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Leprosy in a Mangabey Monkey—Naturally Acquired Infection[1]

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In 1873, in Bergen, Norway, Hansen first observed the leprosy bacillus (*Mycobacterium leprae*) by conventional light microscopy of unstained fresh mounts of tissue fluid from a lesion of a patient with lepromatous leprosy. This pivotal discovery in medical microbiology established that leprosy was infectious, and it launched the search for a suitable animal model. In 1960 Shepard reported reproducible lesions of experimental leprosy in the foot pads of normal mice ([36]). Although the infections in the foot pads of normal mice are highly useful for studies on the etiologic agent (e.g., viability and drug sensitivity of isolates of *M. leprae*), the lesions are not similar to any clinical or histopathologic form of leprosy in humans, and thus are of limited usefulness in studying the pathogenesis and natural history of leprosy. The transmission of leprosy to nine-banded armadillos (*Dasypus novemcinctus*) ([15]) was an important advance in the search for an animal model. Many features of the experimentally induced disease in armadillos resemble leprosy in humans, but there are noteworthy differences: the infection is rapidly fatal, usually in 1½–3 years, nearly all animals have lepromatous (anergic) disease, and neuropathic deformity has not been reported. The best animal model for leprosy would duplicate a maximum number of features of leprosy in humans. The chances for achieving this goal would be expected to be

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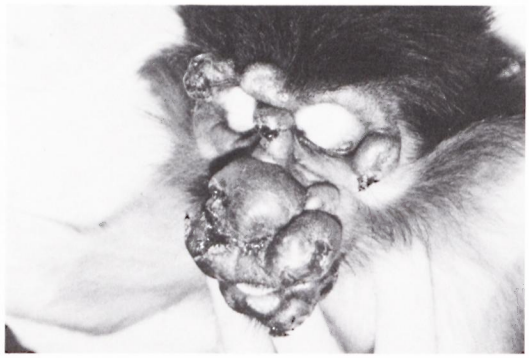

FIG. 1. Mangabey monkey with leprosy, soon after diagnosis in January 1980. Note extensive infiltrations of the skin with ulcers over the muzzle and periorbital area, and the nodular thickening of the ears. AFIP Neg. 81-17362.

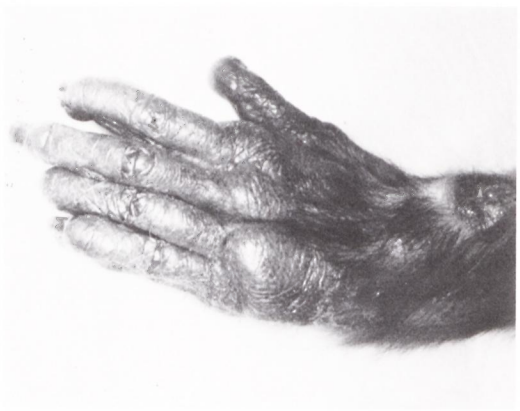

FIG. 2. Hand of the mangabey monkey with leprosy, December 1980. There are large lepromas and ulcers on the dorsum of the hand and wrist, with extensive infiltration of the skin of the fingers and early resorption of the tips of the fingers. AFIP Neg. 81-14384.

greatest in animals that are phylogenetically nearest to humans. Accordingly, there have been many attempts to establish leprosy in nonhuman primates without regular success ([3]). However, Gunders successfully infected one chimpanzee ([10]); Waters, *et al.*, a white-handed gibbon ([43]); and Leininger, *et al.* observed a chimpanzee with naturally acquired leprosy ([17,18]). We report here our observations on naturally acquired leprosy in a sooty mangabey monkey (*Cercocebus atys*), and the results of studies on the etiologic agent.

MATERIALS, METHODS, AND RESULTS

Clinical observations and laboratory studies

This female mangabey monkey was imported from West Africa in 1975 and was estimated to be five years old (wt 4.5 kg) when, in September 1979, clinicians first observed infiltrations of the skin. A biopsy specimen of skin taken in December 1979 revealed typical features of multibacillary leprosy (AFIP Accession No. 1724396). The animal, then housed at Gulf South Research Institute, New Iberia, Louisiana, U.S.A., was on dietary cholesterol studies and had never been experimentally inoculated with *M. leprae*. After transfer to Delta Regional Primate Research Center, Covington, Louisiana, a detailed clinical examination was performed in January 1980. At this time, there were extensive firm infiltrations in the skin of the forehead, periorbital area, muzzle, and lower lip (Fig. 1). The ears were thickened and nodular, and there was slight thickening of the skin over the extensor surfaces of the forearms. All remaining body surfaces were normal, and there were no palpable peripheral nerves of paralytic deformities of the hands or feet. Examination of the eyes with a loupe did not reveal any abnormalities. The larynx was normal by direct laryngoscopy.

Biopsy specimens of skin from the face contained up to 3.9×10^{10} acid-fast bacilli (AFB) per gram of tissue. All counts were made by the Shepard and McRae method ([37]). Bacterial indices (BI) obtained by the slit-scrape technique and evaluated by the method of Ridley ([33]) gave the following results: ears, face, and both forearms, 6+; two fingers, 3+ and 4+. Smears from the nasal mucosa were 6+. As determined by staining methods in current use at the U.S. Armed Forces Institute of Pathology (AFIP), 20–50% of the AFB in the smears were solidly stained. There was an occasional AFB in buffy coats of peripheral blood. The results of hematologic and blood chemistry studies and urinalysis were normal.

During the ensuing 12 months there was a gradual increase in the cutaneous infiltrations, with spread to other areas of the skin. The face, limbs, and tail were heavily infiltrated (Fig. 2), and there was partial loss of hair in these sites. There was extensive ul-

FIG. 3. Clawing of the toes of the foot, December 1980. The foot is inverted, there are ulcers on the lateral aspect, and early contractures of the toes. AFIP Neg. 81-16709.

