THE NASAL EXCRETION OF *MYCOBACTERIUM LEPRAE* IN LEPROSY[1]

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That acid-fast bacilli can be found in the nasal excretions of many leprosy patients has been known for about 70 years. Goldschmidt was probably the first to observe this, and he reported it in 1891 ([3]). It was soon described by several others, including Robert Koch ([6]), who noted that the nasal smears contained acid-fast bacilli when leprous ulcers were present. Many of the early investigators found that positive smears were much more frequent in "nodular" than in "anesthetic" cases.

The analogy between the positive nasal smear in leprosy and the positive sputum smear in tuberculosis is, of course, a striking one, yet it has led to two areas of confusion. The first of these has to do with the "portal of entry" in leprosy. Many assumed that the nasal mucosa was the site of the first lesion; Sticker was a prominent proponent of this notion ([14]). There has been no observational support for the idea, however, and the primary cases of nasal infections without skin infection have been carefully sought but not found (e.g., [1, 13]). The second source of confusion has to do with the diagnostic significance of the positive nasal smear. Since acid-fast bacilli may occasionally be found in the nasal excretions of nonleprous persons, the nasal smear does not have the same diagnostic significance as the sputum smear in pulmonary tuberculosis. There is general agreement that leprosy should not be diagnosed on the basis of a positive nasal smear alone. Indeed, there seems no need to do so, since it is highly probable that leprosy bacilli would be demonstrable in the skin whenever they are to be found in the nasal excretions.

The existence of these two points of confusion has led to some tendency in more recent years to discredit the importance of the presence of leprosy bacilli in nasal excretions. The findings reported here, which are of a quantitative nature, serve to reemphasize the nasal excretion of leprosy bacilli. It has been found that the number of bacilli excreted per day is roughly the same as the number of tubercle bacilli shed in the sputum per day in pulmonary tuberculosis. The leprosy patient excreting such large numbers is the one with typical untreated lepromatous disease.

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[1] This article was prepared for THE JOURNAL on request of the editor. Part of the data, here revised has appeared elsewhere ([10]).

MATERIALS AND METHODS

The patients studied have been at the U. S. Public Health Service Hospital at Carville, Louisiana, and at the Central Luzon Sanitarium at Tala, near Manila, in the Philippines. The nasal passages were washed with 500 cc. Hanks' balanced salt solution (BSS) by means of a nasal siphon. (The fluid courses up one side of the septum, crosses in the nasopharynx, descends on the other side, and leaves the nose through rubber tubing into a receptacle.) Bovine albumin 0.1 per cent was then added, and the washings centrifuged in a horizontal head at 800 G for 1 hour. The supernate was discarded, and the sediment resuspended in a few cubic centimeters of the BSS. Care was taken not to lose sediment into the supernate, and to recover the sediment completely from the centrifuge vessel. The resuspended sediment was digested in 2 per cent NaOH, by shaking with glass beads for 15 minutes at room temperature. Centrifugation was then performed for 1 hour at 1000 G in an angle head, and the sediment was resuspended in 2.0 cc. BSS.

The number of acid-fast bacilli was counted by a technique that has been modified in certain details during the period of more than 3 years. The current procedure, which in most respects is a modification of techniques described by others, especially that by Hilson and Elek [5], follows:

One volume of the resuspended sediment is mixed with 1 volume of formol-milk [5], and microdrops of 2 microliter are placed on microscope slides with a micropipette having a pointed tip.[2] This is done expeditiously, and the sample is stirred each time the pipette is to be refilled in order to minimize settling of clumps. The drops are put on slides that have been cleaned by vigorous rubbing with an alcohol-moistened towel, and placed on a leveling table. Correction for the fluid on the outside of the pipette tip is achieved by touching the tip to an approximately 2 microliter drop of the sample on a spare slide before the drop to be examined is put in place. Each slide receives about 6 drops, and is left to dry at room temperature on the leveling table.

The preparation is then exposed to formaldehyde vapors in a petri dish for 3 minutes, placed on the lid of a boiling water bath for 2 minutes, coated with phenol-gelatin [5], placed on the lid of the boiling water bath for 2 minutes, exposed to formaldehyde vapors for 3 minutes, and finally put back on the lid of the boiling water bath for 2 minutes. Staining is then done with carbol-fuchsin at room temperature for 20 minutes, destaining in 70 per cent ethanol with 1 per cent HCl, and counterstaining with methylene blue for 1 minute.

