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EDITORIALS

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The Search for Animal Models of Leprosy*

When Hansen observed "rod-shaped bodies" of *Mycobacterium leprae* on 20 February 1873 in Bergen, Norway, leprosy became one of the first diseases to be linked with a microbial pathogen. Notwithstanding the 114 years we have known of *M. leprae,* we have amassed surprisingly little knowledge of it. The reason for this is largely attributable to the organism: it refuses to be cultured *in vitro,* causes markedly variant symptoms in humans, and has only rarely been transmitted to experimental animals.

The long search for an animal model for leprosy has involved almost 30 species of animals, and almost as many protocols as researchers. Following is an extensive, but essentially noncritical, review of the animal model research through 1986.

Although Jeanselme[1] states that Hansen attempted to transmit leprosy to monkeys, cats, and rabbits, recent evidence discloses that 12 rabbits only were inoculated, and there were negative results (personal communication, 10 January 1973 between Drs. E. Waaler and C. H. Binford). In 1879 Hansen injected leprous material into a woman suffering from a less severe form of the disease. She was not harmed, but because he had done so without her permission, he was removed from his hospital position in Bergen.[2]

In 1881, Neisser inoculated 24 rabbits and two dogs with leprous nodules, but observed only a local response around inoculation sites.[3]

Kobner (1882) described numerous attempts to inoculate a Java monkey. Injections were made subcutaneously in the back, in both ears and eyelids, and the lower lip. There were lesions in the internal organs at autopsy. Kobner also inoculated a guinea pig, white rats, rabbits, pigeons, eels, loaches and frogs, with only minimal results in the eels and the loaches.[4]

In 1883, Damsch inserted leprous tissue into the anterior eye chamber of two rabbits and into the abdominal wall of two cats. The rabbits died 139 and 219 days later,

* The opinions or assertions contained herein are the private ones of the author and are not to be construed as official or reflecting the views of the U.S. Department of Defense or the Uniformed Services University of the Health Sciences.

[1] Jeanselme, E. *La Lèpre.* Paris: G. Dobin & Co., 1934, pp. 142–154.

[2] Vogelsang, T. M. The Hansen-Neisser controversy, 1879–1880. Int. J. Lepr. **31** (1963) 74–79.

[3] Neisser, S. Weitere Beiträge zur Aetiologie der Lepra. Virchows. Arch. **84** (1881) 314, in Bayon (n. 31) p. 207.

[4] Kobner, H., in Jeanselme (n. 1) pp. 142–143.

and showed a slight multiplication of acid-fast bacilli. The cats had numerous bacilli surrounding the implanted nodule after 3 months.[5]

Vossius, in 1888, confirmed Damsch's work on rabbits and pigs,[6] as did Maucione in 1924.[7]

In 1885, Arning inoculated rabbits, pigs, rats and pigeons, without success.[8]

Between 1885 and 1886, Melcher and Ortmann inoculated four rabbits using Damsch's methods on cats. They observed dissemination of acid-fast bacilli to spleen, liver, cecum, pleura and pericardium.[9] In 1887 Wesener corroborated their findings in eight rabbits. In two of the animals he found nodules in the lungs, pleura, omentum, liver, lymphatics, spleen, kidneys, cecum and peritoneum. Further, in the eyes there were lesions containing acid-fast organisms. He concluded, however, that the nodules were tuberculous and the ocular lesions were tissue responses to dead bacilli.[10] Barannikow corroborated these results 14 years later in a single rabbit.[11]

In 1893, Tedeschi attempted to transmit the bacillus to a nonhuman primate by inoculating leproma into the dura of a monkey; however, the animal died 8 days later.[12] Also in 1893, Wnoukow found localized lesions considered tuberculous in 14 of 20 rabbits inoculated intraocularly, subcutaneously, or intraperitoneally.[13]

In Bombay, in 1897, Sticker infected six monkeys, attempting to prove that the organism invaded the body via the nasal mucosa. No results were described, but the animals were observed for only 4–5 weeks.[14]

Iwanow inoculated guinea pigs in 1902, with a single instance of internal lesions.[15] Previous work by Klitten[16] and Clegg[17] had generated local lesions.

In 1905, Thiroux inoculated five rabbits, but determined that the resulting lesions and nodules were tuberculous. In this regard, he believed he confirmed previous research: "The attempts of inoculations of animals by Melcher and Ortmann, Damsch, Tedeschi, and a number of other bacteriologists, have all arrived at the result: 'Tuberculosis' . . . . There exists, therefore, a cutaneous tuberculosis which bears a great resemblance to leprosy, and in countries endemic to leprosy, a lesion of the face is difficult to distinguish from leprosy."[18]

Nicolle, in 1906, described work with macaque monkeys. This species was chosen because of previous successes in reproducing other "illnesses long regarded as unique to mankind," including syphilis. He inoculated two monkeys, each in five locations, and although 4 days later the animals had recovered, in 2 months nodules had formed about the point of inoculation. Nicolle biopsied one of the larger nodules and observed small amounts of leprous material. In his opinion, this lack of a large increase in bacilli "constitutes the only noticeable difference between the structure of the leprosy in our monkey and that of human leprosy. Conceivably this is due to a difference in age of the lesions (the monkey's nodule was only fourteen days old at the time of my examination)."[19] In all probability, the response was akin to a lepromin reaction.

In 1906, Babes and Kalinder subcutaneously inoculated a macaque monkey. A month later a nodule developed at the point of inoculation, with lymphatic involvement. Three months later the animal died,

[5] Damsch. Übertragungsversuche von Lepra auf Thiere. Virchows. Arch. **92** (1883), in Bayon (n. 31) p. 208.

[6] Vossius. Z. Vergleichende Augenheilkd. **6** (1888) 1, in Bayon (n. 31) p. 208.

[7] Maucione, L. Risultati delle inoculazioni di frammenti e di emulsioni di lepromi umani nella camera anteriore e nella corneadel coniglio. Arch. Ottal. **31** (1924) 385–408, in McKinley (n. 131) p. 229.

[8] Arning, E., in Jeanselme (n. 1) p. 143.

[9] Melcher and Ortmann. Übertragung von Lepra auf Kaninchen. Berl. Klin. Wochensch. **13** (1885) 293, in Bayon (n. 31) p. 208.

[10] Wesener. Zur Frage der Lepraübertragung auf Thiere. Zentralbl. Bakteriol. **3** (1888) 482, in Bayon (n. 31) p. 208.

[11] Barannikow, in Bayon (n. 31) p. 208.

[12] Tedeschi. Zentralbl. Bakteriol. **14** (1893) 113, in Bayon (n. 31) p. 208.

[13] Wnoukow. Inaug. thesis Kasan (Russian). Zentralbl. Bakteriol. **12** (1893), in Bayon (n. 31) p. 208.

[14] Sticker, in Jeanselme (n. 1) p. 143.

[15] Iwanow, W.-W. Sur le sort des bacilles de la lèpre dans l'organisms des animaux (cobayes). Ann. Inst. Pasteur **10** (1902), in Bayon (n. 31) p. 208.

[16] Klitten, in Soule and McKinley (n. 37) p. 6.