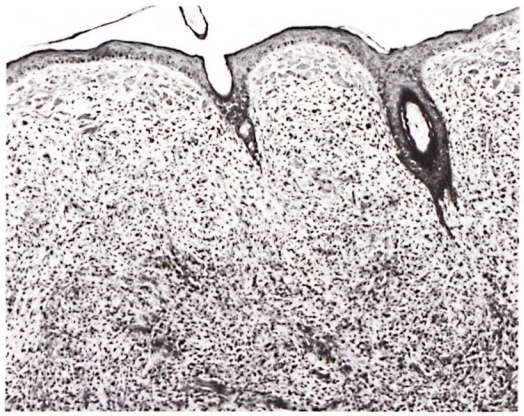

FIG. 4. Specimen of skin from muzzle of mangabey monkey, showing nearly total replacement of dermis by histiocytes mixed with small numbers of lymphocytes. There is a subepidermal clear zone. AFIP Neg. 80-4692 (H&E ×150).

ceration of the skin, with weight loss and anemia. In December 1980 paralytic deformities of the hands and feet were first noted (Fig. 3), and within two months there was paralysis of the intrinsic muscles of both feet and of the extensor digitorum and peroneal muscles of both hind limbs. The toes were clawed and the feet inverted with resulting ulceration of the lateral aspect of the feet and soles. There was paralysis of the intrinsic muscles of the hands, with clawing of the fingers and early absorption of the finger tips. Palpation of the major nerve trunks in the extremities did not reveal thickening at the usual sites of enlargement of peripheral nerves in leprosy patients. Smears taken in February 1981 from the ears, hands, tail, and nasal mucosa were all 6+ for AFB, with a mean of 20% of the organisms being solidly stained. The animal became anorexic, with severe loss of weight, and had extensive ulceration of the skin at many of the infiltrated sites. Antileprosy chemotherapy was started at this time, with a combined regimen of rifampin 10/mg/kg/day orally for one month, and diacetyldiaminodiphenyl sulfone (DADDS) 20 mg intramuscularly every 77 days. After one month, smears from the skin and nasal mucosa remained highly positive but contained no solid-staining AFB. Over the subsequent 16 months there was a gradual resolution of the infiltrations of the skin, but there was progression of the paralytic deformities. In June 1982 there was a recurrence of ulceration around the nares, and smears from this area contained solid-staining AFB. There was an extensive exacerbation of disease clinically, and rifampin therapy was reinstituted in May 1983 with a favorable chemotherapeutic response. Morphological indices (MI) of bacilli in skin smears and mouse foot pad studies did not reveal any viable *M. leprae* in biopsy specimens taken after one month of rifampin therapy. This therapeutic regimen has been maintained and there was no evidence of active leprosy in August 1984.

Histopatholgic and electron microscopic findings

Under ketamine sedation (5 mg/kg), 6 mm biopsy specimens of skin were taken from the ear, face, forearm, and tail, and fixed in buffered formalin or glutaraldehyde. Cut surfaces of the specimens were light tan and the dermis was up to 7 mm thick. Tissues were processed by routine procedures, embedded in paraffin, and cut at a thickness of 5 μ. Hematoxylin-eosin (H&E) stained sections showed a narrow subepidermal clear zone and effacement of the rete-ridges. Infiltrations of vacuolated histiocytes mixed with a few lymphocytes replaced up to 90% of the dermis (Fig. 4). In less extensively involved dermis, the cellular infiltrations tended to be along neurovascular channels. In a few histiocytes there were large vacuoles that contained pale basophilic masses 5–12 μ in diameter (globi) (Fig. 5). The lym-

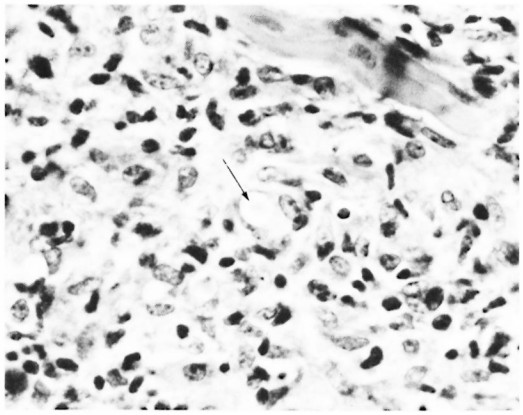

FIG. 5. Specimen of skin from muzzle, demonstrating coarsely and finely vacuolated histiocytes. There is a pale basophilic structure (arrow), a globus of *M. leprae*, in one histiocyte. AFIP Neg. 80-4693 (H&E ×950).

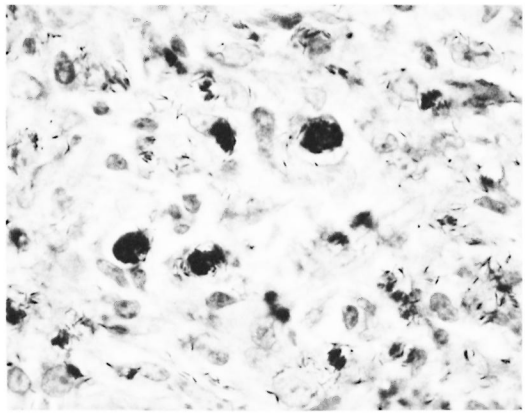

FIG. 6. Acid-fast stained section of skin from muzzle, revealing large numbers of acid-fast bacilli distributed singly, in small clumps, and in globi in histiocytes. AFIP Neg. 81-17785 (Fite-Faraco ×1475).

phocytes were usually scattered randomly in the cellular infiltrations, but in some specimens the lymphocytes were clustered around a germinal center. Sections stained by the Fite-Faraco method revealed large numbers of AFB in histiocytes and a few in nerves (Fig. 6). The AFB were single, in clumps, or in globi. In pre-treatment biopsy specimens, a high percentage of the AFB were solidly stained. Following rifampin and DADDS therapy, the AFB were irregularly stained or granular. There was lamellar thickening or ablation of the perineurium of dermal nerves. Nerves were invaded and sometimes disrupted by cellular infiltrations (Figs. 7 and 8). There were no epithelioid cells or giant cells. According to the Ridley-Jopling classification of leprosy in humans ([34]), the disease in this monkey is in the subpolar-lepromatous to borderline-lepromatous area of the spectrum of the disease.

Electron microscopic studies were performed on specimens of skin fixed in 3% glutaraldehyde prepared in 0.06 M phos-

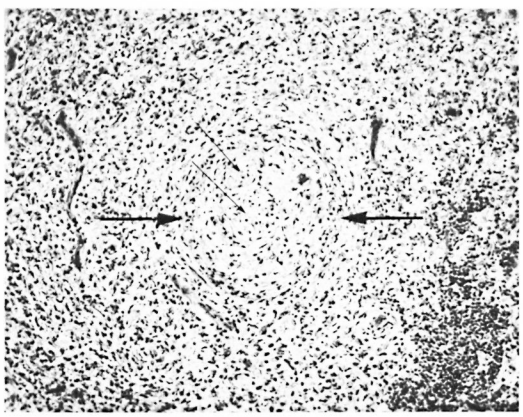

FIG. 7. Specimen from forearm of mangabey monkey showing a nerve in the dermis. There is lamellar thickening of the perineurium (large arrows) and fragmentation of the nerves by infiltrating histiocytes and lymphocytes (small arrows identify two fragments of nerve). At the lower right there is a large focus of lymphocytes. Some of these foci contained germinal centers (not seen here). AFIP Neg. 80-4688 (H&E ×235).

