovic, 1962). The infiltration of tissues with Freund's adjuvant containing mycobacteria, might produce conditions similar to those found in leprosy. Guinea-pigs treated in this way are not only not able to produce cell-mediated immune reactions when actively stimulated with antigen but also have a reduced ability to manifest passively transferred reactivity (Asherson and Allwood, 1969).

All these factors will contribute to a non-specific depression of cell-mediated immunity. However, except in the polar form of lepromatous leprosy, the inability to show cell-mediated immunity is specific to *Myco. leprae* and a non-specific depression does not occur in other parts of the spectrum where bacillary growth may be little controlled. Thus a specific immunological tolerance must play a certain role in the ability to respond to the organism.

Moreover, this tolerance must be in respect of cell-mediated immunity only, as humoral antibody production, far from being depressed, is actually increased. Such "split tolerance" or immune deviation is found in experimental animals following the injection of alum-precipitated antigens (Asherson and Stone, 1965) or by the intraveous injection of soluble antigen (Dvorak *et al.*, 1965) prior to immunization. A similar state of split tolerance involving chemical-contact-sensitivity has been produced in already highly sensitive guinea-pigs (Polák and Turk, 1968). Thus, a cell-mediated immunity can be suppressed after it has developed. This may explain certain aspects of loss of immunity during the exacerbation of leprosy, which may occur when a patient with borderline leprosy swings towards lepromatous leprosy, or a patient at the tuberculoid end of the spectrum develops a disease with certain lepromatous aspects (borderline leprosy).

Therefore, it is probable that the loss of cell-mediated immunity in leprosy could originally be the result of the development of a state of specific immunological tolerance in an individual who already has a basic constitutional defect in cell-mediated immunity. With progressive infiltration of the tissues with *Myco. leprae*, the basic constitutional defect is aggravated so that there occurs a generalized non-specific failure to show certain aspects of cell-mediated immunity such as contact sensibility and homograft rejection. However, this in itself is never complete. Patients with lepromatous leprosy do not die within 6 months from virus and parasitic infections, as do babies with congenital thymic aplasia. A certain degree of cell-mediated immunity is always retained and patients who cannot be sensitized with DNCB can be sensitized with a stronger antigen such as KLH. Moreover, there is no increased incidence in the development of cancer by these patients, as might be expected if there was no ability retained to show cell-mediated immunity. If anything, leprologists have the impression that cancer is somewhat less prevalent in patients with leprosy than it is in the general population.

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The Minimal Inhibitory Concentrations of Sulphadimethozine and Sulphadoxine Against *Mycobacterium leprae*[*]

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Sulphadimethoxine inhibited the multiplication of *Myco. leprae* in the mouse footpad system when given at a dietary concentration of 0.01%, but not at one of 0.001%. Sulphadoxine inhibited the multiplication of *Myco. leprae* at a dietary concentration of 0.04%, but not at one of 0.004%. Determination of the plasma concentrations of the 2 drugs in mice fed with these dietary concentrations of sulphadimethoxine and sulphadoxine indicated that the MICs of these 2 compounds against *Myco. leprae* were between 20 and 35 μg per ml. The significance of these findings is discussed, both in relation to the previously determined MIC of dapsone against *Myco. leprae* and to the use of these 2 long-acting sulphonamides in the treatment of human leprosy.

Sulphadimethoxine (2,6-dimethoxy-4-sulphanilamido-pyrimidine) and sulphadoxine (5,6-dimethoxy-4-sulphanilamido-pyrimidine) are 2 long-acting sulphonamides that have been shown to prevent the multiplication of *Mycobacterium leprae* in the mouse footpad test (Rees, 1965; Gaugas, 1967). Both compounds have been used in the treatment of human leprosy (Opromolla, 1962; Languillon, 1964; Currie, 1966; Gaind *et al.*, 1966; Languillon, 1969). Since *Myco. leprae* cannot be cultivated *in vitro*, the minimal inhibitory concentration (MIC) of an anti-leprosy drug can only be established *in vivo* by determining its concentration in the serum or plasma of mice fed with the minimum dose of the compound that effectively inhibits the multiplication of *Myco. leprae* in the mouse footpad. So far these combined techniques have been used to determine the MIC of only one anti-leprosy drug, 4,4[1]-diaminodiphenyl-sulphone (dapsone, DDS) (Shepard *et al.*, 1966; Rees, 1967*a*). In this paper we describe the methods used and the results obtained in determining the MICs of sulphadimethoxine and of sulphadoxine.

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DETERMINATION OF THE MINIMUM DOSES OF SULPHADIMETHOXINE AND SULPHADOXINE REQUIRED TO INHIBIT THE MULTIPLICATION OF *MYCO. LEPRAE*

The methods used for infecting and determining the multiplication of *Myco. leprae* in the footpads of mice were based on those previously described (Rees, 1965) and subsequently modified for chemotherapeutic studies (Rees, 1967*b*). Briefly, the hind footpads of female P-strain albino mice, in groups of 6, were inoculated with 10^4 acid-fast bacilli derived from homogenates of skin nodules from previously untreated patients with active lepromatous leprosy. In the present studies, 2 strains of *Myco. leprae* from previously untreated patients were used. One of these

strains was also shown to be sensitive to inhibition by 0.0001% dapsone in the diet, demonstrating directly that its sensitivity to dapsone was similar to that of all the other strains of *Myco. leprae* isolated from previously untreated patients (Shepard, 1967; Rees, 1967*a*; Shepard *et al.*, 1969). This standard procedure would be expected to result in the multiplication of *Myco. leprae*, yielding a 100-fold increase in acid-fast bacilli 6 months later in untreated animals. Such multiplication was confirmed by determining the number of acid-fast bacilli in the homogenates of both individual hind footpads from one of the mice in the untreated group killed 6 months after infection. With this confirmation, all the mice from both the untreated and treated groups of animals were killed within the next 2 months, and the number of acid-fast bacilli determined in homogenates from individual hind footpads from all the animals. The quantitative bacteriological methods used in these experiments registered a minimum count of 5×10^4 acid-fast bacilli per footpad homogenate.

Treatment was started on the day of infection and continued throughout the experiment by providing 5 g per mouse per day of powdered 41B diet (Bruce, 1950). The drugs in powder form were incorporated in the diet by thorough mixing in a mechanical food-mixer. Previous data (Rees, 1965) had shown that sulphadimethoxine and sulphadoxine inhibited the multiplication of *Myco. leprae* in the mouse footpad test at 0.1 and 0.04% of the drug in the diet, respectively. Therefore, in the present studies designed to determine the minimum doses of these compounds that would inhibit the multiplication of *Myco. leprae*, these 2 doses together with doses one-tenth and one-hundredth of the above were used. Other groups of 6 mice were fed for 14 days with either 0.001% or 0.01% or 0.1% sulphadimethoxine or 0.004% or 0.04% sulphadoxine in the diet, and the plasma concentrations of the sulphonamides determined in individual mice by the fluorometric and colorimetric methods described below.