[17] Clegg, M. T., in Soule and McKinley (n. 37) p. 6.

[18] Thiroux. Quelques tentatives d'inoculation de la lèpre. Ann. Hyg. Med. Colon. **8** (1905) 148.

[19] Nicolle, C. Arch. Inst. Pasteur (Tunis) **1** (1906) 45–47.

but the authors did not describe bacillary multiplication.[20]

Marchoux and Bourret (1907) surgically implanted a small piece of leprous tissue behind the left ear of a chimpanzee. When the animal died 96 days later, the existing nodule was the size of a split pea. Histopathologically, the human tissue was surrounded by two layers—an inflammatory one with mononuclear leukocytes containing acid-fast bacilli, and a fibrous layer with some bacillary clumps but no bacillary invasion.[21, 22]

One of the most careful and best described early studies was performed by Stanziale in 1909. Thirty-one rabbits were inoculated in the eye, of which eight showed leprous lesions, sometimes as early as hours later. Negative results were obtained by grafting leprous material into the cornea or by injection into the abdomen. There was no mention of dissemination.[23] Also in 1909, Sugai injected Japanese dancing mice intraperitoneally. There were granulomatous lesions containing acid-fast organisms in the peritoneum and bronchial glands.[24] Passage of these tissues were unsuccessful. In the same year Kitasato published a brief paper with few details on transmission to an orangutan's cornea.[25]

In 1911, Duval and Gurd reported their studies with Japanese dancing mice. They proposed that, since they had "succeeded infecting mice by rubbing cultures into the nares after gentle scarification of the mucous membrane . . . the chief portal of entrance of *M. leprae* to the human body is by way of the nasopharynx."[26] However, the description of successful cultivation of the bacillus on a variety of media renders their results tenuous in hindsight.

In the same year, Couret used Duval's cultured strain to inoculate tadpoles, frogs, turtles, snakes, goldfish, and assorted saltwater fish. In no instance was there any evidence of generalized transmission.[27] Serra confirmed transmission of the bacillus to the anterior eye chamber of rabbits in this year.[28]

Nicolle and Blaizot published articles in 1910 and 1911, continuing the former's earlier work with macaques. In the afterword to their 1910 paper they sounded hopeful as to their technique: "These experiences show that one can obtain in inferior monkeys the reproduction, at the point of inoculation, of lesions resembling human leprosy. The avenue we will take using repeated virulent inoculations may permit the realization in these animals of a general infection of human leprosy."[29] By the next year their enthusiasm had faded: "We had hoped, by the repetition of virulent inoculations, to obtain a better result and perhaps a generalized leprosy. It hasn't happened. A chimpanzee, inoculated in the same fashion, has responded exactly like the macaques . . . it is possible that, if in man the same methods of inoculation produce the same negative results—and this is a strong possibility—leprosy is not contracted by external contact."[30]

Bayon experimented in 1912–1913 on both rats and rabbits. Of four rats injected, two had cutaneous nodules near the site of inoculation, but there were no lesions in internal organs. Of 24 rabbits inoculated intraocularly, 20 survived 5 months, and all developed significant corneal lesions. In discussing these the author stated: "I consider the results of Melcher and Ortmann, Wesener, Wnoukow, and possibly Stanziale (to transmit leprosy) to have been successful.

[20] Babes, V. and Kalinder, in Jeanselme (n. 1) p. 144.

[21] Marchoux, E. and Bourret, G. Essai d'inoculation de la lèpre au chimpanze. Bull. Soc. Pathol. Exot. **1** (1908) 416.

[22] Marchoux, E. and Bourret, G. Recherches sur la transmission de la lèpre. Ann. Inst. Pasteur **23** (1909) 513.

[23] Stanziale, R. Inoculazioni di materiale leproso nella camera anteriore dei conigli. G. Ital. Mal. Ven. **5** (1910) 1, in Bayon (n. 31) pp. 208–209.

[24] Sugai, T. Nachtrag zu gelungenen Übertragungsversuchen mit Lepra bei Säugetieren. Lepro **8** (1909) 203, in Bayon (n. 31) p. 208.

[25] Kitasato. Die Lepra in Japan. Z. Hyg. **63** (1909) 507, in Bayon (n. 31) p. 208.

[26] Duval, C. W. and Gurd, F. B. Studies on the biology of and immunity against the bacillus of leprosy. Arch. Int. Med. **7** (1911) 230.

[27] Couret, M. The behavior of *Bacillus leprae* in cold-blooded animals. J. Exp. Med. **8** (1911) 576–588.

[28] Serra, A. Inoculation de culture du bacille de Hansen dans l'oeil du lapin. Lepro **12** (1911) 1–14.

[29] Nicolle, C. and Blaizot, L. Reproduction expérimentale de la lèpre chez les singes inférieurs. C. R. Soc. Biol. **69** (1910) 231–233.

[30] Nicolle, C. and Blaizot, L. Essais de reproduction de la lèpre chez le chimpanze et les singes inférieurs. C. R. Soc. Biol. **70** (1911) 991–993.

Spontaneous tuberculosis in laboratory animals is extremely rare." Despite the inability to develop generalized infections in laboratory animals up to this time, Bayon's work is marked by a hopeful philosophy which would permeate the next 70 years' research: "I should like to observe that the apparent non-success in transmitting leprosy to animals does not, in any way, prove that this feat cannot be achieved. The time is well within the memory of the great majority of medical men . . . when similar views were held regarding syphilis. During my student days I had the intention of trying to inoculate animals with syphilis, and was sternly reproved by my teacher for attempting such useless experiments. Should this meet his eye, and he still remembers his caustic remarks, he may muse on what he may have made me miss."[31]

In discussing his work with a monkey (*Cercopithecus*) in 1914, Verotti noted a subcutaneous nodule at the site of inoculation in the left arm; the animal had also been given an intercardiac inoculation. Two weeks from the appearance of the first nodule, another appeared on the right wrist and contained acid-fast bacilli.[32]

In 1919, Bradley made two intramuscular implantations in the right buttock and one subcutaneously in the right breast of a macaque. The animal died unexpectedly 2 months later, and there were bacilli in lymph nodes in both axilla, in the left inguinal region, and in splenic connective tissue. Besides these and the localized lesions around the area of inoculation, there were no bacilli in the spleen, liver, or kidneys.[33]

In 1924, Limousin injected a cell-free suspension of nasal mucus from patients with leprosy into the anterior chamber of the eye of an albino rabbit at 6-week intervals. After waiting 22 months the animal was sacrificed, and dissemination to internal organs was observed. The author credited his success to the reinfection and the long waiting period.[34]

In 1925, human subjects were again inoculated intradermally by Mariani. Both virulent and avirulent material was used, but with no evident lesions.[35]

The following year Reenstierna inoculated three macaques and four rhesus monkeys. Lesions began forming around the inoculated area after 37–45 days, and persisted for another 40–62 days. One cutaneous nodule remained 7 months post-inoculation, and some animals were reinoculated, but the author could show no evidence of internal organ involvement.[36] Soule and McKinley observed the same results in seven rhesus and five *Cebus olivaceus* monkeys in 1932.[37] Again, these were probably lepromin (Mitsuda) reactions.

