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Editorial

THE MORPHOLOGICAL INDEX

The morphological index (MI), or percentage of solid-staining *Mycobacterium leprae* in a smear stained by an acid-fast technique, is an index with a well-substantiated theoretical basis. The solid-staining state has been correlated with the viability of the organism as assessed by its ultrastructure (McFadzean and Valentine, 1960; Rees and Valentine, 1962) and with viability as assessed by mouse footpad inoculation (Shepard and McRae, 1965). In theory, the MI is a fairly accurate indication of the proportion of viable organisms on one or more lesions, and it has been widely and readily accepted as a good index of the activity of an infection. There have, however, been a few criticisms. Bechelli and Guinto (1970) have given a timely reminder that there is no final proof of a relationship between the MI and contagiousness. This is a measure of present-day dependence on this index. An MI of zero indicates only that no solid-staining bacilli were seen among the bacilli actually examined, often 100, sometimes more. It could not be taken as evidence that no bacterial activity at all was taking place, bearing in mind the vast number of organisms in the body, even if the MI gave a true indication of the viability of all the bacilli examined. In fact, it is not claimed that it can do so. There is evidence that a small proportion of dead bacilli remain solid, at least temporarily (Shepard and McRae, 1965). A more serious difficulty is the uncertainty surrounding very short bacillary rods, which could be no more than fragments of whole organisms and which are therefore regarded as non-solid, though Chang and Andersen (1969) have demonstrated that some very short rods of *Myco lepraemurium* are capable of elongation and multiplication; some of their other criticisms of the morphology-viability concept appear to be less well founded.

The MI remains a tenable concept, therefore, within limits. In practice, its reliability is marred to some extent by inadequate standardization, not so much in definition as in technique, and the results obtained depend on a number of factors. A simple estimate of the percentage of solids in a smear does not give quite the same result as a count of the "solid ratio" as defined by Shepard and McRae (1965). On the other hand, it is probable that some workers who are now using the latter criteria retain the older term "morphological index" which has been in use since the early 1960's by Browne (1966, 1968), Goodwin (1963), and others. It would avoid confusion if there were only one index with an agreed definition. For a technician to be able to obtain reproducible MI's requires both skill and training, and these are not quickly acquired. To ensure that 2 workers regularly obtain the same answers is still more difficult. Technique affects the result at every stage. A smear contaminated with blood is difficult to stain, and is likely to provide misleading results. The MI is also affected by the method of fixation and by almost every part of the staining procedure, subjects that are dealt with in a separate paper in this issue (Ridley and Ridley, 1971, see p. 88).

Finally, the result will be influenced by the choice and number of sites examined, depending on whether there is selection of 1 or 2 active lesions, or whether a site on the ears alone or multiple sites are used to estimate the index. There can be at least a 10-fold difference from one site to another (Levy and Murray, 1969). These questions require further thought and study. Another problem is that when 2 or 3 smears are taken simultaneously from one site for the purpose of comparative study, it has been found that the 2nd and 3rd smears often give an MI different from that from the first smear; this may be either because of contamination with blood, or because the amount of material obtained is too sparse or it dries on the scalpel. If multiple smears are needed for study, they should be impression smears made from a biopsy.

Standardization could be improved, but it seems unlikely that it will ever be perfected. This does not invalidate the primary use of the MI, but it does call for a reconsideration of its use and its limitations. As an index of the bacterial response to chemotherapy in the early stages of treatment (Waters and Rees, 1962) there is no substitute for the MI, which is of particular value in comparing the rates of response to two or more drugs or combination of drugs. In this connection, nothing that has been said above is of any consequence provided the same conditions are maintained throughout the period of the trial. On the other hand, to stipulate that for acceptance to a drug trial a patient must have an MI of 25 or 10 or only 4 (U.S. Leprosy Panal Protocol, 1967) could be seriously misleading without a very high level of skill and critical perception on the part of the technician and observer. As a supplementary method of assessing activity, biopsies of 1 or 2 of the more active-looking lesions have advantages that are not sufficiently appreciated. The cytology of the granuloma and the indications of infiltration or expansile spread are useful pointers to be considered in conjunction with the morphology of the bacilli. Furthermore, even a small proportion of solid organisms is significant if they are situated in groups. It is doubtful if the bacilli located in nerve bundles, which tend to be solid staining, are at all frequently extracted in the preparation of smears. But here again one assumes a good measure of skill on the part of the histologist and also of the clinician who selects the lesions.

Finally, there is the question of the routine management of patients. The MI is, of course, quite valid provided the conditions remain constant, but there is something to be said for using the SFG index (Ridley, 1971; see p. 96) as an alternative. In spite of its apparent complexity, a technician can be trained to get reproducible results with this index more quickly and easily than he can with the MI. Equally important, it can with practice be estimated almost at a glance. There is, therefore, no difficulty in estimating the SFG index on smears from all sites, which allows a better chance of detecting a relapse in its early stages than does an MI based on only 1 or 2 lesions.

The above considerations may not only serve as a background to the interesting observations of Leiker appearing in this issue (see p. 121), but should stimulate more precise investigation of this aspect of leprosy that has most important clinical, therapeutic, and microbiological repercussions.

D. S. RIDLEY

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News and Notes

LEPROLOGISTS NEEDED

From time to time requests arrive through various channels for the services of leprologists. There is at the moment a great dearth of experienced doctors in this field. Governments, mission bodies, and non-sectarian voluntary agencies publicize their needs in the advertisement columns of the medical press, through government bureaux, and in this specialized journals.

Leprosy Review does not offer itself as an employment agency or throw open its pages to "appointments vacant" and "offer of service", but nevertheless would wish to indicate the continuing need for doctors experienced in leprosy. At the moment, the governments of the Arab Republic of Libya, of the Gambia, and of the Solomon Islands seek such men.

(1) Dr. O. Kadiki, Director General, Ministry of Health, Tripoli, is eager to engage an experienced leprologist for Libya.

(2) The British Leprosy Relief Association (LEPRA), 50 Fitzroy Street, London, W.1 would act as the intermediary for the vacancy in the Gambia, and assist financially.

(3) The Director of Medical Services, Honiara, British Solomon Islands Protectorate, or Dr. MacGregor (now at 74 Glasgow Road, Perth, Scotland) would supply particulars of the vacancy in the Solomon Islands.

(4) The Schieffelin Leprosy Research Institute, Karigiri (near Vellore, Tamil Nadu, India) urgently requires an epidemiologist and a well-qualified physician for research and teaching.

The Secretary-General of ELEP (The Federation of European Anti-leprosy Associations) of 196 Rue Stévin, Brussels 4, Belgium, and the Medical Missionary Association (31 Bedford Place, London, W.C.1) receive information of vacancies in institutions wholly or partially concerned with leprosy.

DAMIEN-DUTTON AWARD

On 16 February, 1971, Dr. Chapman H. Binford, Medical Director of the Leonard Wood Memorial, was presented with the Damien-Dutton Award for his many contributions to leprosy research. At present, Dr. Binford—sprightly and energetic despite his 70 years—is registrar for leprosy at the Armed Forces Institute of Pathology in Washington, and head of its special Mycobacterial Diseases Branch. He served in the United States Public Health Service from 1930 until he retired in 1960. Since then, he has been identified with the Leonard Wood Memorial. His many researches into the microbiology and histopathology of leprosy, including successful attempts to induce multiplication of *Myco. leprae* in the earlobes and testicles of hamsters, have placed him among the foremost investigators of the disease. His work as Councillor of the International Leprosy Association and Chairman of its Finance Committee has earned him a high place in the regard of all leprologists.

Leprosy Review adds its sincere congratulations to Dr. Chapman Binford on this well-merited award.

ANNUAL REPORT INSTITUT MEDICAL EVANGELIQUE, KIMPESE, DEMOCRATIC REPUBLIC OF CONGO

The Annual Report of this centre for 1969-70 includes references to the work of the Kivuvu Leprosarium, where 58 in-patients and 391 out-patients were treated during the year, 65 of them being newly admitted to treatment. The medical programme includes health education, physiotherapy, occupational therapy, and shoemaking. Research is proceeding on low-dosage sulphone therapy and on *Myco. ulcerans* infection (Buruli ulcer).

LEPROSY IN AFGHANISTAN

Precise information of the size of the leprosy problem in Afghanistan is not available. The figure of 7600 cases estimated by the World Health Organization in 1964, was admittedly based on incomplete information, and reflected the assumption that the prevalence of leprosy in Afghanistan was likely to be similar to that of neighbouring countries. A total of 100,000 for the whole country, representing a prevalence rate of 5 per 1000, would not be unrealistic. However, a few school surveys and selected population surveys among people of Mongoloid extraction in the mountainous Hazarajat area in Central Afghanistan, indicate a higher prevalence.

Dr. S. G. Browne recently visited Afghanistan at the invitation of the Medical Assistance Programme (M.A.P.) of the International Afghan Mission to investigate and advise on the leprosy problem. The Ministry of Public Health of the Royal Afghan Government has invited M.A.P. to assume the responsibility of organizing and supervising a medical service—including leprosy—over an area of 50,000 miles^2 (130,000 km^2) containing a population of some 2 million. At present, even basic medical services are lacking in this area. The implementation of the official policy of domiciliary treatment for leprosy must be seen in the context of general medical need and the dispersal of the population in narrow fertile valleys surrounded by the massive peaks of the Hindu Kush range.

There may thus be 20 000 leprosy patients needing treatment in the Hazarajat. Nodular lepromatous leprosy is characterized by advanced lesions on the face, while the trunk and limbs are relatively spared. Madarosis occurs early, especially in patients with Mongoloid facies. These observations may be correlated with constant exposure of the face to the extremes of weather—bright sunlight, high elevation, and very low winter temperatures. Tuberculoid leprosy is commonly macular and appears as very large lesions covered with a branny desquamation, less pigmented than the adjacent normal and unaffected Caucasian skin. Peripheral nerve damage may be severe and widespread, involving all the nerves of the limbs (except the radial nerve) and the face.

In the clinics already operating, men outnumber women by 15 to 1, a fact that suggests that many women suffering from leprosy do not come for diagnosis and treatment.

Although leprosy in the Hazarajat constitutes a major health problem affecting perhaps 1% of the population, it is probably widespread throughout the country.

LEPROSY IN AUSTRALIA

The Annual Report for 1968-69 of the Director-General of the Commonwealth Department of Health, Australia, refers to the fact that during the year under review some 68 people were diagnosed as having leprosy, most of them in the Northern Territory. Patients who are no longer contagious receive treatment as out-patients. The shortened period of "isolation" now in vogue, coupled with the obvious success of modern treatment and the benefits of reconstructive surgery, have resulted in early self-reporting and hence reduction in the foci of active disease.

LEPROSY IN INDIA

WORK OF THE HIND KUSHT NIVARAN SANGH

The Annual Report (for 1969) of the Hind Kusht Nivaran Sangh gives a very readable and interesting account of the work accomplished through the Sangh itself in close co-operation with the Government of India, the Departments of Health of the various component States, and the voluntary agencies. The National Leprosy Control programme now comprises no fewer than 1130 S.E.T. (survey, education, treatment) centres.

The Sangh stimulates research and teaching, and actively engages in popular education about leprosy through its admirable series of posters, brochures, leaflets and films. Special emphasis was given to World Leprosy Day, 30 January, 1970, coinciding as it did with the observance of the Gandhi Centenary.

LEPROSY IN MALTA

The Annual Report of the Chief Government Medical Officer of the Maltese Department of Health for 1969 reveals that the total number of patients with leprosy under treatment was 219, of whom 134 were males and 85 females; 45 received treatment as in-patients at St. Bartholomew Hospital during the year. In Malta itself, there are 155 patients who are treated as out-patients. From the hospital good results have been reported with clofazimine (Lamprene, Geigy), and thalidomide. The usual treatment of leprosy is with dapsone, or with thiambutosine as an alternative where indicated. According to a statement made by the British Secretary of State in answer to a question in Parliament, 5 persons coming from Malta between 1964 and 1969 have been notified as suffering from leprosy in Britain.

DANISH SAVE THE CHILDREN ORGANIZATION

LEPROSY CONTROL PROJECTS AT POGIRI AND ASKA

The latest report of the two excellent leprosy control programmes in India, supervised by the Danish Save the Children Organization with the technical help of the World Health Organization and assistance from UNICEF and the Central and State Governments, gives statistics up to the end of September, 1970. No fewer than 47,487 leprosy patients have been registered for treatment in the Pogiri scheme (since 1962), and 22,043 in Aska (since 1966). So far, 16,055 patients have been released from control in Pogiri, and 861 in Aska.

The organizers of these two programmes, ever cost-conscious, derive some satisfaction from the figures now available. The cost per year per patient registered works out (in U.S. dollars) at $3.06 for Pogiri, and $4.66 for Aska, and the leprosy service represents a charge of $0.07 and $0.06 respectively per head of population in the areas covered (about 1,465,000 and 1,113,000 respectively).

ELEP MEDICAL COMMISSION

The Medical Commission of the European Federation of Anti-Leprosy Associations (ELEP) met in Brussels on 19 March, 1971, to examine projects submitted to it and to approve the text of a statement on leprosy research intended to give helpful guidance to voluntary organizations. The Commission has learned with real regret that because of ill-health, Dr. L. P. Aujoulat (*Inspecteur-Général de la Santé Publique*) has had to relinquish the position of Chairman, which he has filled with such distinction. Dr. S. G. Browne was elected in his place.

ELEP

The Annual Report for 1970 of the General Secretary of ELEP (the European Federation of Anti-Leprosy Associations), presented at a meeting of the General Assembly held in Bologna (Italy) in April, 1971, contains several items of information of interest to readers of this journal.

During the year, a total of about £2,200,000 was raised by the member-associations and distributed to 505 centres in 70 countries. This sum represented an increase of 7% on the 1969 figures. Among the receiving countries in Asia, India benefited most, and in Africa, Uganda. In India, the grant per patient treated was just under £1, whereas in Uganda the comparable sum was £3.30. Per patient treated, South Korea, Paraguay, and West Pakistan each received sums somewhat, or considerably, above this figure. Four joint projects, sponsored and financed by several of the member-organizations, are already under way—all in India: at Dharmapuri, Kumbakonam, Aska, and Pogiri.

The Medical Commission, now to be strengthened by the inclusion of Médecin Général P. Richet, will continue to examine projects submitted for its advice, and will attempt to guide the associations in the disbursement—to the best advantage, and in accordance with the principles and priorities of ELEP—of the considerable sums they raise every year.

The Chairman of the Medical Commission (Dr. S. G. Browne) in presenting the Annual Report of the Commission to the General Assembly, indicated that during the next decade all voluntary agencies concerned with the world-wide attack on leprosy would have seriously to consider their strategy and the nature of their fund-raising activities, striving to see their work in the context of other endemic diseases, undernutrition and overpopulation. The integration of their commendable antileprosy programmes into general schemes for raising the health of individuals and communities would go far to ensure that the person suffering from leprosy would have a fair deal and a square deal in the world of tomorrow.

DR. ESMOND R. LONG HONOURED

The Emeritus Editor of *The International Journal of Leprosy and other Mycobacterial Diseases,* ex-professor of pathology, Dr. Esmond R. Long, has

Dr. Long (left) receiving the gold-headed cane

recently been awarded the highest honour given to pathologists in the United States of America and Canada—the Gold Headed Cane. The cane, a replica of one carried by British Royal Physicians from 1689 to 1825, is awarded to a physician representing "the highest ideals in pathology and medicine". It has been granted only 19 times in 52 years.

Dr. Long succeeded Dr. H. W. Wade as Editor of *The International Journal of Leprosy* after the 8th Leprosy Congress, held in Rio de Janiero in 1963. Entering the field of leprosy editing after a very rich and fruitful experience in tuberculosis, general pathology and medical history, and armed with a ready and precise pen, Dr. Long not only maintained but enhanced the high standards of the official publication of the *International Leprosy Association.*

Now, at the ripe old age of 81, he still brings an unusual degree of mental alertness, perspicacity, and rare critical abilities to bear upon the world of leprosy. Readers of *Leprosy Review* would wish to congratulate Dr. Long on the Award, and to thank him for his varied contributions to their special field of interest.

THE WORK OF WHO IN 1970

The Annual Report for 1970 of the Director-General of the World Health Organization (WHO) mentions (p. 10) that over half-a-million patients with leprosy have been registered in the previous 5 years in 75 countries from which returns are available, and that 132,000 patients have been "released from control". It concludes, in the continued absence of complete and reliable statistics, that the total number of persons in the world suffering from leprosy was in 1970 substantially similar to that in 1965.

WHO provided help in 24 projects, and also gave technical guidance in several programmes assisted by UNICEF. Field or laboratory studies were conducted or supported by WHO in 12 countries. The prevailing trends in leprosy control are briefly reviewed, with stress on the need to conduct antileprosy activities as integral parts of national general health services, rather than as separate and isolated programmes.

A short report (p. 185) on the Kampala Seminar (9-14 March, 1970) refers to the main points discussed, viz., the epidemiology and control of leprosy in the African Region, treatment, planning, the evaluation of control programmes and their integration into the public health services.

The seminar on Leprosy Control held in Aska (Orissa, India) from 12 to 31 January 1970, brought together medical officers engaged in leprosy control work in South-east Asia, with the object of acquainting them with recent developments in the epidemiological and immunological aspects of the disease.

LEPROSY OR HANSENIASIS?

The following letter has been received from Dr. A. Rotberg. The "Technical Norm No. 3" referred to in the first paragraph is apparently an administrative instrument that officially substitutes—in the State of São Paulo, Brazil—a revised nomenclature to replace the word "leprosy" and its cognates:

I have the pleasure of enclosing the Technical Norm No. 3 issued by the Secretary of Health of the State of São Paulo, Brazil, ratified by the State Council of Health, referring to the new terminology headed by the word "hanseniasis".

The inconvenience of the old terminology ("leprosy" and derivatives) and the interest in a substitution were pointed out in the recommendations or conclusions of the XVII Brazilian Congress of Hygiene (Bahia, 1968), of the "Seminar on Leprosy (Hanseniasis)" of the XVIII Brazilian Congress of Hygiene (São Paulo, 1970) and of a group of participants in the Seminar on Administrative Methods for Leprosy Control Programs, of the Pan-American Health Organization (Guadalajara, Mexico, 1968).

This new terminology is also expected to help in eliminating the stigmatizing word "leper", condemned by the Conference of Manila (1931) and the Congresses of Leprology of Havana (1948) and Madrid (1953), but still widely employed, in great part because of the term "leprosy" and its derivatives, permitted up to date.

Both the new and old terms will be used jointly by the Secretary of Health in correspondence and other documents destined to other states or countries. In São Paulo the same policy may also be adopted for some time, when necessary to establish synonymy.

DR. A. ROTBERG
Coordenador

A letter from Dr. M. J. Mallac under the heading "It is hanseniasis and not leprosy" appeared in the *Far East Medical Journal* (1971) 7, 108, in which the following passages occur:

I read with great interest in the July issue of your Journal the details under "Australians Help Lepers in South East Asia" and those pertaining to "Hansen's Disease and Hearing". It struck me—as a former WHO hansenologist still committed to this remarkable and challenging disease—that some of your readers are probably unaware of the world-wide movement which, under the aegis of the "Departmento Dermatologia Sanitaria", Brazil (Director: Professor A. Rotberg), is presently underway with a view to relinquishing the word "leprosy" and its linguistic derivatives in favour of hanseniasis, hence correcting a long overdue yet tragic medical mistake and securing—it is hoped—a more rational, if not more enlightened public attitude. The proposed changes are as follows:

TERMINOLOGY

New one	*Present one*
Hanseniasis	Leprosy
Hansenology	Leprology
Hansenologist	Leprologist
Hansenic	Leprotic, Leprous
Hansenoid	Leproid
Hansenoma	Leproma
Hansenide	Lepride
Virchow's cell	Lepromatous cell
Virchowian infiltration	Lepromatous infiltration
Virchowian Hanseniasis	Lepromatous Leprosy
Tuberculoid (T),	Tuberculoid (T),
Indeterminate (I),	Indeterminate (I),
Dimorphous (D)	Dimorphous (D)
Hanseniasis	Leprosy
Mitsuda's antigen	Lepromin
Hansenian or Hanseniasis patient	Leprosy patient

To this letter from Dr. M. J. Mallac, the Secretary-Treasurer of the International Leprosy Association has sent a reply couched in the following terms:

The letter from Dr. M. J. Mallac in your April issue has been brought to my attention. I have of course been long aware of the activities of the excellent Brazilian doctors concerned with the persistence in many countries of the stigma attached not only to the disease caused by *Mycobacterium leprae,* but also to those who suffer from it. I have yet to learn, however, of any "world-wide movement which is presently underway" to abolish the word "leprosy" in favour of the term "hanseniasis".