DETERMINATION OF THE CONCENTRATION OF SULPHADIMETHOXINE AND SULPHADOXINE IN MOUSE PLASMA

Standards consisted of normal mouse plasma to which sulphadimethoxine or sulphadoxine had been added to a concentration of 75 μg per ml. Solvents and reagents were of analytical grade and the ethyl acetate was redistilled before use. Aliquots of mouse plasma (0.2 ml) were pipetted into small stoppered centrifuge tubes together with 2.8 ml of water and 1.0 ml of M/15 pH 7.0 phosphate buffer, and extracted by shaking with 6.0 ml of ethyl acetate. The phases were separated by centrifugation, and the ethyl acetate extract decanted, using a Pasteur pipette, and dried by shaking with 0.5 g of anhydrous sodium sulphate. Sulphadimethoxine was then determined fluorimetrically by measuring the fluorescence of the dried ethyl acetate extract in an Aminco-Bowman spectrophotofluorometer at 332 mμ, the excitation wavelength being 280 mμ. In a similar way, sulphadoxine was determined by measuring the fluorescence of the ethyl acetate extract at 288/330 mμ. Then 3 ml of the ethyl acetate extract was extracted by shaking with 1 ml of 2 N hydrochloric acid, and the sulphadimethoxine and sulphadoxine determined colorimetrically in the 2 N hydrochloric acid extract by a modification of the procedure of Bratton and Marshall (1939). In this modification 0.3 ml of ethanol was added to 0.3 ml of the 2 N hydrochloric acid extract and reacted with 0.01 ml of 1% (w/v) aqueous sodium nitrite. After 5 min the nitrite was destroyed by the addition of 0.01 ml of 10% (w/v) aqueous ammonium sulphamate; after another 5 min 0.01 ml of 2% (w/v) N-1-naphthyl-ethylene-diamine-dihydrochloride was added in acetone/water (1 : 1 by volume) and the optical density measured at 545 mμ after a further 15 min.

RESULTS AND DISCUSSION

The results are summarized in Table I and illustrated in Fig. 1. Little diurnal variation in the plasma concentrations of these compounds was expected, in view of the fact that Böhni and

TABLE 1

Concentrations of sulphadimethoxine and sulphadoxine in mouse plasma and their inhibition of the multiplication of *Myco. leprae*

Drug	*Dose* (% *in diet*)	*Activity*	*Concentration in mouse plasma* (μg/ml) *Fluorimetric method*	*Colorimetric method*
Sulphadimethoxine	0.001	0	12.4±3.4*	7.7±3.0
	0.01	+	37.1±5.1	30.6±6.7
	0.1	+	77.5±11.6	73.9±11.6
Sulphadoxine	0.0004	0	———†	———
	0.004	0	22.3±8.8	20.2±2.7
	0.04	+	62.1±8.9	60.9±11.3

+ = full activity. 0 = inactive. * Mean ± S.D. observations. † Not measured.

her collaborators (1969) found the half-lives of sulphadimethoxine and sulphadoxine in the mouse to be 28 and 38 hr, respectively. Furthermore, after 14 days' continuous feeding in the diet, both the drugs should have reached equilibrium concentrations.

The principal metabolites of these sulphonamides, their N^4-acetyl derivatives and glucuronide conjugates, are devoid of antimicrobial activity (for a recent review, see Böhni *et al.*, 1969). It was anticipated that these metabolites would not be determined by either the fluorometric or the colorimetric methods, since the glucuronides would not extract into ethyl acetate at pH 7.0 and the N^4-acetyl derivatives would not be expected to fluoresce, and cannot be diazotized and coupled by the Bratton and Marshall procedure. The fact that similar results were obtained using both the fluorometric and colorimetric methods supports the conclusion that both methods specifically measured the microbiologically-active unchanged sulphonamides.

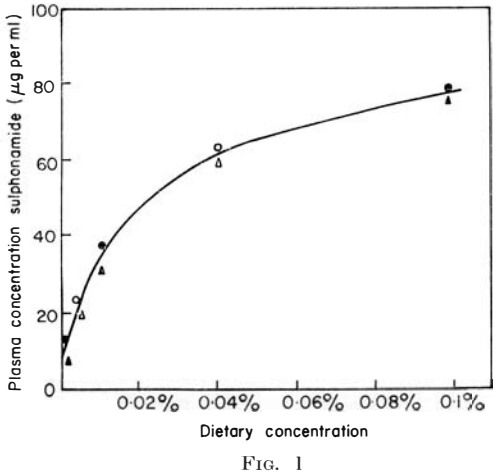

FIG. 1

● Sulphadimethoxine (fluorometric method).
▲ Sulphadimethoxine (colorimetric method).
○ Sulphadoxine (fluorometric method).
△ Sulphadoxine (colorimetric method).

It will be noted that, with both drugs, the plasma concentrations did not increase proportionally to the amount of drug given in the diet. Although it is conceivable that the absorption of sulphadimethoxine and sulphadoxine is less complete at the higher dietary concentrations, Böhni *et al.* (1969) had previously obtained evidence that when sulphadoxine is administered orally to the mouse in therapeutic doses it is almost completely absorbed. A more likely explanation of our results is that the half-life of these 2 sulphonamides decreases with increasing size of dose. Thus, while sulphadoxine has a half-life of 38 hr in mice when given at a dose of 8.7 mg per kg body-weight (Rieder and Böhni, 1964), after a dose of 200 mg per kg its half-life is only 20 hr (Rieder and Böhni, personal communication). The fact that the data for both sulphadimethoxine and sulphamethoxine can be conveniently plotted on the same curve (see Fig. 1) suggests that equal doses of these drugs would give rise to very similar plasma concentrations in the mouse.

Since the multiplication of *Myco. leprae* in the mouse footpad is inhibited by a dietary concentration of 0.01% sulphadimethoxine but

not by a concentration of 0.001%, and since these dietary concentrations result in plasma concentrations of 34 μg per ml and 10 μg per ml of the drug respectively, it was concluded that the MIC of sulphadimethoxine against *Myco. leprae* is about 20 μg per ml. Similarly the multiplication of *Myco. leprae* was inhibited by sulphadoxine in a plasma concentration of 61 μg per ml, but not by one of 21 μg per ml. The MIC of sulphadoxine against *Myco. leprae* is therefore about 35 μg per ml. However, since the dietary concentrations used for testing sulphadimethoxine and sulphadoxine were widely separated, it cannot be inferred that the MICs of these 2 compounds against *Myco. leprae* differ significantly.

The average weight of the mice was 25 g. Dosage with 0.01% (active) and 0.004% (inactive) of these drugs was therefore equivalent to daily dosage with 20 and 8 mg per kg body weight, respectively, of these sulphonamides. The 50% curative dose (CD_{50}) of these compounds against *Myco. leprae* in the mouse must therefore lie between these 2 doses. The results reported by Gaugas (1967), who found that the multiplication of *Myco. leprae* in the mouse footpad was completely inhibited by daily oral administration of single doses equivalent to 75 mg per kg body weight (sulphadimethoxine) and 25 mg per kg body weight (sulphadoxine), are in accord with these conclusions. These CD_{50}s are similar to that obtained by Böhni *et al.* (1969) for sulphadimethoxine against infections of *Escherichia coli* in the mouse, and are from 2 to 20 times greater than the CD_{50}s obtained for both compounds against infections with *Staphylococcus aureus*, *Streptococcus haemolyticus*, *Proteus vulgaris* and *Klebsiella pneumoniae*.