In 1927, Roffo, in experiments on various African monkeys, produced injuries "considered to be a localized infection of experimental leprosy," but observed no generalized reaction. Of note, *Erythrocebus pata* did not exhibit a localized response.[38]

In the next year, Naar used a control injection of normal skin to compare with leprous insertions into the anterior eye compartment of rabbits. While the experimental animals developed localized granulomas, the early inflammation in the control animals subsided rapidly.[39]

In 1930 Franchini described a peculiar incident in his research using macaques. A monkey had been inoculated in the skin of the right eyebrow. After the nodule had regressed, reformed, regressed, and reformed again, the monkey's health failed, with hindlimb paralysis developing 39 months post-inoculation. Autopsy showed no general-

[31] Bayon, H. The transmission of leprosy to animals by inoculation of the human "virus." S. Afr. Med. Rec. 11 (1913) 207–211.

[32] Verotti, G. Atti. Accad. Med. Chir. (Naples) **67** (1914) 175–185, in Martin, *et al.* (n. 127) p. 209.

[33] Bradley, B. Preliminary note on the apparent transmission of leprosy to a macaque monkey. Med. J. Aust. **2** (1919) 414–416.

[34] Limousin, H. Inoculation de la lèpre humaine au lapin. C. R. Séances Acad. Sci. **178** (1924) 599–600.

[35] Mariana, G. Nouve osservazioni sulle reazioni provocate speriment almente con materiale lebbroso nell'uomo. G. Ital. Dermatol. Sif. **66** (1925) 402–426, in McKinley (n. 131) p. 299.

[36] Reenstierna, J. Reproduction expérimentale de la lèpre chez les singes inférieurs. Ann. Inst. Pasteur **40** (1926) 78–88.

[37] Soule, M. H. and McKinley, E. B. Cultivation of *B. leprae* in monkeys. Am. J. Trop. Med. Hyg. **12** (1932) 1–36.

[38] Roffo, A. H. Sobre la transmission de la lepra a los monos inferiores. Bol. Inst. Med. Exp. **4** (1927) 64–91.

[39] Naar, in Jeanselme (n. 1) p. 142.

ization of infection and provided no other reason for the decline in health.[40] Also in 1930, Schöbl, Pineda and Miyao used repeated intradermal inoculations in an attempt to allow the infection to establish itself in monkeys. This method was based on previous successes in transmitting yaws via superinfection and reinfection. The researchers described the "allergic stage of tissue reactivity" in one of the animals, wherein an ulcer developed around the site of inoculation and then healed. Further inoculations caused progressively smaller local reactions. There was no investigation of the internal organs.[41]

Tanimura and Sakurane (1930) inserted nodules into the brain and kidneys of rabbits. Despite the usual localized reactions and healing, they found the lesions persisted longer in the brain than in the kidneys, but elicited a larger inflammatory reaction in the kidney.[42]

The Surgeon General of the United States mentioned ongoing leprosy research in his annual report for 1931. Intravascular injections of white rats caused lesions whose "histology . . . simulated that found in human leprosy." Further, subcutaneous injection into kittens produced granulomas, but observations were confined to the 21 days post-inoculation period. The third research approach was inoculation of rats by "dropping into the nose, without injury to the mucosa, a suspension of either of the organisms of human or rat leprosy." Dissemination was found to the cervical lymph node, lungs and spleen. Specifically, in 15 of 23 mice, bacilli were found in the cervical or mesenteric nodes as quickly as 17–19 hours post-inoculation.[43] Unfortunately, it is not stated whether the lesions were due to *M. leprae* or *M. lepraemurium*.

In the same year de Souza-Araujo described intraperitoneal injections in white rats, white mice, guinea pigs, and monkeys, carried out over the previous 3 years. His results were considered to be superior to Sugai's, with acid-fast bacilli in the organs or peritoneal fluids of the mice (92%), rats (67%), and guinea pigs (85%). Of note, he made extensive use of material rendered avirulent by various methods: ethyl alcohol, 10% Formalin, or boiling for an hour. In the latter two cases, the results were not widely disparate from that with live bacilli.[44]

In 1932, Cantacuzene and Longhin described dissemination to lymph nodes and omentum of white rats which died 5–6 months post-treatment. Inoculation was by intraperitoneal injection of leprous emulsion, 1–2 days after intraperitoneal injection of disodium phosphate and calcium chloride. The "precipitate of phosphate and calcium . . . affixed itself rapidly on the omentum," presumably aiding infection by the bacilli.[45] The authors also spoke of the role of an "ultra-virus" in these transmission studies, pointing to earlier work wherein the filtrate of a lepromatous emulsion had caused bacillary multiplication and lymphadenopathy.[46] In the same year, Ota and Sato inoculated white rats, guinea pigs, and rabbits. The most interesting finding was of a single rat which developed a sterile abscess 6 months after a subdermal inoculation in the back. The rat had been on a vitamin B-deficient diet. Unfortunately, upon its death it was "devoured by one of its companions in the cage, and was unable to be studied." After a study of 12 sets of visceral or cutaneous lesions elicited in other rats, the authors concluded that leprosy was transmissible.[47]

In 1936, Sellards and Pinkerton found no significant lesions in mice or two monkeys as late as 2 years after intracranial inocu-

[40] Franchini, G. Arch. Ital. Sci. Med. Colon. **2** (1930) 1–4, in Martin, *et al.* (n. 127) p. 209.

[41] Schöbl, O., Pineda, E. V. and Miyao, I. Clinical skin lesions in Philippine monkeys resulting from experimental inoculation with human leprous material. Phillip. J. Sci. **41** (1930) 233–243.

[42] Tanimura and Sakurane, in Jeanselme (n. 1) p. 151.

[43] Surgeon General, U.S. *Annual Report of the Surgeon General of the Public Health Service of the United States for the Fiscal Year 1931.* Washington, D.C.: Government Printing Office, 1900, pp. 32–36.

[44] de Souza-Araujo, H. C. Experimental leprosy. Trans. R. Soc. Trop. Med. Hyg. **24** (1931) 577–598.

[45] Cantacuzene, J. and Longhin, S. Transmission expérimentale de la lèpre humaine au rat blanc. C. R. Séances Acad. Sci. **195** (1932) 533–535.

[46] Cantacuzene, J. and Longhin, S. De l'existence d'un ultra-virus chez le bacille de la lèpre humaine. C. R. Soc. Biol. **109** (1932) 107.

[47] Ota, M. and Sato, S. Reproduction de la lèpre chez les animaux par l'inoculation de cultures du *Mycobacterium leprae.* C. R. Soc. Biol. **109** (1932) 29–32.

lations.[48] Shiga, in the same year, described success in intracerebral inoculation of a single white mouse out of over a thousand inoculated. "Success" involved only granuloma formation in the liver and spleen and acid-fast bacilli in the cerebral capillaries and reticuloendothelial system. Splenectomy, thyroidectomy, and low-vitamin diets were investigated in attempts to increase the animals' susceptibility; but none gave consistent evidence of increased bacillary multiplication.[49] Attempts to enhance infection by lowering the host immune response was to become a new school of thought and research in years to follow.