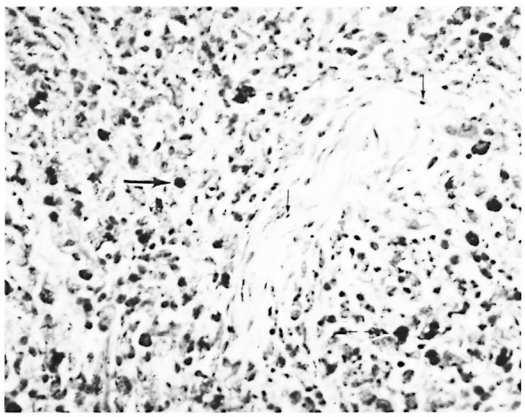

FIG. 8. Section of dermis of forearm demonstrating infilitration of histiocytes and lymphocytes. There are numerous clusters and globi of acid-fast bacilli (AFB) in the histiocytes (large arrows). A few AFB have invaded the nerve (small arrows). AFIP Neg. 81-17786 (Fite-Faraco ×600).

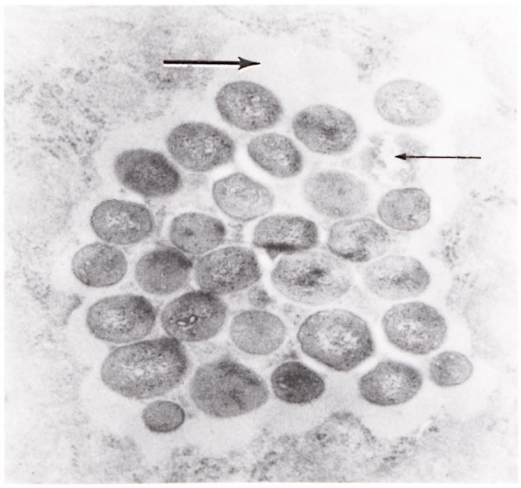

FIG. 9. Ultrathin section of an intracytoplasmic foamy structure in a phagolysosome in a histiocyte from a biopsy specimen of the muzzle skin. Note the relatively electron-transparent zone around the bacilli (large arrow). Only one of the 30 bacilli seen in cross section here shows advanced degeneration of the cytoplasm (small arrow). AFIP Neg. 81-17787 (×29,550).

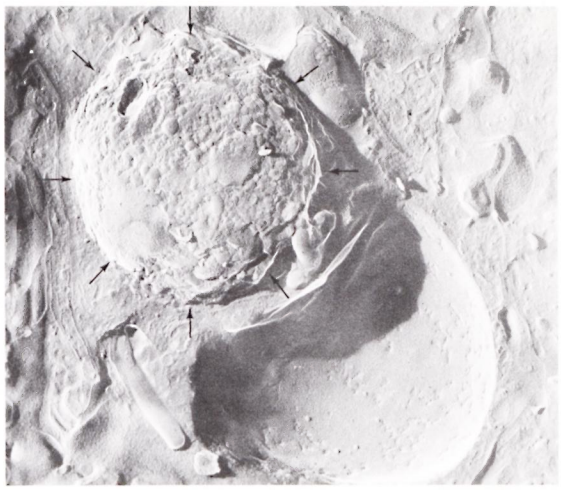

FIG. 10. Freeze-etching preparation showing external features of an intracytoplasmic foamy structure in a phagolysosome in a histiocyte of the same biopsy specimen as in Figure 9. Limits of the foamy structure are marked with arrows. A few segments of bacilli are noted, but the surface of the foamy structure is largely composed of small spherical droplets. AFIP Neg. 83-8056-1 (×11,825).

phate buffer. For thin section studies, the specimen was washed in water and post-fixed in 2% OsO_4, embedded in Spurr low-viscosity medium, cut, and stained with uranyl acetate and lead citrate. Tissues for freeze-etching studies were fixed under the same conditions and immersed in 20% glycerol for 1–2 days at 4°C. The specimens were fractured in a vacuum and shadowed by platinum-carbon and carbon evaporation by the method of Nishiura, *et al.* ([31]). The ultrathin sections of macrophages in the infiltrations contained large numbers of bacilli in phagolysosomes surrounded by an electron-transparent zone (Fig. 9). Freeze-etched preparations demonstrated foamy structures containing bacilli and large numbers of spherical bodies in histiocytes (Figs. 10 and 11). These features are typical of multibacillary leprosy in humans ([31]), nude mice ([9]), and armadillos ([8]).

Immunologic studies

Skin tests. Standard Mitsuda-Hayashi-Wade lepromin, 0.1 ml, prepared from human tissue, was injected intracutaneously in the forearm of the monkey in January 1980. This lepromin contained 160 million bacilli per ml. Both the Fernandez (48 hr) and the Mitsuda (28 days) responses were non-reactive. The intrapalpebral PPD skin test for tuberculosis was likewise non-reactive.

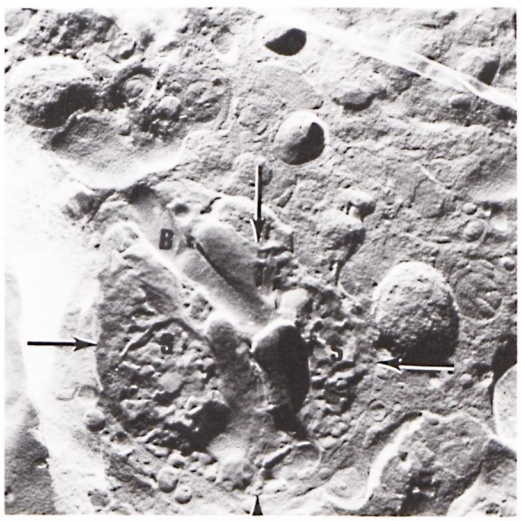

FIG. 11. Freeze-etching preparation of internal features of an intracytoplasmic foamy structure in a phagolysosome in a histiocyte. Limits of the foamy structure are marked with arrows. Within the structure there is a bacillus (B) and many spherical droplets (S). AFIP Neg. 83-8056-2 (×17,750).

TABLE 1. *Mitogen responses of lymphocytes from normal mangabeys and from the infected mangabey before and during treatment.*[a]

Animal	Control	Mitogen stimulation[b]					
		PHA		PWM		ConA	
		E − C	E/C	E − C	E/C	E − C	E/C
				Normal mangabeys			
A022	3,461	78,726	24.0	28,441	9.3	184,567	54.9
A023	2,631	136,621	52.9	36,791	14.9	246,081	94.5
A024	20,777	21,635	2.0	1,068	1.1	50,608	3.4
A038	11,088	13,080	2.2	1,814	1.2	29,288	3.6
A039	1,854	93,569	53.7	45,761	25.7	137,713	75.3
A040	3,906	5,382	2.4	2,416	1.6	9,231	3.4
A043	3,724	29,956	10.2	39,570	10.0	107,635	33.9
Mean	6,771	54,138	21.1	20,837	9.1	109,303	38.4
S.D.	±2,604	±18,687	±8.8	±7,074	±3.4	±32,749	±14.2
				Infected mangabey			
A015, before treatment[c]							
Mean	5,339	32,000	7.1	12,998	3.5	59,158	12.1
S.D.	±1,001	±3,965	±0.4	±1,811	±0.9	±16,176	±2.7
A015, during treatment[d]							
Mean	7,059	30,783	5.8	7,926	2.3	56,710	10.1
S.D.	±1,179	±4,253	±0.9	±1,542	±0.4	±7,204	±2.2

[a] Data are expressed as the difference between the cpm ^{3}H-TdR incorporated by mitogen stimulated and control cultures (E − C) or as the ratio between the cpm ^{3}H-TdR incorporated by mitogen stimulated and control cultures (E/C).