While it is undeniable that in languages derived from or related to Latin, the word "leprosy" may have overtones that suggest uncleanness or ritual defilement, the vast majority of persons suffering from the disease live in countries where non-Romance languages are spoken.

By all means, let us strive to do anything that will reduce the hurt and harm caused by prejudice and ignorance, but a mere change of name affecting less than one-twentieth of the sufferers from this disease in the world will have little impact on the disease itself, or the majority of the sufferers.

Even if the international and national nomenclatures of diseases were—at one fell swoop—to replace the accepted terminology by acoustically awkward, linguistically hybrid, and eponymously cumbrous new terms, we should still be left with the vast problem of treating individual patients and controlling the disease throughout the world.

I can assure Dr. Mallac that The International Leprosy Association is among the foremost in trying to remove the stigma still attached to the word, the disease, and the sufferer. It has pioneered attempts to outlaw the word "leper" from scientific and from popular writings. As an association, it continues to include the word "leprosy" in its title, and the Council has made no move to change the title.

I would also remind your correspondent that the World Health Organization still has its *Leprosy* Expert Committee, and a *Leprosy* section in the Division of Communicable Diseases; it still assists governments in their *leprosy* control projects, and sends its *leprosy* consultants far and wide.

All honour to Hansen, Virchow, Mitsuda and the others, ancient and modern, who have contributed to the scientific study of the disease of leprosy; all honour to those—Muir, Cochrane, Follereau, Rotberg and many others—who have done something towards removing the sting and the stigma of leprosy. Let us try to destigmatize the word "leprosy" where this is necessary, and resolutely face both the word and the disease it stands for, with the scientific and humanitarian resources of the twentieth century.

27 April, 1971 SECRETARY-TREASURER

LEPROSY IN CEYLON

According to the Report of the Director of Health Services in Ceylon for the year 1966-67 (which has just been received), there are 4337 leprosy patients registered in the island. Of these, 783 are treated as in-patients in the two leprosy hospitals, at Hendala and Mantivu, and the rest are treated as ambulatory patients. The cost per head per year is Rupees 2117 for the in-patients, and Rupees 66 for the others; 210 new cases were registered during the year. The average prevalence rate for the whole country is about 0.37 per 1000, the Western Province having the highest rate (of 0.71 per 1000) apart from the Colombo Municipal Area (1.05 per 1000).

According to the World Health Organization figures, the estimated number of sufferers from leprosy in Ceylon is 10,300. Attempts have been made to form a Leprosy Association of Ceylon along the lines of the active Ceylon National Association for the Prevention of Tuberculosis.

FIRST NATIONAL PAKISTAN LEPROSY CONGRESS

Thanks to the inspiration coming from the very active Karachi Branch of the Pakistan Leprosy Relief Association and the dynamic leadership of Dr. Zarina Fezelbhoy, the First National Pakistan Leprosy Congress was held in Karachi from 12 to 14 February, 1971, on the "Control of Leprosy in Pakistan".

Representatives of the Central and State Governments, the diplomatic corps, medical and social organizations in Pakistan, the World Health Organization, the university medical schools, together with doctors and paramedical workers, numbering altogether about 500, attended the opening ceremony. Dr. S. G.

Browne, Secretary-Treasurer of the International Leprosy Association, was the invited guest; other visitors from abroad were Dr. Grace Warren (Hong Kong) and Monsieur Pierre van den Wijngaert, the Secretary of ELEP (the Federation of European Leprosy Associations).

Messages of greeting and good wishes were read from the President of Pakistan and leaders in politics and Government, Her Majesty the Queen of Iran, and also from The Leprosy Mission, the British Leprosy Relief Association (LEPRA) and the International Leprosy Association. The official opening speech was made by the Chairman, the Governor of Sind, and the keynote address was given by Dr. Stanley Browne on "Priorities in leprosy control in Pakistan". After the proceedings the Governor of Sind opened the excellent exhibition, to which participants and the public had access.

The two full days of scientific sessions provided some first-class papers, provoking lively discussions. A most gratifying feature was the presence of doctors from the four states of the West Wing of Pakistan and the East Wing. The active participation of leading professors from the medical schools and of specialists in ophthalmology, plastic surgery, infectious diseases, and tuberculosis served to emphasize the scientific interest of leprosy.

Accurate figures of the prevalence of leprosy in Pakistan are not available, but all the indications point to a low rate of between 2 and 5 per 1000, which certainly still constitutes a health hazard. There are probably more leprosy sufferers in the East Wing than in the West, though in areas in Swat and the North-West Frontier Province rates of 30 per 1000 have been reported. So far, only about 1 in 20 of the 250,000 suffering from active disease are able to get treatment. Excellent pioneer work has been done in Karachi itself by Dr. Ruth Pfau and her devoted colleagues, and paramedical workers trained at the Marie Adelaide Leprosy Centre are being seconded to control schemes all over the West Wing.

The Congress ended with the passing of several hard-hitting recommendations, which should not only stir official consciences and influence Government planners, but should also mobilize public opinion in Pakistan for the practical help of the leprosy sufferer.

The surgical and social aspects of leprosy were not neglected. After the Congress, some 60 participants attended an orthopaedic workshop conducted by Dr. Grace Warren and Dr. Kleese at Manghobir Hospital, a few miles from the centre of Karachi.

The voluntary agencies have played a long and honourable part in awakening the public to the problem of leprosy, and it is now time for the Government to assume its rightful rôle of directing an overall plan for leprosy control in the country, of integrating this plan with the attack on other serious endemic diseases, and of continuing to welcome the active collaboration of voluntary agencies—both from within Pakistan itself and beyond its borders—in the campaign. The training of paramedical workers and the diffusion of knowledge of leprosy to the medical profession and the public will be the rôles that the voluntary agencies are, by experience and motivation, most fitted to assume.

Letter to the Editor

The intent of my first letter (*Leprosy Review,* April 1970, p. 128) was not to imply that a deliberate attempt was made to use prophylactic DDS, but rather to suggest what in fact may have occurred. Both the unintentional prophylaxis and the reduction of the infectious reservoir, as pointed out by Dr. Crawford in his letter of reply, probably were important in reducing the leprosy incidence and prevalence.

Other factors, however, may well also have come into play. Not the least of these, and seldom—if ever—mentioned, is the actual status of the epidemiology of the disease. At what point on the epidemic curve was the survey made? Without better epidemiological data than are usually available and careful analysis of those data, we must be cautious in drawing conclusions about what the effect of any introduced variable may have been. It must also be taken into consideration that Dr. Ross made his survey in 1952, that is, at or after the zenith of the epidemic had been passed. What followed, therefore, may have been due more to what was occurring in the epidemic than to whatever influence the DDS may have had.

In any event, congratulations to Dr. Crawford for re-doing the survey made by Dr. Ross and bringing this very interesting event to light. Let us hope that further studies of this nature will uncover long-sought-after information to aid in better leprosy control efforts.

31 March, 1971

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Stain Techniques and the Morphology of *Mycobacterium leprae**

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A comparative study of acid-fast stain techniques has shown that some methods in common use give divergent values for the MI, and smaller but significant discrepancies in the BI. The methods used by Rees and Valentine and by Shepard to equate solid staining with viability are in fairly good agreement.

The heating of carbol-fuchsin stain is the factor which causes the most variation in the MI, but fixation, time of staining, and method of differentiation all have some bearing on the results. It is thought that all the effective staining procedures produce some alteration of bacterial morphology.

The need for standardization and the suitability of certain methods for routine application are discussed.

Introduction

Although the morphological index (MI) or solid ratio is now widely employed as an index of viability of leprosy bacilli, it is not always recognized that the appearances are affected to a considerable extent by stain techniques. This was demonstrated in the case of *Mycobacterium lepraemurium* by Rees and Valentine (1962*b*) who found that with this organism a solid-stain morphology could be correlated with electron microscopic assessment of viability only when the time and temperature of the Ziehl-Neelsen stain were modified, though with *Myco. leprae* the correlation had been established while using Ziehl-Neelsen stain for 2 min (Rees and Valentine, 1962*a*). Shepard and McRae's (1965) correlation of morphology with viability as indicated by mouse footpad inoculation experiments was made by the use of a Ziehl-Neelsen procedure that was specifically devised for use with washed suspensions of bacilli (Shepard, 1962). It is also a cold method, and when used for routine smears it might be expected to give a lower MI than the hot techniques. The methods by which acid-fast staining has been correlated with viability appear to be relatively "weak", whereas for the routine screening of patients and the assessment of the bacterial index (BI) "strong" methods are needed.

The present study was undertaken to determine how the MI and BI are influenced by the various stain techniques in common use, and to discover whether it is possible to recommend a general-purpose method for routine use.

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Material and Methods

Skin biopsies were available from 11 patients suffering from lepromatous leprosy, and whose skin contained numerous bacilli; 5 of the patients had active infections and 6 were under treatment. In all, 50 impression smears made from each biopsy were used for the estimation of the MI by the method of Shepard and McRae (1965), and of the BI by the method of Ridley (1964). In addition, 2 "slit and scrape" smears were available from each of 6 to 8 sites from a further 10 patients with rather scanty leprosy bacilli. These were not suitable for determination of the MI because of the small numbers of bacilli, but they were a necessary supplement to the BI study, since they provided cases at the lower end of the BI scale. The results with this latter group, however, were unsuitable for mathematical analysis.

Five basic staining methods were compared, and with most of them a number of modifications were tested. Wherever possible, 3 identical smears were stained concurrently by each modification, one to be examined immediately, one to be examined after 24 h, and the 3rd after rinsing in absolute alcohol.

Allowances were made in times of differentiation for the thickness of slit and scrape smears; there was no optimal time. Impression smears required less differentiation because of the lack of contamination, and for the same reason the times could be standardized as stated below. To prepare carbol-fuchsin, 50 ml of 6% alcoholic basic fuchsin was mixed with 450 ml of 5% phenol in distilled water, filtered, and kept for one day in a dark bottle before use.

The following methods and modifications were evaluated.

1.(a) The Ziehl-Neelsen method. Heat-fixed smears were stained in carbol-fuchsin, differentiated in 1% acid-alcohol (1% HCl in 70% ethanol) and counterstained with 1% methylene blue. Unless otherwise stated staining was performed at 60°C for 15 min, and differentiation was carried out for 5 sec. The following time and temperature modifications of the Ziehl-Neelsen technique were employed: staining temperatures of 50°, 42°, 37° and 22°C (room temperature), and staining times of 5 min and 18 h (overnight).

(b) Modifications in differentiating agents and times in the Ziehl-Neelsen method were tested as follows: 1% acid alcohol for 3 min; 10% sulphuric acid for 3, 10 and 20 min; 25% sulphuric acid for ½ min, and 5 and 15 min.

The method of Rees and Valentine (1962*b*) consists in the Ziehl-Neelsen method (1a) as described but with staining at 37°C overnight.

2. Difficulty was experienced in adapting for use with smears the technique of Shepard (1962), which was devised for use on washed suspensions. The phenol gel caused a messy deposit of stain which obscured the bacilli. The gel was therefore omitted, but for the rest, Shepard's technique using formalin vapour fixation and staining at 22°C was followed exactly. As a further modification the smear technique was carried out at a staining temperature of 42°C. This method is referred to here simply as "formalin fixation".

3.(a) Shepard's method. Shepard (personal communication) informed us that his 1962 technique could be applied to smears without any modification. All that was required was that, after application of the freshly prepared phenol gel (gelatin 0.5 g, phenol 0.5 g, distilled water 100 ml) to the smear, the surplus was drained off before re-exposure to formalin vapour; but neither at this stage nor at any

other time must the gel be allowed to dry. This method was followed exactly and compared with some of the other methods referred to; but a different batch of smears was used for this subsidiary trial because by the time Shepard's communication was received the original batch of means had been exhausted. When the results were analysed and the smears calculated, it was necessary to make small adjustments to bring them into line with the mean values obtained in the main trial.

(b) A modification of Shepard's method was tried in which 0.5% phenol was substituted for phenol gel. In all other respects the method was the same. As only a few cases were tested in this way the results are not tabulated.

4.(a) Aubert's (1950) technique. Heat-fixed smears were stained with a carbol-fuchsin-Tween-80 mixture at 22°C for 3 min. The stain was prepared as follows: basic fuchsin, 3.5 g in 12.5 g of pure phenol, was heated to 80°C and 25 ml of alcohol was added. It was allowed to cool, mixed, and made up to 300 ml with water, stirring well, after which 30 drops of Tween-80 were added slowly and mixed well. The stain was filtered before use. Differentiation was in 5% nitric acid followed by a wash in 70% alcohol.

(b) As a modification of Aubert's method, smears were stained for 10 min and differentiated in 1% acid alcohol.

5. Fluorescent methods. (a) According to Kuper and May (1960). The stain, an auramine-rhodamine mixture, was used at 60°C, with differentiation in 0.5% HCl in water.

(b) According to Mansfield (1969). Fixation was by heating in an oven at 65°C for 1 h, followed by formalin vapour for 15 min. The stain was phenol-auramine: 4 ml of phenol + 6 ml of glycerol poured over 0.3 g of auramine-C and mixed, to which 90 ml of distilled water was then added and mixed daily for 3 days. It was allowed to stand in the dark for 4 days before use. Staining was at 30°C for 15 min. Differentiation was in 0.5% acid alcohol and counterstaining in 0.5% potassium permanganate. For fluorescence, BG 12 excitation filters were used in conjunction with a 500 barrier filter.

Results

For the study of the MI the cases fell conveniently into 2 groups: 5 biopsies with MI's in the range 25-75 by the Ziehl-Neelsen (1a) method (Group I); and 6 biopsies with MI's in the range 1-20 (Group II). The means of the results when stained by various methods and modifications are given in Table 1. With Group I, the individual results with the different methods fell into a consistent pattern in line with the means. But with Group II, individual results were erratic and inconsistent with some (though not all) methods. The means, therefore, give only an approximate indication of staining performance in Group II.

The BI results, as already explained, were unsuitable for tabulation, but comments are made on the effect of each particular stain. In general the differences in the BI were smaller than those in the MI.

Temperature of staining. The one single factor which seemed to affect the MI considerably was heating in carbol-fuchsin: the higher the temperature, the higher the MI. The means of the 2 groups stained at various temperatures by the Ziehl-Neelsen method are shown in the table. In each group the MI was significantly higher at 60° than at 22°, but the difference was more pronounced

TABLE 1

Effect of temperature and special stain techniques on the mean MI

Smear group	Stain time	Technique and staining temperature (°C)											
		Ziehl-Neelsen (1a)					Formalin fixed (2)		Shepard (3)	Aubert (4a)	Aubert (4b)	Fluorescent (5a)	Fluorescent (5b)
		22°	37°	42°	50°	60°	22°	42°	22°	22°	22°	60°	30°
Group I	5 min	33		52		56				27			
Group II	5 min	0.2		3.5		6				0.3			
Group I	15 min	41	47	54	59	62	39	51	51		50	65	58
Group II	15 min	1.2	1.6	4.3	7	11	0.3	2.4	3.8		3.8	15	8
Group I	18 h	48	54	59		61							
Group II	18 h	3.5	4	7		11							

Group I = smears with high MI (5 cases).
Group II = smears with low MI (6 cases).
Rees and Valentine's method = ZN (1a) at 37°C for 18 h.

in the group with the low MI. A point which is not brought out by the table is that some fragmented organisms are apparently "reconstituted" by staining plus heat, whereas granular organisms remain granular. This was particularly true with mild heat (37°C) for short periods, which gave unpredictable results in the MI range 15-30.

Examination after staining at room temperature was always difficult because of pallor, which caused a low MI. At this temperature, the BI was also low especially in cases where bacilli were few, and some were missed altogether. Examination after 24 h showed a further fall in both the MI and BI due to fading. This did not occur after staining and heating.

Time of exposure to carbol-fuchsin. The influence of the staining time on the MI was dependent on the temperature of the stain (see Table 1). At 42°C there was a minimal difference in the result whether the staining time was 5 or 15 min. But at room temperature there was a marked lowering of the MI in Group II smears when these were stained for 5 instead of 15 min. Prolonged exposure to the stain resulted in higher MI values, especially in Group II.

The time of staining was more important in assessing the BI. At 5 min some bacilli failed to stain at all, and others, being poorly stained, were difficult to detect; staining at higher temperatures corrected this to some extent. Prolonged exposure to warm stain caused much dropping out of bacilli and skin-scrape smears were difficult to handle. This was the disadvantage of Rees and Valentine's method.

Differentiation. Neither the time of differentiation nor the choice of the agent (whether 1% acid alcohol or 10% or 25% sulphuric acid) caused much variation in the BI or MI as long as smears were stained in steaming carbol-fuchsin for a minimum of 15 min, and provided large numbers of bacilli were present. The time of differentiation was critical only when either of these requirements was not met. The BI fell after prolonged differentiation in either acid alcohol or 25% sulphuric acid following staining at 22°C and/or rinsing in absolute alcohol.

With sulphuric acid differentiation, there was a fall of 5-10% in the MI when examination was made after 24 h. The stain resulted in a purplish background, against which scanty bacilli were often difficult to detect. Over-differentiation was relatively easy when bacilli were scanty. We preferred 1% acid alcohol.

Padma's (1963) 3% acid alcohol was, in our experience, too strong a differentiator for the "weak" stain produced after staining at room temperature (22°C) as recommended. A quick rinse in absolute alcohol before drying gave a cleaner result with more precise morphology.

The above results all relate to the Ziehl-Neelsen method, though they probably are of more general application. The staining quality of other methods, and the effect on the MI and BI of the special features associated with these methods, are dealt with separately.

Formalin fixation. Fixation in formalin vapour (Method 2) caused some slight fall in the MI compared to heat fixation (see Table 1). At low levels the MI was very inconsistent, often falling to zero. Bacilli were pale, there was much dropping out of bacilli, "ghost" forms were seen, and identification was difficult. The BI was considerably lower than that seen in heat-fixed preparations; when bacilli were few, none of them might be stained. Examination after 24 h showed a further fall in the MI as compared with heat-fixed smears.

Shepard's method (3a), although it differed from Method 2 only in the use of phenol gel, Shepard's method gave a much better staining quality and the MI was

higher and more consistent. When bacilli were scanty and granular, however, they were pale and not easy to detect.

The substitution of phenol for phenol gel (3b) produced brightly stained bacilli and granular organisms were better stained. In a small series of smears the MI was not significantly different from those obtained by the use of Shepard's method (3a).

Aubert's method (4a). Using Tween-80 in the stain, had one serious drawback, namely the deposition of the stain in oily blobs, which made it difficult to distinguish globi with certainty. Overwashing in water after staining removed much of the colour from the bacilli, although the slide was somewhat cleaner. This was a critical step and had to be controlled carefully. The bacilli were pale, the MI was low, and there was much "dropping out". At low values, the BI also was lower than in the Ziehl-Neelsen method. There was much loss through fading due to the nitric acid differentiation, and examination had to be made at once.

A modification (4b), using Aubert's stain for 10 min and differentiating in acid alcohol, was easier to control and showed much improvement on Aubert's method (4a). Bacilli were bright and the morphology easy to study. The MI was higher than with method 4a and almost identical to that in Shepard's method. There was still some dropping out of bacilli from the slide, but this did not affect the BI to the same extent as in Aubert's method (4a). The result was still decidedly messy with slit and scrape smears.

Fluorescent methods. The method of Kuper and May (5a) resulted in brightly fluorescent bacilli with clear precise morphology easily distinguishable on both impression smears and skin-scrape smears. The MI was exceptionally high relative to all other methods, particularly at low values. The BI was in the same range as that by the other methods, and examination of routine smears with scanty bacilli was very easy. However, it could not be stated with certainty that the fluorescing organisms were *Myco. leprae* and not skin contaminants. One case showed some fluorescence of non-alcohol-fast organisms.

