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The Significance of *Mycobacterium leprae* in the Nasal Mucosa, with special reference to Chinese Leprosy Patients

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INTRODUCTION

The nasal mucosa as a convenient site for bacteriological sampling in leprosy patients has enjoyed varying popularity during the last 70 years. Following the observations of Arning (cited by *Lancet*, 1886), Goldschmidt (1891) and Koch (1896) that acid fast bacilli, presumably *M. leprae*, could be found in the nasal passages of leprosy patients, great emphasis was placed on examining the nasal mucosa in the diagnosis of leprosy (Sticker, 1897; Jeanselme and Laurens, 1897). This opinion remained prevalent (Manson-Bahr, 1954) and is still widely held (Marshall, 1964). However, the fact that leprosy bacilli can be found in leprosy skin lesions whenever they are found in the nose, and in many patients when they cannot be found in the nose was reported from Hawaii (Brinckerhoff and Moore, 1909), the Philippines (Gomez, 1923), India (Dharmendra and Sen, 1948) and Vietnam (Chaussinand, 1955). These studies and others (Ryrie, 1948; Maxwell and Kao, 1952; Browne, 1966) also refute the suggestion of early workers that the nose is the site of the initial lesion in leprosy. In the absence of skin or nerve lesions of leprosy the finding of acid fast bacilli in the nose is of highly doubtful significance (Karlinski, 1906; Johnston, 1917).

Scraping of the nasal mucosa is a more successful method of obtaining leprosy bacilli than swabbing the mucosa (Lowe and Christian, 1932). Samples from the nasal mucosa must be carefully taken and adequately decolourised to avoid confusion that could be caused by mycobacteria other than *M. leprae* (Cochrane, 1947a) or even diphtheroid organisms (Goodwin, unpublished data). The possibility that mycobacteria other than *M. leprae* are frequently present in the nose of leprosy patients is discounted by the studies of Shepard (1960, 1962) who reported that acid fast bacilli found in large numbers in nasal washings would not grow on artificial media or in tissue culture, but mouse foot-pad inoculation with acid fast bacilli from nasal washings resulted in lepromatous granulomata. A bacillary lepromin antigen prepared from mouse foot-pads produced the same reaction in leprosy patients as standard human lepromin (Shepard and Guinto, 1963) indicating that the bacilli obtained from the nose were *M. leprae*.

Conflicting reports of the comparative value of sampling leprosy skin lesions or the nasal mucosa, to determine the weight of infection and for reliable bacteriological assessment of response to chemotherapy, are probably due to ethnic variations. In Congolese and Nigerian patients the density of leprosy bacilli in the nose is usually greater than the density in skin lesions, and leprosy bacilli persist for a longer period in the nose than the skin (Browne, 1959; Davey, 1959). By contrast, rapid disappearance during chemotherapy of nasal leprosy bacilli, with bacilli persisting in skin lesions has been found in patients in the U.S.A. (Byers and Wolcott, 1954), in Morocco (Rollier, 1959), in Vietnam (Chambon and Pestel, 1960), in East Africa (Allan, 1961), in Russia (Torsuyev *et al.*, 1962), in India (Ram, 1963) and in South America (Opromolla, 1966).

Note: This study formed part of a thesis accepted for the degree of M.D. of Cambridge University.

The importance of distinguishing evenly stained forms of *M. leprae* in scraped incision smear preparations from the skin and nasal mucosa was recognised many years ago by workers in West Africa (Davey, 1958, 1959; Brown, S. G., personal communication). The observation that irregularly stained leprosy bacilli are almost certainly non-viable (Rees and Valentine, 1962) is supported by mouse foot-pad cultivation studies of *M. leprae* (Shepard and McRae, 1965). Following the suggestion of Waters and Rees (1962) that the percentage of evenly stained, morphologically normal forms of *M. leprae* should be calculated in routine Ziehl-Neelsen stained preparations, the term 'Morphological Index' was adopted (Goodwin, 1963), and has been accepted (Pettit and Rees, 1964; Browne, 1965).

