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Editorial

REALISM IN LEPROSY CONTROL

The Director of one of the most efficiently run leprosy control projects in India, reviewing progress over a 17-year period, said recently, "After a few years work by survey, education, and treatment methodology, the decline in incidence comes to a halt, suggesting a new ecological balance between the host and the infective agent. Even though the quantum of (leprosy) infection is reduced, new cases go on appearing in almost equal numbers every year. This static condition may give way if more potent drugs and modified methods of work are discovered" (Nilakanta Rao, 1973*a*).

This experience is shared by many other workers in India. Indeed, Dr K. C. Das, Senior Administrator in the Government national leprosy control programme, reviewing progress over the country as a whole during the past 25 years, said, "Reduction in incidence can only be expected after 15–20 years, but the quantum of infection is reducing, and new cases are mostly of non-infective type" (Das, 1973).

Leprosy control policy in India is directly in line with that recommended by the Expert Leprosy Committee of the World Health Organization. Clearly, employing present internationally accepted procedures, the road to leprosy control in India is going to be hard, long and expensive. India is not at all unique in this. On the other hand, examples do exist where the same techniques, applied with no greater skill and dedication, have produced a very substantial and continuous decline in leprosy incidence, e.g. in Nigeria (Davey, Ross and Nicholson, 1956; Davey, 1957) and Thailand (Pakdi, Sanayakorn and Seal, 1974). Such examples usually relate to static and well disciplined populations.

Clearly, discouraging factors are operative in some countries which do not apply in the same way in others. As communications improve and population mobility increases, discouraging factors are likely to become more dominant. Experience in both E. Nigeria and India has convinced the writer that these are concentrated far more in the spheres of sociology and economics than in biophysics and public health as generally understood (Davey, 1969). It is not realistic to blame a sustained incidence of clinical leprosy on the prolonged incubation of infections arising before chemotherapy was started. The basic problem confronting us is that all too often patients are not coming forward to take chemotherapy in numbers sufficient to have a substantial effect within reasonable time on the spread of the disease. Many surveys give evidence that early infections are concealed. At established treatment clinics, attendance is all too frequently very low. Nilakanta Rao (1973*b*) gives an average attendance of lepromatous cases of 44%, non-lepromatous 28%. Our own figures, at a centre 200 miles further south, were similar. Ekambaram (1974) at the ELEF project in Tamil Nadu gives 57% attendance out of total recorded patients. K. C. Das (1973)

covering India as a whole states that in June and September 1972 although the percentage of recorded cases actually registered for treatment was 91.2 and 92.2 respectively, the percentage who actually took treatment during a three month period was 39.2% in June and 36.1% in September. This problem is shared by other countries. In the Dominican Republic, Herrara (1973) gives 40% of patients as uncontrolled or lost.

In Tanzania, where 32% of patients defaulted for 1 year or more, Hertroijs (1973) found that defaulters occurred more in the lower age groups, in unmarried patients, in those with tuberculoid leprosy, in non-deformed and non-reactive patients, in patients with a short history. Most arose in the first year of treatment, and especially among those treated at wayside clinics. Other factors were, the farming season, mobility of population, and lack of confidence in modern treatment.

The lesson to be drawn is very obvious. It is that unless patients feel that their leprosy is a menace to life and health, they will not put themselves out to treat it, and assess their economic and social concerns as of higher priority in daily living. This universal human reaction has serious implications in leprosy control, because it is the patient with almost insignificant early lepromatous leprosy who may be discharging from his nose huge numbers of viable *Myco. leprae* into the environment. What holds good in Zambia also applies in India, with the added very serious problem that the primary menace of leprosy to personal happiness and well-being is seen less in its potential for physical economic disability than in its effect on social acceptance and stability. The results of this in the concealment of overt leprosy are very serious.

Two fresh factors have recently entered into the situation. First comes convincing evidence from two centres of high reputation, that where patients who do attend for treatment are given tablets of dapsone to use at home, up to about 50% of them may not be taking their tablets regularly (Low and Pearson, 1974; Ellard, Gammon and Harris, 1974). This is obviously not something new. It exposes the same truth, that the dapsone in which we trust, is not being taken by the numbers of patients we hoped for, nor in the dosage we expect. In dealing with chronic illness we cannot expect enthusiasm to be sustained, but personal forgetfulness and indifference are only two small facets of a much more widespread and serious situation. We may make dapsone freely available on the widest possible scale, but it does not follow that patients are going to take it in a way calculated to lead to the control of leprosy in the forseeable future.

A second factor arises from the work of Godal (1974) and Myrvang, Negassi and Lofgren (1973) which shows that leprosy is more contagious than was earlier thought, and that in endemic areas there exists a reservoir of latent infection wider than usually believed. While, as Browne (1974) has shown, many slight infections may be self healing, the balance in others between latent and overt disease may be fine, and easily disturbed by such things as puberty and parturition. There is more leprosy infection than we think. This discovery makes even more pressing the need to find and apply methods of leprosy control which will be widely acceptable.

In practice the central problem is not an economic one. If patients cannot afford to lose a day's wage in order to attend a treatment clinic, it is up to us to circumvent the problem. If, for the sake of work, patients move from place to place, that in itself should be no deterrent to treatment. Much more important than any economic factor is ignorance, and the stubbornly persistent fear of

leprosy and its destructive social consequences, which determine personal and community attitudes and behaviour.

Clearly the order of our priorities needs to be changed. The aims of case finding and case holding by the offering of acceptable chemotherapy and care are still fundamental, but the situation calls for antecedent steps which need to be given the highest priority. Four of these are readily identifiable.

(1) First undoubtedly is a new approach to the leprosy education of the whole exposed population in endemic areas, and *especially people in authority, administrators, doctors and teachers.* Amateur methods are no longer acceptable. The approach must be professional, comprehensive, and utilize the mass media. The Gandhi Memorial Leprosy Foundation is a pioneer in this, and doing work of the utmost importance, including courses for the training of educators at all levels. A similar centre is needed in every country where leprosy presents serious social problems.

(2) Any approach to leprosy control and treatment which singles out leprosy for special attention with a separate organization and staff, except at consultant level, is in many places to be thought of as discredited and out of date. It is now of high priority to integrate leprosy into the general health services *at ground level,* so that chemotherapy can be offered at primary health centres and dispensaries where patients can attend without drawing attention to themselves. This measure at once removes a major source of discouragement to patients. A corollary to it is that other sources of discouragement need to be studied and identified, and the will of patients to take treatment thereby strengthened.

(3) Prophylaxis by the inoculation of a suitable vaccine must now be regarded in principle as of the highest priority, to be applied along with other preventive inoculations, without singling out leprosy as a special disease. The discovery of such a vaccine should soon become a practical possibility. Pending its development, we can at least make use of BCG as an interim measure. Chemoprophylaxis may have limited usefulness, but cannot be generally recommended, because once again it introduces the personal decision to take or not to take the tablet offered.

(4) Finally, there needs to be more dynamism and flexibility, accepting that special situations may call for ways of approach elsewhere unacceptable. Thus in India, there are large numbers of patients who possess literally nothing, whose ties with their village homes have been permanently broken, and who swell the slums in large cities. Orthodox attitudes to leprosy control would frown on the idea of gathering such people together and resettling them into resident, supervised communities outside cities, where they have land for agriculture, but in fact this is a practical and effective solution to a most intractable problem, in the face of which doctrinaire ideas become irrelevant. Sharma (1973) has described a well organized example of this approach.

The need for changing priorities is well expressed in a recent World Health Organization publication. "Health services are too often tied down by definitions of 'environmental health factors' which underline biological and physical factors as opposed to social and economic aspects; the latter entailing changes in human relationships. The conventional structure of many health services at all levels, be they national, regional, or local, are still geared to deal almost exclusively with biophysical hazards and nuisances. *Little time has been found up to now to deal with psycho-social and psycho-economic factors which influence the life and health of people*" (Levi, 1974).*

* Italics mine. T.F.D.

So it is with the leprosy patient. When planning his welfare, it is all too easy to think of him as the pawn in the game, who will fit into a pattern of play without question. He is in fact a person, who will make his own choices whatever we plan, and it behoves us to study his real situation, and devise ways of helping him which preserve his personal dignity and relationships, at the same time as attacking in the community the infection from which he is suffering.

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T. F. Davey

Erratum

A serious misprint occurs in Dr Rees's Editorial "Growing Points in Leprosy Research", on page 2, line 32 of *Leprosy Review* Vol. 45, Number 1. The manuscript stated "does occur". This has been printed as "does not occur". We apologize for the error.

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Leprosy Programmes in the Context of Endemic Disease Control*

S. G. BROWNE

Leprosy Study Centre, London, W.1.

The present disillusionment regarding the general lack of success of most leprosy treatment programmes in effecting a progressive decline in incidence of the disease, makes imperative a critical examination of the methods adopted in various countries for dealing with the problem of leprosy. Local circumstances will determine the details of the best practicable plan, but economic and social factors must not be overlooked.

The time is ripe for a critical and objective reassessment of the effectiveness of leprosy programmes as part of operational research into the control of the major endemic diseases. The main dangers are two: to underestimate, and to overemphasize the importance of the leprosy component of the total endemic disease problem. Health planners and physicians have to avoid the Scylla of neglect of leprosy and the Charybdis of exaggerating its seriousness. The general situation is, at present, far from satisfactory: existing knowledge is not being applied (Browne, 1968), and the best practicable plan for most countries has not yet been decided or put into action. Despite good results registered in some treatment programmes, control of leprosy seems as distant as ever.

Several reasons may be adduced for the growing disillusionment: the early enthusiasms for mass campaigns, based on the expectation of quick results from sulphone treatment, were ill-founded; experiences in Africa were not applicable to countries facing a leprosy problem different in many ways; insufficient attention was accorded to the social component of leprosy; the population explosion in countries of high prevalence nullifies good efforts and exacerbates the problem. Premature attempts at the integration of leprosy programmes into general health programmes have proved illusory.

Certain inherent difficulties in leprosy control have not yet been adequately countered. Leprosy is but one of many morbidity-producing diseases in developing countries. Since its diagnosis may be difficult (and no easy test is available for mass screening), its treatment lengthy and of slow apparent effectiveness, and since the end-points of infectiousness and clinical cure are not easy to establish, the disease, not surprisingly, has generally a low priority and a high cost/effectiveness. Serious absenteeism and failure to take treatment for long enough, coupled with the concealment of infectious patients, have brought

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well-conceived programmes into disrepute. Leprosy still suffers from the overenthusiastic and emotional advocacy of its friends, the prejudice of its enemies, the indifference of politicians and the ignorance of medical workers.

Because most leprosy programmes are primarily concerned with treatment, they lack such objective criteria as falling incidence rates. Moreover, since such programmes—for good historic or philanthropic reasons, as well as because of social prejudice—have mostly developed independently of measures adopted for the control of other endemic diseases, they have failed to profit from available epidemiological knowledge. Leprosy programmes today present a great range of diversity, reflecting as much a widespread uncertainty as a pragmatic adaptation to local needs and pressures:

(1) *Segregation* of all cases diagnosed, whatever the form or the infectiousness, has been practised in South Africa (Schulz and Pentz, 1970), Brazil, Japan (Yoshie, 1970) and Nepal. Selective segregation was adopted during the last century in Norway (Irgens, 1973), and nocturnal segregation has been attempted in certain areas in India. Where the prevalence rate is low, the population co-operative and adequate finance assured, the desirability of some kind of segregation cannot be *a priori* ruled out. The segregation practised in southern Europe, North Africa and the Near East is not for various reasons effective as a control measure.

Some francophone African countries are introducing temporary segregation of infectious patients "*librement consenti*" (Labusquiere, 1969).

The provision of units for the specialized care of sufferers from leprosy (as in U.S.A., Spain, England) is not to be regarded as segregation in the public health sense.

(2) The "*segregation villages*" of the former Eastern Nigeria and Uganda depended on the co-operation of patients and the traditional authorities, and the availability of land for farming. In the pre-sulphone days, the humane separation of patients from their communities for a time was followed by a considerable reduction in incidence of new cases of leprosy.

(3) A *special leprosy service* is provided where the population is dense, the prevalence high and the stigma serious.

In India, the S.E.T. (Survey, Education, Treatment) plan in the National Leprosy Control Programme is in operation in areas where the prevalence of leprosy is moderate; in hyperendemic zones, special Leprosy Control Units are advised. Altogether, some 968,053 patients are at present receiving treatment for leprosy (including voluntary agency programmes) out of an estimated number (1972) of 3.1 million.

In Ethiopia, a "market saturation" technique has been established with a clinic in each important market and smaller clinics at 10 km distance. Where, however, the population density is low, no separate leprosy service is provided (Browne, 1974).

Mobile clinics, which call at central villages at predetermined intervals, are assured by cyclists in francophone West Africa and by Land Rover in the LEPRA project in Malawi.

Foot patrols are necessary in the mountainous regions of Papua New Guinea.

(4) A *co*

former Belgian Congo, where static dispensaries treated patients with leprosy as part of their polyvalent activities (Browne, 1972).

(5) *Combined programmes* have not been widely adopted. Tuberculosis obviously lends itself to such a scheme, and the results in Zambia (McDougall and Drake, 1970) and the plans for central Malawi will bear examination and imitation elsewhere.

The French West Africa *Service contre les Grandes Endémies* has in some countries attempted to include leprosy in a programme that covers tuberculosis, trypanosomiasis, trachoma, etc. In the Philippines, leprosy is treated in "Skin Clinics", and in Thailand and parts of India, family planning clinics have been organized in conjunction with leprosy.

(6) "*Auto-traitement*", which consists of the provision of 6 months treatment for the patient, with opportunity for replenishment at the hands of a literate traditional leader (Nebout, 1972), seems the only practicable method of ensuring treatment where communications are poor (or non-existent for many months at a time), the population scattered, the general health services fragmentary, and the risk of serious leprosy reaction is low. Hence, good results are reported from Chad, Upper Volta, Niger and Senegal. It is also being tried, *faute de mieux,* in Nepal, where patients may have to walk for weeks over mountains to seek treatment.

In any measures taken for the control of leprosy, the following factors should be considered: the natural history of the leprosy endemic, the socio-economic status, the standards of oral, nasal and general hygiene, and the attitude of the population to the disease. The "felt needs" of leprosy patients (ulceration of extremities, deformities, stigmatizing conditions such as madarosis and wasting of the first interosseous space) should be recognized and met, otherwise measures related to control will not be accepted.

At present, BCG vaccination under certain conditions may enhance potential resistance, and dapsone prophylaxis may have a limited usefulness (*Int. J. Lepr.,* 1973). A specific vaccine made from certain moieties of the cell-walls of the organism may become available from quantities of *Myco. leprae* present in the experimentally infected armadillo. Its use might stimulate an existing capacity to mount a cell-mediated immunity against leprosy challenge, and thus prevent tuberculoid leprosy developing with its consequential peripheral nerve damage. If it can also induce, in the individual without such a capacity the development of immunity, then the susceptibles will be protected and the incidence of new cases of multibacillary leprosy will be reduced to a trickle.

The most satisfactory way at present known of reducing incidence rates, however, is to render non-contagious all patients with multibacillary disease by means of standard treatment with bacteriostatic drugs. In addition, the treatment of patients with paucibacillary disease will reduce the possible dissemination from these patients of viable forms that may be non-stainable by ordinary techniques.

With many resources becoming available in poor developing countries for the prevention of measles, tetanus, smallpox, trachoma and trypanosomiasis, and the health teams working on nutrition, child welfare, malaria control, rehabilitation and family planning, it is not too much to expect that the grafting of approved leprosy treatment/control procedures on to such schemes will help to reduce costs, remove stigma, and bring leprosy within the purview of transmissible diseases that are diagnosable, treatable and controllable. Medical auxiliaries may need further training to make them polycompetent, and separate leprosy clinics will be absorbed into the larger service. The voluntary agencies have a continuing role to play in the new situation, bringing their financial help to research as well

as to the service activities in which they have taken a leading part. With their initiative and flexibility, their concern for the social milieu of the leprosy sufferer, and their appreciation of the importance of health education, they may yet make a significant impact on the control of leprosy within the context of endemic disease.

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Some Results from Sixteen Years of Leprosy Control Work in the Khon Kaen Province of N.E. Thailand

ANAN C. PAKDI
Leprosy Control Division, Department of Health, Thailand

CHAIYAN K. SANAYAKORN
Department of Biostatistics, Faculty of Public Health, Mahidol University
and
K. S. SEAL
World Health Organization

The changes in the epidemiological situation of leprosy in the Province of Khon Kaen in N. E. Thailand are assessed from the results of two comparable stratified leprosy surveys conducted at an interval of 10 years. A marked decline in the overall prevalence of the disease is demonstrated despite the persistence of a large reservoir of unregistered cases. It is considered that intensification of certain operational and investigatory measures could reduce further the transmission of the disease.

Introduction

The Khon Kaen Province is one of the largest and most important of the 15 provinces comprising the North Eastern Region of Thailand. This Region, which is a low plateau bounded on its northern and eastern flanks by the Mekong River, has been recognized since the early 1950s as being highly endemic for leprosy. Because of its strategic position in the Region and its available institutional facilities, Khon Kaen Province was chosen in 1956 as the pilot area for a leprosy control project, initiated by Government in association with the World Health Organization and the United Nations Children's Fund (Fig. 1).

Within 5 years of the establishment of treatment services and case-finding procedures, which included the examination of all case contacts and selective village surveys, the registration of patients exceeded 9000 in a population of 700,000, although some 20% of the patients were in fact, not strictly within the bounds of the province, having been attracted there by the prospect of treatment. Considerable reliance was placed on the provision of services through mobile treatment units, although static centres were established at some of the Government rural health units, at that time very limited in number.

First Survey

In 1962, 6 years after the inception of the project the WHO Headquarters Leprosy Advisory Team (L.A.T.) conducted a stratified random sampling survey

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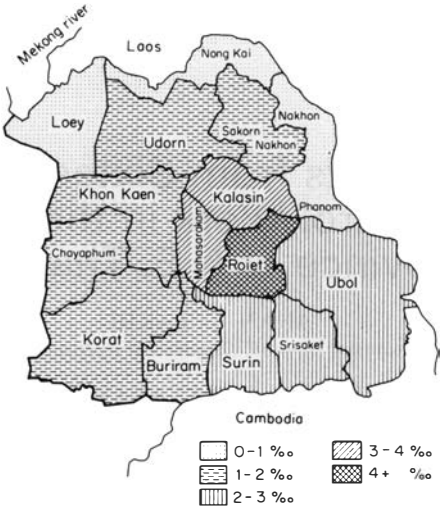

Fig. 1. North-eastern Thailand leprosy rate per 1000 by registered cases 1972—showing position of Khon Kaen Province.

to assess the epidemiological situation and to evaluate certain aspects of the project operations. The provincial population of 720,000 was stratified by its nine districts or blocks, and 16,860 persons in 33 sampling units were screened.

The results showed an overall point prevalence rate of 12.37 per thousand and a lepromatous rate of 4.58. The prevalence rates in the districts varied widely from 3.4 to 22.79 per thousand. The conclusion was reached, that in addition to the 6100 patients resident in the province and receiving treatment, there were probably 2800 undetected cases, that is 31% of the total estimated cases.

Almost 3 years later, two important events occurred which changed the conduct of the work in the province. Firstly, a special campaign was mounted for medical officers to make a thorough clinical review of all the registered patients, to establish more accurate clinical data, and to release from control the inactive cases who fulfilled the criteria for release currently recommended by the WHO Expert Committee on Leprosy. Thus by the end of 1965 the registered cases had been reduced to 3200. Secondly, and concurrently with the review, there was a reorganization of the field operations entailing the abandonment of mobile teams and the establishment of additional static centres, and of particular importance, associated subcentres, to bring the leprosy services as close as possible to the village patients.

The reduction of the case load and the provision of subcentres effected two vital improvements in the efficiency of the control organization.

(a) There was a progressive improvement in the attendance regularity of patients for treatment, so that by 1968 the overall figure of patients having treatment regularly (i.e. 75% of possible treatments) was 72% and that for lepromatous cases 78% (Table 1).
(b) A reasonably adequate surveillance was being exercised on the contacts of all forms of leprosy from 1966 (Table 2).

TABLE 1

Annual rates of regular treatment for period 1965-71 by form and overall rates (annual reports)

Year	I and T			L			Total I-L-T		
	Total patients	Regular	%	Total no.	Regular	%	Total no.	Regular	%
1967	929	440	47	1269	755	70	2198	1195	54
1968	965	627	65	1247	973	78	2212	1600	72
1969	827	563	68	1137	848	75	1964	1411	72
1970	831	599	72	1087	846	78	1918	1445	75
1971	43	32	74	1011	812	80	1745	1360	78

Regular patients are those receiving 75% or more of the treatments.

TABLE 2

Proportions of contacts examined and attack rate annually, all forms

Year	All forms							
	Registered	Examined	%	Cases found				Per mille
				I	L	T	Total	
1966	5925	4484	76	?	?	?	29	6.4
1967	8333	4665	56	6	4	5	15	3.2
1968	8993	6495	72	—	3	10	13	2.0
1969	8225	6507	79	2	5	1	8	1.2
1970	7842	5634	71	2	1	4	7	1.2
1971	7116	5630	75	4	1	2	7	1.3

Second Survey

The second survey of the province was undertaken in 1972 by the authors. The statistical plan adopted was in every way comparable to the earlier survey, using the same frame of blocks, but introducing a substratum by defining all the units for sampling as villages with or without known leprosy cases. A total of 24,010 persons were screened from 44 sampling units, an approximately 2.3% sample and a 98% coverage of the same population was obtained.

During the interval between the two surveys the population had grown to more than a million. The results of the survey revealed a reduction in the overall prevalence from 12.37 to 3.75 per thousand, the rate for registered patients being now 2.0 and the unregistered patients 1.75 per thousand. This latter rate for unregistered patients suggested that there might be as many as 1840 patients in the province needing to be brought under treatment.

The nature of the cases found on survey are set out in Table 3. Of the unregistered cases found in the sample, nine were lepromatous (L) or borderline (B) and of these one lepromatous case had relapsed after release and had not sought further treatment, while three others (2L and 1B) were out of control.

TABLE 3

Clinical classification of leprosy patients found in sampled units

	Indeterminate	Lepromatous	Borderline	Tuberculoid	Total
Registered	–	29	–	19	48
Unregistered	2 (2)	6 (3)	3 (2)	31 (27)	42 (34)
Total	2	35	3	50	90

Figures in brackets are new cases.

Hence it was estimated that the number of cases to be detected for the first time could be 1490 out of the total estimated cases for the province of 3290–i.e. 38% compared with 31% in 1962.

District Prevalences

A reduction in prevalence has occurred in each district (Fig. 2). The variation is still wide, from 0.82 to 6.22 per thousand, and the reduction is by no means uniform and appears to be related in part to the ease of communications and access to treatment services.

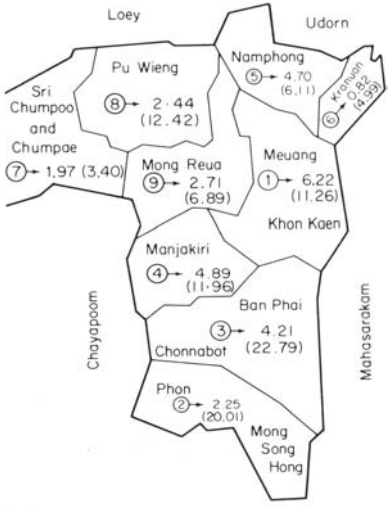

Fig. 2. Khon Kaen Province–showing leprosy rates by block, 1962 and 1972. ○, Numbered sampling block; →, leprosy prevalence (per 1000) in each sample block–1972; figures in brackets, leprosy prevalence–1962.

The Ban Phai and Phon blocks (each now two administrative districts) show the most marked change; Ban Phai from 22.79 (1962) to 4.21 (1972) and Phon 20.01 (1962) to 2.25 (1972). These two districts are traversed by the main north highway, and with the special attention being given by the Government to the

development of this region, improved communications by feeder roads and the extending of the health and social services has been a feature in the last 10 years. Ban Phai District in particular, with the district capital an important road and rail junction has probably the best coverage with rural health units in the province. Partial integration of the leprosy services into the general health services has already in fact been achieved here. Two of the outlying districts of Sri Chumpoo and Kranuan, which are the least densely populated and had a relatively low endemicity have also shown a steady decline in cases.

The poorer responses in Khon Kaen District and Namphong District are thought to be due to a more fluid population. Khon Kaen Town is virtually the Regional Capital, and there is a steady influx of persons from other provinces seeking employment. Moreover Namphong is the site of a large dam and developing irrigation project which has caused a flow of people to the area, as to Khon Kaen.

Case Detection Rates

It is not possible to establish the annual incidence rates for the province from survey data, and reliance must be placed on the annual rate of newly registered patients (or case detection rate per 100,000) to give further indications of the trend of the disease. The progressive decline in detection rates, both overall and for lepromatous cases is shown in Table 4. The field staff in the province has been

TABLE 4

New cases detected and registered annually – 1965-71 by age-group and form of leprosy and detection rates per 100,000 population (derived from monthly reports)

Year	New cases						Grand total	Annual Detection Rate (ADR)	ADR L Cases
	Adults			Children					
	I	L	T	I	L	T			
1965	2	58	100	3	4	7	174	19.2	6.8
1966	–	67	92	4	3	11	177	18.8	7.4
1967	1	53	83	12	1	14	164	16.8	5.5
1968	1	51	86	3	4	17	162	16.0	5.4
1969	1	30	44	4	–	7	86	8.2	2.9
1970	5	24	76	3	1	9	118	10.9	2.3
1971	1	34	47	3	3	3	91	8.4	3.4

reduced from 1970 onwards but the decline in the detection of new cases cannot be attributed to reduced efficiency for supervision from the Regional Headquarters has been good. The relatively poor detection rates among children needs comment. School surveys for leprosy have been the weakest feature of the control organization although routine examinations of school-children are regarded as the normal duty of field staff. In the 1972 survey it is noteworthy that although the age-group 0-14 years represent some 44% of the sample population, the number of children found to be suffering from leprosy was only 6, or 6.7% of the total number of cases, and none were lepromatous. The overall detection rates show a proportion of lepromatous to the combined indeterminate and tuberculoid cases

of never less than 1 : 3, except in 1970 when the usual modes of detection were supplemented by a number of village surveys. This exemplifies the general experience that lepromatous cases seek help more readily than do I and T cases. In this present survey the proportion in newly detected cases is almost 1 : 7 because of the proportionately large number of tuberculoid cases who have not sought treatment voluntarily. This suggests that the lepromatous detection rate may indicate trends in incidence more sensitively than the overall detection rate.