In 1937, Adler described research using splenectomized Syrian (golden) hamsters. A leprous nodule was surgically implanted subcutaneously, followed by an intraperitoneal injection. Within 6 weeks, in addition to localized multiplication, there were bacilli in a liver smear of one animal and in the inguinal lymphatics of another.[50]

In 1938, Burnet showed lymphatic bacillary spread in a splenectomized hamster following subcutaneous implantation. Thirteen other hamsters and six other assorted rodents showed no reaction. Nevertheless, in his conclusion he stated: "The hamster is receptive to human leprosy. A perfected technique can be developed to make this reception more constant. It is a decisive first step in the experimental reproduction of leprosy in a laboratory animal."[51] His results could not be confirmed by Dubois and Gavrilof in 1940.[52] In the same year, Dharmendra and Lowe were unable to confirm either Adler's or Burnet's findings, using intraperitoneal injections and surgical implantations in 23 hamsters. Dharmendra and Lowe made a most important observation in explaining the discrepancy in these observations: "Dead bacilli have extraordinary powers of persistence in tissues of the living animal . . . . We ourselves have found slight lesions and many bacilli in rats examined one year or more after being inoculated with human leprosy bacilli killed by heat . . . . In interpreting results of animal inoculation care must be exercised in order to avoid the mistake of taking the presence of the lesions and bacilli as evidence of a progressive infection. We have, therefore, examined our results critically with this fallacy in mind."[53]

In 1939, Cochrane, *et al.* continued this line of research by following splenectomy of monkeys with the attachment of a leprous nodule to the splenic stump. While recommending surgical procedures, the authors were quite frank that individual variance of resistance could be a major determinant of the amount of infection produced, and that different animals would react uniquely.[54] Five years later, Dharmendra and Mukherji varied this procedure by inoculating splenectomized monkeys intraperitoneally, but confirmed the previous conclusion in that no acid-fast bacilli were found on autopsy.[55]

In 1939, Nojima delivered three subcutaneous injections of bacillary suspension to mice, following three injections of human placenta. Organ involvement was noticed as early as 2 months post-inoculation, including liver, spleen, adrenals, testes, epididymis, and kidney.[56] In the same year Suzuki implanted leprous material into mice on a diet of buckwheat grits and raw potato. If the animal survived 77 days it was reinoculated. There was no evidence of transmission.[57] In the same year, Yamamoto in-

[48] Sellards, M. A. W. and Pinkerton, H. Résumé d'expériences sur la propagation de la lèpre murine et humaine à des animaux considéres comme réfractaires. Bull. Soc. Pathol. Exot. **29** (1936) 847–851.

[49] Shiga, K. Intracerebral infection with lepra bacillus. Kitasato Arch. Exp. Med. **13** (1936) 1–8.

[50] Adler, S. Inoculation of human leprosy into Syrian hamster. Lancet **1** (1937) 714–715.

[51] Burnet, E. Inoculation positive de la lèpre humaine au hamster; inoculation négative à divers autres rongeurs. Arch. Inst. Pasteur (Tunis) **27** (1938) 327.

[52] DuBois, A. and Gavrilof, W. Essais d'inoculation de la lèpre humaine au hamster non splénectomise. Arch. Inst. Pasteur (Tunis) **29** (1940) 170–173.

[53] Dharmendra and Lowe, J. Attempts at transmission of human leprosy to Syrian hamsters. Indian J. Med. Res. **28** (1940) 61–69.

[54] Cochrane, R. G., Pandit, C. G. and Menon, K. P. A preliminary note on inoculation of monkeys with human leprosy material after splenectomy. Int. J. Lepr. **7** (1939) 377–381.

[55] Dharmendra and Mukherji, N. Attempts to transmit human leprosy to splenectomized monkeys. Indian J. Med. Res. **32** (1944) 197–200.

[56] Nojima, T. Lepro **10** Suppl. (1939) 67, in Mukherjee (n. 132) p. 81.

[57] Suzuki, R. Tohoku J. Exp. Med. **36** (1939) 146, in Mukherjee (n. 132).

jected mice repeatedly in the back, but detected only chronic inflammatory changes in the lungs, with bacilli demonstrable only occasionally.[58] Also in 1939, Mitsuda published the results of intratesticular inoculation of small pigs. There was only a local response.[59]

Ota in 1939,[60] and Ota and Sato in 1940,[61] published results of inoculations into the chest muscles of fowls. Of 100 animals, about one half developed local granulomas at 3 months which persisted for 6 months to a year. Very few bacilli could be found. In 1939, Burnet and Jadford fed a golden hamster leprous human liver for 12 days. The animal died 9 months later, and the lung, liver, and lymph nodes were involved.[62]

In 1939–1940, Oberdoerffer[63] and Collier[64, 65] fed monkeys sapotoxin-containing plants in an attempt to depress adrenocortical activity, thereby lowering immunity. In four monkeys in the first study, there were persistent nodules near the site of inoculation, with 20–30 bacilli in nasal smears 9 months post-inoculation. In the second study, of over 30 monkeys treated, variable symptoms "similar to those seen in leprosy in humans" were observed, and these included thickening of the ulnar nerve in one monkey. These claims could not be substantiated by Cochrane in 1947.[66]

In 1940 as well, Nonaka attempted transmission to chickens by inoculation in the chest muscles. Reactions were described but bacillary proliferation was doubtful.[67] This lack of proliferation was confirmed by Lobo and Carvalho in chickens and pigeons.[68]

In 1941, Ota and Nitto claimed that "when the leproma emulsion is injected in admixture with siliceous sinter, trypan blue and potassium iodide, it is possible to obtain positive results without exception." Their animals of choice were fowls of unmentioned species. Six passages were accomplished, although the lesions described were limited to the area of inoculation.[69] In the same year, de Souza-Araujo directed his attention to white rats. Three of the rats were inoculated subcutaneously in the axilla with pus from an inguinal lymph node of a patient with leprosy. Two rats developed visceral lesions after 15–17 months, and the third had no lesions after 18 months.[70] Also in 1941 Chaussinand gave 18 inoculations to a cynomolgus monkey and reported that the repeated injections "progressively immunized the monkey against future inoculations," because, after the fourteenth series, no locule nodules formed, despite higher doses. The monkey died 13 weeks after the final series, with few disseminated bacilli observable at autopsy.[71]

In 1945 Barman reported visceral organ involvement in white rats a year after the last of repeated inoculations.[72] In the next year, Fielding published observations on the

[58] Yamamoto, M. Lepro **10** Suppl. (1939) 7–8, in Mukherjee (n. 132) p. 82.

[59] Mitsuda, K. Demonstration von tuberkuloidem Gewebe im Schweinchenhoden nach Impfung mit Leprabazillen. Jpn. J. Dermatol. Urol. **46** (1939) 68. Abstract in Int. J. Lepr. **9** (1941) 267.

[60] Ota, M. Ueber Impfversuche der menschlichen und Rattenlepra auf Tiere, Haushühner und Vögel. Transactions of the 12th Meeting of the Japanese Leprosy Association, 1938. Lepro **10** Suppl. (1939) 27. Abstract in Int. J. Lepr. **9** (1941) 266.