This study in Chinese leprosy patients includes an analysis of the incidence, density and morphology of leprosy bacilli in the skin and nose in the different forms of leprosy before and during anti-leprosy chemotherapy, together with an investigation into the relation of the length of history of the disease and the lepromin reaction to bacilli in the nose. A spectrum of 5-forms of leprosy was identified in 1961 and written into the protocols of this study. This classification, based on histological features of a skin lesion, is almost identical to that of Ridley and Jopling (1962, 1966). Statistically significant differences, both bacteriological and immunological between the various forms, support the validity of this classification (Goodwin, 1967).

MATERIALS

A detailed study was made of 187 unselected Chinese leprosy patients admitted to the Hong Kong Leprosarium of the Leprosy Mission between May 1962 and May 1964 and treated there. Every patient on admission was subjected to a group of tests for *M. leprae* in the skin and nasal mucosa. Patients in whom leprosy bacilli were found in the nasal mucosa were sampled at 3-month intervals until nasal leprosy bacilli could no longer be found, or until May 1964 if nasal bacilli were still present. The patients consisted of 136 males and 54 females, with ages ranging from 7 to 76 years.

METHODS

(*a*) *Bacteriological.* The whole body surface was examined for leprosy skin lesions and 6 sites were selected to ensure complete coverage of the body. These sites included one of the ear-lobes, the face, the back or chest, one arm, one leg and one buttock. At each site a leprosy lesion, if possible, was selected and a scraped incision (Wade, 1935; Cochrane, 1964) made at the edge of the lesion. The dermal tissue obtained was spread on a slide as a 'smear' to obtain uniform thickness. The nasal mucosa was sampled by scraping on both sides of the septum, the mucosa first being swabbed clean (Browne, 1966). Skin and nasal smears were stained by the Ziehl-Neelsen method modified by the use of 3% hydro-chloric acid in 95% alcohol for decolourisation (Davison, 1960; Padma, 1964). The density of acid fast bacilli in skin and nasal smears was graded from 0 to 6 according to the logarithmic grading of Ridley (1964). The average of the 6 skin sites was taken as the skin Bacterial Index (B.I.) and the average of the 2 nasal smears as the Nasal Bacterial Index (N.B.I.).

(*b*) *The Morphological Index.* Using a powerful light source and a magnification of ×1250, 100 to 200 individual bacilli in each skin and nasal smear preparation were examined and the percentage of evenly stained, morphologically normal leprosy bacilli in a preparation was estimated. From the 6 skin smear sites the average percentage was calculated, and taken as the skin Morphological Index (M.I.). In the smears from 2 sides of the nasal septum the average percentage of evenly stained, morphologically normal bacilli was taken as the Nasal Morphological Index (N.M.I.). The M.I. and N.M.I. were estimated from March, 1963.

(*c*) *Histological.* From every patient on admission a biopsy specimen was taken from the edge of an active, usually raised skin lesion. The specimen was fixed in Ridley's Formol-Zenker Fixative (Ridley, 1957). One section was stained with haematoxylin and eosin, and one

by the Ziehl-Neelsen method modified by the use of 10% sulphuric acid for decolourisation. The histological features identified to distinguish each of the 6 forms of leprosy have been fully described (Goodwin, 1967). In addition to the recognised forms of lepromatous, borderline, tuberculoid and intermediate leprosy, the form intermediate between lepromatous and borderline was termed 'atypical lepromatous' leprosy, and the form intermediate between borderline and tuberculoid leprosy was termed 'atypical tuberculoid'.

(*d*) *The Lepromin Test.* Intradermal inoculation of 0.1 ml. of standardised bacillary lepromin antigen (Dharmendra, 1941) gave rise to a variable induration, which was measured 21 days after inoculation (Lowe and McNulty, 1953; Leiker, 1961). A reading of 2 mm. or more was regarded as a positive reaction. (145 patients were included in an analysis of this test.)

(*e*) *Assessment of the duration of the disease after treatment.* Because of the social stigma associated with leprosy in the minds of Chinese, the first recognisable manifestation of the disease leaves a profound impression in the patient's memory (Maxwell and Kao, 1952). Detailed questioning accompanied by examination was conducted by the author and a Chinese doctor.