[b] The optimal stimulatory doses determined in preliminary experiments were used: PHA, 25 μg/ml; PWM, 50 μg/ml; ConA, 12.5 μg/ml.

[c] Data represent the mean of determinations on three separate dates prior to the initiation of treatment.

[d] Data represent the mean of determinations on five separate dates beginning three months after treatment was initiated.

Antimycobacterial antibodies. Using methods described by Harboe, *et al.* ([11]), crossed immunoelectrophoresis studies and radioimmunoassays were performed to detect antibodies to *M. leprae* antigens. There was strong antibody activity against *M. leprae* antigens 5 and 7, and lower but distinct antibody activity against *M. leprae* antigen 2. Similar patterns are regularly seen in the sera of patients with lepromatous leprosy and of armadillos with established *M. leprae* infection.

Mitogen stimulation studies. The blastogenic responses of peripheral blood lymphocytes (PBL) cultured with phytohemagglutinin (PHA), concanavalin A (ConA), or pokeweed mitogen (PWM) were studied. Mononuclear cell preparations enriched for PBL were separated from heparinized blood (10 U/ml) by a modification of the Ficoll-Hypaque procedure ([22,39]). PBL (2×10^5 cells) were cultured in flat-bottomed microtiter plate wells (Linbro, Flow Laboratories, Hamden, Connecticut, U.S.A.) in 0.2 ml RPMI 1640 (Grand Island Biological Company, Grand Island, New York, U.S.A.) supplemented with 15% fetal bovine serum, penicillin (100 U/ml), streptomycin (100 μg/ml), and L-glutamine (2 mM). Various concentrations of each mitogen or medium alone were added to triplicate PBL cultures, and the plates were incubated for 3 days in a humidified incubator with an atmosphere of 5% CO_2 in air. The incorporation of 1 μCi tritiated thymidine (^{3}H-TdR), added during the final 18 hr of culture, was measured by scintillation counting. Preliminary experiments determined that optimal doses for the stimulation of PBL from normal monkeys were 12.5 μg/ml for PHA, 50 μg/ml for PWM, and 6.25 μg/ml for ConA.

Mitogen-induced blastogenic responses of PBL from the infected mangabey, before and after treatment was initiated, are compared to responses for PBL from seven normal mangabeys in Table 1. There was considerable variation in mitogen responsiveness among the normal mangabeys. PBL

TABLE 2. *Profile analysis of peripheral blood mononuclear cells of mangabey monkeys.*

	Date	Monoclonal antibody								$T4^+/T8^+$
		OKTa	OKT3	OKT4	OKT5	OKT6	OKT8	OKT11	OKM1	
					Normal mangabeys					
A024	4/22/82	25.1[a]	1.7	20.3	31.3	5.7	30.7	77.6	10.7	0.7
	6/31/82	46.9	4.4	21.6	36.9	6.2	54.0	65.6	8.3	0.4
A039	4/22/82	11.3	1.6	21.9	23.4	4.6	14.4	79.1	11.4	1.5
	6/31/82	20.9	4.4	22.7	28.1	4.7	32.2	66.2	6.4	0.7
A043	4/22/82	25.1	2.6	3.9	31.7	5.5	29.6	69.7	14.4	0.13
	6/31/82	51.8	15.6	17.4	54.8	17.0	67.4	73.4	31.3	0.26
					Infected mangabey					
A015	4/22/82	23.6	1.9	14.0	18.9	4.6	23.4	55.7	9.9	0.6
	6/31/82	52.8	3.4	12.9	51.9	4.5	60.1	75.6	7.3	0.2
Human		13.6	58.7	36.0	17.9		17.4			2.1

[a] All values in % of mononuclear cells.

from some animals incorporated relatively low amounts of ^{3}H-TdR in unstimulated control cultures and incorporated relatively high amounts in mitogen stimulated cultures. PBL from other animals incorporated relatively large amounts of ^{3}H-TdR in control cultures and the stimulation by mitogens was less. Each animal tended to be consistent in its responses on repeated testing (not shown). PBL from the infected mangabey prior to treatment were able to respond to both T and B cell mitogens, and the responses were not significantly different from the responses of normal mangabeys. During the 15 months of treatment the responses did not differ significantly from pretreatment values. In human lepromatous leprosy, lymphocyte responses to mitogens may be nonspecifically depressed ([5]), perhaps depending on the bacterial load in the patient ([30]). In view of the variation in mitogen responsiveness among the normal mangabeys, it is difficult to determine whether the responses in the infected mangabey were affected by the disease. Effective therapy in humans often returns depressed mitogen responses to normal ([5]), but the return may require prolonged therapy ([30]).

Surface marker analysis of peripheral blood mononuclear cells. Mononuclear cell preparations from 10–15 ml of heparinized whole blood were isolated on a Ficoll-Hypaque gradient (Litton Bionetics, Kensington, Maryland, U.S.A.). The mononuclear cells were suspended in Hanks' balanced salt solution containing 0.1% sodium azide and 0.1% bovine serum albumin, and incubated with the appropriate dilution of each monoclonal antibody (OKT series, OkIal and OkMl; Ortho Diagnostic Systems, Inc., Raritan, New Jersey, U.S.A.) for 30 min at 4°C. The cells were then washed three times and incubated with fluorescein isothiocyanate-labeled goat anti-mouse IgG (Litton Bionetics) for 30 min at 4°C. The cells were then washed again three times, fixed with 2.0% paraformaldehyde and analyzed on a fluorescence-activated cell sorter (FACS IV; Becton, Dickinson, Sunnyvale, California, U.S.A.).

Monoclonal antibodies against human T and B cell surface markers crossreacted with the mangabey mononuclear cells (Table 2), with the exception of OKT3. The most striking finding was the wide variation in the numbers of the various T cell subsets from one experiment to another, making it difficult to detect any trends in T cell subset alterations between infected and noninfected monkeys. The $T4^+/T8^+$ ratio for mangabey mononuclear cells ranged from 0.2 to 1.5, compared to 2.1 for humans. There appeared to be a consistent reduction of the $T4^+$ helper/inducer subset in the infected monkey when compared to the noninfected controls. No similar change was seen in the $T8^+$ subset. Low $T4^+/T8^+$ ratios are a common finding in subhuman primates when tested with monoclonal antibody to human T cell antigens ([12]).