[61] Ota, M. and Sato, S. Lepröse Veränderungen an der Leber mit menschlichen bzw. Rattenlepramaterial intramuskulär inokulierten Hühnern. Jpn. J. Dermatol. Urol. **47** (1940) 41. Abstract in Int. J. Lepr. **9** (1941) 266.

[62] Brunet, E. and Jadford H. Transmission of human leprosy to the hamster by the digestive tract. Bull. Acad. Med. **109** (1939) 383. Abstract in Lepr. Rev. **11** (1940) 150.

[63] Oberdoerffer, M. J. Uebertragung von Lepra auf sapotoxingefuetterte Affen. Dermatol. Wochnschr. **2** (1939) 1407–1411. Abstract in Int. J. Lepr. **8** (1940) 413.

[64] Collier, D. R. Inoculation of monkeys with leprosy following a diet of pauk (*Colocasia*). Thai. Sci. Bull. **2** (1940) 101–108. Abstract in Int. J. Lepr. **8** (1940) 549–550.

[65] Collier, D. R. Inoculation of monkeys with leprosy following a diet of pauk (*Colocasia*). Lepr. Rev. **11** (1940) 135–140.

[66] Cochrane, R. *A Practical Textbook of Leprosy.* London: Oxford University Press, 1947, pp. 7–9.

[67] Nonaka, N. Saikingaku Zasshi Nos. 528 and 529 (1940), in Mukherjee (n. 132) p. 81.

[68] Lobo, V. X. and Carvalho, B. Results of the inoculation of emulsion of human lepromata in chickens and pigeons. Lepr. India **18** (1946) 22–23.

[69] Ota, M. and Nitto, S. The serial transmission of human leprosy in fowls, continued for seven generations. Int. J. Lepr. **9** (1941) 299–304.

[70] de Souza-Araujo, H. C. Mem. Inst. Oswaldo Cruz **36** (1941), in Mukherjee (n. 132) p. 82.

[71] Chaussinand, R. Contributions a l'étude de la lèpre. Inoculation du bacille de Hansen au singe. Int. J. Lepr. **9** (1941) 203–207.

[72] Barman, J. M. Rev. Med. Rosario **35** (1945) 101, in Mukherjee (n. 132) p. 82.

use of human feces as an inoculum of rats. He described light infections in addition to localized lesions, and felt that "in some rats [there was] evidence that partial immunity had developed."[73]

Sato (1949) inoculated 15 types of animals with various methods of inoculation, with negative results. The animals were gerbils, goldfish, frogs, toads, paddy birds, canaries, parrots, love-birds, hens, mice, guinea pigs, rabbits, dogs and Japanese monkeys.[74]

Chaussinand and Besse (1951) inoculated four rainbow perch, detecting lesions containing acid-fast bacteria in the liver of one fish that died 20 months later.[75]

In 1955, Lai described studies using three methods of inoculation in 21 *Macacus cyclopis* monkeys: a) intramuscular injections, b) dual subcutaneous implantations 2 months apart, c) and subcutaneous implantations "every two or three weeks until the animal died." Only the multiple implantation showed dissemination, with a successful transfer 7 out of 17 times.[76]

In 1953, Tanimura and Nishimura described 12 years of work by their team in Osaka, confirming the specificity of *M. leprae* for humans. Only local reactions were noted, except when injected into Descemet's membrane, where no reaction was noted.[77]

In 1954, Nakagawa and Nakamura met with no success using various protocols, including intraperitoneal injection of cobra venom prior to inoculation of bacilli into the yolk sac of developing chick embryos.[78] Also in this year, Wilkinson obtained "positive results" by adding hyaluronidase to the leprous suspensions.[79]

It was in 1956 that Binford guided the direction in which much future research would go by proposing that the bacillus had a preference for the cooler parts of the body.[80] From this jumping-off point, many future researchers would be more successful than their predecessors.

Bergel attempted to alter host immune response in his 1957 study where he described work with eight white rats on a pro-oxidant diet. Rats injected intratesticularly showed large quantities of acid-fast bacilli in the testes when sacrificed at 10–11 months. The results prompted the author to comment, "We have demonstrated for the first time in the history of leprosy the most important of Koch's postulates: the transmission of Hansen's bacillus to animals."[81] Follow-up work in 1959 varied the dietary conditions and observed similar results, especially in a low vitamin E, high rancid linseed oil diet.[82]

In 1958, Chatterjee reported results with 106 selectively bred black mice and 48 golden hamsters, using inoculum which had been rendered tissue-free by differential centrifugation and diluted in saline to render a known count per unit volume of bacilli (1×10^9 for mice, 3×10^9 for hamsters). This marked the first time that attention had been paid to the number of bacilli inoculated, a practice which has been continued to the present. In the 51 black mice which survived 6 months or more, each of 43 autopsied showed bacilli in the spleen, liver, kidney, glands, testes/ovaries, nerve, skin, and spinal cord. Only 4 of the 16 sur-

[73] Fielding, J. W. Observations on human leprosy: infection in rats with human excretal organisms. Med. J. Aust. **2** (1946) 578–584.

[74] Sato, S. Lepro **18** (1949) 19, in Mukherjee (n. 132) p. 80.

[75] Chaussinand, R. and Besse, P. Inoculation du bacille de Hansen et du bacille de Stefansky à la perche arc-en-ciel. Rev. Bras. Leprol. **19** (1951) 4–7.

[76] Lai, S. Experimental studies on transmission of human leprosy to monkeys. Int. J. Lepr. **23** (1955) 48–51.

[77] Tanimura, T. and Nishimura, S. A review of recent animal inoculation studies with human and murine leprosy bacilli. Int. J. Lepr. **21** (1953) 335–345.

[78] Nakagawa, W. and Nakamura, M. Lepro **23** (1954) 293, in Mukherjee (n. 132) pp. 82–83.

[79] Wilkinson, F. F. Dia. Med. **26** (1954) 189, in Mukherjee (n. 132) p. 81.

[80] Binford, C. H. Comprehensive program for the inoculation of human leprosy into laboratory animals. Public Health Rep. **71** (1956) 995–996.

[81] Bergel, M. Inoculation del *Mycobacterium leprae* a ratas alimentadas con dietas pro-oxidantes. Sem. Med. **111** (1957) 1148–1180.

[82] Bergel, M. Influence of various pro-oxidant nutritional conditions on the growth *in vivo* of *M. leprae*. Lepr. Rev. **30** (1959) 153–158.

viving hamsters showed a generalized infection.[83, 84]

In the same year, Gunders described disseminated experimental leprosy in one of two inoculated chimpanzees. This is probably the first well-documented transmission of disseminated leprosy to a nonhuman primate. A temporal inoculation with a biopsy needle, an inoculation of the left ulnar nerve, an intraperitoneal injection, and an intravenous injection were followed 11 months later by nodules on the ears, hands, feet and legs, with large areas of depigmentation. In three more months the nodules had regressed, leaving the areas of depigmentation.[85] Biopsy specimens of skin showed active borderline leprosy. One of the drawbacks of this study is that there was no follow-up to determine the course of the disease.

