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SPECIAL ARTICLE

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Leprosy in the Americas region (AMRO)

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Background information for activities to be implemented with Sasakawa Memorial Foundation aid (Japanese shipbuilding industry funds).

I. Introduction

Leprosy is an imported endemia in the Americas. All evidence points out that the indigenous population never had the disease before the arrival of the settlers (Spanish, Portuguese, French and Dutch), and later African Negroes imported as slaves.

Several leprosy foci have developed in the last four centuries, always related to the migratory pattern of the settlers. The most important focus is the South American Amazon region, a recent one, since it was only populated and settled during the last quarter of the nineteenth century.

As a general pattern, the leprosy problem is of median size when compared with Asian or African foci. The geographical distribution of cases is not uniform. There is a trend towards concentration in well-defined foci, some with high prevalence rates (20–30 per 1,000).

Until the 1950s it was estimated that most of the cases (about 70%) came from the rural area (villages of less than 2,500 inhabitants or scattered population), but now the situation is inverse due to the phenomenon of 'fast urbanization' in several American countries.

Other important patterns of the disease are:

(a) With few exceptions, half of the detected cases are multibacillary (lepromatous and borderline).
(b) The percentage of detected cases in the group 0–14 years is less than 15%.

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Table 1. Leprosy cases on the active register by country, estimated number of patients, prevalence rates and cases under surveillance, 1976 or most recent year (American region)

Country or territory	Estimated population (in 1,000 s) 30 June 1976	Registered cases		Total estimated		Cases under surveillance	
		Year	Number	Number	Rate per 1,000	Number	(%)
PAHO/WHO Area I							
Countries							
Venezuela	11,632	1975	12,734	19,101(a)	1.6	8,923	46.7
Surinam	411	1973	2,311	4,044(b)	9.8	2,311	57.2
Guyana	774	1976	665	1,164(b)	1.5	642	55.2
Trinidad & Tobago	1,070	1976	906	1,359(a)	1.3	869	63.9
Barbados	244	1975	33	66(c)	0.3	33	50.0
Grenada	96	1975	94	282(d)	2.9	94	33.3
Jamaica	2,008	1975	366	549(a)	0.3	348	63.4
Bahamas	197	1974	1	3(d)	0.0	1	33.3
British territories							
St. Vincent	96	1968	13	39(d)	0.4	13	33.3
St. Lucia	107	1973	204	408(c)	3.8	204	50.0
Montserrat	12	1975	2	6(d)	0.5	2	33.3
Antigua	70	1976	48	144(d)	2.1	10	6.9
St Kitts–Nevis–Anguilla	65	1975	4	12(d)	0.2	4	33.3
British Virgin Islands	10	...	...	10(e)	1.0	...	...
Dominica	74	1975	11	33(d)	0.5	11	33.3
Cayman Islands	11	...	...	10(e)	0.9	...	...
Turks & Caicos Islands	–	1974	...	10(e)	–	...	...
Bermuda	55	1975	2	6(d)	0.1	2	33.3
French territories							
French Guiana	58	1971	957	1,436(a)	24.8	749	52.2
Guadeloupe	349	1975	2,033	3,050(a)	8.7	1,802	59.1
Martinique	358	1973	2,180	3,270(a)	9.1	1,204	36.8
Netherlands Antilles	238	...	...	20(e)	0.1	...	...
Subtotal	17,935		22,564	35,012	1.9	17,222	49.1
PAHO/WHO Area II							
Mexico	58,118	1975	14,775	25,857(b)	0.4	10,742	41.5
Cuba	9,090	1975	4,554	6,831(a)	0.8	4,417	64.7
Dominican Republic	4,562	1976	3,739	6,544(b)	1.4	3,349	51.2
Haiti	5,414	1974	270	810(d)	0.2	94	11.6
Subtotal	77,184		23,338	40,042	0.5	18,602	46.5
PAHO/WHO Area III							
Guatemala	5,328	1977	186	558(d)	0.1	72	12.9
El Salvador	3,980	1975	1	300(e)	0.1	1	0.3
Nicaragua	2,084	1973	291	873(d)	0.4	140	16.0
Costa Rica	1,921	1976	444	777(b)	0.4	375	48.3
Panama	1,618	1975	167	251(a)	0.2	167	66.5
Honduras	2,933	1974	263	526(c)	0.2	98	18.6
Belize	136	1971	1	10(e)	0.1	1	10.0
Subtotal	18,000		1,353	3,295	0.2	854	25.9

PAHO/WHO Area IV							
Bolivia	4,688	1976	1,705	5,629	1.2	1,107	19.7
Colombia	22,807	1975	18,625	37,250(c)	1.6	16,693	44.8
Ecuador	6,951	1974	2,801	5,602(c)	0.8	2,542	45.4
Peru	15,383	1973	2,708	5,416(c)	0.4	1,638	30.2
Subtotal	49,829		25,839	53,897	1.1	21,980	40.8
PAHO/WHO Area V							
Brazil	110,124	1976	150,840	242,273	2.2	91,984	38.0
Subtotal	110,124		150,840	242,273	2.2	91,984	38.0
PAHO/WHO Area VI							
Argentina	26,056	1967	9,627	14,852	0.57	6,122	41.2
Chile	10,655	1975	36		–	13	36.1
Paraguay	2,647	1976	5,160	–	1.9	3,157	36.1
Uruguay	2,782	1976	492	556	0.2	492	88.4
Subtotal	42,140		15,315	15,408	0.4	9,784	63.5
Area I total	17,935		22,564	35,012	1.9	17,222	49.1
Area II total	77,184		23,338	40,042	0.5	18,602	46.5
Area III total	18,000		1,353	3,295	0.2	854	25.9
Area IV total	49,829		25,839	53,897	1.1	21,980	40.8
Area V total	110,124		150,840	242,273	2.2	91,984	38.0
Area VI total	42,140		15,315	15,408	0.4	9,784	63.5
All the Americas Grand total	315,212		239,927	389,927	1.2	160,426	41.1

(a) + 50%; (b) + 75%; (c) + 100%; (d) + 200%; (e) estimated, 10 cases.

II. Summary of the situation by PAHO/WHO areas

Table 1 shows the number of known patients in American countries or other political units, percentage under surveillance and an estimate of the total number of cases.

1. PAHO/WHO AREA I

1.1. Political units included in Area I

1.1.1 Countries: Venezuela, Surinam, Guyana, Trinidad & Tobago, Barbados, Grenada, Bahamas, and Jamaica (Total: 8).

1.1.2 Territories:

1.1.2.1 *English:* St Lucia, St Vincent, Dominica, Montserrat, St Kitts–Nevis–Anguilla, British Virgin Islands, Antigua, Cayman Islands, Turks & Caicos Islands, and Bermuda (Total: 11).

1.1.2.2. *French:* Guadeloupe, Martinique, French Guiana and St Maarten (1/2) (Total: 4).

1.1.2.3 *Dutch (Netherlands Antilles):* Curacao, Aruba, Bonaire, St Eustace, Saba and St Maarten (1/2) (Total: 6).

1.2. Size of the problem

Leprosy endemicity is highly distinct in this area. Some countries or territories have prevalence rates relatively high (from 9 to 25 per 1,000: French Guiana, Guadeloupe, Martinique and Surinam), however, the multibacillary rates are lower than elsewhere in the area.

With respect to the English-speaking countries and territories, the main leprosy problem is in St Lucia (prevalence rates over 4 per 1,000), Trinidad & Tobago, Guyana and Grenada (rates over 2 per 1,000).

In Venezuela, the estimated prevalence rate is less than 2 per 1,000. Over the past 25 years there has been a small reduction of prevalence, perhaps because over 50% of patients, and similarly in most of the South and Central American countries, are multibacillary cases which, normally, are not released from control. However, case detection rates of all forms of leprosy (incidence) have declined over the same period of time by 75%.

Leprosy prevalence in other countries and territories seems to be low.

1.3. Operational management, facilities and activities

In Surinam, Guyana and Jamaica there are well-organized control programmes, integrated into general health care units but with central policy-making and supervisory departments. In Trinidad & Tobago control actions (case detection and case holding) are the responsibility of a vertical programme but with good co-ordination with general health structures. The organizational aspects of leprosy control in the other English-speaking territories are in the planning stage.

In Venezuela, the service situation is generally well developed, although there are some local variations. The national service for skin diseases has policy-making functions (planning, evaluation) while programme implementation is decentralized and under the responsibility of the state health service.

Standard treatment in all countries and territories is Dapsone, oral or parenteral, but there is a general disposition to adopt combined schedules if Clofazimine, Rifampicin or other drugs are available.

2. PAHO/WHO AREA III

2.1. Countries included in Area II

Mexico, Cuba, Dominican Republic and Haiti.

2.2. Size of the problem

The leprosy cases shown in Table 1 have a patching distribution that can be summarized as follows:

(a) Mexico – 70% of the patients are from the Pacific states (Guanajato, Sinaloa, Najarit).
(b) Cuba – more than half the cases live in the eastern side of the island (Camaguey and South-east provinces).
(c) Dominican Republic – two interesting epidemiological patterns deserve mention, one is the urban distribution of cases (more than 50% live in Santo Domingo) and the other is that most of the patients are women.
(d) Haiti – the situation is practically unknown. A few cases were detected in only one service, the University Hospital at Port-au-Prince.

2.3. Operational management, facilities and activities

In Mexico, policy-making and evaluative functions are centralized in a 'Chronic Skin Diseases Program', and leprosy control implementation is the responsibility of the local health services. Evaluation is assisted by a well kept data registration and information system.

In the Dominican Republic, the leprosy control programme is, by government delegation, the responsibility of a private institution – the Instituto Dermatológico, with policy-making and evaluative functions (there is a written manual of technical and administrative policies). Programmed activities are implemented with the Ministry of Health infrastructure and utilize paramedical personnel to execute the main control actions (screening examinations of contacts and general population for case detection and administration of treatment).

The treatment schedule adopted in Mexico and Haiti is Dapsone monotherapy, generally in low doses (50–100 mg weekly) but in Cuba and the Dominican Republic the administration of combined treatment (DDS and/or Rifampicine and Clofazimine) is routine.

PAHO/WHO AREA III

3.1. Countries included in Area III

Guatemala, Honduras, El Salvador, Costa Rica, Nicaragua, Panama and Belize.

3.2. Size of the problem

This area, considered globally, has the lowest prevalence rate in the Americas (excluding the United States and Canada). The main foci are located in the

Pacific coast (provinces of El Salvador, Honduras and Nicaragua around the Fonseca gulf, Azuero peninsula in Panama). Only two foci are known on the Atlantic coast (Limones in Costa Rica and Bocas del Toro in Panama). The situation in El Salvadore is practically unknown, but more than 200 cases were detected in the past (1958–67). It is interesting to note that in Belize only one case of leprosy was detected, an old lepromatous arrested case.

3.3. Operational management, facilities and activities

In three countries (Honduras, Costa Rica and Panama) there are well organized control programmes, integrated into the general health structure (with social security in Costa Rica). In Nicaragua and Guatemala, there are practically no control activities, except the care of a few in-patients (leprosaria). In El Salvador a few patients (not officially notified) are treated in the dermatological ward of Rosado Hospital in San Salvador.

With the exception of Costa Rica that has adopted combined regimens and parenteral Dapsone, the rule is DDS monotherapy.

The need of training and motivation in the area is evident.

4. PAHO/WHO AREA IV

4.1. Countries included in Area IV

Colombia, Ecuador, Peru and Bolivia.

4.2. Size of the problem

The most important problem is Colombia with around 2,000 cases under treatment. The main focus (70% of the cases) lives in the departments of the oriental branches of the Andean 'Cordillera', that is, some important foci are in altitudes above 2,000 m (6,000 feet). In Ecuador most of the detected cases came from the Pacific coast provinces (Guayas, Los Rios and El Oro) and very few cases from the 'Sierra' or Andean 'Cordillera'. In Peru more than 80% of the cases are infected in the Amazon rain forest, mainly on the Ucayali river and its tributaries.

In Bolivia, leprosy apparently does not propagate in the Andean highlands, but the situation is serious in the Santa Cruz and Chuquisaca departments (south-east of the country) on the slopes of the Andes and the far north-east – the Amazon basin.

An intensive survey (Vallegrande province, Santa Cruz department 32,000 inhabitants) has shown a point prevalence of 14 per 1,000 (1974).

4.3. Operational management, facilities and activities

Colombia, since 1958, has a structured vertical programme implemented by 32 regional units. By decision of the Ministry of Health all facilities and personnel were decentralized (October 1977) to the Department of Health Services, and a policy of integration is to be implemented in the next years. The first one will be Santander and a plan for this aim was drafted.

About 4,000 cases are treated as in-patients (leprosaria).

Ecuador, since 1975, has integrated its leprosy control activities into the general public health services. The results are satisfactory in Guyas province where a well-developed health infrastructure exists, but not in other endemic areas like Los Rios, Bolivar and El Oro where the health centres or posts are not staffed or managed as expected.

In Peru, leprosy control activities are integrated into the health service of the Amazon departments (Loreto and San Martin), The main structure for primary medical care of the scattered population is the 'Posta Sanitaria' staffed with only one auxiliary health worker. Most of these auxiliaries are trained for diagnosis of suspicious leprosy cases and administration of treatment.

Leprosy control in Bolivia is centered around four focal points.

(a) Jorochito Hospital near Santa Cruz city – 90 beds (for tropical diseases in general) and out-patients ward, but no field activities.
(b) Vallegrande Province (Santa Cruz Department) is a field station of CENETROP (Centro Nacional de Enfermedádes Tropicales) where a comprehensive health care programme is being implemented and leprosy control is an important component.
(c) Monteagudo Hospital and out-patient clinic. There are 15 beds (only for leprosy patients) but the main activities (case detection and treatment delivery) are implemented in the foci by travelling teams (doctor and auxiliary personnel).
(d) Beni Department (Amazon basin) – patients are cared for in a dermatological ward in Trinidad (Department capital– and those who live far from this centre are visited (no more than once a year) by personnel from the haemorrhagic fever programme which is headed by the chief of leprosy control.

Treatment policy is Dapsone monotherapy but the Ministry of Health is aware of the need for combined regimens.

5. PAHO/WHO AREA V

5.1. Country included in Area V

Brasil.

5.2. Size of the problem

More than 60% of the known leprosy cases in the Americas are from Brazil, but as observed in almost all other American countries, the geographical distribution of the endemia is uneven. A breakdown of the prevalence rates for the region (December 1975) is:

	per 1,000
Amazon	2.94
North-east	0.30
South-east	1.68
Midwest	2.01
South	0.97

The Amazon region has 6.9% of the general population of the country and 15.6 of the registered cases, while for the south-east region the figures are 42.2% and 55.1% respectively.

In 1975 the incidence of detected new cases was 8.68 per 100,000 inhabitants (9,300 patients) and 48.7% lepromatous and borderline forms.

5.3. Operational management, facilities and activities

All activities for leprosy control are integrated into the state general health services, but policy-making and general evaluation is centralized in the Ministry of Health (Division of Sanitary Dermatology).

In Brazil there are 29 leprosaria or colonies with 15,689 in-patients (December 1975).

General treatment policies include Dapsone monotherapy (oral or repository) and particularly in the last year combined regimens (Rifampicine, DDS and Clofazimine).

Recently the federal government decided to formulate and implement a special leprosy control programme under the responsibility of the sanitary dermatology division to cope with the problem of Acre State and the Amazon territories and Para State bordering the transamazon highway.

6. PAHO/WHO AREA VI

6.1. Countries included in Area VI

Argentina, Uruguay, Paraguay and Chile.

6.2. Size of the problem

Argentina: The known cases are concentrated in the north-eastern provinces and also in the metropolitan area of 'Great Buenos Aires' where about 70% of the cases are not autochthonous but immigrated from hyperendemic areas.

Paraguay has the highest prevalence in South America (estimated at 3.3 per 1,000), and almost all cases detected in the eastern provinces and the capital, Asuncion.

In Uruguay the only information available shows that there are 514 leprosy patients under treatment in Montevideo.

Chile presents a unique epidemological situation – no cases (except a few imported from Argentina) and a high prevalence (30 per 1,000) in Easter Island, about 5,000 miles from the Chilean Pacific Coast, and with a population ethnically polynesian.

6.3. Operational management, facilities and activities

Leprosy control in Argentina was integrated into the general health services in 1969, a policy that could not be implemented due to many difficulties encountered in co-ordinating the field work.

The Ministry of Health has recently decided to formulate a new programme with PAHO/WHO assistance. The general strategy will be the implementation of a vertical specialized control programme, taking full advantage of existing resources and structures which have proved their usefulness in detecting and treating leprosy cases (about 25% of the patients are being treated by private doctors).

In Paraguay there is a well planned and supervised control programme which utilizes paramedical personnel for case detection (screening examination), patient care and emphasizes close co-ordination with the general health structure.

An important source of funds for programme implementation came from ILEP (German Leprosy Relief Association).

Uruguay is just starting to evaluate its problem; many patients are now being treated and a request was presented to PAHO/WHO to assist in the formulation of a programme.

In Chile the few patients detected in Easter Island are under treatment. A survey of the total population (1,200 inhabitants) will be made in May 1978 by a PAHO/WHO adviser and control measures will be recommended.

In Area VI there are 5 leprosaria (Argentina, 1,048 patients; Paraguay, about 350 patients).

