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Experimental Leprosy in the Mangabey (*Cercocebus atys*): Necropsy Findings[1]

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Spontaneously occurring and experimentally induced multibacillary leprosy have recently been described in the mangabey monkey (*Cercocebus atys*) ([4, 11, 13–15, 21]). Briefly, a female sooty mangabey imported from West Africa and housed at Gulf South Research Institute, New Iberia, Louisiana, U.S.A., was found to have skin lesions with typical histopathological features of multibacillary leprosy. This animal was transferred to the Delta Regional Primate Research Center (DRPRC), Covington, Louisiana, for further study. Acid-fast bacilli (AFB) from this animal met all available criteria for identifying *Mycobacterium leprae*. The disease continued to progress, and *M. leprae* collected from the cutaneous lesions were used to experimentally inoculate additional mangabeys, resulting in successful transmission of the disease. The spontaneously infected monkey was eventually placed on treatment and the disease is now clinically inactive.

Clinically, the spontaneous and experimental disease in mangabeys closely resembles lepromatous leprosy in human beings. The clinical findings in infected mangabeys include progressive nodular and diffuse thickening of the skin of the face, ears, and distal parts of the extremities. The cutaneous lesions often ulcerate. Nasal smears become positive for AFB, although buffy coat smears have been only rarely positive for AFB. Neurologic motor deficits develop in the intrinsic muscles of the hands and feet, with clawing of the fingers and toes and inversion of the feet. Skin biopsies reveal histologic changes similar to human leprosy at the lepromatous end of the disease spectrum according to the Ridley-Jopling classification for human leprosy ([18]). One of the experimentally infected mangabeys which had developed severe generalized lesions died 46 months after experimental infection and was necropsied. This provided the first opportunity for complete pathological evaluation of a mangabey with disseminated leprosy. The necropsy findings in this animal are the subject of this report.

MATERIALS AND METHODS

An adult male mangabey monkey was acquired from the Yerkes Regional Primate Research Center (YRPRC), Atlanta, Georgia, U.S.A., in January 1980. The animal was born at the YRPRC in September 1972, had no relevant clinical problems, and had numerous negative tuberculin tests. The monkey was individually caged in the isolation facility at the DRPRC and was well nourished and clinically normal.

The mangabey was sedated with ketamine HCl (10 mg/kg) for inoculation as well as for all other procedures (biopsies, blood samples, etc.). The inoculum was prepared by homogenization and differential centrifugation of cutaneous lepromas from the naturally infected mangabey. Bacilli were counted using standard methods ([19]). The monkey was inoculated on 20 March 1980 as follows: 1.2×10^9 AFB intravenously and 3×10^8 AFB intradermally at each of five sites (brow, lip, calf, and both ears) for a total inoculation of 2.7×10^9 AFB. Cutaneous nodules appeared at inoculation sites by four months post inoculation. By ten months post inoculation, there were small cutaneous nodules on the scrotum, an uninoculated site. The experimentally induced disease progressed and disseminated over

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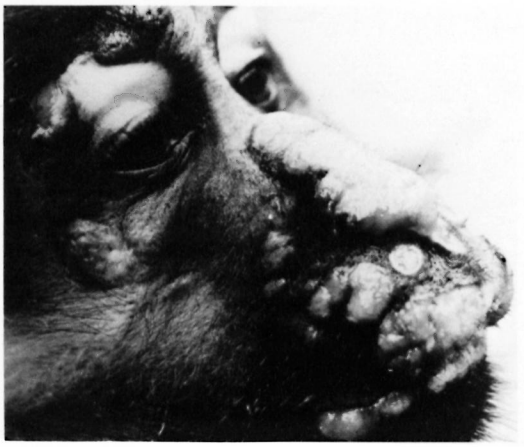

FIG. 1. Face of experimentally infected mangabey with lepromatous leprosy. Note nodular and diffuse dermal thickenings, many of which are ulcerated.