Peripheral blood leukocytes do not always reflect profound changes in lympho-

TABLE 3. *Plasma protein fraction levels in mangabey monkeys.*

Fraction	Normal human	Date (mo/yr)	Normal adult mangabeys			Infected mangabey
			A024[a]	A039	A043	A015
IgG	800–1800	3/82	1450[b]	1340	1650	1710
		6/82	1125	860	1050	2000
IgM	90–450	3/82	160	78	140	160
		6/82	128	78	78	78
IgA	54–268	3/82	240	147	240	240
		6/82	240	75	240	240
C3	55–120	3/82	86	98	74	69
		6/82	63	60	51	9
C4	20–50	3/82	6	23	11	54
		6/82	2	7	10	10
Fibrinogen	200–450	3/82	119	166	158	119
		6/82	111	119	143	170
CRP	0.06–8.2	3/82	2.9	7.9	3.6	4.2
		6/82	2	2	3.2	2

[a] Individual animal designations.
[b] All values in mg/dl.

cyte populations in disease ([2]). Others have reported that the peripheral blood $T4^+/T8^+$ ratio is not altered in lepromatous leprosy in humans, except in patients with erythema nodosum leprosum in which the $T4^+/T8^+$ ratio is increased ([1]). The cellular infiltrations in cutaneous lesions of leprosy reflect specific patterns of T cell numbers and distributions in relation to the clinical and histologic forms of the disease ([28, 40]).

Plasma protein fraction levels. Radial immunodiffusion assays (RID) for plasma immunoglobulins, C-reactive protein (CRP), C3, C4, and fibrinogen were performed on RID plates (Calbiochem-Behring, La Jolla, California, U.S.A.) ([21]). Plates were allowed to stand at room temperature for 20 min before placing protein standards and test samples (10 μl or 20 μl volumes as required) in the agarose wells. The plates were closed and incubated at 27°C for 72 hr. The diameters of the precipitin zones were determined with calipers and compared with standard curves (Table 3). All assays on both the March 1982 and June 1982 specimens were performed simultaneously. Thirteen (March 1982) to 16 (June 1982) months after treatment was initiated, the IgG levels in plasma from the infected mangabey were higher than the levels in any of the normal samples. The plasma IgM, C3, and C4 concentrations decreased markedly in the infected monkey between 13 and 16 months after treatment was initiated. In human leprosy, increased serum levels of IgG, IgM, and IgA are frequently observed; the serum IgM levels decrease during effective therapy, but the IgG and IgA levels tend to remain elevated during prolonged therapy ([5]). The present results in the monkey are consistent with these immunologic variations in humans with lepromatous leprosy.

Studies on the etiologic agent

Cultivation. Approximately 10^7 AFB isolated from biopsy specimens of skin taken prior to chemotherapy were inoculated onto three tubes of Lowenstein-Jensen and 7H10 media and incubated at 37°C, 32°C, and room temperature for up to 8 months. There was no growth of mycobacteria in any of the tubes.

Staining properties. The AFB in tissue sections processed by routine histopathologic procedures and embedded in paraffin stained much more consistently and intensely by the Fite-Faraco ([7]) method than with the Ziehl-Neelsen technique. Exposure to pyridine abolished the acid-fastness of the AFB ([6]). These properties are consistent with the staining characteristics of *M. leprae* in contrast to those of other mycobacteria.

DOPA-Oxidase activity. Purified suspensions of AFB oxidized D-DOPA when tested by the method of Prabhakaran ([32]).

Lepromin reaction. Lepromin (160×10^6

AFB/ml) was prepared by the Mitsuda Hayashi-Wade technique from biopsy specimens of heavily infiltrated skin from the muzzle and ears of the monkey, and tested in leprosy patients at the Institut Medical Evangelique, Kimpese, Zaire, by Dr. G. Stenstrom and staff. All patients gave informed consent to participate in the study. The initial classification of all patients was made according to the criteria of Ridley and Jopling ([34]), including clinical and histopathologic evaluations and response to lepromin. Following procedures previously described ([24]), comparative testing of the mangabey lepromin (Lepromin-M) and standard lepromin of human origin (Lepromin-H) was performed. This consisted of the intracutaneous inoculation at the same sitting of 0.1 ml of each of these preparations on the volar aspect of the forearms, and the diameter of the infiltrated area was measured 28 days after inoculation. The differences of the means of the reactions to Lepromin-M and Lepromin-H in the two groups of patients (Table 4) are not significant by the "*t*-test," and when the values for Lepromin-M are plotted against those for Lepromin-H, the points were not only collinear (statistically) for both groups, but the overall correlation coefficient was 0.93, indicating a very strong relationship between the responses to the two reagents.

TABLE 4. *Comparisons of Mitsuda reactions to lepromin-M (mangabey monkey origin) and lepromin-H (human origin).*

Lepromatous patients[a]		Nonlepromatous patients[b]	
Lepro-min-M	Lepro-min-H	Lepro-min-M	Lepro-min-H
4[c]	5	15	18
2	4	12	13
2	4	9	12
5	3	11	11
0	3	7	8
3	0	7	4
0	0		
0	0		
2.0	2.4	10.2	11.0

[a] Includes lepromatous (LL) and borderline-lepromatous (BL) patients.
[b] Includes tuberculoid (TT) and borderline-tuberculoid (BT) patients.
[c] mm of induration.

Inoculation into mice. Sixty-five BALB/c mice were inoculated in the hind foot pads with 5×10^3 AFB isolated from biopsy specimens from the monkey. This large number of mice was employed to provide a reserve source of the etiologic agent and to determine the growth pattern. The mean foot pad count obtained from 10 mice killed eight months after inoculations was 6.6×10^6 AFB. No gross changes were noted in the foot pad, and there was no dissemination of the infection. Successive passage of this organism in BALB/c mice yielded mean foot pad counts of 4.1×10^6, 3.17×10^6, and 5.4×10^6 AFB at harvest.

Inoculation into armadillos. Twelve nine-banded armadillos (*Dasypus novemcinctus*) that had been screened for naturally acquired leprosy and found to be free of disease were inoculated with suspensions of AFB isolated from biopsy specimens of skin from the mangabey monkey. Five of the armadillos received 1.3×10^9 AFB subcutaneously at each of two sites on the abdomen, and the remaining seven armadillos received 2.6×10^8 AFB intravenously in addition to the subcutaneous inoculations. Four of the 7 animals inoculated both subcutaneously and intravenously, and 3 of the 5 animals inoculated subcutaneously only, developed disseminated disease. All animals with disseminated leprosy were necropsied. The clinical, gross pathologic, and histopathologic findings in all seven armadillos were like those seen in experimental leprosy in nine-banded armadillos. Three armadillos died of causes unrelated to leprosy, and the two remaining animals never developed leprosy.