The first drops of phenol-gelatin and carbol-fuchsin are placed gently but directly on the microdrops to prevent lifting of the material from the dry slide by the spreading meniscus, but care is taken at all later steps to direct the stress of solutions away from the drops. It has been found essential to prevent overheating of the slide, both during fixation and staining. Serious decreases in numbers of acid-fast bacilli were noted on slides that had been overheated. An extensive comparison of steaming carbol-fuchsin with the cold procedure given above showed that brighter staining followed cold staining.

Round microdrops were selected with a hand lens. The acid-fast bacilli in a strip across the equator of a drop was carefully counted, and the diameter of the drop measured in terms of microscope fields. The number of acid-fast bacilli counted in the equatorial strip is multiplied by D/1.27 (where D is the diameter of the drop expressed in microscope fields), to give the estimate of the number of acid-fast bacilli in the microdrop (2 microliter). At least 2 microdrops are counted for each specimen. Each microscope field is examined in its entire depth of focus with Köhler illumination and apochromat objectives. Optical resolution allows acid-fast bacteria to be distinguished readily

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[2] Specialized Instrument Company, Belmont, California, part No. 300-816.

out to the edge of the field. With less ideal microscopic conditions the field can be stopped down to the portion that does allow adequate resolution by inserting a diaphragm into the eyepiece.

In a typical example, 56 and 49 acid-fast bacilli were counted in two microdrops which were 34 and 38 fields wide, respectively. Accordingly, the number of acid-fast bacilli in the 2 cc. washing was

$$(56 + 49) \times \frac{34 + 38}{2} \times 10^3 = 2.98 \times 10^6.$$

The reproducibility of the result appears to be within 20 per cent if enough organisms are counted to lessen the effect of sampling variation. The approximate lower limit of detectability, representing one acid-fast bacillus seen in 2 drops, is about 3×10^4 organisms in the 2 cc. Further details on technique and reproducibility have been published ([10, 11]).

The studies were initiated at Carville, and the experience gained there allowed a more intensive study of 24 routine admissions at Tala in November and December 1958. There the patients were interviewed and examined in cooperation with the professional staff, and a classification of the disease was made by them based on clinical appearances and results of a preliminary bacteriologic examination. In the nasal examination a head-light and nasal speculum were employed. The smears reported in Table 1 were made and graded by one experienced technician. The sites chosen were earlobe, nasal septum, and two optional locations. The smears represented material removed after the surface had been wiped clean. Lepromin tests were made at one time with a single batch of lepromin.

The study has been continued at Carville. The type diagnoses were those entered on the patients' charts. Most of the data, other than nasal washings, were also taken from the patients' charts. It was not feasible for me to see the patients at the time of admission, but they were interviewed and examined during occasional visits to Carville.

RESULTS

Relationship of nasal count to clinical features.—The results with the patients at Tala are arranged according to diagnoses and nasal counts in Table 1. The patients with counts greater than 1×10^6 had advanced lepromatous leprosy with ulcers in the nose, had experienced distinct nasal symptoms for long periods, and were completely nonreactive to lepromin at 22 days. Of 14 patients with a diagnosis of lepromatous leprosy, 8 had counts of 1×10^5 or greater. Of 7 patients with a clinical diagnosis of borderline, and 3 with tuberculoid leprosy, none had counts greater than 1×10^5.[3] The nasal counts predicted the clinical findings more closely than did either the skin and septum smears, or the lepromin reaction.

The results from the patients at Carville are classified in Table 2 according to diagnosis and amount of antileprosy treatment. As in the Tala group, counts greater than 1×10^5 were seen only with patients with lepromatous leprosy.

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[3] The cases diagnosed as tuberculoid were not of typical mild kinds which are treated as outpatients, but were severe and active cases, with relatively extensive lesions, which had been admitted for hospital treatment.

TABLE 1.—*The patients studied at Tala, arranged according to diagnosis and numbers of acid-fast bacilli in the nasal washings.*[a]