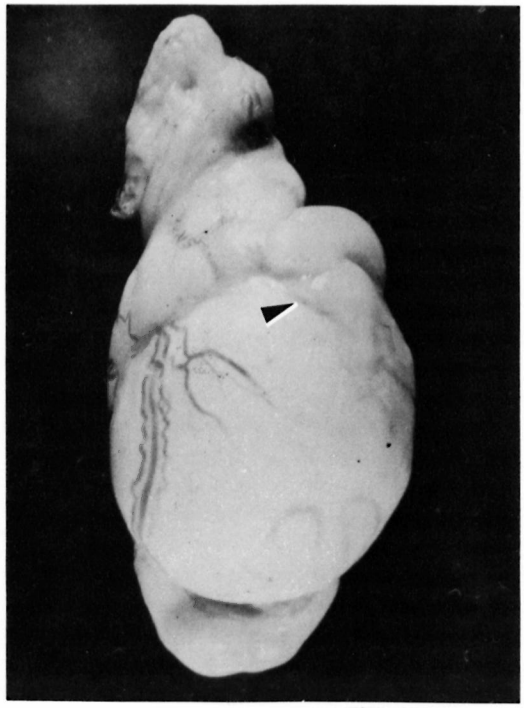

FIG. 2. Testicle of experimentally infected mangabey. Note nodules on tunica albuginea and around epididymis (arrowhead).

the next four years until the skin of the face, ears, scrotum, and distal extremities was extensively involved with nodular and diffuse thickenings, many of which became ulcerated. Peripheral nerves were not noticeably enlarged. Numerous biopsies from various sites indicated a disease histologically similar to human subpolar lepromatous leprosy (LLs) according to the Ridley-Jopling classification system. The animal had repeated episodes of anemia which responded to symptomatic treatment. In 1982 the monkey was lepromin tested using Mitsuda-Hayashi-Wade integral lepromin containing 1.6×10^8 AFB/ml and was lepromin negative. The animal became increasingly debilitated. There was profound anemia (hemoglobin 5.8 g %), probably primarily from extensive cutaneous ulceration and blood loss. The animal died 1 February 1984 (46 months after inoculation) following anesthesia given to collect biopsy specimens.

A complete necropsy was performed approximately 3 hours postmortem. Representative samples of all tissues were fixed in 10% neutral buffered formalin, processed, and embedded in paraffin for routine light microscopy. Sections were cut at 5 μ and stained with hematoxylin and eosin (H&E) and Fite-Faraco acid-fast stains.

RESULTS

Gross findings

Gross changes were limited to the skin, peripheral nerves, lymph nodes, and testicles. The animal had lost about 15% of its initial body weight and was thin, but not emaciated. The skin of the muzzle, including the upper and lower lips, was diffusely thickened. There were multiple confluent cutaneous nodules, most of which were ulcerated, covering the dorsum of the muzzle and the margins of the lips. There were a few scattered nodules measuring 0.5–2.0 cm in diameter elsewhere on the muzzle. The skin of the nares was thickened and ulcerated, causing partial obstruction of the nasal passages. There was nodular and diffuse thickening of the skin over the frontal crest, zygomatic arch, and beneath the eyes (Fig. 1). The pinnae were diffusely thickened and had multiple confluent ulcerated nodules along the margins. The skin of the neck, chest, abdomen, and back was unremarkable. There was no gynecomastia. The skin of the scrotum was diffusely thickened and had a few nodules of approximately 1 cm in diameter. The scrotal skin was extensively ulcerated, in part from recent biopsy incisions. The skin of the upper arms was unremarkable, but from the elbow distally,

the skin of both arms was irregularly thickened and contained multiple, often confluent and ulcerated nodular areas. The dorsum of the hands and fingers was infiltrated and ulcerated while the palmar surfaces were spared. The skin of the upper legs was unremarkable, but a few patchy areas below the knees were thickened. The skin of the dorsal and lateral surfaces of the feet and toes was thickened and contained numerous ulcerated nodules. The plantar surfaces of the feet were spared. The toenails were elongated and distorted. The skin of the tail was diffusely thickened and contained numerous nodular and ulcerated areas throughout its entire length.