Treatment is mainly Dapsone monotherapy, except in Argentina where combined regimens are being planned.

7. CONCLUSIONS AND RECOMMENDATIONS

7.1

Leprosy in the Americas could be considered as 'median prevalence'. Nevertheless, there are some limited foci where transmission seems to be comparable with some African or Asian foci.

7.2

In many American countries the leprosy problem does not have priority related to the size of the problem and relative public health importance.

7.3

One of the main constraints to implementation of control programmes is a general shortage of funding sources coupled with deficient training (and motivation) of the personnel.

7.4

The financial assistance offered by the Japanese shipbuilding industry will be welcome by all interested countries and will help to remove many of the observed constraints if used judiciously.

The general stategy proposed for utilization of JSIF funds through WHO is:

7.4.1 Reinforcement and upgrading of training and training capability. Priority must be assigned to countries of fewer resources and more important problems.

(a) Some funds could be used to facilitate mobility of trainees and staff in field work demonstration at CEPIALET (PAHO/WHO Panamerican Center for Research and Training in Leprosy and Tropical Diseases – Caracas, Venezuela.)

(b) Awarding of fellowships for training abroad (CEPIALET).

(c) Assistance in national courses or seminars.

(d) Assistance for regional or subregional workshops on selected matters of control policies.

(e) Funding of STC expenses for educational technology for CEPIALET.

7.4.2 Funding of advisory services (PAHO/WHO STC) for programme formulation.

7.4.3 Assistance to selected countries or areas in implementation of field work (transportation and subsistence expenses).

7.4.4 Supplies and equipment – mainly drugs for combined regimens (Rifampicin, Clofazimine, DADDS).

7.4.5 Some allocations to reinforce PAHO/WHO duty travel expenses (regular staff).

The annexed tentative budget (1978–81) was drafted taking into account this proposed strategy.

Lepr Rev (1980) **51**, 295–301

Special Article

THE IMMUNOGENICITY OF KILLED MYCOBACTERIA

Introduction

The question of the immunogenicity of killed mycobacteria has become of enormous importance in relation to the future design of vaccines for leprosy. Much attention has been focused on the fact that a single subcutaneous injection of relatively large numbers of leprosy bacilli killed by heat or by irradiation induces in mice a state of delayed skin-test positivity, which can be elicited with soluble antigen a few days later.[1, 2] Moreover, the killed organisms evoke resistance to live leprosy bacilli,[3] resistance to an intravenous challenge with *Mycobacterium tuberculosis* and local resistance to *Listeria monocytogenes.*[1] There is an increasing tendency to regard this immunogenicity of killed organisms as exceptional and as an indication of some bizarre property unique to *Mycobacterium leprae.* This tendency is a consequence of a widespread misconception that other species of mycobacteria lose their immunogenicity when killed, whereas this is in fact true only of a minority of species, in certain strains of mice.

The immunogenicity of killed mycobacteria in guinea-pigs and rabbits

The first report of the induction of positive tuberculin reactivity in guinea-pigs with killed *Mycobacterium tuberculosis* was published by Baldwin in 1911 and subsequently discussed by Petroff[4] in 1923. This author extended the studies of Baldwin and observed that from 1–3 intraperitoneal injections of 12.5 mg (dry weight) of dead tubercle bacilli, even if autoclaved at 121°C at 15 lb in^2, was as effective a way of inducing cutaneous tuberculin-test positivity as was a virulent infection. Using this protocol, Petroff and Stewart[5] subsequently demonstrated that guinea-pigs immunized with killed bacilli were also indistinguishable from infected ones on the basis of susceptibility to delayed fatal haemorrhagic tuberculin shock and showed a similar degree of protection from an intracutaneous challenge with virulent organisms.

These results were obtained in spite of the fact that the intraperitoneal route is not an efficient one, but very large doses of killed organisms were

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required. Freund and Opie (1938),[6] working with rabbits, tried various routes and were able to show that a far smaller dose of killed organisms (0.2 mg at weekly intervals) injected subcutaneously or intracutaneously was as effective as BCG, although repeated injections were required for most animals. Thus, in their own words (p. 296): 'Rabbits (and human beings) differ widely in the rapidity with which they undergo sensitization with heat-killed tubercle bacilli, but after repeated injections all animals become sensitized.'

Similarly Wilson *et al.* (1940)[7] injected guinea-pigs intramuscularly with various doses of heat-killed *M. tuberculosis* (at 5–7-day intervals for 12–20 weeks) and reported that after a variable number of injections all the animals became skin-test positive, some of them exquisitely so. Highly significant protection from subsequent intramuscular challenge with from 12–300 live *M. tuberculosis* organisms was also seen in those animals with weak or moderate responses.

Numerous other examples could be quoted (see Weiss, 1959, for 263 refs)[8] but these suffice to indicate that in rabbits and guinea-pigs killed *M. tuberculosis* is immunogenic. Moreover, the method used to kill the organisms seems to be of secondary importance since workers using heat, ultra-violet light, prolonged storage, formalin, urea or phenol, reported essentially similar findings.[8] However, large doses, or repeated injections, or both, are needed in order to evoke responses comparable to those evoked by li e organisms. (In the early years this quantitative difference was less obvious because even live organisms were used in quite unnecessary doses, often of several milligrams.) At first sight the explanation for this difference between the immunogenicity of live and dead organisms seems likely to be a trivial one. Thus live organisms proliferate and synthesize more antigen and so constitute a far greater and more prolonged immunogenic stimulus than an equivalent number of killed organisms. Large or repeated injections of the latter clearly compensate for this difference. However, the situation is in reality rather more complex. Although killed organisms injected into a large number of outbred guinea-pigs will evoke in at least some of the animals all of the phenomena associated with the response to live ones (necrotic or non-necrotic skin-test responses, tuberculin shock and protection), it is clear from studies by Raffel[9] that the percentage of the animals showing the *necrotic* type of skin-test reactivity is lower when killed organisms are used, even when skin-test reactions of a similar size are obtained. Moreover, the relationship between skin-test antigen dose and reaction size is different. Killing *M. tuberculosis*, therefore, has qualitative as well as quantitative effects on its immunogenicity.

The immunogenicity of killed mycobacteria in mice

The significance of these observations becomes clearer when one considers the immunogenicity of killed mycobacteria in mice.

Our present concepts of the cellular basis for immunity to facultative or obligate intracellular parasites originate in the studies of immunity to *Listeria monocytogenes*, *Brucella abortus* and *Salmonella typhimurium*, in mice reviewed by Mackaness.[10]

These organisms do not evoke protective immunity when injected dead and Mackaness suggested that this is because they lack 'built-in' adjuvanticity. This view is supported by the more recent observation that certain adjuvants, such as polyanions, or the cationic surface-active agent dimethyl, dioctadecyl ammonium bromide, when mixed with killed *Listeria monocytogenes*, can render it protective.[11] However, Mackaness also made the point that mycobacteria are different from these other genera, in that they *do have* 'built-in adjuvanticity' and many are immunogenic in mice when killed, though as in rabbits and guinea-pigs larger doses are required than when live organisms are used.[10, 12] Numerous examples in mice were also reviewed by Weiss.[8] In this respect many mycobacterial species resemble *Corynebacterium parvum*, which has a similar adjuvant-active cell wall and is routinely used killed.

More recently workers who use mice have lost sight of this well-established immunogenicity of most killed mycobacteria and have begun to make misleading generalizations such as that 'immune reactivity to mycobacteria or other intracellular parasites develops only when bacilli multiply in the phagocyte'.[13] Such statements are obviously in direct conflict with a huge amount of published evidence. How has this apparent disagreement come about? One possibility, for which there is good evidence, is that killing does not have the same effect on the immunogenicity for mice of all mycobacterial species. It is often possible to predict whether or not killing an organism will reduce its immunogenicity for a particular mouse strain, by studying the type of response evoked in that mouse strain by the same organisms injected live. Thus delayed skin-test (foot-pad test) responses in mice have distinct 24-hour and 48-hour components.[2, 13, 14] Those organisms which evoke little skin-test positivity during the first 2–3 weeks, but then cause a response with a powerful 48-hour component, are the ones which are not immunogenic when killed. The best examples of this are *Mycobacterium kansasii*,[2] *M. lepraemurium*,[13] some *M. avium* strains and some, but not all, strains of BCG (own published observations) or *M. tuberculosis*. These, although a minority of mycobacterial species, are the most commonly used organisms and therefore the view that killed mycobacteria are not immunogenic has dominated. Indeed these organisms, when injected killed, sometimes not only fail to evoke skin-test positivity or protective immunity, but actually trigger mechanisms which allow increased proliferation of a simultaneously administered live inoculum.[13]

Other organisms, however, evoke mainly the 24-hour component. This type of response more closely resembles that induced by *Listeria monocytogenes*,[10] or *Corynebacterium parvum*. It appears within a few days of infection, reaches a maximum or plateau by about 10 days and peaks 18–24 hours after skin-test

challenge with soluble antigen. (The 2–3-week delay is not seen and the 48-hour component is weak or absent.) Those mycobacteria which evoke this '*Listeria*-like' response, when injected live, will do so equally well when injected dead if a dose of at least 2×10^7 organisms is used. This property, which is characteristic of *Mycobacterium vaccae*[2] and *M. nonchromogenicum*, is shared by Glaxo BCG, *Corynebacterium parvum*, *Mycobacterium leprae*,[2] and probably other, non-pathogenic species.

These types of response also differ by several other criteria but it is not yet clear whether the basis for the differen
level.[2] Both types of response can correlate with protection, though under different circumstances (Rook, Bahr and Stanford, submitted for publication). Thus the 48-hour type of response correlates very strongly with protection against *dissemination* of *M. avium* (McIntyre and Rook, unpublished observations) or *M. lepraemurium*[14] from superficial sites. On the other hand, it is the '*Listeria*-like' response which correlates with the best protection against systemic challenge with these organisms. Moreover, the '*Listeria*-like' response gives full protection against foot-pad challenge with *M. Kansasii* (Rook and Stanford, submitted for publication) or *M. leprae*. The importance of these findings lies in the fact that, as discussed above, killing mycobacteria preferentially reduces the 48-hour component in mice, and the necrotic or 'Koch' component in guinea-pigs (perhaps these are analogous). We clearly need to understand better the protective relevance to man of the human equivalents of these types of response.

The immunogenicity of killed mycobacteria in man

During the first 25 years of this century approximately 23,000 individuals in Italy were vaccinated with heat-killed *Mycobacterium tuberculosis* by scarification.[15] Subsequently more than 50,000 children were vaccinated with intradermal injections of formalinized organisms (1.5 mg, wet weight.[15] Unfortunately these trials were almost entirely uncontrolled, and evidence for their success is anecdotal. Nevertheless, this evidence is suggestive and the disappearance of the killed vaccine appears to have been due not so much to its own failure, as to the proven success of BCG in well-organized trials. However, two 'defects' of the killed vaccine were noted. First, 30–50 times as many organisms were required, and these, even when given in divided doses, often resulted in distressing lesions. Secondly, there was a theoretical objection to the fact that unlike BCG, the killed vaccine caused little local lymph-node involvement. It was felt that the ideal vaccine should mimic the 'primary complex' of a tuberculous infection. This led to the interesting suggestion that killed organisms failed to reach the draining node, and that greater lymph-node involvement could be achieved by adding hyaluronidase to the vaccine (reviewed[15]).

Surprisingly, our knowledge of the immunogenicity for man of killed *M. leprae* is even more fragmentary. It is still not clear whether Lepromin acts as an unusual skin-test reagent, or as an immunizing stimulus, or both. There are of course numerous reports that repeated Lepromin tests can cause positive Mitsuda responses in previously negative individuals,[16] and that Mitsuda positivity correlates with a decreased risk of disease, particularly of the lepromatous type.[17] However, these studies have involved small selected groups, already exposed to living leprosy bacilli, and do not constitute proof that one or more doses of killed *M. leprae* will evoke either Mitsuda positivity or protection in normal, previously unexposed populations.

Is killed *Mycobacterium leprae* unique?

It is obvious from the work reviewed above that the immunogenicity of killed *M. leprae* in mice is not in itself unique. On the other hand, the studies of Shepard and his colleagues point to two ways in which *M. leprae* does appear to differ from all the other species which have been studied, though we cannot at present be certain that unusual properties of the available preparations of *M. leprae* bacilli are not due to unusual methods used in this preparation. First, only *M. leprae* causes *chronic* lymph-node enlargement when injected killed.[3, 16] This contrasts with the relative failure of killed *M. tuberculosis* to cause a lymph-node reaction, which was discussed earlier. It will be interesting to know whether this property of killed *M. leprae* should be attributed to a tendency to localize in the draining node, or to very slow degradation. The latter could be explained by the unexpectedly high proportion of glycine in the wall of *M. leprae*[19] which may be related to an unusual peptidoglycan structure of extreme stability.

The second possibly unique feature of the immunogenicity of *M. leprae*, is that killing may a
any property of an organism which affects the way in which its antigens are presented to the host's antigen-recognizing cells and which is dependent on the viability of the organism, will be relevant to the consequences of killing it. Live *M. leprae* can escape from phagosomes, and it gets into the cytoplasm of many cell types, including macrophages, muscle cells and fibroblasts. Moreover, it can get into nerves, which may constitute an 'immunologically privileged site'. Antigens leaking from such sites into the blood-stream have been shown to evoke suppression rather than immunization.[20] (This mechanism has been discussed recently in relation to leprosy.)[21] When killed, the leprosy bacillus presumably does not get into these 'hiding places', but rather into phagosomes of phagocytic cells, capable of 'processing' antigen and presenting it to the antigen-recognizing system. Thus, killing would be expected to increase immunogenicity.

Conclusion

The variable effect of killing on the immunogenicity of different mycobacterial species is clearly trying to tell us something fundamental about the biology of the organisms. It is therefore essential to build up an accurate picture of how *Mycobacterium leprae* compares with other species.

(1) The immunogenicity of killed *M. leprae* is not in itself unique (although it has unusual features). There is a huge neglected literature on the ability of killed mycobacteria to evoke *both* skin-test positivity *and* protection.

(2) It is possible that killing *M. leprae* causes *qualitative* changes in the type of response evoked. This is true of pathogenic members of the slow-growing subgenus, which, when killed, evoke less of the necrotizing component. We therefore need to know more about the relevance to protection of these different components. However, the response to killed *M. leprae* in mice resembles that evoked by several non-pathogenic members of the fast-growing subgenus, rather than the response to pathogenic slow-growers.

(3) We know that BCG, a living vaccine, can protect man against leprosy. There is at present no evidence that killed *M. leprae* is immunogenic (skin-test positivity or protection) in people not previously exposed to living leprosy bacilli, but a review of the literature involving killed mycobacteria suggests that it will be. It remains, nevertheless, an act of faith.

G A W ROOK

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Preliminary immunological studies in search of correlates of protective immunity carried out on some Iranian leprosy patients and their families

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Summary Multiple skin-testing, lymphocyte transformation tests and enzyme-linked immunosorbent assay of antibodies to mycobacterial antigens have been carried out on patients and their healthy children living in Baba Baghi Leprosy Sanatorium in Iran. The data reported shows a remarkable correlation between responses to *Mycobacterium leprae* and *M. vaccae* in all 3 test systems.

The percentage of positive responders to skin tests with Leprosin A amongst the children is higher than has previously been found and BCG has been shown to enhance the capacity of the individual to recognize *M. leprae* in this way. Finally, the majority of a small number of children considered to be protected from leprosy have been shown to possess lymphocytes that transform in the presence of *M. leprae* and *M. vaccae* antigens, but little antibody to *M. leprae* by the enzyme-linked immunosorbent assay. Of the 3 types of test assessed here only skin-testing appears to be of any value as a measure of protection, but whether even this will prove useful at the individual level is far from certain.

Introduction

The purpose of this study was to evaluate 3 tests as correlates of protection from mycobacterial disease. It was carried out at Baba Baghi Leprosy Sanatorium near Tabriz in Iranian Azerbaijan. None of the children born and

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brought up in the Sanatorium have developed leprosy in the 20 years that families have lived there, despite their frequent contact with new bacilliferous patients and a marked tendency amongst a few of the patients not to take their drugs. The majority of the children must therefore have protective immunity since most are in daily contact with bacilliferous cases of the disease. Since it is not possible to define a control group without protection we make a comparison between healthy children and the patients with whom they live. Of the 47 families living at Baba Baghi, 45 agreed to take part. Each person was skin tested and subsequently blood samples were obtained from individuals selected on the basis of skin-test results. These were used for lymphocyte transformation studies and enzyme-linked immunosorbent assay of antibodies.

Materials and methods

THE FAMILIES

Forty-two of the families consisted of leprous parents and from 1 to 5 healthy children. One family consisted of the husband and 2 wives, all with leprosy and 3 healthy children. Two families consisted of a mother with leprosy, each with a single healthy child.

A grandfather lived with one of the families, an uncle with another family and an uncle and aunt with a third family. In each case these extra family members had leprosy. One of the healthy children was herself married to a leprosy patient.

Apart from a few children who were staying with relatives away from the Sanatorium or who had got married and left, and one child who went on holiday before the skin tests were read, every family member was skin tested. Some of the children had accompanied parents from their village when they first came to the Sanatorium, but most were born in the Sanatorium and had spent all their lives there. The total number of parents and other relatives studied was 94 and the total number of children studied was 113. (Additionally some skin-test results for other patients in the leprosarium have been added to Table 1.)