Transmission by "Open" Cases

The 1972 survey revealed that about 76% of the estimated lepromatous cases are under treatment, and a definite decline in prevalence is proceeding at this level of control.

Three sources of continued transmission were identified by the survey, apart from the appearance of new "open" cases.

(a) Three of the six "out of control" cases seen were lepromatous.
(b) One released lepromatous case had relapsed and had failed to return for further treatment.
(c) Because of the high proportion of lepromatous cases in the sample who have shown persistently positive slit skin smears even after seven years of treatment, an analysis of all case records for the province was undertaken. This revealed that 111 (14%) out of 777 lepromatous cases who had received treatment for periods ranging from 7 to 13 years were still bacteriologically positive by standard skin smears.

    It is considered that a continued reduction in the transmission of the disease and of the disease prevalence level could be achieved by widening the operational measures to include permanent surveillance on all previously released cases, out of control cases and by the investigation in hospital if necessary of persistently positive cases to ascertain the reason for the poor response to treatment, and to take appropriate action.

In the general assessment it was encouraging to find by a review of the case records of all registered cases in the province that 45% of the indeterminate and tuberculoid patients were clinically inactive, and that 55% of the lepromatous cases were "inactive" as indicated by negative standard slit skin smears; and thus the epidemiological importance of these patients has been greatly reduced.

In conclusion, the results of the surveys show that the overall point prevalence has declined by 69% and clearly indicates the valuable effect that the control services are exerting on the endemicity of the disease.

Acknowledgements

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An Approach to Urban Leprosy Control

The Greater Madras Leprosy Treatment and Health Education Scheme (Greamaltes) Sponsored by the German Leprosy Relief Association

W. GERSHON

Regional Secretary for India
German Leprosy Relief Association

Introduction

Gremaltes is in tune with the National Leprosy Control Programme. The designers of this have categorized the Indian population into two groups—the rural and the urban population—and have prescribed two different methods to cover these two different groups. While the rural population is to be covered by the S.E.T. pattern (Survey, Education and Treatment) with leprosy control units and S.E.T. centres, the urban population is expected to be covered by Health Education.

It is against this background the Greater Madras Leprosy Treatment and Health Education Scheme ventured to take up the urban work with slight modifications, additions and improvements on the basic scheme. According to the census figures the population of Madras City comes to three million. Gremaltes visualized an attack on the leprosy problem from three angles:

(1) Slums, with an approximate 30% of the total population, with intensive mass survey.
(2) School children, who constitute another 25 % of the total population, with a yearly skin check-up.
(3) The rest of the population, with Health Education.

A flashback of the history of the slums in Madras reveals a steady increase in the number of slums side by side with the development of the city. The slums which were only 189 in 1933 shot up to 306 in 1953 and 548 in 1961. From 1961 onwards the State Housing Board took over the re-housing attempts from the Corporation and now the Slum Clearance Board is in the field. Their attempts are showing fruitful results and their work is laudable. The 1971 census still shows about 600 slums, in which live approximately 700,000 people of the city population. Naturally, the bad housing, overcrowding, ignorance, poverty and unhygienic conditions form a breeding place for a host of infectious diseases, leprosy being no exception. Inferential evidence suggests a high prevalence of leprosy in the slums, but the greatest obstacle to leprosy control is that the patients who are living in the slums have to go to hospital for treatment. It is a fact from experience that slum dwellers never care to avail themselves of the treatment facilities of a nearby hospital, because of factors like long distance, loss

of a day's work, etc. For a successful campaign of leprosy treatment in the slum areas, the medicines have to be taken to the slums and treatment provided then and there, with health education in leprosy carried out simultaneously with treatment. This intensive double faceted programme of treatment and health education will make people of the slums leprosy conscious. If this consciousness is developed among the people of the slums, cases of leprosy will come voluntarily for treatment. Elsewhere, population leprosy survey of the city will not be feasible for practical reasons, but through intensive Health Education it is hoped to bring out the major number of old and new cases for treatment.

Organization Set-up

The objective of the project, as explained earlier, is to control leprosy in the city of Madras in the most efficient way. In order to achieve this, case finding methods have been planned so as to detect the maximum number of cases as early as possible. Special stress is laid upon case holding. Maximum benefit is derived from the use of trained Paramedical Workers to replace medical personnel. Procedures in the project are standardized, and recording and reporting are limited to what is essential for supervision, periodical evaluation and assessment.

Structurally, the project is divided into peripheral field units, supervised, coordinated and controlled by the Headquarters.

Area of operation

The project envisages the overall coverage of the Madras City population with the three tier programme of Slum Survey, School Survey and Health Education within a period of 6 years. For organizational and operational efficiency, the city was divided into North and South. Then realizing the density of the slums we selected North Madras, comprising 63 Municipal divisions, having an approximate population of 16 lakhs (1.6 million) as our initial area of operation.

Field units

The initial project area of North Madras was divided into five convenient zones, each zone having an approximately equal population. Zones are divided into control units manned by two trained paramedical workers, one being the senior. Wherever local circumstances make it necessary the size of the control unit is adjusted to the local geographical conditions, leprosy prevalence, and population density.

Each paramedical worker living in the area of the unit is responsible for case finding among the population of the area, and for treatment, follow-up and further management of the cases detected.

Clinics

The clinics are held once a week but treatment is given to each patient for a period of 2 to 4 weeks. No treatment for other common diseases is given but patients needing it are referred to a general hospital. In the clinic the worker is supervised by the Medical Officer and the senior staff members who visit the clinic regularly from the Headquarters. Utmost importance is given to the maintenance of a satisfactory attendance rate. The worker, who must always maintain good relationships with the patients, contacts the absentees, and stresses the need for regular and systematic treatment.

Headquarters

Planning, supervision and evaluation of the campaign are done at the Headquarters. A well established laboratory for diagnostic investigations is under the guidance of a well trained laboratory technician.

Statistics

Records and reporting are simplified. The basis of the statistics is the individual treatment card, supported by the following records: contact survey cards, school survey forms and bacteriological report forms. Workers finalize their individual reports and present the same to the senior worker in the headquarters each week. Senior workers consolidate the report of the project.

Physiotherapy unit

A physiotherapy technician visits the clinics at regular intervals and assesses the disabilities and deformities of the patients. The patients are educated in general about the care of anaesthetic limbs. The technician also contacts the patients individually and gives advice to them. Patients who need special physiotherapy treatment are referred to Dayasadan Centre. Arrangements are being made to refer patients for surgical treatment to Christian Medical College Hospital, Vellore.

Laboratory

A laboratory technician visits the peripheral clinics and takes smears for diagnostic purposes from all infectious cases, and also cases with doubtful classification. Smears taken in the field are brought to laboratory for bacteriological investigation and the results are recorded in a book and in the bacteriological report form, which once again is transcribed by the zonal worker on to the treatment card. Smears are taken twice a year for infectious cases. Since the pressure of work is increasing, arrangements are being made that in future leprosy auxiliary workers will take the smear and send it to the headquarters laboratory.

Methodology and Accomplishments

The principles of leprosy control campaigns are laid down in the reports of the meetings of World Health Organization Expert Committee on Leprosy. They were also clearly expressed at the International Leprosy Congress at Tokyo 1958, e.g. "from the epidemiological point of view it is more advantageous to reduce infectiousness in many patients than to eliminate infectiousness in few". The first development is the recognition of out-patient treatment clinics as the principal centre for attack on leprosy. Therefore in India according to the generally accepted Survey, Education and Treatment (S.E.T.) pattern, first priority is given to treatment facilities, and is immediately accompanied by an intensive educational campaign, gradually increasing by intensive surveys. Furthermore, the programme is aimed at using the most efficient ways of case finding and achieving maximum case holding.

Case Finding

Case finding mainly relies on health education, the examination of school children, contact surveys and mass surveys of selected areas. Locally, these four

methods have been made the standard methods of case detection. On the starting of a clinic in the zonal area after careful reconnaisance of the area and collection of demographic data, an intensive education campaign is launched with film shows and group meetings with leaders of the slums, describing the main symptoms of leprosy and announcing the availability of the treatment. The paramedical worker following this visits slums in his area and talks to prominent leaders and elders. The personal contacts of the worker during his survey visits spread the message further. In this way the great majority of persons learn to recognize the symptoms and will come forward voluntarily for examination and treatment. Experience shows that patients motivated and coming voluntarily for registration are more regular for treatment than cases detected by other methods. Since the start of the project up till the end of August 1973, out of 4360 known cases 1370 (32%) cases have come voluntarily. This is an indication that there is an increase in leprosy consciousness among the population. Whenever the worker visits the area for survey, or for contacting absentees, people do come in large numbers and request him to examine them. At the beginning of the project, workers observed that all persons who reported voluntarily were suffering from leprosy, but now in old unit areas out of ten such persons, only two were suffering from leprosy. Here is another indication as to the awareness of leprosy in the population.

Mass Survey

Intensive house to house examination of slum areas has been carried out. Out of 183,471 population enumerated, 153,615 have been examined with an examination rate of 83%, and 2900 cases detected with a leprosy rate of 19 per thousand.

School Survey

The school population is an easily accessible part of the population, examination of which can be done in a short time without major difficulties. Periodic visits to the schools by the workers and other staff members provide the opportunity to educate both students and the teachers about leprosy. In general, excellent co-operation is given by the school authorities.

Out of 59,362 children on School rolls, 47,933 have been examined and 812 cases detected, with a leprosy rate of 17 per thousand. It is a matter of great interest to note that all detected cases in the schools were suffering from early forms of leprosy.

Contact Survey

All the healthy contacts of registered patients are recorded in contact survey cards, kept under surveillance, and examined periodically once a year. 7400 contacts are under observation.

Case Holding

In all treatment campaigns in which long term treatment is necessary, case holding is of the utmost importance. Failure in this, makes the investment in the case finding worthless.

Right from the day cases are detected and registered for treatment a very good friendly relationship is maintained by the worker with the patients. Whenever the patients are absent, workers contact them in their houses and explain to them the necessity of regular and systematic treatment. Even so, it is not unusual for a patient after a few months of treatment to become an absentee. This is mainly because patients are disappointed over the lack of quick results, or else after one or two years when the obvious symptoms are disappea 'ng, they neglect treatment.

In-patient Treatment Factilities

After launching the project in the city, and when the number of patients started increasing, we faced the problem of finding a place where patients who require specialized intensive hospital care could be admitted and treated. Dayasadan, the beggar home run by the North Indian Business Community came to our rescue. Dayasadan is situated in the centre of the city and the authorities placed at our disposal a ward with ten beds for hospitalizing patients from the project area. With the permission of the management of Dayasadan we have put up one more ward with ten beds and so at present we have 20 beds in Dayasadan for patients from the project areas.

Medical Aid to Care Homes

In Dayasadan there are 100 beggar leprosy patients, who are fed and looked after by the management. Medical care of these inmates is undertaken by the medical team of "Gremaltes". Medical care is also given to the 250 leprosy inmates of Pope John's Colony at Madhavaram, as well as to the trainees of Gabriel Rehabilitation Centre.

Health Education and In-project Training

Health education in leprosy, as all workers know, is divided into two branches—education of the patients and education of the public. In "Gremaltes", the work of educating the patients is shared by the paramedical workers and the physiotherapist. Educating the public, forms an important ingredient in the Gremaltes recipe, since the coverage of half of the city population rests in publicity. Moreover, in the modern concept of leprosy control work, the stress is shifted from the medical aspect to the social aspect and the leprosy problem is generally conceived now more as a social problem than a medical problem. Hence a transformation of the old concepts, entertained by society, has to be achieved through a planned educational scheme. Gremaltes being a novel project, was able to introduce a novelty in health education, deviating a bit from conventional health education methods. In order to produce the maximum impact on the public, we resolved to resort to the two most powerful media in publicity—the film and the radio. Arrangements are complete to produce a short advertisement—like film strip and put on the Air a continuous "lepra publicity".

Resorting to modern mass communication media does not mean a complete departure from ordinary methods of health education. We have all along been utilizing the usual methods of health education.

Prior to school survey, the school teachers are given a lecture on leprosy, adding a request that the information should be passed on to their students. A

class on leprosy to the teacher-trainees has formed a regular course every year in every Teacher Training College in the city. This also will enable the younger generation to form a different cult about leprosy. A lecture class on leprosy by the Gremaltes staff to the students of all the three social work teaching institutions has almost formed a part of their syllabus.

An orientation course for 250 doctors in Madras City was organized and arrangements exist for further such courses. It has become a routine for every batch of the Youth Service Corps Volunteers of the Tamil Nadu Government, undergoing training in the Madras School of Social Work, to attend demonstration classes and discussion classes in leprosy. So far 6 batches, 50 in each batch, have attended. Besides, the Youth Corps Volunteers, placed in leprosy institutions, get a preliminary training in our project. Five such teams have come to us so far. Apart from this, the Madras School of Social Work also deputes its regular students for field training. Other Social Workers attached to different welfare organizations are sent to us for short-term training in leprosy.

Conclusion

While presenting this preliminary report with pride and pleasure, we, in all humility acknowledge the loopholes in our work. We are always ready to benefit from advice and experience. Constructive criticism will always be welcomed with a smiling face and a plastic mind. Our request is only to perceive the whole work as a maiden experiment in urban leprosy control work.

Appendix

Figures at a glance up to the end of August 1973

Present Project Area:	North Madras
Total population:	16 Lakhs
Slum population:	5 Lakhs 31%
School children:	4 Lakhs 25%
Other population:	7 Lakhs 44%

Statistics up to the end of August 1973

Total population enumerated		244,488
From slum population	183,471	
From school children	59,362	
From self-reported population	1,655	
Total population examined		203,203
From slum population	153,615	
From school children	47,933	
From self-reported population	1,655	

	L	N	N?L	Total
Total no. of cases detected up to the end of this report	120	2751	119	2990
Through slum survey cases	115	1978	85	2178
Through school survey cases	5	773	34	812
Total no. of cases voluntarily reported	228	1033	109	1370
Total no. of known cases recorded up to	348	3784	228	4360
Deletions from known cases up to August	57	337	37	431

Total no. of known cases on roll at the end	291	3447	191	3929
No. of cases registered for treatment up to the end of this report	321	2849	200	3370
Deletion from regd. cases up to August	56	291	34	381
Total no. on roll at the end of report	265	2558	166	2989
Attendance for the month of August 1973	Lepromatous	78%	All	62%
Unregd. cases in the slum cases	24	687	14	725
Unregd. cases in the school cases	2	202	11	215
Total no. of unregd. cases up to the end of this report	26	889	25	940
Total no. of healthy contacts under observation				7400

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Do Leprosy Patients Take Dapsone Regularly?

S. J. M. LOW
Oxford University Medical School, England

and

J. M. H. PEARSON*
Medical Research Council Leprosy Project, All Africa Leprosy and Rehabilitation Training Centre, Addis Ababa, Ethiopa

Urine dapsone/creatinine (D/C) ratios were determined repeatedly in 15 hospital patients on a supervised daily dosage of dapsone. Figures for the group as a whole covered a wide range, but those for individual patients clustered within that range and were much less variable.

D/C ratios were also determined in 89 out-patients prescribed daily dosage of dapsone. Thirty-nine patients gave figures lower than any found in the supervised group, and it was estimated that this group of patients had taken about 42% of their prescribed dosage in the previous 24 to 28 h.

Estimation of the D/C ratio can be utilized to assess regularity of drug taking by a group of patients. Individual patients however can be reliably monitored only if their D/C ratios on supervised treatment are known.

The majority of patients with leprosy are treated outside hospitals, and often in very simple and remote clinics where close supervision of treatment is virtually impossible. Furthermore, the staff who administer treatment have usually themselves undergone only elementary medical training, and the patients may be the first in their area to be exposed to "western" treatment, as leprosy control often pioneers rural health services. Under these circumstances it would be surprising if treatment regimens were rigidly adhered to.

Nevertheless it is valuable in the evaluation of leprosy control programmes to know if patients are taking treatment in the prescribed dosage and frequency; if they are only taking half the prescribed dosage, this fact should be known—and the reasons investigated. In addition it is sometimes important to know whether individual patients are taking dapsone regularly. Any patient, for instance, whose urine tests regularly confirm he is absorbing the drug, but who nevertheless deteriorates clinically must be harbouring dapsone resistant organisms.

The majority of leprosy patients are treated with dapsone (4′4-diamino-diphenylsulphone, DDS). This drug offers few technical problems in analysis or interpretation of results. It is almost completely absorbed from the gastro-intestinal tract, and up to 90% is excreted in the urine (Israili *et al.*, 1973): the

* Requests for reprints should be addressed to JMHP at the National Institute for Medical Research, London NW7 1AA, England.

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half life of about one day is not affected by the variable rates at which dapsone is acetylated (Ellard *et al.*, 1974). Thus estimation of the amount of dapsone in the urine should indicate whether a patient is taking treatment in the prescribed dosage and regularity.

When urine tests however are taken from a group of patients who received the same dosage of dapsone, say 100 mg given 24 h previously, there is a very wide range of concentrations of dapsone in the urine. The major factor accounting for this is the urine concentration, for the more a patient drinks, the more dilute is the urine and the lower will be the dapsone concentration.

The wide range of dapsone concentration makes it impossible for tests of urine dapsone concentration to give a reliable indication of regularity of treatment. However this problem can to some extent at least be overcome if the urine creatinine concentration is also estimated, and the result expressed as the ratio of dapsone to creatinine (D/C ratio). Creatinine is a breakdown product of striated muscle, and for any individual the creatinine output per 24 h is very constant. Thus if the urine is concentrated the urine creatinine concentration is increased; with dilute urine it is decreased. The concentration of dapsone in the urine varies in much the same way, and so the D/C ratio varies less than does the dapsone concentration.

This paper describes the results of urine tests carried out on patients attending the hospital service and a leprosy control clinic of the All Africa Leprosy and Rehabilitation Training Centre (ALERT).

Patients and Methods

The study was divided into two parts. In the first stage patients receiving dapsone daily under full supervision provided urine specimens immediately before swallowing their next tablets. These patients were in the hospital service of ALERT; the tablets were administered by nursing staff and seen to be swallowed. These tests provided information on the range of figures to be expected 24 h after various doses of dapsone. Control urines were also obtained from patients and staff not receiving dapsone.

In the second stage of the study patients attending an ALERT leprosy control clinic were requested to provide a urine specimen, but not informed of the reason; the majority agreed to do so. They were receiving daily dosage, but attended usually once a month for routine examination and issue of tablets. The results of these tests gave some indication of the overall regularity of treatment attained at this leprosy control clinic.

Dapsone was estimated by a modification of the method of Bratton and Marshall (1939); creatinine was estimated by the alkaline picrate method. Further details are given elsewhere (Ellard, Gammon and Harris, 1974). A duplicated sheet describing the actual technique in step by step detail is available (request as for reprints).

Results

(1) *Control urines and specimens from patients under fully supervised treatment*

The urine dapsone levels of subjects not receiving dapsone (control blanks) and of patients receiving different fully supervised dapsone dosages are shown in Fig. 1. There is considerable overlap of the dapsone levels at different dosages: a

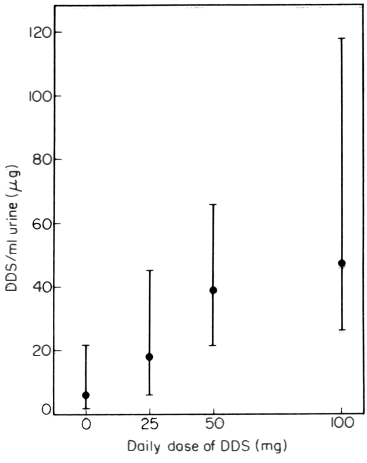

Fig. 1. Urine dapsone concentrations of patients receiving different dosages of dapsone under supervision.

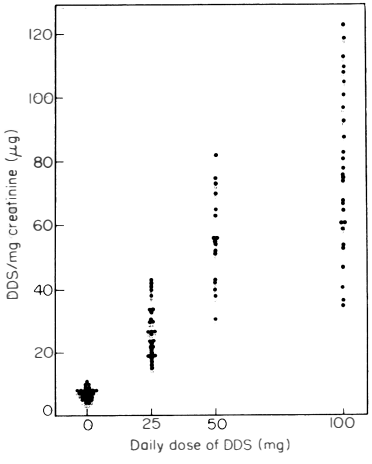

Fig. 2. Urine dapsone/creatinine ratios of patients receiving different dosages of dapsone under supervision.

patient with 40 μg dapsone/ml of urine, for instance, could have taken 25, 50, or 100 mg of dapsone 24 h previously. By contrast, the D/C ratios (Fig. 2) show much better separation. In particular there is complete separation between the control and other groups, and almost complete separation of the 25 mg and 50 mg groups.

The D/C ratio for 4 or more repeated tests on individual patients (in hospital) on different dosages are shown in Fig. 3. The range for a single patient is much less than that of the group as a whole.

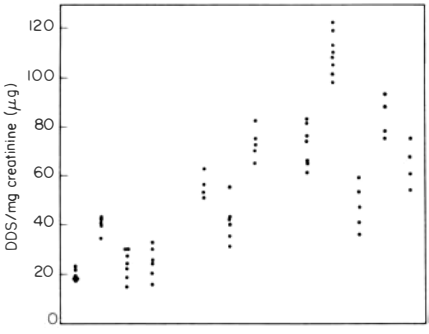

Fig. 3. Urine dapsone/creatinine ratios of individual patients receiving different dosages of dapsone under supervision.

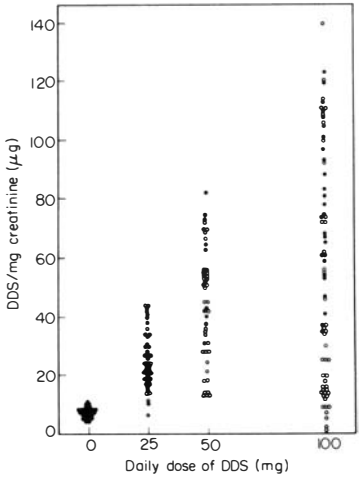

Fig. 4. Urine dapsone/creatinine ratios of patients receiving different dosages of dapsone; ●, under supervision in hospital; ○, prescribed as outpatient treatment.

(2) *Patients attending an ALERT leprosy control clinic*

These patients, though receiving daily dosage of dapsone, were seen usually once a month. Results of their urine tests, taken at routine clinic attendance, are shown in Fig. 4. The patients should have taken their tablets 12 to 24 h previously, but only one test was higher than any under supervised dosage. Thirty-nine of the patients however gave lower figures than the lowest seen in their control groups: and statistical analysis indicated that the group as a whole had taken about 42% of their prescribed dapsone dosage in the previous 24 to 48 h.

Discussion

In the field of leprosy, regularity of clinic attendance is usually considered (at least for the purposes of annual reports) as adequate indication of regularity of

drug taking. This assumption might be considered reasonable in view of the virtual absence of unpleasant side effects of dapsone *per se* in currently accepted dosage (i.e. not greater than about 100 mg daily). Nevertheless the majority of experienced field workers appear to consider that leprosy patients as a group are unreliable, and cannot be trusted to take tablets except under the closest supervision. Thus, one of the benefits of weekly dosage of dapsone is considered to be that the patient (if he can reach the clinic each week) can be "fully supervised", and need not be given tablets to take away, which he may or may not swallow.

The results of this study support those who think that patients often fail to take treatment regularly. Out of 89 patients tested, 39 who should have taken a tablet in the previous 24 h had probably not done so. The attendance rate at this clinic is good (80% are regular attenders) but it is clear that, in this clinic at least, regularity of attendance is no guarantee of regularity of dosage. Similar findings have been reported from the only other control programme where these tests have been performed (Ellard *et al.*, 1974). Both these studies were performed in Africa. However random home visits to check on tablet consumption (carried out in an Indian leprosy control programme) showed that about three-quarters of patients visited had the correct number of tablets remaining (Cap, 1974).

In tuberculosis irregular drug treatment is liable to give rise rapidly to drug resistance. In leprosy this is not the case: dapsone resistance develops only in a small proportion of patients with lepromatous leprosy, and not at all in borderline or tuberculoid cases. Irregular treatment has, however, been shown in leprosy to prolong the period of treatment required for patients to become smear negative (Cap, 1974); and it may also give rise to more complications, probably including an increased risk of dapsone resistance. Moreover it is clearly impossible to evaluate the benefits and hazards of different drug regimes if it is uncertain whether patients are adhering to them.

The results of these tests point clearly to the existence of a group of patients who, though willing to attend clinics regularly, are not taking the prescribed treatment. The reasons for this pattern of behaviour are obscure; possible explanations include:

(1) The patients are sharing out or selling some of their tablets.
(2) They may come to the clinic, but their main interest in doing so may be to obtain other things than medication, such as shoes or clothes, which meet their felt needs. They may not be particularly interested in dapsone.
(3) They may not see their disease improving, and therefore lose trust in their supervisor and interest in their treatment. Patients such as beggars may also have little motivation to be "cured".
(4) They may just forget to take their tablets, or lose them, or find they have crumbled up, and are afraid to say what has happened.