DNA studies. DNA homologies on the mangabey monkey agent were done according to methods previously described ([14]). From 17 g of spleen obtained from one of the armadillos described above, 500 mg (wet wt) of AFB yielding 670 μg of bacterial DNA was isolated. This DNA was 100% homologous with the DNA obtained from AFB purified from the spleen from an armadillo that had been experimentally infected with *M. leprae* (kindly supplied by Dr. E. E. Storrs, Florida Institute of Technology, Melbourne, Florida, U.S.A.). The DNA of the mangabey monkey agent showed only 19% homology with the DNA of *M. lepraemurium* prepared as described elsewhere ([13]), indicating their remote genetic relatedness.

TABLE 5. *Sensitivity of the etiologic agent of leprosy in the mangabey monkey to DDS in the foot pads of BALB/c mice.*[a]

Study	Level of DDS in diet			
	None	0.01%	0.001%	0.0001%
I	3.17×10^6 (3)[b]	0[c] (5)	1.3×10^5 (3)	1.7×10^5 (3)
II	5.4×10^6 (6)	0 (8)	5.0×10^4 (8)	1.5×10^6 (8)

[a] Study I = DDS started at time of inoculation. Study II = DDS started three months after infection. All harvests were at eight months postinoculation.

[b] Values are numbers of AFB/foot pad, and figures in parentheses are numbers of animals in each group.

[c] 0 = No AFB found.

Dapsone sensitivity. In two separate assays, the sensitivity of the mangabey agent to dapsone (diaminodiphenylsulfone, or DDS) was tested in the normal BALB/c mouse foot pad. Table 5 shows that the mangabey agent was inhibited completely by 0.01% dietary dapsone and partially inhibited by 0.001% and 0.0001%. The agent is thus partially resistant to dapsone.

DISCUSSION

"Experiments of nature" have contributed much to the understanding of disease. Apropos to natural phenomena, Rous, in 1942 ([35]), observed that, "Every one who deals with the phenomena of pathology soon comes to know that nature often speaks her secrets with a still, small voice out of a dense thicket of happenings. He who would hear and comprehend can have no pride of intellect, no fixed preconceptions; he can only listen intently and ask himself what he may have heard." This quotation was cited recently by Leader and Padgett ([16]) in recognition of the importance of animal models, and they further added that, "The discovery, recognition, and exploitation of an animal model to contribute ideally to human and animal welfare should accomplish the end of eliminating the disease in question." The potential for our understanding of leprosy has been greatly enhanced by two previous experiments of nature: indigenous leprosy in wild nine-banded armadillos in the southern United States ([4, 27, 42]), and naturally acquired leprosy in a chimpanzee captured in Sierra Leone ([17,18]). The results of the studies reported here justify the addition of the sooty mangabey monkey to the list of known animal species that have acquired leprosy in nature.

All of the clinical, histopathologic, immunologic, and bacteriologic findings accumulated in the study support the diagnosis of leprosy in this sooty mangabey monkey and the identification of the etiologic agent as *M. leprae*. These findings are summarized in Table 6. The currently accepted components of the criteria for the identification of candidate organisms as *M. leprae*, established at the IX International Leprosy Congress in 1968, are thus satisfied ([19,23]). Two important, additional, recently recognized criteria for the identification of this etiologic agent as *M. leprae* have also been satisfied, i.e., typical foamy structures in macrophages in lesions ([8, 9, 31]), and DNA homology.

The circumstances that led to the infection of this mangabey monkey in nature are not known. The known facts about the animal are: 1) the monkey was imported from Africa in 1975; 2) it was securely housed at Gulf South Research Institute in Louisiana; 3) it had never been experimentally inoculated with *M. leprae*; and 4) the first clinical signs of leprosy were observed in September 1979. The possibility that the animal acquired the disease in the United States is so remote that we are convinced the monkey was infected while in Africa, most likely by contact with a patient with active leprosy, or with fomites from such a patient. Exposure to leprosy could have occurred during the period of captivity while awaiting shipment, or the animal or its parents could have been pets in a village. Because of its tractable disposition, the sooty mangabey is reported to be a favorite pet of townspeople and inhabitants of villages, at least at Liberia ([38]). The partial resistance of the etiologic agent to dapsone suggests that the infection may have been acquired from a patient who had been under sulfone thera-

TABLE 6. *Features of disease in the mangabey monkey consistent with leprosy.*

A.	Clinical findings
	1. Lesions favor the cool areas of the host.
	2. Peripheral neuropathy.
	3. Response to antileprosy chemotherapy.
B.	Histopathologic findings
	1. Typical infiltration of histiocytes containing acid-fast bacilli.
	2. Invasion of dermal nerves by acid-fast bacilli.
	3. Acid-fast bacilli stain more consistently by the Fite-Faraco method than by the Ziehl-Neelsen method.
	4. Electron microscopic demonstration of macrophages with phagolysosomes that contain foamy structures composed of bacilli typical of *M. leprae* surrounded by spherical droplets.
C.	Immunologic response
	1. Clinical form of disease (lepromatous) is consistent with a negative lepromin reaction (Mitsuda).
	2. Antibodies to *M. leprae* antigens 2, 5, and 7 in serum.
D.	Studies on etiologic agent
	1. No mycobacterial growth on Löwenstein-Jensen or 7H10 media with repeated attempts.
	2. Acid-fastness is abolished by exposure to pyridine.
	3. Oxidizes D-DOPA.
	4. Elicits typical pattern of lepromin reactions in leprosy patients.
	5. Growth in the normal mouse foot pad is typical.
	6. Produces disseminated lesions of lepromatous leprosy in armadillos.
	7. DNA sequence is homologous to that of *M. leprae.*
	8. Intermediate dapsone resistance.

py, or who was infected with a primary dapsone-resistant strain of *M. leprae.*

Whether or not there is enzootic leprosy in sooty mangabey populations is unknown but is of great interest, especially because of the anecdotal evidence that leprosy can be a zoonotic disease ([20, 41]). Detection of disease in wild monkeys would require careful evaluation of the animals, preferably by histopathologic study and serologic testing for antimycobacterial antibodies. Animals with disseminated leprosy, especially those with deformities, probably would not survive long in the wild, and thus may go undetected in superficial surveys. The longevity of mangabey monkeys in captivity averages 20.5 years ([29]), but in nature longevity is probably much shorter.

Studies on the usefulness of the sooty mangabey monkey as a model for leprosy are in progress in our laboratories. Early findings suggest that the mangabey is regularly susceptible to leprosy when given large inocula of *M. leprae* ([25, 26]). This animal offers potential as a model for lepromatous leprosy because: 1) The animal is a primate. 2) The disease in the mangabey reproduced many features of the lesions of leprosy in humans. Peripheral neuropathic lesions are of particular interest. 3) The animal is large enough to provide sufficient repeated specimens of peripheral blood and other tissues in sufficient quantities for most studies. 4) Many reagents used for the study of immunologic parameters in humans readily crossreact with the mangabey. 5) The longevity in captivity of 20.5 years permits prolonged observations on the natural history of leprosy and on the effects of chemotherapy and immunotherapy.