The nasal mucosa was thickened and had several small areas of ulceration. The nasal passages were partially constricted and contained a mucoid exudate.

The testicles were of normal size and consistency but had multiple tan, smooth, firm nodules from 0.5–1.0 cm in diameter adherent to the surface of the tunica albuginea. The head and tail of the epididymis were engulfed in nodules (Fig. 2). A tan smooth infiltrate was present within the parenchyma of the peripheral area of each testicle. The central portions of the testicles were spared. The tunica albuginea was infiltrated in several areas.

The peripheral nerves of the right limbs were examined. The left limbs were preserved intact for other studies. Proceeding distally from near the head of the fibula, the right superficial peroneal nerve gradually enlarged to about three times its normal diameter (Fig. 3). The enlarged area of the nerve was slightly yellow. Other nerve trunks were normal.

The lymph nodes in the inguinal area and within the pelvic cavity were moderately enlarged and tan. Other lymph nodes were grossly unremarkable.

FIG. 3. Lower leg of experimentally infected mangabey. Note enlarged superficial peroneal nerve (arrowhead).

Histologic findings

Skin. The affected areas of the skin were ulcerated at many sites. Within the dermis there was a dense focal and diffuse inflammatory infiltrate. In some areas there was a well-defined subepidermal clear zone while in other areas the infiltrate reached the basal lamina. The infiltrate was composed predominantly of histiocytes which had a modest amount of finely vacuolated cytoplasm (Fig. 4). A few lymphocytes and occasional plasma cells and eosinophils were intermixed with histiocytes. Numerous neutrophils were adjacent to areas of ulceration. In less severely affected areas, the infiltrate was concentrated around dermal adnexae and neurovascular bundles. While some small dermal nerves were unremarkable, others were midly to severely infiltrated with lymphocytes and histiocytes and had a thickened perineurium (Fig. 5). The walls of some dermal arterioles were infiltrated with lymphocytes and the endothelium was hyperplastic. Within the toes the tissue between the nail bed and the underlying phalanx was completely infiltrated while the epithelium of the nail matrix was unremarkable. The Fite-Faraco acid-fast stain revealed numerous AFB, singly and in large masses, within dermal histiocytes. Some were beaded or fragmented, but a high percentage were solidly stained (Fig. 6). AFB were also present in dermal nerves (Fig. 7), the walls of dermal arterioles, and in the epithelium of hair follicles. A cutaneous leproma contained 9.6×10^9 *M. leprae*/g. The

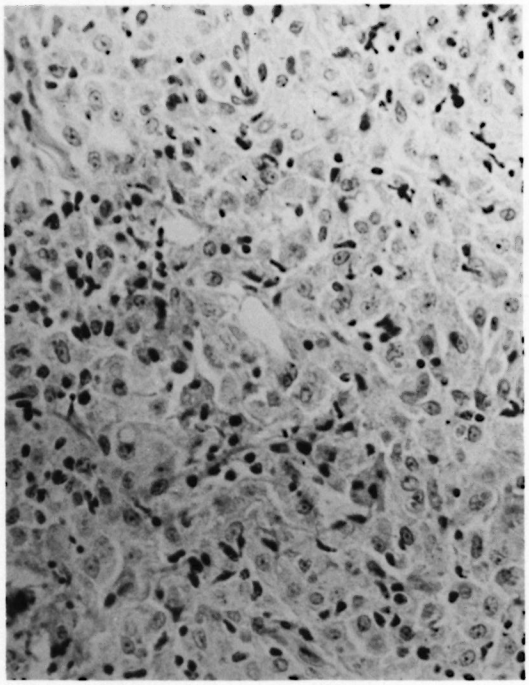

FIG. 4. Dermal infiltrate in experimentally infected mangabey. Infiltrate consists predominantly of histiocytes (H&E ×250).