Estimation of the D/C ratios of a group of patients makes it possible to determine whether irregular drug taking is a problem in a clinic or control scheme. Similarly the test can be used to monitor the regularity of an individual patient provided his normal range is first determined by several tests during a period of fully supervised treatment. Evaluation of the medical, social, and personality differences between regular and irregular takers should make it possible to discover why some patients do not take treatment regularly, and to institute preventive measures. Further studies along these lines are planned.

Acknowledgements

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The Application of Urine Tests to Monitor the Regularity of Dapsone Self-administration

G. A. ELLARD AND PATRICIA T. GAMMON

Medical Research Council's Unit for Laboratory Studies of Tuberculosis, Royal Postgraduate Medical School, London W12 0HS, England

and

JUNE M. HARRIS

Somerville College, Oxford OX2 6HD, England

Simple methods are described for determining the regularity of dapsone (DDS) self-administration by out-patients. These methods are based on a comparison of the ratios of the concentrations of DDS to those of creatinine in urine samples collected from out-patients and those from controls given the same daily dose of DDS under strict supervision. These methods were applied to urine samples collected from patients attending some of the mobile clinics in Malawi. They showed that the patients had taken only about half of their prescribed DDS doses in the days immediately preceding their attendance at the clinic. The therapeutic importance of these findings is discussed, and the value of extending these studies to obtain estimates of the regularity of DDS self-administration through the whole treatment period by applying such methods to urine samples obtained by surprise visits to the homes of the out-patients is emphasized.

The mean rate of elimination of DDS determined among a small group of Malawian patients was equivalent to a half-life of about 31 h. Significant differences were found between patients in the rates at which they eliminated DDS but these differences were unrelated to the extent to which they acetylated the drug.

Introduction

Dapsone (DDS) remains the drug of choice for the world-wide treatment of leprosy (Ellard, 1974). Since most patients receive their treatment from out-patient clinics, the successful mass treatment of leprosy is therefore very largely dependent on the regularity with which out-patients take the DDS tablets they are given. Experience in the treatment of other diseases indicates that self-medication is often unreliable (Fox, 1962; Porter, 1968). Thus recent studies of the treatment of pulmonary tuberculosis in East Africa, Hong Kong and India have all demonstrated that drug regimens that are highly successful in controlled clinical trials often fail when used routinely in large-scale treatment because

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patients fail to take their drugs (Fox, 1968, 1972; Kent *et al.*, 1970; Hong Kong Treatment Services/British Medical Research Council, 1972). Similar problems inevitably occur in the mass treatment of leprosy. For instance Pettit and Rees (1964) showed that 4 out of 7 patients who apparently failed to respond to sulphone treatment of over 13 years duration, still harboured DDS-sensitive *Mycobacterium leprae*. During much of this period these patients presumably had often failed to take their prescribed treatment, and when their dapsone treatment was fully supervised a satisfactory therapeutic response was obtained.

In a number of recent controlled clinical trials, simple qualitative urine tests have been used to assess the regularity with which tuberculosis patients took their prescribed medicaments (British Medical Research Council Co-operative Study, 1973; Hong Kong Treatment Services/British Medical Research Council, 1972; Nazareth *et al.*, 1971; Singapore Tuberculosis Service/Brompton Hospital/British Medical Research Council Investigation, 1971; Tuberculosis Chemotherapy Centre, Madras, 1970, 1973; WHO Collaborating Centre for Tuberculosis Chemotherapy, Prague, 1971). In several of the studies patients with higher proportions of negative urine tests were shown to have had a significantly poorer response to chemotherapy.

We have recently evaluated the potential use of several urine-test methods for monitoring the regularity of DDS self-administration (Ellard *et al.*, 1974*a*). Methods based on the detection of diazotizable compounds in the urine or on their quantitative determination were found to be unsatisfactory because of the marked effects of diuresis on the urinary concentrations of DDS, its diazotizable metabolites and of natural diazotizable compounds. The effects of diuresis could however be largely overcome by determining the ratios of the urinary concentrations of diazotizable compounds (as DDS) to those of creatinine, a normal urinary constituent whose rate of excretion in subjects varies little from day to day or from hour to hour. By comparing the ratios of the concentrations of DDS/creatinine in urine samples from patients self-administering their DDS with those from patients receiving the same daily dose of DDS under strict supervision, and from controls not taking DDS, the proportion of self-administered DDS doses actually being taken can be calculated (Ellard *et al.*, 1974*a*). For this purpose urine samples should if possible be obtained by means of surprise visits to the homes of out-patients self-administering their DDS. Because of the slow elimination of DDS from the body and the large range of DDS/creatinine ratios found in urine samples from different patients receiving the same daily dose of DDS, the result of a single urine test cannot establish conclusively whether or not a particular patient actually took their last prescribed dose. This question can however be answered by making a comparison with the results obtained when the patient is given the same dose under supervision.

This paper describes the results obtained when the DDS/creatinine ratios of urine samples collected from out-patients in Malawi who were self-administering their daily DDS doses, and from in-patients receiving the same DDS dosage under supervision, were determined, using simple colorimetric methods. It also describes an alternative procedure for determining the proportion of DDS doses actually being taken by the out-patients, in which pools were prepared from urine samples from the two groups of patients and the DDS/creatinine ratios of the pools then determined using a specific fluorimetric method for DDS. The rates of elimination of DDS from the plasma by a small group of Malawian patients were also determined.

Methods

Collection of samples

Control urine samples were collected from each of 12 patients in the leprosy wards of the LEPRA Control Centre, Blantyre, Malawi, 24 h after they had taken at least four consecutive supervised daily doses of 25 mg DDS, and from 12 healthy staff who were not taking DDS. A urine sample was also collected from each of 206 male patients attending the fortnightly out-patient clinics served by the Land Rover circuits administered by the LEPRA Control project, Blantyre ("mobile clinic" samples). At each visit to these clinics patients are given a fortnight's supply of 25 mg DDS tablets for daily self-administration. Each patient was questioned as to when his last DDS dose had been taken. Thirty-seven patients stated they had taken their last DDS dose earlier in the day, and since possible absorption of DDS from this dose would have invalidated the comparison with the control urines, these samples were subsequently discarded, leaving a total of 169 for analysis. Nine other patients failed to produce a urine sample. Similar urine samples were also obtained from 15 patients attending the Blantyre out-patient clinic. Each urine sample was preserved by the addition of approximately 0.5 volumes of 2 N-HCl and stored without refrigeration until analysis in London three months later.

Four blood samples were collected from each of 17 leprosy patients in the LEPRA Control Centre, Blantyre, at 4 and 24 h respectively after two consecutive daily doses of 50 mg DDS had been taken on an empty stomach. The blood was taken into heparinized tubes, the plasma rapidly separated off and then stored at -20° C until analysis in London.

Analyses

Urinary concentrations of total diazotizable compounds (as DDS) and of creatinine were determined by the modifications of the Bratton and Marshall (1939) and alkaline picrate methods described previously (Ellard *et al.*, 1974*a*). Plasma concentrations of DDS and monoacetyl-DDS (MADDS) were determined fluorimetrically by a simplified procedure based on our previous method (Ellard and Gammon, 1969). In this modification 3 ml of serum was extracted with 15 ml ethyl acetate after the addition of 1 ml M-tri-sodium citrate. Ten ml of the ethyl acetate extract was then washed successively by shaking in the same stoppered centrifuge-tube with 1 ml aliquots of 0.1 *N*-sodium hydroxide, 0.1 N-HCl and M-sodium citrate, respectively. After drying the washed ethyl acetate extract with 1 g anhydrous sodium sulphate the concentration of DDS was determined from the fluorescence at 298/345 nm, and that of MADDS from the fluorescence at 298/420 nm, after allowing for the contribution due to DDS. The fluorescence of the ethyl acetate extracts were also determined at 295/324 nm.

Analyses of special samples

The possibility that some of the patients might have been taking sulphonamides concomitantly was suggested by the fact that several of the urine samples had considerably higher DDS/creatinine ratios than those encountered in urine samples from Malaysian patients receiving 25 mg DDS daily under strict supervision (Ellard *et al.*, 1974*a*). To clarify the situations further analyses were undertaken on all the urine samples that were originally estimated to contain

more than 40 μg DDS/mg creatinine. The colorimetric creatinine and DDS determinations were first repeated and the reaction product obtained in the DDS method scanned in a recording spectrophotometer. Aliquots of the acidified urine samples were also neutralized and extracted by the procedure used for the fluorimetric determination of DDS (Ellard and Gammon, 1969) and the extracted DDS determined both fluorimetrically and colorimetrically (Ellard *et al.,* 1971).

Analyses of representative pools of urine samples

Pools were constituted either by mixing equal volumes of urine, or by mixing volumes of urine containing equal amounts of creatinine as determined colorimetrically by the alkaline picrate method. Pools were prepared in this way from urine samples obtained from the healthy staff not taking DDS, from the control patients receiving supervised daily doses of 25 mg DDS and from the out-patients served by the mobile clinics. One ml aliquots of these pools were made alkaline by the addition of 1 ml *N*-sodium hydroxide and DDS determined fluorimetrically (Ellard and Gammon, 1969). Their creatinine concentrations were also determined.

Results

Analyses of special samples

The repeat colorimetric determinations carried out on the 13 urine samples found to have ratios of greater than 40 μg DDS/mg creatinine confirmed the original results in every case. In five of the samples, with apparent DDS/creatinine ratios of 61-135 (mean 99), there was evidence for the presence of significant concentrations of diazotizable compounds other than DDS and its metabolites, presumably because the out-patients concerned had been taking sulphonamides. Thus when these five samples were reacted directly by the modified Bratton and Marshall procedure, the absorption of the reaction product at 550 nm was more than twice that of 600 nm in direct contrast to the results from the other eight samples with DDS/creatinine ratios of over 40 (range 41-79, mean 53) and the 11 samples from the supervised controls with ratios of less than 40 (range 18-36, mean 27). Furthermore the concentrations of fluorimetrically-determined DDS in these five samples averaged only about 10% (range 3-15%) of the concentrations of diazotizable compounds determined by the direct colorimetric method, compared to an average of about 50% (range 30-70%) for the other samples. In two of the five samples the interfering sulphonamide did not appreciably extract in the solvent system used for the fluorimetric determination of DDS and the extracts obtained reacted and fluoresced in an identical fashion to DDS. The other three samples contained sulphonamides that were partially extracted into ethyl acetate and thence into 1.2 N-HCl. On diazotization and coupling they gave a sharp peak at 550 nm unlike the flatter peak given by DDS at 570 nm, and they were less fluorescent in ethyl acetate than DDS, their maximal fluorescence occurring at 275/340 nm instead of at 298/345 nm as is found for DDS.

Colorimetrically-determined DDS/creatinine ratios

The results obtained, after excluding the values for the five samples for which convincing evidence of interfering sulphonamides was obtained, are summarized in Table 1. The DDS/creatinine ratios of the 164 urine samples from the

TABLE 1

Ratios of DDS/creatinine determined colorimetrically in urine samples from out-patients and controls

Origin of samples[a]	Number of subjects	DDS/creatinine ratios Range	Mean[b]	Estimated % doses taken[c]
Controls not on DDS	12	2.6-10.9	5.2 ± 0.7	0
Supervised controls on DDS	12	17.6-45.5	28.6 ± 2.3	100
Out-patients				
No default admitted	101	3.7-78.6	21.1 ± 1.3	68
Default admitted	63	3.1-40.2	11.8 ± 1.1	28
All out-patients	164	3.1-78.6	17.6 ± 1.0	53
Taking DDS regularly[a]	82	15.1-78.6	26.4 ± 1.2	91
Taking DDS irregularly[a]	33	10.0-14.9	12.3 ± 0.3	30
Taking DDS grossly irregularly[a]	49	3.1- 9.6	6.1 ± 0.2	4

[a] For details and definitions see text.
[b] Mean ± standard error of mean (μg/mg).
[c] $\frac{\text{Mean test ratio} - \text{mean blank ratio}}{\text{Mean control ratio} - \text{mean blank ratio}} \times 100$.

out-patients attending the mobile clinics averaged 17.6 ± 1.0 compared to 28.6 ± 2.3 for the controls receiving the same daily dose of DDS under strict supervision and 5.2 ± 0.7 for the healthy subjects not taking DDS. It was therefore calculated that in the immediate period before the urine samples were collected only about 53% (17.6-5.2/28.6-5.2 x 100) of their prescribed DDS doses had been taken by the out-patients. A similar proportion (57%) was calculated from the ratios of the 15 samples from out-patients attending the Blantyre clinic. When the urine samples collected at the mobile clinics from patients who claimed to have taken their last DDS dose on the previous day were considered, the estimated proportion of doses taken rose to 68%, in contrast to a value of only 28% for those who admitted failing to take a dose of DDS on the previous day. There was however no significant difference between the results from the 11 patients who admitted to only a single missed dose and those from the 52 who confessed to more serious default.

By analogy with the results from the controls, it was considered probable that the 82 out-patients (50% of the total) with DDS/creatinine ratios of greater than 15 had taken their prescribed DDS doses regularly in the days immediately prior to the collection of the urine samples, that the 33 (20%) with ratios of 10-15 might have been irregular, and that the 49 (30%) with ratios of less than 10 might have been grossly irregular in their drug taking. The proportions of prescribed DDS being taken by these three groups calculated from their mean DDS/creatinine ratios were about 91%, 30% and 4%, respectively (Table 1).

Fluorimetric analysis of representative pooled urines

The pools prepared from the urine samples collected from the 12 healthy staff not taking DDS yielded ethyl acetate extracts with negligible fluorescence at the wavelengths used to determine DDS, while the pools prepared from the controls

given supervised DDS doses and the out-patients served by the Land Rover circuits yielded extracts whose fluorescence characteristics were identical to those of DDS. The ratios of fluorimetrically determined DDS/creatinine in these pools are summarized in Table 2. Almost identical results were obtained whether the pools were prepared by mixing equal volumes of urine or by mixing aliquots containing equal amounts of creatinine. Furthermore it was found that the results were unaffected by the inclusion of the five samples containing sulphonamides in the pools. From these results it was calculated that the out-patients had been taking only 52-53% of their prescribed DDS doses during the period immediately before the urine samples were collected. Analyses of the urine pools prepared from samples with DDS/creatinine ratios of less than 10, between 10 and 15, and over 15, respectively, revealed that in this period about 6% of the prescribed DDS doses had been taken by the "grossly irregular" patients, 33-36% by the "irregular" patients and 91% by the "regular" patients. These findings were in remarkable agreement with those calculated from the means of the individual colorimetrically-determined ratios for the three groups.

Plasma concentrations of DDS and MADDS

There were highly significant differences between patients in the ratios of the plasma concentrations of MADDS/DDS attained at 4 and 24 h after dosage with 50 mg DDS, eight of the patients being slow acetylators with mean MADDS/DDS ratios of 0.16 (range 0.12-0.18) and nine rapid acetylators with mean MADDS/DDS ratios of 0.46 (range 0.34-0.55). In any one patient however the ratios attained at 4 or 24 h were very similar. The mean half-lives of DDS of the 17 patients calculated from the fall in DDS plasma concentrations from 4-24 h after giving two successive doses of DDS averaged 31 h (range 25-43 h). Significant differences were found between patients in the rates of elimination of DDS ($P = 0.025$), but these differences were unrelated to the extent to which the patients acetylated DDS. The fluorescence of the ethyl acetate extracts of the plasma samples at 295/324 nm equalled that due to the calculated concentrations of DDS and MADDS, indicating that significant concentrations of diacetyl-DDS (DADDS) were not present.

Discussion

The remarkable agreement between the estimated proportions of the prescribed DDS doses being taken by the out-patients served by the mobile clinics, whether calculated from the means of the individual colorimetrically-determined DDS/creatinine ratios or from the ratios of fluorimetrically-determined DDS/creatinine of the representative pools of the urine samples (Table 2), indicates the validity of either method for monitoring the regularity of DDS self-administration. To prepare truly representative urine pools, volumes of urine containing equal amounts of creatinine should be mixed. However, since no correlation was found between the colorimetrically-determined DDS/creatinine ratios of the urine samples and their creatinine concentrations, it is understandable that virtually identical results were obtained whether the pools were prepared by mixing aliquots of equal creatinine content or much more conveniently by mixing aliquots of equal volume. It is of interest that the colorimetrically-determined DDS/creatinine ratios of the Malawian controls not taking DDS and of those taking supervised daily doses of 25 mg DDS were

TABLE 2

Comparison of the results of three different methods of assessing the percentage of DDS doses actually being taken

Sources of samples[a]	Method					
	Individual estimations (DDS colorimetric)[c]		Equal volume pool (DDS fluorimetric)[c]		Equal creatinine pool (DDS fluorimetric)[c]	
	DDS/creatinine	% doses taken	DDS/creatinine	% doses taken	DDS/creatinine	% doses taken
Controls not on DDS	5.2[b] ± 0.7	0	<0.1	0	<0.1	0
Supervised controls on DDS	28.6 ± 2.3	100	13.1	100	13.0	100
Out-patients						
All out-patients	17.6 ± 1.0	53	6.8	52	6.9	53
Taking DDS regularly[a]	26.4 ± 1.2	91	11.9	91	11.8	91
Taking DDS irregularly[a]	12.3 ± 0.3	30	4.7	36	4.3	33
Taking DDS grossly irregularly[a]	6.1 ± 0.2	4	0.8	6	0.8	6

[a] For details and definitions see text.

[b] Mean ± standard error of mean (μg/mg).

[c] $\frac{\text{(Mean) test ratio}-\text{(Mean) blank ratio}}{\text{(Mean) control ratio}-\text{(Mean) blank ratio}} \times 100.$

approximately double the corresponding values obtained from Malaysian patients (Ellard *et al.*, 1974*a*). This finding emphasizes the importance of comparing the results from out-patients with appropriately matched controls.

The apparent ingestion of sulphonamides by some 3% of the out-patients slightly complicated this investigation. Fortunately the results obtained indicated that such interference could simply be established by measuring the absorption of the reaction product in the colorimetric DDS method at 600 nm as well as at 500 nm.

The calculated proportion of prescribed DDS doses taken by the out-patients will be a true estimate if the timing of the collection of the samples from the out-patients was the same as that in the controls and if the proportion of DDS doses taken by the out-patients in the days immediately previous to the collection of urine samples was typical. The studies carried out on Malaysian patients showed that after giving a single dose of DDS, DDS/creatinine ratios fell in the urine at similar rates to plasma DDS concentrations (Ellard *et al.*, 1974*a*; Gelber and Rees, 1974). It may therefore be concluded that differences in timing between the collection of the urine samples from the out-patients served by the mobile clinics (possibly 12-30 h after the previous day's dose) and the control in-patients (24 h after the previous dose) could have overestimated the proportion of DDS doses being taken by the out-patients by 5-10%.

A more serious reservation to interpreting the results obtained in this study rests on the fact that representative urine samples were not obtained by means of surprise visits to the out-patients' homes. It might be argued that the stimulus of preparing to attend the clinic might have reminded many of the patients to have taken their DDS in the immediately proceeding days. Conversely it could however be reasoned that if patients either lost of disposed of their tablets, or missed a clinic attendance, the resultant deficit to their stock of DDS tablets would be most likely to result in a failure of self-administration in the days immediately before the next clinic. It would however seem highly probable that the regularity of DDS self-administration by the out-patients who provided the urine samples analysed in this study was superior to that of the other out-patients who failed to attend at the mobile clinics.

Although attempting to categorize the patients according to their regularity of DDS self-administration is necessarily arbitrary, the results did indicate that only about half of the out-patients attending the mobile clinics had taken their DDS tablets regularly in the 2-3 days immediately preceding the clinic. A further 20% of the patients had probably taken only about a third of their prescribed doses during this time, while the remaining 30% had taken virtually no DDS at all during this period. *If* the irregularity of DDS self-administration by the patients studied in this investigation, revealed in the period immediately before the urine samples were collected, was typical of the whole treatment period, it would be likely to seriously jeopardize hopes of curing the great majority of the patients and of eliminating leprosy from the area within the foreseeable future.

The MADDS/DDS ratios of the slow and rapid acetylators among the 17 Malawian leprosy patients studied were very similar to those found among a small group of dermatitis herpetiformis patients being treated with DDS in Britain (Ellard *et al.*, 1974*b*) and confirm the previous studies of Gelber *et al.* (1971) and Peters *et al.* (1972) which showed that DDS, like isoniazid, sulphadimidine and certain other hydrazides and sulphonamides, is polymorphically acetylated in man. The demonstration of significant differences in the rates of elimination of

DDS by different subjects confirms the investigation of Peters *et al.* (1972) while it is of interest that the mean half-life of DDS in the 17 Malawian patients investigated in our study (31 h) is very similar to that of 28 h found among 21 African patients in Zaire by Peters *et al.* (1974). The finding that the half-life of DDS is unrelated to the extent of DDS acetylation also confirms evidence obtained in other investigations (Peters *et al.*, 1972; Gelber and Rees, 1974). The absence of significant concentrations of DADDS in the plasma of DDS-treated patients is also in accord with the studies of Murray *et al.* (1971) who used an extremely specific and sensitive chromatographic-fluorimeter procedure to determine DDS and its acetylated derivatives.

The results obtained in the present study suggest the following procedure for monitoring the self-administration of DDS by leprosy out-patients. Urine samples should be collected from approximately 20 healthy subjects not taking DDS, from 50-100 out-patients, and from about 20 in-patients receiving the same daily dose of DDS under strict supervision. If possible urine samples should be collected from the out-patients by means of surprise visits early in the morning to their homes. The patients should be questioned as to when they had taken their previous DDS dose. Their stock of remaining DDS tablets should also be inspected since a significant excess over the correct number would indicate failure of DDS self-medicated in the previous period, while a shortage would normally result in supplies being exhausted before the next clinic. Surprise home visits would obviously be much more difficult to organize in rural areas such as those served by the mobile clinics in Malawi than in the semi-urban environments of Madras and Hong Kong where many of the controlled antituberculosis trials described earlier were held. Urine samples would be collected from the in-patients 2-4 h and 24 h after their fourth supervised daily dose of DDS and strict precautions taken to ensure that during this period they ingested no other drugs. Pools would then be prepared from 1 ml aliquots of the urine specimens from the control groups from the out-patients who stated they had taken their last DDS dose on the previous day, and from those who stated they had taken their last dose at least 2 h earlier in the day. Samples from patients having taken a dose of DDS within 2 h would be unsuitable for analysis on account of uncertainty concerning the proportion of the DDS dose absorbed during this period. Aliquots of the pools would then be preserved without refrigeration by the addition of 0.5 vol. 2 N-HCl, and their DDS/creatinine ratios determined using a sensitive and specific fluorimetric method for the determination of DDS. Interference from other fluorescent compounds is very unlikely and in any case their presence would almost certainly be revealed by examining the fluorescence excitation and emission spectra of the extracts.

Such a study should reveal the overall regularity with which DDS was being taken by the out-patients. If it were considered that the irregularity of DDS self-administration was likely to have unacceptable therapeutic consequences, the DDS/creatinine ratios of the individual urine samples should then be analysed by the simple colorimetric methods described. A comparison of the results from the out-patients with those from the controls, taken together with evidence of the possession of grossly incorrect stocks of DDS tablets, would indicate which patients were likely to be the most serious defaulters in self-medication. Specific efforts could then be made to encourage such patients to take their DDS tablets more regularly, further home visits made and urine samples analysed to establish or indicate whether or not the exhortations had been successful. If consistently

low ratios of DDS/creatinine were still found in samples from individual patients, the regularity of their drug-taking could be established by comparing the results with those obtained after they had been given fully supervised doses of DDS. In many situations such studies would not be feasible for individual patients, and the only practical response to a serious overall level of defaulting might be to consider replacing unsupervised daily chemotherapy by some form of *fully supervised* intermittent chemotherapy.

It might be argued from this Malawian study that questioning of the patients was in itself an effective method of discovering irregular DDS self-medication since significiantly lower DDS/creatinine ratios were found among those patients who admitted missing doses. Nevertheless the DDS/creatinine ratios of urine samples from 13 of the 101 out-patients who denied missing DDS doses were not significantly different from those of the controls who had taken no DDS. Obviously strenuous effort should first be made among those patients admitting default to encourage them to take their DDS doses more regularly. However such efforts might well result in these patients insisting on a future occasion that they were taking their DDS regularly, even if this were not the case. Hence the importance of basing action in individual cases on evidence provided by determinations of urinary DDS/creatinine ratios.

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Electronmicroscopic Demonstration of *Myco leprae* in Axons

C. K. JOB

Schieffelin Leprosy Research Sanatorium-632106, Karigiri, Via. Katpadi, Tamil Nadu, S. India
and
Department of Pathology, Christian Medical College and Hospital, Vellore-632004, Tamil Nadu, S. India

and

R. VERGHESE

Schieffelin Leprosy Research Sanatorium-632106, Karigiri, Via. Katpadi, Tamil Nadu, S. India

Biopsies from 14 lepromatous nerves were examined. Unequivocal evidence for the presence of *Myco. leprae* in axonal cytoplasm is found in four biopsies. In the nerves the organisms are present much more frequently and in large numbers in Schwann cells, macrophages and perineurial cells and therefore the intra axonal bacilli may not have much part to play in the destructive process of the nerve. However it is suggested that axons are very likely sites for bacilli to remain protected from the bodily defence mechanisms and drugs resulting in relapse of the disease.

The affinity of *Myco. leprae* to peripheral nerves and especially to Schwann cells is quite well known and has been well established through several studies using the light microscope (Khanolkar, 1955; Weddell *et al.*, 1963; Lumsden, 1964). Nishiura *et al.* (1957, 1958, 1960) and later Imaeda and Convit (1963) in their electromicroscopic studies have reported the finding of *Myco laprae* in axons and have suggested that Schwann cell infiltration by *Myco. leprae* is a sequelae to axonal invasion. However, Job (1970) and later Dastur *et al.* (1973) studying lepromatous nerve biopsies using the electronmicroscope have clearly demonstrated and confirmed the observations of Weddell *et al.* (1963) and Lumsden (1964) that *Myco. leprae* primarily affect the Schwann cells. Although we are convinced that the target cell of *Myco. leprae* in the peripheral nerve is the Schwann cell, we would like to record here the finding of *Myco. leprae* in axons in four of the lepromatous nerve biopsies studied.