The sooty mangabey breeds readily in captivity but had no demonstrated usefulness in medical research until now. Because of this lack of demand for utilization for research, sooty mangabey monkeys are currently in short supply in the United States. We believe, however, that a suitable supply will become available now that there is an awareness of the potential of this animal as a model for leprosy.

SUMMARY

Naturally acquired leprosy was detected in an otherwise normal "sooty" mangabey monkey (*Cercocebus atys*). This animal was imported from West Africa in 1975 and developed clinical symptoms of leprosy in 1979. Histopathologic findings were those of subpolar-lepromatous to borderline-lepromatous leprosy in the Ridley-Jopling classification. The disease was progressive, with crippling neuropathic deformities of

the hands and feet. The disease regressed under specific therapy. The etiologic agent was identified as *Mycobacterium leprae* by the following criteria: invasion of nerves of host, staining properties, electron microscopic findings, noncultivable on mycobacteriologic media, DOPA-oxidase positive, lepromin reactivity, infection patterns in mice and armadillos, sensitivity to sulfone, and DNA homology. We believe the animal acquired the disease from a patient with active leprosy. The mangabey monkey offers promise as a primate model for leprosy, and adds a third reported species to animals with naturally acquired leprosy.

RESUMEN

Se descubrió un caso de lepra adquirida de manera natural en un mono mangabey "sooty" (*Cercocebus atys*). Este animal fue importado del Africa Occidental en 1975. Los hallazgos histopatológicos fueron del tipo lepromatoso subpolar o lepromatoso intermedio, según la classificación de Ridley y Jopling. La enfermedad fue progresiva con alteraciones neuropáticas deformantes en pies y manos. La enfermedad pareció revertir siguiendo al tratamiento específico. El agente etiológico se identificó como *Mycobacterium leprae* en base a los siguientes criterios: invasión de los nervios del huésped, propiedades tintoriales, hallazgos al microscopio electrónico, incapacidad de crecer en medios micobacteriológicos, actividad positiva de DOPA-oxidasa, reactividad de lepromina, patrones de infección en ratones y armadillos, sensibilidad a las sulfonas, y homología del DNA. Pensamos que el animal adquirió la enfermedad de un paciente con lepra activa.

El mono mangabey promete ser un modelo primate para la lepra y se constituye en la tercer especie animal reportada que puede adquirir lepra por infección natural.

RÉSUMÉ

Une lèpre naturellement acquise a été décelée chez un singe mangabey (*Cercocebus atys*) qui par ailleurs paraissait normal. Cet animal avait été importé d'Afrique occidentale en 1935. Il a développé des symptômes cliniques de lèpre en 1979. Les observations histopathologiques ont révélé un type intermédiaire entre la lèpre lépromateuse intra-polaire et la lèpre lépromateuse-dimorphe, selon la classification de Ridley-Jopling. La maladie a présenté une évolution progressive, avec des difformités neurotrophiques entraînant des déformations des mains et des pieds. La maladie a bien répondu à la thérapeutique spécifique. L'agent étiologique a été identifié comme *Mycobacterium leprae,* sur la base des critères suivants: invasion des nerfs de l'animal atteint, propriétés de coloration, aspect à la microscopie électronique, absence de culture sur des milieux pour mycobactéries, réaction positive à la DOPA-oxydase, réactivité à la lépromine, transmission par inoculation à la souris et au tatou, sensibilité aux sulfones, et homologie de l'ADN. On pense dés lors que l'animal a acquis l'infection auprés d'un malade atteint de lèpre active. Le singe mangabey se révèle dès lors comme un modèle prometteur de la lèpre chez le primate. Une troisième espèce animale est donc ainsi identifiée comme étant susceptible d'acquérir naturellement la lèpre.

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REFERENCES

1. Bach, M.-A., Chatenoud, D., Wallach, F., Tuy, P. D. and Cottenot, F. Studies on T-cell subsets and functions in leprosy. Clin. Exp. Immunol. **44** (1981) 491–500.
2. Basten, A., McCaughan, G. W., Adams, E. and Callard, R. E. Human immunoregulation: A commentary. Immunology Today **3** (1982) 178–180.
3. Binford, C. H. The inoculation of human leprosy in the chimpanzee. Initiation of a long-term project. Int. J. Lepr. **33** (1965) 666–668.
4. Binford, C. H., Meyers, W. M., Walsh, G. P., Storrs, E. E. and Brown, H. L. Naturally acquired leprosy-like disease in the nine-banded armadillo (*Dasypus novemcinctus*). Histopathologic and microbiologic studies of tissues. J. Reticuloendothel. Soc. **22** (1977) 377–388.
5. Bullock, W. E. Immunobiology of leprosy. In: *Comprehensive Immunology. Immunology of Human Infection,* Part I. Good, R. A. and Day, S. B., eds. New York: Plenum Publishing Corp., 1981, p. 369.