RESULTS

(i) *The incidence of M. leprae in the nose.* In all untreated patients with lepromatous, atypical lepromatous and borderline leprosy, *M. leprae* could be found in the skin. In 47 of 50 lepromatous patients (96%), in 13 of 18 atypical lepromatous (72.3%) and in 7 of 15 borderline patients (46.6%) leprosy bacilli were found in smear preparations from the nasal mucosa. The density of leprosy bacilli in skin lesions (the B.I.) in the 15 patients with no nasal leprosy bacilli, was on average 1.84, with a range of 0.2-4.7. In the 3 patients with tuberculoid leprosy, bacilli were found neither in the skin nor in the nose. After 18 months chemotherapy, leprosy bacilli were not found in the nose of any patient with atypical lepromatous leprosy and in only 32% of lepromatous patients, while bacilli could be found in skin lesions in 100% of patients with both forms of leprosy. In 37 lepromatous patients at the time when the Nasal Bacterial Index (N.B.I.) was 0 the skin B.I. was on average 2.73, and in 15 atypical lepromatous patients the B.I. was 2.25.

(ii) *The value of nasal sampling in assessing bacteriologically the severity of the disease process.* The superiority of skin sampling is demonstrated by an analysis of the density of leprosy bacilli before and during treatment in the different forms of leprosy (Table 1). (The difference between the numbers of patients sampled at 0 and 3 months is because some patients prior to admission had received a few weeks chemotherapy. After 6 and 9 months treatment the patients sampled included only those who had a positive N.B.I. at the previous sampling.) In lepromatous and atypical lepromatous patients nansal tests gave a false impression of the success of chemotherapy.

(iii) *The Lepromin reaction, and the duration of the disease.* In all the lepromatous patients the lepromin reaction was negative, but 10.5% of 38 atypical lepromatous patients had a positive reaction. A correlation was evident between the density of nasal leprosy bacilli and the size of the lepromin reaction. All patients with a density index of nasal bacilli (N.B.I.) greater than 3.0 had a negative lepromin reaction, while in patients with a moderate lepromin reaction of 2 mm. to 6 mm. a low N.B.I. was found; and when the lepromin reaction was greater than 6 mm. the N.B.I. was 0. Inasmuch as a positive lepromin reaction indicates a relative tissue immunity and an ability to localise the infection, it would appear that the nasal mucosa is parasitised when the infection is disseminated. Such a dissemination would be expected to be more frequent the longer the duration of the disease before treatment. This is supported by the observations that among the lepromatous patients the only 2 who had no nasal bacilli had a history of less than one year, and the greatest density of nasal leprosy bacilli was in patients with the longest history (Table 2). The density of the bacilli in the skin was unrelated to the duration of the disease.

TABLE 1

A comparison of the density of leprosy bacilli in the skin and the nose before and during treatment

	Treatment (months)	*No. of Patients*	*Skin B.I.*		*N.B.I.*	
			Range of B.I.	*Average B.I.*	*Range of N.B.I.*	*Average N.B.I.*
Lepromatous	0	50	2.8-5.5	4.30**	0-6	2.85**
	3	76	2.3-6.0	4.18	0-6	2.53
	6	64	1.7-5.5	3.69	0-5	1.87
	9	48	1.7-5.3	3.38	0-5	1.43
	12	41	1.2-4.7	3.18*	0-4	1.36*
	15	24	1.3-4.2	3.02	0-4	1.46
	18	14	0.3-4.5	2.86	0-3	1.50
	21	7	1.3-4.2	2.64	0-2	1.26
Atypical Lepromatous	0	18	0.5-4.5	3.12**	0-5	1.58**
	3	40	0.3-5.0	3.07	0-4	0.85
	6	20	0.3-4.5	2.37*	0-2	0.60*
	9	8	1.0-3.0	2.20	0-2	0.62
	12	4	0.3-2.7	1.62	0-1	0.50
	15	2	0.2-3.2	1.70	0-2	1.00
Borderline	0	15	0.2-4.5	1.99	0-5	1.07
	3	17	0.3-4.8	1.70	0-4	0.80
	6	6	0.8-2.3	1.35	0-3	1.50
	9	2	0.3-1.0	0.65	0-1	0.50

* The difference between the Average B.I. and N.B.I. is significant at the 0.1% level.
** The difference between these 2 means is significant at the 1% level.