DISCUSSION

These results may be contrasted with those obtained with dapsone, which consistently inhibits the multiplication in the mouse footpad of strains of *Myco. leprae* derived from untreated patients, at a dietary concentration as low as 0.0001% (Shepard *et al.*, 1966, 1969; Shepard, 1967; Rees, 1967*a*, *b*). Estimation of the serum or plasma concentrations of dapsone in mice fed with 0.001 to 0.1% of the sulphone in the diet, indicated that a dietary concentration of 0.0001% would give rise to plasma concentrations of the drug of only about 0.01 to 0.015 μg per ml (Shepard *et al.*, 1966; Rees, 1967*a*; Ellard *et al.*, 1971). Thus, it would appear that while *Myco. leprae* is exquisitely sensitive to dapsone, it is some 2000 times less sensitive to sulphadimethoxine and sulphadoxine.

The MIC of dapsone against *Myco. leprae*, as determined in the mouse footpad system in this way, not only explains the effectiveness of standard doses of 100 to 600 mg of dapsone per week in the treatment of human leprosy, but has been used to predict the efficacy of doses of as little as 1 mg of dapsone per day (Waters and Rees, 1971). It is pertinent, therefore, to consider the clinical implications of these determinations of the MIC of sulphadimethoxine and of sulphadoxine against *Myco. leprae*.

When used for the treatment of human leprosy, sulphadimethoxine is normally given in doses of either 0.75 g on alternate days (Languillon, 1964) or 1.5 g daily (Opromolla, 1962). The half-life of sulphadimethoxine in man is about 36 hr (Brandman *et al.*, 1959; Madsen and Iversen, 1964). Consequently, daily administration of the drug results in a progressive rise in plasma concentrations until after about a week a plateau is reached. Madsen (1961) found that the plasma concentration of sulphadimethoxine immediately before daily dosage with 0.5 g of the drug rose to about 60 μg per ml after 5 days and remained constant thereafter. Brandman *et al.* (1959) found peak blood concentrations of about 60 μg per ml 4 hr after dosage with 1 g of the drug, and after repeated daily dosage a plateau of about 70 μg per ml immediately before the next daily dose. Assuming that sulphadimethoxine, like sulphadoxine, does not penetrate into the blood red cells to any significant extent (Böhni *et al.*, 1969), such a plateau blood concentration would be equivalent to a plasma concentration of about 120 μg per ml. It would therefore appear that

repeated daily dosage with 1.5 g of sulphadimethoxine would lead to the attainment of plasma concentrations fluctuating between about 180 and 300 μg per ml.

Sulphadoxine is usually given in a dosage of 1 to 1.5 g per week when used for the treatment of human leprosy (Currie, 1966; Gaind *et al.*, 1966; Languillon, 1969). Its half-life in man is about one week (Portwich and Büttner, 1964; Böhni *et al.*, 1969), and repeated daily dosage with 1 g of sulphadoxine eventually gives plasma concentrations that fluctuate between about 100 and 200 μg per ml (Böhni *et al.*, 1969).

Thus the plasma concentrations of these 2 sulphonamides that are achieved in man at the dosages usually given in the treatment of leprosy range from about 150 to 300 μg per ml. This is between 4 and 15 times their MICs against *Myco. leprae* as determined in the mouse footpad test. It is therefore suggested that the treatment of human leprosy with sulphadimethoxine and sulphadoxine is comparable to the use of dapsone in doses of less than 15 mg per day or of giving injections of 225 mg 4,4[1] diacetyl diamino diphenyl sulphone (DADDS) every 11 weeks (Shepard *et al.*, 1968). Great care should therefore be taken to establish whether or not relapse can occur during treatment with these 2 long-acting sulphonamides because of the appearance of drug-resistant mutants of *Myco. leprae* (Rees, 1967*a*, *b*; Shepard *et al.*, 1969). Since cross-resistance has been demonstrated in one strain of *Myco. leprae* between dapsone and sulphadimethoxine (Adams and Waters, 1966) and between dapsone, sulphadimethoxine and sulphadoxine in 4 other strains of this organism (Rees, 1967*a*) patients who relapse during treatment with sulphadimethoxine or sulphadoxine, owing to the emergence of drug resistance, may fail to respond to subsequent treatment with dapsone.

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Management of Steroid-dependency with Clofazimine [Lamprene or B 663 (Geigy)]

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One of the main complications of leprosy confronting the clinician is the state known as reaction or acute exacerbation of the disease. Whether the patient presents in progressive lepra reaction or with repeated episodes of erythema nodosum leprosum (ENL), corticosteroids are often needed to control the symptoms. Indeed, high fever, severe peripheral neuritis, swollen and painful glands, or painful joints can often be controlled only by the use of these drugs. The danger is that such patients may become steroid-dependent. (Steroid dependence implies that the patients need to take corticosteroids continuously to control their symptoms, and that cessation of such treatment is followed by recurrence of the symptoms.) The side-effects of prolonged treatment with these drugs or with ACTH (corticotrophin) have been clearly pointed out by Jopling (1962), West (1962), and others.

Another related problem, of course, is the control of active disease. All anti-leprosy drugs, even in very small doses, are liable to precipitate an episode of acute exacerbation. The patient's condition may steadily deteriorate in spite of his taking steroids, because he cannot tolerate any drug that would control the active disease. It has been reported that clofazimine (Lamprene or B 663 (Geigy)) has an antibacterial action as well as an anti-inflammatory action (Barry and Conalty, 1965; Browne, 1965, 1966; Imkamp, 1968).

In October, 1967, a supply of Lamprene (B 663) in 100-mg capsules was made available by courtesy of Messrs. Geigy S.A. Basle, and the first 7 patients were chosen to take part in a trial of the drug; since then, 23 patients have been treated with the drug at this hospital. Of these original 23 patients, one absconded but, being later persuaded to return for treatment, was brought into the trial again, another absconded and did not return, and one died, possibly of adrenal failure.

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CHOICE OF PATIENTS

Patients for treatment with Lamprene were chosen for the following reasons: (1) Dependency on corticosteroids to control ENL or progressive lepra reaction [exacerbation nodules, or ENL, according to Ridley's classification of reactions (Ridley, 1969)]. (2) Inability to tolerate even small doses of anti-leprosy drugs, especially dapsone. Some patients had had full courses of other drugs, such as thiambutosine or thiosemicarbazone, but because of the danger of developing drug resistance it was considered unwise to continue giving these drugs. (3) The occurrence of severe peripheral neuritis, arthritis, or enlargement and suppuration of lymph nodes. (4) Increasing deformity. (5) A stationary or rising bacillary index (BI) and/or morphological index (MI). (These findings might imply the appearance of drug-resistance, but no steps were taken to verify this possibility.)

All but 2 of the 23 patients had been given corticosteroids at one time or another for short or long courses, and all such treatment had been given in hospital. One had received inadequate doses as an out-patient some years previously, before hospital facilities were available, while another had treated himself with corticosteroids and had been admitted to hospital as an emergency with steroid-induced haematemesis. Of the 23 patients in the trial, 14 were on frequent or almost continuous corticosteroid

therapy when treatment with Lamprene was begun. In none of the patients was the dosage of anti-leprosy drugs sufficient to control the disease, 10 of them being in exacerbation of the progressive type of lepra reaction, and 12 liable to repeated episodes of ENL. The bacillary and morphological indices of 11 of the patients were either stationary or rising, and 4 had severe peripheral neuritis with increasing limb deformity.