SKIN TESTS

The reagents used were a range of new tuberculins prepared from live organisms grown on Sauton's agar, or, in the case of Leprosin A, from irradiated organisms harvested from armadillo tissues and freed from material of armadillo origin by the method of Draper (in Shepard *et al.*, 1980). In each case the harvested organisms were broken with ultrasound. The sonicates were sterilized by serial filtration, the protein content measured, and the final dilutions

Table 1. The results of skin-testing leprosy patients and their children with a series of new tuberculins

Reagent	Concentration	Patients + ve	Children + ve	Significance (Chi^2)
Leprosin A	10 μg/ml	18/127	71/113	$P < 0.0001$
Vaccin	20 μg/ml	3/58	16/24	$P < 0.0001$
Nonchromogenicin	20 μg/ml	17/43	18/25	$P < 0.01$
Scrofulin	2 μg/ml	21/48	12/24	ns
Xenopin	2 μg/ml	2/31	5/25	ns
Rhodesin	2 μg/ml	0/19	6/25	ns
Marinin	2 μg/ml	28/40	19/25	ns
Diernhoferin	2 μg/ml	0/7	0/4	
Neoaurumin	2 μg/ml	0/6	3/8	
Gilvin	2 μg/ml	1/6	3/4	
Duvalin	2 μg/ml	0/6	2/4	
A*-in	2 μg/ml	10/19	4/5	
Aviumin C	2 μg/ml	2/7	1/5	
Gordonin	2 μg/ml	9/21	3/5	
Chitin	2 μg/ml	2/6	2/7	
Skinsnes'reagent	2 μg/ml	13/39	19/31	$P = 0.02$
ICRC reagents	2 μg/ml	5/12	10/15	ns
Kanasin	2 μg/ml	17/26	–	
Aviumin A	2 μg/ml	3/12	–	

In this table + ve refers to responses of 2 mm or more of induration to the skin-test reagents shown.

prepared in a tween containing borate buffer containing tween (Paul *et al.*, 1975). The concentrations used for skin testing were 20 μg/ml in the cases of Vaccin and Non-chromogenicin, 10 μg/ml in the case of Leprosin A, and 2 μg/ml for all the other reagents.

Every person was skin tested by the intradermal injection of 1 μg of Leprosin A and all but 11 of the youngest children (aged 1–3 years) were skin tested with 2 or 3 other reagents at the same time. Reactions were recorded as mean diameters of induration at 72 hours and reactions of 2 mm or more were considered positive. The list of reagents, numbers tested and numbers producing positive responses are shown in Table 1.

Ninety-two of the children had received BCG vaccination, a number of them on several occasions. This was confirmed by examination of their arms for scars. The distribution of the children by age, BCG status and response to Leprosin A is shown in Table 2.

IN VITRO TESTS

Blood samples were taken from 23 carefully selected children. Fourteen children who did not respond to skin test with Leprosin A agreed to be bled and in 9 cases there was a suitable Leprosin A-positive brother or sister who

Table 2. The effect of BCG and age on positivity to skin tests with Leprosin A

Age	BCG − ve Leprosin A + ve	BCG + ve Leprosin A + ve	
(A) *Baba Baghi children*			
1	0/8		
2	0/6	3/4	
3	0/3	1/3	
4		3/6	
5	0/1	2/5	
6		0/4	
7		2/3	11/25 (44%)
8		2/2	
9	0/1	5/7	
10	1/1	10/11	
11		10/12	
12		7/8	34/40 (85%)
13	0/1	7/7	
14		6/6	
15		6/8	
16		4/4	
17		1/1	
18			
19			
20		1/1	25/27 (93%)
	1/21 (4.8%)	70/92 (76%)	
(B) *Children living in a town in the leprosy endemic area*			
12	4/9	13/24	
13	3/14	9/27	
14	2/9	16/26	
	9/32 (28%)	38/77 (49%)	

BCG + ve and − ve refer to vaccinated and nonvaccinated children respectively. Leprosin A + ve refers to children producing induration of 2 mm diameter or more to this reagent.

also agreed to be bled. As far as possible pairs from each family were of the same sex, roughly the same age and with the same BCG status. Five children were either single or had brothers or sisters too young to be bled for comparison. In every case the children came from families with at least one lepromatous parent. Blood samples were collected from 27 male leprosy patients (mainly lepromatous) for comparison with the children.

Lymphocyte transformation tests were carried out on cells separated with methyl cellulose in a system suitable for simple laboratory conditions as described by Rook and Stanford (in preparation). Cells were cultured in

RPM1 medium in the presence of 10% autologous serum. Leprosin A, Vaccin, Tuberculin and Nonchromogenicin were used as antigens at a final concentration of 20 μg/ml.

ENZYME-LINKED IMMUNOSORBENT ASSAY

This assay was performed as described by Voller *et al.* (1979) using peroxidase conjugated rabbit antisera to human immunoglobulin heavy chains (Dako). The antigens were the preparations used for skin-testing, diluted to 1 μg/ml and all sera were tested at a dilution of 1/800. The substrate was O-phenylene diamine. Data are presented as geometric means of absorbance values and statistical analysis was performed using log.-transformed absorbance values and Student's t test.

Results

SKIN TESTS

The results of all skin tests carried out on the patients and their children are shown in Table 1 and the significance of differences between results for the 2 groups are also indicated (Chi^2).

From Table 2 it can be seen that BCG has a considerable effect on development of Leprosin A positivity and so too does age. Thus of 21 children who had not received BCG only 1 was Leprosin A positive and of 92 BCG recipients 70 were positive. Among the BCG recipients, of the 25 aged 7 or less, 11 were Leprosin A positive, of the 40 aged 8–12, 34 were positive and of the 27 aged 13–20, 25 were positive. These results and the corresponding figures for Nonchromogenicin and Vaccin tests are shown together with the results obtained on the leprosy patients themselves in Table 3.

Table 3. The effect of age on response to 3 skin-test reagents in children who had received BCG. The results obtained in leprosy patients are shown for comparison

		Positive responders to		
Patients		Leprosin A 13/94 (14%)	Vaccin 1/17 (6%)	Nonchromogenicin 5/19 (26%)
BCG + ve, Children aged:	2–7	11/25 (44%)	1/5 (20%)	1/1
	8–12	34/40 (85%)	9/11 (82%)	7/12 (58%)
	13–20	25/27 (93%)	6/7 (86%)	8/8 (100%)

Mycobacterium nonchromogenicum and *M. vaccae* were the two species shown to be related to the leprosy bacillus in skin-test studies in East Africa by Paul *et al.* (1975), and *M vaccae* was the species most similar to *M. leprae* in some taxonomic studies (Stanford *et al.*, 1975; 1978). The sizes in millimetres

of responses of the children to Leprosin A are shown in Figure 1. Table 5 shows the mean positive reaction sizes to 5 reagents for patients and their BCG-vaccinated children. Statistical differences determined by Student's t test are indicated.

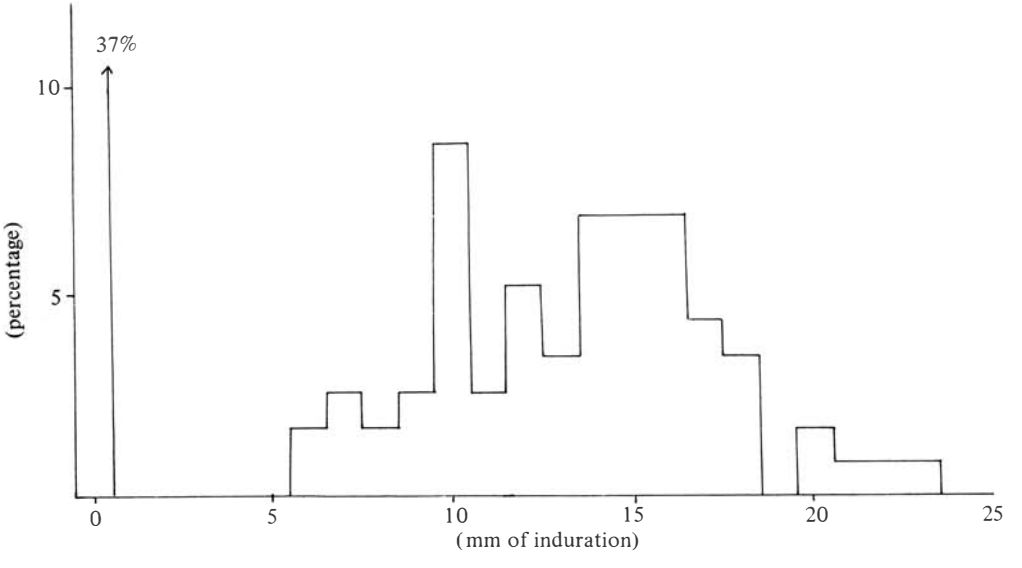

Figure 1. The percentages of children producing responses to Leprosin A expressed as millimetres of induration at 72 hours.

IN VITRO TESTS

The results of the lymphocyte transformation tests are shown in Figure 2 as ratios of uptake of radioactive thymidine by cultures in the presence of antigen and in its absence. The results for Leprosin A skin-test responders (LA + ve) and non-responders (LA − ve) are shown separately.

ENZYME-LINKED IMMUNOSORBENT ASSAY

Patients showed very significantly more IgG antibody to *M. leprae* and *M. vaccae* than either the Leprosin A − ve or the Leprosin A + ve children (Table 6). However, they did not have significantly more antibody than the Leprosin A + ve children, to *M. nonchromogenicum* or *M. tuberculosis*. There was also a tendency, not always significant, for Leprosin A + ve children to have *less* antibody to *M. leprae* and *M. vaccae* and *more* antibody to *M. nonchromogenicum* and *M. tuberculosis*, than the Leprosin A − ve children.

Discussion

The overall percentage of positive responders to Leprosin A of 64% among the 113 children tested is the highest recorded so far with this reagent. Among the 92 children vaccinated with BCG 76% responded to Leprosin A. This association of Leprosin A positivity with BCG vaccination could be explained in one of two ways. Either there is marked cross-reaction between Leprosin and BCG or BCG enhances the individuals capacity to recognize *Mycobacterium leprae* met in the environment. Of these two alternatives the latter is likely to be the true explanation. This is shown in Table 2 by the effect of age on skin-test responses among the BCG recipients. Although not tabulated this same age effect among the BCG recipients is seen for each of the reagents to which a resonable proportion of the children respond, and has been documented in previous studies (Paul *et al.*, 1975; Stanford *et al.*, 1976; Shield *et al.*, 1977; Stanford *et al.*, 1980). However, the effect is not seen with reagents prepared from mycobacterial species absent from the child's environment indicating that it is not cross-reactivity that increases with time after BCG vaccination. Recipients of the same BCG, aged 12–14 and still living in the region from which most of the patients originated, are much less responsive (49% positive) to Leprosin A than are the children reported here, (see the lower section of Table 2). Thus, in an environment where the leprosy bacillus is frequently encountered, BCG administration markedly enhances immunological recognition resulting in development of positivity to Leprosin A. That this is beneficial is suggested by the rarity of leprosy developing in these children. Thus to this extent positivity to skin-testing with Leprosin A is a measure of protection.

Unfortunately this is not likely to be the whole story, if it were then BCG vaccination might be expected to be effective against leprosy everywhere the disease is common. That other organisms are involved in the process of recognition of *M. leprae* was suggested by our earlier studies (Paul *et al.*, 1975) and has been supported by much as yet unpublished data from the field and from animal experiments.

Correlations between the results of testing with Leprosin A and with some of the other test reagents can be made. These are shown in Table 4 under two categories. The total correlations including failures to respond to both reagents and positive responses to both reagents. Positive correlations are also shown in which persons producing positive reactions to pairs of reagents are expressed as a percentage of all persons responding to at least one of the pair. Because too few persons were tested with some of the reagents for meaningful correlations to be derived the results have been pooled. 'Fast growers' include the results for Diernhoferin, Neoaurumin, Gilvin, Duvalin and Chitin; 'Slow growers' include A*-in (made from an as yet unnamed species), Aviumin C and Gordonin. The reagents prepared from 9 of Professor Skinsnes' strains grown from leprosy patients fall into 2 clusters, a group of 6 reagents showing little correlation

Table 4. The relationships between skin-test responses to Leprosin A and to the other reagents tested. See text for an explanation of the correlations shown

'Other' reagent	Leprosin A + ve	'Other' reagent + ve	Correlations	
			Total	Positve
Vaccin	19/41	17/41	39/41 (95%)	17/19 (89%)
Nonchromogenicin	22/44	22/44	33/44 (75%)	17/27 (63%)
Marinin	23/51	37/51	34/51 (67%)	22/38 (58%)
ICRC 1204	15/27	13/27	21/27 (78%)	10/18 (56%)
ICRC 1203	15/27	9/27	20/27 (74%)	8/16 (50%)
Skinsnes × 6	26/92	8/92	64/92 (70%)	2/32 (6%)
Skinsnes × 3	20/38	14/38	32/38 (84%)	14/20 (70%)
Fast growers	16/46	11/46	37/46 (80%)	9/18 (50%)
Slow growers	9/36	14/36	29/36 (81%)	7/16 (44%)
Scrofulin	19/41	16/41	30/41 (73%)	12/23 (52%)
Rhodesin	22/44	6/44	27/44 (61%)	6/22 (27%)
Xenopin	22/44	6/44	25/44 (57%)	4/24 (17%)

with Leprosin A and a group of 3 reagents showing much better correlation. Much the best correlation is shown by *M. vaccae* which appears to have some special relationship with *M. leprae*. Of the 17 individuals producing positive responses to Vaccin all produced positive responses to Leprosin A, although there were 2 responders to Leprosin A who did not respond to Vaccin. Thus positivity to Vaccin may also be a measure of protection from infection. Other species also show a correlation with Leprosin A, although to a much smaller extent. It is interesting to note that reagents prepared from 3 of Professor Skinsnes' isolates from leprosy tissues show 70% of positive correlation with Leprosin A. More results are needed with these strains to confirm this particularly since the concentration of reagents used may not have been optimal. The nature of the relationships reflected by correlations of skin-test results is not clear and again simple cross-reactivity does not appear to be the explanation. In unpublished experiments on mice, Vaccin positivity can be shown to enhance the animals capacity to develop positive reactions to Leprosin A after challenge with small doses of leprosy bacilli. Observations in animals of a relationship between *M. leprae* and *M. vaccae* have also been made by Watson *et al.* (1979). Among groups of people, mean positive reaction sizes may be of importance. Thus the children tend to produce larger-sized responses to Leprosin A than do the patients (Table 5), and the same is true of Vaccin. The reverse is the case for Marinin prepared from *M. marinum*, an organism apparently frequently met by patients and children. The results of lymphocyte transformation tests with Leprosin A (Figure 2) do not correlate with the skin-test results. Although they show some differences between the children and lepromatous patients. Of the 23 children tested, 3 show markedly greater transformation than the others. Transformation in the presence of Vaccin produced results remarkably similar to those with Leprosin A and the highest results occur in the same 3 children.

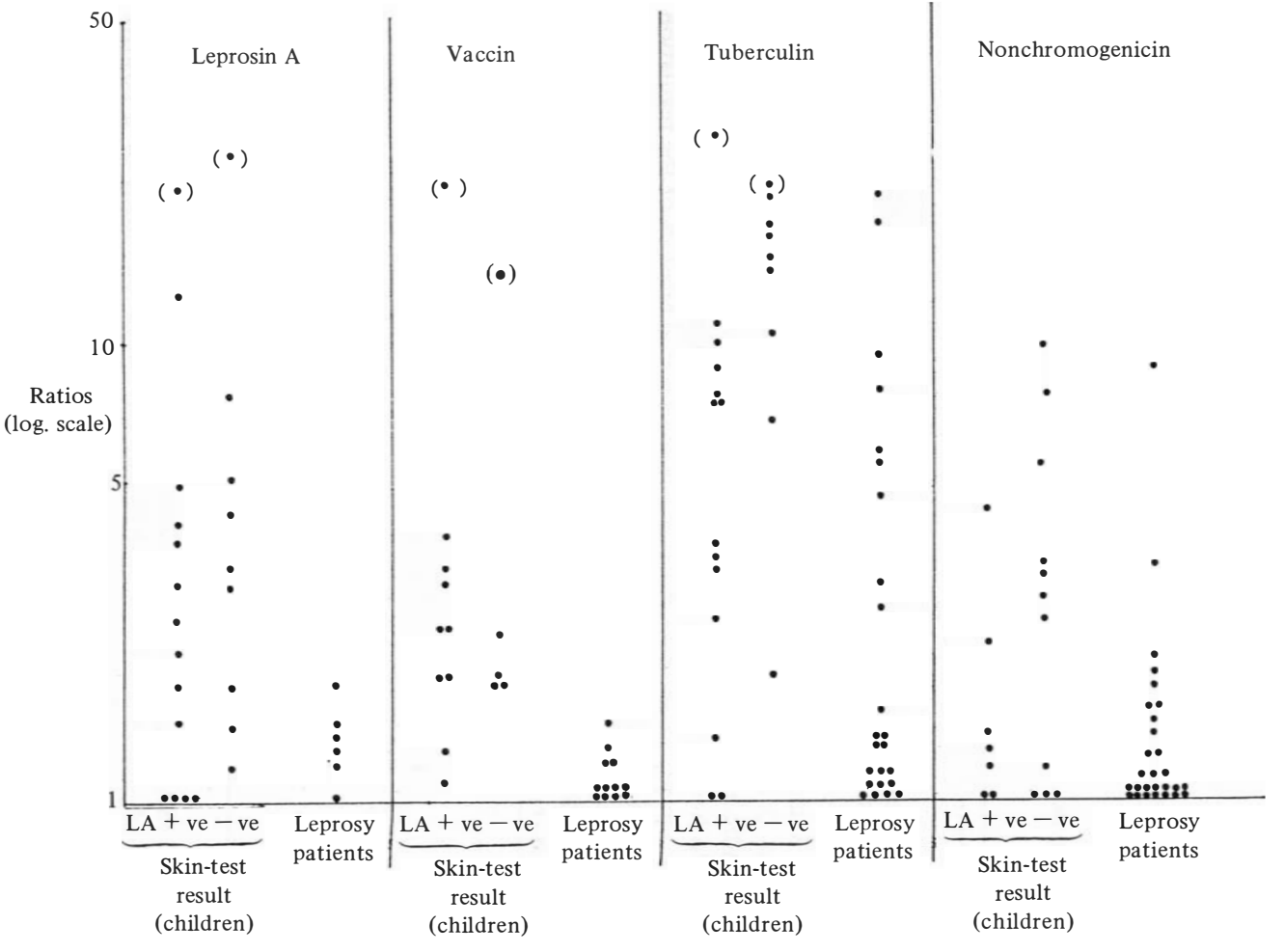

Figure 2. Lymphocyte transformation test results.