TABLE 2

The relation of the duration of the disease to the density of skin and nasal leprosy bacilli in lepromatous leprosy

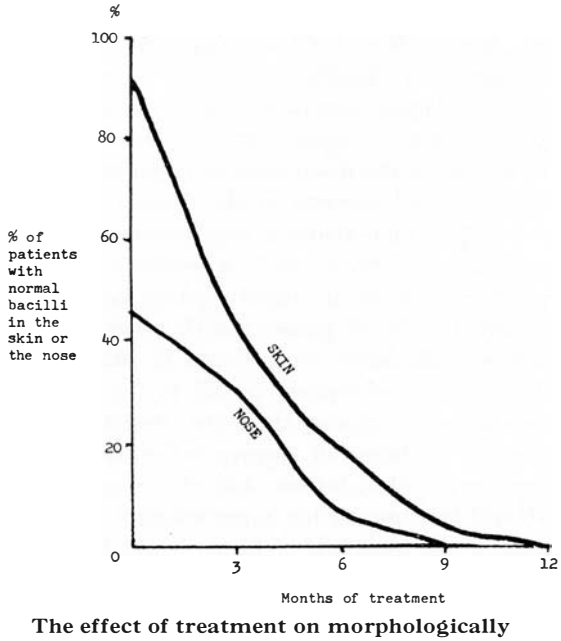
Duration of Disease	*No. of Patients*	*Range of B.I.*	*Average B.I.*	*Range of N.B.I.*	*Average N.B.I.*
Less than 1 year	7	2.8-4.7	4.0	0-4	2.0
1-4 years	25	3.7-5.5	4.84	1-5	2.94
5-10 years	12	3.0-5.0	3.97	1-4	2.50
More than 10 years	6	3.8-5.5	4.63	3-6	4.16

(iv) *The nasal mucosa as a source of viable leprosy bacilli.* The percentage of evenly stained, morphologically normal bacilli in nasal smears (the N.M.I.) ranged from 40% to 80% in 9 lepromatous patients, while the percentage in skin smears (the M.I.) was never greater than 30%. Of 73 patients with normal bacilli in the skin or nose, 43 (58.8%) had such bacilli in the skin alone. Only 2 out of 15 atypical lepromatous patients had evenly stained bacilli in the nose, the highest being 10%. Evenly stained bacilli persisted for a longer time in the skin than the nose (Table 3).

(v) *Reappearance of nasal leprosy bacilli.* Of 24 patients who consented to nasal sampling for 12 months after nasal bacilli had disappeared, 18 patients continued to respond to chemotherapy as shown by a fall in the B.I. and in these patients no nasal bacilli were found. In the remaining 6 patients the skin B.I. rose temporarily and nasal bacilli reappeared; but with a later fall of B.I., nasal bacilli could not be found.

(vi) *A comparison of the effect of Dapsone and Thiambutosine on nasal leprosy bacilli.* Of 49 lepromatous patients who could be sampled for 18 months, 35 were treated with Dapsone

TABLE 3



The effect of treatment on morphologically normal leprosy bacilli in the skin and the nose in 32 lepromatous patients

and 14 with Thiambutosine. After 12 months treatment nasal bacilli were found in 54.3% of the Dapsone group and in 57.2% of the Thiambutosine group. After 18 months the percentage with nasal bacilli in the Dapsone group was 28%, but in the Thiambutosine group was 43%, confirming previous reports that some patients may develop resistance to Thiambutosine after 12 months treatment.

DISCUSSION

In Chinese leprosy patients leprosy bacilli were found in the nasal mucosa in relation to the severity and length of duration of the disease. In pure lepromatous patients with a history of longer than one year, leprosy bacilli were found in every patient. A high incidence of nasal leprosy bacilli in patients with a longer history was reported by Rogers and Muir (1925), who found that 68% of established patients, but only 37% of early patients had nasal bacilli. In 600 lepromatous patients in India, Cochrane (1947b) found nasal bacilli in 100% of very advanced patients, 85.9% of moderately advanced patients and in only 36.8% of early patients of the disease. In the Philippines, 35 children with early leprosy had bacilli in skin lesions, but only 40% of the children had bacilli in the nose (Solis and Wad, 1925). The earliest sign or symptom of leprosy has been variously found to be an area of anaesthetic skin (Gomez, 1923), skin macules or a thickened nerve (Ryrie, 1948) and neuritic symptoms (Maxwell and Kao, 1952). These reports support the proposition that in most patients the nasal mucosa is the site of multiplication of leprosy bacilli at a later date than the skin or nerves.