Material and Methods

In a period of three years 14 lepromatous nerve biopsies have been studied using the electronmicroscope. Thirteen of them were from the radial cutaneous nerves and one from the greater auricular nerve. They were cut into small pieces

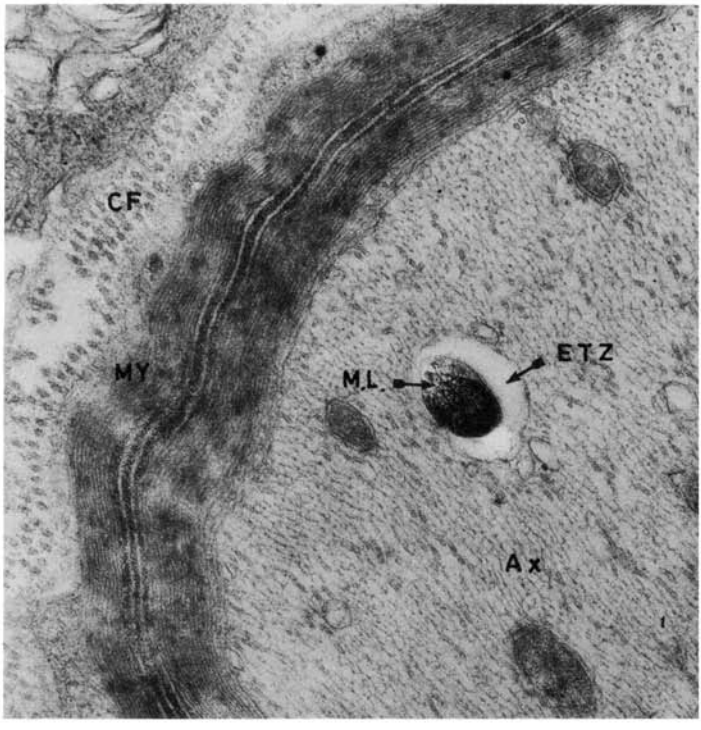

Fig. 1. Electronmicrograph to show a myelinated nerve fibre containing the cross section of a bacillus (ML) inside an axon (AX). Note electron transparent zone (ETZ) surrounding it. MY, Myelin; CF, Collagen fibres.

of about 3 mm long and 1 mm thick and were fixed in 5% glutaraldehyde solution in phosphate buffer at pH 7.2 for 4 h at 4° C. Later they were washed in buffer solution and treated with 2% osmium tetroxide, processed in grade alcohol followed by propylene oxide and embedded in araldyte. In a few biopsies part of the tissue was fixed in Dalton's solution instead of glutaraldehyde. Ultra-thin sections were prepared, stained with lead citrate and uranyl acetate and were examined under an electronmicroscope.

Findings

The results of the study of most of the lepromatous nerve biopsies have been reported earlier in two communications (Job, 1970, 1971). The nerve parenchyma in most instances was partly, and in some largely, replaced by collagen fibrils, macrophages, plasma cells, lymphocytes and fibroblasts. The number of Schwann cells with axons were much reduced. Perineurium was thickened with infiltrating collagen fibrils and proliferating perineurial cells. *Myco. leprae* were found in large numbers in Schwann cells, macrophages, perineurial cells and endothelial cells.

On searching through many ultra-thin sections we were able to find in four

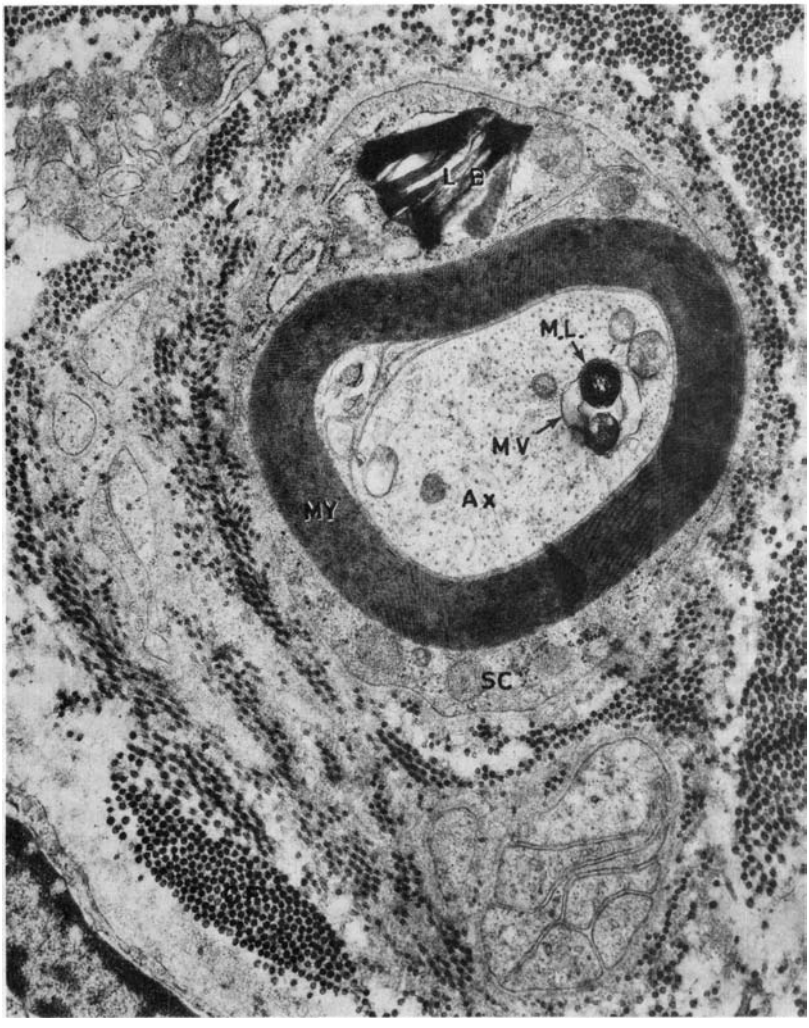

Fig. 2. Electronmicrograph showing a myelinated axon containing two *Myco. leprae* (ML) inside the axon (AX). A double membrane bound vacuole (MV) contain the organisms. SC, Schwann cell; CF, Collagen fibril; LB, a lamellar body.

biopsies very occasional bacilli inside axons (Figs 1 and 2). The cytoplasm of all the intraneurally situated organisms was markedly electrondense as found in solidly staining organisms. The thin electrondense double layers of the plasma membrane were well seen. In some instances the organisms were found in the ground substance of the axon with an electron transparent zone around it (Fig. 1). In other sections there were membrane bound vacuoles which contained the organisms (Fig. 2). The axons which contained the bacilli appeared normal with no evidence of any degenerative changes. The Schwann cell in one of them

contained a large vacuole with bacillary debris and in another there is a lamellar body (Fig. 2) which normally occurs in Schwann cells.

Discussion

In this paper unequivocal evidence for the intra-axonal presence of *Myco. leprae* is presented in four different lepromatous patients. In some the bacilli were present in the ground substance of the axonal cytoplasm and in other sections the bacilli were present in membrane-bound vacuoles. There were also electron transparent zones surrounding the organisms as seen in Fig. 1.

Khanolkar (1955) had suggested earlier that bacilli enter the nerve through the naked axons in the skin, proliferate in the axonal tissue and are taken up by the Schwann cells only later. Nishiura *et al.* (1957, 1958, 1960) in their electronmicroscopic studies had sought to confirm these findings. However, Dastur *et al.* (1973) were unable to see organisms in axons. Job (1970) reported findings of organisms inside axons in two patients but suggested that they could be situated in intra-axonal Schwann cell processes. The electronmicroscopic evidence in this study leaves no doubt as to the intra-axonal presence of the bacilli. The interaction between the bacilli and the axonal cytoplasm had produced the electron transparent material around it and this is very similar to what was described in the cytoplasm of macrophages and Schwann cells (Imaeda, Convit and Lapenta, 1963; Job, 1974).

It is interesting to note that in all the four patients the bacilli were seen in myelinated fibres. Bacilli could enter the myelinated axon at the internodal sites. Intra-axonal proliferation of the bacilli is quite possible but we are not able to demonstrate it. The significance of the presence of bacilli inside axons is yet to be determined. Compared with the number present in other components of the nerve, the bacilli in axons are very uncommon and therefore it is reasonable to infer that it is only in a very rare instance bacilli enter the axoplasm. Once they enter the axons they are very much protected. No lysosomal granules are present in axons and therefore intra-axonal degradation and dissolution of bacilli may not be possible as in other cells. No fragmented bacilli were seen in the axons and all those seen were solidly staining and electron dense and therefore they could be considered viable. Axons may be sites where organisms can be protected from enzymic and other protective action of the body's defence mechanism and drugs and can remain there long enough to be responsible for relapses. If they do multiply in axons, which is also reasonable to expect, disintegration of axons directly by the organism is a possibility (Imaeda and Convit, 1963). However, this process would indeed be a rare occurrence.

Acknowledgements

This investigation was supported by funds from the U.S. Government grant SRS-IND-31-67 from PL 480 funds, a grant from The Leprosy Mission, London, and American Leprosy Mission, New York. Excellent technical assistance was provided by Mr Shantha Kumar Rhenius and Mr B. Joseph. Secretarial assistance was provided by Mr J. Ramamurthy.

Addendum

Since submitting this paper for publication we have come across two papers on intra-axonal *Myco. leprae*, one by Yoshizumi *et al.* and another by J. Boddingius, and their references are given below:

Yoshizumi, M. O. and Asbury, A. K. (1974). Intra-axonal bacilli in lepromatous leprosy—a light and electron microscopic study. *Acta Neuropath. (Berl.)* **27**, 1-10.

Boddingius, J. (1974). The occurrence of *Mycobacterium leprae* within axons of peripheral nerves. *Acta Neuropath. (Berl.)* **27**, 257-270.

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Dastur, D. K., Ramamohan, Y. and Shah, J. S. (1973). Ultrastructure of lepromatous nerves—neural pathogenesis in Leprosy. *Int. J. Lepr.* **41**, 47-80.

Imaeda, T. and Convit, J. (1963). Electronmicroscopic study of cutaneous nerves in leprosy. *Int. J. Lepr.* **31**, 188-210.

Imaeda, T., Convit, J. and Lapenta, P. (1963). Electronmicroscopic study of Borderline leprosy. *Int. J. Lepr.* **31**, 389-417.

Job, C. K. (1970). *Mycobacterium leprae* in nerve lesions in lepromatous leprosy—an electronmicroscopic study. *Arch. Path.* **89**, 195-207.

Job, C. K. (1971). Pathology of peripheral nerve lesions in lepromatous leprosy—a light and electronmicroscopic study. *Int. J. Lepr.* **39**, 251-268.

Job, C. K. (1974). Schwann cell changes in lepromatous leprosy—an electronmicroscopic study. Submitted for publication.

Khanolkar, V. R. (1955). Perspectives in pathology of leprosy. *Indian J. Med. Sci.* **9** (Suppl. 1), 1-44.

Lumsden, C. E. (1964). Leprosy and the Schwann cell *in vivo* and *in vitro*. In *Leprosy in Theory and Practice*, 2nd ed. (Eds R. G. Cochrane and T. F. Davey), pp. 221-250. Bristol: John Wright & Sons Ltd.

Nishiura, M. (1960). The electronmicroscopic basis of the pathology of leprosy. *Int. J. Lepr.* **28**, 357-400.

Nishiura, M., Harada, N. and Imaeda, T. (1957). Electronmicroscopy of ultra-thin sections of lepromatous peripheral nerves. *Int. J. Lepr.* **25**, 323-328.

Nishiura, M., Harada, N. and Imaeda, T. (1958). Electronmicroscopic study of the ultra-thin sections of leprous peripheral nerves. *Acta Neuroveget (Vienna)* **18**, 411-423.

Weddell, A. G. M., Palmer, E., Rees, R. J. W. and Jamison, D. G. (1963). Experimental observations related to the histopathology of leprosy. In *The Pathogenesis of leprosy*, (Eds G. E. W. Wolstenholme and M. O'Connor), Ciba Foundation Study Group No. 15, pp. 3-15. London: J. & E. Churchill.

Lepr. Rev. (1974) **45**, 240-243

All India Leprosy Workers' Silver Jubilee Conference

T. F. DAVEY

In October 1973, 500 leprosy workers from all parts of India met at Sevagram to pay tribute to the memories of Armauer Hansen and Father Damien, and also celebrate 25 years of association and cooperation in their specialized profession. It was entirely fitting that the Jubilee Conference should be held at Sevagram, near Wardha, a place of hallowed memory for all Indian patriots, for it was Gandhiji who called together here the first national gathering of leprosy workers in India, and it was in his own compassionate approach to leprosy patients that they found the inspiration for concerted action.

The Jubilee Leprosy Conference was the largest ever held in India, and from beginning to end the spirit and example of Gandhiji seemed to pervade it; in its simplicity, some might even say austerity, in its excellent fellowship and goodwill, and not least in the vegetarian catering, served with astonishing efficiency and expedition by a crowd of students rendering voluntary service.

The Conference was inaugurated by the Honourable Shri R. K. Khadilkar, Union Health Minister. While covering all the main aspects of leprology, and devoting special sessions to commemorating the centenary of Dr Hansen's discovery of *Myco. leprae* and Father Damien's arrival in Molokai, the Conference had its own Indian flavour in the emphasis given to problems surrounding epidemiology, control, and rehabilitation. At each session a high quality of contribution was assured by a rigorous curtailment of individual papers, most of the time available being filled by original papers from selected main speakers and shorter invited papers, with ample time for discussion. The following is an outline of material presented.

Epidemiology

Dr Dharmendra saw dangers in the assumption that a patient with zero M.I. is in a state of non-infectivity to others. Even if all non-solidly staining leprosy bacilli are non-viable, a zero M.I. means no more than that the number of non-viable bacilli in the body has fallen below the level at which they can be identified by standard techniques. Their numbers may however still be enormous. While the same limitation applies to a zero reading of the B.I., the criterion is much stricter, *viz.* no bacilli at all, solidly or non-solidly stained, being found in multiple skin smears in repeated examinations. Existing criteria of non-infectivity should not be abandoned. *S. K. Noordeen,* describing a longitudinal study over six

years in a geographically limited rural community highly endemic for leprosy, reported a very high incidence of minimal lesions of tuberculoid type, with spontaneous regression a common feature, not only among children, but among adults. In a small proportion of patients showing regression, subsequent relapse occured. *B. R. Chatterji, Jacob Thomas, C. E. Taylor and G. N. Naidu* reported a study of a population of 7500 in a highly endemic area of West Bengal, kept under intensive epidemiological surveillance for six years. Standard routines included the examination of the ear lobes of asymptomatic individuals, contacts and others, for AFB, with the careful follow-up of all persons giving positive findings. The incidence rate of clinical leprosy was six times higher among such persons than in the general population. Incidence rates among nuclear family contacts of lepromatous/borderline leprosy were not significantly higher than in those of tuberculoid/indeterminate leprosy. Economic and crowding indices had no correlation with leprosy prevalence, but incidence rates were higher in the low income groups. The findings indicate that frequent examination with high population coverage is essential to define the basic epidemiology of leprosy. *R. J. W. Rees and T. F. Davey* presented recent clinical and bacteriological studies which emphasized the high prevalence and very high content of viable *Myco. leprae* in nasal discharges in early cases of lepromatous leprosy, and also indicated the capacity of *Myco. leprae* discharged from the nose to survive in a dried condition, 100% for 24 h, significant numbers for 1.75 days, and a few for up to 7 days.

Leprosy Control

M. S. Nilakanta Rao presented data extending over 17 years of intensive leprosy control work organized by the Gandhi Memorial Leprosy Relief Foundation in selected areas. He concluded that after a few years of work by S.E.T. methodology the decline in incidence comes to a halt, suggesting a new ecological balance between the host and the infecting agent. Even though the quantum of infection in the community is reduced, new cases go on appearing in almost equal numbers every year. This static position may give way if more potent drugs and modified methods of work are discovered. *P. Vijay Shanker* described a three-tier programme for attacking the formidable problem of urban leprosy control in Madras city, namely, full survey in slum areas covering approximately 33% of the city population, school surveys covering another 25%, and health education covering the remaining 42%. Health education methods suitable to various categories of people are an essential ingredient everywhere, aiming to prepare the community to accept leprosy on the same terms as other public health problems. Demonstration centres where leprosy is treated in integration with other health problems are being established. A paper on organization by *D. D. Banerjee, Gangadhar Sharma and N. V. Nagabhusanam* stressed the importance of health education and greater effort in the discovery of open cases.

Experimental Medicine

W. F. Kircheimer, guest of the Conference, described at first hand the work which has established the nine banded armadillo as a valuable animal model for the study of human leprosy. *K. V. Desikan* reported findings in mouse footpads harvested at varying intervals from 1-90 days after inoculation. A steep fall was

found in the number of bacilli within the first week of inoculation, the yield falling to 20% of the original number.

Immunology

Tore Godal, guest of the Conference, described the detection of sub-clinical infection in leprosy by means of the lymphocyte transformation test (LTT) and the leucocyte migration inhibition test, and concluded that leprosy is more highly infectious than indicated by the prevalence of leprosy, and that sub-clinical infection commonly follows exposure to *Myco. leprae.* The relatively low response among contacts of active lepromatous patients was a surprising observation, which suggests that in such contacts "super exposure" to *Myco. leprae* can bring about a decrease in host resistance. *N. H. Antia* considered that the transfer of immunity by sensitized lymphocytes opens up new possibilities in the treatment of lepromatous leprosy resistant to the drugs available. *Kunal Saha, M. M. Mittal, H. B. Maheswari and Col. R. N. Dutta* reported some encouraging results in this direction in patients who received viable lymphocytes, but not in patients who received transfer factor only. *I. Nath, J. Curtis, L. K. Bhutani, V. Mehra and G. P. Talwar* found an increase in B-lymphocytes in most patients with lepromatous leprosy studied, but that a sub-population of T-lymphocytes was diminished.

Impact of Leprosy Control on the Trend of Leprosy

Summarizing the achievements of the past 25 years, *Dr K. C. Das,* Assistant Director General of Medical Services (Leprosy), emphasized six aspects.

(1) The national leprosy control programme has now covered a population of 99 millions. 1.2 million cases of leprosy have been detected out of an estimated total of 3.2 millions for India as a whole.

(2) Through early diagnosis and treatment, large numbers of patients have been prevented from becoming infectious, and much deformity has been prevented.

(3) Reduction in incidence can only be expected after 15-20 years, but the quantum of infection is reducing at many centres and new cases are mostly of non-infective type.

(4) Leprosy is not dislocating the lives of patients on the same scale as formerly. Health education has led to less deformity and less social stigma.

(5) Research and training services have developed enormously and are of international importance.

(6) The ground work has been laid for a decisive nationwide attack, with the hope of controlling leprosy within 15 years.

C. Vellut stressed early and regular domiciliary treatment of all cases as the condition for success. Health education is of great importance in encouraging this. *V. Ekambaram* emphasized the greater interest being shown in the rehabilitation of patients, eye care, and the social aspects of the disease.

Rehabilitation

In an extremely valuable session, *Anthony Swamy* and *Mrs Doraiappan* both emphasized that rehabilitation must always be in the forefront of our thinking in

every approach to patients from the very beginning, its primary object being to minimize disturbance to family and community life. *Capt. Scott* described much useful experience at an industrial rehabilitation centre, with his approach, "Judge by the abilities of a patient rather than by his disabilities". *R. S. Sharma* described an interesting experiment in agricultural rehabilitation at Dattapur. Occupational therapy was discussed by *Monica Hopkins* and *Mrs Doraiappan.*

Health Education

M. S. Mehendale pointed out the urgent need for proper orientation and training in health education relating to leprosy for health workers at all levels. Only through systematic training will vagueness, imbalance and inconsistency be eliminated and essential objectives be pinpointed. *S. W. Ghokale* saw health education as a self-help process, instilling a sense of responsibility for one's health and betterment, and so for that of the community as a whole.

Social Welfare

Papers by *S. D. Ghokale, Paul Karipurath* and *Jagdish Deen* all stressed the need for greater and united effort in promoting the social welfare of patients.

Full sessions of the Conference were devoted to medical and surgical management, and covered recent developments in both fields. In addition moving tributes to Armauer Hansen and Father Damien were paid by *Prof. Johs Boe* and *T. N. Jagadisan.*

This Conference amply demonstrated the wealth of experience, talent, and dedication found among the great body of leprosy workers in India, and which offer some hope that the formidable problems of leprosy control in India can be solved. The profound thanks of all concerned are due to the Organizing Committee of the Conference, and especially to Mrs Susheela Naya, Dr Nilakanta Rao and his staff. Their kindness, thoughtfulness and imperturbability made the occasion most memorable.

Lepr. Rev. (1974) **45**, 244-272

Reprinted Article

We are happy to include the following important reports
by the courtesy of the World Health Organization.

Immunological Problems in Leprosy Research*: 1

This Memorandum reviews the present status of knowledge of the immunology of leprosy, with particular attention to developments since the publication of a similar review in 1970. The different types of lepromin reaction and their significance in healthy contacts and in patients with tuberculoid and lepromatous leprosy are discussed. The immunological responsiveness of patients with leprosy is also considered, with special attention to *in vitro* methods for evaluating this response. Part 2 of the Memorandum will cover possible mechanisms of altered immune response in leprosy (including a tentative scheme to explain the possible genesis of the lepromatous lesion); genetic, nutritional, and hormonal factors; the possibility of vaccination; attempts at immunotherapy; and areas in which further research is needed. A detailed protocol for evaluating the effect of transfer factor in leprosy will be included as an appendix.

Although leprosy is one of the principal public health problems of the world, the leprosy bacillus appears to be relatively innocuous and causes damage to host cells only after extensive proliferation. Immune responses protect the host against unlimited multiplication of bacteria and in most exposed individuals probably terminate the infection at a subclinical level (Godal *et al.*, 1973). Paradoxically, however, immune responses appear to play a major role in producing the various structural and functional disturbances that characterize leprosy. The analysis of immune mechanisms in persons infected with *Myco. leprae* is therefore complex and must range from the consideration of protective responses to intracellular microorganisms to the analysis of the mechanisms of immunologically-mediated tissue injury.

Leprosy presents a clinicopathological "spectrum" of disease between two polar forms (tuberculoid and lepromatous). Tuberculoid leprosy, the highly resistant form, presents with a few well defined skin lesions that histologically resemble typical delayed-type hypersensitivity granuloma. The cellular reaction consists of focal collections of epitheloid cells surrounded by large numbers of lymphocytes. Bacilli are not found by routine histological examinations or are very rare.

In lepromatous leprosy, the "low resistant" form, however, the lesions are multiple and diffuse and consist predominantly of macrophages with an undifferentiated (histiocytic) or "foamy" appearance. The number of

* This Memorandum was drafted by the signatories listed at the end of Part 2.

lymphocytes present is insignificant. Bacilli are present in large numbers in the macrophages.

Between these two forms a continuous range of clinical and histopathological features is seen. Outside this range, early leprosy may manifest itself as a single vague skin lesion, which has been called "indeterminate" leprosy. Histologically, loose collections of histiocytes and lymphocytes, often associated with dermal nerves, are seen. A few bacilli may sometimes be seen in histiocytes, dermal nerves, and erector pilae muscles.

An earlier paper on this subject (*Bull. Wld Hlth Org.*, 1970) emphasized the importance of a detailed clinical and histopathological classification of leprosy so that immunological data could be adequately compared. At that time the Ridley and Jopling (1966) scale* appeared to provide additional histological criteria that had assisted immunologists with the clinicopathological classification of patients for research purposes.

During the past two years the Ridley-Jopling classification has shown good correlation between the clinical-histological scale and the immunological status as assessed *in vitro* by lepromin sensitivity and lymphocyte reactivity to *Myco. leprae*.

An international collaborative study involving the exchange of tissue slides from patients whose immunological status has been determined by *in vivo* and *in vitro* studies would serve to resolve existing differences in the classification of patients with leprosy.†

Physiological and Antigenic Properties of *Myco. leprae*

Bacteriology and antigenic composition

Myco. leprae is an obligate intracellular parasite. It has not been cultivated on artificial media, and only irregular and limited growth has been reported in tissue culture. It has been grown in mice and other small rodents and in armadillos. Nearly all the bacteriological studies have been carried out in mice. The rate of growth during the logarithmic phase shows a doubling time of 12-13 days (Shepard and McRae, 1965), irrespective of the immunological competence of the mouse. The rate of growth has not changed on continued passage in mice during periods up to 14 years.

Isolates have been found to differ slightly in certain growth characteristics and have been classified as "fast" and "slow" (Shepard and McRae, 1971); "fast" strains have somewhat shorter incubation periods, faster average rates of growth between inoculation and harvest, and higher average bacterial populations at plateau. The distribution of various isolates is continuous between the two extremes of "fastness" and "slowness". The trait is stable on passage in mice and repeated isolations from the same patient have the same characteristic. There is no relation to drug resistance.

Isolates from different parts of the world and from patients in the range LL-BT have behaved similarly in mice. Studies of the tissue distribution of the bacilli in

* The Ridley-Jopling scale is as follows: TT, polar tuberculoid; BT, borderline tuberculoid; BB, borderline; BL, borderline lepromatous; LL, polar lepromatous.