Table 5. Mean positive reaction sizes (2 mm or more induration at 72 hours) of leprosy patients and their BCG-vaccinated children to 5 skin-test reagents. Statistical significance for the different reaction sizes between the patients and the children has been calculated by Student's t test. Numbers of positive reactors are shown in parentheses

Reagent	Mean positive reaction sizes		Significance (t test)
	Patients	BCG + ve children	
Leprosin A	11.67 mm (18)	13.53 mm (70)	$P < 0.08$
Vaccin	5.3 mm (7)	8.33 mm (16)	$P < 0.03$
Nonchromogenicin	10.12 mm (17)	10.0 mm (16)	ns
Scrofulin	11.8 mm (21)	11.8 mm (12)	ns
Marinin	16.3 mm (26)	12.7 mm (19)	$P < 0.004$

BCG + ve refers to children who have been vaccinated with BCG.

Nonchromogenicin and Tuberculin induced a considerable amount of transformation amongst some of the lepromatous patients and did not discriminate between any of the groups. The 3 children producing the highest results with Leprosin A and Vaccin were not tested with Nonchromogenicin, but with Tuberculin, 2 of the 3 produced the highest results of their groups. Our results do not indicate that lymphocyte transformation tests of the type described here would have any value as a measure of protection. However, recent studies (Bahr *et al.*, in preparation) suggest that more detailed analysis of the regulation of such lymphoproliferative responses may provide valuable information.

Table 6. The results of ELISA of immunoglobulin to the antigens of 4 mycobacterial species. The results are expressed as absorbance values

	M. leprae	*M. vaccae*	*M. nonchromogenicin*	*M. tuberculosis*
Patients	0.29	1.93	0.189	0 42
	$P < 0.0001$	$P < 0.0001$	$P = 0.004$	
Leprosin A + ve children	0 07	0 59	0.24	0.33
	$P = 0.1$		$P = 0.0002$	$P = 0.002$
Leprosin A − ve children	0.107	0.65	0.087	0.18

The ELISA results (Table 6) again pointed to a relationship between *M. leprae* and *M. vaccae,* and also indicated that the development of a positive leprosin A response is not associated with an increased level of antibody against these organisms. Skin-test positivity may even be associated with lower levels of antibody to *M. leprae* and related species, in which case measurement of antibody to selected antigenic components may prove to be of value in the future.

The lack of development of overt leprosy lesions in these children with

considerable exposure to *M. leprae* over their entire lifetimes provides evidence of their lack of susceptibility to the disease. Thus the observations made are likely to reflect the immunological status of the protected individual. Probably the 2 or 3 children differing from the others have recently undergone or are presently experiencing some mycobacterial challenge. Whether they will successfully overcome it and their immunological parameters return to that of their fellows, and whether their fellows will in turn experience similar immunological disturbances we hope to discover by retesting the same individuals over a number of years.

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Dapsone-resistant leprosy in a population of Bamako (Mali)

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Summary Prevalence of dapsone resistance among 105 previously multibacillary patients, living in the vicinity of the Marchoux Institute in Bamako, Mali, was 5.7%. Patients had been treated for 10–29 years with a mean of 21 years. It is possible that although the amount of drug administered was only 56% of that prescribed, these long incubation times are the result of a year-long practice of administering dapsone by injections. It is possible that the technique of patient selection did not detect the appearance of resistant relapses at the earliest stages. The need for training in the early diagnosis of relapses is stressed.

Introduction

The Marchoux Institute was founded in 1933 as a research, teaching and reference centre for leprosy in French West Africa. Since 1960 it has been integrated into the Organisation de Coordination et de Coopération pour la lutte contre les Grandes Endémies (OCCGE) whose headquarters are in Bobo-Dioulasso (Upper Volta). Member states are Benin, Guinea, Ivory Coast, Mali, Mauritania, Niger, Senegal, Togo, Upper Volta and France.

During the years a great number of leprosy patients were hospitalized in the Institute or lived for shorter or longer periods in one of the 90 houses belonging to the Institute. After discharge, many patients went back to their home villages, but a number of them settled with their families in the vicinity of the Institute, creating a new village on the outskirt of the city. Patients continue their treatment at the out-patient clinic of the Marchoux Institute. In 1978 a patient from this population developed a relapse of lepromatous leprosy proven

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to be dapsone resistant (Pattyn *et al.*, 1979). It was then decided to conduct a dapsone-resistance survey among the remaining patients from this population.

Materials and methods

Starting in early 1979, smears were prepared from both earlobes and the nasal mucosa of all originally multibacillary patients treated for more than 5 years. If the bacterial index (average of the three smears) was 2 or more, a possibly active skin lesion was biopsied and sent on ice by air to the Institute for Tropical Medicine, Antwerp, where the bacilli, if present in sufficient numbers, were inoculated into mouse foot pads and screened against a full range of dapsone concentrations in the mouse diet: 0.1%, 0.001% and 0.0001%. Concentrations were checked for each new batch of food.

At 6 months after inoculation a control mouse was examined. If the count reached 5.10^5 acid-fast bacilli per foot, 4 more control mice were examined and 5 mice of each of the drug-treated groups. If in the control group no or insufficient multiplication was observed at 6 months, new harvests were performed at 7–9 and 12 months.

Results

Of the 105 originally multibacillary patients living in the village, who had a BI $\geqslant$ 2, 12 were biopsied. Five of these biopsies were not inoculated into mice because in smears prepared from the suspensions only 1–5 acid-fast bacilli (AFB) were observed. Six biopsies contained a mean of 6.10^7 AFB per gram of tissue (range $3.7\ 10^7 - 1.10^8$) and a seventh $9.2\ 10^5$ AFB per gram of tissue. These were inoculated into mouse foot pads. Table 1 shows the results. Bacilli from the biopsy with the lower bacterial load did not multiply in the control mice. One strain was fully dapsone sensitive. No low-grade resistance, e.g. multiplication only in mice fed dapsone at 0.0001% in the diet and in the controls, was observed; 3 strains were resistant to the 0.001% and 2 strains to the 0.01% dapsone concentration. In some instances only part of the mice fed a given concentration of dapsone showed bacterial multiplication with scores of 2 or 3 positive mice out of 5 examined. This points to the existence in the biopsy specimen of mixed populations of sensitive and resistant bacteria to the particular concentration of dapsone. For the final analysis these results were interpreted as resistant.

Discussion

In this study the primary selection of patients was based on the results of the bacteriological examination of the earlobes and the nasal mucosa. This resulted

Table 1. Results of mouse foot-pad tests for dapsone resistance in 7 patients

Degree of resistance	Number	Mean BI	Duration of therapy (yrs)	
			Mean	Range
Resists dapsone 0.01% in mouse diet	2	4.5	22	15–29
Resists dapsone 0.001% in mouse diet	3	4.5	20	10–26
Sensitive to dapsone 0.0001% in mouse diet	1	4		22
No multiplication of *Mycobacterium leprae*	1	3		28

in the discovery of 12 patients revealing a BI $\geqslant$ 2 (in fact of 4–4.5, see Table 1), 5 of which harboured dapsone-resistant *Mycobacterium leprae* in their skin.

Clinical examination of these patients at the end of 1979 revealed that all showed relapsing skin lesions. It is impossible to know when these clinical relapses started. These patients present themselves more or less regularly to the out-patients clinic to collect their drug, and certainly their relapsing lesions were not noted by the paramedical worker responsible. At the end of 1979, 3 patients, the two with high dapsone-resistant *M. leprae* and one of those with intermediate resistance (3 positive mice out of 5 fed 0.001% dapsone), had multiple small skin nodules. The two remaining patients had solitary skin lesions. It was furthermore noted that all the relapsing lesions had a rose to red colour, whereas normally lepromatous lesions on a black skin are black.

The patients whose skin biopsy contained only very few AFB, and were therefore not inoculated into mice, had a BI lower than the skin biopsy positive group: mean 2.8, range 2–3.5, they had been treated for 5, 6, 8, 20 and 20 years respectively. Examination of these patients in December 1979 did not reveal any active skin lesions. It would have been interesting to isolate in mice the bacilli excreted through the nose.

The patient with dapsone-sensitive *M. leprae* had a BI of 4 and had been treated since 1957. The patient whose bacilli did not multiply had a BI of 3 and had been treated for 28 years (Table 1). The only explanation possible is that both must have stopped their treatment for some time in the past, the second having restarted his treatment some months before the biopsy was taken. Since no urine tests to detect dapsone were performed it is impossible to support this hypothesis.

The shortest incubation time for the appearance of resistance was 10 years after the start of treatment, 2 occurring within 15 years and 3 between 15 and 29 years. All the patients had received dapsone injections once every 2 weeks, 1.25 g in chaulmoogra oil since 1971–72, and dapsone tablets distributed once every 2 weeks since 1977–78. Examination of the files, taking into account the injections of dapsone only, allowed to calculate that on an average 56% of the drug had been administered with a range of 41–67%. For comparison, regularity of treatment was calculated in the same way for 10 randomly selected patients treated for more than 10 years but showing no evidence of

relapse or drug resistance, the percentage of drug administered in this group was on the average 45% with a range of 27–58%.

There was no significant difference in the bacterial indices nor in the duration of treatment between the fully resistant (0.01% dapsone in the diet corresponding to the administration of 100 mg to man) and the medium-resistant strains (see Table 1).

Two patients, one with fully resistant and one with sensitive *M. leprae* had belonged in 1958 to an experimental group (described by Languillon & Clary, 1959) who had been given a combination of dapsone and thiosemicarbazone injections for an unknown period.

The prevalence of dapsone resistance among this population, taking into account the one case studied previously (Pattyn *et al.*, 1979), is 5.7% and should be compared with figures obtained elsewhere: 2.5% in Malaysia (Meade *et al.*, 1973), 6.8% in Costa Rica (Peters *et al.*, 1976), 3.7% in Israel (Levy *et al.*, 1977), 3% per year in Ethiopia (Pearson *et al.*, 1976). Our results do not allow a calculation of the incidence of dapsone resistance. Compared with the other prevalence studies mentioned, it seems that in the population studied, resistance developed after longer incubation times. It is possible that this is the result of the year-long practice of dapsone injections, which produce discontinuously high peaks of serum dapsone levels (Schneider *et al.*, 1959). Pearson *et al.* (1979) showed evidence that patients with various levels of dapsone resistance were still improving clinically for several years when given injections of dapsone (375 mg once weekly).

It should also be mentioned that the technique of patient selection followed in the present study may not have detected some early cases of dapsone resistance if resistant relapses start as solitary skin lesions from where bacilli invade the whole body producing, at a later stage only, high BI values in ear lobes and nasal mucosa. This point deserves further study.

Our results also illustrate the need for additional training of the paramedical workers for the earlier detection of relapses and clinical suspicion of dapsone resistance.

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Assaying dapsone in mouse diets

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Summary A simple colorimetric method is described for checking that dapsone-containing diets have been correctly prepared for mouse foot-pad evaluation of the dapsone sensitivity of strains of *Mycobacterium leprae*.

Introduction

The mouse foot-pad technique was first used by Pettit and Rees in 1964 to demonstrate that lepromatous patients could relapse after many years of sulphone treatment due to the emergence of dapsone-resistant strains of *Mycobacterium leprae*.[1] Numerous other strains of *M. leprae* with acquired dapsone resistance have since been isolated from relapsed lepromatous patients from many different parts of the world.[2–9] Furthermore, strains have recently been isolated with primary dapsone resistance from previously untreated patients in an area where acquired dapsone resistance is now common.[10] In order to estimate the sensitivity of strains of *M. leprae* to dapsone, bacilli are inoculated into mice fed with normal diet and to others given dapsone at concentrations of 0.0001%, 0.001% and 0.01% in the diet. Multiplication of *M. leprae* in both the control mice and in those fed with dapsone-containing diets indicates that the leprosy bacilli are dapsone resistant, since strains from previously untreated patients were inhibited by feeding 0.0001% dapsone in the diet in the original investigations when dapsone-resistant leprosy was very rare and primary resistant strains had not yet been isolated.[3] In view of the serious threat to hopes of controlling leprosy with chemotherapy based on dapsone-containing regimes posed by the spread of dapsone-resistant strains of *M. leprae*,[9] studies of the prevalence of primary and acquired dapsone resistance are currently being undertaken in a number of countries. As a consequence we have recently had requests from several newly-established mouse foot-pad laboratories for help in assaying their dapsone-containing mouse diets to check that these had

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been correctly prepared and that mixing of the drug in the powdered diet was uniform. We therefore believe that a description of the simple colorimetric method that we have used over the past 6 years to assay mouse diets might be of value to other workers contemplating such estimations. This method, which is based on previous adaptations[11, 12] of the Bratton and Marshall procedure,[13] is somewhat simpler than that described by Levy and Peters.[14]

Method

Two gram amounts of powdered diet are extracted by shaking for 1 minute with 20 ml ethyl acetate in a stoppered centrifuge tube on a vortex mixer. After centrifugation and filtration, 10 ml of the ethyl acetate extract is then extracted by shaking with 2 ml 2N HCl. Prior to extraction with HCl, ethyl acetate extracts from the diets containing the highest concentrations of dapsone are first diluted with ethyl acetate to give a calculated concentration of about 1 μg/ml dapsone (e.g. 10-fold for diets supposedly containing 0.01% dapsone). Aliquots of the 2NHCl extracts (1 ml) are then diluted with an equal volume of ethanol and reacted by the successive addition at 5-minute intervals of 2 drops (0.01 ml) of freshly prepared 1% (w/v) aqueous sodium nitrite, 10% (w/v) aqueous ammonium sulphamate and 1% (w/v) N-l-naphthyl-ethylene-diamine-dihydrochloride in acetone/water (1 : 1 by volume). Fifteen minutes later the extinction of the reaction product is measured at 570 nm and the concentration of dapsone in the mouse diet calculated by comparison with the results obtained by extracting 10 ml ethyl acetate containing 1 μg/ml dapsone with 2N HCl and reacting in the same way. Results should be corrected for any Bratton–Marshall positive compounds extracted from normal unmedicated diet. The amounts of such compounds present in the powdered 41B diet used in the studies of Rees and his colleagues were extremely small and equivalent to less than 0.00002% dapsone.

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Prevalence of leprosy among in-patients in general hospitals—A survey in Bombay*

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Summary Screening of 11,505 adult in-patients admitted in various general hospitals (for complaints other than leprosy) revealed that 101 had leprosy with a prevalence of 8.8 per 1,000. Ten of these were found to be smear positive (prevalence rate 0.9/1,000). Such surveys provide a quick and convenient method of screening the urban population, especially adults who usually are not available during mass surveys.

Introduction

The National Leprosy Control Programme in India is based on the survey, education and treatment (SET) technique. Our experience during surveys in urban slums shows that a large section of the adult male population is not available for examination during house-to-house visits, because the subjects are away at work. Further, it is amongst the adult male population that the highest prevalence of the disease and bacteriological positivity are encountered (Ganapati *et al.*, 1977). We have therefore examined in-patients admitted to various hospitals in Bombay, to determine the prevalence of leprosy amongst them. Surveys for leprosy in factories and industrial establishments are difficult to conduct because if any worker is found to be suffering from bacteriologically positive types of leprosy he is liable to lose his job or remain on long leave till he becomes negative.

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Material and methods

Six large hospitals in Bombay, including three catering for industrial workers (under the Employees State Insurance Scheme–ESIS), were selected for the study. One large tuberculosis hospital was also included. None of these hospitals admitted patients for the treatment of leprosy *per se*. All the in-patients except seriously ill and post-operative cases were examined at the bedside for evidence of leprosy by medical and paramedical teams, trained and experienced in leprosy. Each hospital was surveyed twice after an interval of about 4 months to cover freshly admitted patients. All the suspected infectious cases were subjected to bacteriological examination at different leprosy treatment centres in the city.

Observations and discussion

Table 1 shows the population examined and the number of patients detected.