In 1959, Binford described the results of 3 years of studies on over 1500 golden and albino hamsters, white mice, rats, guinea pigs, and hairless mice. Only the testes and the ears of golden hamsters with 18-month incubation periods showed bacillary multiplication and nerve involvement. Total body irradiation did not affect infection, and those animals treated with cortisone died too early for meaningful results.[86] In 1966, Waters and Niven published work involving the inoculation of the ears and foot pads of golden hamsters. They found a limited multiplication, but described "very scanty" mycobacteria in the sciatic nerve of one of the hamsters in contrast to Binford's heavier neural involvement.[87]

In 1960, Shepard succeeded in achieving bacillary multiplication in the foot pads of CFW mice, including 22 of 22 inoculations with nasal washings from patients with leprosy. There were some unique aspects of this research: a) The incubation period varied from 1–2 months for an inoculation of $10^{5.5}$–10^{6} bacilli to 6 months for 10^{3}. b) Regardless of the number of bacilli in the inoculation, the number of bacilli harvested was a maximum of 10^{6}. c) Passage to new mice was successful 11 out of 12 times. These results were less obvious in Syrian and Chinese hamsters and Mongolian gerbils.[88] Confirmation of these findings was made by Shepard 2 years later,[89] by Rees in 1964,[90] and Pattyn and Janssens in 1965.[91] Also in 1965, Hilson reproduced Shepard's findings in the foot pads of white rats.[92]

McFadzean and Ridley, in 1961, tested the efficacies of X-rays in reducing the immune response in long-tailed macaques prior to inoculation. Although the rate of disappearance of the localized lesion was slower in the irradiated monkeys, there was no evidence of bacillary multiplication. Both intradermal and intravenous inoculations were used, although the intravenous results were considered unsatisfactory.[93]

In 1962, Sengupta inoculated prenisolone-treated monkeys subcutaneously in the forehead. Only a local reaction was observed, and when a persistent lesion was

[83] Chatterjee, K. R. Experimental transmission of human leprosy infection to a selected, laboratory-bred hybrid black mouse. Int. J. Lepr. **26** (1958) 195–203.

[84] Chatterjee, K. R. Experimental transmission of human leprosy in laboratory-bred selected hybrid black mice and Syrian hamsters. Bull. Calcutta Sch. Trop. Med. **6** (1958) 83–85.

[85] Gunders, A. E. Progressive experimental infection with *Mycobacterium leprae* in a chimpanzee. J. Trop. Med. Hyg. **61** (1958) 228–230.

[86] Binford, C. H. Histiocytic granulomatous mycobacterial lesions produced in the golden hamster (*Cricetus auratus*) inoculated with human leprosy. Lab. Invest. **8** (1959) 901–924.

[87] Waters, M. F. R. and Niven, J. S. F. Experimental infection of the ear and footpad of the golden hamster with *Mycobacterium leprae*. Br. J. Exp. Pathol. **47** (1966) 86–92.

[88] Shepard, C. C. The experimental disease that follows the injection of human leprosy bacilli into foot pads of mice. J. Exp. Med. **112** (1960) 445–454.

[89] Shepard, C. C. Multiplication of *Mycobacterium leprae* in the foot-pad of the mouse. Int. J. Lepr. **30** (1962) 291–306.

[90] Rees, R. J. W. Limited multiplication of acid-fast bacilli in the footpads of mice inoculated with *Mycobacterium leprae*. Br. J. Exp. Pathol. **45** (1964) 207–218.

[91] Pattyn, S. R. and Janssens, P. G. Experiences with mouse footpad inoculation of leprosy bacilli originating from the Congo. Ann. Soc. Belg. Med. Trop. **45** (1965) 9–16.

[92] Hilson, G. F. R. Observations on the inoculation of *M. leprae* in the footpads of the white rat. Int. J. Lepr. **33** (1965) 662–665.

[93] McFadzean, J. A. and Ridley, D. S. Studies on the inoculation of *Mycobacterium leprae* into monkeys. Trans. R. Soc. Trop. Med. Hyg. **55** (1961) 235–238.

biopsied 3 months later, no acid-fast bacilli were detected.[94]

Convit described in 1962 the results of 2 years' work using over 1000 hamsters, mice, rats, guinea pigs, and rabbits. The emphasis of the research lay in the intradermal inoculation of cooler body parts and used material from patients with different forms of the disease. Only the hamsters produced localized lesions, and the most marked results were generated by material with a low initial bacillary count from a patient with borderline leprosy.[95] When the subsequent data were published in 1964, over 2500 animals had been inoculated, and the author confirmemd earlier findings in hamsters. Curiously, the author stated in this paper: "I had the opportunity to see the preparations from the biopsies of the footpad lesions of Shepard's mice. They differed greatly from the lesions in Chatterjee's mice and in our hamsters in Venezuela . . . ."[96] In a summary article, their conclusion was that "a new variant of *M. leprae* has been produced by mutation. This concept is supported by the observation under the electron microscope of differences between the human and hamster lesions and their bacilli and by differences in the immunologic properties of the human and the hamster strains."[97]

Also in 1964, Fite, Wrinkle and Sanchez inoculated 177 *Anolis* lizards, 203 painted turtles, 5 alligators, and 12 fish. Without referencing Couret,[27] they confirmed his findings, stating, "the important feature of this work lies in the elimination of reptile as a possible home for *M. leprae.*"[98]

Rees and Path, in 1965, made a comparative study of inoculations into mouse foot pads of leprous material from untreated patients in four different parts of the world, and got identical results in each case. Further, "bacilli derived from patients treated for 12 to 16 months with DDS (diaminodiphenylsulfone) always failed to multiple." The authors also made the first attempt to meld cooler body parts with diminished whole-body resistance by inoculating thymectomized-irradiated mice in the foot pads. Their preliminary results indicated that so treating the animals increased bacillary multiplication.[99] This was corroborated by Rees in 1966,[100] Gaugas in 1967,[101] and Rees, *et al.* in 1967.[102] In fact, the last authors reported "the unexpected observation that 12 months after inoculation not only had the local infection increased in intensity, but specific sites elsewhere in the body had become infected—for example, the nose and forepaws." Further, systemic infections in mice inoculated intravenously were described.

In 1968 antilymphocytic serum (ALS-IgG) was first used to further lower thymectomized mice's resistance. The bacillary yield for the experimental mice was about 30 times higher than the control group. When this high yield was considered with certain advantages of ALS over irradiation, Gaugas felt that this new technique would "provide a hitherto elusive means for detailed study of the pathogenesis of nerve and tissue damage as well as the antimicrobial therapy of leprosy."[103]

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[94] Sengupta, P. C., Mukherjee, N., Majumdar, D. S., *et al.* Attempt at transmission of human leprosy to the rhesus monkey: preliminary observations. Bull. Calcutta Sch. Trop. Med. **10** (1962) 157–159.

[95] Convit, J., Lapenta, P., Ilukevich, A. and Imaeda, T. Experimental inoculation of human leprosy in laboratory animals. I. Clinical, bacteriologic, and histopathologic study. Int. J. Lepr. **30** (1962) 239–253.