Mansfield's modification of Kuper and May (5b) overcame the problem of non-specific staining by lowering the staining temperature. Staining was bright and examination easy. The MI was slightly lower than with (5a), but the BI was about the same.

Reproducibility of smears. It was noted that impression smears gave reproducible results with the MI, but that there was some variation in the BI because different amounts of material were used in making the smears. With slit and scrape smears, a pair of smears made from a knife blade after a single scrape were not always uniform as regards either the MI or BI, presumably because of drying on the knife before making the second smear. When 2 separate scrapes were made from the same slit in order to make a pair of smears, the MI and BI were more or less identical if there was heavy infiltration and plenty of dermal fluid in the lesion, but the results were apt to be discordant when the lesions were small and dry. Separate slits made close together within the same lesion gave reproducible results for MI and BI in one patient, but were found too irksome for the patient to be generally acceptable. They were not employed in this study.

Discussion

The object of determining the BI is to indicate the density of *Myco. leprae* in a smear; the higher the value, the better the stain technique. As regards the MI, our

results confirm the finding of Rees and Valentine (1962*b*) that heating in carbol-fuchsin causes a redistribution of acid-fast material in the body of the bacillus and so raises the MI; our results also confirm the observation of Nakamura *et al.* (1968) that heat fixation gives a higher MI than formalin gas. There are 2 possible objective assessments of any MI, whatever its apparent level: Rees and Valentine's (1962*a*) comparison with electron-microscopic morphology (using *Myco. lepraemurium*) and Shepard and McRae's (1965) comparison with footpad viability. In our experience in this study, the acid-fast stain techniques used by Shepard and by Rees give fairly good agreement, though the MI is somewhat lower by Shepard's method. Some of the other methods in regular use gave widely divergent results, especially when the MI was low.

Any stained specimen is an artefact, and this applies no less to acid-fast stains than to others. As regards Shepard's method, we had at first assumed that the object of the phenol-gel was to make the washed bacilli adhere to the slide. Our results prove, however, that phenol-gel is an essential component of the technique, without which bacilli are pale and the MI is low. The technique requires that, after application of the phenol-gel, while the gel is still moist, the slide is warmed on the lid of a boiling water-bath for several minutes. This reproduces precisely the conditions which, according to Rees and Valentine, caused a re-distribution of acid-fast material in the substance of the bacillus, with the artificial production of "solids", namely, heat in the presence of phenol solvent. All the methods that we have found to produce MI levels of the sort that have been equated with viability employ heat in the presence of phenol, with 2 exceptions: Aubert's (4b) method uses Tween-80, which might well facilitate the liquefaction or mobility of acid-fast substance. The other satisfactory relatively cold (30° C) method is the fluorescent method of Mansfield (5b), which incorporates glycerol. The mechanism here is not quite clear. It seems likely therefore that a small re-distribution of acid-fast substance during staining is necessary in order for the MI to be equated with viability, using the present criteria for estimating the MI. The difficulty is to achieve just the right degree of adjustment. Weak staining methods which could not possibly alter acid-fast morphology would be unsatisfactory for general use, since the staining is pale and some granular organisms are not stained at all.

Rees and Valentine's method is not convenient for routine use, and was indeed never intended for this purpose. A very similar result can be obtained using the Ziehl-Neelsen (1a) procedure, with staining at 42° C for 15 min. The only disadvantage of this is that in order to produce the temperature accurately a special water-bath or incubator is required. With experience, however, the correct amount of direct heat can be judged fairly well: the slide is heated until the stain just begins to steam. We found Shepard's method at first impossible to apply to skin smears, but the difficulty was overcome. The only practical disadvantage of this method is that fresh phenol gel has to be prepared each day. This problem could be overcome by substituting 0.5% phenol for phenol gel; and from a small series of tests it seems that this modification would make a good alternative to Ziehl-Neelsen at 42° C and would be suitable for routine use. Any of these 3 methods give reasonably good staining quality and a fairly reliable BI. But for the certain staining of all leprosy bacilli there is no substitute for the Ziehl-Neelsen method using hot stain (60° C). The modification of Aubert's method (4b) can be recommended as simple and reliable, and it gives results almost identical to those

by Shepard's method; it also has advantages under field conditions. But with slit and scrape (as opposed to impression) smears, the result is messy.

For the routine management of patients it is not necessary for the MI to be absolutely equated with viability, but in view of the importance attached to the MI in drug trials, standardization of technique is important.

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The SFG (Solid, Fragmented, Granular) Index for Bacterial Morphology*

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The morphological index (MI) has become almost universally adopted as an indication of the viability of leprosy bacilli. This is unquestionably the correct procedure in drug trials and in many forms of research, but the determination of the MI is unnecessarily time-consuming for the routine management of patients, and also demands a high level of skill and experience on the part of the technician if reproducible and meaningful results are to be achieved.

The reason why the granularity index (Ridley, 1960) has not become more popular is probably that its simplicity is not at first sight appreciated. Dr. Tin Shwe has pointed out to me that it is also unnecessarily difficult to explain and comprehend because the values are the reverse of those of the MI. To obviate this small obstacle, it is suggested that the index values should be inverted, so that 10 represents all solid bacilli and 0 all granular bacilli. Accordingly, the index is redescribed here in inverted form, with a new name to avoid confusion. This index has been in regular routine use at this hospital for 12 years, and the technicians who have been taught its use have without exception preferred it to the morphological index. The SFG index cannot be directly equated with the MI, but it has been found entirely adequate for its purpose by clinicians.

(As a matter of historic interest, it may be mentioned that both Lowe and Davey, working in the Leprosy Research Unit, Uzuakoli (in the former Eastern Nigeria), regularly employed a similar classification of the morphology of bacilli present in routine smears.)

The SFG index. Bacilli are conveniently divided into 3 classes: (1) "solid" (S), i.e. solid-staining unbroken rods; (2) "fragmented" (F), i.e. bacilli in which the acid-fast substance is interrupted at one or more points, but at least one fragment displays an elongated form; also single very short rods; and (3) "granular" (G), i.e. round granules either in line or in clumps. It is difficult to estimate the exact percentage of bacilli in each class, but their approximate ratios can be estimated almost at a glance.

A value is assigned to the bacilli of each class in a smear: 2 if they appear numerous (over 20% of all bacilli); 1 if few (1-20%) or 0 (if less than 1%). Thus the relative proportion of bacilli in the 3 classes SFG (in this order) are represented by one of the permutations of 2-1-0. These combinations have been placed in order of descending granularity from 2-0-0 (all solid) to 0-0-2 (all

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SFG value	SFG index
2-0-0	10
2-1-0	9
2-2-0	8
2-1-1 (= 1-2-0)	7
2-2-1	6
1-2-1 (= 2-2-2)	5
1-2-2	4
1-1-2 (= 0-2-1)	3
0-2-2	2
0-1-2	1
0-0-2	0

(The values 2-1-2, 2-0-2 and 0-2-0 are very seldom found. All give an index of 5.)

granular) to give an index, as shown in the accompanying column. The order is not obvious. Based on the ratios of solid to granular bacilli it was determined by plotting the various SFG values in the form of crude distribution curves on squared paper and counting the squares on either side of the "F" values. If several smears are available the mean index is taken.

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Four Years' Experience with Dapsone as Prophylaxis Against Leprosy*

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Dapsone has been used as a prophylaxis against leprosy in the schools in Samia (trial area). The results obtained suggest that the drug is effective in protecting children against leprosy. This agrees well with the findings of Dharmendra *et al.* (1965). The major limitations are those of administration. No side-effects were noted during the trial.

Introduction

The value of dapsone as a prophylaxis against leprosy has been advocated by some leprosy workers (Dharmendra *et al.*, 1965; Wardekar, 1967) and denied by others. Since *Mycobacterium leprae* have been found in human mammary glands and in human milk (Pedley, 1967, 1968), more evidence concerning the efficacy of dapsone as a prophylactic against leprosy is badly needed.

The East African Leprosy Research Centre, therefore, planned a controlled trial in the two adjacent areas of Samia and Bunyala Locations (Fig. 1) to ascertain whether dapsone is an effective prophylactic agent against leprosy. This paper discusses the trial carried out in schools in these two areas, covering the period between February, 1963, and December, 1967.

Method

An intensive survey was carried out in 1963 in Samia and Bunyala Locations, in which 28,873 people of all ages were examined clinically for signs of leprosy, special attention being paid to the schoolchildren.

The names of the children in primary classes I to IV in the two areas were recorded in special registers. In Samia there were 20 schools with a total of 3380 children; and in Bunyala Location, 14 schools, and 1393 children.

In Samia Location, dapsone was issued twice a week by the headmasters of the schools to all the pupils. No dapsone was given in Bunyala schools. All the schools in the two areas were regularly visited by a research officer from the Leprosy Research Centre and fortnightly during the period of the trial.

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Fig. 1. Map showing locations of Samia and Bunyala. Treatment and control areas are marked.

Results

Among the 28,873 persons examined in the preliminary survey, 1860 were found to have leprosy, giving a prevalence rate of 64.4 per 1000; 73.8% of these patients had tuberculoid leprosy and the remainder lepromatous or borderline forms.

The prevalence rates among schoolchildren in the two locations were similar, namely 6.5 per 1000 in Bunyala and 6.2 per 1000 in Samia. At the beginning of the trial there were 9 known cases of leprosy in the Bunyala schools and 21 in the Samia schools. During the period of the trial, out of the 3366 children in the Samia schools who had regularly taken prophylactic dapsone, 4 contracted leprosy, whereas 13 out of 1370 among the non-protected children in the Bunyala schools were diagnosed as having contracted leprosy. The incidence rates for the 3 years are thus 1.2 per 1000 and 9.5 per 1000 respectively, an 8-fold difference

attributable, in our opinion, solely to the protective value of the dapsone regularly administered to the former group.

All patients diagnosed in the schools before and after the trial had tuberculoid leprosy (Table 1). All the patients discovered were given treatment.

TABLE 1

Showing two groups of children (in trial and control areas)

Location	No. of children before trial	No. of children at the follow-up	No. with leprosy at beginning of trial	No. developing leprosy during trial
Samia (trial group)	3380	3366	21	4
Bunyala (control group)	1393	1370	9	13

Discussion

The results obtained confirm that dapsone does protect children against leprosy. In their study, Dharmendra *et al.* (1965) concluded that the prophylactic value of dapsone begins to show 9 months after starting to take the drug, but that once infection has taken place, dapsone confers no protection against leprosy. It is possible that the children in the present trial who were found to have leprosy had been infected before the trial began.

The original prevalence of leprosy in the schools in the two groups in Bunyala and Samia was similar. However, at the end of the trial, the differences in the two areas were statistically significant ($\chi^2 = 7.743$ and $0.01 > P\ 0.005$). The questions remaining are: How long has the drug to be taken–for life? Will there be any toxic effects?

In the trial period of 3 years and 10 months, no toxic effects were observed. Kidney and liver function tests on 52 children selected at random in the Samia trial gave normal values.

It would appear from the results that dapsone can be used to protect children whose mothers have leprosy. Since the effect of the drug begins to show after about 9 months, it would be better to give the child a small dosage (25 mg twice a week) as soon as it is weaned, since during lactation the child is apparently protected by the dapsone excreted in the mother's milk.

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Studies on the Determination of the Minimal Inhibitory Concentration of 4,4'-diamino-diphenyl-sulphone (Dapsone, DDS) against *Mycobacterium leprae**

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Plasma DDS concentrations were determined fluorometrically in mice fed continuously with 0.01% of the drug in their diet. They averaged 0.74 μg per ml.

(2) Since it is known that the multiplication of *Myco. leprae* in the mouse footpad system is inhibited by feeding 0.0001% DDS but not by 0.00001% DDS in the diet, it was concluded that the MIC of DDS against *Myco. leprae* must be less than 0.01 μg per ml.

(3) The half-life of DDS in the mouse was determined after intraperitoneal dosage and found to be 2.7 h after dosage with DDS at 10 mg per kg and 3.7 h after 50 mg per kg.

(4) DDS serum concentrations in patients who were being successfully treated with 1 mg of DDS a day averaged 0.018 μg per ml 3 h after daily dosage, and a similar proportion of the drug was excreted in the urine as was found with doses some 50 to 300 times as large.

(5) The significance of these findings is discussed both in the relation to the treatment of leprosy with DDS, and to the use of the mouse footpad system in predicting the likely efficacy of other potential antileprosy compounds.

Introduction

When diamino-diphenyl-sulphone (DDS) was first introduced for the treatment of leprosy, the doses used were chosen empirically since at that time no laboratory method was available for measuring the sensitivity of *Mycobacterium leprae* to

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† This laboratory is designated a WHO Regional Reference Centre for *Myco. leprae*.

the drug. Daily doses of up to 100 mg DDS by mouth or twice weekly doses of 300 mg by injection were found to be reasonably well tolerated by most patients, and to be therapeutically effective. For these reasons weekly dosage with 600 mg of DDS gradually became accepted as the standard treatment.

The discovery by Shepard (1960) that bacteria from leprosy patients multiply to a limited extent in the footpads of mice made it possible to test experimentally the effectiveness of drugs in inhibiting the multiplication of strains of *Myco. leprae* from previously untreated patients. The activity of DDS in preventing multiplication of the organisms in the mouse footpad system was first demonstrated by Shepard and Chang (1962). In this first study, DDS was fed at a dietary concentration of 0.1%. Two years later they showed that its antileprosy activity was still maintained when it was given in a dietary concentration of 0.01% (Shepard and Chang, 1964). These findings were confirmed by Rees (1965) and Pattyn and Royackers (1965). Since these initial studies, there have been several investigations to determine the minimal effective dose of DDS necessary to inhibit the multiplication of *Myco. leprae* in the mouse footpad system. The results that have been obtained are summarized in Table 1. All the strains of *Myco. leprae* were inhibited by feeding DDS at a dietary concentration of 0.0001%, and with but two exceptions, they were not inhibited by a dietary concentration of DDS of 0.00001%. The minimal effective dose of DDS against *Myco. leprae* is therefore somewhere between these two limits.

TABLE I

Activity of DDS against M. leprae *in the mouse foot-pad system*

% DDS in the diet				
0.0001[a]	0.00005	0.000025 and 0.00003	0.00001	References
A (1)[b]		A (1)	A (1)	Shepard *et al.* (1966)
A (2)			IA (4)	Shepard (1967*a*)
A (9)			IA (5)	Shepard (1967*b*)
A (2)			IA (2)	Rees (1967*a*)
A (2)			A (1) IA (1)	Rees (1967*b*)
A (11)				Shepard *et al.* (1969)
A (12)	A (1)	A (1)	IA (5)	Shepard (1969)[c]
A (25)			IA (24) A (1)	Rees (1970), current data.

A = Active, inhibition of multiplication.
IA = Inactive.
[a] = All concentrations above 0.0001% active.
[b] = Number of strains in parenthesis.
[c] = Personal communication.

Since it is still not possible to cultivate *Myco. leprae in vitro,* the minimal inhibitory concentration (MIC) of DDS against the organism cannot be measured directly. However, it can be estimated by measuring the concentration of DDS in the serum or plasma of mice fed with the minimal effective dose of the drug required to inhibit the multiplication of *Myco. leprae.* Since DDS is a relatively

non-polar, uncharged, lipid-soluble compound, it would be expected that it would readily penetrate cell membranes so that tissue concentrations would parallel the concentrations of the drug in the blood. Its rapid absorption in man (Glazko *et al.*, 1968) and its penetration into the tissues in mice (Shepard and Chang, 1964; Rees, 1967*b*) and in man (Chatterjee and Poddar, 1957) are in accord with these assumptions.

In this study we have measured the plasma concentrations of DDS using a new fluorometric method which is considerably more sensitive and potentially more specific than the colorimetric methods previously employed. This method has also been used to determine two potential metabolites of DDS, namely, *N*-acetyl-DDS (MADDS) and *N N'*-diacetyl-DDS (DADDS, acedapsone). The earlier colorimetric studies indicated that the concentration of DDS in the serum of mice receiving 0.0001% of the drug (the minimal effective dose) was approximately 0.01 μg per ml, or only about one-hundredth of the minimal serum concentrations achieved in man after daily dosage with 100 mg of the drug. A pilot clinical trial was therefore undertaken to establish whether a dose of as little as 1 mg of DDS a day was also effective in the treatment of lepromatous leprosy (Waters *et al.*, 1968). This clinical trial has now been completed and has established the effectiveness of this dose of DDS (Waters and Rees, 1971). In order to facilitate a direct comparison between the results achieved in the clinical study and the previous experimental studies using the mouse footpad system, we also measured the concentrations of DDS in the plasma of the patients participating in this clinical trial, using the same fluorometric method employed to determine the MIC of the drug against *Myco. leprae* in the mouse.

Methods

MEASUREMENT OF THE CONCENTRATIONS OF DDS, MADDS AND DADDS IN THE PLASMA OF MICE

Plasma was obtained between 09.00 and 11.00 h from a total of 13 female P-strain albino mice who had been fed continuously with 0.01% DDS in the diet for periods ranging from 6 to 10 months. It was estimated that continuous feeding with DDS should result in steady-state plasma concentrations of the drug being achieved within one day. The mice were exsanguinated individually, following cardiac puncture under ether anaesthesia. Plasma was also collected from 2 groups each of 15 mice which had been dosed with DDS, 10 and 50 mg per kg body-weight respectively, by intraperitoneal injection. Each mouse received the drug dissolved in 1 ml of ethanol/polyethylene glycol 300/0.85% sodium chloride (1 : 3 : 6 parts by volume), and the mice, in groups of 3, were killed 1, 2, 4, 6 and 24 h, respectively, after dosage. Initial experiments demonstrated that the vehicle was well tolerated by the mice, as were doses of DDS of up to 50 mg per kg in the vehicle; doses of 100 and 200 mg per kg in the vehicle, however, were lethal.

Approximately 0.3 ml of plasma was obtained from each mouse. This was diluted to 3 ml with water and the concentrations of DDS, MADDS and DADDS determined by the fluorometric method of Ellard and Gammon (1969). Standards, which were extracted in duplicate, consisted of 3 ml of water, normal mouse plasma, aqueous 1 μg per ml DDS, MADDS, and DADDS, respectively. For these studies, DDS was extracted into 3 ml of 1.2 N-HCl, rather than 2 ml as in the original method.

The plasma DDS concentrations in mice which had been dosed intraperitoneally with DDS, 50 mg per kg, were also determined, using a modification of the Bratton and Marshall (1939) colorimetric procedure (Ellard *et al.*, 1970). Fifteen minutes after reaction the absorption spectrum was scanned in a Unicam SP 800 spectrophotometer and the concentration of DDS calculated from the extinction at 570 mμ. Standards which were extracted in duplicate consisted of 3 ml of water, normal mouse plasma, and aqueous 10 μg per ml DDS, respectively.

MEASUREMENT OF THE SERUM CONCENTRATION AND URINARY EXCRETION OF DDS IN PATIENTS PARTICIPATING IN A THERAPEUTIC TRIAL OF 1 mg OF DDS DAILY

The design and conduct of the clinical trial has been described elsewhere by Waters and Rees (1971). Serum samples and 24-h urine collections were both obtained from the patients immediately before commencing treatment and thereafter alternating every 3 weeks during treatment. Blood samples were taken 3 h after dosage with DDS during the 6th, 12th, and 18th weeks of treatment. Glazko *et al.* (1968) have shown that DDS is rapidly absorbed in man after oral dosage and that peak serum concentrations occur 2 to 4 h after dosage. During this period DDS serum concentrations are almost constant, and thereafter fall slowly, with a half-life of about 21 h.

Urine collections were made during the 3rd, 9th, 15th and 18th weeks of treatment. Each 24-h urine collection was diluted to 2.4 litres and an aliquot was preserved by the addition of 1% (by volume) glacial acetic acid (giving a pH of between 3 and 4). The urine and serum samples were stored at −20° C until despatch on ice by air from Malaya to England for analysis. It had previously been shown that DDS was stable in serum for at least 6 months when stored at −20° C.