ADMINISTRATION OF STEROIDS

Corticosteroids had been given by two methods: (1) short course starting with a daily dosage of 40 mg of prednisolone, which was then reduced rapidly every other day, the total duration of the course being not more than 10 days; or (2) a longer course adjusted to the patient's symptoms. This longer course consisted of 30 to 40 mg of prednisolone given on alternate days and gradually tapering off as the patient's symptoms improved. All steroid-dependent patients were on the alternate-day régime. Some patients were also receiving ACTH, either in occasional doses of 40 units or once or twice weekly. At the beginning of the trial, the patients on prednisolone were receiving from 5 to 20 mg of the drug daily. The 14 patients who were steroid-dependent when starting the treatment with Lamprene were weaned from steroids in periods varying between 2 weeks and 7 months.

ADMINISTRATION OF LAMPRENE

Lamprene was given in a starting dose of either 100 mg on alternate days, or 100 mg daily, the dosage being gradually increased every 2 or 3 weeks until there was a definite clinical response. At the same time, the dosage of prednisolone was progressively decreased. Most patients received a maximum dosage of 300 mg of Lamprene daily, but some needed 400 mg daily, and one as much as 600 mg daily. When there was no further evidence of symptoms of reaction, and when the patient had been completely weaned from corticosteroids, the dosage of Lamprene was gradually reduced to a level that controlled the symptoms. Most patients were maintained on a daily dosage of 100 mg, some received 100 mg on alternate days, but a few needed 200 mg daily. When the patient's BI and MI had both fallen, dapsone was reintroduced in low doses, which were slowly increased at monthly intervals. Lamprene was stopped after a 6-month overlap with dapsone. Up to the time of writing, these patients have remained free of complications since the reintroduction of dapsone; 4 patients of the original 23 are now back on dapsone therapy.

CLINICAL RESPONSE

Many of the patients showed an almost immediate amelioration of symptoms. After Lamprene was given, some improved dramatically and were soon well enough to do things they had been unable to do for years. But those who were steroid-dependent were often slower to respond; in these cases, the dosage of both Lamprene and steroids had to be carefully adjusted to the individual's needs. A few became worse for a brief period after being on Lamprene for 6 to 10 weeks, with in some cases a severe exacerbation of symptoms. But they would then suddenly begin to improve and thereafter make steady progress. An intercurrent infection during treatment could also precipitate an acute exacerbation, but the latter usually improved as appropriate treatment controlled the precipitating infection.

BACTERIAL RESPONSE

The Bacterial Index (BI) and Morphological Index (MI) were determined at the beginning of the trial, and thereafter every 3 months (not always by the same technician).

All patients showed a steady response to treatment, as far as the leprosy infection was concerned. Of the 16 patients who have completed one year's treatment with Lamprene, all had a raised BI at the beginning of the trial, and all but 4 had viable bacillary forms in the smears. At the end of a year, only 6 had a raised BI and none had viable forms in the smears. Of those who had been steroid-

dependent, 6 still had a raised BI after one year, but in all of them the MI was zero.

HAEMATOLOGICAL FINDINGS

Some degree of anaemia was present in most of the patients before treatment with Lamprene, but as their general condition improved so also did their haemoglobin levels. Our aim was to raise the haemoglobin level to at least 75% (11 g). Malaria and hookworm infection were controlled by appropriate treatment.

A leukocyte count was made each month. Initial counts were all high, ranging from 15,000 to 30,000 per mm^3. As the clinical picture improved, so the count gradually returned to normal. If an exacerbation occurred during treatment, it was found that the white cell count increased. During treatment, 7 of the patients showed a mild eosinophilia. In no case, however, did the count fall to an abnormally low level, and in all cases it was normal after 6 to 8 months' treatment. The urine of all the patients was examined weekly for protein, but none of them developed any kidney complications.

SKIN PIGMENTATION

No patient objected to the pigmentation of the skin that developed. All turned a deep red, and the lesions later became black. Even black lesions on the face did not seem to embarrass the patients. The wide range of skin colour among the population in Thailand may account for the ready acceptability of a change in skin pigmentation due to the drug. Out of the 21 patients still taking Lamprene, only one Chinese patient had difficulty with his family because of the change in his appearance.

All the patients were admitted to hospital at the beginning of treatment with Lamprene. When weaned from steroids, and as their health improved, they all asked to continue treatment as out-patients. This they were allowed to do; they returned to hospital each month for examination, some travelling considerable distances, well over 100 km (62 miles). They came by public transport, but pigmentation did not seem to constitute a social problem.

DISCUSSION

All 23 patients in this series treated with Lamprene responded well to treatment. After about 4 to 6 weeks, 12 patients had a further episode of acute exacerbation of their symptoms, but this yielded to an increase in the dosage of the drug. Once the patient began to improve, he usually made good and steady progress. Intermittent infections provoked an occasional episode of acute exacerbation, but this improved as the infection was controlled. Some patients needed a short course of steroids at this time.

Side-effects were minimal; 2 patients complained of nausea, and a few of itching of the skin. Pigmentation of the skin was obvious in all the patients, the normal skin turning brick red and the lesions black. But this did not prove to be a problem. Patients who had been steroid-dependent were treated as out-patients once they had been weaned from dependence, and those who were not taking corticosteroids were given Lamprene as out-patients. The drug had no apparent effect on enlarged, painful, or suppurating lymph nodes.

In all the patients the BI and MI showed a steady improvement and normal bacilli disappeared rapidly from smears taken routinely. No kidney complications were seen. White cell counts, which were high—20,000 to 30,000 in most cases—fell steadily and remained between 7000 and 11,000.

CONCLUSION

All patients in the series treated with Lamprene had severe complications of leprosy. Out of 23 patients, 14 were steroid-dependent when they started to take Lamprene. None had had adequate treatment with any antileprosy drug.

Treatment with Lamprene had the following results: (a) patients could be weaned from dependence on steroids; (b) the acute exacerbation was brought under control; (c) normal bacillary forms disappeared rapidly from routine smears, and the BI fell consistently; (d) peripheral neuritis was controlled; (e) return to dapsone treatment became possible, and 5 of the patients are now taking full doses of DDS.

Both patients and staff were impressed with the efficacy of Lamprene. Apart from skin pigmentation (which as shown did not constitute a problem here), there were no side-effects. For patients who are steroid-dependent, or who have intractable peripheral neuritis or persistent severe exacerbations, Lamprene is the drug of choice.

The author's results were subjected to statistical analysis, based on the 11 patients who completed the whole course of treatment, i.e. first with other anti-reactional drugs and then with clofazimine.

For each patient the following variables were used for the statistical evaluation, viz. the duration of the treatment period, the number of reactions occurring during the period, and the duration of such reactions. The two periods (i.e. before clofazimine treatment and during treatment with this drug) were then compared in 3 ways: the mean number of reactions in the 2 periods (number of reactions per month of the period); the mean duration of the reaction in the 2 periods; the relative duration of all reactions in the period according to the length of the period. Because of the low number of patients (11) completing the trial, parameter-free methods were considered appropriate for the statistical analysis.