† Since 1970, WHO has planned collaborative studies involving nine laboratories in connexion with the WHO International Reference Centre for Histological Identification and Classification in Leprosy (Instituto Nacional de Dermatologica, Caracas, Venezuela).

mice and in men at several constant ambient temperatures indicate that there is no optimum temperature for the growth of *Myco. leprae.*

Recent experience with mice in the tropics has shown that temperature control (e.g. air conditioning) is not necessary for experiments with *Myco. leprae,* although it is preferable for the health of the mice and for constant results at different seasons of the year.

Heat-killed suspensions of *Myco. leprae* fail to react as a skin-test antigen in patients with lepromatous disease. This property is not shown by any other mycobacterium. Immunodiffusion tests show that *Myco. leprae* shares many antigens with other mycobacteria. However, one specific protein antigen ("nodular extract"), which is inactivated by heat and trypsin, has been isolated so far (Abe, 1970; Abe *et al.,* 1973). The specificity of this antigen for *Myco. leprae* was demonstrated by immunodiffusion and immunofluorescence tests with rabbit antisera against nodular extract and a wide range of mycobacteria (Abe, 1971). It is present in the tissues of lepromatous nodules and in the bacterium. Observations suggest that it is a protoplasmic antigen. In a dose of 10 μg, it gave positive 48 h skin reactions in patients with tuberculoid leprosy but negative reactions in those with the lepromatous form of the disease (30 of each). It is recommended that the specificity of this antigen in human skin tests be fully investigated.

Another specific antigen, which is heat-stable, has been demonstrated by lymphocyte transformation, and its antigenic relationship to nodular extract should be investigated by means of the lymphocyte transformation test.

Detailed histological studies in man and in mice indicate that all leprosy infections are systemic. The tissues that contain the largest numbers of bacilli in lepromatous disease are: the skin (exclusive of the axillae, creases, etc.), the nasal mucous membrane, the peripheral nerves, and the reticuloendothelial system, including the bone marrow. There is a continuous bacteraemia in patients with untreated lepromatous disease. Tuberculoid disease shows few bacilli; they are largely concentrated in the dermal nerves and erector pilae muscles of the lesion and in the peripheral nerves. In the evolution of experimental disease, the invasion of nerves by bacilli is a late manifestation, and therefore in man may also be secondary to extraneural infection. Peripheral neuropathy in leprosy is important but the means by which the bacilli enter the nerves is unknown.

The route of transmission of leprosy to man is not established. The portal of exit has commonly been assumed to be the skin or the nasal mucosa. The number of bacilli excreted from the skin is small, whereas the output from the nose is large (comparable with that from the lungs in open cases of tuberculosis). The portal of entry also has usually been assumed to be the skin or the nasal mucosa, but is, in fact, unknown, and the bacillus might well enter the body in inhaled air.

*Lepromin reaction**

Type and standardization of lepromin. The original lepromin of Mitsuda (1923) and Hayashi (1933) is a suspension of the whole autoclaved homogenized leproma, including some tissue elements. This is sometimes called "integral" lepromin. Purified bacillary suspensions have been made, more or less completely freed from tissue elements, and are sometimes called "bacillary" lepromins. Antigens that consist of the soluble proteins of the bacilli, with or without

* For reaction scale, see *Bull. Wld Hlth Org.* (1970).

proteins of the leproma not coagulated by heating, elicit only the early reaction. For distinction, such antigens should be called "leprolins". The "defatted" bacillary suspension devised by Dharmendra (1942) is used especially for testing the early reaction; it gives only a weak late reaction. Because this material is obtained by the chemical extraction of bacilli, it is neither a lepromin nor a leprolin as defined above, and therefore should be referred to as "Dharmendra antigen". The fundamental difference between lepromins and leprolins is that the latter elicit only the early (Fernandez) reaction after 48 h (Fernandez, 1940) and do not themselves sensitize; the former also elicit the early reaction to a variable extent and in addition induce the late (Mitsuda) reaction after four weeks. The test, being a form of "microvaccination", could affect the immunological status of the individual. One consequence is that persons negative to a first test may give positive reactions to a second or subsequent test.*

The injection of autoclaved normal skin or homogenates has given negative to weak reactions in healthy persons and in those with tuberculoid leprosy. From the practical point of view, therefore, there is a possibility that weak positive reactions may be caused by the excess of tissue components in the lepromin.

The Mitsuda reaction is of prime importance for prognosis, particularly in patients with indeterminate and borderline leprosy. Some investigators think it is useful for evaluating the relative resistance of contacts and populations. In order to obtain comparable data from different countries, the standardization of lepromin is necessary.

Recent research has been directed towards the production of an improved lepromin and the investigation of Mitsuda reactions to diluted lepromins. It is hoped to retain all significant reactions to the bacilli while decreasing the number of false positive (1+) reactions, which have been attributed to tissue components. An improved lepromin should possess three properties: (1) bacilli that have been subjected to minimal, controlled mechanical trauma, (2) a uniform range of bacterial clump sizes, and (3) freedom from visible, rapidly settling tissue particles. These qualities would increase the reliability of bacteria counts as a primary means of standardization and ensure the injection of uniform doses of bacteria with minimal tissue component.

Healthy persons in endemic areas. The lepromin test is usually negative in the first months after birth. Before the age of 1 year, approximately 50% of children may present a weak (1+) reaction. The proportion of positive reactions and the degree of positivity increase steadily with age, and at the age of 15 years and over most persons give a 2+ or 3+ reaction.

Maturation and/or exposure to *Myco. leprae, Myco. tuberculosis,* and possibly other mycobacteria may be the cause of such reactivity. The injection of lepromin can also act as a sensitizer. Some believe that prior sensitization with either *Myco. leprae* or *Myco. tuberculosis* does not offer an adequate explanation for the occurrence of natural reactivity to lepromin in some areas. The cause of such reactivity is in fact unknown.

The *in vitro* test of lymphocyte responsiveness to *Myco. leprae* appears to give a high proportion of positive results in the contacts of patients with leprosy, while

* Such studies are being carried out by the WHO regional reference centres for standardization of lepromin (Johns Hopkins University, Leonard Wood Memorial, Department of Pathobiology, Baltimore, USA, and the National Institute for Leprosy Research, Tokyo, Japan) and by the Instituto de Leprologia, Rio de Janeiro, Brazil. These laboratories are able to provide lepromin for research purposes.

most of those who are not contacts give negative results (Godal *et al.*, 1973). On the other hand, no association could be found between the intensity of the Mitsuda reaction and the *in vitro* lysing activity of the derived monocytes of healthy individuals.

Healthy persons in nonendemic areas. Several studies have shown that populations in areas where leprosy is not endemic (Europe and the USA) show a high proportion (about 80%) of positive Mitsuda reactions. The porportion of positive Fernandez reactions is low, even in patients with tuberculosis.

Macroscopic and histological appearance. It has been shown that positive late lepromin reactions are characterized histologically by a tuberculoid-type granuloma, while negative and doubtful reactions (0, ±) are associated with a nonspecific cellular response. Weak positive (+) reactions are associated with a nonspecific cellular response in approximately 15% of contacts, while in tuberculoid and indeterminate cases of leprosy they are usually associated with a tuberculoid-type granuloma.

Further histological studies are required to determine whether the greater refinement in the preparation of lepromin would reduce the frequency of nonspecific histological responses in + late lepromin reactions.

Repeated reactions in healthy persons. It is well established that repeated Mitsuda tests—often even a second test—can convey lepromin positivity. This type of conversion has been observed even in children who are tuberculin-negative. The reaction may also intensify (+) in a relatively high proportion of individuals. Thus lepromin may act as a sensitizer in persons who have the potentiality to react. Rees (1964) has reviewed the significance of the lepromin reaction in man.

White Cell Responses to Infections

The main leucocytes involved in responses to chronic infections, including leprosy, are lymphocytes and macrophages, although polymorphonuclear infiltrations are found in acute exacerbations such as *erythema nodosum leprosum.* Collaboration between lymphocytes and macrophages appears to be the main defence mechanism against organisms, such as *Myco. leprae,* that multiply within cells.

Origin and activation of lymphocytes

This subject has been discussed in detail by the WHO Scientific Group on Cell-Mediated Immunity and Resistance to Infection (1973). Lymphocytes are of two main types—thymus-derived (T cells) and bone-marrow-derived (B cells). T cells are generated within the thymus from precursor cells seeded from the bone marrow. Some of these T cells migrate to special regions of the lymph nodes and spleen (thymus-dependent areas) and proliferate following contact with the appropriate antigen. T cells are characterized by an extensive capacity for circulation from one lymphoid organ to another via the thoracic duct and blood and can freely penetrate most tissues, often returning to lymph nodes in the afferent lymph.

B lymphocytes also arise from precursors in the bone marrow and migrate to defined areas of the lymph nodes and spleen. These cells also proliferate following antigenic stimulation, and some B cells can circulate although most appear to remain localized at their site of production in the lymphoid tissues.

In the peripheral lymphoid organs, the main proliferative stimulus for T and B

cells is antigenic stimulation. This is a highly selective process since individual lymphocytes are stimulated only by one or a limited number of antigens. The individual, or restricted, responsiveness of lymphocytes is a property acquired by these cells in the thymus and bone marrow in a process involving selective gene derepression and synthesis of antigen receptors—which, in the case of B cells, are immunoglobulins that can be demonstrated on the plasma membrane.

While lymphocyte circulation ensures a regular distribution of lymphocytes to the tissues, this process is amplified when local deposits of antigens or microorganisms are present in a tissue. Reaction between antigen and the occasional circulating lymphocyte with specific receptors for that antigen causes lymphocyte activation and the local release of soluble factors, which may bring about the localization of additional leucocytes that are not necessarily specifically reactive. Since B and T cells responding to antigens become more adherent and less mobile than unstimulated cells, with the passage of time there may be a tendency towards selective accumulation of specifically reactive cells at local sites of antigen deposition and in the draining lymph nodes.

The clonally expanded populations of T and B lymphocytes that develop following antigenic stimulation have distinct immunological functions. B lymphocytes and their specialized variants, the plasma cells, secrete specific immunoglobulins (antibodies: Ab). T lymphocytes are the effector cells of cell-mediated immune responses and in humoral responses to most antigens T cells collaborate with B cells, helping the latter to proliferate and secrete antibody.

T lymphocytes responding to specific antigens undergo blast transformation and proliferate. Activated T cells can kill target mammalian cells with which they come into contact—e.g. foreign cells with antigens against which the T cells are specifically sensitized. This may possibly include target cells containing microorganisms if products of the latter reach the cell membrane. There is no evidence so far that T cells can kill microorganisms by such direct contact. However, activated lymphocytes can increase the capacity of macrophages to kill microorganisms (see below) and also release macrophage-immobilizing factors.

B lymphocytes responding to specific antigens secrete immunoglobulins, which, in conjunction with complement, are bacteriolytic for some bacteria and can opsonify many microorganisms, thereby facilitating their phagocytosis by leucocytes. No information yet exists as to whether antigen-stimulated B cells release other soluble products that affect the localization and functional activity of other leucocytes.

Macrophage production, localization and activation

Most tissue macrophages are derived from blood monocytes, which originate from precursor cells located mainly in the bone marrow, with smaller numbers in the spleen (Nelson, 1970; Metcalf and Moore, 1971).

Following localization in different organs and exposure to varying stimuli, macrophages can adopt a wide variety of morphological forms. The resulting pleomorphism is well exemplified in leprosy where macrophages may appear as epithelioid, multinucleate, histiocytic, or foamy cells. In culture, clonally-derived macrophages can assume a fibroblastic appearance, and this may also happen in tissues.

Macrophages and granulocytes are closely linked in origin and regulatory control. They share the same precursor cells (*in vitro* colony-forming cells) and their proliferative activity is controlled by the same regular system—the

glycoprotein colony-stimulating factor. Selective entry of the proliferating cells into the granulocytic or monocyte–macrophage pathway is determined by the concentration of this factor and by the operation of a complex of serum lipoproteins termed "colony-stimulating-factor inhibitors". Macrophage formation is favoured by relatively low or high levels of inhibitor.

Following viral or bacterial infections, or the injection of bacterial antigens, there is a rapid increase in tissue synthesis of stimulating factor and a subsequent rise in the levels of this factor in the serum. This response is radioresistant and not T-cell-mediated. Specifically preimmunized animals fail, on challenge, to exhibit this response and their depressed stimulating-factor reactivity is transferable to normal animals by immune sera. The rise in the levels of colony-stimulating factor following antigenic stimulation is followed by increased proliferative activity of granulocyte and monocyte-macrophage precursors, particularly in the spleen (Metcalf, 1972).

Under certain conditions of antigenic stimulation–e.g. after the use of complete adjuvants including mycobacteria–a limited degree of proliferation can occur in local tissue macrophages (see Nelson, 1970). Under normal conditions, a slow turnover of tissue macrophages results from the seeding of blood monocytes into the tissues and a limited degree of relocation of tissue macrophages among different organs. The latter process could result in the dissemination of organisms from one tissue to another–e.g. in leprosy, where the macrophages contain viable organisms. In the lepromatous form of the disease dissemination may be favoured by frank bacillaemia.

The localization of macrophages at sites of antigen deposition may be amplified by the release from reacting lymphoid cells of macrophage chemotactic and immobilizing factors.

Macrophages play a number of important roles in immune response (WHO Scientific Group on Cell-Mediated Immunity and Resistance to Infection, 1973). On the afferent side they allow (a) phagocytosis and modification of particulate antigens with the release of more antigenic determinants; and (b) long-term retention of antigen on the cell membrane in an accessible and correctly oriented form that is capable of activating potentially reactive lymphocytes. This latter process may be facilitated by the capacity of macrophages to allow attachment of the Fc portion of immunoglobulins at the cell membrane. Macrophage-associated antigen is especially efficient in eliciting cell-mediated immune responses.

On the efferent side of the immune response, the activation of macrophages–e.g. by exposure to products of interactions between antigens and T lymphocytes–increases the capacity of these cells to kill or inhibit the multiplication of certain pathogenic organisms. Macrophages so activated can kill *Listeria monocytogenes* and inhibit the multiplication of *Myco. microti* and *Myco. lepraemurium,* but their role in relation to *Myco. leprae* has not yet been investigated. The survival of some intracellular organisms (e.g. *Myco. tuberculosis*) may be favoured by the failure of lysosomes containing hydrolytic enzymes to fuse with phagocytic vacuoles containing living bacteria, but in macrophages infected with *Myco. leprae* and *Myco. lepraemurium* such fusion appears to take place. Whether *Myco. leprae* can be digested by macrophages is also unknown. If so, the process must be very slow since organisms remain demonstrable for years in the tissues of patients with lepromatous leprosy who are under treatment. Possibly the ultimate loss of organisms from lesions follows the relocation of macrophages containing indigestible cell walls.

Macrophages can also collaborate with fibroblasts in chronic inflammatory reactions. In silicosis, particles that are ingested by and damage macrophages stimulate collagen synthesis and extensive fibrosis. The presence of indigestible mycobacterial constituents or of immune complexes in certain types of leprosy may favour fibrogenesis by analagous mechanisms.

The functional efficiency of the extreme morphological variants of macrophages seen in leprosy lesions is unknown. The possibility that activated macrophages may release factors that modify the proliferative or functional activity of lymphoid or other cells has not yet been investigated, although macrophages exposed to mycobacterial and other adjuvants appear to increase lymphocyte proliferation in immune responses (WHO Scientific Group on Cell-Mediated Immunity and Resistance to Infection, 1973).

Lymphocyte function tests

Distinguishing lymphocyte subpopulations. Principal lymphocyte subpopulations can be identified and distinguished by virtue of their surface markers and by their response *in vitro* to certain nonspecific stimuli. B lymphocytes are known to have surface immunoglobulins that can be identified by direct or indirect immunofluorescence using polyvalent antisera to human immunoglobulins. In addition, B lymphocytes have a complement receptor that can be detected by rosette formation (termed the "EAC rosette test") with sheep erythrocytes coated with a rabbit anti-Forssman antibody (i.e. amboceptor) plus mouse complement (or human complement in sublytic concentrations). They also have a receptor for the Fc fragment of human immunoglobulin that has been either bound to antigen in the form of a soluble immune complex or heated at 60° C. Because this last method requires the use of isotopically labelled immunoglobulins or antigen, the EAC rosette test is more generally applicable. There is much evidence that T lymphocytes form spontaneous rosettes (termed "E rosettes") with washed sheep erythrocytes. Clearly, T lymphocytes do not have surface receptors for immunoglobulin or for complement; nor do they have a receptor for the Fc fragment of human immunoglobulins. It has been possible to separate T and B subpopulations of human peripheral blood lymphocytes by physical methods using these properties. In addition, T lymphocytes can be recognized by the blast-cell transformation test using phytohaemagglutinin (PHA test) or allogeneic lymphocytes (MLC test); here lymphocyte stimulation is detected by morphological enumeration of blast cells or by their ability to incorporate isotopically labelled thymidine.

Specific immunological reactivity of cultured lymphocytes. A number of *in vitro* tests have been and are currently being developed to detect specific immunological reactivity of T and B lymphocyte subpopulations. This discussion is limited to the two tests that have been most extensively applied to man. Antigen-induced blast-cell transformation is thought to detect primarily T-cell sensitization, although there are situations in which B cells are thought to respond. In order to assess the significance of lymphocyte transformation responses it is desirable to test reactivity to a panel of common antigens with which most individuals in the population may have had contact (e.g. tuberculin PPD, streptokinase-streptodornase, *Candida,* mumps). A positive lymphocyte transformation test in inferred when the number of blast cells in antigen-stimulated cultures exceeds by 3% the number in the control cultures or when there is a twofold (or greater) increase in thymidine incorporation relative

to the control cultures. The second widely used test of lymphocyte reactivity is that in which antigen inhibits the outward migration of leucocytes from cell suspensions packed in capillary tubes. In this test it is thought that specific antigen stimulates the lymphocytes to generate a migration inhibition factor whose action in this sytem is recognized by the inhibition of polymorph migration. A positive leucocyte migration test in inferred when specific antigen reduces leucocyte migration areas by 20% (or more) with respect to control cultures. In this test it is important to delineate toxic (i.e. nonimmunological) effects of antigens on the migration of leucocytes; accordingly, antigen preparations should be used at concentrations below those that inhibit leucocyte migration from individuals known not to be sensitized. As with lymphocyte transformation, the significance of leucocyte migration inhibition is best assessed by reference to other common antigens to which individuals may be expected to have developed sensitivity.

Technical considerations. For distinguishing T cells and B cells it is desirable to work with purified washed lymphocytes. Peripheral blood lymphocytes are best obtained by centrifugation through a solution containing "Ficoll" (a sucrose polymer) and sodium metrizoate (or sodium diatrizoate or diodone) at a specific gravity of 1.078 (Böyum, 1968); cells purified in this way can also be used for lymphocyte transformation studies. In the assessment of lymphocyte transformation it is desirable to employ a range of antigen concentrations and to determine the time course of response, and dose-response relationships should similarly be obtained in leucocyte migration. In lymphocyte transformation tests, the measurement of radiolabelled thymidine uptake is preferable owing to its greater precision and sensitivity; however, morphological assessment of blast cells correlates well with thymidine uptake if the cells are counted by an experienced observer. It is recommended that individual tests of lymphocyte transformation be carried out in duplicate or triplicate, and that in leucocyte migration six replicate capillaries be used at each antigen concentration. Both tests are now available in a miniaturized form that permits the use of smaller quantities of blood, making replicate tests and paediatric applications possible.

Immunological Responsiveness in Leprosy

While the humoral immune responses, as gauged by serum immunoglobulin levels and the ability to form antibodies to bacterial vaccines such as TAB, are unimpaired in patients with leprosy, there is increasing evidence of a depression of cell-mediated immunity in certain categories of patients suffering from leprosy. The degree of depression varies from the TT to the LL end of the spectrum in relation to the clinical, bacteriological, and histopathological status of the subject. The maximum impairment is noticeable in untreated patients with the polar form of lepromatous leprosy. As will be specified below, it appears that the depression of cell-mediated immune response is both specific and nonspecific. Peripheral leucocytes from untreated cases of lepromatous leprosy show poor transformation with PHA and *Myco. leprae* antigens. Upon treatment, the mitogenic response to PHA is improved, while anergy to *Myco. leprae* antigens is maintained over many years of treatment with sulfones. The status of immunological responses in various categories of patients with leprosy as shown by *in vivo* and *in vitro* tests is considered below.

In vivo *studies on cell-mediated immunity*

In vivo studies on cell mediated immune processes in leprosy have included the following tests: (1) reactivity to killed (autoclaved) suspensions of whole *Myco. leprae* bacilli (bacillary lepromin) and Dharmendra lepromin; (2) delayed hypersensitivity to other bacterial antigens, such as tuberculin (PPD); (3) active sensitization to 1-chloro-2,4-dinitrobenzene and 2-chloro-1,3,5-trinitrobenzene ("picryl chloride"); and (4) skin allograft rejection. The findings may be summarized as follows.

Lepromin testing. Patients with tuberculoid leprosy give uniformly strong reactions of the late type (Mitsuda reaction) but a variable degree of reactivity of the early type (Fernandez reaction). Patients with polar lepromatous disease give uniformly negative results in both tests. Patients with borderline leprosy show a variable degree of reactivity, depending on their place in the pathological spectrum—i.e. those with the BT and TT forms react similarly, while untreated patients with the BL form usually reveal a negative response (Myrvang, 1972).

Delayed hypersensitivity to PPD and other microbial antigens. In contrast to late lepromin, the reactivity to PPD does not bear a consistent relationship to the spectrum of disease in patients with leprosy. Variable proportions of positive and negative reactors are found in patients with tuberculoid, borderline, and lepromatous disease (Myrvang, 1972). Similar observations have been made with other microbial antigens (Turk and Bryceson, 1971).

Active sensitization. A high proportion of patients with lepromatous disease have been found not to respond to 1-chloro-2,4-dinitrobenzene, in contrast to healthy persons and those with the tuberculoid form of the disease (Turk and Bryceson, 1971). Similar findings have been noted with 2-chloro-1,3,5-trinitrobenzene (Bullock, 1968).

Skin allograft rejection. Patients with the lepromatous and, to a lesser degree, the tuberculoid form of the disease have revealed a delayed rejection of allogeneic skin grafts as compared with healthy individuals. The degree of impairment in patients with lepromatous leprosy was found to be correlated to bacillary concentration of the skin at the site of transplantation (Han *et al.*, 1971).

Assessment of lymphocyte function in vitro

The following methods have been used to assess cell-mediated immune response in patients with leprosy: (1) blast transformation of peripheral leucocytes in the presence of whole *Myco. leprae,* leprolin, other mycobacteria, and PHA; (2) mixed leucocyte culture, and (3) tests of the ability of sensitized lymphocytes to produce biologically active products.

Blast transformation. The method of lymphocyte transformation has recently been established for the study of lymphocyte reactivity to *Myco. leprae in vitro* (Bullock and Fasal, 1971; Godal *et al.*, 1971). While patients with TT leprosy respond quite strongly, those with the LL form of the disease regularly give negative results (Bullock and Fasal, 1971; Godal *et al.*, 1971). Those with BT leprosy show a variable degree of responsiveness, while those with untreated BL disease are usually negative (Myrvang, 1972). The failure of lepromatous lymphocytes to respond to *Myco. leprae* appears not to be reversed even after many years of chemotherapy (Godal *et al.*, 1972*a*).

Although lepromatous leucocytes fail to transform in the presence of *Myco. leprae,* they respond to a varying degree of other mycobacterial antigens, such as whole BCG and PPD (Myrvang, 1972). The level of reactivity appears to depend

on the status of treatment—i.e. untreated patients with lepromatous leprosy have revealed lower reactivity than those who received prolonged chemotherapy (Godal *et al.*, 1972).

Phytohaemagglutinin in solution induces the blast transformation of T cells and can thus be used as a measure of T cell responsiveness. The test can be performed on a micro scale with 50 μl of blood. Well-standardized methods that permit sensitive and repeatable measurements have been described (Mehra *et al.*, 1972). The mitogenic response of peripheral leucocytes to PHA is observed to be depressed in untreated cases of lepromatous leprosy, while tuberculoid and DDS-treated lepromatous cases show a normal response (Talwar *et al.*, 1972).

Peripheral leucocytes from untreated cases of lepromatous leprosy manifest low transformation when cultured in autologous as well as in AB serum (Mehra *et al.*, 1972). Furthermore, serum from patients with lepromatous leprosy fails to inhibit *Myco. leprae*-induced transformation of leucocytes from patients with the tuberculoid form of the disease, suggesting that transformation inhibitory factors are absent from these sera (Godal *et al.*, 1972).

Mixed leucocyte culture. The blast-cell responsiveness of lepromatous leucocytes (L x L) appear to be of similar degree to that of tuberculoid (T x T) leucocytes in mixed leucocyte cultures (Godal *et al.*, 1971). The response to phytomitogens, as compared with allogeneic cells, has so far not been studied in individual patients.

Production of lymphocyte factors. The supernatant fluid from cultures of *Myco. leprae* with lymphocytes from patients with untreated lepromatous leprosy does not aggregate guineapig macrophages (MAF) (Talwar *et al.*, 1972) or affect their migration (MIF) (Katz *et al.*, 1971). Moreover, the migration of lepromatous leucocytes is not inhibited when they are cultured in the presence of *Myco. leprae* (Myrvang, 1972). Patients with tuberculoid leprosy give a positive response in all these tests.

Humoral responses in leprosy

Immunogloublins. Increased levels of IgG, IgA, and IgM are frequently found in cases of lepromatous leprosy and *erythema nodosum leprosum,* although many patients have values within normal limits. IgD levels are within normal limits but IgE levels are sometimes higher than those in control samples from the same areas. The levels are somewhat higher in lepromatous leprosy than in the tuberculoid form of the disease, although there is considerable overlap. The production of antibodies to unrelated antigens such as typhoid/paratyphoid vaccines appears to be normal in patients with tuberculoid and lepromatous leprosy (Sheagren *et al.*, 1969; Jha *et al.*, 1971).