The concentration of DDS in the serum and urine samples was determined using a modification of the method of Ellard and Gammon (1969). Serum standards, which were extracted in triplicate, consisted of 3 ml of water, normal serum, and normal serum containing 0.01, 0.1 and 1.0 μg per ml DDS, respectively. Urine standards, a 24-h collection which were extracted in quadruplicate, consisted of 10 ml of water, normal urine (from a healthy subject, G.A.E.) diluted to 2.4 l after the addition of 24 ml of glacial acetic acid, and normal urine containing 0.01, 0.1 and 1.0 μg per ml DDS, respectively.

Next, 3 ml of serum was extracted by shaking with 8 ml of ethyl acetate and 1 ml of M sodium citrate in a stoppered centrifuge tube; 10-ml aliquots of urine were extracted by shaking with 10 ml of ethyl acetate, 2 ml of M sodium citrate and 8 g of ammonium sulphate. Thereafter the serum and urine extracts were treated identically. Of the ethyl acetate extract 6 ml was washed by shaking with 1 ml of 0.1 N sodium hydroxide and 5 ml of the washed extract further washed by shaking with 1 ml of 0.1 N hydrochloric acid; 4 ml of this washed ethyl acetate extract was then extracted by shaking with 3 ml of 1.2 N hydrochloric acid. Duplicate 1-ml aliquots of the 1.2 N hydrochloric acid extract were then pipetted into small centrifuge tubes. To the first, 0.1 ml 1% (w/v) aqueous sodium nitrite was added to destroy the fluorescence of any DDS present, presumably through diazotization of the aromatic amino groups, and 5 min later the nitrite was destroyed by the addition of 0.1 ml of 10% (w/v) ammonium sulphamate. Two ml M sodium citrate was then added and the mixture extracted by shaking

with 2 ml of ethyl acetate. The second 1-ml aliquot was extracted by shaking with 2 ml of M sodium citrate and 2 ml of ethyl acetate without the prior addition of nitrite and sulphamate. Each ethyl acetate extract was then dried by shaking with 0.5 g of anhydrous sodium sulphate.

The concentration of DDS was determined by measuring the fluorescence of the second ethyl acetate extract at 298/345 mμ and subtracting from it the fluorescence of the first nitrite-treated extract. In this way each sample also provided its own blank. The fluorescence of the extracts was also measured at 298/420 mμ and 295/324 mμ, respectively.

Results

PLASMA DDS CONCENTRATIONS IN MICE

The results obtained are summarized in Tables 2, 3 and 4. That the fluorometric method employed specifically measured unchanged DDS in the plasma of the mice was apparent for 4 reasons: (1) plasma from normal mice gave

TABLE 2

Concentrations of DDS in the plasma of mice fed continuously with 0.01% DDS in the diet

Experiment	Concentrations of DDS (μg/ml)	Fluorescent characteristics	
		Ratio[a] 420/345 mμ	Ratio[a] 324/345 mμ
1	0.73, 0.39	0.26 (0.24)[b]	0.39 (0.40)
2	0.65, 0.99, 1.19, 1.00, 0.90	0.22 (0.26)	0.34 (0.36)
3	0.35, 0.57, 0.69, 0.24, 0.54, 1.38	0.33 (0.32)	0.32 (0.35)
Mean[c]	0.74 ± 0.10	–	–

[a] Ratio of fluorescence at 298/420 mμ and 295/324 mμ, respectively, compared with that at 298/345 mμ.
[b] Ratios for 1 μg/ml aqueous DDS standards in brackets.
[c] Mean ± standard deviation of mean.

TABLE 3

Concentrations of DDS in the plasma of mice after 10 mg/kg DDS intraperitoneally

Time (h)	Plasma DDS concentrations (μg/ml)		Fluorescent characteristics	
	Individual	Mean[a]	Ratio[b] 420/345 mμ	Ratio[b] 324/345 mμ
1	6.59, 5.83, 4.59	5.61	0.32	0.32
2	2.45, 5.41, 3.69	3.66	0.35	0.34
4	2.51, 2.72, 2.46	2.56	0.33	0.34
6	1.30, 1.42, 1.65	1.45	0.34	0.35
24	0.18, <0.10, <0.10	–	0.32	0.68
1 μg/ml aqueous DDS standard (3 ml)			0.32	0.35

[a] Geometric means.
[b] Ratio of fluorescence at 298/420 mμ and 295/324 mμ, respectively, compared with that at 298/345 mμ.

TABLE 4

Concentrations of DDS in the plasma of mice after 50 mg/kg DDS intraperitoneally

Time (h)	Plasma DDS concentrations (μg/ml)		Flourescent characteristics	
	Individual	Mean[a]	Ratio[b] 420/345 mμ	Ratio[b] 324/345 mμ
1	18.6, 24.6, 29.8	23.9	0.34	0.36
2	15.3, 22.1, 19.2	18.7	0.32	0.34
4	16.0, 14.5, 12.5	14.3	0.33	0.33
6	7.77, 9.92, 9.76	9.10	0.33	0.35
24	<0.10, 0.43, 0.15	—	0.63	0.47
1 μg/ml aqueous DDS standard (3 ml)			0.32	0.35

[a] Geometric means.

[b] Ratio of fluorescence at 298/420 mμ and 295/324 mμ, respectively, compared with that at 298/345 mμ.

extracts whose fluorescence did not differ significantly from those of the aqueous blanks, so that the fluorescence of the extracts from the treated mice must have been due to compounds derived from DDS; (2) the fluorescent characteristics of the extracts from the treated mice were very similar to those of the DDS standards and unlike those of MADDS, which also extracts to a small extent in this solvent system; (3) when duplicate 1-ml aliquots of the 1.2 N hydrochloric acid extracts were heated for 1 h at 100°C prior to subsequent extraction, they gave ethyl acetate extracts whose fluorescent characteristics were identical to those of DDS, and whose absolute fluorescence was only about 6% greater than those of the unheated extracts; and (4) specificity studies showed that DDS-N-glucuronide does not extract into ethyl acetate and it is known that DADDS does not extract into 1.2 N hydrochloric acid (Ellard and Gammon, 1969).

From the variation in the fluorescence of replicate extracts from the aqueous blank and normal mouse plasma, it was concluded that the method could be used to measure concentrations as low as about 0.1 μg per ml DDS in 0.3 ml of plasma.

The plasma concentrations found in mice which were being continuously fed with 0.01% DDS in their diet are summarized in Table 2. These concentrations ranged from 0.2 to 1.4 μg per ml and averaged 0.74 μg per ml.

The concentrations of DDS in the plasma of mice after intraperitoneal dosage with 10 and 50 mg of DDS per kg, respectively, are summarized in Tables 3 and 4 and illustrated in Fig. 1. The half-life of DDS in the mouse was calculated from regression analysis of the logarithms of the DDS plasma concentrations at 1, 2, 4, and 6 h after dosage. These analyses showed that the plasma concentrations of DDS fell exponentially throughout this period at rates equivalent to a half-life of 2.7 h after a dose of DDS of 10 mg per kg and 3.7 h after 50 mg per kg. The difference between these half-lives was significant ($P < 0.0001$). If the fall in DDS concentrations had continued exponentially at each dose, the concentrations at 24 h would be expected to be 0.01 μg per ml and 0.33 μg per ml, after doses of 10 and 50 mg per kg, respectively. The results obtained are in reasonable agreement with these predictions and do not suggest any major change in the rate of fall of the plasma concentrations of DDS at these later times.

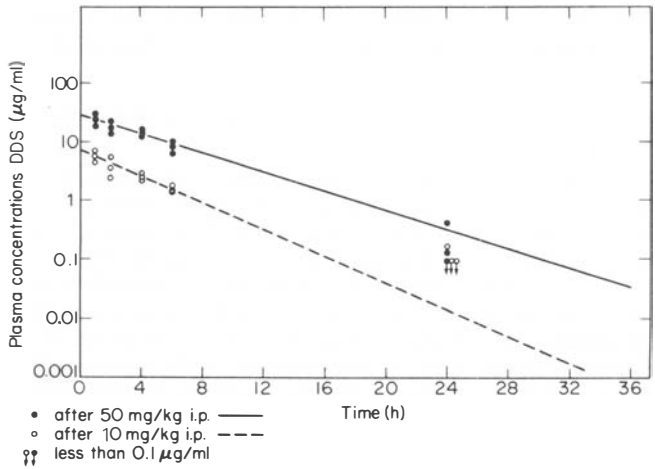

Fig. 1. Concentrations of DDS in mice after dosage with 10 and 50 mg per kg.

From the regression analyses it was concluded that the replicate errors were equivalent to about ±18% after 50 mg per kg DDS and ±24% after 10 mg per kg DDS. Since these coefficients of variation are composite error terms that include errors in dosage, timing, and the fluorometric DDS determinations, as well as variations between individual mice, it is probable that the individual mice did not vary greatly in the rates at which they metabolized or excreted DDS.

PLASMA MADDS AND DADDS CONCENTRATIONS IN MICE

Convincing evidence for the presence of MADDS in the plasma of mice was not obtained. The fluorometric MADDS determinations and the estimated amounts of acid-hydrolysable DDS extracted into 1.2 N hydrochloric acid indicated that after intraperitoneal dosage with 50 mg of DDS per kg the MADDS concentrations could not have exceeded a sixth of the concomitant plasma DDS concentrations. DADDS was not detected in these plasma samples and it was concluded that the concentrations of DADDS could not have exceeded 0.2 μg per ml or 1% of the concomitant DDS concentrations.

PLASMA DDS CONCENTRATIONS IN MICE DETERMINED BY THE COLORIMETRIC METHOD

The reacted extracts showed similar extinction profiles to that of DDS with its characteristic hump and maximal absorption at 570 mμ. This is presumably because both the aromatic amino groups of DDS can be diazotized and coupled with N-1-naphthyl-ethylene-diamine. The ratio of the extinctions at 570 mμ to those at 545 mμ averaged 1.05 for the extracts from the DDS-treated mice compared with 1.08 for the DDS standard. By contrast, MADDS and most other aromatic monoamines show a near symmetrical extinction profile, with a peak absorption at about 545 mμ (for MADDS the ratio of the extinction at 570 mμ to that at 545 mμ was about 0.75). Although the absorption spectra of the reacted extracts were thus very similar to that of DDS, the absolute concentrations of DDS determined colorimetrically in this way were about 25% greater than those determined using the more specific fluorometric method. A comparison of the

results obtained by the two methods indicated that the sensitivity of the fluorometric method was at least 5 times greater than that of the colorimetric method.

SERUM CONCENTRATIONS AND URINARY EXCRETION OF DDS IN PATIENTS BEING TREATED WITH 1 mg OF DDS DAILY

Regression analysis showed that the fluorescence of the extracts from both the serum and urine standards included in the analyses of the serum and urine samples from the patients treated with 1 mg of DDS per day, was directly proportional to the concentration of DDS over the range 0.01 to 1.0 μg per ml. In each case the error of the method in this concentration range was estimated at ± 0.010 μg per ml. The fluorescence of the extracts of the normal serum and urine used to prepare the standards, and of the nitrite-treated extracts from the DDS standards, was not significantly greater than the fluorescence of the aqueous blank extracts.

The fluorescence of the extracts of the pre-treatment serum samples from the patients did not differ significantly from that from the aqueous blank, but the extracts from the serum samples obtained 3 h after dosage with 1 mg of DDS showed significant fluorescence at 298/345 mμ ($P < 0.001$). The mean fluorescence of these extracts was equivalent to a concentration of DDS of about 0.018 μg per ml. However, the error of the method (equivalent to ±0.010 μg per ml DDS) was such that it was not possible to measure individual DDS serum concentrations. Nevertheless, it was calculated that the serum concentrations of DDS in the patients never exceeded 0.05 μg per ml. Since tablets of DDS that might have been available locally to the patients would have contained at least 50 mg of DDS, it was concluded that surreptitious self-medication with alternative supplies of DDS could not have occurred in any of the 4-day periods prior to the serum collections. The fluorescent characteristics of the compound extracted from the serum of these patients was, considering the inaccuracies of the method at these low concentrations, fairly similar to that of DDS (Table 5).

Unlike the ethyl acetate extracts from the urine used for setting up the DDS standards, the extracts from the patients' pre-treatment urine samples showed

TABLE 5

Concentrations of DDS in human serum 3 h after daily dosage with 1 mg DDS

Serum DDS concentrations (μg/ml)	Fluorescent characteristics	
	Ratio[a] 420/345 mμ	Ratio[a] 324/345 mμ
0.018 ± 0.003[b]	0.40	0.37
0.1 μg/ml standard	0.25	0.41
1 μg/ml standard	0.26	0.41
0.4 – 4.1[c]	0.28	0.41
1 μg/ml standard	0.27	0.39

[a] Ratio of fluorescence at 298/420 mμ and 295/324 mμ, respectively, compared with that at 298/345 mμ.

[b] Mean ± standard deviation of mean.

[c] For comparison, from a group of dermatitis herpetiformis patients being continuously treated with 50-300 mg of DDS per day, analysed in a similar way (Ellard and Gammon, 1969).

significant fluorescence. Part of this fluorescence was due to compounds that were destroyed by treatment with nitrous acid. The fluorescence characteristics of these extracts were markedly different from those of DDS (Table 6), but were similar to those from urine samples collected from other patients in the Sungei Buloh leprosarium who were either temporarily untreated or else being treated with drugs other than DDS. The fluorescence of the extracts of the urines obtained during daily treatment with 1 mg of DDS was significantly greater than that of the pre-treatment extracts ($P = 0.01$) and the increase in fluorescence at 298/345 mμ was equivalent to the urinary excretion of about 0.18 mg of DDS per day. Further, the fluorescence characteristics of the treatment extracts were more like that of DDS than were those of the pre-treatment extracts. The fluorescence ratios of the compound excreted during daily dosage with 1 mg of DDS were calculated for each patient by subtracting the fluorescence of the pre-treatment extract from that of each treatment extract. It will be seen from Table 6 that the mean fluorescence ratios were similar to those of DDS. For comparison, the results that were obtained from a group of patients with dermatitis herpetiformis who were being treated with some 50 to 300 times this dose of DDS (Ellard and Gammon, 1969) are also shown.

Discussion

The concentrations of DDS found in the serum or plasma of mice, together with determinations of its half-life in the mouse, found in this study are compared in Table 7 with the results obtained by other workers. The mean concentration of DDS in the plasma of mice which had been continuously fed with 0.01% DDS in the diet averaged 0.74 μg per ml. This is in good agreement with the results obtained by Shepard *et al.* (1966) and Bushby and Rees (Rees, 1967*b*) using colorimetric methods for the determination of DDS. In the latter study serum was adjusted to pH 1 and heated for 1 h at 100° C. After cooling the pH was adjusted to 8 and DDS extracted into methyl isobutyl ketone and thence into 2 N hydrochloric acid, and determined by a modification of the Bratton and Marshall (1939) procedure (Bushby, personal communication). Tissues were incubated with papain at 56° C overnight and then treated in the same way as serum. Our results are also similar to those obtained by Glazko and Shepard (personal communication) and Ozawa *et al.* (1971) using either the fluorometric method of Glazko *et al.* (1968) or that employed in this study (Ellard and Gammon, 1969).

After intraperitoneal dosage, the half-life of DDS in the mouse was found to be 2.7 h after dosage with 10 mg per kg, and 3.7 h after 50 mg per kg. These results are similar to those obtained by Gordon *et al.* (1970) after dosing mice intraperitoneally with DDS, 1 mg per kg, and by Ozawa *et al.* (1971) after terminating continuous dosage with 0.01% DDS in the diet.

The concentrations of DDS at time zero after intraperitoneal DDS dosage with 10 and 50 mg per kg were calculated by extrapolation as being 6.8 and 28 μg per ml, respectively. The fact that the plasma concentrations of DDS fell exponentially from the earliest time of collection (1 h) indicated the absorption of DDS from the peritoneum, and its subsequent distribution throughout the body was extremely rapid. Its volume of distribution in the mouse would appear to be about 1.6 times the body weight. Somewhat similar results were obtained by Francis and Spinks (1950), using an oral dose of DDS of 100 mg per kg, and Gordon *et al.* (1970) after an intraperitoneal dose of 1 mg per kg.

TABLE 6

Urinary excretion of DDS (μg per ml)

Urine Samples	Dose (mg DDS/day)	Apparent concentration (μg DDS/ml)	Excretion (mg/day)	Excretion (% dose)	Fluorescence characteristics	
					Ratio[a] 420/345 mμ	Ratio[a] 324/345 mμ
Pre-treatment	nil	0.043 ± 0.013[b]	–	–	0.42	0.95
Treatment	1	0.117 ± 0.012	–	–	0.32	0.67
Treatment	1	increment 0.074 ± 0.012	0.178 ± 0.029	18	0.23	0.51
Standards	–	(0.1-1.0)	–	–	0.23	0.41
Treatment[c]	50-300	(0.8-13.5)	(2-32)	17	0.26	0.41
Standards	–	(1.0)	–	–	0.25	0.39

[a] Ratio of fluorescence at 298/420 mμ and 295/324 mμ, respectively, compared with that at 298/345 mμ.
[b] Mean ± standard deviation of mean.
[c] For comparison, from a group of dermatitis herpetiformis patients analysed in a similar way (Ellard and Gammon, 1969).

TABLE 7

Concentrations of DDS in the serum, plasma or blood of mice

% DDS in the diet													
0.2	0.1	0.05	0.025 and 0.03	0.016	0.01	0.006	0.003	0.001	0.0001	Single dose (mg/kg)	Half-life DDS (h)	Method[a]	Reference
				3.9[b]						248	5-6[b]	C	Titus and Bernstein (1949)
		9.1										C	Francis and Spinks (1950)
										100	9-10[c]	C	Francis and Spinks (1950)
	15.3											C	Francis (1953)
22.1[d]	19.0	2.4	5.2		2.6							C	Shepard and Chang (1964)
			3.6[e]		0.93[e]		0.49[e]	0.14[e]				C	Shepard *et al.* (1966)
	12.5		3.3		0.89	0.55		0.15				C	Rees (1967b)
					1.00			0.063	0.007			F	Glazko and Shepard (1969)[h]
										1[f]	2.6	F	Gordon *et al.* (1970)
					0.79			0.093	0.011			F	Ozawa *et al.* (1971)
											3.6-5.0[g]	F	Ozawa *et al.* (1971)
					0.74							F	This study
										10[f]	2.7	F	This study
										50[f]	3.7	F	This study

[a] Methods: C = colorimetric; F = fluorometric.
[b] Calculated from the data illustrated in Figs 1 and 2 of Titus and Bernstein (1949).
[c] Calculated from the data illustrated in Fig. 6 of Francis and Spinks (1950).
[d] Food consumption reduced.
[e] Means calculated from data in Table 1 of Shepard *et al.* (1966) weighted with number of mice whose blood was pooled for analysis.
[f] Administered intraperitoneally.
[g] After terminating continuous dosage with 0.01% DDS in the diet.
[h] Personal communication.

The acute toxicity of DDS was manifest within a few minutes after intraperitoneal dosage with 100 mg per kg. This would have been expected to result in plasma concentrations of DDS of about 50 μg per ml. Boyer (1951) has previously found the LD^{50} of orally administered DDS in the mouse to be 600 mg per kg, although it is possible that under some conditions of oral dosage DDS may be rather less rapidly absorbed (Titus and Bernstein, 1949). Nevertheless, these latter workers did obtain peak blood DDS concentrations of about 100 μg per ml 7 h after dosage with 248 mg per kg without reporting the occurrence of acute toxicity. It should be noted that Francis (1953) has obtained evidence that suggests that old, large mice are less likely to develop nervous symptoms due to DDS on account of its poorer penetration into their brains than are young, small mice.

Mice tend to eat continuously throughout the day and night. The mice used in these studies had an average weight of 25 g, and consumed 5 g of diet a day (Rees, 1967*a*). If it were assumed that the mice eat continuously at the same rate throughout the day and night, then the formula derived by Wagner (1969) for a "one compartment open model" with a constant rate of infusion can be used to calculate the "steady state" DDS plasma concentrations that should be attained. This formula states that:

$$C = \frac{R}{V \cdot K}$$

where R is the rate of infusion, V the volume of distribution and K the first order rate constant for the overall loss of DDS in the mouse. Thus feeding mice with 0.01% DDS in the diet, with a drug half-life of 2.7 to 3.7 h would be expected to result in an average steady state plasma concentration of DDS of 0.20 to 0.28 μg per ml. Although this calculated value is only approximately one-third of the actual value found experimentally, it does at least provide an explanation for the fact that continuous dosage with 0.001% DDS in the diet of the mouse (equivalent to about 2 mg of DDS per kg per day) gives rise to DDS plasma concentrations that are less than a tenth of those achieved with an equivalent dose in man (100 mg per day) in whom the half-life of the drug is about 21 h. This discrepancy had previously been noted by Rees (1967*a*).