It was demonstrated that: (1) reactions occurred less often during the period of clofazimine treatment; (2) the mean duration of each reaction was shorter during treatment with clofazimine; and (3) the total duration of reactions occurring in the clofazimine treatment period was less than during treatment with other anti-reactional drugs. It is pointed out that the 2 periods were not strictly balanced, clofazimine being given only in the later period, and also that results were available for only 11 of the 14 patients. Nevertheless, they are considered to be "*an indication* of the superiority of treatment" with clofazimine.

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Iridocyclitis in Lepra Reaction Treated with Thalidomide*

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Thalidomide is shown to be a useful drug in patients with lepromatous leprosy who develop iridocyclitis during a lepra reaction. It should not be given, however, to women in their child-bearing years.

Iridocyclitis is one of the more severe manifestations of the lepra reaction (Gomez Orbaneja and Garcia Perez, 1953; Duke-Elder, 1954; Cochrane and Davey, 1964). It may appear spontaneously or while the patient is under treatment.

Before corticosteroids became available, the management of iridocyclitis was unsatisfactory. Local or systemic steroid therapy has, however, considerably improved the outlook. High doses of these drugs are sometimes necessary, and their prolonged use incurs the risk of the well-known side-effects.

Recently, thalidomide has been reported to be effective in control of the general manifestations of the lepra reaction (Sheskin, 1965, 1970; Sheskin and Sagher, 1970). In this report, the effect of thalidomide on the iridocyclitis of patients suffering from reaction in lepromatous or dimorphous leprosy is recorded.

Case 1. A patient, age 59, developed skin lesions in 1955. Six years later, numerous nodular lepromata of the scalp, face, chest and extremities were found. There were no signs of ocular involvement. Smears taken from the ears, nose and one hand revealed acid-fast bacilli, and a biopsy of one of the lesions showed a lepromatous granuloma with numerous acid-fast organisms. The lepromin test was negative. Three months after the institution of sulphone treatment, a reaction consisting of lesions of erythema nodosum leprosum (ENL) appeared on the upper limbs and thighs. The reaction improved gradually with the daily administration of 1.5 mg of dexamethasone and a bi-weekly dose of 20 units of ACTH gel.

In December, 1964, a severe reaction occurred, with polyarthritis and polyneuritis. This was accompanied by severe photophobia and pain in both eyes, caused by bilateral iridocyclitis. Numerous keratitic precipitates and a well-marked flare in the anterior chamber were seen in both eyes. Treatment was continued with 50 mg of sulphone daily and 400 mg of thalidomide daily for a week. All manifestations of the lepra reaction, including the iridocyclitis, resolved during this time. For the last 8 years the patient has received a daily maintenance dose of 100 mg of thalidomide and 50 to 100 mg of sulphone, and no further reaction has occurred. For the last 4 years, smears taken from the patient have been bacteriologically negative.

Case 2. A widow, now aged 35, had suffered from lepromatous leprosy and lepra reaction for the past 23 years.

In October, 1967, at the first examination, diffuse lepromatous infiltration of the face, trunk, and extremities was seen. Atrophy of the thenar and hypothenar eminences, and partial absorption of the distal phalanges, were also present. There was cicatricial entropion of the left eye; the right eye was blind, the pupil was occluded, and numerous posterior synechiae

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were present. In November, 1967, while the patient was receiving a daily dose of 50 mg of sulphone, her temperature rose to 39°C (102.2°F) and erythema nodosum leprosum, polyarthritis, and severe left iridocyclitis appeared.

The eye showed numerous keratitic precipitates and a very pronounced flare in the anterior chamber. The condition improved gradually as the patient was given 20 mg of meticorten daily and a twice weekly dose of 20 units of ACTH gel; the condition recurred, however (without iridocyclitis), and the dose of meticorten was reduced to 10 mg daily, plus 20 units of ACTH gel weekly. In January, 1968, another similar reaction (including acute iridocyclitis) occurred. Steroids were withdrawn and topical 1% atropine drops were given, with 400 mg of thalidomide daily for a week; sulphone treatment was meanwhile continued. The reaction resolved within 3 days, and the signs of iridocyclitis disappeared within a week. As long as the patient contined to take a maintenance dose of 100 mg of thalidomide daily there was no recurrence of reaction, but stopping the drug was followed by the reappearance of symptoms of reaction, which however improved when thalidomide was again given. Thalidomide has now been given for 2 years, with no recurrence of iridocyclitis.

Case 3. A 52 year old woman who had suffered from lepromatous leprosy for 12 years, was first seen in April, 1969. She presented with nodular lepromata of the forehead and of the extremities. Sulphone treatment, 25 mg daily, was initiated. Two months later signs of reaction developed, the temperature rose to 38.5°C (101.3°F) and lesions of erythema nodosum leprosum appeared on the face and hands. These signs disappeared rapidly after treatment with 300 mg of thalidomide daily. A recurrence, 2 months after the withdrawal of thalidomide, was controlled by further thalidomide therapy.

In December, 1969, during treatment with clofazimine (Geigy B 663) the patient developed incipient reaction, together with iridocyclitis in the right eye. Keratitic precipitates and a moderately strong flare in the anterior chamber were noted. Dexazone, 2 mg daily, and the instillation of 1% atropine solution, led to improvement. Within 48 hr of 400 mg daily thalidomide being given, the reaction subsided; the iridocyclitis resolved within 6 days. Since then a maintenance dose of 100 mg daily of thalidomide, together with 25 mg sulphones, have been given and no further reaction has occurred.

COMMENT

Acute iridocyclitis in leprosy is characterized by ocular pain, ciliary injection and an exudative reaction in the anterior chamber. The keratitic precipitates and aqueous flare are difficult to differentiate from infective inflammation of diverse origin (Duke-Elder, 1954). Acute reaction may precipitate a reactivation of a former iritis or trigger the onset of a new inflammatory process in the anterior chamber. In 3 patients suffering from lepromatous leprosy, iridocyclitis either appeared for the first time or recurred during a lepra reaction.

A patient with indeterminate leprosy who had never shown signs of lepra reaction, developed an acute iridocyclitis. The usual dose of thalidomide and atropine during 10 days failed to improve the condition. Treatment was altered to Meticorten, 30 mg daily, plus 20 units ACTH weekly, and local application of a 1% atropine solution. Improvement followed.

All the reactional lesions in lepromatous leprosy (including neuritis, polyneuritis, erythema nodosum leprosum, orchitis, and iridocyclitis) improved with thalidomide; they failed, however, to improve in a patient suffering from dimorphous leprosy in reaction, and in a patient with indeterminate leprosy in whom iridocyclitis was present. In the 3 patients with lepromatous leprosy described above, the iridocyclitis quickly improved on thalidomide treatment on 4 occasions. Iridocyclitis and other manifestations of the lepra reaction did not recur while the patients were receiving a maintenance dose of this drug.

No explanation can as yet be offered of the pharmacological effect of thalidomide, but it is thought to be immunosuppressive. In no case were signs of toxicity observed. Side-effects were mild, and in no case was the withdrawal of treatment necessary.