Diagnosis	Case No.	Duration disease (yrs)	AFB count	Smear skin	Nasal septum	Lepromin 2d.	Lepromin 3w.	Nasal examination	Duration of nasal symptoms (yrs) Obstruction	Duration of nasal symptoms (yrs) Bleeding
L3	1	5	8.4×10^6	4+	4+	0	0	Ulc.	5	–
	2	4	3.6×10^6	vs, 1+	4+	0	0	Ulc.	1	0
	3	6	2.0×10^6	2+	4+	–	–	Ulc.	1	1
	4	5 mo.	1.6×10^6	4+	4+	2	0	Ulc.	2	2
L2-3	5	2	2.7×10^6	vs, 4+	4+	4	0	Ulc.	9 mo.	9 mo.
	6	10	5.0×10^5	3+, 4+	2+	2	0	Ulc.	1	0
L2	7	5	6.8×10^5	2+	3+	2	0	Ulc.	1	1
	8	8	8.2×10^4	2+, 3+	2+	2	1	Nod.	1 mo.	1 mo.
	9	2 mo.	7.4×10^4	2+	3+	2	0	0	0	0
	10	11	4.8×10^4	1+, 2+	3+	2	0	Ulc.	1 mo.	0
	11	1	$<3 \times 10^4$	2+	2+	–	–	0	0	0
	12	4	$<3 \times 10^4$	3+, 4+	4+	2	1	Nod.	0	0
L1	13	2	2.2×10^5	vs, 1+	2+	0	1	Ulc.	0	0
	14	6 mo.	$<3 \times 10^4$	1+	1+	2	3	0	0	0
B3	15	10 mo.	$<3 \times 10^4$	3+	4+	1	2	Nod.	5 mo.	0
	16	2	$<3 \times 10^4$	vs	1+	3	4	Nod.	3 mo.	0
B2-3	17	11	$<3 \times 10^4$	1+, 2+	2+	5	6	0	1 mo.	0
B2	18	6 mo.	4.8×10^4	vs, 1+	2+	3	2	Er	0	0
	19	2	$<3 \times 10^4$	vs, 2+	3+	3	4	Nod.	0	0
	20	11	$<3 \times 10^4$	2+	2+	1	0	Nod.	2 mo.	0
B-12	21	10 mo.	$<3 \times 10^4$	vs, 2+	2+	3	0	Er.	0	0
T2	22	3 mo.	$<3 \times 10^4$	—, 2+	vs	2	0	0	0	0
	23	7 mo.	$<3 \times 10^4$	vs	2+	7	7	Ulc.	1 mo.	0
	24	8 mo.	$<3 \times 10^4$	—, 2+	—	3	2	0	8 mo.	0

[a] The diagnosis is given as L (lepromatous), B (borderline), or T (tuberculoid); the clinical severity as 3 (advanced), 2 (moderately advanced), or 1 (slight). The AFB (acid-fast bacilli) count gives the number recovered in the nasal washings. The numbers of bacilli in smears of skin and nasal septum were graded 1+ to 4+; vs means very scanty bacilli. The lepromin reaction is given in mm. induration. The results of the nasal examination indicates whether ulcers were seen (Ulc.), nodules only (Nod.), erosion only (Er.), or no pertinent findings (O). $<3 \times 10^4$ means "negative" in the sense that no acid-fast bacteria were seen during the standard microscopic search.

The division between lepromatous and borderline is somewhat arbitrary in some patients, and two leprologists will not always agree as to the classification. The impression has been gained that diffuseness of the margins of the lesions and the convexity of those lesions with definable borders have predictive value for high nasal counts.

It was possible to correlate the facial appearance with the nasal counts by the simple procedure of arranging admission photographs of Carville patients according to nasal counts. The patients with counts greater than 1×10^6 had the diffuse infiltrative changes with rounding of the face and few or no nodular changes ("lepra bonita") or the typi-

cal leonine facies. Either of these types of involvement, both characteristic of advanced lepromatous leprosy, were seen only in patients with high counts, or in patients whose treatment had been underway a few months—i.e., long enough to render the nasal count negative.

Effect of treatment on nasal counts.—In Table 2 are presented the results with the Carville patients according to diagnosis and history of treatment. Nearly all of the lepromatous patients without treatment (17 of 20) had counts greater than 10^5. In contrast, none of the 16 lepromatous patients who had been treated for more than 2 months had counts at this level. Those treated for less than two months, or with reactivated lepromatous disease (that usually associated with irregular intake of drug), had varying counts.

After several months of treatment, 10 Carville patients were recalled for a second washing with the results shown in Table 3. The

TABLE 2.—*The patients studied at Carville classified according to diagnosis and amount of antileprosy treatment.*[a]

20 lepromatous patients without treatment		*7 lepromatous patients with less than 2 months treatment*
2.2×10^7	6.7×10^5	1.5×10^7; 3-4 tabs. diasone
2.0×10^7	6.6×10^5	3.1×10^6; 1 tab. DPT/dy, 1 mo.
1.6×10^7	5.1×10^5	6.4×10^5; 14 days
1.6×10^7	4.2×10^5	3.2×10^4; 7 weeks
1.0×10^7	3.8×10^5	$<3 \times 10^4$; 14 days
7.2×10^6	1.3×10^5	$<3 \times 10^4$; 1½ month
5.0×10^6	10^5	
1.7×10^6	3.4×10^4	
1.2×10^6	$<3 \times 10^4$	
9.9×10^5	$<3 \times 10^4$	