The results obtained by Shepard *et al.* (1966), Bushby and Rees (Rees, 1967*b*), Glazko and Shepard (personal communication) and Ozawa *et al.* (1971) (Table 7) indicate that the plasma concentrations of DDS in the mouse are approximately proportional to dose over the dosage range 0.0001 to 0.01% DDS in the diet. Thus since the minimal effective dose of DDS against *Myco. leprae* in the mouse is less than 0.0001% in the diet (Table 1), the average minimal inhibitory concentration of DDS against *Myco. leprae* in the mouse footpad system must be less than 0.01 μg per ml. It should be emphasized that these calculations are based on the average concentrations of DDS expected in mice during the day. The actual concentrations probably vary above and below these values throughout the day according to the feeding habits of the mice. Such factors are probably a major cause of the variation between the results from different mice shown in Table 2.

The fact that MADDS concentrations in the mouse were less than one-sixth, and DADDS concentrations less than one-hundredth, of the concomitant DDS concentrations is in accord with the results of Ozawa *et al.* (1971) and other workers. Thus Glazko and Shepard (personal communication) found similar "total acid-hydrolysable" and "free" DDS plasma concentrations in mice after

dosage with DDS, and Baukema, Chang and Glazko (personal communication) found only traces of MADDS in the urine after dosage with 7 mg per kg of ^{35}S-labelled DDS. Furthermore, Gordon *et al.* (1970) were unable to detect MADDS in the plasma of mice which had been dosed intraperitoneally with either DDS at 1.0 mg per kg or MADDS at 1.2 mg per kg, and concluded that the dose of MADDS had been entirely deacetylated to form DDS.

The serum concentrations of DDS in the patients who were being treated with 1 mg of DDS daily averaged 0.018 μg per ml 3 h after dosage. By comparison, using the same fluorometric method, we have previously found a DDS concentration of 1.8 μg per ml in the serum of a patient with dermatitis herpetiformis 4 h after repeated daily dosage with 100 mg of DDS (unpublished results). Glazko *et al.* (1968), using their fundamentally similar fluorometric method, found peak DDS plasma concentrations of about 1.2 μg per ml in a group of 5 volunteers after a single dose of 100 mg of DDS. Thereafter the DDS plasma concentrations fell exponentially, with half-values averaging 20.6 h. Similar results have been obtained by Gelber *et al.* (1971) using the fluorometric method of Peters *et al.* (1970). On the basis of these data it was calculated that daily dosage with 100 mg of DDS would eventually lead to peak DDS plasma concentrations of 2.2 μg per ml, and trough values of 1.0 μg per ml. These results suggest therefore that in man, serum or plasma concentrations after daily dosage with 1 mg of DDS are almost exactly one-hundredth of those obtained after daily dosage with 100 mg DDS.

The urinary excretion of DDS by the patients who were being treated with 1 mg of DDS daily averaged 0.18 mg, or 18% of the dose. Since these urine samples had been preserved by the addition of acetic acid, it may be assumed that a considerable proportion of any DDS-N-glucuronide that had been excreted would have already been converted to DDS before analysis. The results may be compared with an excretion of 17% DDS by a group of subjects who were being treated with 50 to 300 mg of DDS a day, or with the excretion of about 10% DDS and 15% DDS-N-glucuronide after single 100-mg doses of the drug (Ellard and Gammon, 1969; Gelber *et al.*, 1971).

Considering both the DDS serum concentrations of the patients who were being treated with 1 mg of the drug daily, and the amount of the unchanged drug they excreted in the urine, it would appear that the absorption, metabolism and excretion of DDS when given at this dosage is similar to that found with doses 50 to 300 times as great. This would suggest that the trough values of the DDS serum concentrations in the patients being treated with 1 mg of the drug daily probably averaged about 0.010 μg per ml.

In the mouse footpad system, the multiplication of *Myco. leprae* was prevented by dosing the animals with 0.0001% DDS in the diet, which it was calculated gave rise to serum/plasma concentrations of DDS that averaged less than 0.01 μg per ml throughout the day. The fact that patients with lepromatous leprosy were successfully treated with 1 mg of DDS daily (Waters and Rees, 1971), a dose that resulted in serum concentrations of the drug ranging from about 0.01 to 0.02 μg per ml, suggests that with this drug the results obtained in the mouse footpad system can be used directly to predict the efficacy of novel DDS regimens.

Treatment with 1 mg of DDS daily appeared, from the fall in the morphological indices, to be as effective as 50 mg of the drug twice-weekly (Pearson and Pettit, 1969), or 300 mg of the drug given twice-weekly by intramuscular injection (Waters, 1963; Waters and Pettit, 1965) in rendering

Myco. leprae non-viable in lepromatous patients, at least during an initial period of 4½ months (Waters and Rees, 1971). The rate of fall of the morphological indices was also similar to that achieved with clofazimine (Pettit *et al.*, 1967). The evidence at present available indicates that at concentrations near their minimal inhibitory concentrations (MIC's) the sulphonamides are bacteriostatic drugs *in vitro* and that they show bactericidal activity only at much higher concentrations. It would therefore seem probable that at all the doses of DDS used in these clinical trials, which have ranged from an average of 1 to 100 mg per day, the drug has primarily acted by preventing the multiplication of *Myco. leprae* in the patients, and that the fall in the morphological indices was due to killing by the cell-mediated defence mechanisms of the patients. The fact that in many series only about 35% of the bacilli in untreated lepromatous patients are viable, provides direct evidence of the capacity of the host to kill *Myco. leprae.* Furthermore when leprosy is treated with rifampicin, a drug known to show bactericidal activity against other organisms, the rate of fall of the morphological index is more rapid than that encountered with DDS or clofazimine (Rees *et al.*, 1970).

The results obtained in this study are also in accord with the findings of Shepard *et al.* (1968), who demonstrated the therapeutic effectiveness of intramuscular injections of 225 mg of DADDS given once every 77 days, and showed that such treatment resulted in the urinary excretion of amounts of acid-hydrolysable DDS equivalent to daily release in the tissues of about 2.5 mg of DDS.

It should be emphasized, however, that all these clinical studies have been of too short a duration to detect the potential emergence of DDS-resistant strains, which would, by analogy with tuberculosis, be expected to be apparent only after several years' treatment.

In the studies carried out using the mouse footpad system, compounds are generally tested for their potential antileprosy activity by giving them in the diet. If it is true that the mice feed continuously throughout the day and night so that approximately constant tissue and plasma concentrations of the compounds are achieved, then the MIC's determined by measuring these steady state concentrations at the minimal effective doses of the drugs in question are obviously analogous to MIC's determined *in vitro* against cultivatable pathogens.

In the treatment of tuberculosis there is, on the whole, a good correlation between the activities of drugs *in vitro* and *in vivo.* Thus, those compounds whose toxicity is low enough for them to be given in doses that result in plasma concentrations that are many times their MIC's *in vitro* are generally more powerful antituberculosis drugs than those whose tolerable plasma concentrations in man are only 2 to 4 times greater than their MIC's against *Myco. tuberculosis.*

The MIC's of 2 other drugs, the long-acting sulphonamides sulphadimethoxine and sulphadoxine, have also been determined recently against *Myco. leprae,* using the mouse footpad system (Ellard *et al.*, 1970). Like DDS (half-life about 21 h) these drugs also have relatively long half-lives in man (about 36 h and 1 week respectively). It should therefore be possible to predict with a reasonable degree of certainty the likely efficacy of all 3 compounds in the treatment of human leprosy. The plasma concentrations of sulphadimethoxine and sulphadoxine that are achieved in man at the dosages normally employed are about 4 to 15 times their MIC's against *Myco. leprae* as determined in the mouse footpad system (Ellard *et al.* 1970). Initial treatment with these drugs should therefore be

expected to produce results similar to that achieved with doses of 1 to 100 mg of DDS per day. However, Rees (1967*a*) has suggested that the effectiveness of long-term treatment with doses of DDS averaging about 100 mg per day, doses which produce concentrations of DDS in the body of over 100 times the MIC of DDS against *Myco. leprae,* could be due to the rarity of natural mutants of *Myco. leprae* with these degrees of resistance. If this hypothesis is true, long-term chemotherapy with much lower doses of DDS, or with sulphadimethoxine and sulphadoxine may be less successful, and the possibility of relapse due to the appearance of drug-resistant mutants of *Myco. leprae* after several years' treatment must be entertained.

The fact that the half-life of DDS in the mouse (about 3 h) is much shorter than that in man (about 21 h) may have important implications for experimental studies designed to investigate the potential effectiveness of intermittent DDS chemotherapy in the treatment of human leprosy. Rees (1967*a*) has already demonstrated the effectiveness of once-weekly dosage with 0.01% DDS in preventing the multiplication of *Myco. leprae* in the mouse. Studies on the effectiveness of the treatment of experimental and human tuberculosis with isoniazid given intermittently have shown that the extent to which doses can be spaced out before chemotherapy breaks down is directly related to the half-life of isoniazid in the body. Thus in man, when the interval between the isoniazid doses was extended from 3 or 4 days to 7 days, treatment began to break down in the rapid inactivators (isoniazid half-life about 1 h) but not in the slow inactivators of the drug with an isoniazid half-life of about 3 h (Tuberculosis Chemotherapy Centre, Madras, 1970). Isoniazid has a similar half-life in the guinea-pig to that in human rapid inactivators, and in guinea-pigs intermittent chemotherapy with an equivalent dose of isoniazid also broke down when the interval between the doses was extended from 4 to 8 days (Dickinson *et al.,* 1968). It would therefore seem likely that if a given intermittent regimen was effective in inhibiting the multiplication of *Myco. leprae* in the mouse footpad system, then the corresponding intermittent regimen in man (with same mg per kg dose of DDS) should be at least as effective in the treatment of human leprosy.

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Trial of High Dosages of Dapsone in the Treatment of Tuberculoid Leprosy*

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Two groups, each of 100 patients suffering from tuberculoid leprosy, were treated with different dosages of dapsone, the control group being given a conservative dosage of the drug and the study group a high dosage (1200 mg weekly). The patients in the high-dosage group showed more rapid regression of the disease, a lower incidence of relapse and early regression of the tingling, enlargement and tenderness of the nerve involved. Dapsone in a dosage of 1200 mg weekly was well tolerated by these patients with active tuberculoid leprosy.

Introduction

Although the sulphones are toxic when taken in high doses, dapsone is the drug of choice in leprosy. The use of dapsone (DDS) may be followed by anaemia, which may be due to haemolysis, iron deficiency, or lack of some nutritional factor (Brownlee, 1948). Psychosis is another complication which may be encountered with high doses of the sulphones.

The low toxicity and therapeutic activity of dapsone as demonstrated by McEwen *et al.* (1941) encouraged Cochrane and fellow workers (1949) to use dapsone in the treatment of leprosy. The drug was thereafter given, but generally in low doses and some apprehension still persists regarding its use. However, Chang *et al.* (1952) reported a high safety level in the sulphones. Cochrane (1952) suggested that the basis of their proper use is to start with small doses of dapsone and gradually increase them to the maximum dose. The question that arises is: need dapsone be started in low dosage if the patients tolerate the drug well? If treatment is begun with the maximum therapeutic dose, it is conceivable that the disease would be arrested and cured more rapidly and complications averted, without allowing sequelae to develop.

The aim of the present study was to evaluate the response of patients with tuberculoid leprosy to a higher dosage schedule of dapsone than that conventionally used at present.

Materials and Methods

The 200 patients selected for the trial all had the tuberculoid type of leprosy and one or more infiltrated patches over the face, elbow, or leg, with enlargement

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and tenderness of the regional nerve. They were of similar age-group and of both sexes and, for the purposes of the trial, patients with comparable disease and of similar age and sex were allotted to one of 2 groups of 100 each. In all cases the duration of the lesion was 6 months or more. Routine investigations of the urine and the blood (total white and red blood cell counts, differential count, and haemoglobin estimations) were done before starting treatment, and every 4 months thereafter. The lepromin test was positive in all patients, and examination of skin smears and nasal mucosa smears did not disclose any acid-fast organisms.

The first group of 100 patients was treated as follows: Dapsone, 50 mg, was given twice weekly, with 25 mg increments every 2 weeks until 100 mg twice a week was reached. The dose was then raised by 100 mg every month until 300 mg twice a week was reached. Then 100 mg was given daily except Sunday. Tablets containing iron and a vitamin mixture were prescribed for all patients.

The patients in the second group were given the following treatment: Dapsone, 100 mg twice daily, morning and night (except on Sundays). This dose was continued for 12 months. The same iron-vitamin tablets were given as in the first group. The patient was declared cured when the lesions had completely disappeared, together with nerve tenderness, or when repigmentation was almost complete.

After treatment for 12 months, the patients were examined every 4 months for 2 years, the routine investigations being repeated. The patients were instructed to report at once if they noticed any signs of relapse, or nerve tenderness, or the appearance of a new lesion.

Results

The patients on the higher dosages of dapsone showed marked improvement after taking the treatment for 6 weeks; in all patients, the infiltration in the lesions was the first abnormality to subside; then the erythema disappeared, and last of all pigmentation of the hypopigmented area returned. Nerve tenderness and enlargement diminished more slowly. In all the patients the lesions disappeared in 6 to 9 months. In regard to side-effects, only 9 patients complained of headache, loss of appetite, loss of taste, abdominal pain or anaemia. There was no relapse. In no patient did deformity develop. The urine remained normal throughout. Neuritis did not get worse during treatment.

In the group of patients receiving the conventional dosage schedule, the regression of the disease took longer, and it was only after 3 months of treatment that any improvement could be detected, with decrease in the erythema. The infiltration of the lesions took a year to disappear, and nerve tenderness even longer. Eight patients returned with relapse within 3 to 6 months of stopping treatment, 5 patients could not tolerate the drug, and 4 patients developed wasting of the small muscles of the hand.

In both groups only negligible changes occurred in the blood picture. The urine remained normal. No sensitivity rashes occurred, and no exacerbation of the leprosy.

Discussion

Though dapsone has been used in the treatment of leprosy since 1941, the best dose of the drug is still a matter of dispute. It is generally advocated that the

treatment should begin with a low dose, which is gradually increased until the therapeutic level is attained. Recently the trend has been to give lower doses of dapsone. Leiker and Carling (1966) gave 200 mg, 400 mg and 800 mg of dapsone weekly to 3 groups of patients respectively. None of the patients became bacteriologically negative. The frequency of lepromatous reactions remained unchanged in the patients given 800 mg and 400 mg weekly, while they increased in the group given 200 mg weekly. Though admittedly based on a small number of cases, the study suggests that with small doses of dapsone, the results are in no way better than with larger doses, nor are the reactions in lepromatous leprosy lessened.

In the present study of patients with active tuberculoid leprosy, dapsone was given from the beginning in the high dose of 1200 mg weekly. The drug was well tolerated, and the disease was rapidly controlled. Evidence of toxicity and intolerance to these high doses was found in only 9% of cases.

The advantages accruing from this high-dose regime are the following: (1) More rapid regression of the disease. (2) Rapid improvement in the subjective sensory sensations, such as tingling, and rapid reduction in observable enlargement and tenderness of the nerve involved. (3) Lower incidence of relapse. (4) Reduced incidence of toxicity and intolerance. (5) Less frequent occurrence of deformity.

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Assessment of Bacteriological Changes in Leprosy, Based on Serial Biopsies

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After a discussion of some of the advantages and disadvantages of the smear technique in estimating the bacteriological changes that occur in patients with leprosy, the author then describes in some detail his method of assessing these changes by the examination of sections of serial biopsy specimens. Although the method, which is fully described, is difficult and cannot be regarded as ideal, he concludes nevertheless that the results are far superior to those of assessment based on smears.

Introduction

The bacteriological status of leprosy patients is usually assessed by taking smears. The advantages are that the technique is relatively simple, that sophisticated laboratory facilities are not required, and that several smears from different sites can be taken in one session without too much inconvenience to the patient. If, however, biopsies of a patient suffering from a paucibacillary form of leprosy (whose smears may not reveal any bacilli) are examined, bacilli are not infrequently found in the sections, occasionally in the infiltrates in the corium or subcutaneous tissues, but more frequently in nerve twigs, vessel walls, and in smooth muscle tissues. Such bacilli would rarely be present in the material obtained by the usual technique of taking smears.

It has also been found that the Bacterial Index (BI) in smears from one lesion may differ considerably when the smears are taken by different technicians, and even when smears from the same lesion are taken after a brief interval by the same technician. This observation should not cause surprise, since many variables may vitiate the results. For instance, the total number of bacilli observed depends on the actual amount of dermal tissue obtained and the area on the slide on which the material is spread. The latter can be standardized, but the amount of tissue taken may vary considerably. The latter depends on the length and the depth of the incision, on the number of times the incision is scraped, on the force used, on the shape and sharpness of the knife, and on the physical condition of the tissue. The latter varies from site to site and may change with time (e.g. owing to fibrosis or influenced by a reactional phase).

Variables in staining technique and in assessment of the BI and the Morphological Index (MI) may be avoided by sending the unstained smears to one investigator for processing and examination. Because of the variables in the taking of smears, however, the results of examination of smears from different centres do not appear to be comparable. Many of these disadvantages disappear, or at

least decrease, if the assessment of the bacteriological changes is based on serial biopsies taken from the same lesion.

Technique of Collecting Specimen

A lesion sufficiently large to allow the taking of several biopsy specimens should be selected. If such a large lesion is not present, more than one lesion, all of the same age and clinically similar, should be selected. The successive biopsies are taken from a site adjacent to the first biopsy, just avoiding the scars of previous biopsies. The punch-biopsy method (using a 4- to 6-mm punch) has the advantage that the technique is simple and that several biopsies can be taken from one moderately large lesion. The punches should be sharp, otherwise a considerable part of the small specimen is seriously damaged. The specimen should include subcutaneous tissue and be handled at the outer edges only with a forceps with fine teeth. Fixation in 4% neutral formalin solution (10 times diluted commercial stock solution) is to be preferred to other more complicated fixation techniques because of the smaller chance of error. The risk of damage to the specimen and the costs of mailing (postage) are greatly reduced by using plastic containers (5-ml serum tubes with lid, sealed with tape).

In our laboratory, the Triff method is used for routine staining. Although this method gives excellent results in the hands of experienced technicians, the morphology of bacilli is less readily determined than in sections stained according to the Fite-Faraco-Wade technique. The assessment of bacteriological changes is therefore based on the latter.

One advantage of this method is that there are fewer variables in the taking of biopsy specimens than in taking skin smears; the results of examination of material from different centres by one examiner are thus more likely to be comparable. This consideration is important in view of the increasing shortage, in many centres, of patients suitable for drug trials. A second advantage is that the examination of successive biopsies by an independent assessor provides a check on the selection of patients for trial; in particular, patients exhibiting some borderline features can thereby be excluded from a trial designed to embrace only those with lepromatous leprosy.

Technique of Assessment

The Bacteriological Index is found by counting the numbers of bacilli in a number of fields of infiltrated parts of the section, if the BI is found to be below Grade 5+ (Ridley's decimal scale). The difference between Grade 5+ (100-1000 bacilli per field) and Grade 6+ (more than 1000 bacilli per field) is estimated. Bacilli in globi are not counted, but estimated. Bacilli in bacilliferous macrophages are counted only if the separate bacilli can be clearly distinguished.

The decimal grading system proposed by Ridley has one disadvantage. The highest grade is 6+, meaning 1000 or more bacilli per field. Frequently, the number of bacilli in sections is 10, or even more, times higher than 1000 per field. The difference between 10 000 and 1000 per field is not recorded, and changes in the BI are not apparent until the number of bacilli has decreased to less than 1000 per field. Because the elimination of dead bacilli is a slow process in patients with lepromatous leprosy, one or more years may elapse before any decrease in the BI becomes apparent. Because the number of bacilli in smears is on the average

smaller than in histological sections, this disadvantage is less serious if the BI is based on smears. It may be useful to extend the decimal scale to 10+ (Gaffky), although the grades above 5+ can only be estimated.