The results observed in these 3 patients justify further trials of thalidomide in iridocyclitis accompanying acute reaction in patients suffering from lepromatous leprosy.

SUMMARY

Signs of iridocyclitis accompanying other lesions of lepra reaction were seen in 3 patients suffering from lepromatous leprosy—twice in one of the patients and once in each of the others. In a fourth patient with indeterminate leprosy signs of iridocyclitis were found in the absence of a systemic lepra reaction.

Thalidomide therapy in doses of 300 to 400 mg daily, added to the existing DDS treatment, rapidly improved both the ophthalmic condition and the systemic signs of the reaction in the 3 patients with lepromatous leprosy, and daily maintenance doses of 50 to 100 mg of thalidomide (again added to DDS) prevented recurrences of the iridocyclitis. In the patient suffering from indeterminate leprosy, the iridocyclitis was not improved by thalidomide, and it was necessary to administer steroids both locally and systemically to alleviate the condition.

The favourable results of thalidomide administration in the lepra reaction of lepromatous leprosy and iridocyclitis justify further trials, from which, of course, female patients of child-bearing age should be excluded.

ACKNOWLEDGEMENT

Thanks are due to Professor J. Landau, M.D., for his helpful co-operation.

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Lepr. Rev. (1970) **41**, 236-237

Spina Bifida Occulta Causing Plantar Ulceration*

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The differential diagnosis of the underlying cause of plantar ulceration will include such well-recognized conditions as diabetes and yaws, and the relatively rare diseases of tabes dorsalis and syringomyelia. Plantar ulcer is the commonest serious foot lesion associated with leprosy, but anaesthesia and intrinsic muscle paralysis due to other diseases causing nerve damage must always be remembered. The following history of chronic plantar ulcer in a boy aged 16 years serves as a salutary reminder of this fact.

A deep plantar ulcer, 3 cm in diam., was present on the right heel (Fig. 1). Its base was clean and covered with thin granulation tissue. The sole of the foot was flattened, the foot being inverted (Fig. 2). There was loss of sensation to light touch, pin-prick, and thermal stimulation in the skin around the ankle and the outer aspect of the heel. No leprosy lesions were found in the skin, and all the peripheral nerves were normal. The Achilles tendon was shortened. There was no family history of leprosy. The patient was said to have started walking late and in a clumsy way.

Radiological examination showed early rarefaction of the os calcis. When the patient was stripped, a tuft of hair was noticed over the 4th and 5th lumbar vertebrae (Fig. 3). A small depression, suggestive of an underlying bony defect, was palpated at this site and confirmed by radiological examination, which showed defects of the 5th lumbar vertebra and the upper margin of the sacrum. There was pronounced atrophy of the glutei and of the muscles of the right thigh and leg. There was marked scoliosis, and knee and ankle jerks on the affected side were diminished. Bowel and bladder functions were normal. The results of laboratory investigations were as follows:

(1) Skin smears for *Myco. leprae*	Negative
(2) Swab from ulcer, examined for *Myco. leprae* .	Negative
(3) Wassermann reaction .	Negative
(4) Lepromin test (Mitsuda reaction) .	10 mm

The case was, therefore, diagnosed as *spina bifida occulta*. In this condition the vertebral arches fail to fuse, but there is no protrusion of the coverings of the cord. The defect is usually occupied by fibrous tissue to which the cord or dura is adherent and which is itself attached to the skin. As the bony vertebral column grows in length, traction is exerted on the cord, leading to paralyses and neuropathic ulcerations of various kinds.

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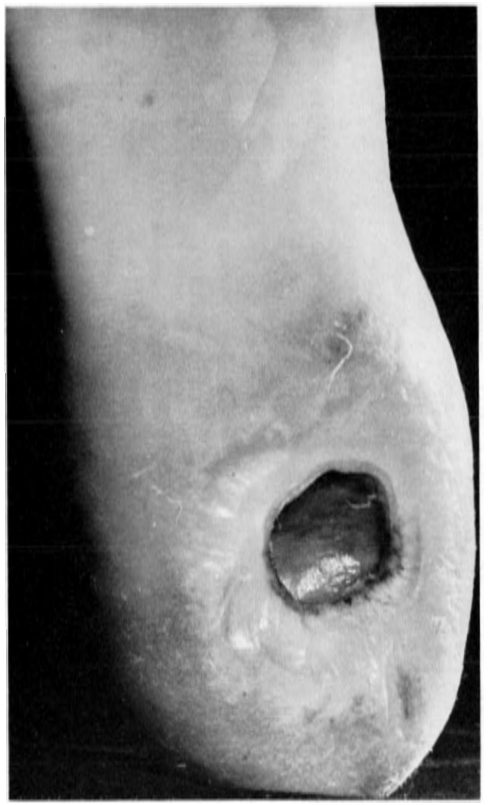

FIG. 1
Plantar ulcer on the right heel.

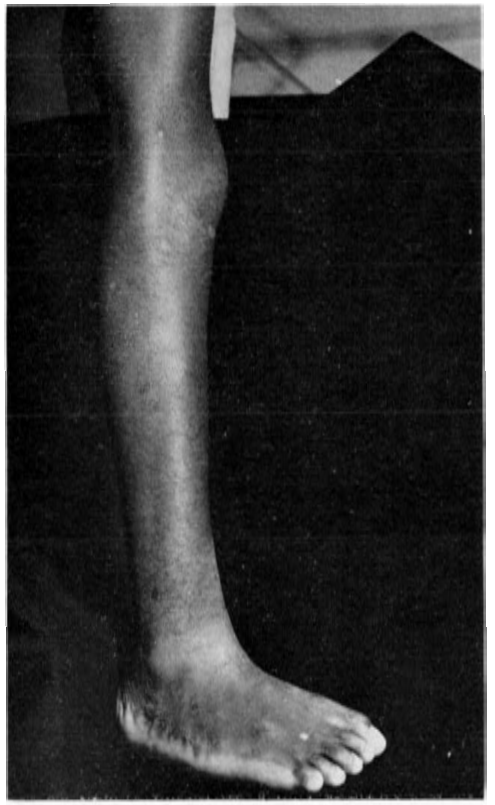

FIG. 2
Right foot inverted with dropping of the big toe.

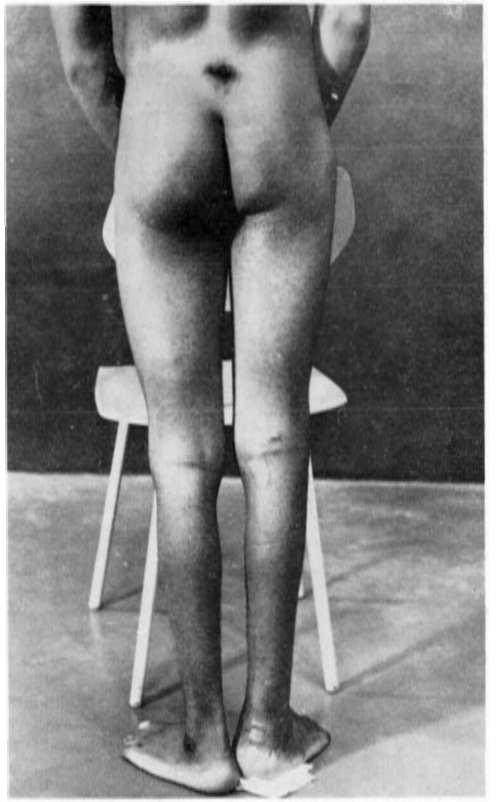

FIG. 3
The back of the patient showing tuft of hair over the lumbosacral area, atrophy of the right hip, thigh and leg muscles and swelling of the right ankle.