Table 1. Population examined and prevalence rates

	Male adult	Female adult	Child population	Total
Enumeration	8,197	3,800	830	12,827
Examination	7,880	3,625	799	12,304
Percentage of coverage	96%	95%	96%	95%
Total cases	76	25	2	103
Prevalence per 1,000	9.6	6.8	2.5	8.4
Smear + ve cases	9	1	–	10
Prevalence of smear + ve cases per 1,000	1.1	0.2	–	0.9

A total of 12,304 in-patients (out of 12,827) were examined. One hundred and three leprosy cases were detected, giving an overall prevalence of 8.4 per 1,000 with smear positive case prevalence of 0.9 per 1,000. Table 1 shows that:

(1) Ninety-six per cent of male adult subjects could be examined (as compared with our experience of 60% coverage of this group in the slums).
(2) Maximum prevalence rate (9.6 per 1,000) was found in the male adult group.
(3) Maximum number of smear positive cases, i.e. 9 (1.1 per 1,000), was also found in the above group.

An analysis of leprosy cases detected among 11,505 adults examined in various hospitals is shown in Table 2.

The highest prevalence was found in the ESIS hospitals which admit industrial workers. In one such hospital situated in North Bombay, we

Table 2. Prevalence rates in various hospitals

	General hosptials	TB hospitals	ESIS hospitals	Total
Total cases	48	12	41	101
Prevalence rate per 1,000	7.5	5.3	14.5	8.8

encountered 19 cases among 716 subjects examined (prevalence of 26.5 per 1,000). Since ESIS hospitals admit industrial workers residing in areas in their vicinity, such surveys may provide useful epidemiological data about leprosy among industrial workers. However, the sample in this study is small and such findings deserve confirmation on studies on larger series. Twenty-two patients (22%) had pulmonary tuberculosis besides leprosy. More extensive surveys in tuberculosis wards can be expected to reveal useful data on the co-existence of these two diseases.

Table 3 indicates the deformity status of the patients detected among the adult group.

Table 3. Deformity status of leprosy cases

Deformity	No. of cases	(%)
Nil	75	74
Grade I	13	13
Grade II	13	13
Total	101	100

No patients were found to have gross (WHO grade III) deformities, while 13 (13%) had grade II deformity. This perhaps indicates the fact that patients with obvious gross deformities could not gain admission into general hospitals for their general complaints not related to leprosy.

Sixty (59%) leprosy patients were detected in the general medical and surgical wards, while 17 (17%) were in the tuberculosis wards, the remaining being found in the gynaecological, orthopaedic and ophthalmic wards (one case was found in the skin ward). Only in three instances were the patients admitted for complications arising out of leprosy (two in orthopaedic and one in skin wards). It is interesting to note that out of 103 cases detected only 8 were known to the hosptial staff.

In the above investigations, one trained worker was able to examine on an average 26 subjects per hour and 95% of the inpatients could be examined. Therefore, it appears to us that such surveys provide a quick and convenient method of screening male adult population for leprosy, especially since they are carried out in a hospital set-up. The hospitals selected in this study predominantly cater for the low- and middle-income groups. Since data on prevalence of leprosy among the high-income group are not available, such

surveys if carried out in the private hospitals may provide useful information on these lines.

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The histology of the Mitsuda reaction and its significance

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Summary The Lepromin test was done on 38 leprosy patients belonging to the various classifications of the disease. The 'delayed' or 'Mitsuda' reaction was assessed clinically and histologically at 21 days. The tuberculoid, borderline tuberculoid and all but one of the indeterminate patients showed a tuberculoid histology on Lepromin biopsy. The agreement between the histological reaction to Lepromin and the histopathology of the skin lesions was near complete in tuberculoid and borderline tuberculoid patients. In indeterminate leprosy the tissue response to Lepromin gives a clear indication of the progress of the disease in that patient. The histology of Lepromin in the lepromatous and borderline lepromatous groups was non-specific and demonstrated large numbers of the injected bacilli. Further, in these patients minimal nodular reaction may be produced by a non-specific response of fibroblastic proliferation. In addition to the clinical reading, histologically evaluated Lepromin reaction is an important procedure to assess the immunological status of a leprosy patient.

Introduction

The development of the Lepromin test began when Yoshinubu Hayashi (1918) attempted skin testing of leprosy patients with a suspension of leprosy bacilli in Ringer's solution. He found that lepromatous patients gave few positive and many negative responses. The matter was taken up by the Japanese

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Leprologist, Kensuke Mitsuda (1919) who developed the prototype of Lepromin as it is used today and described the peculiar delayed reaction which appears at 3–4 weeks and which bears his name.

The 'late' or 'delayed' Lepromin reaction, also referred to as the 'classical' Mitsuda reaction, consists of a nodular infiltration which begins after the first week following the injection, reaches its maximum about the fourth week and later regresses, frequently leaving atrophy or a scar. Intensely strong reactions may result in ulceration (Sixth International Congress of Leprosy, 1953).

A positive Mitsuda reaction is generally equated with a pre-existing or a stimulated delayed hypersensitivity to injected antigen (Skinsnes, 1964; Meyers *et al.*, 1975). Histologically, it consists of a dense grouping of epithelioid cells with giant cells and lymphocytes – a typical tuberculoid picture (Mitsuda, 1919; Hayashi, 1933; Schujman, 1936; Rabello & Rotberg, 1937; Rodriguez, 1938; Fernandez, 1940; Nolasco, 1940; Rodriguez, 1950; de Faria, 1954; Neves, 1963). The negative reaction, on the other hand, may show a fibrotic, foreign body or histiocytic response (Nagai, 1940; Nolasco, 1940; Rodriguez, 1950; Azulay *et al.*, 1960; Kuper, 1964). Epithelioid cell granulomas are absent.

The histological evaluation of the Mitsuda reaction gives an indication of the state of tissue reactivity to antigens of *Mycobacterium leprae* and hence is an index of the resistance of the patient to the disease. Although several workers have described the histological appearances of positive and negative Mitsuda reactions (Mitsuda, 1919; Hayashi, 1933; Schujman, 1936; Rabello & Rotberg, 1937; Rodriguez, 1938; Fernandez, 1940; Nagai, 1940; Nolasco, 1940; Rodriguez, 1950; de Faria, 1954; Azulay *et al.*, 1960; Neves, 1963; Kuper, 1964), to our knowledge a systematic study of the tissue response to Lepromin antigen in the entire spectrum of leprosy patients is not available. This study, therefore, attempts a detailed histological characterization of the reaction in the entire spectrum of leprosy. The relationship between the Lepromin histology and the histology of the skin lesions, the presence of the injected bacilli and the extent of agreement between the clinical and histological responses are also assessed.

Materials and methods

Thirty-eight patients were selected for the study from the Schieffelin Leprosy Research and Training Centre, Karigiri, North Arcot District. They were classified on the basis of clinical, bacteriological and histopathological criteria into five groups, according to the method of Ridley and Jopling (1966): lepromatous (resolved), 9; borderline lepromatous, 4; borderline tuberculoid, 12; tuberculoid, 4; indeterminate, 9. All the patients belonging to the lepromatous group were bacteriologically negative.

The Lepromin test was performed using the Mitsuda – Hayashi antigen as modified by Wade (Report of the WHO Expert Committee on Leprosy, 1953). The suspension was standardized to give a bacillary concentration of 1.6×10^8 baccilli per millilitre. 0.1 ml of this antigen was injected intradermally on the flex or surface of the forearm in an area free of leprosy infiltration or other skin lesions. The location of the test site with respect to anatomical landmarks was recorded.

The clinical reaction was read at the end of 21 days and graded according to the criteria adopted by the Sixth International Congress of Leprosy (1953).

All reactions whether positive or negative were biopsied. The tissue was fixed in Formol–Zenker solution for 4 hours, transferred to 70% alcohol, routinely processed and paraffin embedded. Five micron sections were stained by Haematoxylin and Eosin and by the modified Fite–Faraco method for acid-fast bacilli (Fite *et al.*, 1947).

Results

The ages of the patients ranged from 9 to 71 years. Thirty-one of them were males and 7 females. Table 1 shows the results of the late Lepromin reaction in the various groups of patients.

TUBERCULOID GROUP

Clinical readings

At 21 days, a clinical reaction was observed in all 4 patients and varied in size from 8.0 to 21.5 mm. Three of the nodules showed ulceration.

Histological reactions

The histology of the lepromin nodules in all 4 tuberculoid patients was characterized by the presence of large and often confluent granulomata composed of organized nodules of epithelioid cells and well-formed Langhans' giant cells bounded by a dense infiltrate of lymphocytes (Figure 1). Ulceration of the overlying epidermis was noted in three biopsies with a caseous abscess in the centre of the reaction in one of them (Figure 2) and an acute abscess in another (Figure 3). Acid-fast bacilli were not identified.

BORDERLINE TUBERCULOID GROUP

Clinical readings

In 3 of the 12 patients, the Lepromin nodules were less than 5.0 mm in diameter. In the remaining 9 patients, nodular reactions 5.3–18.0 mm in diameter were observed. Ulceration was noted in 4 of these nodules.

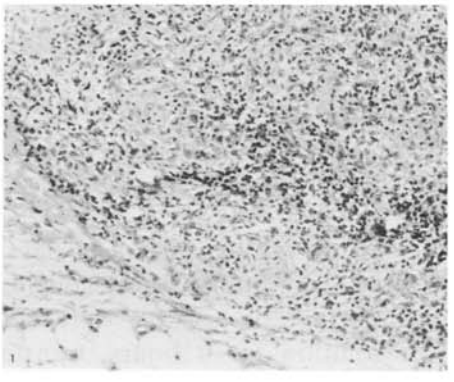

Figure 1. Tuberculoid leprosy (TT). Late Lepromin reaction. A large granuloma composed of epithelioid cells and numerous lymphocytes. H & E × 480.

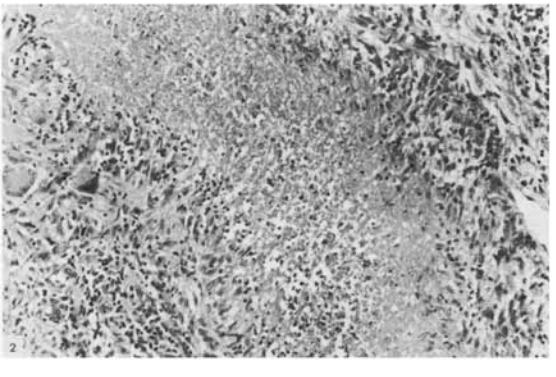

Figure 2. Tuberculoid leprosy (TT), Late Lepromin reaction. Caseous necrosis in the centre of the reaction bonded by epithelioid cells, Langhans' giant cells and lymphocytes. H & E × 480.

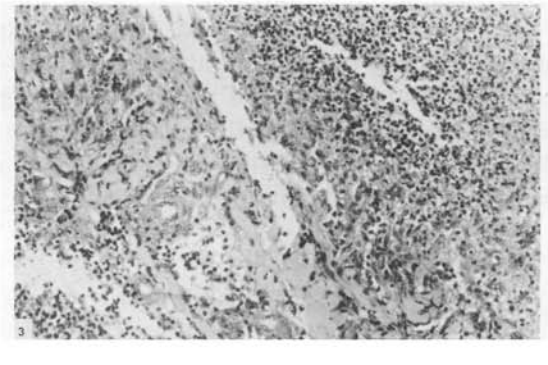

Figure 3. Tuberculoid leprosy (TT). Late Lepromin reaction. A large abscess in the centre of the reaction. Towards the periphery of the abscess numerous epithelioid cells are seen H & E × 480.

Histological reactions

The histological picture was essentially uniform in all 12 biopsies and consisted of granulomata made up predominantly of epithelioid cells with variable numbers of giant cells and lymphocytes. The later were often admixed among the epithelioid cells in the granuloma (Figure 4). The granulomas were generally smaller than those seen in the tuberculoid group. Acid-fast bacilli were not detectable in 5 patients, rare in 2 others and in the remaining 5 patients moderate numbers of bacilli were identified within the granulomas.

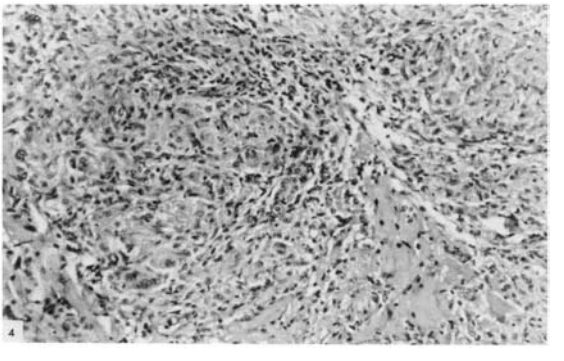

Figure 4. Borderline tuberculoid leprosy (BT). Late Lepromin reaction. Photomicrograph of the granuloma to show large collections of epithelioid cells and a few admixed lymphocytes. H & E × 620.

BORDERLINE LEPROMATOUS GROUP

Clinical readings

No clinical reaction was appreciated at 21 days in 2 of the 4 patients. In the 2 others, there was minimal induration less than 1.0 mm in diameter.

Histological reactions

A cicatricial reaction with micro-nodular proliferation of connective tissue and small aggregates of macrophages and lymphocytes was noted in 3 of the 4 biopsies (Figure 5). A few foreign-body giant cells were also present. Acid-fast bacilli were present in moderate numbers within macrophages, foreign-body giant cells and plump mononuclear cells resembling fibroblasts in the stroma. In the fourth patient belonging to this group, no histological reaction or acid-fast bacilli were demonstrable at the biopsy site.

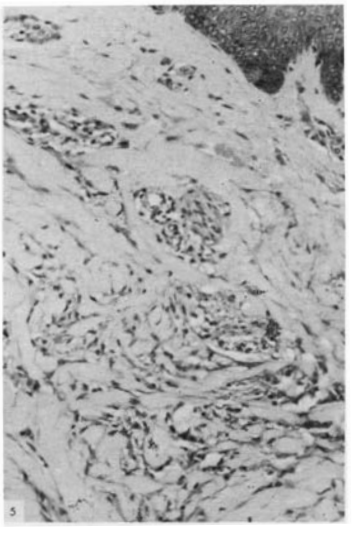

Figure 5. Borderline lepromatous leprosy (BL). Late Lepromin reaction. Diffuse fibroblastic proliferation in the dermis. H & E × 480.

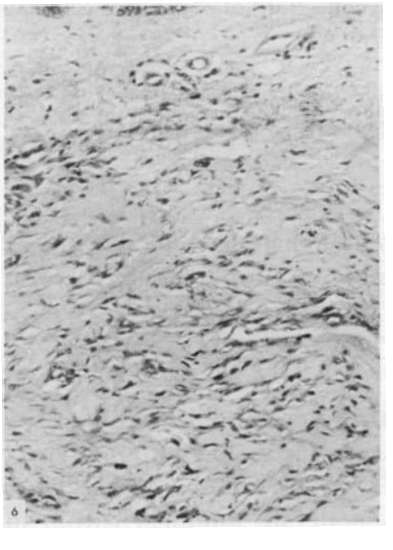

Figure 6. Lepromatous leprosy (LL). Late Lepromin reaction. Fibroblastic proliferation in the dermis. H & E × 480.

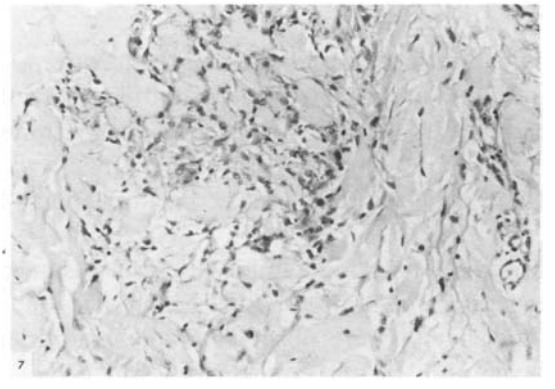

Figure 7. Lepromatous leprosy (LL). Late Lepromin reaction. Fibrotic nodule containing foreign-body giant cells. H & E × 480.

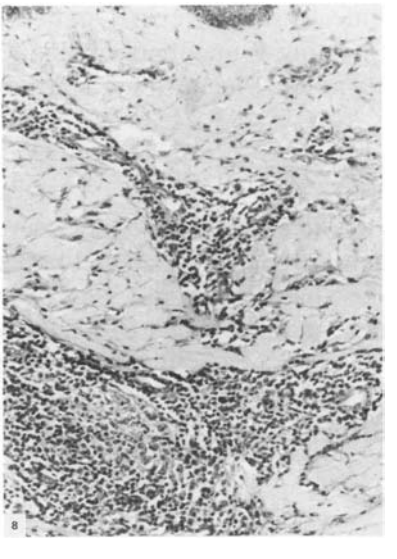

Figure 8 Lepromatous leprosy (LL). Late Lepromin reaction. Large focal aggregates of lymphocytes in the dermis. A small collection of histiocytes is also seen. H & E × 480.

LEPROMATOUS GROUP

Clinical readings

No reaction was observed in 5 patients. In the remaining 4 members of this group, there was minimal induration less than 1.0 mm in size.

Histological reactions

A variety of histological patterns were noted in this group.

Three of the patients demonstrated micro-nodular fibroblastic proliferation with a few small aggregates of macrophages at the periphery of the fibrous nodule (Figure 6).