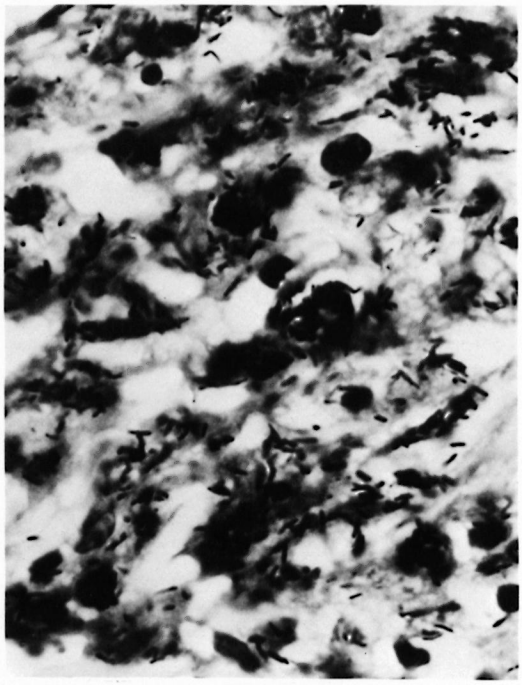

FIG. 6. Acid-fast bacilli within dermal infiltrate (Fite-Faraco ×1000).

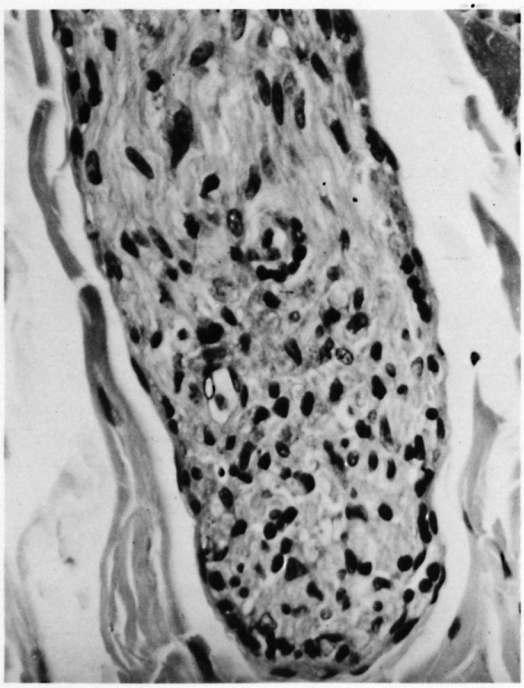

FIG. 5. Small dermal nerve containing inflammatory cells (H&E ×400).

clinically uninvolved skin from the chest, abdomen, axilla, and inguinal areas was unremarkable histologically.

Respiratory tract. The nasal mucous membrane was focally ulcerated and covered in some areas by a pseudomembrane consisting of proteinaceous material, neutrophils, and cellular debris. The intact mucosa was often hyperplastic with areas of squamous metaplasia. There was a dense lepromatous infiltrate in the nasal submucosa (Fig. 8). Many submucosal glands were displaced or destroyed by the infiltrate. Fite-Faraco staining revealed numerous AFB. The pharynx and tonsillar area, larynx, and trachea were unremarkable. Rare AFB were noted within the alveolar septae, presumably in endothelial cells.

Cardiovascular system. There were no changes related to leprosy in the cardiovascular system other than lesions of the small vessels described elsewhere.

Lympho-reticular system. The splenic white pulp was markedly atrophic, with only small remnants of lymphoid nodules re-