Lepr. Rev. (1970) **41**, 238-241

Abstracts

1. **Leprosy disability in Yimbo and its economic effects,** by H. J. Chum and Y. Otsyula. *East African med. J.*, 1970, **47**, 389.

The authors conducted a survey of patients with leprosy in Yimbo Location, in the Central Nyanza district of Kenya, with special reference to disability attributable to leprosy. A complete house-to-house census of the extremely scattered population was undertaken. Of the total number of people counted (5225), 92% (4731) attended for medical examination. Among these, 63 patients (24 male, 39 female) were diagnosed as suffering from leprosy (classification and activity not indicated), of whom 26 had some disability—cutaneous anaesthesia (14 patients), neuropathic ulceration (6), madarosis, claw-hand and footdrop. A third of the females had some degree of disability, and 13 males (out of 24).

S. G. Browne.

The following 2 abstracts are reprinted, with permission, from *Trop. Dis. Bull.*, 1970, **67**, 7:

2. **Evaluation of the earlobe in leprosy. A clinical and histopathological study,** by R. E. Mansfield, M. A. Storkan and I. S. Cliff. *Arch. Derm.*, 1969, **100** (4), 407-12.

Since the ear-lobe is a commonly chosen site for skin smears, a study was made of the histopathological lesion in the ear in 54 patients with leprosy (34 lepromatous, 10 dimorphous and 10 borderline).

There was found to be good correlation between clinical and histological findings in patients with lepromatous leprosy, and erythema nodosum leprosum was detected in the biopsy specimen whenever it was present clinically. In tuberculoid leprosy, however, the ear biopsy specimen showed only a chronic dermatitis, while in patients with the dimorphous type the biopsy results were unpredictable, and a characteristic granuloma was found in only 2 patients. There was good correlation between bacterial and morphological indices in scrapings and biopsies.

D. S. Ridley.

3. **La microradiographie et la microscopie de fluorescence appliquées a l'étude des lésions osseuses de la lèpre** (Application of microradiography and fluorescent microscopy to the study of bone lesions of leprosy), by L. Coutelier and A. Renders. *Ann. Soc. Belg. Méd. Trop.*, 1969, **49** (5), 427-56.

Conscious of the fragmentary nature of knowledge of specific bone changes in leprosy, the authors had the happy idea of applying the modern techniques of microradiography and fluorescent microscopy to the simultaneous study of 42 specimens of bone (mainly phalanges) removed at operation from adult patients with leprosy. By giving tetracycline to these patients at known periods before operation it was possible to deduce which of the observed bony changes occurred after deposition of the "calcium-tetracycline complex", which is radiographically opaque.

Examination of microradiographs of specimens 70 μ thick provides earlier and much more precise information of bone destruction and new bone deposition than is shown on standard X-ray plates, and the juxtaposition of the microradiographs with the pictures obtained by fluorescent microscopy reveals the timing of the osteological changes. In lepromatous leprosy, both external destruction of the bony tube and internal bone deposition in the medullary cavity proceed apparently in one of two ways—either slow and regular, or rapid, irregular and exuberant. The information given in the text and the excellent reproduced microradiographs may be mentally correlated with existing knowledge obtained by conventional radiographic procedures.

[This paper should be studied in the original.]

S. G. Browne.

The following 4 abstracts are reprinted, with permission, from *Trop. Dis. Bull.*, 1970, **67**, 8:

4. **Deux cas d'abcès lépromateux aigus du nerf cubital** (Two cases of acute lepromatous abscess of the cubital nerve), by A. Carayon, J. Languillon, L. Maydat, I. Faye and M. Bourges. *Bull. Soc. Méd. Afri. Noire Lang. Fr.*, 1969, **14** (4), 659-62.

"Tuberculoid cold abcesses of the nerves are encountered in 2 to 3% of the cases. The lepromatous acute abscess is very uncommon. Two cases are presented; one in the course of a relapse, 20 years after 'recovery'; the other in a treated lepromatous patient (3 nerves affected, temperature at 41°C that fell only after draining off)."

5. **Auto-traitement de la lèpre** (Self-treatment in leprosy), by J. Dutertre. *Méd. Trop.*, 1969, **29** (4), 490-96.

The author reviews an experiment he conducted in 1962 in the Upper Volta, where the climate, the scattered nature of the population and the difficulty of maintaining itineraries by motor vehicles rendered the regular treatment of patients with leprosy almost impossible. He abandoned, in turn, such methods as: quarterly visits by car to fixed centres in order to give to each patient enough tablets for the following 3 months; quarterly visits by cyclist for the same purpose; providing a supply of tablets to some responsible

person in the villages for distribution to patients with leprosy. The method finally adopted—and advocated in the article—was the provision of tablets at central dispensaries from which patients could draw their quarterly supplies on any day they chose. The author concludes that this practicable method ensured continuity of treatment by enlisting good patient co-operation and the goodwill of medical auxiliaries. He answers objections raised by critics of this method, for example—that medical control of the patients and the dose of drug taken is no longer possible; that, in the absence of a doctor, patients with leprosy cannot be diagnosed or receive treatment; that the paper packets containing tablets for 3 months do not stand up to wear and tear; and that the patients are denied the psychological incentive that comes from meeting together on the same day at a central place to receive treatment.

[This article contains many forthright and practical suggestions for bringing leprosy treatment to the many areas in the world where essentially similar conditions militate against the application of conventional (and perhaps idealistic) methods of mass treatment.]

S. G. Browne.

6. **La lutte contre la lèpre en Afrique Centrale** (The leprosy campaign in Central Africa), by R. LABUSQUIERE. *Méd. Trop.*, 1969, **29** (4), 479-89.

The author provides a salutary answer to some recent over-pessimistic pronouncements about the world leprosy situation. With good planning and conscientious execution of mass control schemes, the prevalence of leprosy has been halved in some of the ex-French colonies of Central and West Africa. But where such schemes have been applied half-heartedly, or abandoned before they could show results, no similar reduction in prevalence has been noted. The segregation of patients in old-type leprosaria (as in Cameroun) is both costly and ineffective, and the consequences of the virtual discontinuance of the mobile treatment circuits (as in Gabon) are now evident.

The excellent coverage of leprosy in many of the ex-French territories not only emphasizes the possibility and practicability of leprosy control, but also gives point to the less optimistic reports from countries where regular whole-population surveys and mass treatment campaigns are non-existent or in their infancy, and where patients registering voluntarily for treatment are already suffering from advanced leprosy and deformities.

S. G. Browne.

7. **Morphological changes of *Mycobacterium lepraemurium* grown in cultures of mouse peritoneal macrophages,** by Y. T. CHANG and R. N. ANDERSEN. *J. Bact.*, 1969, **99** (3), 867-75.