In two others, there were micro-nodules containing many foreign-body giant cells (Figure 7).

In the 4 remaining patients, the cellular reaction was more pronounced and consisted of focal aggregates of lymphocytes (Figure 8).

Numerous acid-fast bacilli were observed in all 9 biopsies, localized within macrophages, giant cells and stromal connective tissue cells.

INDETERMINATE GROUP

Clinical readings

The late reaction was clinically negative in 1 patient and 3.1 mm in size in a second patient. In the remaining 7 members of this group, the nodules ranged from 6.5 to 15.5 mm in diameter. Ulceration was not noted.

Histological reactions

In 8 of the 9 patients in whom clinically, nodules were present, epithelioid cell granulomas with few giant cells and variable numbers of lymphocytes were p (Figure 9). Acid-fast bacilli were not identified in 5 biopsies, they were scarce in 1 and in 2 others small numbers of bacilli were found in the granulomas. There was no correlation between the size of the clinical reaction and the persistence of bacilli. In the last member of this group no histological reaction or acid-fast bacilli were observed at the biopsy site.

Discussion

The 'tubercle' which has been described as the hallmark of a histologically positive late lepromin reaction is the characteristic manifestation of cell-mediated immunity (Bryceson, 1976). A 'tuberculoid' response to injected lepromin is thus indicative of an effective cell-mediated immune system against antigens of *Mycobacterium leprae*.

The results of this study have shown that patients belonging to the polar tuberculoid group demonstrate a typical tuberculoid histology on biopsy of the late Lepromin nodules. There was also complete destruction and removal of the injected bacilli by 21 days. The reaction in the borderline tuberculoid group

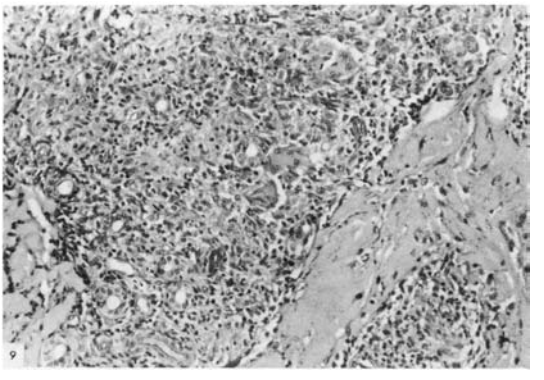

Figure 9. Indeterminate leprosy. Late lepromin reaction. A granuloma composed of epithelioid cells, giant cells and few lymphocytes, H & E × 480.

is essentially similar to that observed in tuberculoid patients with a few minor differences such as the smaller size of the granulomas and less marked lymphocytic infiltration. However, clearance of the injected bacilli was not as complete or effective as in the tuberculoid patients and persisting bacilli were observed in 7 of the 12 patients.

In the polar lepromatous group, although induration was observed clinically in some patients, the histological reaction consisting of fibroblastic proliferation, macrophages and foreign-body giant cells was non-specific in character. This indicates the importance of histological evaluation in detecting false positive clinical reactions.

Focal collections of lymphocytes were present in the biopsies from 4 of the lepromatous patients. The significance of this finding depends on whether these lymphocytes are of the B-cell or T-cell type. If they happen to be largely T-cells, their presence would suggest a partial restoration of the cell-mediated immune response in lepromatous patients with long period of disease inactivity. Further investigation is required to evaluate the significance of this finding.

The persistence of large numbers of the injected bacilli in all of the lepromatous patients is an expression of the extreme lack of sensitivity to the bacilli in these patients.

The Lepromin reaction in borderline lepromatous patients was very similar to the polar lepromatous group. In the borderline group of leprosy patients, the separation of the tuberculoid and lepromatous varieties is of great importance as prognosis and duration of treatment greatly differ in the two groups. The Lepromin reaction is a good and reliable ancillary confirmatory test in making this differentiation.

Eight of the 9 patients with indeterminate leprosy demonstrated a tuberculoid histology on biopsy of the Lepromin nodule. This reaction resembled that seen in borderline tuberculoid patients and consisted of epithelioid granulomas with few giant cells and moderate lymphocytic infiltration. However, the destruction and dissolution of bacilli was variable and would reflect the degree of resistance of the patient.

In patients with indeterminate leprosy, the Lepromin reaction is of great significance as it gives an indication of how the disease would evolve if allowed to progress. In patients with a strongly positive Mitsuda reaction with characteristic tubercles and no bacilli, the disease may show spontaneous healing, remain unchanged or become tuberculoid. In contrast, those with a negative Lepromin reaction have a tendency to develop the lepromatous form of the disease (Schujman, 1953). Further, in those indeterminate patients who show tuberculoid histology in their Lepromin reactions, but many acid-fast bacilli even after 21 days, it is possible that they may progress to the lepromatous spectrum if not adequately treated.

In this study, a nodule greater than 5.0 mm in diameter was classified as a positive clinical reaction and it was found that in the tuberculoid, the borderline lepromatous and lepromatous groups, there was total agreement between the clinical and histological reactions. In 3 borderline tuberculoid patients and in 2 patients with indeterminate leprosy, the clinical reactions were less than 5.0 mm in size. However, a tuberculoid histology characterized the reactions in all 5 patients. In 2 patients with borderline lepromatous leprosy and in 4 others with lepromatous leprosy, although minimal induration was noted at the site of biopsy, the histological reactions were non-specific. The clinical reading of the Lepromin reaction, although most often reliable, may occasionally be misleading and a histological evaluation is important to confirm the positivity or the negativity of the reaction. Azulay and co-workers (1960) found agreement between macroscopic and microscopic reactions in only 75.3% of 73 positive reactors. In 2 patients, one of the indeterminate and another of the borderline lepromatous group, no bacilli or cellular reaction were seen in the Lepromin biopsy. It may be that they are negative reactions and the site was missed during biopsy in spite of all the care taken to identify the site of lepromin infection.

There is an active search for a vaccine which would protect individuals exposed to leprosy. One of the methods to find out if a person or an animal has developed immunity to leprosy and thereby determine the efficacy of the vaccine is to assess his or its reaction to lepromin before and after the administration of vaccine. In these cases, to ensure accuracy, the Lepromin reaction should be interpreted clinically and histologically. It is hoped that this histological characterization of the Lepromin reaction will increase the value of the test in the evaluation of vaccines.

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Failure to detect *o*-diphenoloxidase in cultivable mycobacteria obtained from feral armadillos

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Summary We reported earlier that *Mycobacterium leprae* separated from lepromatous human as well as armadillo tissues contains an unusual form of *o*-diphenoloxidase which oxidized several diphenols, including D- and L-DOPA (3,4-dihydroxyphenylalanine) to quinones *in vitro*. It was not known whether any other species of mycobacterial separated from infected armadillo tissues would show *o*-diphenoloxidase activity. Recently, a few feral armadillos with mycobacterioses caused by cultivable bacilli became available. The data presented in this report demonstrate that cultivable mycobacteria obtained from the tissues of wild-caught armadillos did not contain the enzyme. Two species of nocardia tested converted DOPA to pigment, but this reaction was found to be non-enzymatic, being unaffected by heating. On the other hand, *o*-diphenoloxidase of the leprosy bacilli was sensitive to higher temperatures. Visual evidence on the occurrence of the enzyme in *M. leprae* is also presented.

Introduction

We have shown earlier that suspensions of *Mycobacterium leprae* purified from infected human or armadillo tissues contain the enzyme *o*-diphenoloxidase (EC 1.10.3.1), which actively oxidizes a variety of phenolic substrates including D- and L-DOPA (3,4-dihydroxyphenylalanine) (Prabhakaran & Kirchheimer, 1966;[1] Prabhakaran *et al.*, 1975).[2] In its wide substrate-specificity, resistance to inhibition by reducing agents and certain metal chelators, and in its kinetic properties, the bacterial enzyme was different from the *o*-diphenoloxidase occurring in vertebrate melanocytes (Prabhakaran, 1971;[3] Prahhakaran *et al.*, 1976).[4] At the time of our previous studies (Prabhakaran *et al.*, 1975),[2] we did not have any controls using other species of mycobacteria separated from armadillo tissues. Recently, a few wild-caught animals were found to have

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mycobacterioses caused by cultivable organisms. The mycobacteria separated from the infected tissues of feral armadillos and the organisms grown in culture did not contain *o*-diphenoloxidase. Data are also presented on the enzymatic nature of the oxidation of D-DOPA by *M. leprae* and the absence of the enzyme in two species of nocardia.

Materials and methods

ORGANISMS

Mycobacterium leprae was separated from the infected spleen or lymph nodes of armadillos, as described earlier (Prabhakaran *et al.*, 1976).[4] The organs were removed aseptically during autopsy and held at 0°C, before being homogenized in 0.2 M sucrose, using a Braun Model 853-202 homogenizer. The bacilli were separated by differential and density-gradient centrifugations in solutions of sucrose (0.2 M and 0.3 M) and KCl (1.5 M). The bacterial suspension was washed twice with cold saline and twice with deionized, glass-distilled water. All the operations were carried out at 0–4°C. The organisms were counted by the method of Hanks *et al.* (1964).[5] The same procedure was used for separating the cultivable mycobacteria from the spleen, lymph node or liver of feral armadillos. The bacilli were grown in Dubos' medium at 37°C for 3 weeks, harvested by centrifugation, and washed with saline and water. Four species of mycobacteria have been isolated from the tissues of the armadillos: *M. simiae, M. scrofulaceum, M. avium* and *M. terrae. Nocardia asteroides* (ATCC 19247) was grown for 2 weeks in Dubos' medium at 25°C, and *N. brasiliensis* (ATCC 19296) at 37°C. The cultured organisms remained viable and showed good respiratory activity.

CHEMICALS

DL-DOPA (G-^{3}H), 250 mCi/mmol was purchased from Amersham, Arlington Heights, Ill, USA; glass counting vials from Beckman, Fullerton, Calif, USA; the scintillation solution, Aquasol from New England Nuclear, Boston, Mass, USA, and D-DOPA from ICN, Cleveland, Ohio, USA. Other chemicals used were of the highest purity commercially available.

ENZYME ASSAY

Oxidation of D-DOPA was assayed spectrophotometrically by measuring the absorbance of the quinone formed in the reaction (Prabhakaran *et al.*, 1976).[4] The reaction system was as follows: Na_2HPO_4-KH_2PO_4 buffer (pH 6.8), 0.1 M (final concentration); D-DOPA, 0.002 M; bacilli, 5×10^9; volume, 3 ml;

temperature, 37°C; time, 60 minutes. The oxidation of ^{3}H-DOPA was determined as described before (Harris & Prabhakaran, 1975),[6] where the activity of tritiated water formed from labelled DOPA was measured in a liquid scintillation counter. The organisms were heated at 100°C for 15 minutes, and cooled in an ice bath. To study the heat-sensitivity of *o*-diphenoloxidase of *Mycobacterium leprae*, the bacterial suspensions were exposed to different temperatures in an oven; the maximum duration of the exposure was 15 minutes to ensure complete inactivation of the enzyme. Each experiment was done at least 3 times, except in the case of bacilli separated from the tissues of feral armadillos. With these organisms, the experiments were done once each, because only limited amounts of material were available. However, the assays were repeated when the mycobacteria were grown in culture. For each bacterial suspension, the results did not show variations exceeding 10%; the data presented are for representative experiments.

Results

CULTIVABLE MYCOBACTERIA FROM FERAL ARMADILLOS

The bacilli separated from the tissues of feral armadillos and the same organisms grown in culture failed to oxidize DOPA (Table 1). Sufficient bacteria for the assay were recovered from the tissues of 3 armadillos. However, the bacilli

Table 1. Oxidation of DOPA by mycobacteria from feral armadillos: absorbance 480 nm ($X10^{-3}$)

Bacilli	Unheated bacilli, + DOPA	Heated bacilli, + DOPA	DOPA
Separated directly from:			
Spleen	8	12	8
Liver	8	12	10
Lymph node	10	15	14
Grown in culture from			
Spleen (*M. simiae*)	20	30	10
Liver (*M. scrofulaceum*)	15	21	12
Lymph node (*M. terrae*)	20	22	10
Lymph node (*M. avium*)	8	12	10

were cultured from the tissues of many more animals. The slight activity observed with the bacteria was apparently not enzymatic, since heating the organisms failed to inactivate the reaction. This is in contrast to what was observed with *Mycobacterium leprae* where the activity was completely lost on heating. The data show that cultivable mycobacteria obtained from host tissues do not contain *o*-diphenoloxidase. The results corroborate our earlier finding

that *o*-diphenoloxidase of *M. leprae* is not due to tissue components adsorbed by the organisms, but is an intrinsic property of the bacilli.

Even with the highly sensitive radiometric method using labelled DOPA, little activity was observed in a culture of *M. scrofulaceum* isolated from the lymph node of an armadillo (Table 2). However, *M. leprae* readily oxidized the tritiated substrate.

Table 2. Oxidation of tritium-labelled DOPA by *Mycobacterium leprae* and *M. scrofulaceum*

Bacilli	p mol ^{3}H-DOPA oxidized*
M. scrofulaceum	0.35
M. leprae	59.70

*Values corrected for auto-oxidation of DOPA

NON-ENZYMATIC OXIDATION OF DOPA BY NOCARDIA

The oxidation of DOPA by *M. leprae* was compared to that observed with two species of nocardia. After centrifugation of the reaction mixtures, the spectra of the supernatant fractions were measured. The pigment formed from DOPA by nocardia gave a broad peak in the region 540–580 nm. The quinone formed from DOPA by *M. leprae* showed maximum absorbance at 475–480 nm (Table 3). The reaction observed with nocardia was not inactivated by heat, indicating that it is a non-enzymatic process, stimulated by inorganic ions. *o*-Diphenoloxidase which is heat-labile is the only exzyme that oxidizes DOPA to pigment. With *M. leprae*, heated bacilli showed no oxidation of DOPA.

Table 3. Comparison of the oxidation of DOPA by *Mycobacterium leprae* and *Nocardia* sp.: (absorbance X10^{-3}).

Organisms	Wavelength (nm)	Unheated organisms + DOPA	Heated organisms + DOPA	DOPA
N. asteroides	560	60	55	12
N. brasiliensis	560	60	64	10
M. leprae	480	73	10	12

VISUAL EVIDENCE OF *o*-DIPHENOLOXIDASE IN *M. leprae*

Unheated or heated *M. leprae* suspensions were incubated with or without DOPA. In the tube containing unheated bacilli and DOPA, the reaction mixture developed an intense dark colour, due to oxidation of the diphenol to pigment (Figure 1a). The tubes containing unheated bacilli or heated bacilli with or

without DOPA showed little colour change. Under the experimental conditions used, the substrate did not undergo auto-oxidation.

After incubation for 60 minutes, the reaction mixtures (except the one with DOPA, which remains in solution) were centrifuged at 25,000 *g* for 30 minutes. The precipitate in the tube containing unheated bacilli and DOPA was black; part of the pigment formed from DOPA sedimented with the bacilli (Figure 1b). The precipitate in the tubes containing unheated or heated bacilli alone or heated bacilli with DOPA showed no colour change. Evidently the *o*-diphenoloxidase of *M. leprae* is not catalysed by inorganic ions.

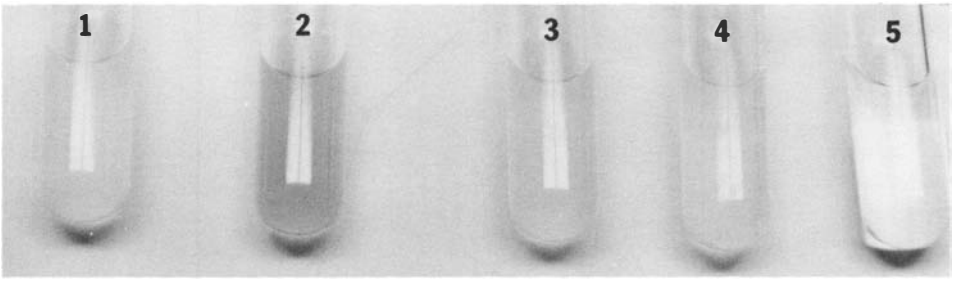

Figure 1a. *o*-Diphenoloxidase in *Mycobacterium leprae*: before centrifugation of the reaction mixtures. From left to right: 1, unheated bacilli; 2, unheated bacilli + DOPA; 3, heated bacilli + DOPA; 4, heated bacilli; 5, DOPA.

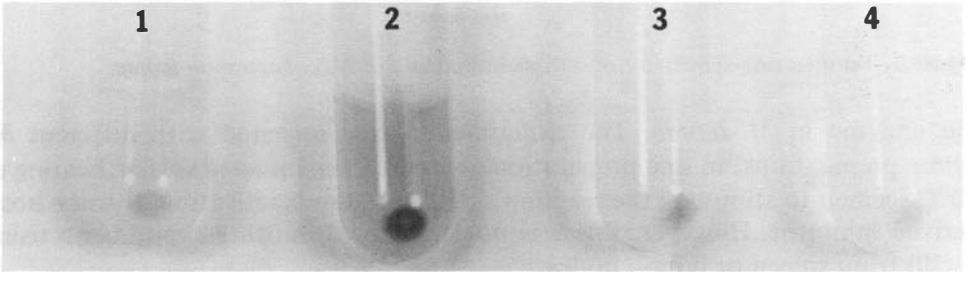

Figure 1b. *o*-Diphenoloxidase in *Mycobacterium leprae*: after centrifugation of the reaction mixtures. From left to right: 1–4 same as Figure 1a; sample 5 being a solution, was not centrifuged.