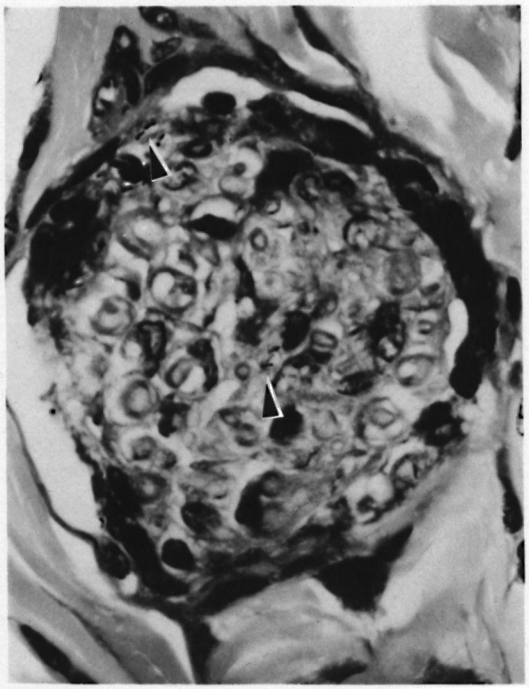

FIG. 7. Acid-fast bacilli (arrowheads) within small dermal nerve (Fite-Faraco ×630).

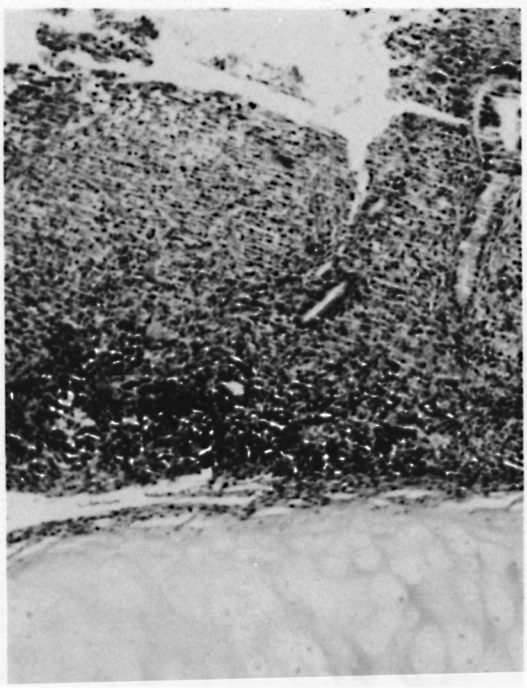

FIG. 8. Nasal mucosa with diffuse lepromatous infiltrate and ulceration. Nasal septum below (H&E ×100).

maining. There were a few small focal accumulations of macrophages near the lymphoid nodules. Many of the central arteries were thickened and hyalinized. The sinusoidal lining cells were slightly hypertrophic in some areas. Fite-Faraco stains revealed a few intracellular AFB in the red pulp. The spleen contained 8×10^6 *M. leprae* per g.

Peripheral and pelvic lymph nodes, as well as a bronchial node, contained numerous, slightly foamy histiocytes within the sinusoids (Fig. 9). Acid-fast stains revealed numerous AFB within the histiocytes. Peripheral lymph nodes contained $2.2–4.7 \times 10^9$ *M. leprae*/g. Mesenteric lymph nodes were unremarkable.

There was marked hyperplasia of all elements of the bone marrow accompanied by an absence of fat. Fite-Faraco staining of decalcified sections revealed a few AFB scattered within the marrow.

Liver. There were minimal periportal lymphoid infiltrates within the liver. Some Kupffer cells were slightly hypertrophic, and there were a few minute focal accumula-

FIG. 9. Lymph node with sinusoids filled with histiocytes (H&E ×160).

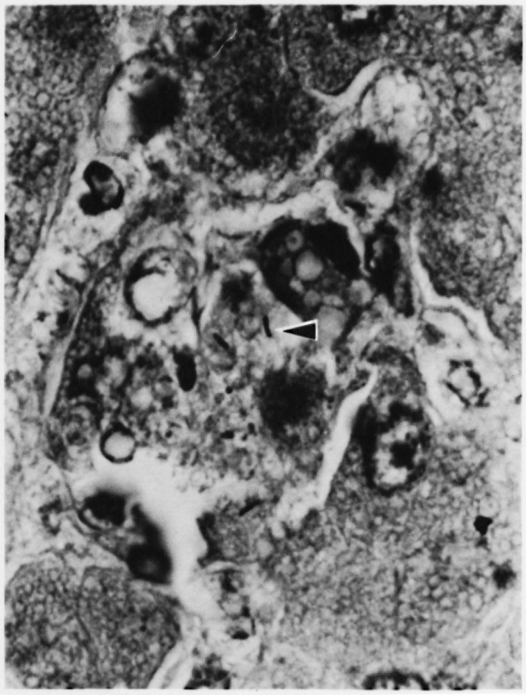

FIG. 10. Acid-fast bacilli (arrowhead) within microgranuloma in hepatic sinusoid (Fite-Faraco ×1000).