The Granularity Index (percentage of granular bacilli) is found by examining the morphology of 100 to 200 bacilli, excluding those in globi, but including those in bacilliferous histiocytes if the individual bacilli can be clearly distinguished.

Because it is more difficult to determine the morphology of the bacilli in sections as compared with smears, 3 categories of bacilli are distinguished: (1) Bacilli that are completely and evenly stained are recorded as intact (I); they are regarded as viable. (2) Bacilli that are somewhat unevenly stained, but are not yet granular, are recorded as fragmented (F); this is seen to be a mixed group of viable and non-viable bacilli, since growth in the mouse footpad has been obtained from biopsy specimens containing no intact bacilli but about 10% fragmented bacilli. (3). Bacilli that appear as granules are recorded as granular (G); such bacilli are regarded as non-viable. Actual counts are made of the percentages of the 3 categories of bacilli, using a tabulator similar to those used for differential counts of white blood cells. The Morphological Index corresponds to some extent, but not completely, with the total of intact and fragmented bacilli. In order to express the bacteriological status of the patient in one figure, the Granularity Index is preferred.

Discussion

Because it is more difficult to assess the BI and the morphology of the bacilli on sections as compared with smears, the method cannot be regarded as an ideal one. In our hands, however, the method was found to be far superior to the assessment based on smears alone. It has been found that a technician is able to achieve uniform and dependable results after some months of experience. Control is by counting blindly a number of sections for a second time after an interval of a week or two. The results of a second technician were not accepted for trial work until his counts in a series of sections were comparable with those of the first technician. Unexpected changes in the MI or the morphology of the bacilli were always checked; with few exceptions, the findings of a trained technician were confirmed. The results of blind assessment of thousands of sections in the past years have been very consistent, which is regarded as evidence of the relative reliability of the method.

It has been found that the proportion of completely intact bacilli is higher in smears as compared with sections of the same lesion from the same patient. It is probable that the staining properties of the bacilli are affected by the more elaborate processing technique of preparing sections. Because the error is constant and in the same sense, the reliability of the method for assessing bacteriological changes is not impugned.

It has been objected that valid conclusions cannot be drawn from the examination of only one biopsy specimen. And it is admitted that the BI in lesions of different age or from different sites of the body may indeed differ significantly.

In this respect a series of smears undoubtedly gives a more accurate picture of the BI. This disadvantage may to some extent be reduced by selecting a fairly advanced lesion for biopsy examination. Much more important than the BI is

usually the morphology of the bacilli. The examination of one biopsy specimen gives a reasonably accurate picture of the morphological status of the bacilli of the whole patient, since the changes in bacillary morphology in the various lesions of the skin and the mucosa are very similar. There is one exception however. The pattern may be different in different lesions of patients during an exacerbation or a relapse of the disease, for example, due to the development of drug resistance.

In spite of some disadvantages, the method described is regarded as the most satisfactory for the assessment of the bacteriological effect of treatment, and in particular for drug trials.

Lepr. Rev. (1971) **42**, 125-130

Bacteriological Effect of Lamprene (Clofazimine) in Lepromatous Leprosy*

(Report of one year's treatment of 44 patients with 100 mg of Lamprene daily)

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The treatment of 44 lepromatous patients with 100 mg of clofazimine (Lamprene, Geigy) daily resulted in a rapid increase in the percentage of granular bacilli during the first 3 months of treatment, reaching 94% after 6 months of treatment. The bacterial index decreased in 1 year from 5.4 to 4.5. Three patients responded rather slowly. In only 1 patient was there no bacteriological improvement after 1 year of treatment. It is concluded that clofazimine (Lamprene) is an effective, rapidly-acting drug in lepromatous leprosy, and that the bacteriological effect is comparable with that of sulphones.

Introduction

The bacteriological effect of giving 100 mg of clofazimine (Lamprene) daily to patients with lepromatous leprosy has been investigated by a group of workers in different centres. All patients were classified on clinical and bacteriological grounds as lepromatous; patients with borderline features were excluded. This

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TABLE 1

Bacteriological progress in 44 lepromatous patients treated with 100 mg of Lamprene daily, showing Bacterial Index (BI) and percentage of bacilli intact (I), fragmented (F) and granular (G)

A. *Reactive patients* (18)

Biopsy / Patient	Onset				3 months				6 months				9 months				12 months			
		Percentage				Percentage				Percentage				Percentage				Percentage		
	BI	I	F	G	BI	I	F	G	BI	I	F	G	BI	I	F	G	BI	I	F	G
793	4	4	34	62	3	0	3	97	2	0	0	100	4	0	7	93	3	0	4	96
1417	4	3	40	57	3	0	2	98	3	0	0	100	3.5	0	5	95	4	0	6	94
1432	5.5	2	24	74	3	0	1	99	4	0	0	100	5	0	3	97	4	0	3	97
1378	4	0	21	79	2	0	5	95	3	0	4	96	2	0	3	97	2	0	0	100
1392	4.5	2	47	51	4	0	0	100	3	0	5	95	3.5	0	1	99	4	0	1	99
1049	3.5	0	26	74	2	0	1	99	3	0	6	94	3	0	0	100	3	0	6	94
1331	6	9	85	6	5	0	6	94	4.5	0	1	99	4.5	0	3	97	5	0	6	94
2073	5.5	0	32	68	5.5	0	4	96	4	0	10	90	3	0	16	84	2	0	7	93
14/67	4.5	0	23	77	6	0	31	69	6	0	6	94	5.5	0	2	98	5.5	0	2	98
32/67	6	3	36	61	6	0	2	98	6	0	1	99	6	0	6	94	5.5	0	30	70
94/67	5	8	46	46	5	0	20	80	5	0	3	97	5	0	4	96	5	0	0	100
102/67	6	3	75	22	5	0	11	89	6	0	3	97	6	0	15	85	6	0	0	100
7/68	4.5	0	28	72	5	0	29	71	4	0	1	99	5	0	0	100	4	0	3	97
LT	4	0	38	62	4,5	0	19	81	5	0	4	96	4	0	1	99	2	0	1	99
Z	6	3	90	7	6	0	28	72	6	0	16	84	6	0	3	97	4.5	0	11	89
S	5.5	3	26	71	5	0	8	92	5	0	6	94	4.5	0	3	97	4	0	0	100
LS	6	1	45	54	6	0	35	65	6	0	15	85	6	0	9	91	6	0	3	97
R	6	0	64	36	5	0	32	68	4	0	0	100	4	0	2	98	3.5	0	3	97
Average	5.0	2	44	54	4.5	0	13	87	4.3	0	5	95	4.5	0	5	95	4.1	0	5	95

Table 1 continued

B. *Non-complicated patients* (26)

2252	6	0	41	59	4	0	11	89	6	0	5	95	4	0	16	84	4.5	0	8	92
2254	6	0	22	78	6	0	20	80	6	0	11	89	5	0	11	89	6	0	3	97
2255	5	0	41	59	4.5	0	7	93	4.5	0	2	98	4	0	4	96	4.5	0	11	89
2262	6	1	36	63	6	0	18	82	5	0	11	89	3	0	7	93	1	0	0	100
2263	6	0	26	74	6	0	21	79	6	0	7	93	4	0	8	92	5	0	1	99
2265	5	1	38	61	6	0	12	88	6	0	3	97	5	0	4	96	4	0	0	100
2269	6	1	22	77	5.5	0	23	77	5.5	0	13	87	5	0	30	70	3.5	0	4	96
2270	5.5	0	35	65	6	0	16	84	5.5	0	1	99	4	0	9	91	4	0	1	99
45/67	6	4	60	36	6	0	5	95	5	0	2	98	5.5	0	2	98	5	0	8	92
80/67	6	0	28	72	5.5	0	12	88	6	0	6	94	6	0	3	97	6	0	9	91
85/67	5.5	2	66	32	5.5	0	9	91	5	0	8	92	5	0	5	95	4.5	0	12	88
93/67	6	1	38	61	6	0	7	93	6	0	0	100	6	0	0	100	6	0	0	100
138/67	6	2	43	55	6	0	12	88	5	0	2	98	5.5	0	0	100	4	0	1	99
151/67	6	3	61	36	6	0	1	99	5	0	1	99	6	0	2	98	6	0	4	96
V	5	2	75	23	4.5	0	17	83	6	0	6	94	5	0	4	96	5	0	2	98
P	6	0	50	50	6	0	31	69	6	0	11	89	6	0	28	72	4.5	0	53	47
L	5	1	64	35	5	0	24	76	5	0	28	72	5	0	32	68	5	0	26	74
BR	6	0	40	60	6	0	21	79	6	0	2	98	6	0	3	97	6	0	3	97
FO	6	4	50	46	6	2	27	71	6	0	4	96	6	0	6	94	5	0	5	95
FN	6	1	44	55	6	2	61	37	6	0	40	60	5	0	18	82	5	0	10	90
BO	5	3	52	45	6	0	19	81	5	0	2	98	5	0	2	98	5	0	1	99
478	6	8	86	6	4	0	4	96	6	0	0	100	4	0	2	98	4	0	1	99
1234	6	0	44	56	6	1	82	17	6	0	9	91	6	0	2	98	6	0	1	99
2739	4	1	54	45	5	0	3	97	5	0	7	93	3	0	4	96	3	0	6	94
2755	5	1	71	28	6	0	39	61	6	0	2	98	5	0	1	99	6	0	0	100
951	6	0	72	28	4.5	0	36	64	6	0	1	99	6	0	1	99	5	0	0	100
Average	5.6	1.5	49	50	5.5	1	24	75	5.6	0	7	93	5.0	0	8	92	4.7	0	7	93
Total average	5.4	2	46	52	5.1	0.1	18	82	5.1	0	6	94	4.8	0	6	94	4.5	0	6	94

report deals with 44 patients who at the beginning of the trial had a minimum Bacterial Index (BI) of 4+ (Ridley-Cochrane scale) and at least 20% solid staining bacilli. The patients were treated for 12 months with 100 mg of clofazimine daily, and this treatment, with few exceptions, was continued during periods of reaction.

The patients are divided into 2 categories: Group A (18 patients) had shown repeated severe reactions in the year prior to the beginning of treatment with clofazimine, while Group B (26 patients) consisted of patients who were not suffering from any complications.

Assessment

The bacteriological evaluation was based on the examination of serial biopsies, taken at the beginning of the trial and thereafter at 3-monthly intervals. In each patient the biopsy specimens were taken from the same lesion. All biopsies were processed in one laboratory and were examined by the same investigator, who, at the time of examination, did not know the bacterial counts of previous biopsies.

With this method of assessment, it is considered justifiable to include in one trial patients from several centres. Such a course would, in our opinion, be impracticable if the assessment was based on the examination of smears, because of differences of technique in different centres. A double-blind trial was not considered appropriate or necessary, partly because of lack of suitable patients, but also because the deposit of clofazimine in the infiltrates is easily recognizable in sections.

Results

The bacteriological effect of clofazimine on the groups of patients is shown in Figs 1 and 2. The effect on the individual patients is shown in Table 1.

Figure 1 shows that there is little difference between the 2 groups in the rates of increase in the percentage of granular bacilli and in the decrease of the bacteriological index. In both groups a rapid increase in the percentage of granular bacilli is seen in the first 3 months of treatment. The rate decreases in the second 3 months, while after 6 months, about 95% of the bacilli have already become granular. Further increase in this percentage is very slow.

The table shows that this result is applicable to the majority of patients. Out of 44 patients, only 4 showed a slower response to treatment (Group B: 2269, P.L.F.). Only 1 patient (P) did not show improvement after 1 year of treatment with clofazimine. This patient had advanced diffuse lepromatous leprosy. Failure cannot be ascribed to lack of intake or absorption of the drug, since the skin became markedly pigmented.

Occasionally fluctuations in the percentage of granular bacilli have been seen in this and in similar trials. These sections were re-examined in order to exclude possible errors in counting, but it was found that the counts were generally correct. It was not possible in most cases to establish whether the temporary increase in the percentage of fragmented bacilli was to be ascribed to a temporary increase of viable bacilli or to the fact that the biopsies were taken from a different part of the same lesion. Because the increase in fragmented bacilli was not accompanied by an increase in completely intact bacilli, the second possibility is the more likely.

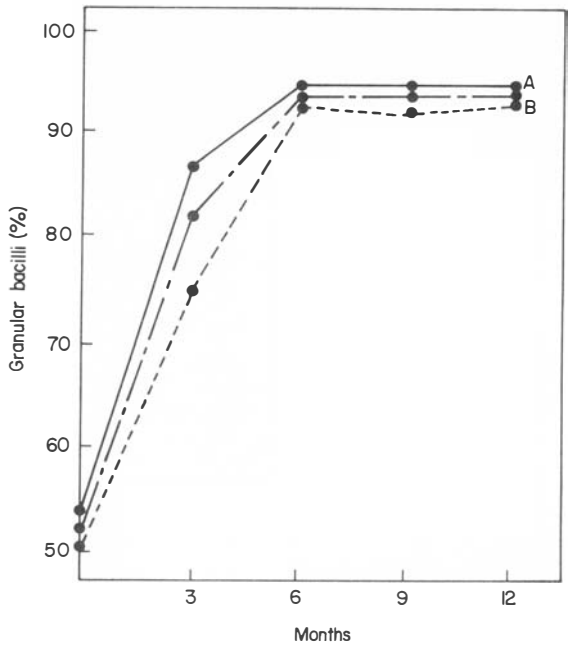

Fig. 1. Percentage of granular bacilli in 44 lepromatous patients treated with 100 mg of Lamprene (clofazimine) daily. – – – – Reactive patients A. ———— Uncomplicated B. – – – – – – All patients.

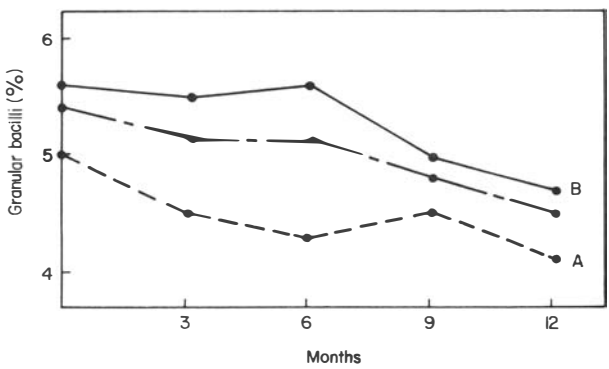

Fig. 2. Bacterial Index in 44 lepromatous patients treated with 100 mg of Lamprene (clofazimine) daily. – – – – Reactive patients A. ———— Uncomplicated B. – – – – – – All patients.

The decrease in the bacteriological index (Fig. 2) seems to be relatively slow. It should be kept in mind, however, that a high proportion of the patients started the trial with a BI of 6+ (indicating 1000 or more bacilli per microscopic field). Only after the number of bacilli has decreased below 1000 per field is a decrease in BI evident in this notation. The fall in the BI from 5.4 to 4.5 after 1 year of treatment is about the same as that found in patients treated with sulphones.

It is concluded that clofazimine (Lamprene) is a rapidly acting drug in leprosy and that the bacteriological effect of treatment is comparable with that of the sulphones.

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The Blinding Lesions of Leprosy

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Introduction

Estimates of the frequency with which the eyes are affected by leprosy have varied considerably in reports from widely separated places during the century or so in which the nature of the disease has become more clearly apparent. Climate, race and skin pigmentation are prominent among the factors which have been thought possibly to be responsible for these differences, and there can be no doubt of course that ocular involvement is everywhere seen more frequently among patients with lepromatous or near lepromatous leprosy (Choyce, 1969; Hobbs, 1971). Another factor which appears to us to be of probable importance in giving rise to these differences is the method of ocular examination employed.

Some workers fail to indicate how their observations were made; others have used a magnifier, but in diffuse light when definition is less good. Only a minority have been able to employ biomicroscopy with the slit-lamp microscope and, not surprisingly, the incidence of ocular lesions detected by them has been higher than in others examined by less critical methods (Beretti and Cahuzac, 1970). With this instrument the detection of early ocular lesions with ease and certainty has become routine practice in the ophthalmic clinic. Realization of the increased gravity of eye complications, especially in India and the East, makes it highly desirable that the most accurate means of diagnosis should be available where it is most needed, that is, in the leprosarium.

Two types of ocular lesion, in general very different in their clinical presentation, can be distinguished as the causes of the vast majority of blindness attributable to leprosy. The first type comprises chiefly conditions involving the superficial tissues of the eye and eyelids. Lagophthalmos from involvement of the facial nerve in the tuberculoid form of leprosy is the commonest. Impaired corneal sensation, due to trigeminal nerve damage, frequently aggravates the effects of inadequate corneal protection from this cause; but whether or not this is the case, exposure keratitis with vascularization and opacification lead to loss of vision. Intrinsic corneal lesions—corneal lepromata or interstitial keratitis occurring either alone or in association with other ocular disease—are also to be included in this group, the common characteristic of which is the fact that the abnormalities are apparent to the naked eye. Iritis, when it occurs with severe

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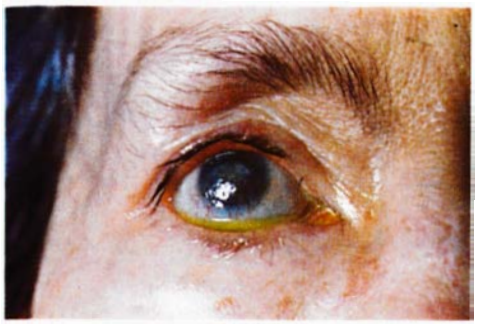

Fig. 1. A mature, complicated cataract resulting from long-standing iritis in a patient with inactive lepromatous disease. There is no history of ocular pain, no ciliary injection and the posterior synechiae which are present are invisible to the naked eye. Vision is reduced to the ability to perceive light only. (Coincidental lagophthalmos and exposure keratitis also.)

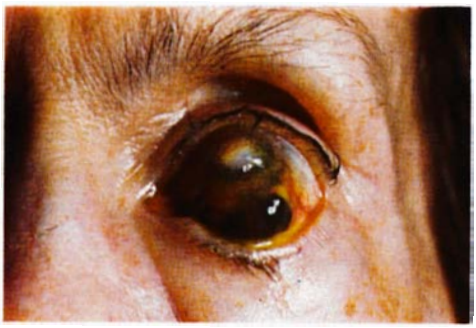

Fig. 2. The sightless, painless, degenerating fellow-eye of the patient depicted in Fig. 1. Total synechiae have been followed by secondary glaucoma and then by thinning and degeneration of the ocular coats. Herniation of the ciliary body ("ciliary staphyloma") has finally resulted in the swelling seen below the cornea. (The eye is directed upward.)

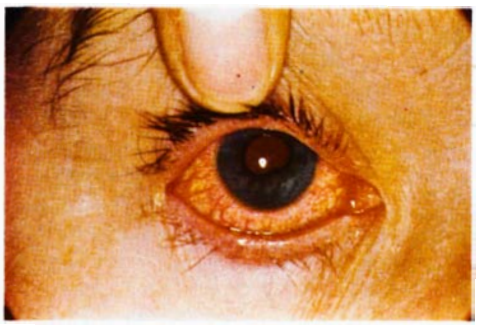

Fig. 3. Acute iritis in a patient in reaction. Severe pain, with intense ciliary injection and diminished vision, attract the attention of patient and doctor at once. The pupil is partly dilated with atropine and a posterior synechia—invisible until then—is seen. The slit-lamp microscope displayed a dense aqueous flare.

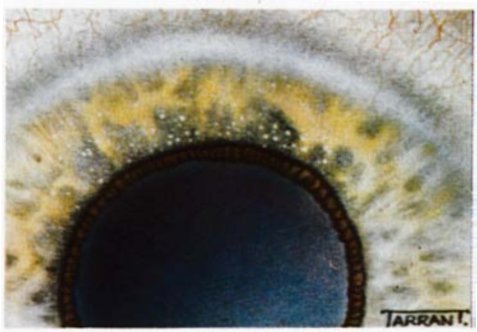

Fig. 4. Iris "pearls" in a patient with controlled lepromatous leprosy who was visually symptomless. Unless they are accompanied by other evidence of ocular disease these remarkable deposits may remain for long periods without giving rise to complications which threaten sight.