Antimycobacterial antibodies. Circulating antibodies against the polysaccharide antigen shared by *Myco. leprae* and other mycobacteria have been demonstrated by immunodiffusion, immunofluorescence, and indirect haemagglutination tests. A very high proportion of patients with lepromatous leprosy and a minority of those with the tuberculoid form of the disease have these antimycobacterial antibodies, and the antibody levels are higher in the former than in the latter. Specific antibodies against *Myco. leprae* have also been demonstrated by indirect immunfluorescence with sera from patients with lepromatous disease absorbed with cardiolipin and mycobacterial polysaccharide (Abe *et al.*, 1972*b*). These antibodies seem to react with protein antigens of *Myco leprae,* because the reaction is greatly reduced by autoclaving or trypsin digestion of bacilli. The

antibodies compete with rabbit antibodies against "nodular extract" antigen as judged by immunofluorescence reactions.

The antibodies specifically reacting with *Myco. leprae* are found in IgG and IgM classes, but none has yet been found in the IgA (Abe *et al.*, 1972). It would be of special interest to know whether IgA antibodies to *Myco. leprae* are present in secretory fluids such as nasal fluid.

Although irradiated or ALS-treated thymectomized mice can produce antibodies against heterologous proteins, the specific antibody response of these immunosuppressed mice against *Myco. leprae* and its relationship with the development of disease are not yet fully clarified. Antibodies against *Myco. leprae* have not yet been demonstrable in the immunosuppressed mice.

It would be interesting to investigate, using the model disease in mice, the possibility that a humoral response against *Myco. leprae* may inhibit the development of cell-mediated immune responses. Some intact mice infected with *Myco. leprae* develop a lepromatous type of disease after a long incubation period, and antibody against mycobacterial polysaccharide antigens has been observed by immunodiffusion. The contrast with T-cell-deprived mice suggests that a T-cell helper effect may be required for the formation of antibody against mycobacterial antigens. The presence of antimycobacterial antibodies in patients with lepromatous leprosy suggests the presence of T-cell function.

Autoimmunity. Since mycobacteria exert adjuvant effects, they might be expected to stimulate the formation of many autoantibodies. There have been many reports of increased autoantibodies in patients with lepromatous leprosy, but the findings have not been uniform. Cryoglobulins are common in India and Italy but are rare in Japan and Malaysia. The incidence of antinuclear factors, rheumatoid factors, antithyroglobulin antibodies, and cold autoantibodies is raised in patients with lepromatous leprosy in some countries but not in others. False positive results of serological tests for syphilis are common, and may be related to the presence in *Myco. leprae* of a cardiolipin antigen. Cytotoxic antibodies that react with lymphocytes from a panel of normal subjects at 15°C and lyse these cells in the presence of complement have been found in patients with lepromatous and, to a lesser extent, tuberculoid leprosy. Further comparative observations on autoimmune reactions in leprosy would be of interest, especially the evaluation of the possible role of autoantibodies against lymphocytes in the generalized depression of T-cell function often found in the lepromatous form of the disease. Such antibodies should be tested against lymphocytes from the same patient rather than from other subjects. It would also be of interest to know whether autoimmune reactions against nervous tissue contribute to peripheral nerve injury in a manner analagous to experimental allergic neuritis.

Allergic reactions and nerve damage in leprosy

Allergic reactions. Two types of allergic reaction in leprosy were described in *Bull. Wld Hlth Org.* (1970)—*erythema nodosum leprosum* and reversal reactions.

Erythema nodosum leprosum occurs in highly bacilliferous patients (BL-LL) and both the local and systemic manifestations observed suggest that immune complexes are involved in the pathogenesis of these lesions. Moreover, IgG and β1c/β1a (components of C3) have been demonstrated in the lesions by immunofluorescence (Wemambu *et al.*, 1969). Further support for this concept has recently been provided by the finding of immune complexes in the serum of

such patients as demonstrated by precipitation with Clq (Moran *et al.,* 1972; Estrada-Parra, 1973). However, as a proportion of patients with lepromatous leprosy but without *erythema nodosum leprosum* also show complexes by this technique, further studies–including more detailed characterization of the antigen and the antibody in these complexes–are needed. There appears to be no difference in the immunoglobulin levels in patients with *erythema nodosum leprosum* and in those without this condition but with lepromatous leprosy; however, complement (C2 and C3) is raised (*Bull. Wld Hlth Org.,* 1970).

Reversal reactions may occur throughout the spectrum of leprosy, with the exception of the two polar groups as defined by the Ridley-Jopling classification. Such reactions often appear "spontaneously" in untreated patients, but may also be precipitated by chemotherapy against *Myco. leprae.* Some observations suggest that BCG vaccination or exposure to *Myco. tuberculosis* may precipitate such reactions.

Present evidence indicates that reversal reactions are due to a rapid increase of cell-mediated immune response to *Myco. leprae* for the following reasons: (1) reversal reactions are associated with a shift in histological classification, both in lesions and in lymph nodes, towards the tuberculoid end of the scale (Souza Lima and Cerqueira, 1946; Souza Lima and Rath de Souza, 1949; Souza Lima, 1953; Ridley, 1969; Turk and Waters, 1971); (2) patients with reversal reactions reveal strong responses to *Myco. leprae in vitro* as measured by lymphocyte transformation and leucocyte migration inhibition (Godal, 1972); and (3) mice with high bacillary loads develop, following injections of syngeneic lymphoid cells, changes in their lesions that resemble the reversal reaction in man (Rees, 1970; Gaugas *et al.,* 1971).

Nerve damage. Peripheral nerves are affected in all forms of leprosy and during allergic reactions. In tuberculoid leprosy the histological lesion in the nerve is the same as in the skin, but can also sometimes produce caseation ("abscess"), both resulting in destruction of nerve fibres with subsequent fibrosis. In lepromatous leprosy nerves are heavily infected with bacilli, mainly in Schwann and perineurial cells. Although the infected nerves are not damaged initially in lepromatous leprosy, neuritis eventually develops in a high proportion of such patients, and the perineurium is mainly affected. In patients developing reversal reactions or *erythema nodosum leprosum,* cellular changes corresponding to those in the skin occur in nerves, leading to severe damage to nerve fibres.

Experimental leprosy in the mouse also results ultimately (8-30 months) in nerve involvement and has provided a model for studying in detail the evolution of leprosy neuritis and the effect of "reversal reactions" on infected nerves (Rees and Weddell, 1968; Weddell *et al.,* 1971). Thus, in reversal reactions, oedema formation within the nerves is an important cause of nerve fibre destruction. In mice with leprosy the perineurial cells are frequently damaged and later there is also increased permeability of the capillaries in the nerves (Boddingius *et al.,* 1972).

The observations in the mouse suggest that leprosy neuritis may be due to a change in the endoneural environment following a defect in the blood and/or perineurial nerve barrier. Further comparative observations on human leprosy neuritis would be of interest, especially evidence of such a defect in man and for an immunological origin of the defect.

Immunological Problems in Leprosy Research: 2

Part 2 of this Memorandum covers possible mechanisms of altered immune response in leprosy (including a tentative scheme to explain the possible genesis of the lepromatous lesion); genetic, nutritional, and hormonal factors; the possibility of vaccination; attempts at immunotherapy; and areas in which further research is needed. A detailed protocol for evaluating the effect of transfer factor in leprosy is included as an appendix.

Possible Mechanisms of Altered Immune Responsiveness in Leprosy

Depression of specific immunological responses

Humoral and cell-mediated immunity. In complete immunological tolerance there is a failure to mount immune responses to a particular antigenic determinant together with a normal ability to respond to other antigens. This can be induced by exposure to a wide variety of antigens in high dosage or, in the case of soluble protein antigens, by the repeated administration of low doses. High-dose tolerance appears to involve the elimination or inactivation of both B and T cells with specificity for the specific antigen. In contrast, low-dose tolerance appears to involve the selective inactivation of specifically-reactive T cells leaving B-cell function intact. Immunological tolerance may not always be absolute, and various degrees of suppression of specific cell-mediated immune responses and specific antibody formation have been described. The duration of tolerance depends on the dose of antigen used to induce the unresponsive state.

Certain individual animals are genetically incapable of mounting an effective immune response to particular antigenic determinants. Genetic unresponsiveness is controlled by "immune response" (Ir) genes, which may be linked to genes controlling major histocompatibility antigens or to genes controlling immunoglobulin structure (e.g. allotypy of immunoglobulins). Other types of similar unresponsiveness may be under multifactorial genetic control.

Selective suppression of cell-mediated immunity. A form of partial immunological unresponsiveness that primarily affects T-lumphocyte function can result from the administration of protein or microbial antigens by a route or in a physical form that does not normally produce cell-mediated immunity. This phenomenon of antigen-mediated depression has been referred to as "immune deviation". While the mechanisms underlying this phenomenon have not been fully established, it is possible either that the T cells are selectively rendered tolerant or that prior induction of antibody deviates antigen away from engaging T cells. In contrast, "immunological enhancement" refers to the situation in which sensitized lymphocytes present in an individual are unable to effect cell-mediated immunity because of blocking by antibodies or antigen-antibody complexes. The simplest mechanism is that in which antibody or antigen-antibody complexes cover antigens on target cells, thereby rendering them inaccessible to sensitized T cells. These forms of immuno-depression can be differentiated by ascertaining the extent of T-cell desensitization and the ability of serum from immunized donors to block specific T-cell function *in vivo* or *in vitro*.

Desensitization of cell-mediated immunity can be achieved by the injection of antigen in large or repeated doses. It is believed that sensitized T cells are activated in the circulation, exhausted of their capacity to produce mediators, and

unable to respond when they encounter antigen in the tissues. Unless antigen is administered repeatedly, sensitization usually reappears.

In one type of immunodeficiency, and in some collagen autoimmune diseases, depression of T-cell function has been related to the presence of circulating lymphocytotoxic autoantibodies. In this situation, cell-mediated immune responses to a wide variety of antigens are suppressed.

Selective suppression of antibody. Under physiological conditions, the formation of specific antibody is under feedback control. This is mediated by IgG antibodies that selectively suppress the formation of IgG and IgM antibody directed against the same antigenic determinants. The immunization of animals with low doses of antigen can lead to the formation of small amounts of high-affinity antibody that can suppress antibody formation on subsequent exposure to larger amounts of the same antigen.

T cells not only exert a "helper" function in antibody formation but also exert selective feedback inhibition of specific antibody formation (e.g. allotype and autoantibody suppression).

Antigenic competition. There is experimental evidence that cell-mediated and humoral responses to a given antigen may be suppressed by the prior or simultaneous injection of unrelated antigens. This phenomenon is referred to as "antigenic competition" and is thought to result from the preemption of T and B cells or from competition for pathways of antigen processing in macrophages. Experiments have been described in which antigenic competition may take place between different determinants on the same antigenic macromolecule.

Mechanisms by which specific immune responses are increased

Adjuvants. The administration of antigen with adjuvants may increase antibody formation, cell-mediated immune responses, or both. Some adjuvants, such as alum or vitamin A, selectively increase antibody formation but not cell-mediated immunity. Killed mycobacteria in oil (Freund's complete adjuvant) or, under certain circumstances, BCG increase cell-mediated immunity to a greater extent than antibody formation, although this effect is not always predictable. Recent studies suggest that bacterial and other adjuvants such as poly-A: poly-U exert effects directly on macrophages and indirectly through the proliferation of T lymphocytes (Allison, 1973). Such adjuvant effect disappear in animals deprived of T lymphocytes. Certain adjuvants such as poly-A: poly-U added *in vitro* to leucocyte cultures increase reactions carried out by T cells, such as mixed lymphocyte reactions and responses to tuberculin.

While it would be of great importance to have adjuvants that could increase cell-mediated immunity in patients with lepromatous leprosy, the possible hazards involved in the use of adjuvants should be recognized. Among these are the danger of producing *erythema nodosum leprosum*, increasing antibody formation rather than cell-mediated immunity, and the possible precipitation of autoimmune complications, such as allergic neuritis.

Composition and chemical modification of antigens. The antigenic determinants recognized by T and B lymphocytes are often different. Certain antigens, such as chemical contact sensitizers, certain basic proteins of membranes, and schistosome egg antigens, preferentially stimulate cell-mediated immune responses. The chemical modification of antigens—e.g. the aceto-acetylation of proteins (Parish and Lieuw, 1972) or coupling with fatty acids—selectively stimulates cell-mediated immune responses rather than antibody

formation. Analogous modification of *Myco. leprae* or cross-reacting antigens might be used to stimulate cell-mediated immune responses in patients with leprosy.

The administration of very small doses of antigen, or of antigen-antibody complexes in the correct proportions, can also favour cell-mediated immunity rather than antibody formation. The route of administration may also affect the outcome. Chemical sensitizers induce cell-mediated immune responses when applied to the skin, whereas administration by the oral or intravenous route can prevent the eliciting of contact sensitivity on subsequent application to the skin.

Lymphoid cell transfer. The injection of viable lymphocytes from specifically sensitized donors into nonsensitized recipients of the same species can confer both specific cell-mediated immunity and enhanced specific antibody production on the recipient. The extent and duration of these responses is usually greater if donor and recipient are syngeneic. The ability of such recipients to manifest cell-mediated immunity (e.g. delayed hypersensitivity) is thought to depend on adequate survival of the donor's T cells; thus cell transfer of cell-mediated immunity is ineffective between animals of different species. The injection of both sensitized lymphoid cells and the specific sensitizing antigen may confer on syngeneic recipients on ability to give enhanced cellular and humoral responses to an unrelated antigen. This can also occur if sensitized or non-sensitized lymphoid cells are injected into allogeneic recipients; this phenomenon, termed the "allogeneic cell effect", is thought to be mediated by activation of the recipient's T cells and possibly macrophages also.

Injection of sensitized lymphoid cell extracts. Cell-free extracts of peripheral blood leucocytes from human subjects with delayed hypersensitivity to bacterial antigens can apparently confer a state of specific delayed hypersensitivity when injected into nonsensitized human subjects. Transferred sensitivity may persist for some weeks or months, and its duration is thought to be extended by the repeated injection of leucocyte extract. The active principle, termed "transfer factor", is found in RNA-rich dialysate fractions of human leucocyte extracts; dialysable human transfer factor is not active when injected into experimental animals, nor can such transfer factor be generated by species other than man. However, resistance to certain experimental tumours has been stimulated by the injection of macromolecular RNA derived from cells from specifically immunized donor animals. There is some doubt as to the specificity with which cell-mediated immunity is stimulated; thus an adjuvant-like effect of transfer factor (in man) or RNA preparations (in animals) has not been excluded.

Tentative scheme for the possible genesis of leprosy lesions

Studies in mice suggest that an infection with *Myco. leprae* can be established with about five viable bacilli. After a long period these grow exponentially with a doubling time of 12-13 days for a limited period, after which the rate of growth declines, even in immunosuppressed mice. It is likely that the undetected early stages of the human infection are similar. Many infections probably show spontaneous cure before the number of organisms is sufficiently large to produce a clinically detectable lesion. Since tuberculoid lesions can show spontaneous cure, it seems reasonable to postulate that a microtuberculoid reaction is involved in the recovery from infection at the subclinical level, and the demonstration of specific lymphocyte transformation with *Myco. leprae* antigens in contacts of leprosy cases supports this view.

One possiblity is that in the absence of any immunological reaction the number of organisms can ultimately become sufficiently large to produce clinically detectable lesions. These would be the indeterminate cases, in which it may be postulated that lesions result from simple infiltration with macrophages, in a manner analogous to a foreign-body reaction without any immunological component. Infiltrating macrophages become infected and there may be local depigmentation.

Immune responses to *Myco. leprae* antigens somehow occur later in the course of bacterial multiplication. If there is effective cell-mediated immunity, a clinically apparent tuberculoid lesion develops while the number of organisms is relatively small. The predominant feature of such lesions is cell-mediated immunopathology.

There are two possible explanations for the evolution of a polar lepromatous lesion from an indeterminate one: (1) that as the number of infected macrophages increases the character of the lesion changes without any local allergic reactions; and (2) that there is a chronic antibody-mediated immunopathological reaction somewhat analogous to extrinsic allergic alveolitis in the lung. The generation of small amounts of immune complexes over a long period might facilitate the recruitment of macrophages to produce a pure macrophage granuloma with mild fibrogenesis. Experience with the lung shows that in chronic antibody-mediated immunopathology, bound immunoglobulin and complement are not usually detectable in lesions, so that it may be difficult to ascertain directly whether there is any immunopathological component in the lepromatous lesions.

Irrespective of its role in lepromatous lesions, antibody-mediated immunopathology probably makes an important contribution to *erythema nodosum leprosum*. Various possible explanations exist for the susceptibility of some but not other patients with lepromatous leprosy to this complication. One is that the quantity or nature of the antibodies against *Myco. leprae* antigens is different in patients who develop *erythema nodosum leprosum*. For instance, they might have antibodies belonging to a complement-fixing subclass of immunoglobulin, whereas insusceptible patients do not. Alternatively, susceptible patients might have IgE as well as IgG antibodies. Reactions of *Myco. leprae* antigen with IgE might increase vascular permeability, thereby promoting local accumulation of IgG or IgM antibodies, which on reaction with antigen might induce polymorph infiltration, degranulation, and other signs of Arthus-type hypersensitivity.

In borderline cases of leprosy, both cell-mediated and antibody-mediated immunopathological reactions may be present in varying degrees, depending on the position of the case in the range between the polar forms of the disease.

Immunopathological reactions are of special interest in relation to the mechanisms of nerve damage in leprosy. Cell-mediated immunopathological reactions are of major importance in the tuberculoid forms of the disease and during the course of reversal reactions. Chronic antibody-mediated immunopathology may play a role in stimulating fibrogenesis in peripheral nerves in chronic lepromatous disease. Antibody-mediated allergic reactions in a patient with *erythema nodosum leprosum* may cause acute aggravation of nerve damage.

It is useful to make a distinction between the presence of systemic immune responses, humoral and cell-mediated, and localizing factors that ensure that these are manifested at the sites of bacterial multiplication. Little is known about initial localizing factors in cell-mediated immunity, although once a local reaction occurs

it may be increased through autocatalytic events by which more specifically sensitized T cells enter reaction sites. Perhaps reactions to BCG or other antigens might be used to facilitate localization of sensitized T cells in lesions. The possibility should also be considered that antibody-mediated reactions may help to localize T cells that are reactive against *Myco. leprae* antigens. These concepts are illustrated in Fig. 1.

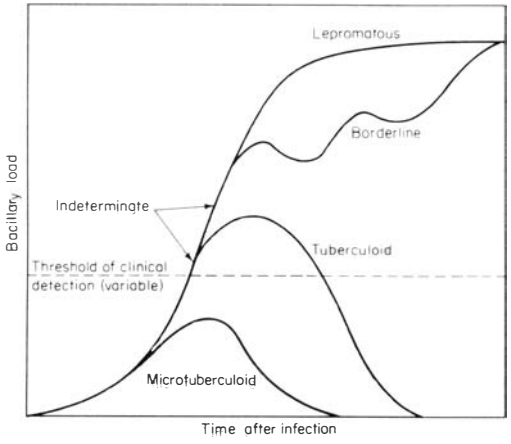

Fig. 1. Tentative scheme of the possible genesis of leprosy lesions.

Evaluation of immunological mechanisms in lepromatous leprosy

Of the possible mechanisms of selective unresponsiveness in lepromatous leprosy, complete tolerance of B and T cells to all antigens of *Myco. leprae* can be excluded, since high levels of antibodies to some antigens of *Myco. leprae* are frequently detectable in the sera of patients with lepromatous leprosy. However, it is still possible that complete tolerance is induced to a small number of *Myco. leprae* antigens that are important with respect to cell-mediated immunity.

Immunological enhancement appears unlikely since (1) serum from patients with lepromatous leprosy fails to inhibit the *in vitro* response of lymphocytes from patients with tuberculoid disease to *Myco. leprae*, and (2) lymphocytes from patients with lepromatous leprosy do not respond to *Myco. leprae* in normal human serum. The desensitization of cell-mediated immunity also appears unlikely because patients with lepromatous leprosy fail to respond to *Myco. leprae*, even when the antigenic load has been reduced or eliminated by treatment.

The available data do not permit a distinction between selective T-cell tolerance or antibody-induced deviation of T-cell sensitization.

Genetic, Nutritional and Hormonal Factors

A recent study on 102 twin pairs indicated that among monozygotic twins there was a high concordant incidence (59.7%, 37/62) of leprosy in both siblings—i.e. both twins were affected. In the dizygotic pairs, the concordant incidence of leprosy was lower (20%, 8/40). These observations suggest that genetic constitution is a predisposing factor to leprosy.

These studies also showed that in 40% of monozygotic twins one member of the twin pair remained free of any clinical manifestation of the disease. Moreover, the type of leprosy in five identical twins was different. These results indicate that factors other than genetic composition—e.g. intensity and type of infection, nutritional status, and other systemic factors—may be important. Some recent studies have pointed to a notable effect of protein-calorie malnutrition on immune responses (WHO Scientific Group on Cell-Mediated Immunity and Resistance to Infection, 1973). Other systematic factors, such as hormones, have also been observed to affect lymphoid organs (Pandian and Talwar, 1971; Talwar, 1972).

Studies on the interaction of genetic, hormonal, nutritional, and other predisposing factors should be extended with a view to defining their role in the susceptibility of a subject to leprosy.

Vaccination

Vaccination in man

The development of a specific vaccine for the prevention of leprosy in handicapped by the present inability to culture *Myco. leprae in vitro*, and vaccination efforts have been restricted to trials with BCG.

The United Kingdom Medical Research Council trial in Uganda (September 1960-September 1970) (Brown and Stone, 1966; Brown *et al.*, 1968*a*, *b*, 1969) was confined to child contacts and relatives of known cases of leprosy (BCG group, 8085; control group, 8065). By the end of the fourth follow-up examination (1970), 201 cases of leprosy had been diagnosed in the unvaccinated children of the primary (main) intake, and 41 cases in the BCG-vaccinated children. This represents a reduction in incidence of 80% attributable to the BCG vaccination. The efficacy of BCG does not appear to be associated with any of the following factors: sex, age at intake, and type of leprosy (lepromatous or non-lepromatous) in the index case. Findings also showed that there is a slight falling-off in the efficacy after the second follow-up among the children of the main intake.

In Karimui (New Guinea), a study has been carried out on about 5000 persons of all ages. The findings (Scott *et al.*, 1966; Russell *et al.*, 1968) indicated, with regard to leprosy incidence (1963-1969), that the efficacy of BCG vaccination was 46% for the total population, 47% for males (all ages), 47% for females (all ages), 44% for children aged 0-9 years, 56% for children aged 5-14 years, 56% for the age group 20-29 years and 25% for persons aged over 30 years.

The WHO trial in Burma, started in August 1964, is concerned with a child population, mainly not exposed at home (BCG group, 14,108; control group, 14,112) (Bechelli *et al.*, 1970). The findings up to the end of June 1971, which related to six annual follow-up examinations, were 285 cases of leprosy in the BCG group and 325 cases in the control group. The incidence rates in the age group 5-9 and 10-14 years were similar in the vaccinated and unvaccinated groups, irrespective of tuberculin reaction. However, in the group aged 0-4 years at intake, BCG-vaccinated children had a lower rate than those of the control group—a protection rate of 44.2%. The proportions of indeterminate, tuberculoid, and tuberculoid in reaction cases were similar in both trial groups.

The findings concerning the incidence in the three trials are strikingly different and relate only to early cases of leprosy. The interpretation of these results

requires further data on: forms of leprosy, bacterial status, lepromin reactivity, evolution of cases, and level of endemicity. Once this information is available, it should be possible to appraise the preventive effect of BCG in leprosy and the impact the vaccine may have on the trend of the disease.

Experimental immunity in mice

Protection is observed as a reduction in the number of *Myco. leprae* that grow in vaccinated mice. Heat-killed vaccines prepared from various mycobacterial cultures have been compared and *Myco. tuberculosis* has been found to confer the greatest degree of protection. Living BCG was at least as effective, and the effect was not much reduced when the BCG organisms were heat-killed. The route of administration has been studied with living BCG, the intravenous route being most effective, although the interdermal and intraperitoneal routes were not much inferior; a low dose given in the foot was sometimes highly effective. The degree of protection was dose-dependent and was still detectable with 1×10^6 BCG bacilli given intradermally. Vaccine given after challenge was effective.

Reinfection experiments with *Myco. leprae* given in a new site (the other footpad) have usually failed to indicate any immunity, and even though the first infection showed a plateau, the mice usually developed a second infection that was fully comparable to a primary one.

Mice experimentally inoculated with *Toxoplasma gondii* develop chronic infection with persisting antigen and delayed hypersensitivity. Their macrophages are activated, as demonstrated morphologically and by the ability of the macrophages to kill *Listeria.* Such mice have been found to be partially resistant to *Myco. leprae.* The immunity is increased if *Toxoplasma* antigen is given locally in the footpad.

Attempts at Immunotherapy

Attempts at immunotherapy have consisted chiefly in the injection of peripheral leucocyte suspensions and of leucocyte extracts in an attempt to increase cell-mediated immunity in cases of lepromatous leprosy.

Injection of allogeneic lymphocytes

Three groups of workers have reported the intravenous injection of allogeneic lymphocytes into patients with lepromatous leprosy. Paradisi *et al.* (1969) observed the acquisition of Fernandez sensitivity in four of 13 patients who received leucocytes from normal healthy subjects with intense positive tuberculin reactions. Saha *et al.* (1971) injected, on three successive occasions, peripheral lymphocytes from healthy donors with strong Mitsuda sensitivity into four patients who showed dapsone intolerance; the patients showed clinical, histological, and bacteriological improvement. Improvement in clinical status was maintained for 2½ years. One patient developed Fernandez sensitivity after five months but none developed Mitsuda reactions (Saha *et al.*, 1972). In a third study Lim, Fusaro and Good (personal communication) treated 15 patients with lepromatous, mixed, or tuberculoid leprosy with 8-10 successive weekly injections of leucocytes from different healthy donors either presumed to be lepromin positive or shown to be lepromin negative and tuberculin negative. Each leucocyte infusion was given from a separate donor, and donors and recipients were deliberately mismatched to facilitate reactions with donor cells and to avoid early

immune elimination of the cells. Patients of all three groups showed clinical, histological, and bacterial improvement. The improved clinical status was maintained for four months. With respect to the prolonged response to leucocyte injections, the second and third of the reports noted above are similar.