Mouse peritoneal macrophages were infected with either "short forms" of *Mycobacterium lepraemurium*, "moderately long" forms or killed bacilli. There was a lag phase of 5 weeks before bacillary multiplication was observed. During the first 2 weeks after infection the proportion of irregularly stained organisms increased from 31 to 66 or 77%. "The average length of the non-solid bacilli increased from 2.3 to 3.6 μm in 1 week", and there was a "rapid disappearance of very short organisms with the simultaneous increase of longer ones". It is pointed out that Waters and Rees (*Trop. Dis. Bull.*, 1963, **60**, 1123) considered short forms of *Myco. leprae* as degenerate or dead bacilli. Eight different types of appearances of irregularly staining bacilli are described, and exactly similar appearances were seen with the killed bacilli, but in the latter case "no elongation could be measured". The authors suggest that their study "does not support the current hypothesis that all non-solid acid-fast organisms are non-viable". They discuss previous studies of irregularly staining *Myco. leprae* and state that in these studies non-solid organisms obtained from the growth phase were not tested for viability [but their own experiments did not include such a test. Further studies are clearly needed to elucidate this important point.]

C. S. Goodwin.

The following 7 abstracts are reprinted, with permission, from *Trop. Dis. Bull.*, 1970, **67**, 9:

8. **Occurrence of leprosy in U.S. veterans after service in endemic areas abroad,** by M. L. BRUBAKER, C. H. BINFORD and J. R. TRAUTMAN. *Publ. Hlth Rep.*, 1969, **84** (12), 1051-8.

Basing their report on information obtained from the United States Public Health Service Hospital at Carville, Louisiana, and State medical authorities, the authors provide interesting (though admittedly incomplete) figures of known instances of leprosy almost certainly contracted by "veterans" (ex-service men) during military service in countries where leprosy is prevalent. Patients who may have contracted the disease during previous residence in areas of the United States where leprosy is endemic are excluded. Before 1940, 30 cases were attributed to such infection, out of a total of 83 reported cases. From 1940 to 1968, out of a total of 240 cases of leprosy reported in United States veterans, 46 were considered to have been almost certainly contracted during military service abroad. All but one of these 46 patients were exposed to leprosy before 1960. A brief history and clinical details are given of each patient.

It is considered likely that within the next few years instances of leprosy infection will come to light among those serving in the armed forces in Vietnam, where the prevalence of leprosy is of the order of 5 per 1000, and also among members of the Peace Corps working in countries of known high prevalence.

The authors give a salutary reminder that leprosy is a contagious disease. A returned veteran had signs of leprosy for 4 years before the disease was suspected and diagnosed. In the meantime, his wife and 3 of his 4 children had contracted leprosy. In the United States

[and elsewhere] leprosy is overlooked by physicians and pathologists. It is hidden by patients for fear of loss of employment and social ostracism. Healthy persons who have had leprosy at some time are not allowed to serve in the armed forces. The authors advocate a revision of antiquated laws and regulations to bring them into line with modern conceptions of the disease, its low infectivity and its curability.

S. G. Browne.

9. **Cell-mediated immunity in patients with leprosy,** by J. L. Turk and M. F. R. Waters. *Lancet*, 1969, Aug. 2, 243-6.

There have been several accounts of absent delayed hypersensitivity reactions in patients with lepromatous leprosy. However, unlike babies with complete deficiency of cell-mediated immunity, patients with leprosy are not specially prone to secondary infection except of anaesthetic ulcerated areas.

On testing with a simple contact agent, 2,4-dinitrochlorobenzene (DNCB), 12 out of 24 patients with lepromatous leprosy could not be sensitized. Seven out of 7 patients with tuberculoid leprosy and 3 out of 4 in a borderline group developed delayed hypersensitivity. Of the 12 patients with lepromatous leprosy who did not react with DNCB, 10 subsequently showed delayed hypersensitivity when tested with keyhole limpet haemocyanin (KLH).

Epitrochlear lymph glands from patients who could be sensitized to DNCB, and from those who could not, were similar histologically and showed massive replacement of the paracortical areas by actively phagocytic histiocytes. Some small lymphocytes were however present.

It is suggested that failure of cell-mediated immunity in these patients was primarily directed against *Mycobacterium leprae* but was followed by a partial non-specific failure due to infiltration of the paracortical areas of lymph glands by histiocytes containing mycobacteria. Sufficient cell-mediated immunity was thought to be left to enable these patients to deal normally with other viral, fungal, mycobacterial and protozoal infections.

KLH appears to be a more potent inducer of delayed hypersensitivity than DNCB but the picture is confused by the fact that two-thirds of the patients tested showed an immediate hypersensitivity reaction to KLH, suggesting previous experience of a cross-reacting antigen.

A. A. Glynn.

10. **Syphilis and biologic false positive reactors among leprosy patients,** by A. T. Scotti, D. M. Mackey and J. R. Trautman. *Arch. Derm.*, 1970, **101** (3), 328-30.

Sera from 206 patients with leprosy [unclassified] at Carville, Louisiana, U.S.A., were tested with the Venereal Disease Research Laboratory (VDRL) slide test and the rapid plasma reagin (RPR) card test. Sera positive in either of these tests were tested with the treponemal immobilization (TPI) test and the fluorescent treponemal antibody absorption (FTA-ABS) test. Eighty-two sera were positive in the VDRL test, but 58 of these were negative in the RPR test. Twenty-three of the 24 sera positive in the RPR test were positive in the TPI or FTA-ABS test. Of the 82 positive sera 24 were positive in both the TPI and FTA-ABS tests, 7 being positive in the latter alone, and 2 in the TPI test alone. Thus 33 (16%) of the 206 patients tested were considered to have serological evidence of a treponematosis and 24% of the sera showed "false-positive" tests for syphilis. Many of the patients included in this study had lived in or visited areas in which treponematoses other than syphilis are endemic. "We must assume that at least some of the patients with reactive treponemal tests [TPI or FTA-ABS] are syphilitic." Socio-economic factors and host susceptibility are discussed as possible contributory causes for this high rate of 16% of probable infection with syphilis among leprosy patients.

C. S. Goodwin.

11. **Experimental and clinical studies on rifampicin in treatment of leprosy,** by R. J. W. Rees, J. M. H. Pearson and M. F. R. Waters. *Br. med. J.*, 1970, Jan. 10, 89-92.

After establishing the efficacy of rifampicin (Rifadin) in mouse footpad infections with *Mycobacterium leprae*, the authors treated 6 patients suffering from lepromatous leprosy who had not received previous treatment. The antibiotic was administered in a single oral dose of 600 mg before breakfast. Observing a rapid fall in the Morphological Index, much more rapid than would have been expected with dapsone, they enlarged the trial to include an additional group of 10 patients, some of whom had been previously treated with dapsone; those in this group who weighed less than 35 kg were given 450 mg daily.

In both groups, rifampicin was found to be highly active against *Myco. leprae*, and the rapid fall in Morphological Indexes indicated that it was acting bactericidally. Reactions occurred in some patients, but were neither more frequent nor more severe than would have been expected with dapsone. No toxic effects were encountered.

[See also *Lepr. Rev.*, 1970, **41**, 25-30.]

W. H. Jopling.