HEAT-SENSITIVITY OF *o*-DIPHENOLOXIDASE IN *M. Leprae*

The bacterial suspensions were exposed to different temperatures and tested for oxidation of D-DOPA. There was a gradual loss of activity from 37°C to 80°C, a sharp drop at 90°C, and at 100°C the enzyme was inactivated

completely. The decrease in activity was 21.6% at 60°C, 39.2% at 80°C, and 72.5% at 90°C (Figure 2). The bacilli were heated for varying periods at 100°C. There was complete loss of *o*-diphenoloxidase even when the exposure time was as low as 2 or 3 minutes, indicating the extreme temperature-sensitivity of

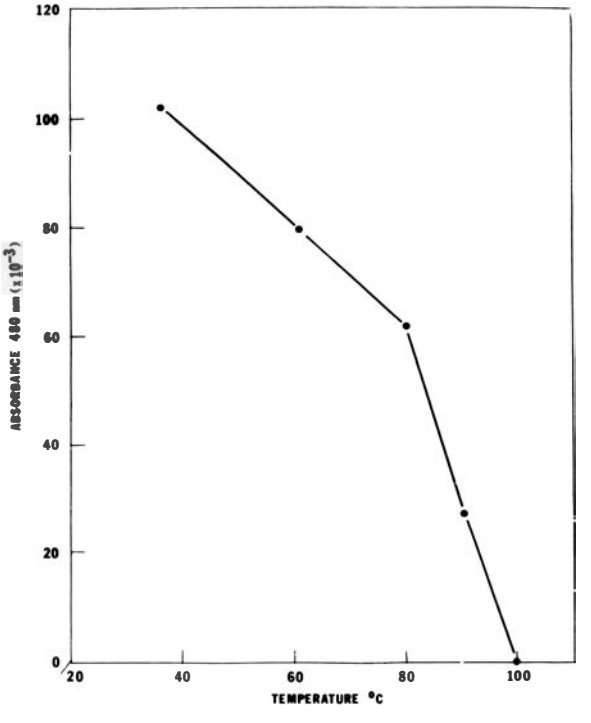

Figure 2. Temperature-sensitivity of *o*-diphenoloxidase of *Mycobacterium leprae.*

the enzyme in *M. leprae*. The experiments were repeated with different *M. leprae* preparations. In one preparation obtained from infected spleen, heating at 60°C seemed to stimulate the reaction, indicating the inactivation of some host-derived inhibitor. However, this was not confirmed in other experiments using bacilli from spleen or lymph nodes.

Discussion

For the first time, we show that cultivable acid-fast bacteria separated from the organs of feral armadillos with mycobacterioses, do not possess *o*-diphenoloxidase. As such, the oxidation of phenolic substrates by *Mycobacterium leprae* prepared from the organs of experimentally infected armadillos could not be due to host tissue elements adsorbed on the bacilli. The

oxidation of DOPA by *M. leprae* is catalysed by *o*-diphenoloxidase which is a temperature-sensitive enzyme. The non-enzymatic conversion of the diphenol to pigment by the cultured organisms is probably caused by inorganic ions adsorbed from the media; the activity was not abolished on heating.

Certain precautions have to be observed to demonstrate *o*-diphenoloxidase in *M. leprae* obtained from lepromatous tissues. The tissues have to be held at 0°C or below and the preparative procedures done in the cold to prevent enzyme denaturation. When the armadillo tissues are stored for over 2 months, the bacilli gradually lose the enzyme activity. If the bacterial preparation is contaminated with host tissue material, no enzyme activity would be detected (Prabhakaran *et al.*, 1979).[7] Some suspensions of *M. leprae* prepared from infected armadillo liver might have a dark-brown or greenish tinge, indicating the presence of bile pigments. Such preparations do not oxidize DOPA, while bacilli separated from the spleen or lymph nodes of the same animal would be active. Probably, this phenomenon depends on the degree of liver damage in the individual animal. Occasionally we have found that leprosy organisms separated from the subcutaneous nodules of armadillos are hard to purify and would show very little enzyme activity. We have not encountered such discrepancies in *M. leprae* derived from human tissues. Cultures of mycobacteria used for testing DOPA oxidation have to be grown in liquid media and washed thoroughly to exclude false positive results. Control experiments using heated bacilli should be done, to determine non-enzymic conversion of DOPA to pigment by metal ions. To detect enzymatic oxidation of dopa by *M. leprae*, prolonged incubation of the reaction mixtures at 37°C is unnecessary. The substrate would undergo little auto-oxidation if the incubation conditions are properly controlled. In our experience, quinone formation from DOPA can be observed within 15 minutes if the bacterial suspension is enzymatically active. Failure to observe these precautions would, in fact, lead to anomalous results (Binford *et al.*, 1977;[8] Kato *et al.*, 1976).[9]

In our previous experiments where *M. leprae* was derived from frozen tissues, the pigment produced from DOPA gave a peak at 540 nm, characteristic of indole-5,6-quinone (Prabhakaran *et al.*, 1976).[4] In recent experiments where bacilli separated from fresh tissues were used, the peak was near 480 nm, characteristic of dopachrome. The enzyme was heat-labile in both cases. A decarboxylation step is involved in the conversion of dopachrome to indole-5,6-quinone. Probably, a decarboxylase is associated with the bacilli from frozen tissues. The reason for this variation remains to be elucidated.

We have shown earlier that β-glucuronidase detected in *M. leprae* preparations is adsorbed from the host tissue (Prabhakaran *et al.*, 1978).[10] However, *o*-diphenoloxidase seems to be a constitutive enzyme of the bacillus. So far, we have tested *M. leprae* for *o*-diphenoloxidase after five passages in the armadillo, and the bacilli have retained the activity unimpaired. Our efforts to induce the enzyme in two species of cultivable mycobacteria did not succeed

(Prabhakaran *et al.*, 1969).[11] The species-specificity of DOPA metabolism for *M. leprae* (Kirchheimer & Prabhakaran, 1968)[12] has been corroborated by other workers and is being used as an identification test of the bacillus (Ambrose *et al.*, 1974;[13] Hall & Rightsel, 1978;[14] Kohsaka *et al.*, 1978).[15] Melanocytes do not lose *o*-diphenoloxidase in spite of repeated passages *in vitro* (Prabhakaran *et al.*, 1975);[2] it is likely that the leprosy bacteria would retain the activity, even if it were possible to grow the organisms in a bacteriological medium.

Acknowledgements

We thank Gregory T McCormick for growing the nocardias; Rita M Sanchez and J P Pasqua for providing the mycobacterial cultures.

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Lepr Rev (1980) **51**, 351–353

Leprosy and the Community

1981: International Year of Disabled Persons

WHO press release WHA/13 of 23 May 1980 contains the following paragraph:

Year of disabled persons: Statistics show that there are now about 450 million disabled in the world. In view of this difficult situation, the UN General Assembly has proclaimed 1981 International Year of Disabled Persons. In a statement to the World Health Assembly, Mrs Zala L N'Kanza, Executive Secretary of the Year, pointed out that the general theme of the Year would be full participation and equality of the disabled. WHO will focus its activities relating to prevention and treatment of invalidity on the responsibility and solidarity of the communities within which the disabled persons live.

Institute of Child Health, London: Child-to-Child Programme, and the International Year of Disabled Persons

A free copy of *Child-to-Child,* published by the Macmillan Press, and a set of Child-to-Child activity sheets will be sent to anyone sending a real life description of how a child helps his or her disabled brother or sister or the disabled child of a neighbour. These stories are *urgently* needed for the Child-to-Child Programme in the International Year of Disabled Persons (1981).

Please send an account, long or short, to:

Duncan Guthrie,
Child-to-Child Programme,
c/o Institute of Child Health,
30 Guilford Street,
London WC1N 1EH,
England.

ILEP (International Federation of Anti-Leprosy Associations): 13th GENERAL ASSEMBLY

London, June 1980. Twenty-four major voluntary agencies representing 21 countries, all Members of the International Federation of Anti-Leprosy Associations, met in London from 17 to 22 June for the 13th General Assembly. Observers from WHO and the International Union Against Tuberculosis were also welcomed.

The meetings were to ensure continuing co-operation between the agencies in leprosy work in more than 100 developing countries, and to enable the best use of resources by avoiding competition and duplication.

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The General Assembly invested as President Mr W Thomassen, President of Nederlandse Stichting voor Leprabestrijding (Netherlands Leprosy Relief Association) for the period 1980–82, in the place of the out-going President Mr A D Askew, of The Leprosy Mission (International).

The main business of the plenary sessions included a debate on proposals for closer co-ordination of field activities, the transfer of the ILEP headquarters and co-ordinating bureau to London, and the receiving of annual and financial reports from the General Secretary. The Federation decided to strengthen its permanent Medical Commission by the appointment of a doctor as full-time Secretary.

The report of the General Secretary highlighted a budget for 1980 of £20 million sterling, and an expenditure in 1979 of £14.3 million in 786 projects in 86 countries.

A new member association was received into membership – the Leprosy Trust Board of New Zealand.

Meetings of the Medical Commission took place, and also working groups on special interests, notably 'Statistics', 'Leprosy in Europe', 'Training in Leprosy', 'Social Aspects', 'Health Education', 'Combined Leprosy and Tuberculosis Programmes'.

Two new working groups were also created, one on Primary Health Care, the other on Publicity.

World Directory of Medical Schools, fifth edition. WHO 1979. Price £11.00 in the UK

This up-to-date and valuable paperback from WHO runs to 358 pages and measures 2 × 16 × 24 cm. We have previously drawn attention to this remarkable source of information on medical schools in all parts of the world, but do so again now with the object of ensuring that all who are interested in the proper education of medical students in leprosy-endemic areas may be fully aware of its existence. Two of its annexes are worth quoting in full:

Annex 2. Example of medical school's general objectives (based on the curriculum of the M.D. programme of McMaster University, Hamilton, Ontario, Canada, June 1972).

Graduates will have acquired or developed the knowledge, abilities, and attitudes necessary to qualify for further education in any medical career. The general goals of the programme are to endow the future physician with the following skills:

(1) To identify and define health problems, and search for information to resolve or manage these problems.

(2) To examine the physical or behavioural mechanisms underlying a given health problem. (A spectrum of phenomena might be included – from molecular events to those involving the patient's family and community.)

(3) To recognize, maintain, and develop the personal characteristics and attitudes required for professional life. These include:

(*a*) awareness of personal assets, potential, limitations, and emotional reactions;

(*b*) responsibility and dependability:

(*c*) ability to relate to and show concern for other individuals.

(4) To develop the clinical skills and methods required to define and manage the health problems of patients, including their physical, emotional, and social aspects.

(5) To become a self-directed learner, recognizing personal educational needs, selecting appropriate learning resources, and evaluating progress.

(6) To be able critically to assess professional activity related to patient care, health care delivery, and medical research.

(7) To be able to function as a productive member of a small group which is engaged in learning, research or health care.

(8) To be aware of and to work in a variety of health care settings.

Annex 3. Definition of 'integration' in the medical curriculum.

The term *integration* signifies an organization of the teaching/learning process whereby, to a specific extent, different courses are correlated or instruction centres around problems or applications rather than on discrete disciplines or specialities.

More specifically, integration implies a process of curriculum development and implementation characterized by:

(1) *delineation* of themes or problems involving the application of knowledge from more than one of the traditional health disciplines;

(2) *development* of interdisciplinary curricular components based on such themes or problems;

(3) *teaching* of these components by teams in which representatives from more than one dicipline participate; and

(4) *evaluation* based on student ability to relate individual disciplinary elements to the theme or problem.

To those who are concerned with the distribution of teaching and learning material, or of leprosy journals, it should also be added that this WHO Directory lists all the medical schools of India under one heading (pages 137–47; a total of no fewer than 106 schools affiliated to 56 universities), together with those of Africa (under separate countries), UK, USA, South America and the Far East – an up-to-date check-list of addresses which it is hard to find elsewhere.

OXFAM: The Field Directors' Handbook, 1980 Edition

The newly revised *Oxfam Field Directors' Handbook* summarises the objectives and strategies utilized by Oxfam field staff in assessing projects and provides advice based on project experience for the information of field staff, project holders and others. The main sections of the handbook are – objectives and procedures, agriculture, health, social development, humanitarian and disaster relief.

It is also hoped to produce in due course a series of booklets based upon individual sections or sub-sections of the Handbook. Further information on these booklets will be sent on request.

The *Oxfam Field Directors' Handbook* was published on 28 March, 1980. Cased in its own strong ring binder, the Handbook contains 460 pages and costs £10.00 or $20.00 plus 15% postage. Apply to OXFAM, 274 Banbury Road, OXFORD OX2 7DZ.

Lepr Rev (1980) **51**, 354–355

Field Workers' Forum

TECHNICAL GUIDE FOR SPUTUM EXAMINATION FOR TUBERCULOSIS BY DIRECT MICROSCOPY

The International Union Against Tuberculosis: 3 rue Georges Ville, 75116 Paris, France

Rather than review it under the usual section of this journal, we take this opportunity to describe at some length the content of a booklet of great practical value, which should be of interest to those engaged in combined tuberculosis/leprosy programmes, and which may hopefully stimulate the production of a full and detailed guide of a similar type for slit-skin smears in leprosy.

The Preface by Professor V Farga and Dr Annik Rouillon (Editors of the Bulletin of IUAT) reads as follows:

> This Guide is based upon one initiated as early as 1969 by Dr J Holm (then Executive Director of the International Union Against Tuberculosis). It was felt that the auxiliary personnel, especially in developing countries, needed a simple guide for collection, storage and transport of sputum specimens and for examination for tuberculosis by direct microscopy.
>
> This document, the third edition, has been carefully examined and revised by the members of the two IUAT Scientific Committees on Bacteriology/Immunology and Diagnostic Methods; account was also taken of suggestions made by other experienced authorities, as well as those of workers who have been using the Guide in the field.
>
> The Guide is intended for field laboratories which may often have very limited facilities and personnel. It presents the basic general principles for collection, transportation, and examination by smear of sputum possibly containing tubercle bacilli.
>
> While the Guide provides basic procedures for the detection of infectious tuberculous patients, it is recognized that local modifications of methods may be both desirable and appropriate.
>
> To clarify all points of procedure or detail all possible modifications of methods would be prohibitive. It is anticipated that some users of this Guide, in consultation with colleagues, supervisors, and central laboratory personnel, may modify certain procedures to accommodate local facilities and equipment.

The inside cover contains a quotation from the Ninth Report of the *WHO Expert Committee on Tuberculosis* (*Technical Report series*, 1974, No. 552) which is worth recording in full, if only because of its relevance, with the change of a few words, to the control of leprosy:

> The object of tuberculosis control is to break the chain of transmission of infection. This can be achieved by detecting the sources of infection as early as possible and rendering them non-infectious by chemotherapy. Transmission is maintained in the community particularly by subjects whose sputum is so heavility positive that tubercle bacilli can be detected by smear microscopy.

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The subject matter of the booklet is dealt with under the following main headings:

I. Collection of sputum specimens.
II. Storage and transport of sputum specimens.
III. The laboratory.
IV. Reception and registration of sputum specimens.
V. Preparation of smears.
VI. Staining technique.
VII. Examination by microscopy.
VIII. Results of examination.
IX. Recording at a microscopy centre.
X. Disposal of examined slides.
XI. Dispatch of results of examination.
XII. Formulation of reagents.

There are 21 figures which illustrate with great clarity the equipment needed and the technical procedures involved in making, fixing and correctly staining slides for the detection of tubercle bacilli. Dr S G Browne, in The Leprosy Mission publication *Partners*, has already described, with excellent line drawings, the basic steps in the taking and examination of slit-skin smears for leprosy, but there is nevertheless a place for a full technical guide, comparable in scope and length to this one from IUAT. It is in fact a matter of concern that such a booklet, on a laboratory procedure of vital importance to leprosy control, has not long ago been produced and circulated, in several languages.

EDITOR

Lepr Rev (1980) **51**, 356–358

News and Notes

DR J WALTER, WHO, GENEVA

Dr J Walter MD, Medical Officer in the Leprosy Unit of the Division of Communicable Diseases, World Health Organization, Geneva, retired on 31 May 1980 after 10 years service in the unit which seeks to co-ordinate leprosy control efforts in the world and to provide, through its Regional Offices, technical co-operation with member states in their leprosy control activities.

Dr Walter's interest in leprosy arose out of his studies at the London School of Hygiene and Tropical Medicine. Proceeding to Paraguay in 1952 he was initially engaged in general medical duties but in 1954 entered upon full-time leprosy control work, firstly with one of the voluntary agencies and latterly as Government Regional Leprologist. He joined the Ghana Medical Service in January 1960 as leprologist in charge of the N Ghana Leprosy Service. Then followed two appointments as a WHO Field Adviser on Leprosy, in Indonesia (1964–67) and Thailand (1967–80) before his selection for the post in Geneva. His zealous interest and wide experience in three continents made him a wise choice for a unit which is pursuing the Organization's purposes of making the benefits of research widely available and guiding health administrations in developing rational strategies for their leprosy control.

A person of strong convictions and vision, he has during a decade of unprecedented activity both in research and in the development of national leprosy programmes, worked strenuously to ensure that leprosy activities are given their rightful place within national health plans.