Infection of leucocyte extracts (transfer factor)

Bullock *et al.* (1972) prepared dialysable leucocyte extracts from lepromin-sensitive healthy subjects and injected the preparations subcutaneously into nine patients with lepromatous disease on one occasion. Within seven days, six of the patients developed weak Fernandez reactions and experienced transient inflammation of leprous skin infiltrates. Lymphocyte transformation to *Myco. leprae* antigen remained negative and there was no long-term improvement in clinical status. Mittal *et al.* (unpublished data, 1972) prepared nondialysed leucocyte extracts from healthy Indian subjects and injected the extracts intravenously into four patients with lepromatous leprosy on three occasions at monthly intervals. During a 5-month period, two patients developed positive Mitsuda tests, but no clinical, histological, or bacteriological improvement was obtained.

Areas for Further Research

(1) Animal experiments should be undertaken using killed mycobacteria and isolated mycobacterial antigens—e.g. "nodular extract" and cell walls—antigen being administered (e.g. intravenously, in low doses, by repeated feeding, and intranasally) so as to bring about preferential stimulation of antibody formation. Challenge with infectious mycobacteria and skin-test antigens would indicate whether prior antibody formation can suppress cell-mediated immune responses to these antigens as predicted by an immune deviation model.

(2) The transfer of lymphoid cells to non-immuno-suppressed "lepromatous" mice with high levels of *Myco. leprae* antibodies would indicate whether T cells can function in this situation. The occurrence of "reversal reactions" induced by the transferred cells would suggest a state of T-cell tolerance; the failure of the cells to induce such reactions would also be consistent with immune deviation.

(3) The class and subclass of antibodies to *Myco. leprae* in the serum, infected tissue, and nasal secretions of patients with all forms of the disease should be determined. This information may help to predict which lepromatous patients are more likely to develop *erythema nodosum leprosum*—e.g. whether only patients with antibodies in complement-fixing classes or subclasses are susceptible to this complication. Regional differences in susceptibility to the condition may be explicable on this basis. These studies may also help to clarify the role of local antibody formation in the immunopathology of leprosy. A search should be made for reaginic antibodies to *Myco. leprae* antigens. Likewise, the presence of such antibodies may contribute to susceptibility to *erythema nodosum leprosum*.

(4) It would be of interest to determine whether the failure of some patients with indeterminate leprosy to become lepromin positive following treatment is related to the level and class or subclass of antibodies before and following treatment. A correlation between the presence of such antibodies before or during treatment and failure to develop cell-mediated immunity would be most consistent with the immune deviation model.

(5) It is important to use modern techniques to characterize more fully the nature of the cellular defect in lepromatous leprosy. The number of lymphocytes,

the proportion of T cells (E rosettes) and B cells (EAC rosettes), and responsiveness in the lymphocyte transformation test to a battery of common antigen should be determined.

(6) Immunological techniques recently applied to leprosy (particularly the specific lymphocyte transformation test) should be widely employed (a) in field studies on the reactivity of contacts and non-contacts of patients with leprosy in a geographically-defined endemic leprosy area; and (b) in population samples in the three BCG trial areas.

(7) Further *in vitro* studies are needed on the interaction between lymphocytes and macrophages in relation to host resistance to *Myco leprae.* The studies should be designed so that the role of macrophages, lymphocytes, and products of activated lymphocytes in both the induction and expression of immunity may be separately analysed. It is recommended that recently established *in vitro* methods for studying lymphocyte-mediated modification of antibacterial activity of macrophages be used.

(8) It would be of interest to establish whether *in vitro* transformation of human lymphocytes by *Myco. leprae* antigens is due to T lymphocytes. Selective enrichment or depletion of T cells by E-rosette formation, antisera reacting specifically with T cells, or removal of B cells by immunoabsorbants would help to decide the point.

(9) A possible role for T-cell inhibition of T-cell reactivity in the lepromatous form of the disease might be evaluated in mixed lymphocyte cultures. Positive suppression would be expected if the leucocytes of an identical twin with tuberculoid leprosy were cultured in the presence of antigen together with leucocytes of the other twin with lepromatous leprosy. Sufficient numbers of identical twins who are discordant for leprosy type are available to allow this distinction to be made.

(10) The availability of concordant and discordant leprosy cases amongst identical twins should also provide a useful opportunity to test the hypothesis relating to specific and non-specific depression of cell-mediated immunity.

(11) The regulation of macrophage proliferation should be investigated in patients with various types of leprosy by measuring serum levels of colony-stimulating factor and its inhibitor. The agar-culture technique should be used to determine the incidence and proliferative activity of macrophage precursors in the bone marrow, blood, and local lesions of patients with leprosy.

(12) Further studies are needed on the biochemical mechanisms by which macrophages kill and degrade intracellular *Myco. leprae.* Attempts should be made to establish whether human macrophages activated by products of activated lymphocytes or colony-stimulating factor are able to inhibit the multiplication of *Myco. leprae.* The use of colony-stimulating factor and newly-developed media may facilitate the establishment of long-term human mononuclear phagocyte cultures which are required for this purpose. Such studies may help to explain the mechanism by which the body eliminates mycobacteria in tuberculoid leprosy, although not lepromatous leprosy.

(13) It would be of interest to determine whether a primary nasal infection is associated with a greater risk of developing lepromatous leprosy than a primary skin infection. In practice, nasal infection could be sought in contacts of patients with lepromatous leprosy and in early indeterminate cases. The incidence of persisting lepromin negativity in such cases as compared with those lacking evidence of nasal infection could be determined. Such studies may indicate

whether routes of infection other than through the skin predispose to lower cell-mediated immunity.

(14) It would be of interest to follow the lepromin reactivity *in vivo* and *in vitro* of babies born of mothers with lepromatous leprosy where *Myco. leprae* had been found in the placenta or cord blood. While little is known about the induction of immunological tolerance in neonate humans, the failure of such children to become Mitsuda positive would be consistent with the immunological tolerance model.

(15) While assessing the role of BCG in protection against leprosy it would be of interest to compare the effect of BCG inoculated directly into skin lesions with that of inoculation at other sites. Intra-lesion inoculation may help to localize T-cell effects at the sites of lesions.

(16) Further studies on autoimmunity in patients with leprosy from different populations should be undertaken, with special reference to evaluating the possible role of autoimmune reactions in nerve damage.

(17) The possible presence of autoantibodies that are cytotoxic for lymphocytes should be explored, particularly by comparison between patients with lepromatous leprosy showing unimpaired cell-mediated immunity to unrelated antigens and those with a generalized nonspecific depression of cell-mediated immunity.

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Abe, M. *National Institute of Leprosy Research, Tokyo, Japan.*
Allison, A. C. *Clinical Research Centre, Northwick Park Hospital, Harrow, England.*
Bechelli, L. M. *Ribeirão Prête Faculty of Medicine, University of São Paulo, Brazil.*
Bloom, B. R. *Department of Microbiology and Immunology, Albert Einstein College of Medicine, New York, N.Y., U.S.A.*
Dumonde, D. C. *The Mathilda and Terence Kennedy Institute of Rheumatology, London, England.*
Godal, T. *Armauer Hansen Research Institute, Addis Ababa, Ethiopia.*
Goodman, H. C. *World Health Organization, Geneva, Switzerland.*
Hopwood, B. E. C. *The Wellcome Trust, London, England.*
Metcalf, D. *Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Parkville, Victoria, Australia.*
Olakowski, T. *WHO Regional Office for South-East Asia, New Delhi, India.*
Pervuhin, J. V. *Research Institute for the Study of Leprosy, Astrakhan, U.S.S.R.*
Rees, R. J. W. *National Institute for Medical Research, London, England.*
Saha, K. *Department of Bacteriology, G. B. Pant Hospital, New Delhi, India.*
Sansarricq, H. *World Health Organization, Geneva, Switzerland.*
Shepard, C. C. *Leprosy and Rickettsial Diseases Unit, Centre for Disease Control, Atlanta, Ga., U.S.A.*
Talwar, G. P. *Department of Biochemistry, All India Institute of Medical Sciences, New Delhi, India.*
Torrigiani, G. *World Health Organization, Geneva, Switzerland.*

Résumé

Problèmes d'Immunologie dans la Recherche sur la Lèpre

Le présent document fait le point des connaissances actuelles en matière d'immunologie de la lèpre et expose les progrès accomplis dans ce domaine depuis la publication d'une revue similaire en 1970.

L'épreuve la plus anciennement utilisée pour évaluer l'état immunitaire de l'organisme à

l'égard de *Myco. leprae* est la réaction tardive à la lépromine. Les renseignements les plus significatifs donnés par cette réaction sont indiqués.

Des méthodes plus récentes permettant d'analyser l'état de l'immunité à support cellulaire, telles la transformation lymphoblastique on l'inhibition de la migration des leucocytes, ont été appliquées à l'étude de la lèpre. Il est de plus en plus démontré que l'immunité à support cellulaire est déprimée dans certaines formes de lèpre. Le niveau de l'immunité à support cellulaire baisse du type polaire tuberculoïde au type polaire lépromateux selon une gradation continue correspondant aux divers degrés d'atteinte clinique et bactériologique. La dépression est à son maximum chez les malades lépromateux non traités. Cette dépression de l'immunité à support cellulaire possède deux composantes, l'une spécifique de *Myco. leprae*, l'autre non spécifique. En ce qui concerne la composante spécifique, il est probable qu'elle se développe par un mécanisme de "déviation immunitaire" mais il n'est pas possible pour l'instant de préciser si la tolérance à *Myco. leprae* est sélectivement induite au niveau des lymphocytes T ou si des anticorps circulants prélablement formés réagissent avec les antigènes, les empêchant ainsi d'entrer en rapport avec ces lymphocytes T.

On a pu montrer l'existence d'anticorps circulants dirigés contre un antigène polysaccharidique commun à *Myco. leprae* et à d'autres mycobactéries. Ces anticorps sont trouvés chez la plupart des lépromateux et chez quelques cas tuberculoïdes. Des anticorps spécifiques de *Myco. leprae* appartenant aux IgG et aux IgM ont été également mis en évidence dans les sérums de lépromateux.

La présence d'autoanticorps chez les malades lépromateux a été maintes fois signalée, mais les différents autoanticorps ont une fréquence variable suivant les pays.

Des crises d'érythème noueux lépreux apparaissent chez certains malades lépromateux très bacillifères. L'hypothèse selon laquelle elles sont déclenchées par la formation d'immunocomplexes, suggérée par les manifestations cliniques, est étayées par la mise en évidence d'IgG et de certains composants du complément au niveau des lésions, ainsi que d'immunocomplexes dans le sérum. Toutefois, on observe aussi des immunocomplexes dans le sérum de lépromateux ne présentant pas d'érythème noueux lépreux. De nouvelles recherches sont de ce fait nécessaires pour déterminer l'origine précise et le mécanisme exact des crises d'érythème noueux lépreux.

Quant à la réaction d'inversion (*reversal reaction*), elle est liée à une rapide augmentation de l'immunité à support cellulaire comme le montrent les modifications histologiques observées au niveau des lésions et des ganglions lymphatiques, l'apparition de tests positifs pour ce type d'immunité et certains faits observés chez la souris d'expérience.

Il est possible que des mécanismes immunologiques jouent, dans le développement des lésions nerveuses de la lèpre, un rôle beaucoup plus large que celui avanché jusqu'ici.

Certains faits suggèrent que des facteurs génétiques prédisposent à contracter la lèpre, tandis que le rôle possible de facteurs nutritionnels et hormonaux demande à être étudié.

Alors que les résultats de trois essais contrôlés sur le terrain du vaccin BCG dans la prévention de la lèpre sont jusqu'ici nettement différents entre eux, chez la souris le vaccin BCG et d'autres vaccins mycobactériens confèrent un certain degré de protection contre *Myco. leprae* inoculé dans le coussinet plantaire.

Le dernier chapitre formule des recommandations touchant aux nouvelles recherches qui devraient être effectuées. La majorité des problèmes proposés à l'attention des chercheurs ont trait aux rôles respectifs des différents facteurs intervenant pour créer les situations immunologiques diverses rencontrées dans le cadre de la lèpre. D'autres sont des problèmes d'épidémiologie, de pathogénie, ou même de bactériologie auxquelles certaines techniques immunologiques modernes pourront apporter des solutions.

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Appendix

Protocol for Evaluating Transfer Factor in Leprosy

Purpose

(1) To ascertain whether transfer factor engenders immune reactivity to *Myco. leprae* antigens *in vivo* and *in vitro* and, concomitantly, whether it changes the pathological classification and/or increases the rate of clearance of bacteria.

(2) To determine whether transfer factor prepared from donors with a high degree of hypersensitivity to *Myco. leprae* is more efficacious than that prepared from non-sensitive donors. Once this determination has been made, it would not be necessary to include transfer factor from both sensitized and non-sensitized donors in future protocols.

Rationale. (a) The ultimate goal of immunotherapy in patients with lepromatous leprosy is both to terminate their infection and to prevent recurrence. However, evaluation of the effects of transfer factor in terms of marked clinical change would require a large study with a long period of observation. A more limited trial to assess the effects of transfer factor on the immunological and histopathological status of these patients is therefore recommended.

(b) While most clinical trials of transfer factor have used material prepared from highly sensitized donors, the possibilities that it acts non-specifically and that when prepared from normal donors may be equally effective have not been explored. There are a number of instances in which transfer factor from a donor positive to several antigens has conveyed only some reactivities to recipients, particularly to those antigens with which they have had contact. There are other instances in which recipients have shown conversion in response to antigens to which the donor was negative. These findings justify testing the possibility that transfer factor acts primarily as a non-specific immunological adjuvant. Were this demonstrated to be the case—i.e. that transfer factor from non-sensitive donors acted as effectively as that from positive donors—then the availability of transfer factor would be vastly increased since blood-bank blood from normal donors could be used in many situations. This might also lead to the development of synthetic adjuvants for use in man.

Patient groups

(1) *Clinical-histopathological status.* It is recommended that previously untreated patients with lepromatous leprosy be studied, and that transfer factor from sensitized donors and chemotherapy be given at the same time. The patients should be in the BL/LL and LL categories.

Controls would be matched for classification, and for locale, age, and sex where possible. There would be two control groups, one receiving non-sensitive transfer factor and the other no transfer factor but a slightly irritating placebo. All patients would receive chemotherapy. Patient groups could be established from an initial large pool, or consecutive patients could be assigned to each group. Between 12 and 20 patients should be included in each group.

Histopathological and bacteriological specimens and pretreatment sera must be saved for later reference, and the morphological and bacteriological indices should be determined.

(2) *Immunological status.* Recipients should be negative in the lepromin skin test (Fernandez and Mitsuda) and in the lymphocyte transformation test. They should also give normal phyto haemagglutinin (PHA) responses, or at least two-thirds of the average normal stimulation, and should be reactive to at least one other common antigen—e.g. PPD, streptokinase-streptodornase, *Candida*, or mumps, *in vivo* or *in vitro*.

The IgG levels should be less than 20 g/l.

Rationale. (a) For this protocol the untreated BL/LL (LI) group has been selected. There is a relatively low incidence of these patients spontaneously moving up the scale within the test period and *erythema nodosum leprosum* reactions would possibly be lower in this group. While patients with indeterminate leprosy (I) have a sufficiently low bacterial load and immune response to be considered for such a study, since only a minority convert to the lepromatous form, large numbers of recipients would have to be included in order to obtain a statistically significant result. Further, it would not be ethical to withhold chemotherapy in this group for any considerable period, and chemotherapy alone would terminate the disease and eliminate bacilli in the skin in such patients. The disadvantages of using patients in the borderline lepromatous group for study are that the frequency of reversal reactions with chemotherapy is greater and that too high a percentage may move up the scale with chemotherapy alone.

(b) It is essential that patients selected for this trial have adequate T-lymphocyte function—i.e. react well to PHA and unrelated antigens. In addition, they must be specifically nonresponsive to *Myco leprae*.

(c) Normal serum levels of IgG are preferable to minimize possible inhibitory effects of antibodies to mycobacteria on development of cell-mediated immunity and to diminish the likelihood of *erythema nodosum leprosum*.

Transfer factor donors

(1) *Sensitized donors* must have strong positive skin tests (Fernandez) and show lymphocyte transformation *in vitro*, preferably to a single pool of *Myco. leprae* bacilli. Ideally, they should all be negative for a known control antigen—e.g. histoplasmin, coccidioiodin.

(2) *Nonsensitized donors* must be negative *in vitro* to a single test pool of *Myco. leprae* and *in vivo* to BCG, and to PPD (second strength). Where possible, Fernandez tests can be given to these donors retrospectively, i.e. after the blood leucocytes have been collected. Ideally they should be strongly positive to the second test antigen, e.g. histoplasmin, coccidioiodin.

(3) All donors should be in good physical condition and negative for hepatitis B antigen.

(4) The transfer factor should be prepared in a limited number of centres using either fresh or frozen leucocytes sent by mail. The final pools of transfer factor must be tested for sterility and must be negative for hepatitis B antigen in the most sensitive test available. In addition biochemical analysis should be made. Pools should be coded before being sent to the appropriate clinical centres for testing.

Rationale. (a) The protocol is designed using sensitivity to two antigens so that donors sensitive to *Myco. leprae* would be unexposed and negative to one other

antigen, and control donors would be negative for reactivity to *Myco leprae* but positive to the second antigen. The recipients should be negative to both antigens. In this way the specificity of transfer factor for two antigens can be tested simultaneously.

(b) The study should be double blind—i.e. the transfer factor preparations must be coded and the code not broken until the end of the study.

(c) The lepromin-positive donors should preferably be recovered tuberculoid patients (TT or BT/TT) since they have demonstrated adequate levels of true cell-mediated immunity, although highly-positive contacts could be employed.

Treatment of recipients

(1) An essential prerequisite for such a study is the availability of close medical supervision and hospital facilities, so that the necessary treatment can be provided should reactions occur.

(2) Coded transfer factor should be administered subcutaneously at monthly intervals for 12 months. The code should not be broken until the end of the study.

(3) Recipients should receive the equivalent of 400×10^6 leucocytes (obtained from approximately 500 ml of blood) but the dose may be increased if reactions are not encountered, or lowered if reactions are severe.

All patients, whether in hospital or out-patients, must be seen at least weekly. If any patients respond to several injections with substantial clinical deterioration, they must be dropped from the study.

Rationale. In previous studies some lepromatous leprosy recipients of transfer factor showed "flare" reversal reactions, and, in at least one case, *erythema nodosum leprosum.* Since in studies in patients with other conditions the effect of transfer factor has been transient, it seems likely that in patients with lepromatous leprosy repeated doses would be required for histopathological changes and clearance of bacteria from the tissues to be observed.

Evaluation of the results

(1) All patients should be examined weekly for clinical status, special attention being given to neurological and ophthalmological symptoms.*

(2) At least three skin tests to lepromin and to the unrelated antigen should be made approximately 8-10 days after the first administration of transfer factor and 6 and 12 months. Biopsies should be taken from these sites.

(3) The lymphocyte transformation test or other *in vitro* tests for cell-mediated immunity should be performed at monthly intervals.

(4) At least two skin biopsies should be taken after 6 and 12 months. In addition biopsies should be obtained from affected areas whenever reactions are observed. Several centres should be available for expert histopathological evaluation of tissue samples. In addition serum samples must be obtained at the beginning of the study, and at 6 and 12 months, as well as during any periods of *erythema nodosum leprosum* reaction that may occur.

(5) Bacteriological indices and morphological indices should be taken from 6-12 areas on a monthly basis. Again, several centres should be available for expert evaluation of slides.

* The necessary clinical protocol can be obtained, on request, from Immunology, World Health Organization, 1211 Geneva 27, Switzerland.

(6) After one year, the results, including clinical, histopathological, and immunological data, should be reviewed and evaluated by experts convened by WHO.

(7) The clinical status of all patients should be followed up for at least one additional year.

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News and Notes

INTERNATIONAL JOURNAL OF LEPROSY CENTENNIAL FESTSKRIFT

The centenary of the discovery of *Myco. leprae* has been made the occasion by the *International Journal of Leprosy* for a most interesting commemorative issue which covers a century of progress in the field of leprosy. This, Volume 41, Number 2 (April-June, 1973) is in two parts. In addition to reviews of the century by outstanding contributors, a unique feature is a photographic gallery and brief appreciations of senior leprologists both past and present, whose life and work have contributed materially to present progress. Here are many well known and well loved faces of our former teachers and senior colleagues, and there is unalloyed pleasure in handling this volume, which brings together so distinguished a gallery, and holds so many treasured memories. Dr Skinsnes and his colleagues are to be congratulated on an outstanding production.

A single bound volume of the Festskrift is now available at a cost of $5.00 from the International Journal of Leprosy Business Office, P.O. Box "G", Madison Sq. Station, New York, N.Y. 10010, U.S.A.

PAPUA NEW GUINEA MEDICAL JOURNAL

Readers of the Review may be interested to know that small supplies of the June 1973 issue of the *Papua New Guinea Medical Journal* based on leprosy [see *Leprosy Review* **45**(1)] are available from the editorial office of the Journal, P.O. Box 1174, Boroko, Papua New Guinea, at the cost of $1.00 (Aust.).

PERSONAL

We are happy to join with many other friends in congratulating Mr G. Newberry Fox, International General Secretary of the Leprosy Mission, who has been awarded the O.B.E. During the nine years of his General Secretaryship, the budget of the Mission grew from some £600,000 a year to nearly £1,000,000, and its activities in the field developed both in size and scope, particularly with growing emphasis on disease prevention and early detection, especially with the use of mobile clinics and travelling teams. In the same period supporting auxiliaries were established in a number of European countries. On his retirement, Mr Fox is succeeded by Mr A. D. Askew, and we offer both of them our cordial good wishes.

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Leprosy and the Community

THE GANDHI MEMORIAL LEPROSY FOUNDATION: REPORT FOR 1972-3

The Gandhi Memorial Leprosy Foundation is an astonishing multi-faceted organization which, inspired by the spirit of Gandhiji, spreads from its centre in the heart of India its beneficient influence in all directions. In his report for 1972-3, the Director, Dr Nilakanta Rao, covers the following activities of the Foundation.

(1) *Control units*

These, the first activity of the Foundation, were started in 1951 to test the efficacy of DDS by S.E.T. methods in a rural area. Out of 10 original units, six have either closed or been handed over to other organizations. Efficient work, and a high population coverage in repeated surveys at the remaining four units, have after 21 years produced a situation where out of 2986 registered patients, 2104 are now cured or inactive. New cases detected during the year totalled 181, of which only 14 were of lepromatous type.

(2) *Health education units*

A novel feature of the Foundation is the establishment of seven health education units, each located in populous areas of India, and staffed by a trained paramedical officer, whose one and only task is to educate the general public at all levels in the facts about leprosy and encourage rational attitudes. Doctors and teachers are a special objective, and great experience has now been gathered in effective ways of approach to people. During 1972-3, over 2000 private interviews, 221 group meetings, 20 public meetings, lectures to over 12,000 teacher training students and over 10,000 college students, refresher courses for 311 doctors, exhibitions and film shows complete this very interesting aspect.

(3) *Training*

The Foundation is one of the leading leprosy training centres in India, with regular substantial courses for paramedical workers and courses in health education.

(4) *Chemoprophylaxis project*

In 1962, the Foundation, supported by the Indian Council for Medical Research, started an experiment to study the effect on prevalence of intensive prophylactic treatment of entire healthy population groups below 25 years of age. A population of 40,000 was selected, one half of which was retained as a control group. After 10 years, and nine post-prophylaxis surveys, a marked reduction in leprosy incidence in the age group selected has been consistently found as compared with control groups.

(5) *Work among doctors*

In addition to lectures and refresher courses attended by 311 doctors, a novel feature of the Foundation is the orientation courses in leprosy arranged for Professors of medical colleges, and attended by 12 Professors from various disciplines during three week-end courses in 1972-73.

(6) The central hospital and laboratory, orientation courses for Gandhian workers, coordination work among various agencies in Maharashtra, and the organization of a large Seminar on Leprosy sponsored by the Indian Association of Leprologists, all add up to an outstanding contribution to leprosy control.

ELEP LEPROSY CONTROL PROJECT, DHARMAPURI, S. INDIA 6TH ANNUAL REPORT, 1973

This major project consists of three control units covering five out of six Taluks in the Dharmapuri District of Tamil Nadu, and out of a population of 1,493,084 (1971 census), 853,009 have been covered by a survey, education and treatment programme following the lines of the National Leprosy Control Programme. The project, based at Dharmapuri, has a total staff of 112, including four doctors and 76 supervisory and paramedical workers. At the end of 1973, out of 14,893 known leprosy patients, 12,663 were registered for treatment, with 8346 actually attending during December 1973. 2502 new cases were detected during 1973, 37% by survey, 47% by voluntary reporting of patients, 8% by the detection of cases among healthy contacts. No falling off is taking place in cases detected. 6.6% of new cases were of lepromatous type, 88.8% non-lepromatous, and 4.6% N?L. Physiotherapists and laboratory technicians are attached to each control unit, and hospital facilities are operating at two of them. The entire cost of the project works out at Rs.62 *per annum* per patient. The progress of the project owes much to the dynamism of Dr V Ekambaram.