8 lepromatous patients with reactivated disease or irregular treatment		*16 lepromatous patients with greater than 2 months treatment*	
3.4×10^6	$<3 \times 10^4$	$<3 \times 10^4$; 2 mo.	$<3 \times 10^4$; 1 yr.
5.7×10^5	$<3 \times 10^4$	$<3 \times 10^4$; 3 mo.	$<3 \times 10^4$; 1¼ yr.
4.8×10^5	$<3 \times 10^4$	$<3 \times 10^4$; 3 mo.	$<3 \times 10^4$; 1½ yr.
1.4×10^5	$<3 \times 10^4$	$<3 \times 10^4$; 3 mo.	$<3 \times 10^4$; 3 yr.
		$<3 \times 10^4$; 4 mo.	$<3 \times 10^4$; 3 yr.
		$<3 \times 10^4$; 6 mo.	$<3 \times 10^4$; 6 yr.
		$<3 \times 10^4$; 1 yr.	$<3 \times 10^4$; 9 yr.
		$<3 \times 10^4$; 1 yr.	$<3 \times 10^4$; 9 yr.

6 borderline patients	*4 tuberculoid patients*
3.4×10^4; no treatment	$<3 \times 10^4$; no treatment
$<3 \times 10^4$; no treatment	$<3 \times 10^4$; no treatment
$<3 \times 10^4$; no treatment	$<3 \times 10^4$; no treatment
$<3 \times 10^4$; no treatment	$<3 \times 10^4$; 1½ yr.
$<3 \times 10^4$; no treatment	
$<3 \times 10^4$; 13 days	

[a] The number of acid-fast bacilli in the nasal washing and the length of treatment is given for each patient.

counts had dropped markedly during sulfone therapy, in some cases in 3.5 months.

More recently it has been possible to follow several patients with washings every few weeks, and the counts have become negative ($<3 \times 10^4$) after 3 months of sulfone therapy (unpublished data).

Effect of repeated washings on nasal counts.—Three patients of

TABLE 3.—*Decrease in number of acid-fast bacilli recoverable in nasal washings during sulfone therapy.*

Case	Original count	Count after treatment	Months of treatment
1	1.6×10^7	8×10^3	3.5
2	1.6×10^7	4×10^3	3.5
3	1.5×10^7	3.6×10^5	3
4	1.7×10^6	$<1 \times 10^3$	11.5
5	6.7×10^5	4.5×10^4	3
6	4.2×10^5	1×10^3	12
7	3×10^3	4×10^3	3.5
8	$<1 \times 10^3$	1×10^3	3
9	$<1 \times 10^3$	3×10^3	8
10	$<1 \times 10^3$	1.2×10^4	8

TABLE 4.—*Repeated nasal washings did not lower the number of acid-fast bacilli recovered.*

Date of washing	Patient		
	Gal	Sa	Ca
Nov. 24	8.4×10^6	1.6×10^6	
Nov. 27			2.2×10^5
Dec. 3	1.6×10^7	3.9×10^6	2.3×10^5
Dec. 4	8.2×10^6	1.3×10^7	8.1×10^5
Dec. 5	8.6×10^6	1.6×10^5	5.1×10^5
Dec. 6	7.3×10^6	6.9×10^6	6.3×10^5

Table 1 were selected for repeated nasal washings. In addition to the washings shown in Table 4, one additional washing was made on December 1 or December 2 for shipment to the base laboratory. Repeated washings did not significantly lower the numbers of acid-fast bacilli recovered. The numbers observed apparently represent a minimal estimate of the number of organisms excreted per day.

Results of inoculation of foot-pads of mice.—The multiplication of *M. leprae* from nasal washings and from skin-biopsy specimens is described more fully in another paper ([12]).

DISCUSSION

These studies of nasal washing were originally undertaken for the chief purpose of developing a new source of *M. leprae* for attempts at isolation of the agent. It seemed to me that continuous excretion of the

bacilli from an open, draining lesion provided evidence that they were viable. This was in contrast to the situation in the typical closed lesion of the skin, where there was no assurance that the bacilli were anything more than an accumulation of nonviable acid-fast "skeletons."[7] However, leprosy bacilli from both these sources have now been observed to multiply when injected into mouse foot-pads. The consistency of the result was greater with bacilli from nasal washings, but the differences in viability between those from nasal excretions and from skin specimens are obviously not of an all-or-none nature.