6. Convit, J. and Pinardi, M. E. A simple method for the differentiation of *Mycobacterium leprae* from other mycobacteria through routine staining technics. Int. J. Lepr. **40** (1972) 130–132.
7. Fite, G. L., Cambre, P. J. and Turner, M. H. Procedure for demonstrating lepra bacilli in paraffin sections. Arch. Pathol. Lab. Med. **43** (1947) 624–625.
8. Fukunishi, Y., Meyers, W. M., Walsh, G. P., Johnson, F. B., Binford, C. H., Okada, S. and Nishiura, M. Ultrastructural features of macrophages of armadillos infected with actively multiplying *Mycobacterium leprae.* Int. J. Lepr. **52** (1984) 198–202.
9. Fukunishi, Y., Okada, S., Nishiura, M. and Kohsaka, K. Ultrastructural features of the multiplication of human and murine leprosy bacilli in macrophages of nude mice. Int. J. Lepr. **50** (1982) 68–75.
10. Gunders, A. E. Progressive experimental infection with *Mycobacterium leprae* in a chimpanzee. A preliminary report. J. Trop. Med. Hyg. **61** (1958) 228–230.
11. Harboe, M., Closs, O., Rees, R. J. W. and Walsh, G. P. Formation of antibody against *Mycobacterium leprae* antigen 7 in armadillos. J. Med. Microbiol. **11** (1978) 525–535.
12. Haynes, B. F., Dowell, B. L., Hensely, L. L., Gore, I. and Metzgar, R. S. Human T cell antigen expression by primate T cells. Science **215** (1982) 298–300.
13. Imaeda, T., Barksdale, L. and Kirchheimer, W. F. Deoxyribonucleic acid of *Mycobacterium lepraemurium*: Its genome size, base ratio, and homology with those of other mycobacteria. Int. J. Syst. Bacteriol. **32** (1982) 456–458.
14. Imaeda, T., Kirchheimer, W. F. and Barksdale, L. DNA isolated from *Mycobacterium leprae*: Genome size, base ratio, and homology with other related bacteria as determined by optical DNA-DNA reassociation. J. Bacteriol. **150** (1982) 414–417.
15. Kirchheimer, W. F. and Storrs, E. E. Attempts to establish the armadillo (*Dasypus novemcinctus* Linn.) as a model for the study of leprosy. I. Report of lepromatoid leprosy in an experimentally infected armadillo. Int. J. Lepr. **39** (1971) 693–702.
16. Leader, R. W. and Padgett. G. A. The genesis and validation of animal models. Am. J. Pathol. **101** (1980) 511–516.
17. Leininger, J. R., Donham, K. J. and Meyers, W. M. Leprosy in a chimpanzee: Postmortem lesions. Int. J. Lepr. **48** (1980) 414–421.
18. Leininger, J. R., Donham, K. J. and Rubino, M. J. Leprosy in a chimpanzee: Morphology of the skin lesions and characterization of the organism. Vet. Pathol. **15** (1978) 339–346.
19. Long, E. R. and Tepper, B. S. Workshop on cultivation of *M. leprae.* Int. J. Lepr. **36** (1968) 559.
20. Lumpkin, L. R., III, Cox, G. F. and Wolf, J. E., Jr. Leprosy in five armadillo handlers. J. Am. Acad. Dermatol. **9** (1983) 899–903.
21. Mancini, G., Carbonara, A. O. and Heremans, J. F. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry **2** (1965) 235–254.
22. Martin, L. N., Leslie, G. A. and Hindes, R. Lymphocyte surface IgD and IgM in non-human primates. Int. Arch. Allergy Appl. Immunol. **51** (1976) 320–329.
23. Meyers, W. M. and Binford, C. H. Identification of *Mycobacterium leprae.* (Letter to the Editor.) Arch. Pathol. Lab. Med. **100** (1976) 506.
24. Meyers, W. M., Kvernes, S. and Binford, C. H. Comparison of reactions to human and armadillo lepromins in leprosy. Int. J. Lepr. **43** (1975) 218–225.
25. Meyers, W. M., Walsh, G. P., Binford, C. H., Brown, H. L., Wolf, R. H., Gormus, B. J., Martin, L. N. and Gerone, P. J. Multibacillary leprosy in unaltered hosts, with emphasis on armadillos and monkeys. Abstract in Int. J. Lepr. **50** (1982) 584–585.
26. Meyers, W. M., Walsh, G. P., Brown, H. L., Binford, C. H., Gerone, P. J., Wolf, R. H., Gormus, B. J. and Martin, L. Leprosy in the mangabey monkey (*Cercocebus torquatus atys,* "sooty" mangabey). Abstract in Int. J. Lepr. **49** (1981) 500–502.
27. Meyers, W. M., Walsh, G. P., Brown, H. L., Rees, R. J. W. and Convit, J. Naturally acquired leprosy-like disease in the nine-banded armadillo (*Dasypus novemcinctus*). Reactions in leprosy patients to lepromins prepared from naturally infected armadillos. J. Reticuloendothel. Soc. **22** (1977) 369–376.
28. Modlin, R. L., Hofman, F. M., Taylor, C. R. and Rea, T. H. T lymphocyte subsets in the skin lesions of patients with leprosy. J. Am. Acad. Dermatol. **8** (1983) 182–189.
29. Napier, J. R. and Napier, P. H. *A Handbook of Living Primates.* New York: Academic Press, 1967, pp. 95–99.
30. Nath, I., Curtis, J., Sharma, A. K. and Talwar, G. P. Circulating T-cell numbers and their mitogenic potential in leprosy—correlation with mycobacterial load. Clin. Exp. Immunol. **29** (1977) 393–400.
31. Nishiura, M., Izumi, S., Mori, T., Takeo, K. and Nonaka, T. Freeze-etching study of human and murine leprosy bacilli. Int. J. Lepr. **45** (1977) 248–254.
32. Prabhakaran, K. A rapid identification test for *Mycobacterium leprae.* (Letter) Int. J. Lepr. **41** (1973) 121.
33. Ridley, D. S. Bacterial indices. In: *Leprosy in Theory and Practice.* Cochrane, R. G. and Davey, T. F., eds. 2nd ed. Bristol: John Wright & Sons, Ltd., 1964, p. 620.
34. Ridley, D. S. and Jopling, W. H. Classification

of leprosy according to immunity. A five-group system. Int. J. Lepr. **34** (1966) 255–273.

35. Rous, P. Inquiry into certain aspects of Eugene L. Opie. Arch. Pathol. **34** (1942) 1–6.
36. Shepard, C. C. The experimental disease that follows the injection of human leprosy bacilli into footpads of mice. J. Exp. Med. **112** (1960) 445–454.
37. Shepard, C. C. and McRae, D. H. A method for counting acid-fast bacteria. Int. J. Lepr. **36** (1968) 78–82.
38. Strong, R. P., ed. The African Republic of Liberia and the Belgian Congo. In: *The Harvard African Expedition 1926–1927*. Cambridge: Harvard University Press, Vol. II, 1930, p. 587.
39. Thorsby, E. and Bratlie, A. A rapid method for preparation of pure lymphocyte suspensions. In: *Histocompatibility Testing*. Terasaki, A. J., ed. Baltimore: Williams and Wilkins Co., 1970, p. 655.
40. Von Voorhis, W. C., Kaplan, G., Sarbo, E. N., Horwitz, M. A., Steinman, R. W., Levis, W. R., Nogueira, N., Hair, L. S., Gattass, C. R., Arrick, B. A. and Cohn, Z. A. The cutaneous infiltrates of leprosy. Cellular characteristics and the predominant T-cell phenotypes. N. Engl. J. Med. **307** (1982) 1593–1597.
41. Walsh, G. P., Meyers, W. M., Binford, C. H., Gerone, P. J., Wolf, R. H. and Leininger, J. R. Leprosy—a zoonosis. Lepr. Rev. **52** Suppl. (1981) 77–83.
42. Walsh, G. P., Storrs, E. E., Meyers, W. M. and Binford, C. H. Naturally acquired leprosy-like disease in the nine-banded armadillo (*Dasypus novemcinctus*). Recent epizootiologic findings. J. Reticuloendothel. Soc. **22** (1977) 363–368.
43. Waters, M. F. R., Bakri Bin H. J. Isa, M. D., Rees, R. J. W. and McDougall, A. C. Experimental lepromatous leprosy in the white-handed gibbon (*Hylobatus lar*): Successful inoculation with leprosy bacilli of human origin. Br. J. Exp. Pathol. **69** (1978) 551–557.