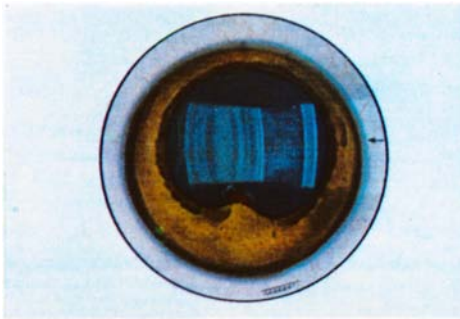

Fig. 5. The anterior chamber of a patient with controlled lepromatous leprosy as seen by slit-lamp microscopy. The sole ocular symptom was vague discomfort; vision was unimpaired and ciliary injection minimal. Nevertheless, iritis of considerable severity is present, since the slit-beam displays dense turbidity of the aqueous from the numerous leucocytes exuded into it from the surface of the inflamed iris. ("Aqueous flare".)

Fig. 6. The slit-lamp microscope picture of the anterior chamber of a normal eye (the "optical section"). The clear aqueous reflects no light from the slit-beam into the observer's eye and hence the interval between cornea and lens appears dark—"optically empty".

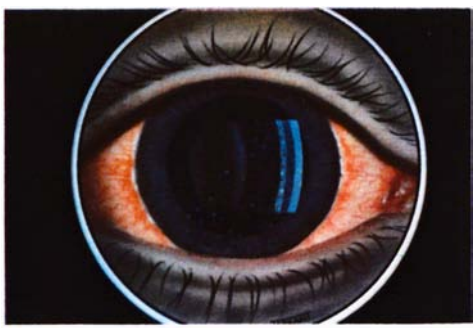

Fig. 7. The optical section in iritis. In this case not only is there aqueous flare, but from the masses of leucocytes in the aqueous many have been deposited as clumps upon the posterior surface of the cornea and are seen as whitish dots—so-called "keratic precipitations" or "K.P."

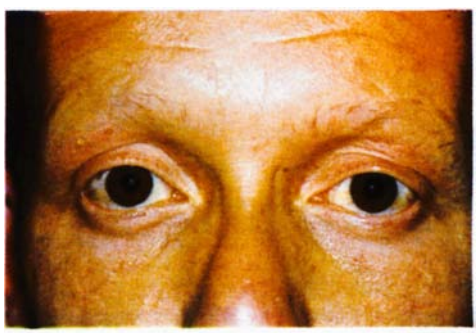

Fig. 8. The eyes of a patient aged 14 who had been under treatment for lepromatous leprosy for some 8 years and in whom, 6 years previously, the condition depicted in Fig. 5 had been detected. Following continuous general and ocular treatment he retains excellent vision and shows no sign of ocular activity.

congestion, pain, and loss of vision (as in the lepra reaction, for example (see Fig. 3)) may be included in this group; and iris pearls and lepromata may also be large enough to attract the attention of the naked eye. Treatment in the majority of these patients can be very effective when they present early to the leprologist, and it is perhaps for this reason that they appear to be making a smaller contribution to the total of those blinded by leprosy.

Detailed analyses of large series of the leprotic blind are few; but recent opinions suggest that the greater proportion of cases of blindness arise from causes in the second group. These consist of purely endogenous ocular lesions—usually anterior uveitis (iritis or iridocyclitis) of an insidious type which, unless it becomes complicated by secondary cataract (Fig. 1) or ciliary staphyloma (Fig. 2), is likely to remain invisible to the naked eye. Certainly, in its early stage when it is readily amenable to simple treatment, naked eye signs are absent (Fig. 5); the muted sensations of the leprotic eye cause the patient little in the way of discomfort in an eye which may appear almost white, and the turbidity of the aqueous humour, the sole observable abnormality at this stage, offers little or no hindrance to vision.

Bull and Hansen (1873) first drew attention to this insidious form of leprotic iritis which they found occurring "without violent symptoms" and often with "exudations around the borders of the pupils and adhesions to the capsule of the lens in patients who have not complained of pain or derangement of sight". Such signs were observed by them in some 30% of their patients. Thereafter the condition seems to have passed largely unnoticed until the development of the slit-lamp microscope provided the ophthalmologist with a diagnostic tool on which he could rely, *inter alia,* for the detection of the earliest signs of uveal inflammation: i.e., the exudation of leucocytes into the normally clear aqueous of the anterior chamber (Figs 6 and 7). Now, with opinions based upon this evidence, ophthalmologists with experience of leprosy see the rôle of this type of uveitis in the production of blindness more clearly. Kirwan (1955) refers to it as "the commonest cause of blindness in leprosy"; Choyce (1964) as "responsible for most of the blindness", and Weerekoon (1969) as "the cause *par excellence* of blindness" in leprosy patients. In contrast to the first group of causes its association is predominantly with the lepromatous or near lepromatous forms of the disease.

Personal Observations

In the ophthalmic clinics of the Hospital for Tropical Diseases, London, and the Hospital and Homes of St. Giles at East Hanningfield in Essex, we detect the early signs of insidious uveitis, sometimes associated with active systemic disease. Not infrequently, however, signs of the latter are absent, skin biopsies are negative, and the disease, on these grounds, would be looked upon as controlled, if not cured. In both instances, however, local treatment with atropine and hydrocortisone drops, as well as any general treatment dictated by the systemic disorder, is needed urgently and when so applied is consistently effective in preventing the blinding complications of secondary cataract and ocular degeneration (Hobbs, 1963). However, unless a leprosarium is equipped with a slit-lamp microscope and the need to employ this in the routine examination of all patients with active disease is realized, these early signs will be overlooked, so

that complications and visual loss will gradually develop. Treatment, if at this stage still possible, may then involve complex surgical procedures.

In these circumstances—evidence, ancient and modern, of the importance of insidious iritis in leprosy and of its clinically "silent" presentation, with the earliest diagnostic signs visible only on bio-microscopy—it appeared at least possible that, in the field, a proportion of patients with signs of iritis might exist unsuspected, and that patients with the complications of the condition might be found to contribute notably to blindness among sufferers from leprosy. The fact that the condition, in its early active stage, had been seen to be readily amenable to simple treatment rendered the problem the more interesting, with its indication of potential therapeutic rewards.

The Sungei Buloh Survey

One of us, therefore (H.E.H.), has recently undertaken a brief survey in the large leprosarium at Sungei Buloh in Malaysia with the primary object of detecting signs of iritis—active, healed or complicated—and of estimating the frequency with which the complications resulted in blindness. The results of this study have been reported in detail elsewhere (Hobbs, 1971).

The table below summarizes the important ocular observations in the series of 507 patients examined.

TABLE 1

Ocular lesions and blindness at Sungei Buloh

No. of patients examined: 507	male 297 female 210
Incidence of ocular lesions of all types[a]	32.5%
Percentage of leprotic eye lesions in the total[b]	50%
Percentage of leprotic eye lesions due to iritis (males 54%, females 44%)	50%
Total no. of blind patients	36 (7.1% of sample)
Blind from leprotic lesions	18 (50% of total)
Blind from leprotic iritis	11 (61% of leprotic lesions)

[a] Pterygium, senile cataract, primary glaucoma and leprotic lesions.

[b] Lagophthalmos, exposure and intrinsic keratitis, corneal leproma, iridocyclitis and its complications.

In the majority of patients the disease was under control and the signs of iritis were those of the old healed or complicated condition, evidence of activity being noted in only a few cases. Such signs were absent in patients below the age of 30, but thereafter the incidence increased from 4.9% in the 30-39 age-group to 11.7% in the 70+ age-group. No complications were seen in patients under the age of 40, but later age-groups showed an incidence of some 7% to 10%. The incidence of blindness from all causes rose steadily from 1.5% in the 30-39 age-group to 22% in the 70+ age-group, but that due to leprotic iritis showed a maximum incidence (6% in the 60-69 age-group) at a slightly earlier age. The known association of lepromatous leprosy with iritis was confirmed, 35 out of a total of 39 cases of iritis (90%) occurring in lepromatous patients. No relationship could be found

between the duration of treatment and the presence of signs of iritis. Choyce's (1970) figures also demonstrate the virtually exclusive association of leprous iridocyclitis (and keratitis) with lepromatous and borderline leprosy.

From these findings it is clear that a large proportion of ocular involvement in this group of patients is as iritis, as has been increasingly emphasized (Somerset, 1962; Kirwan, 1955; McKie Reid, 1966; Weerekoon, 1969; Choyce, 1964; Beretti and Cahuzac, 1970). That in the Sungei Buloh series the iritis should have resolved spontaneously or been controlled by systemic treatment without local ocular measures is probably no more than fortunate, given the general tendency for the condition to relapse and become complicated in its later stages. The complications observed—secondary cataract, secondary glaucoma, ciliary staphylomata and phthisis bulbi—are such as would be expected to follow untreated iritis, whatever its cause. The contribution which these complications make to blindness in leprosy is evidently a considerable one.

Conclusions

The treatment of early leprotic iritis presents difficulties in only a minority of cases—principally those in which episodes of acute exacerbation attract attention during a "reaction", or in which massive destruction of the iris by a localized leproma occurs. In the important group of cases in which insidious iritis leads gradually to loss of vision and eventual blindness, it is early diagnosis which is needed to interrupt this silent and sinister sequence; and for this, the naked eye is insufficient. The modern equivalent of Bull and Hansen's "focal light" and "magnifying glass", i.e. a loupe and lens, or the Hobbs illuminated slit-loupe (Hobbs, 1963) may elicit early signs in practised hands; but for the ophthalmologically inexpert an up-to-date slit-lamp microscope is the most certain way of demonstrating unequivocally the signs of early uveitis.

The need for more expert ophthalmological advice and skill in dealing with problems of potentially blinding leprotic ocular conditions has been stated on many occasions, recently and notably at the Ninth International Leprosy Congress in London in 1968. This need is perhaps emphasized by the high incidence of ocular lesions of various types observed in the Sungei Buloh series, a large proportion of which would now be remediable only by surgery. Our aim here, however, is to draw attention to the at least equally important prophylactic rôle of ophthalmic medical treatment in treating leprotic iritis and preventing its blinding complications. Early and accurate detection of exudation into the anterior chamber from the inflamed iris is the essential preliminary diagnosis here, and in the modern slit-lamp microscope the means to do this are available. It has been suggested that full ophthalmological training is necessary for the use of this invaluable diagnostic tool but, whilst in no way wishing to decry the clinical value of the sophisticated techniques which may be developed in the use of the instrument, we should like to emphasize our belief that for the purpose in mind—primarily the early diagnosis of iritis—only a short course of instruction by a trained observer is necessary.

The installation of these instruments at strategically sited centres where suitable personnel could be trained to make regular surveys of infected individuals and so detect insidious leprotic iritis in its early remediable stage, is, in our opinion, a necessary and important measure in the prevention of blindness in leprosy.

Acknowledgement

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Lepr. Rev. (1971) **42**, 139–148

Abstracts

1. **Odontodysplasia leprosa in Danish mediaeval skeletons**, by K. DANIELSEN. *Tandlae Gebladet* 1970, **74**, 603-625.

This interesting and important paper describes the osteological, and particularly the peri-oral and dental changes found during the examination of about 1000 skeletons from 4 mediaeval Danish leprosy cemeteries. In addition to confirming the now accepted criteria for bony damage attributable to advanced low-resistant leprosy (atrophy of the anterior nasal spine; atrophy and recession of the maxillary alveolar margin, with perhaps loss of the upper central incisors; inflammatory changes on the upper surface of the hard plate), the author found specific changes in the teeth. Thus, in children developing low-resistant leprosy during the first decade of life, characteristic malformations of the permanent maxillary incisors were noted, the tooth diameters being suddenly and concentrically reduced and the pulp cavities showing a similar constriction. Less pronounced changes were also present in the mandibular incisors, and other teeth.

The changes observed are thought to be developmental disturbances occurring during the rapid progress of multibacillary disease in children who subsequently died young. The paper is excellently illustrated with photographs and X-ray pictures of the teeth and affected bones. Clinicians will henceforth be on the lookout for similar changes in young living subjects affected by severe lepromatous leprosy.

S. G. Browne

2. **A propos d'un type de lésion nerveuse non-spécifique chez les lépreux (Report of a type of non-specific nerve lesion in leprosy patients)**, by A. CARAYON, J. LANGUILLON, R. CAMAIN and P. BOBIN. *Bull. Soc. Med. Afr. Noire Lang. Fr.,* 1970, **15**, 186-91.

The authors draw attention to a type of nerve damage in leprosy that appears to be different from the 2 main kinds that have long been recognized, these being destruction of the nerve either by epithelioid infiltrate or by lepromatous granuloma. In one of their 2 patients, in whom clinical and surgical studies were supplemented by arteriographic and histological investigations, the evidence suggested some kind of antigen-antibody reaction. Two alternative mechanisms appear to be possible: either the unusual liberation of normal antigenic constituents from the body cells, or the liberation of extraneous antigens (derived from dead *Myco. leprae*), which stimulate the host to produce antibodies not only against the micro-organisms themselves, but also against the host's own tissues—a suggestion earlier made by Browne (*Int. J. Lepr.,* 1965, **33**, 881).

S. G. Browne

3. **Leprosy in Ceylon**, by L. GREENFIELD. *Illinois Med. J.,* 1970, **138**, 87-91.

The author gives a brief summary of the leprosy situation in Ceylon, based on official reports and his own observations. The number of patients under treatment in the population of 12 million is about 4300, of whom 850 (including 150 females) are in the Hendala Leprosy Hospital, and 150 in Batticaloa.

Through the Social Service Agency, the government gives an allowance of 20 rupees a month to the families of patients with infectious forms of leprosy, and 50 rupees a month to patients

after discharge from 1 of the 2 leprosaria, a sum that is insufficient to encourage a desire to leave the sheltered life of the leprosarium. Out-patient treatment is provided at 9 leprosy clinics and at a "Special Skin Clinic" at the Colombo General Hospital.

The author refers to an apparent lack of concern about leprosy in the Health Ministry, and suggests that serious efforts to control the disease have yet to be made. The social stigma against leprosy remains strong, and physicians lack both knowledge of the disease and desire to co-operate in its detection and management.

S. G. Browne

4. **The surgical treatment of lower facial palsy in leprosy,** by J. B. A. VAN DROOGENBROECK. *Ann. Soc. belge Méd. trop.,* 1970, **50**, 6, 653-88.

Plastic and reconstructive surgeons will find much to interest and instruct them in this well-illustrated and well-documented account of the author's experiences in dealing with the diverse combinations of paralysis of the facial muscles as they occur in patients in Korea. Both upper and lower facial palsies, unilateral or bilateral, and even complete bilateral facial palsies, are by no means uncommon in the Far East. The author's principal surgical attack on these problems is the use of either the temporalis or the masseter muscle, with suitable sling operations for the sagging face.

S. G. Browne

5. **Ocular leprosy in Uganda,** by V. P. EMIRU. *Br. J. Ophthal.,* 1970, **54,** 11, 740.

The author reports the incidence and type of ocular lesions among 890 patients in the main leprosaria of Uganda. Examinations were made by direct inspection in sunlight, with visual acuity estimation and the use of an x10 loupe. The slit-lamp microscope was not employed and fundus examinations were possible in only a proportion of the cases.

Madarosis was the commonest lesion observed in this way (8.2%), while chronic iridocyclitis (3.1%) was the most serious. Exposure keratitis was uncommon (incidence not stated) although lagophthalmos was seen in 5.6% of cases, 4 patients were totally blind, and 12 were blind in 1 eye.

H. E. Hobbs

6. **Drug potentiation of macrophage function,** by MARTIN J. CLINE. *Infection Immunity,* 1970, **2**, 601.

By subjecting cultured leucocytes containing ingested living *Listeria* to various concentrations of oxygen and of clofazimine (B 663, Lamprene (Geigy)), the author shows that in aerobic conditions clofazimine potentiates bacterial killing by the macrophages. The potentiation observed corresponds to concentrations of the drug that are obtained in man when therapeutic doses are given. Even serum concentrations that are not high enough to be directly injurious to the organism used, are somehow rendered bactericidal when the macrophages take up the drug. Clofazimine increased the oxygen consumption of the leucocytes; this increased utilization was not infiltrated by potassium cyanide, an observation that suggests that the mechanism is independent of the mitochondrial system.

S. G. Browne

7. **Lepromatous leprosy and Australia antigen, with comments on the genetics of leprosy,** by B. S. BLUMBERG, L. MELARTIN, R. GUINTO and M. LECHAT. *J. chron. Dis.,* 1970, **23**, 7, 507-16.

The intriguing observation that Australia antigen (Au (1)) is associated with the occurrence of lepromatous leprosy, is further investigated in the studies reported in this paper from Cebu (Philippines).

The serum findings in a larger group of patients confirm the initial conclusions that Au (1) is found in a significantly higher proportion of patients with lepromatous leprosy than in those with tuberculoid leprosy or in the general population; that males have a higher proportion than females; and that the age-group showing the highest frequency is 6 to 9 years.

The significance of these observations is discussed, and the hypothesis advanced that the postulated gene (which in double dose confers increased susceptibility to hepatic virus infections) may also confer susceptibility to other chronic infections, including leprosy. If this is the case, lepromatous leprosy would follow a pattern of autosomal recessive inheritance in areas of high leprosy prevalence. Individuals carrying this gene would, if exposed to leprosy infection, be more likely to develop the lepromatous type of the disease than those not carrying the gene. Susceptibility is not on these grounds absolute, but such factors as age and sex and possibly other genes are also involved.

S. G. Browne

8. **Dapsone and peripheral motor neuropathy**, by A. C. SAQUESTON, A. L. LORINCZ, N. A. VICK, and R. D. HAMER. *Arch. Derm.*, 1969, **100**, 214.

The authors report 2 cases of severe peripheral neuropathy apparently induced by dapsone, and followed by complete clinical recovery after discontinuance of dapsone treatment. In the first case, the 20-year-old male patient was suffering from pyoderma gangrenosum, and the daily dose of dapsone reached 400 mg (given for 10 days): a total of 26 g of dapsone in 2 months. The other patient, a 17-year-old boy, had widespread and long-standing acne conglobata, and he received dapsone in a dosage of up to 350 mg daily (given for 5 weeks)—a total of 48 g over 5 months.

Both patients experienced signs of distal motor weakness about 3 months after the beginning of dapsone treatment. The first patient had signs of ulnar nerve damage and severe symmetrical foot-drop; in the second patient, the damage was confined to the lower limbs.

The mechanism of toxic peripheral neuritis in these instances probably differs from that of the neuritis of leprosy, whether occurring spontaneously or apparently induced by dapsone.

S. G. Browne

9. **Agranulocytosis due to dapsone,** by A. J. OGNIBENE. *Ann. intern. Med.,* 1970, **72,** 521-24.

The author reports 16 cases of agranulocytosis with 8 deaths (mainly from *Pseudomonas* septicaemia) occurring in perhaps 200,000 American servicemen taking dapsone as a malarial prophylactic in Vietnam. Apparently no soldiers had developed agranulocytosis while taking chloroquine-primaquine once weekly, or during standard treatment of falciparum malaria with quinine, pyrimethamine and dapsone.

All the 16 patients had been taking 25 mg of dapsone daily, for periods ranging from 3 weeks to 3 months before the onset of signs of agranulocytosis, and 1 of them had taken no antimalarial medication apart from dapsone. Leucopenia is said to be common during dapsone prophylaxis for malaria (and, in fact, its appearance has been taken as an indication for stopping dapsone), but these are the first cases of agranulocytosis to be reported in association with dapsone prophylaxis.

On the evidence submitted in the paper, it is not possible definitely to incriminate dapsone as the sole and sufficient cause of the observed signs: other drugs, including antimalarials, may have played a determining rôle. Furthermore, the diagnosis in some of the cases would not appear to be convincingly established; for example, 2 patients had a "marked leucocytosis" on the third day, 1 of them with 28,000 white blood cells per mm^3. Another patient also suffered from anaemia due to glucose-6-phosphate dehydrogenase deficiency, possibly precipitated by dapsone.