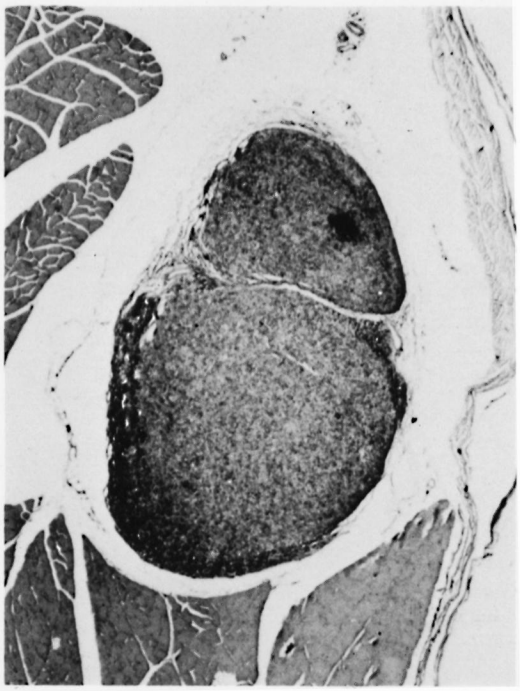

FIG. 11. Cross-section of superficial peroneal nerve which is completely replaced by lepromatous infiltrate (H&E ×20).

tions of histiocytes within the sinusoids. There were occasional AFB in Kupffer cells and in the focal macrophage accumulations (Fig. 10). The liver contained 6×10^6 *M. leprae*/g.

Urinary tract. Many glomeruli were mildly hypercellular and had an increased amount of mesangium. There was scant hyaline droplet change in a few cortical tubules. Rare AFB were seen in the capillary endothelium of interstitial areas.

Endocrine system. There was diffuse follicular atrophy of the thyroid. Other endocrine organs were unremarkable.

Gastrointestinal tract. There were no changes related to leprosy within the gastrointestinal tract.

Nervous system. The central nervous system was unremarkable. Peripheral nerve trunks were unremarkable except for the superficial peroneal and ulnar nerves. The superficial peroneal nerve at the site of thickening previously described was completely replaced by a dense lepromatous infiltrate similar to that described elsewhere (Figs. 11, 12). Numerous well-stained AFB were present within the histiocytes. The ulnar nerve had mild patchy infiltrates of lymphocytes and histiocytes within the nerve. Numerous AFB were also present within this nerve.

Reproductive tract. There were multiple, dense, nodular lepromatous infiltrates on the surface of the tunica albuginea. The tunica albuginea itself was focally infiltrated in many areas. The epididymis was surrounded by the infiltrate, but was otherwise unaffected. Much of the periphery of the testicle was replaced by a dense lepromatous infiltrate. The infiltrate was primarily in interstitial areas and surrounded many of the remaining seminiferous tubules (Fig. 13). Many seminiferous tubules were totally destroyed. Others were markedly atrophic with only Sertoli cells remaining, while unaffected tubules contained active spermatogenesis. The muscular walls of some testicular arteries were hypertrophied. Numerous AFB were present within histiocytes, within cells lining degenerating seminiferous tubules, and within the muscular wall of hypertrophied arteries.

The prostate was diffusely atrophic.