In wishing him a happy retirement, we shall hope that it will still be possible in the future to draw on his fund of knowledge of leprosy control and management and not least of his understanding of patients' needs.

HEISER PROGRAM FOR RESEARCH IN LEPROSY

Beginning post-doctoral research fellowships, research grants, and visiting research awards available in amounts up to $15,000 per year, plus other allowances depending on type of award applied for. Applicants should have MD, PhD, or equivalent degree. Applications by 1 February, 1981, for awards to be activated June–December 1981. For information write to: Heiser Program for Research in Leprosy, 450 East 63rd Street, New York, NY 10021, USA.

PRELIMINARY ANNOUNCEMENT

The 12th International Leprosy Congress of the International Leprosy Association will be held in New Delhi, India, from Monday 21 November till Saturday 26 November 1983.

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Papers will be invited from intending participants dealing with any aspect of leprosy on which the authors have original work to report.

Detailed information will be published shortly.

Dr S G Browne CMG, OBE,
Secretary General,
16 Bridgefield Road,
Sutton, Surrey SM1 2DG.

MARIE ADELAIDE LEPROSY CENTRE, KARACHI, PAKISTAN: TRAINING COURSES

Marie Adelaide Leprosy Centre has been conducting training courses for leprosy technicians, recognized by the Medical Faculty, since 1965. A total of 127 candidates have been trained, of whom 93 are still working in the Leprosy Control Scheme (August 1980).

The particular situation in Pakistan – scattered population and poor communication, poor medical infrastructure in the rural areas where leprosy is common and lack of medical officers willing to join rural health programmes – has created the need for a rather extensive training of 1 year, during which general medical principles (community hygiene, management of common illnesses, nursing procedures, basic laboratory tests, etc.) are likewise taught. In recent years, increasing stress has been laid on the teaching of tuberculosis control as well, since some of the rural control schemes are operating already as combined leprosy–tuberculosis control programmes. After 2 years work in the field, a Senior Course of 4 months duration is offered, admissions to which are on merit case, taking into consideration the needs of the particular control programme in which the applicant is working. During this course, special attention is paid to control methods, and to the health problems peculiar to the respective area (trachoma, goitre, basic health care, immunization).

Recognizing the contribution of the leprosy technicians towards the control of the disease, the government has established a service structure which provides for promotion to Senior Leprosy Technician, District Leprosy Controller, and Provincial Leprosy Field Officer.

CLOSURE OF THE LEPROSY STUDY CENTRE IN LONDON, JUNE 1980

It is with a sense of profound regret that we record the closure of the Leprosy Study Centre, 57a Wimpole Street, London, in June this year. The idea of a centre in London was originally conceived by Dr R G Cochrane, following his return to the United Kingdom in 1951 after many years' service abroad in the field of leprosy. His intention was to establish a centre for study and teaching, while at the same time setting up a registry of histopathology. The Leprosy Research Unit, as it was originally called, was started in Weymouth Street; in 1961 it moved to its present address, and in the same year Dr H J Smyly came to assist with the histopathological examination of biopsies and also to deputize for Dr Cochrane in the following year; the position of Director was taken by Dr S G Browne OBE, CMG, and the name changed to Leprosy Study Centre in 1966. In the period of nearly 3 decades from 1952 to 1980, medical, para-medical and non-medical visitors came to the Centre in large numbers, some for an hour or a few days, others to study histopathology and other aspects of leprosy for several weeks or months. A library was formed and a large number of journals on leprosy and related subjects constantly available. Dr D J Harman arrived in 1961 and soon became increasingly involved in the interpretation and reporting of biopsies from many parts of the world, each report eventually going out by airmail, accompanied by stained

slides, to the doctor who had submitted the material. Dr Harman in co-operation with colleagues who have been privileged to study, learn and work at this remarkable centre, contributed to the medical literature in the field of biopsy and staining techniques; transmission of leprosy; nerve damage; dermal microfilariasis; ocular leprosy; and Clofazimine treatment.

In recent years it became clear that the task of finding a histopathologist with the necessary enthusiasm and expertise to continue the work of examining and reporting biopsies, together with the alarming rise in the rental and running expenses of such a centre in London, would make it impossible to keep the doors open. We thank Dr Browne and Dr Harman for the many years they have devoted to this centre and wish them all possible happiness in retirement. The collection of over 16,000 slides, together with the reports and clinical data, now go to the Hospital for Tropical Diseases in London, and in the near future it is hoped to discuss the most advantageous future use of this material, which is of exceptional quality, for teaching and research.

Lepr Rev (1980) **51**, 359–362

Letters to the Editor

QUANTITATIVE STUDY OF THE SEPARATION OF *MYCOBACTERIUM LEPRAE* FROM ARMADILLO TISSUE

Sir,

Studies using relatively large quantities of *Mycobacterium leprae* at present depend on concentrates of the bacillus separated from lepromatous tissues of experimentally infected armadillos. Since the infected tissues are available only in limited amounts, it is important to recover as much bacilli as possible from them. For metabolic studies, *M. leprae* has to be obtained in a state involving the least damage to its enzymatic activities. For other purposes like the preparation of purified proteins or other fractions, maximum recovery of the organisms would be the main consideration.

In this report the yield of *M. leprae* from 2.0 g samples of liver tissue, obtained from an armadillo experimentally infected with *M. leprae*, was compared using two different separation techniques: (i) differential and density-gradient centrifugation of the tissue homogenate in sucrose and KCl solutions as previously reported by Prabhakaran *et al.*[1] and (ii) a modification of the chloroform extraction procedure introduced by Dharmendra.[2]

The chloroform extraction procedure was done as follows: 2.0 g of liver tissue was homogenized in 20 ml of 0.85% NaCl using a Braun Model Potter S homogenizer. The resulting homogenate was centrifuged at 24,500 × *g* for 30 minutes in a refrigerated centrifuge and the supernatant discarded. The residue fraction consisting of *M. leprae* and cell debris was transferred to a large mortar and the bacilli extracted with five, 10 ml aliquots of chloroform. All of the chloroform extracts were pooled in a large beaker kept on wet ice.

The chloroform was removed by evaporation under a stream of cool air, leaving a residue of bacilli and lipids. The lipids were removed by suspending the residue in 0.1 N NaOH and centrifuging at 24,500 × *g* for 30 minutes. The pellet consisting of acid-fast organisms was washed by resuspension in 20 ml of water and centrifugation at 24,500 × *g* for 30 minutes. The washed pellet was resuspended in 2.0 ml water and the bacilli enumerated by the method of Hanks *et al.*.[3]

Ether can be used instead of NaOH for extracting the lipid material, and may be desirable when preparing antigenic fractions; however, it was difficult to prepare a homogenous suspension from the resultant powder, for bacterial enumeration.

A comparison of the chloroform-extracted tissue residues and the residues normally discarded when using the sucrose–KCl method showed considerably fewer acid-fast rods remaining in the former. Data on the percentage of organisms recovered by the two procedures are given in Table 1. The percentage recovery is based upon bacterial counts in tissue samples taken before processing. We also observed that chloroform extraction after saline washing resulted in the removal of almost all the remaining bacilli in the residue fraction that is discarded from the sucrose–KCl procedure.

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EUGENE B HARRIS
K. PRABHAKARAN

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TABLE 1. Percentage recovery of *M. leprae* from armadillo tissue

Separation method	Experiment			
	1	2	3	$\bar{X}$
Density-gradient centrifugation	26.9	32.7	36.5	32.0
Chloroform extraction	67.3	63.5	63.5	64.8

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ADVANCE NOTICE OF SEMINARS, INTERNATIONAL MEETINGS AND CONFERENCES ON LEPROSY

Sir,

We receive *Leprosy Review* regularly and find it a valuable way to keep in touch. Some of the excellent accounts of international meetings which have taken place prompts me to ask if it would be possible for the Editorial Board to consider the regular publication of forthcoming events in the international field, preferably very well in advance of the relevant date, together with details of application for attendance and cost, etc. I understand that plans are often made for these meetings at least one year in advance: their earliest publication in your journal would be appreciated, especially by those working in remote areas.

J T CASSIDY

Director
Chest & Tuberculosis Service
Public Health Department
17 Murray Street
Perth, Western Australia

DOES CLOFAZIMINE HAVE ANY VALUE IN THE MANAGEMENT OF REVERSAL REACTION?

Sir,

I refer to the letter from W F Ross, *Leprosy Review* **51** (March 1980), 92–3. On 7 May 1980 I returned a questionnaire on Clofazimine to Drs W Vischer and O de S Pinto, Geigy, Basle.

In a separate letter I summarized my comments on Clofazimine. I quote from the letter:

> As for B.663 being anti-inflammatory in borderline leprosy reactions I must say that clinical evidence has not proved this.
>
> I have tried B.663 alone in borderline reactions (cell-mediated immunity reactions), both downgrading and reversal, and could not control the reaction without giving corticosteroids as well.
>
> I have tried to increase and reduce the dosage of B.663 and found that 300 mg B.663 weekly given *with* corticosteroids controls the cell-mediated reaction, but the steroids really are effective and not the B.663. The latter is merely given then as an anti-mycobacterial drug.

I do not think B.663 has an anti-flammatory effect in cell-mediated immunity reactions.

I agree with Ross. The alleged effectiveness of clofazimine in cell-mediated reactions is a myth.

Westfort Hospital
Private Bag X559
Pretoria 0001
South Africa

F M J H IMKAMP

THE SFG (SOLID, FRAGMENTED, GRANULAR) INDEX FOR BACTERIAL MORPHOLOGY

Sir,

We have recently been looking with renewed interest into the various methods which have been described for assessing the morphology of leprosy bacilli in stained smears, using classic Ziehl–Nielsen techniques. In talking to our laboratory technicians, and in the process of checking and supervising their work, we have been impressed with the fact that several of them have difficulty in consistently distinguishing between solid, and non-solid staining bacilli, as in the Morphological Index (MI), whereas they find it easier and quicker to assess a slide using the SFG, as described by Ridley in 1971 (*Leprosy Review* **42**, 96–7). In fact this article begins by saying that the MI is unnecessarily time-consuming on the part of the technician.

We have also been impressed by what seems to be a clear correlation between an increasing percentage of granular bacilli and the progress of regular treatment. Our purpose in writing this letter is to ask if other workers have adopted the SFG index? Should it perhaps be tried out more systematically in assessing the effect of treatment in the field?

Kumi Leprosy Centre
PO Box 9
Kumi, Uganda, Africa

P A M SCHREUDER
L COLPA

LEPROSY: SOCIO-ECONOMIC CONDITIONS AND PRIMARY HEALTH CARE

Sir,

Your reference to Horst Buchmann's publication struck an interesting chord with me. I have been watching the progress of leprosy in this part of the world with interest since 1951, when I started the 'Centre de Salud Menonita, Km 81' in Paraguay. There was an effort to raise the socio-economic level in Paraguay – the Alliance for Progress – during this time, which if it had been successful would have shown this effect that Buchmann points out. This programme did not work out successfully. Now that WHO proposes Dr Buchmann's approach, I find great hopes for improvement in the control of leprosy. As is known by all, control work of leprosy in the world today is not getting anywhere. Drs Jacobson and Hastings in their article in *The Star* (Vol. 39, No. 3) on Hansen's Disease Control state in their conclusion: 'but drastic improvements in this area are unlikely during the 1980s unless a major breakthrough in terms of vaccine development or therapy were to occur'.

It may be of interest to record that we have records of Mennonite refugees who came from Russia, from areas where there was no leprosy, but seven persons developed the disease here in Paraguay within the first 20 years, in spite of not having much contact with the Paraguayan population. None, however, have shown up with it in the last 25 years, in spite of the fact that they have had much more contact with the Paraguayans than they did

the first 20 years. The one main difference that is associated with this is the improved hygiene and standard of living. This same thing has been born out in many places in the world in the past.

For those who are afflicted with Hansen's disease, we must obviously pursue conventional drug and other treatment. For a more meaningful control programme, I contend that our best approach would be to improve socio-economic conditions for those who are at risk, and the Primary Health Care approach so ably described by Horst Buchmann.

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Book Reviews

The Role of the Spleen in the Immunology of Parasitic Diseases
Schwabe & Co AG, Basle, 1979.

This fascinating little book contains the proceedings of a meeting held in Geneva in 1978 under the auspices of the UNDP/World Band/WHO Special Programme for Research and Training in Tropical Diseases. It is the first volume of a new Tropical Diseases Research Series.

Almost the only criticism is the title, which I fear may lead potential readers to dismiss the book as too narrow in outlook. In reality the reverse is true and the book should be read by clinicians and fundamental research workers alike, whether or not their interest lies in tropical disease. The 29 participants cover all aspects of the spleen including structure and physiology, lymphocyte recirculation and sequestration, role in immunity and immunoregulation, regulation of the clearance of circulating material and role in several animal and human parasitic diseases and in the tropical splenomegaly syndrome. All this is covered authoritatively by people who one suspects have never sat down in the same room before. Thus the discussions between papers often make exciting reading and the organizers must be congratulated for arranging such a programme.

Since this is a leprosy journal, it may be appropriate to highlight some points which seemed particularly relevant, though mycobacteria are not mentioned. No doubt other readers will find different gems to suit their own particular tastes.

It is clear that both in mice and man, the mycobacterioses are accompanied by a disturbance of lymphocyte recirculation and by sequestration of antigen-reactive cells. Two chapters, by W L Ford and J Sprent, are devoted to this topic.

There is increasing interest in the possibility that antigens of the leprosy bacilli may trigger immunosuppressive mechanisms by travelling directly to the spleen via the blood stream. J R Battisto discusses such mechanisms.

Leprologists have long been puzzled by the ability of *Mycobacterium leprae* to exist free in the cytoplasm of infected cells. How can they be removed from such a site? Drs Nogueira and Cohn report that when *Trypanosoma cruzi* has 'escaped' into the cytoplasm of macrophages, activation of those macrophages *in vitro* will result in membranes reappearing round the parasites. Is there some kind of recognition system for 'foreign' particles within macrophages?

I hope these few points will suffice to whet the appetite of other potential readers.

G A W ROOK

[This is No. 1 in a series of 3. No. 2 is entitled *The Membrane Pathobiology of Tropical Diseases* and No. 3 *The* in vitro *Cultivation of the Pathogens of Tropical Diseases – Editor.*]

Le Pied du Lépreux (The Foot in Leprosy), by Raymond and Pierre Jaccard. Editions Fondations Raoul Follereau, 33 rue de Dantzig 75015 Paris, France. 113 pp.

0305–7518/80/040363 + 02 $01.00

This very practical manual written especially for workshop technicians making protective footwear for the anaesthetic and deformed feet of leprosy sufferers, but likely to prove of interest to anybody having anything to do with the victims of neglected leprosy, will be an indispensable guide and reference book to those fortunate enough to secure a copy. It is profusely illustrated with line drawings and diagrams. It gives precise instructions for making footwear that uses locally available materials, and avoids expensive imported plastics. This, of course, makes for low cost as well as for acceptability. The wearer of such shoes does not feel himself stigmatized; the closer his shoes are to what his healthy fellows are wearing, the more likely is he to make a habit of wearing the shoes and thus protecting his insensitive feet from damage.

While one can appreciate the rather simplistic attitude to both leprosy and to materials and designs, one can forgive some misunderstanding of the complex pathology of plantar ulceration; for instance, not every ulcer is indicative of an underlying osteitis, nor are arteries compressed by hypertrophic nerves. A leprologist shies away from using the word 'cure' in respect of a plantar ulcer in the sense that cicatrization is the goal of treatment. It is scarcely true that 95% of plantar ulcers are due to ill-fitting footwear, or that 90% of patients requiring protective footwear live in villages.

However, these are minor blemishes to offset the undoubted value of the book for the people it is written for.

While it may still be true that the most important technicians in an old-style leprosarium are the surgeon and the footwear-maker, who both have to cope with the backlog of neglected leprosy, the day should surely dawn when preventible deformity (and plantar ulceration) is actually prevented, and when such a book as *Le Pied du Lépreux* is of historic interest only.

S G BROWNE

Atlas de Histologia del Armadillo de 7-Bandas (*Dasypus hybridus*), by Alberto Cuba Caparo. Centro Panamericano de Zoonosis, Casilla No. 3092, Correo Central, 1000 Buenos Aires, Republic of Argentina, 1979.

This is a paperback book of 166 pages, A4 size, and 1 cm thick. It is written in Spanish and is not stated to be available in other languages. The author, who is a medical pathologist of distinction from the Medical School of Lima, describes the histopathology of this armadillo under the following main headings: skin and adnexae; lymphatic system; respiratory system; circulatory system; nervous system; male and female genital apparatus; endocrine glands; blood and bone marrow. There is an informative introduction covering various aspects of this animal's morphology, biology, genetics, natural diseases, teratogenesis and application in the experimental study of infectious disease. Those who have had experience of the histology of this animal and the opportunity to compare it with *novemcinctus* and *sabanicola* have the impression that there are not important differences between the three. After some initial difficulties with the printing of this book, it is now very well presented, and should be of great interest and value to those who study armadillo tissues at microscopical level. Obtainable from the Office of Publications, PAHO, 525 Twenty-third Street, Washington DC 20037, USA.

A C McDOUGALL

Due to lack of space in this Volume further Reviews and Abstracts will be included in the next issue

Editor

CONTENTS

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