In many Chinese patients with borderline and atypical lepromatous leprosy, *M. leprae* could be found in skin but not in the nasal mucosa. Thus skin lesions and not the nose should be sampled for bacteriological confirmation of the diagnosis of leprosy. Nasal tests may have a value in indicating whether the disease process has become disseminated; and it was in patients with a greater tissue immunity, as evidenced by a positive lepromin reaction, that nasal leprosy bacilli were rarely found.

For the apparently conflicting reports from different countries of the incidence of *M. leprae* in the nose of leprosy patients, three reasons are suggested, the most probable reason being that there is a geographical or racial variation in the pattern of leprosy and nasal lesions in different countries. Secondly, the significance of the duration of the disease in relation to the incidence of nasal leprosy bacilli may not have been realised; if many early patients of leprosy were included in a study, a lower incidence of nasal leprosy bacilli might be found. The third reason is that patients with atypical lepromatous leprosy, who are shown by this present study to have a lower incidence of nasal leprosy bacilli, may be included in the figures for lepromatous leprosy.

In Chinese patients in lepromatous, atypical lepromatous and borderline leprosy, the average density of bacilli in the skin was found to be significantly higher than the average density in the nose, both before and during treatment. The observation that nasal leprosy bacilli disappear after a shorter period of treatment than bacilli

in the skin has been reported from all countries, except West Africa, and has been found in the Anglo-Indian type of patient in England (Goodwin, unpublished data). Skin lesions and not the nasal mucosa should be sampled for a bacteriological assessment of the response to chemotherapy. However, nasal leprosy lesions when they do occur, may be of great importance in the spread of leprosy. Leprosy bacilli in the nose are probably spread to the environment more easily from the nose than from the skin, and in Chinese patients the percentage of evenly stained, presumably viable leprosy bacilli in the nose can be much greater than the percentage in the skin. This has been briefly reported in Nigerian patients (Browne, 1966).

In only a very few patients with atypical lepromatous or borderline leprosy were evenly stained bacilli found in the nose.

Evenly stained bacilli persisted for a longer period in the skin than in the nose, in contrast to observations in Nigerian patients (Browne, 1966).

'Elimination of nasal scrapes is recommended.' This statement was recorded by the panel on Bacteriology at the VIIIth Internal Congress of Leprology in September, 1963 (Rio de Janeiro Congress, 1963). However, the force of the recommendation was weakened by the preceding sentence which stated: 'In routine examination, work can be saved by recalling that ear lobes, the nasal mucosa and margins of active lesions are the sites more frequently and strongly positive, and the last ones to become negative during therapy.' This grouping together of the nose and skin lesions in one generalisation would seem to be confusing, in the light of this report and the others mentioned in this paper. In West African patients, nasal tests may be of great value, but in patients in other countries nasal tests are probably of less value than skin tests. It is agreed that nasal tests should lie more in the province of the research worker.

SUMMARY

187 Chinese leprosy patients were studied during the period May 1962 to May 1964. A 5-form spectrum of classification based primarily on the histological features of an active skin lesion was adopted. Leprosy bacilli were found in scrapings of the nasal mucosa in relation to the duration and severity of the disease. In many borderline and atypical lepromatous patients, especially in those with a positive lepromin reaction, few or no nasal leprosy bacilli were found; but in all patients with other forms of leprosy, *M. leprae* were found in skin lesions. The density of leprosy bacilli in the skin was significantly greater than the density in the nose in all forms of leprosy before and during treatment. Skin lesions and the nasal mucosa should be sampled for bacteriological confirmation of the diagnosis of leprosy, and for assessment of the response to chemotherapy. Nasal tests may have greater value in West Africa than other countries. The nose can be a potent source of infection, as a higher percentage of presumably viable bacilli can be found in the nasal mucosa than in the skin, in Chinese leprosy patients.

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