TANZANIA: NATIONAL REPORT ON LEPROSY FOR 1972

The Report on Leprosy in 1972, issued by the National Leprosy Advisory and Coordinating Committee of Tanzania indicates that leprosy control schemes are in progress in about 38 out of 61 Districts, with 45,500 patients on treatment at the end of 1972 out of an estimated total of 138,000 sufferers from leprosy in the country. Only 2000 patients are living at leprosy centres. The programme may be regarded as yet in its early stages, and is based on mobile teams with "Health Home Visitors" responsible for case finding and routine treatment. A notable feature of the national leprosy control programme in Tanzania is the excellent coordination between Government and Voluntary Agencies. Responsibility for all medical services is borne by the Regional Medical Officers. In ten Regions the leprosy duties have been delegated to a Regional Leprosy Officer. The majority of leprosy control schemes and centres are cared for by voluntary agencies, but in many of them the government shares in the cost, while the whole programme comes under the purview of the National Leprosy Advisory and Coordinating Committee, on which both government and voluntary agencies are represented.

Field Worker's Forum

DRUG RESISTANCE IN LEPROSY

S. G. BROWNE

The Leprosy Mission, 50 Portland Place, London, W.1, England

The publicity that has recently been given to the fact that dapsone-resistant leprosy bacilli are appearing with disturbing frequency in several countries has raised many questions in the minds of those involved in leprosy work, including the staff of The Leprosy Mission and its aided centres. It is therefore hoped that a considered statement, representing a consensus of present-day medical opinion, will help to allay fears and anxieties and to ensure that the patients for whom we are clinically and therapeutically responsible receive the best and the safest treatment.

The somewhat alarmist reports that sulphone-resistance is becoming very common must be viewed in the light of our knowledge of the development of drug-resistance in other chronic infections, such as tuberculosis. It would be surprising if a mycobacteriostatic drug like dapsone did not—when given to millions of people for long periods—provoke the appearance of resistant strains of leprosy bacilli. What is more surprising is that the appearance on a world scale of such cases has been so long delayed.

To judge from other infections, potentially drug-resistant mutants arise in any large bacterial population. The greater the numbers of leprosy bacilli, and the longer the infection remains virtually uncontrolled in the patient, the greater the likelihood of resistant bacilli arising and multiplying in the tissues. Eventually, these resistant bacilli multiply to such a degree that the majority of bacilli present may be drug-resistant. The rest have been prevented from multiplying by the bacteriostatic property of the drug.

Laboratory confirmation of the clinical suspicion of the occurrence of drug-resistance had to await the availability of the mouse footpad inoculation technique. And now that instances of proven drug-resistance have occurred in many countries—and the numbers are in direct relation to the awareness of clinicians and the availability of facilities for experimental investigation—we do well to appraise the situation and formulate practical and practicable recommendations.

To resume:

(1) Drug-resistant leprosy bacilli have been demonstrated in about half of the patients in the U.S.A. who took sulphones more than 20 years ago and in about 200 patients in Malaysia among a total of probably 4000 patients.
(2) Slow clinical and bacteriological response to the sulphones must not be confused with drug resistance.
(3) It is more than probable, to judge from analogy with other infections, that irregular and intermittent treatment, especially when associated with low doses of sulphones, is the commonest factor involved in the development of drug resistance in leprosy.

(4) Crossed resistance between the sulphones and the sulphonamides does occur.
(5) So far, no case of leprosy caused by sulphone-resistant bacilli has been reported, but the possibility exists.
(6) All patients with sulphone-resistant bacilli have responded to either clofazimine or rifampicin, and so far no case of resistance to either of those drugs has been reported.
(7) Sulphone-resistance occurs in those patients who have little or no resistance to leprosy infection; that is, in those with lepromatous or near-lepromatous (BL) leprosy. It has not occurred in those patients who have a well-developed cell-mediated immunity, that is, in those with tuberculoid or near-tuberculoid leprosy.
(8) When sulphone-resistance does occur in leprosy, it develops in a stepwise fashion, so that bacilli that multiply when a daily dose of, say, 10 mg of dapsone is given, will be inhibited when the dose in increased to 50 or 100 mg a day.

The typical history of the development of drug resistance would be as follows: a patient who has been taking a sulphone (dapsone, DDS, would be the commonest) for a variable period and with good clinical and bacteriological results, develops new lesions for no obvious reason. He is usually still taking the drug. The more irregular he has been in taking treatment, and the lower the dose of drug he has been taking, the more likely it is for drug-resistant bacilli to make their appearance. Usually, bacteriological changes precede obvious changes in the skin, and that by several months or even years. Hence the importance of regularly taking smears from every patient who has had lepromatous leprosy, after clinical and bacteriological quiescence has been attained. The material should be taken from a new lesion (if there is one) and from the ear lobe. Such patients should be seen every three months for the first two years, then every six months for the next two years, then every year, and smears should be taken on each occasion. Ideally, these patients should continue to take treatment—at half the therapeutic dose—"for life".

The new skin lesions may resemble the old ones, or may be in the nature of rapidly-developing papules, or a maculo-erythematous eruption reminiscent of a drug rash.

These lesions are sometimes mistaken for those of *erythema nodosum leprosum,* but it should be noted that they are persistent, they are not tender to the touch, and they are not accompanied by any of the systemic signs of acute exacerbation. Furthermore, a slit-smear examination reveals numerous typical acid-fast organisms, many of which are morphologically normal. When corticosteroids are given (as they often are, unfortunately) and anti-leprosy treatment suppressed, the lesions do not tend to diminish or disappear—on the contrary.

The smears which have not shown any morphologically normal bacilli for a variable period—months or years—may show numerous solid-staining and deeply-staining organisms. The most likely site to obtain such bacilli is the small pinkish fleshy papules that may arise on the skin anywhere in the body. If in a patient who has been bacteriologically negative for a variable period, or in whom solid-staining rods have not been found for many months, such organisms begin to be found despite the fact that he is still taking dapsone, then the presumption is that dapsone-resistance has already occurred.

If such a patient is taking a low dose of dapsone (say, for this purpose, 50 mg a

day or less), the bacilli may be resistant to low concentrations of dapsone in the serum, but would be sensitive to higher concentrations. Therefore, increase the dose to 100 mg a day, and examine the smears every two to four weeks. If morphologically normal bacilli disappear from the smears, it is probable that the bacilli were resistant to low dapsone concentrations but sensitive to higher concentrations. However, experience suggests that once resistance does develop, it will increase so that eventually the bacilli will become resistant to the higher serum concentrations corresponding to a daily dose of dapsone of the order of 100 mg.

It has been assumed that the patient is actually taking the drug at the dose suspected, and that the drug is being absorbed from the intestine. Supervision of the actual swallowing of the tablet, and the demonstration that the tablet will readily disintegrate when placed in a glass of water—these are necessary precautions to be taken before concluding that the patient is harbouring sulphone-resistant bacilli.

Since sulphone-resistance does not, for all practical purposes, occur in patients who are suffering from tuberculoid or near-tuberculoid leprosy, and since it is common knowledge that such patients are liable to develop severe peripheral nerve damage—particularly in the case of tuberculoid-borderline leprosy if the dapsone is given in too high a dose at the beginning of treatment, or if a high dose is attained too rapidly—the usual practice of prescribing dapsone at a maximum weekly amount of 200 to 300 mg given in divided doses, has much to commend it.

The dangers of severe, widespread and permanent nerve damage must always be borne in mind when dapsone is given. In some patients, nerve trunks will become enlarged and painful whenever dapsone is given and whatever the dose. Most patients with intermediate forms of leprosy, however, will experience this distressing complication only when the dose of dapsone given is too high.

On the other hand, the more bacilli there are in the routine smears, the greater the need to give as high a dose of dapsone as the patient can tolerate, and this will mean a dose of 100 mg every other day, or even (in the case of patients weighing over 75 kg) of 100 mg a day. Since even 1 mg of dapsone daily will prevent the multiplication of leprosy bacilli, it is evident that doses of this order are considerably above those necessary to produce a minimal inhibitory concentration in the serum.

In the case of suspected sulphone resistance, recourse should be had to clofazimine, to be given at a dose of 100 mg every other day.

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Abstracts

The following Abstracts are reprinted, with permission, from *Trop. Dis. Bull.* 1974, v. 71.

1. AGIUS-FERRANTE, A., DEPASQUALE, G., BONNICI, E., PARIS, C. and GRIMA, W. **The leprosy eradication project of Malta.** *Ztschr. Tropenmed. Parasit.* 1973, v. 24, No. 1, 49-52.

A determined effort is being made to eradicate leprosy from the Maltese Islands. 210 patients out of a total of 225 (in a population of 320,000) are at present under treatment with a triple-drug regimen, composed of rifampicin (10 mg/kg), ethionamide (5 mg/kg), a sulphone-sulphonamide complex (2-20 mg/kg) and isoniazid (5 mg/kg). Whatever their initial bacteriological status of clinical classification, all patients have been placed on this treatment.

Excellent co-operation is reported, and the early results–bacteriological and clinical–are said to be encouraging.

With such a relatively controllable problem, and given adequate financial resources and medical supervision, there is reason to hope that the objects of the programme will be achieved.

S. G. Browne

2. KOHOUT, E., HUSHANGI, T. and AZADEH, B. **Leprosy in Iran.** *Int. J. Lepr.* 1973, v. 41, No. 1, 102-11.

Imprecise estimates of the prevalence of leprosy among the 29 million inhabitants of Iran vary from 0.20 to 0.54 per thousand. Under 5000 patients are at present registered and receiving treatment. The authors quote official figures showing that the bulk of those suffering from leprosy live in the colder northern districts, but the disease is widely distributed, with concentrations even in the dry hot south.

Two leprosaria at Meshed and Tabriz cater for about 1000 in-patients, and an ambitious rural residential project at Behkadeh accepts patients whose leprosy is quiescent. A clinic in Teheran, and a histopathological reference centre, also in Teheran, testify to the growing interest in the endemic.

Case-finding is seriously hampered by the widespread and deep-rooted stigma of leprosy, a view which is shared by doctors as well as by the general public.

S. G. Browne

3. BECHELLI, L. M. *ET AL.* **Some epidemiological data on leprosy collected in a mass survey in Burma.** *Bull. Wld Hlth Org.* 1973, v. 48, No. 3, 335-44.

This paper should be studied in detail. It sets out objectively the most significant findings in the WHO survey of an area of Burma where prevalence rates of leprosy are high or very high. The high lepromatous rates recorded were found in districts where the total prevalence rate and the annual incidence rate were both high. About 3% of such a population is prone to develop lepromatous leprosy if sufficiently exposed to infection. The highest prevalence rates (48.1 per 1000) were found in the 30-39 years age-group, in which the lepromatous rate was 12.5 per 1000. No child under 10 years was found suffering from lepromatous leprosy. This observation was interpreted as an expression of the long silent period and the length of time generally noted before overt signs of lepromatous leprosy appear. Indeterminate leprosy was commonest in children under 14 years, reaching its peak in the 10-14 age-group.

There was a true and significant male predominance: 40.4 cases per 1000 males, and 24.0 per 1000 females. Under 10 years, the sex prevalence was approximately equal.

On the figures presented, multibacillary leprosy (that is lepromatous and borderline forms combined) is only three times as contagious as tuberculoid leprosy, but it may be that contacts of patients with tuberculoid leprosy has also been exposed to persons suffering from the multibacillary forms.

It is noteworthy that, on subsequent surveys, only indeterminate and tuberculoid forms of leprosy were seen in the new cases. Initial lesions were found on thighs and buttocks, less frequently on arms, forearms and legs.

Bacilli were demonstrated in skin smears of patients with tuberculoid leprosy, particularly if recourse was had to histopathological examination. Bacilli were also occasionally found in the nasal mucosa of such patients, and even in ear lobes of patients with clinically inactive tuberculoid leprosy or in the preclinical state.

It is considered that higher prevalence rates of tuberculoid leprosy are indicative of the spread of leprosy rather than of a higher level of resistance among the population. Leprosy can show itself at any age; the most important factor is exposure to infection. Given infectious index-cases, high prevalence rates may occur in any ethnic group, provided that the socio-economic and environmental factors are propitious. (The early diagnosis and adequate treatment of patients suffering from indeterminate leprosy is probably a Utopian ideal, but one worth striving for.)

S. G. Browne

4. BECHELLI, L. M. *ET AL.* **BCG vaccination of children against leprosy: seven-year findings of the controlled WHO trial in Burma**. *Bull. Wld Hlth Org.* 1973, v. 48, No. 3, 323-34.

The results reported in this paper provide factual data for the continuing debate on the possible efficacy of BCG vaccination in conferring protection against leprosy. The total number of children concerned was 28,220, almost equally divided between the BCG-vaccinated and the non-vaccinated groups.

The groups have now been followed for periods up to seven years. Up to June 1971, 285 and 325 new cases of leprosy have been detected in the BCG and control groups respectively, representing incidence rates of 5.2 and 6.0 per 1000 patient-years of observation.

The report provides useful analytical tables relating to the various aspects of the trial, e.g. leprosy incidence according to household contacts, tuberculin status, and age at intake, and the (late) lepromin reaction in new cases of leprosy.

The results so far obtained in this trial indicate that BCG confers no protection on household contacts of open cases of leprosy, nor would it have benefited lepromin-negative contacts of cases of leprosy. The relative infectiousness of multibacillary and paucibacillary index cases—virtually unaffected by BCG vaccination of contacts—is about 3 to 1 in this trial.

The incidence of leprosy in BCG-vaccinated children aged 0-4 years at intake was somewhat lower than that of children in the control group.

It is concluded that this slight reduction in incidence in one age-group would not substantially affect the pattern of the disease in a population comparable to that in Burma where the trial is being conducted. In an area where the prevalence rate is low, i.e. of the order of 1-2 per 1000 or less, BCG vaccination would probably not affect the incidence of leprosy. It is considered premature to recommend BCG vaccination, even to children 0-4 years of age, for the sole purpose of conferring protection against leprosy. To recommend BCG vaccination on the grounds of its proven value in protecting against tuberculosis and its possible protective value against leprosy, would be to induce a false sense of security and perhaps lead to neglect of important leprosy control measures.

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5. PISANI, R. C. B., BEIGUELMAN, B. and OPROMOLLA, D. V. A. ***In vitro* behaviour of blood derived macrophages against killed *Myco. leprae***. *Int. J. Lepr.* 1973, v. 41, No. 1, 14-24.

"A technic is described that renders easier the assessment of the *in vitro* phagocytic and lysing ability of blood derived macrophages against killed leprosy bacilli. Such *in vitro* reactions were

followed and scored at 5 day intervals for 25 days. This technic was applied to 54 leprosy patients (10 LL, 10 TT, 10 BB, 17 II and 7 of uncertain classification) and to 57 healthy individuals (40 non-contacts and 17 contacts of leprosy cases). Three classes of *in vitro* reaction were distinguished among the leprosy cases: lytic, weakly lytic and nonlytic reactions. The lepromatous cases consisted of non-lytic reactors, the tuberculoid patients of lytic reactors, the dimorphous of nine weakly lytic and one lytic reactors, and the indeterminate cases were represented by all three types of reactors.

"The blood derived macrophages of the healthy individuals showed a low rate of *in vitro* phagocytosis and lysis of *Myco. leprae* and none of them disclosed a lytic reaction similar to that observed among the tuberculoid cases. No association could be found between the intensity of the Mitsuda reaction and the *in vitro* lysing activity of blood derived macrophages of healthy individuals.

"It is concluded that with present day technics the *in vitro* test cannot be considered as an advance over the Mitsuda reaction for practical purposes, nor does it yet give evidence as to whether or not the tissue resistance to *Myco. leprae* infection may be regarded as a genetic polymorphic trait."

6. SILVA, C., LIMA, A. O., ANDRADE, L. M. C. and MATTOS, O. **Attempts to convert lepromatous into tuberculoid-type leprosy with blood lymphocyte extracts from sensitized donors.** *Clin. Exp. Immunol.* 1973, v. 15, No. 1, 87-92.

"Two groups of lepromatous patients (L type of leprosy) were injected intramuscularly and respectively with the Lawrence's transfer factor (LTF) or a ribonucleic acid (RNA) extracted from viable blood lymphocytes of healthy donors hypersensitive to lepromin (48-72 h and 20-30 days), tuberculin (PPD), toxoplasmin and histoplasmin. Each patient received a single dose of LTF or RNA obtained from 10^9 lymphocytes and was carefully observed for a period of four months. Histopathology of skin lesions and of lymph nodes were repeatedly performed. The results of intradermal tests with antigens were read at different times up to 120 days after the injection of LTF and RNA. Delayed cutaneous reaction to some antigens was induced in three patients injected with LTF and in two others injected with RNA. The lepromin reaction read at the end of 48-72 h and after 20-30 days remained negative in all patients during 120 days of observation. Clinical and histopathological signs of transformation into tuberculoid leprosy could not be observed in the eight patients tested."

7. MENDES, N. F., KOPERSZTYCH, S. and MOTA, M. G. S. **T and B lymphocytes in patients with lepromatous leprosy.** *Clin. Exp. Immunol.* 1974, v. 16, No. 1, 23-30.

"The percentages of T and B peripheral blood lymphocytes were established in 36 patients with lepromatous leprosy. T lymphocytes were detected by rosette formation with sheep erythrocytes (E) and B lymphocytes were detected by rosette formation with human erythrocytes sensitized with antibody and complement (HEAC). The mean per cent values for both T and B lymphocytes were significantly lower in these patients as compared to mean values from 30 normal subjects ($0.05 > P > 0.01$). Lymph nodes sections treated *in vitro* with E or HEAC from two lepromatous patients which were examined, showed marked depletion of T cells in the paracortical areas and the follicles were slightly reduced in size, but still presenting B cells. Establishing the percentages of T and B peripheral blood lymphocytes and their distribution in lymph nodes may represent an additional method of evaluating the immunologic status of leprosy patients."

8. RIDLEY, D. S. **The pathogenesis of the early skin lesion in leprosy.** *J. Path.* 1973, v. 111, No. 3, 191-206.

"A study of early skin lesions of leprosy patients, many of them with a history known to be of less than 1 year's duration, showed that by the use of serial sections the great majority could be classified on an immunological basis in much the same way as the lesions of established leprosy.

It was also shown that they could be classified according to their predominant distribution as neural, vascular or subepidermal.

"Lesions of the vascular type are multiple and blood disseminated, though they appear to originate in nerves. The subepidermal type are solitary lesions and there is evidence that in many cases transmission is through the epidermis. Intra-epidermal lesions are described. The neural type constitute a mixed group.

"Interpretation of the evidence concerning the route of transmission is complicated by two factors: (1) In the epidermis and subepidermal zone there is a 'single ratio' of tissue reactivity to numbers of bacilli, which is either very high or very low. And (2) there is evidence of a two-way spread of leprosy bacilli, from epidermis to deep nerves and vice versa.

"Bacilli were found at six sites in the skin. The optimum site for multiplication depended on the level of immunity, and to a less extent for a given level of immunity it depended on how well the infection was established. Bacilli were protected against elimination in patients with relatively high immunity not only in nerve but also in the subepidermal zone and to some extent in muscle. But bacilli in the two latter situations may in fact be present in neural elements."

9. RIDLEY, D. S. **A skin biopsy study of lepromatous leprosy**. *Papua New Guin. Med. J.* 1973, v. 16, No. 2, 100-104.

"Out of 51 biopsies of skin lesions from patients in relapse 16 showed very early lesions. The distribution of solid-staining bacilli gave evidence that in most cases the relapse had originated in a dermal nerve of an old lesion; and that in patients in whom new lesions had developed the dissemination had taken place via the blood stream from another skin site. Thirty-five other biopsies showed relapse lesions in an advanced stage. Half of them showed certain histoid features which were attributed to the fact that the lesions were unusually acute, with a high cell turnover in the granuloma.

' Skin biopsy is a sensitive method of detecting relapse provided the site is well chosen."

10. STORRS, E. E. **Leprosy in the nine-banded armadillo**. *Ztschr. Tropenmed. Parasit.* 1973, v. 24, No. 1, 53-65.

"It is concluded that leprosy is a much more severe disease in the armadillo than in man, since leprotic pneumonitis and esophageal and meningeal involvement are found which to our knowledge have not been reported in human leprosy. Also, a leproma taken from an armadillo contained 10^{10} bacilli/g compared to 10^{7} to 10^{8}/g usually found in skin biopsies taken from advanced human cases. The time required for the disease to develop is 10 months or less compared to an estimated 3 to 5 years in man. Preliminary results suggest that the incidence of susceptibility in the armadillo may be as high as 40% compared to an estimated 5% in man. A total of 243 g of lepromas containing 10^{10} bacilli/g was obtained from two armadillos with advanced lepromatoid leprosy. Armadillos have now developed leprosy after inoculation with bacillo obtained from man, the mouse footpad, and another armadillo. The magnification of leprosy observed in the armadillo suggests that this animal model will be useful as a source of leprosy bacilli, for studies on disease transmission and for the evaluation of experimental drugs."

11. PETCHLAI, B., CHUTANONDH, R., PRASONGSOM, S., HIRANRAS, S. and RAMASOOTA, T. **Complement profile in leprosy**. *Am. J. Trop. Med. Hyg.* 1973, v. 22, No. 6, 761-4.

"We investigated complement in leprosy to detect its possible role in pathogenesis. Complement components, C3 proactivator (C3PA), CH_{50} and anticomplementary activity (AC) were determined in sera collected from lepromatous and tuberculoid leprosy. C3 was found increased in lepromatous leprosy and high increased C3PA was found in both groups. Changes in other components lacked specific pattern. Increases in C3PA levels are believed to be due to

aggregation of the increased serum immunoglobulins. C3 levels and CH_{50} levels in the present study agree with those in other reports. Development of AC occurred in lepromatous sera even during 2 days of storage at −80°C. AC found among freshly collected sera was probably due to aggregated immunoglobulins. The present study serves as a groundwork for future investigations."

12. HENNEQUIN, M. La lutte contre la lèpre en République Centrafricaine. [**Leprosy control in the Central African Republic.**] *Méd. Trop.* 1973, v. 33, No. 3, 289-96. English summary.

This paper provides a useful review of the leprosy campaign in the Central African Republic over the past 12 years. In 1958, the Republic had the unenviable distinction of reporting the highest prevalence rates in francophone Africa, i.e. 55 per thousand. The distribution of the disease is low in the hot and dry northwest, but very high in the humid southern districts bordering on Zaïre. The proportions of the different types of leprosy resemble those found in neighbouring countries, only 8.4% of patients having lepromatous leprosy.

Case-finding is mainly by polycompetent teams, to which are attached auxiliaries experienced in the diagnosis of leprosy. These teams cover the whole country (population approximately 3 million) every 2 years. Their work is supplemented by auxiliaries responsible for treatment, who examine patients and their contacts, especially in those years when the survey teams are not at work in the district concerned. Follow-up is considered to be satisfactory.

Most of the patients are given either dapsone or a long-acting oral sulphonamide. Treatment is brought to each village by the regular weekly or bi-weekly visit of an auxiliary cyclist. The few "runs" that have been attempted by motor vehicles have proved disappointing by reason of frequent breakdowns. In addition, a small proportion of patients is treated at 11 all-purpose dispensaries, 22 leprosy dispensaries and 7 private institutions. A small pilot scheme for self-treatment has recently been established; the results of this experiment are awaited with interest.

The author advocates the segregation of patients with "contagious" forms of leprosy, and claims that, after 6 months' treatment with sulphonamides, half the patients in three hospitals were rendered bacteriologically negative. (This controversial opinion will not meet with universal acceptance or approval.)

The results of the campaign over the past 12 years are distinctly encouraging: the numbers of patients under treatment have dropped from 64,719 to 23,070; 32,346 have been discharged symptom-free, and the prevalence has fallen from 57 per thousand in 1959 to 14 per thousand in 1971.

S. G. Browne

13. GANAPATI, R., NAIK, S. S., SANE, A. B. and PARIKH, A. C. **Leprosy among school children in Greater Bombay—results of surveys.** *Lepr. India* 1973, v. 45, No. 3, 151-62.

This interesting and informative paper embodies the results of an extensive leprosy survey of schoolchildren in a randomly selected sample of the municipal and private schools in Bombay, covering all language groups and socio-economic classes and representing about 10% of the school population and hence about 5% of the child population of Bombay.

The overall prevalence rate of leprosy was found to be about 3/1000, with males definitely preponderating, and higher rates found in older children. No fewer than 203 (out of the 209 diagnosed) were suffering from non-lepromatous forms of leprosy, 102 having slightly raised lesions classified as tuberculoid and the others having flat and hypo-aesthetic lesions. In the six with lepromatous or borderline leprosy, skin smears contained acid-fast organisms. Of the nine patients showing evidence of peripheral nerve damage, four had no discernible abnormalities of the skin.

BCG vaccination scars were present in 0.29% (78% out of 27,204) of patients with leprosy, and absent in 0.36 (131 out of 36,885). No information is available concerning the date of BCG vaccination and the onset of the visible leprosy lesion.

14. KUMAR, A., INDRARAN, A., ISSAC, J. J. and GUPTA, S. C. **Age at onset of leprosy in relation to type of leprosy, site of first lesion and occupation—observations based on records of patients in Naini Hospital, Allahabad.** *Lepr. India* 1973, v. 45, No. 3, 167-73.

This study presents an analysis of 1100 patients, diagnosed as suffering from leprosy, who presented themselves during a 12-month period at the Naini Hospital, Allahabad, India. Noteworthy findings in this self-selected group were the apparent late age of onset (average 34 years); the low proportion (10%) of childhood infections, that is, children aged less than 14 years; and the frequency with which the initial lesion was on the hand (31%). Patients with indeterminate lesions were much younger (16 years) than those with borderline leprosy (36.72 years).

Since this analysis of a highly selected and motivated population depends largely on lay suspicion and poor recall, the conclusions are not necessarily typical of the situation in India or elsewhere.

S. G. Browne

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