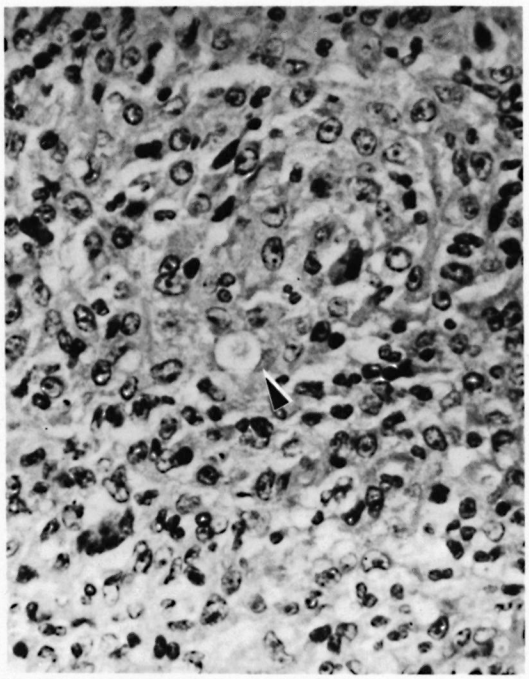

FIG. 12. Higher magnification of Figure 11. Note globus (arrowhead) within infiltrate (H&E ×400).

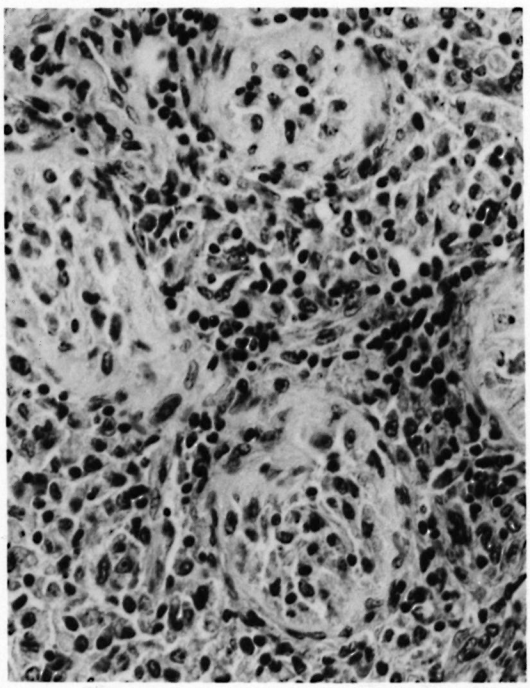

FIG. 13. Testicle. Note lepromatous infiltrate in interstitium and atrophic seminiferous tubules (H&E ×250).

Musculo-skeletal system. Sections of synovial membrane from the knee were unremarkable, as were sections of muscle and bone.

Eye. There was vascularization at the corneal limbus. The area was infiltrated with histiocytes which on Fite-Faraco stain were filled with AFB. Organisms were also seen in the endothelial cells lining the blood vessels and in Schwann cells of the corneal nerves.

DISCUSSION

The experimental attempts to infect nonhuman primates with *M. leprae* have been recently reviewed ([12]). Briefly, numerous attempts have been made by many investigators to infect various nonhuman primate species beginning in the late 1800s and continuing until now. Until recently, progressive experimental infection had been reported only in a chimpanzee ([6]) and a gibbon ([20]). The lesions in the chimpanzee were regressing when last observed at 14 months post inoculation. The gibbon had no clinical evidence of disease, although at necropsy there were early disseminated lepromatous infiltrations visible histologically. In addition, clinical and necropsy findings in a spontaneously infected chimpanzee with disseminated lepromatous leprosy have been described ([2, 9, 10]), as have clinical findings in a naturally infected mangabey ([14]). The lesions in the chimpanzee were similar to those of human lepromatous leprosy and similar to those in the mangabey. Successful experimental production of disseminated progressive lepromatous leprosy, clinically similar to that seen in humans, has recently been reported in mangabeys ([13, 21]).

The mangabey thus represents the first animal model of leprosy in which a form of leprosy closely resembling that in man can be readily produced experimentally.