While most leprosy-control schemes, in which many thousands of patients take the equivalent of at least 25 mg of dapsone daily, do not have the kind of medical supervision

available to the American armed forces in Vietnam, it is arguable that if agranulocytosis were a real hazard of dapsone treatment, some few cases at least would have been reported from one of these control schemes.

The sensitization of the bone marrow to the effect of toxic drugs, or the summation of potentiation of their effects, is however always a possibility. Leprosy workers should maintain a watching brief for suggestive signs and symptoms, and investigate and report any occurrence of unusual toxic phenomena associated with, and possibly attributable to, dapsone.

S. G. Browne

10. **Pseudoleukaemia during recovery from dapsone-induced agranulocytosis,** by P. H. LEVINE and L. R. WEINTRAUB. *Ann. intern. Med.,* 1968, **68**, 1060-1065.

The authors report the case of a patient with agranulocytosis in whom the blood- and bone-marrow pictures were indicative of a leukaemic condition, but in whom complete recovery ensued. The patient, a 40-year-old woman, was given dapsone, 50 mg 4 times daily, for a resistant type of psoriasis. The blood dyscrasia was considered to be directly due to dapsone (since no other drugs had been given), the time lag was consistent, and a challenge with self-administered dapsone provoked the symptoms described by early leprologists as "dapsone fever".

(A similar case was reported by McKenna and Chalmers in 1958 (*Br. med. J.,* 1958, *i,* 324)).

S. G. Browne

11. **Diaminodiphenylsulphone and steroids in the treatment of pyoderma gangrenosum,** by L. D. SOTO. *Int. J. Derm.,* 1970, **9**, 293.

The author gave dapsone at a dose of 100 mg daily, together with 18 mg of paramethasone, to 4 patients suffering from "pyoderma gangrenosum" seen in Mexico City. All 4 had been previously treated for long periods with various combinations of antibiotics, tuberculostatics and corticosteroids, but it was not until dapsone was given (with paramethasone) that definite clinical improvement, leading to complete cure, was achieved.

Although the author is understandably hesitant in attributing this result to dapsone in a disease that often tends to be self-limiting, he suggests that further work should be done along these lines.

S. G. Browne

12. **Transmission of Buruli Disease,** by D. J. P. BARKER, J. K. CLANCEY, R. H. MORROW and S. RAO. *Br. med. J.,* 1970, *iv,* 558.

In a letter to the *British Medical Journal,* the authors (working as a team, The Uganda Buruli Group) give advance notice of the contents of an article that will appear later. They claim that organisms, resembling *Mycobacterium ulcerans* in several respects, have been isolated from various grasses growing in districts where Buruli ulcer occurs, and that 24 other cultures of mycobacteria, differing in certain cultural characteristics, have been also obtained from the grasses in question.

The publication in full of these important observations is awaited with interest. If *Myco. ulcerans* can be found on grasses, and positively identified, the way may be open for *Myco. leprae* to be traced to some similar nidus, just as various fungi pathogenic to human skin have been isolated from soil.

S. G. Browne

13. **Histologic and lipid studies of sural nerves in inherited hypertrophic neuropathy: preliminary report of a lipid abnormality in nerve and liver in Dejerine-Sottas disease** by P. J. DYCK, R. D. ELLEFSON, A. C. LAIS, R. C. SMITH, W. F. TAYLOR and R. A. VAN DYKE. *Mayo Clin. Proc.*, 1970, **45**, 286-327.

A very full biochemical study of nerves in a disease characterized by the clinical features of peripheral neuropathy and gross enlargement of peripheral nerve trunks is of interest to leprosy workers. The following is the authors' summary:

"Analysis of fascicular portions of sural nerves from patients with hypertrophic neuropathy of the Dejerine-Sottas type (HN-DS) has shown a great decrease in cerebrosides in the presence of at least normal amounts of sphingomyelin and possibly increased amounts of sulfatides. The decreased amounts of cerebrosides may parallel the great decrease in amount of myelin but, in view of this great decrease, the normal sulfatide value actually may represent an abnormally large amount of sulfatides. Analysis of a liver specimen from a patient with HN-DS revealed an abnormal lipid profile—a sevenfold increase in ceramide monohexoside sulfates with very low levels of ceramide dihexoside sulfates and of other unidentified lipid sulfates. These findings suggest the existence of a systemic defect in the metabolism of ceramide hexosides and ceramide hexoside sulfates.

Quantitative histologic studies of sural nerves from patients with hypertrophic neuropathy of the Charcot-Marie-Tooth type (HN-CMT) and with HN-DS demonstrated generalized abnormalities of myelinated fibres, but not of unmyelinated fibres. In the sural nerve of the most severe case of HN-DS, myelinated fibres were demyelinated for most of their length, leaving only occasional, short internodes. In sural nerves from persons with typical HN-CMT, approximately 7.5 to 10% of the length of myelinated fibres was demyelinated, but the remainder of the fibre studied also showed evidence of previous demyelination and re-myelination. In both disorders, the diameter of the largest axis cylinders of demyelinated regions was less than the diameter of the axis cylinders of largest myelinated fibres of healthy nerves. An actual decrease in the number of myelinated fibres is inferred from the observation of onion-bulb formations without central fibres, from the known decrease in number of Meissner's corpuscles in skin of persons with these disorders, and from the known presence of fibrillations and increases in amplitude of motor unit potentials in these disorders. The present histologic evidence suggests an abnormality of myelination. The changes in axis cylinders may represent the loss of trophic influences from demyelination or a coincident metabolic abnormality in axis cylinders."

14. **Stigma and the leprosy phenomenon: The social history of a disease in the nineteenth and twentieth centuries**, by ZACHARY GUSSOW and GEORGE S. TRACY. *Bull Hist. Med.*, 1970, **44**, 425-49.

The thesis advanced by the authors, from their wide reading of the relevant literature and their enquiries in the United States, is that the stigma popularly thought to surround leprosy is not so widespread as some people think, and that therefore the considerable efforts put forward by interested laymen and medical workers are not completely congruent with modern scientific knowledge. In the Western world, the stigma is generally believed to be associated with, or derived from the Old and New Testaments; attempts at "destigmatization" often revolve around efforts to show that this association is without base on historical, medical and exegetical grounds.

The authors suggest that these attempts are of relatively recent origin, and are common in the U.S.A. and in Europe. [The categorical statement that "with the exception of a few small endemic foci, leprosy 'disappeared' from the continent of Europe around the 16th century and has remained absent from that continent since" will be challenged by doctors treating some of the 52,000 leprosy patients still living in European countries]. They view with some concern any extensive public education programme on leprosy in the United States or Western Europe,

which might have undesirable effects in creating or increasing anxiety in vulnerable sections of the population.

In an extended historical section, the authors trace the growing awareness among the colonial powers (Britain in particular) of the existence of leprosy and the threat it posed, and indicate the factors concerned with the development of the stigma that now is held to surround leprosy in the Western world. It is noteworthy that in some countries where leprosy is very prevalent, little or no stigma exists, whereas in Europe and North America—not directly threatened by the disease—the stigma and hence the need to rationalize or to remove it are generally accepted by those most closely concerned with leprosy and its victims. "The myth of leprosy as the disease of the 'leper' . . . is still widely disseminated", and the concept stigmatizes by reason of the implied moral connotation of the words.

The authors conclude that the current destigmatization theory has a mythological foundation: "a rational fear of the disease cannot be attenuated through the myth that it is stigmatized by virtue of a faulty association with Biblical references and images." They suggest that the necessarily partial and emotional approach of voluntary agencies must be supplemented by the greater resources and dispassionately scientific attitude of Governments facing the medico-social problems of a slightly contagious disease.

S. G. Browne

The following 3 abstracts are reprinted, with permission, from *Trop. Dis. Bull.*, 1971, **68**, 1:

15. **Studies towards the standardization of lepromin. Progress and prospects**, by J. H. HANKS, M. ABE, T. NAKAYAMA, M. TUMA, L. M. BECHELLI and V. MARTÍNEZ DOMÍNGUEZ. *Bull. Wld Hlth Org.*, 1970, **42**, 5, 703-9.

Lepromin suspensions for intradermal testing are usually prepared from the skin of patients with lepromatous leprosy, and this may contain varying amounts of tissue debris and clumps of leprosy bacilli. An improved lepromin should have the following properties: (1) it should contain leprosy bacilli that have been subjected to minimal, controlled mechanical trauma; (2) there should be a uniform range of bacterial clump sizes, and (3) freedom from visible, rapidly-settling tissue particles. Thus a standard lepromin containing 160×10^6 bacilli/ml can be prepared. An electric blender for initial preparation, and treatment of tissue residues with 7% chloroform to declump the bacilli are recommended. Lyophilized lepromins retain their potency for more than 3 years, while storage at 4°C is less reliable. Dilution of lepromin decreases the frequency of weak reactions, "false-positives", in patients with lepromatous leprosy. A dilution of 1 in 7.5, producing induration of 3 mm, is equivalent to an induration of 5 mm produced by full-strength lepromin. The Fernandez reaction at 48 hours is not reliably observed with diluted lepromin and is considered unimportant. Further trials of dilutions of lepromin 1 in 4 (40×10^6 bacilli/ml) and 1 in 8 are recommended. The diameter of induration at 4 weeks should be recorded in patients and contacts. Four degrees of induration are suggested.

C. S. Goodwin

16. **BCG vaccination of children against leprosy. Preliminary findings of the WHO-controlled trial in Burma**, by L. M. BECHELLI *et al. Bull. Wld Hlth Org.*, 1970, **42**, 2, 235-81.

This eagerly awaited preliminary report, although presented with considerable reservations, will provide useful data for the continuing debate on the protective value against leprosy of BCG vaccination. The purpose of the investigation was to observe, in a densely populated township in a highly endemic area with a high lepromatous/tuberculoid ratio, whether BCG vaccination had any protective effect in children possibly exposed to leprosy infection outside the home.

The authors briefly review a selection of the many publications dealing mainly with the capacity of BCG vaccination to evoke positivity of the lepromin reaction in different populations, different age-groups, exposed or not exposed to leprosy, and exposed or not exposed to tuberculosis and perhaps to anonymous mycobacteria.

They then give a very fair summary of the results reported to date in the other two large-scale and adequately planned trials of BCG vaccination currently in progress, that is, in Uganda and in New Guinea. It would seem that BCG vaccination in Uganda has conferred protection against early forms of leprosy for a period of 3 years or more in about four-fifths of children exposed to intra-familial risk. These findings are not necessarily applicable to communities with different total prevalence rates or with different proportions of lepromatous cases. In New Guinea, BCG vaccination has provided no unequivocal protection of exposed individuals, though preliminary findings were encouraging and suggested that some protection was afforded in persons in the 10-29 years age-group.

In the WHO trial in Burma, the potency of the BCG vaccine used was determined, and considered to be satisfactory from the standpoints of both viability and antigenicity. No distinction was made between household contacts and other children, since attack rates were similar in both groups.

The incidence of leprosy in the 2 comparable groups of children, vaccinated with BCG and unvaccinated, is analysed in a series of useful tables, taking into consideration the degree of tuberculin sensitivity, age, and so on. The findings at each of the 3 annual re-examinations showed no significant differences in the pattern of leprosy incidence in the 2 groups, although inconsistent fluctuations were observed from year to year. Nor did BCG have any appreciable effect on the forms of leprosy that did develop in both groups, which were mainly tuberculoid or indeterminate. The Mitsuda reaction was in general more highly positive in children who had received BCG vaccination, and in the children in this group who developed leprosy. Other interesting facts emerged. In addition to the lack of protection afforded by BCG vaccination to children exposed to leprosy in the home, was the grossly similar risk arising from index patients suffering from lepromatous or from tuberculoid leprosy. Again, naturally occurring tuberculosis infection did not confer any significant protection against the subsequent development of leprosy. In the first annual follow-up, the incidence of leprosy was rather higher in the BCG vaccinated group, suggesting that the vaccination may have had an enhancing or accelerating effect on the appearance of incipient leprosy lesions.

A comparison of the results obtained is made, the methodology and some of the epidemiological aspects of the 3 trials are discussed, and suggestions are offered to explain the considerable apparent discrepancies in their results. [The whole paper deserves detailed study.]

S. G. Browne

17. **Evaluation of leprosy control programmes: some suggestions for operational and epidemiological assessments,** by L. M. BECHELLI and V. MARTÍNEZ DOMÍNGUEZ. *Bull. Wld Hlth Org.*, 1970, **42**, 4, 631-4.

The authors set out clearly and categorically the kinds of information ideally required for the appraisal of the effectiveness of leprosy control programmes. Operational assessment depends on the completeness and accuracy of records furnished periodically to the central authority, and in particular on the efficiency of case-finding procedures and treatment. Salutary advice is offered to offset the frank admission that, in view of the shortcomings of treatment and the continuing lack of an agreed protective vaccination applicable in all situations, the incidence of leprosy is not expected to show an immediate dramatic decline.

S. G. Browne

The following 3 abstracts are reprinted, with permission, from *Trop. Dis. Bull.*, 1971, **68**, 3:

18. **Muscular changes in lepromatous leprosy,** by S. E. MANSOUR, A. MEHASEN and A. F. EL-ARINY. *Trans. R. Soc. Trop. Med. Hyg.*, 1970, **64**, 6, 918-20.

Thenar muscle biopsy specimens from patients with lepromatous leprosy, with no history of myopathy or neuropathy, showed the presence of leprosy bacilli in 13 out of 13 cases. The great majority were in macrophages or Virchow cells but a few were seen lying free in relatively intact muscle fibres.

Histologically there were varying degrees of lepromatous infiltration, forming a granuloma in some cases. The muscle fibres showed reactional changes secondary to the infiltration. When severe this involved destruction of the muscle fibres together with perimysium and endomysium.

[It is very interesting that muscle lesions were found in all 13 cases though nasal smears were positive in only 6. These lesions, however, are not strictly comparable to the multiplication of bacilli in mouse muscle; nor is it safe to assume a similar involvement of deep muscles.]

D. S. Ridley

19. **Australia antigen and lepromatous leprosy. Studies in South India and elsewhere,** by B. S. BLUMBERG and L. MELARTIN. *Int. J. Lepr.*, 1970, **38**, 1, 60-67.

Sera from 552 patients with lepromatous and 384 with tuberculoid leprosy, and from 251 people without leprosy, were collected at Chingleput and in the surrounding district in South India. Two tables give details of the incidence of Australia antigen in the various age-groups and types of leprosy, together with results of comparable studies in Cebu in the Philippines [see this *Bulletin*, 1970, v. 67, abstr. 1548]. In South Indian patients 6.2% of those with lepromatous and 2.1% of those with tuberculoid leprosy had Australia antigen, the highest incidence being in those under the age of 20 years. Among people without leprosy the incidence was 2%, but below the age of 20 the incidence was 7.9%. The difference in the incidence of Australia antigen between the lepromatous leprosy group and the tuberculoid group is significant. Frequency was higher in males, and in younger males than in older males. These age, sex and classification differences are the same as those previously reported for the larger Cebu study. The authors discuss the "inadequacies" in the sample selection in India, namely, that no attempt was made to match the patients and those without leprosy as to caste and location. Patients with lepromatous leprosy were "mostly" living in an institution, and this may have influenced the incidence of Australia antigen in this group. However, in Cebu, institutionalization did not appear to be a factor determining the frequency of this antigen in leprosy patients. The authors postulate that people who are homozygous for a gene which indicates susceptibility to chronic infection with serum-hepatitis virus are also susceptible to chronic infection with other organisms, and may be more likely to develop lepromatous leprosy when infected with *Mycobacterium leprae.*

C. S. Goodwin

20. **The use of flufenamic acid in acute complications of leprosy, and the associated lowering of raised serum transaminase levels,** by C. S. GOODWIN and M. J. WOOD. *Int. J. Lepr.*, 1970, **38**, 1, 68-77.

Sixty leprosy patients suffering from reaction phases received short, high-dosage, tapered courses of flufenamic acid (FFA), and the drug appeared to be effective in relieving the manifestations of reaction in lepromatous leprosy, such as fever, erythema nodosum leprosum (ENL), neuritis and iridocyclitis. Reaction in borderline and tuberculoid leprosy was not so well relieved. These high doses were well tolerated, gastrointestinal symptoms being the commonest side-effect. Temporary neutropenia occurred in 1 of 4 patients who received 28 mg/kg daily,

but the leucocyte count returned to normal 10 days after the drug was stopped. Levels of serum glutamic oxaloacetic transaminase (SGOT) and of glutamic pyruvic transaminase (SGPT) were raised in a number of patients when treatment with FFA was commenced, and in some there was a significant fall during the time when the largest doses were being given.

W. H. Jopling

21. **Patterns of sensory loss in lepromatous leprosy,** by T. D. SABIN and J. D. EBNER. *Int. J. Lepr.,* 1969, **37**, 3, 239-48.

At Carville, Louisiana, U.S.A., the authors compared thermographs depicting skin temperature patterns of normal subjects with the configuration of sensory loss to pinprick in a series of patients with lepromatous leprosy, and found that the pattern of sensory loss tends to involve the cooler skin surfaces earliest and then progresses on the basis of relative skin temperature. Thus the limbs are affected first—the dorsa of feet, the lateral aspects of legs, and the dorsal aspects of hands and forearms—whereas the scalp, axillae, intergluteal fold and the inguinal areas are all warm regions that tend to show normal sensation even in far advanced cases.

W. H. Jopling

22. **Double-blind controlled clinical trial of clofazimine in reactive phases of lepromatous leprosy,** by A. B. A. KARAT, A. JEEVARATNAM, S. KARAT and P. S. S. RAO. *Br. med. J.,* 1970, *i,* 198-200.

A double-blind controlled trial in 24 patients with lepromatous leprosy in reaction showed that clofazimine (B 663, Lamprene) successfully controlled erythema nodosum leprosum and had a useful effect in preventing recurrence once the reaction had been controlled. The dosage of clofazimine was 100 mg 3 times a day, and the authors consider the drug to be safer and more effective than prednisolone. The only side-effect observed was red/black skin pigmentation, and the patients were willing to accept this in return for relief of symptoms.

W. H. Jopling

23. **Dapsone-resistant *Mycobacterium leprae* in a patient receiving dapsone in low doses,** by S. G. BROWNE. *Int. J. Lepr.,* 1969, **37**, 3, 296-301.

A Nigerian man, aged 35 years, suffering from advanced lepromatous leprosy, was treated at the Oji River Leprosy Settlement. The patient's Morphological Index (MI) was 35% initially and the Bacterial Index (BI) 3.3 (maximum 4). Dapsone 50 mg was given twice weekly for 52 months, each dose of the drug being swallowed in the presence of a doctor or a leprosy worker. After 6 months treatment, the MI was 0% and after 35 months "even fragmented bacilli and acid-fast dust were no longer to be seen" in skin smears. After 52 months, small fleshy papules appeared on the skin of the arms and the lumbar region. The histological picture was granulomatous tissue crammed with *Mycobacterium leprae,* 80% of which were morphologically normal. "There was no suggestion of any defect in intestinal absorption" (of dapsone). Apparently normal skin, earlobes and nasal mucosa "remained free from bacilli"[3]. Tissue from one of the papules was injected into the footpads of mice receiving dapsone in their diet. Multiplication of *Myco. leprae* was found in 10 out of 12 footpads of mice receiving dapsone 0.006% in their diet, and 6 out of 12 footpads of mice receiving 0.025% dapsone.

The significance of this case history is discussed, including its relevance to field work by medical auxiliaries. Dapsone given in a "low-dose regimen facilitates treatment. . . . On balance, then, the risk of the emergence of resistant strains is most probably outweighed by the undoubted advantage of a reduced rate of complications" (of low dose dapsone).

[The rate of excretion of dapsone varies widely and may be very rapid. Now that the minimum inhibitory concentration of dapsone for *Myco. leprae* is known approximately, a rational discussion of dapsone dosage and treatment intervals in relation to maintaining satisfactory anti-bacterial blood levels would have added to the value of this paper. That alternative low dosage regimens are rational, such as 10 mg or 25 mg daily, 50 mg thrice weekly or 75 mg or 100 mg twice weekly, should be more widely known.]

C. S. Goodwin

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