[96] Convit, J. Infections produced in hamsters with the human leprosy bacillus; a critique of recent studies. Int. J. Lepr. **31** (1964) 310–321.

[97] Convit, J., Lapenta, P., Ilukevich, A. and Imaeda, T. Experimental inoculation of human leprosy in laboratory animals; III. Int. J. Lepr. **32** (1964) 136–149.

[98] Fite, G. L., Wrinkle, C. K. and Sanchez, R. Inoculations of *M. leprae* in reptiles. Int. J. Lepr. **32** (1964) 272–278.

[99] Rees, R. J. W. and Path, F. C. Recent bacteriologic, immunologic and pathologic studies on experimental human leprosy in the mouse footpad. Int. J. Lepr. **33** (1965) 646–655.

[100] Rees, R. J. W. Enhanced susceptibility of thymectomized and irradiated mice to infection with *Mycobacterium leprae.* Nature **211** (1966) 647–658.

[101] Gaugas, J. M. Effect of x-irradiation and thymectomy on the development of *Mycobacterium leprae* infection in mice. Br. J. Exp. Pathol. **48** (1967) 417–422.

[102] Rees, R. J. W., Waters, M. F. R, Weddell, A. G. M. and Palmer, E. Experimental lepromatous leprosy. Nature **215** (1967) 599–602.

[103] Gaugas, J. M. Enhancing effect of antilymphocytic globulin on human leprosy infection in thymectomized mice. Nature **220** (1968) 1246–1248.

In 1971, Fieldsteel and McIntosh used antithymocytic serum (ATS) in conjunction with neonatal thymectomy, and compared results against thymectomy alone using Lewis and Buffalo rats. The experimental procedure produced marked improvement in bacillary multiplication, including a 57,600-fold increase to 2.88×10^8 organisms per testis in Lewis rats. Buffalo rats did not show testicular involvement, although both breeds were susceptible in their foot pads.[104] The susceptibility of the neonatally thymectomized Lewis rat was replicated by Fieldsteel and Levy in 1980.[105] However, it was determined that some rats, thymectomized as early as 18 hours after bith, responded as normal rats did to the inoculation. When Fieldsteel's group compared circulating T cells versus degree of infection in 1981, the groups of rats with the highest concentration of T cells also experienced moderate-to-severe infection. The authors concluded, "since there was no apparent relationship between T-cell depletion and susceptibility to infection with *M. leprae,* an additional, unknown mechanism was also involved."[106]

The first attempt to inoculate the nine-banded armadillo was described by Kirchheimer and Storrs in 1971,[107] and Kirchheimer, *et al.* in 1972.[108] Dissemination was observed in the skin, bone marrow, liver, spleen, lymph nodes, lung, meninges, and eye. Additionally, leprotic pneumonitis, leprotic meningitis, and esophageal involvement were described—complications not usually observed in human leprosy. The body temperature range for armadillos is 32°–35°C. Further studies in 1974 by Storrs, *et al.* estimated armadillo susceptibility at 40%. The degree of lepromatous leprosy in armadillo is, thus, more severe than in man, with the additional possibility of infections in the central nervous system and the lungs. It was theorized that "armadillos in the late stages of disease become depressed immunologically because of massive invasion of the bone marrow and related reticuloendothelial tissues of leprosy bacilli." The average survival time of the adult animals from inoculation until death from leprosy or its complications appears to be about 31 months.[109]

In 1973, Binford discussed the results of inoculation of 21 different animals at sites of low body temperatures. Mild localized infections were observed in only two species of hamster, in mice, cotton rats and South African white-tailed rats. The other animals included chimpanzee, chinchilla, dog, fruit bat, guinea pig, hairless mouse, hog, lemming, meadow vole, Mongolian gerbil, cynomolgus monkey, rhesus monkey, opossum, and three species of rat (Binford, C. H., personal communication, 1987).

Also in 1973, Lew, *et al.* published an investigation of 7 years' duration using the foot pads of Korean chipmunks. Multiplication of bacilli became apparent at 7 months post-inoculation, with a harvest of 2×10^{10} organisms following the inoculation of 10^6 bacilli 10 months previously. Acid-fast bacillary involvement of dermal nerves was also reported.[110, 111]

Turanov's group (1973) described successful transmission to chimpanzees, although how many of the 12 animals inoc-

[104] Fieldsteel, A. H. and McIntosh, A. H. Effect of neonatal thymectomy and antithymocytic serum on susceptibility of rats to *Mycobacterium leprae* infection. Proc. Soc. Exp. Biol. Med. **138** (1971) 408–413.

[105] Fieldsteel, A. H. and Levy, L. Combined rifampin and dapsone chemotherapy of *Mycobacterium leprae* infection of the neonatally thymectomized Lewis rat. Int. J. Lepr. **48** (1980) 267–276.

[106] Fieldsteel, A. H., Sato, N. and Colston, M. J. Relationship between T-cell population in neonatally thymectomized Lewis rats and susceptibility to infection with *Mycobacterium leprae.* Int. J. Lepr. **49** (1981) 317–323.

[107] 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.

[108] Kirchheimer, W. F., Storrs, E. E. and Binford, C. H. Attempts to establish the armadillo (*Dasypus novemcinctus* Linn.) as a model for the study of leprosy. II. Histopathologic and bacteriologic post-mortem findings in lepromatoid leprosy in the armadillo. Int. J. Lepr. **40** (1972) 229–242.

[109] Storrs, E. E., Walsh, G. P., Burchfield, H. P. and Binford, C. H. Leprosy in the armadillo: new model for biomedical research. Science **183** (1974) 851–852.

[110] Lew, J. and Yang, Y. T. Growth of *M. leprae* in Korean chipmunks. Int. J. Lepr. **41** (1973) 510.

[111] Lew, J., Yang, Y. T. and Pyun, W. S. Experimental infection of the Korean chipmunk with *M. leprae.* Int. J. Lepr. **42** (1974) 193–202.

ulated reacted positively was not mentioned. A 10-year observation period was not necessary, and results were described as relapsing, progressive tuberculoid leprosy. Twenty other kinds of monkeys and 311 rabbits, white mice, white rats, and guinea pigs failed to exhibit generalization of disease.[112]

Congenitally athymic (nude) mice were first used in 1975 by Prabhakaran, *et al.*, who found "despite their proven T-cell deficiency, the nude mice do not promote generalized infection with *M. leprae.*" A 6-month observation period was used.[113] The following year Colston and Hilson decided differently, when a nude mouse which had survived over 322 days post-inoculation showed significant liver and spleen involvement, in addition to the involvement of the foot pads.[114] In 1976, Kohsaka, Mori and Ito observed bacillary dissemination to other low-temperature areas in addition to nerves.[115] Nakamura and Yogi, in 1980, also reported systemic infections after inoculation in the paw and the area of the mystacial vibrissae of the right upper lip.[116] The same authors also found no increase in multiplication with congenitally asplenic mice,[117] but did note an increase when thymus cells were grafted into nude mice 5–7 months after inoculation.[118]

In later research, Storrs, *et al.* were able to transmit leprosy to the seven-banded armadillo in 1975,[119] and Convit, *et al.* described infection of the eight-banded armadillo in 1978.[120]