The necropsy findings reported here further substantiate the mangabey as a suitable animal model for human lepromatous leprosy and represent the first description of necropsy findings in a primate host with clinical disease and a known history of infection. The autopsy findings in human leprosy have been well documented

([1, 3, 5, 7, 8, 16, 17]) and are quite similar to those seen in our mangabey. The selective involvement of the cooler parts of the skin, peripheral nerves, upper respiratory tract, peripheral lymph nodes, and testicles are all typical of what has been described in human lepromatous leprosy, as is the relative lack of involvement of internal organs. Lesions were not observed on the trunk, presumably due to the warmer temperature compared with the affected areas. The slight visceral involvement in the mangabey may be related to the higher temperature in these areas, and also to the relatively short duration of disease. Normal rectal temperature for a mangabey is approximately 38.3°C. The bacterial counts in the liver and spleen compared with those in peripheral lymph nodes and cutaneous leproma reflect the histological findings. The presence of numerous AFB in a bronchial lymph node is unusual and may also be temperature related, although the relative temperature of the peribronchial area is not known. The disease in the mangabey is unlike disseminated leprosy in the armadillo, where lesions are seen over the entire body and the liver and spleen have a bacterial load as large as subcutaneous tissues, due to the cooler body temperature (30–33°C). Unlike the results of most attempts to infect nonhuman primates with *M. leprae,* the disease produced in mangabeys is clinically and pathologically progressive. We did not find histologic evidence of any other infectious disease in this animal which would have predisposed it to progressive infection. The organism with which it was inoculated had been characterized as *M. leprae* by all available techniques. AFB collected at necropsy were noncultivable on mycobacterial media and their acid-fastness was extractable with pyridine. The cause of death was apparently anemia and debilitation secondary to extensive cutaneous ulcers, combined with the stress of general anesthesia. No more specific cause of death was apparent at necropsy.

The mangabey thus offers an animal model of leprosy which clinically and pathologically mimics human lepromatous leprosy very closely. As the model becomes better defined it should provide abundant opportunities to study the transmission, pathogenesis, immunology, reconstructive surgery, and therapy of lepromatous leprosy in a host closely related to man.

SUMMARY

A mangabey monkey (*Cercocebus atys*) was inoculated intravenously and intracutaneously with acid-fast bacilli (AFB) from a mangabey with spontaneously acquired leprosy. It developed generalized lepromatous leprosy and died 46 months after inoculation. Necropsy revealed severe lepromatous infiltrates in the skin, nasal mucosa, peripheral nerves, and testicles. Internal organs were only minimally involved. The lesions seen at necropsy were very similar to those seen in untreated cases of human lepromatous leprosy. These findings further substantiate the mangabey monkey as a suitable animal model for the study of lepromatous leprosy.

RESUMEN

Se inoculó un mono mangabey (*Cercocebus atys*), intravenosae intracutáneamente, con bacilos ácido-resistentes aislados de otro mono mangabey con lepra adquirida espontáneamente. El mono desarrolló lepra generalizada y murió 46 meses después de la inoculación. La necropsia reveló severos infiltrados lepromatosos en la piel, en la mucosa nasal, en los nervios periféricos y en los testículos. Los órganos internos sólo se afectaron en grado mínimo. Las lesiones encontradas en las necropsias fueron muy similares a las observadas en los casos humanos de lepra lepromatosa sin tratamiento. Los hallazgos refuerzan la utilidad de los monos mangabey como modelos animales para el estudio de la lepra lepromatosa.

RÉSUMÉ

Un singe mangabey (*Cercocebus atys*) a été inoculé par voie intraveineuse et par voie intracutanée avec des bacilles acido-résistants recueillis chez un singe mangabey qui avait spontanément contracté la lèpre. Le singe inoculé a développé une lèpre lépromateuse généralisée. Il est mort 46 mois après l'inoculation. L'autopsie a permis de mettre en évidence des infiltrats lépromateux prononcés dans la peau, la muqueuse nasale, les nerfs périphériques, et les testicules. Les organes internes n'étaient que fort peu atteints. Les lésions observées à l'autopsie étaient fort semblables à celles que l'on constate dans des cas de lèpre humaine lépromateuse non traitée. Ces observations confirment, s'il le failait, que le singe mangabey constitue un modèle animal approprié pour l'étude de la lèpre lépromateuse.

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