Evidence on the excretion rate and viability of a pathogen applies to considerations of the natural transmission of the disease. The number of leprosy bacilli in the nasal washings of patients with untreated lepromatous leprosy is of the same magnitude as the number of tubercle bacilli in tuberculous sputum. The mechanism by which the leprosy bacilli are carried to a new host might be the normal movement of hands to nose, face, and environment, in which case entry of the bacilli into the new host through the skin might occur. Another possible mechanism would be the more direct one in air-borne droplets or particles, and in this case entry via the nasal mucosa would seem possible. The nose is an efficient sampler of bacteria in air. Normal breathing at rest passes about 10 liters of air per minute through the nose ([4]), and of the particles in the inspired air a high proportion of those greater than 2μ in diameter are deposited on the nasal mucosa ([8]).

Another human infection, neonatal staphylococcal disease, is marked by the presence of large concentrations of the disease agent in the nasal secretions. During epidemics of this infection, the organism can be readily isolated in the nursery from air, blankets, floor, etc. Recently, Eichenwald *et al.* ([2]) have shown that viral respiratory infections can convert a nondisseminating nasal carrier to a highly infectious "cloud baby," which is their designation for the efficient disseminator that causes high staphylococcus counts in the air and rapid spread of staphylococcus infections to other infants in the nursery.

Considerations of the nasal excretion rate of leprosy bacilli appear also to apply to antileprosy therapy. The results obtained so far are consistent with the idea that sulfone therapy causes a characteristic drop in excretion rate, so that in about 3 months the numbers of bacilli fall to undetectable levels. This phase of the subject remains under investigation.

The evidence that the large numbers of acid-fast bacilli in the nasal washings of leprosy patients are *M. leprae* rather than some incidental organism is as follows: (*a*) the large numbers were associated with lepromatous leprosy, and not with other forms of the disease; (*b*) the numbers were correlated with the severity of the disease; (*c*) the large numbers were found in patients with the nasal symptoms and nasal ulcers characteristic of the disease; (*d*) smears made directly from the

ulcers contained many characteristic organisms; (*e*) the acid-fast bacilli in the washings were frequently present in globi and packets; (*f*) the high counts were reduced after sulfone therapy; and (*g*) the acid-fast bacilli would not grow on artificial media, nor would they grow in tissue culture under conditions found suitable for the growth of the cultivable mycobacterial disease agents. ([9]) Further evidence is presented in another paper ([12]), which describes the growth of leprosy bacilli from biopsy specimens and nasal washings in a characteristic and reproducible fashion in the foot-pads of mice.

The findings described here lend a support to two of the basic tenets of modern leprosy control, first the importance of the lepromatous case as an infectious source, and second the effectiveness of sulfone therapy in rendering patients noninfectious.

SUMMARY

1. A technique is described whereby the leprosy bacilli are washed from patients' nasal passages, then concentrated and counted.

2. Counts of 10^5 to 10^7 bacilli were observed in most of the patients with untreated lepromatous leprosy. The highest counts were observed in patients with advanced lepromatous leprosy and nasal ulcers, and histories of nasal obstructions and bleeding.

3. The counts apparently represent minimal estimates of daily excretion, since repeated daily washings did not lower the numbers significantly.

4. After 2 to 4 months of sulfone therapy, the counts were much lower.

RESUMEN

1. Descríbese una técnica con la que se lavan los bacilos leprosos de las vías nasales de los enfermos, y luego se concentran y cuentan.

2. Se observaron numeraciones de 10^5 a 10^7 de bacilos en la mayoría de los enfermos que tenían lepra lepromatosa sin tratar. Observáronse las numeraciones más altas en los sujetos que tenían lepra lepromatosa avanzada y úlceras nasales e historias de oclusión y hemorragia nasales.

3. Las numeraciones representan aparentemente cálculos mínimos de la excreción diaria, dado que repetidos lavados diarios no rebajaron mayor cosa el número.

4. Al cabo de 2 a 4 meses de sulfonoterapia, las numeraciones eran mucho más bajas.

RESUMÉ

1. Une méthode est décrite pour laver les bacilles de la lèpre des fosses nasales des malades, les concentrer, et les compter.

2. Chez la plupart des malades atteints de lèpre lépromateuse non traitée, on a dénombré de 10^5 à 10^7 bacilles. Les chiffres les plus hauts ont été relevés chez des malades présentant une lèpre lépromateuse avancée et des ulcères nasaux, ainsi que des antécédents d'obstruction des conduits nasaux et de l'epistaxis.

3. Ces chiffres semblent constituer une évaluation minimale de l'excrétion, journalière, car des lavages quotidiens répétés n'entraînent pas une réduction significative de ce nombre.

4. Après 2 à 4 mois de traitement par les sulfones, les chiffres sont beaucoup plus bas.

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