In 1976, Narayanan's group inoculated four wild slender lorises. Although two of the animals had died by the time of publication, yielding no sign of acid-fast bacilli on autopsy, the authors remained hopeful, stating "it will be premature to draw any conclusion at present as inoculation of more slender lorises is being conducted."[121]

Waters, *et al.* (1978) reported transmission of leprosy to a single white-handed gibbon, with an observation period of nearly 15 years. Although this would appear to mirror some incubation periods in humans, the authors' feelings regarding replication were that "the prospect of 15–20 years' follow-up would seem somewhat daunting."[122]

In 1979, Klingmüller reported uncertain results after the inoculation of hedgehogs.[123]

In more recent research, Wolf, *et al.* in 1983 discussed successful transmission to rhesus and African green monkeys, using intravenous and intradermal inoculation.[124] The discovery of naturally occurring leprosy

[112] Turanov, N. M., Studnitsin, A. A., Zalkan, P. M., *et al.* Experimental inoculation of leprosy in the chimpanzee. Int. J. Lepr. **41** (1973) 509–510.

[113] Prabhakaran, K., Harris, E. B. and Kirchheimer, W. F. Hairless mice, human leprosy and thymus-derived lymphocytes. Experientia **31** (1975) 784–785.

[114] Colston, M. J. and Hilson, G. R. F. Growth of *Mycobacterium leprae* and *M. marinum* in congenitally athymic (nude) mice. Nature **262** (1976) 399–401.

[115] Kohsaka, K., Mori, T. and Ito, T. Lepromatoid lesion developed in nude mouse inoculated with *Mycobacterium leprae*; animal transmission of leprosy. Lepro **45** (1976) 177–187. Abstract in Int. J. Lepr. **45** (1977) 403–404.

[116] Nakamura, K. and Yogi, Y. The nude mouse as an experimental lepromatous leprosy model (continued): The lepromatoid lesions in mystacial vibrissae located site of injection. Int. J. Lepr. **48** (1980) 490.

[117] Nakamura, K. and Yogi, Y. The experimental inoculation with *M. leprae* in the asplenic mouse. Int. J. Lepr. **48** (1980) 492.

[118] Nakamura, K. and Yogi, Y. The nude mouse as an experimental lepromatous leprosy model (continued): The enhancing effect of thymus cells in infected nude mice. Int. J. Lepr. **47** (1979) 105.

[119] Storrs, E. E., Walsh, G. P. and Burchfield, H. P. Development of leprosy in another species of armadillo *Dasypus hybridus* (L.): genetic and immunologic implications. J. Trop. Med. **78** (1975) 216–218.

[120] Convit, J., Aranzazu, N. and Pinardi, M. E. Leprosy in the armadillo: clinical and pathological aspects. In: *The Armadillo as an Experimental Model in Biomedical Research.* Pan American Health Organization #366, 1978, pp. 41–46.

[121] Narayanan, E., Manja, K. S., Bedi, B. M. S., *et al.* Experimental transmission of leprosy to animals: a preliminary note on attempt to transmit leprosy to the slender loris, *Loris tardigradus* (Linnaeus). Lepr. India **48** (1976) 36–41.

[122] Waters, M. F. R., Isa, M. D. B., Rees, R. J. W., *et al.* Experimental lepromatous leprosy in the white-handed gibbon (*Hylobatus lar*): successful inoculation with leprosy bacilli of human origin. Br. J. Exp. Pathol. **59** (1978) 551–557.

[123] Klingmüller, G. Inoculation of hedgehogs (*Erinaceus europeaus*) with *Mycobacterium leprae.* Int. J. Lepr. **47** Suppl. (1979) 344.

[124] Wolf, R. H., Martin, L. N., Baskin, B. J., *et al.* Experimental transmission of leprosy in African green monkeys (*Cercopithecus arthiops*) and the rhesus monkey (*Macaca mulatta*). Int. J. Lepr. **51** (1983) 664–665.

in a mangabey monkey[125, 126] sparked interest in that species' potential, and successful transmissions were reported in 1984[127, 128] and 1985.[129, 130] This promises to be the animal of choice for many future modeling attempts, with further research presently under way.

There obviously has been much attention paid historically to the search for an animal model of leprosy. One hundred fourteen years have yielded three species of armadillo; nude mice and rats, black mice, and normal mouse foot pads; Korean chipmunks; and four nonhuman primates (gibbon, rhesus, African green and mangabey). Chimpanzees could be added, but more experimental studies are necessary to establish their utility.

These discoveries are not without controversy. *M. tuberculosis* and *M. lepraemurium* are two of many acid-fast organisms which could have caused confusion for many researchers. Witness the numerous claims of cultured organisms. Further, there were widely variant definitions of "success." Whereas most researchers considered generalized infection to be proof of transmission, many simply felt the localized lesions were evidence enough. Many early researchers also lost sight of the objective in the fervor of the search—it is curious whether a tadpole would have been a suitable model even had the organism "taken."

To confound matters further, researchers developed their own "pet" protocols in the drive to enhance infection. This dispersal of effort along myriad paths detracted from a coherent, stable path of experimental design and collection of experimental data.

Finally, given the wide distribution in the literature, it is unlikely that anyone could keep current on all ongoing work. Especially in the first years, there was often a considerable delay between the scientist's "Eureka!" and the heeding attention of his contemporaries to his cry. However, it is easy to second-guess matters while safely ensconced in 1987, and critique is not the purpose of this treatise.

In defense of the obviously elementary and feeble attempts of the earliest researchers, their efforts took place in the early infancy of bacteriology, using an agent which had only recently been plucked from the miasma. The fact that much headway was made at all is to their credit, considering the many secrets of the organism that remain elusive even to the technology of the 1980s. Given continuing efforts, perhaps today's technology will find the ultimate answer to this 114-year search for a suitable animal model for leprosy.

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[125] Meyers, W. M., Walsh, G. P., Brown, H. L., *et al.* Naturally acquired leprosy in a mangabey monkey (*Cercocebus* sp.). Int. J. Lepr. **48** (1980) 495–496.

[126] Meyers, W. M., Walsh, G. P., Brown, H. L., *et al.* Leprosy in a mangabey monkey—naturally acquired infection. Int. J. Lepr. **53** (1985) 1–14.

[127] Martin, L. N., Gormus, B. J., Wolf, R. H., *et al.* Experimental leprosy in nonhuman primates. Adv. Vet. Sci. Comp. Med. **28** (1984) 201–236.

[128] Meyers, W. M., Binford, C. H., Walsh, G. P., *et al.* Animal models of leprosy. In: *Microbiology 1984.* Leive, L. and Schlessinger, D., eds. Washington, D.C.: American Society for Microbiology, 1984, pp. 307–311.

[129] Wolf R. H., Gormus, B. J., Martin, L. N., *et al.* Experimental leprosy in three species of monkeys. Science **227** (1985) 529–531.

[130] Martin, L. N., Gormus, B. J., Wolf, R. H., *et al.* Depression of lymphocyte responses to mitogens in mangabeys with disseminated experimental leprosy. Cell. Immunol. **90** (1985) 115–130.