INOCULATIONS OF *M. LEPRAE* IN REPTILES[1,2]

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Because of suggestions that *M. leprae* and related microorganisms might prefer a growth temperature lower than that commonly encountered in mammals, experiments were carried out in the inoculation of several species of reptiles and fish, especially of the *Anolis* lizard (*Anolis carolinensis*) and of the young painted turtle (*Chrysemys picta*).

MATERIALS AND METHODS

Anolis lizards were purchased commercially and caged in simple screened boxes containing potted plants. They were watered by keeping the plants drenched, and were fed on live meal-worms or a prepared food composed of dried insects. Deaths from starvation and dehydration (failure to adapt to cage conditions) were numerous, but many animals survived and were in excellent condition when sacrificed 4 to 5 months after inoculation.

Small painted turtles were kept in open aluminum pans containing water and supplied with a wooden out-of-water tray. They were fed the same diet.

Alligators (*Alligator mississipiensis*) were kept in an outdoor-indoor box and fed meat. Some larger turtles indigenous to the Louisiana locale, *Triomyx, Pseudomys, Chrysemys,* and *Kinosternon spp.* were tanked with the alligators. A few fish of native *spp.* (catfish and bream), as well as a Japanese mouth-breeder, were kept in an aerated fresh water tank.

Experiments were carried out over a one-year period. These provided, in the summer, day-night temperatures of 25-35°C, with an average close to 32°C. Indoors, during winter months, the temperature was a standard 22-25°, with a slight nocturnal drop. The fish tank showed little variation from 22°C, winter or summer.

Anolis lizards are easily handled by chilling to 5°C, but this procedure was not found necessary. At this temperature they are wholly inactive. Inoculations were made cutaneously, or intraperitoneally, or both. Microscopic sections were made of tissues of all animals sacrificed and most of those dying. These showed clearly that pure cutaneous inoculations were not affected often. Inoculated material frequently entered the general circulation in quantity by way of lymphatics shortly after inoculation, even when great care was taken and the smallest size needles were used.

Turtles were inoculated "subcutaneously" near the hind leg. These inoculations were frequently found to have entered the peritoneal cavity, or the general circulation, for reasons related to the physical effects of fluid exchange in a hard-shelled animal. Other animals, alligators and fish, were inoculated subcutaneously.

The materials inoculated were homogenized saline suspensions of human leprosy, or of murine leprosy, the usual dose being 0.1 ml. (rarely larger) of a heavy (1-30) suspension.

RESULTS

Inoculation of Anolis *lizards with* M. leprae.—In 10 experiments 177 lizards were inoculated with doses of *M. leprae*, varying somewhat in concentration (Table 1). In 5 of these experiments 8 efforts to trans-

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TABLE 1.—*Inoculation of reptiles and fish with mycobacteria.*

Organism inoculated	Number of trials	Animal	Total animals used	Transfer attempts	Total transfer animals used	Results
M. leprae	10	*Anolis* lizards	177	8	65	Negative
M. leprae	11	Painted turtles	203	15	153	Negative
M. leprae	1	Alligators	5	—	—	Negative
M. leprae	1	Fish	12	—	—	Negative
M. lepraemurium	8	*Anolis* lizards	164	1st 18 2nd 4	157 29	Negative
M. lepraemurium	6	Painted turtles	154	1st 17 2nd 1	182 6	Probably negative
M. lepraemurium	6	Misc. turtles	18	—	—	Probably negative
M. lepraemurium	1	Alligators	5	—	—	Negative
M. lepraemurium	3	Fish	36	—	—	Negative
M. phlei, *Smegmatis*,	2	*Anolis* lizards	80	—	—	Negative
Tuberculosis bovis, and *BCG*	2	Turtles	48	—	—	Negative

fer to fresh groups were made. Survival of animals was good, some living for 4 and 5 months, and frequent survival to 90 days was obtained.

These experiments were completely convincing that *M. leprae* did not propagate in these animals. Although microorganisms could be demonstrated at the sites of inoculation indefinitely, the numbers manifestly diminished, and the visceral lesions never progressed beyond the extent observable microscopically a few days after inoculation. The number of organisms in the tissues was always a direct factor of the number inoculated, and transfers made from more heavily inoculated animals never yielded sufficient organisms for an additional transfer attempt.

The *M. leprae* seen in the granulomas after a few weeks or months still stained vigorously, but the arrangement within the phagocytic cells was always haphazard, suggesting absence of biologic activity. This is unlike the appearance of *M. leprae* in mouse footpad infection, in which there is some "bundling" of bacilli similar to that seen in the active infection in human tissues.

Inoculation of M. leprae *into turtles.*—In 11 experiments (Table 1) 203 small painted turtles were inoculated. These animals survived a little better during the summer months, many living 3, some 5, and a few 6 months. As with the *Anolis*, no progression of infection was seen. In addition, 15 attempted transfers were made in 153 animals.

Surprisingly, many of the transferred suspensions proved toxic to the recipient animals, producing lethargic states lasting 12 to 24 hours after inoculation, with some mortality.

It was found necessary to introduce needles well away from points of inoculation, to prevent fungus abscesses developing along needle tracts. The number of *M. leprae* persisting in lesions was related to the number introduced. In the majority of animals most bacilli disappeared within a few weeks, leaving only a few at inoculation sites or occasional phagocytized accumulations in lung or liver. Nerve infection was never seen.

Inoculation of Anolis *lizards with* M. lepraemurium.—In 8 experiments 164 animals were inoculated, with good survivals from 90 to 165 days. An additional 157 animals were inoculated in transfer attempts, and a further 29 were used in second transfer attempts from these. In one experiment, 14 animals were additionally inoculated with a boiled (10 minutes) suspension, the animals being killed serially at weekly intervals for comparison with those inoculated with living organisms.

It was immediately clear that *M. lepraemurium* survived morphologically in greater degree than *M. leprae* in *Anolis* lizards. Well preserved bacilli were found in those animals receiving live organisms. The quantities of boiled bacilli that persisted were surprisingly large, and it was many weeks before there was evident diminished staining capacity. Even after this developed, however, poorly stained organisms usually remained in the tissues for a long time. In a few animals with live bacilli the stained appearance was better, but in the remainder it was not significant. The persistence of organisms led, at first, to anticipation of possible biologic survival, and 18 attempted first transfers were made in 157 animals, but no evidence of successful passage was obtained.

Inoculation of M. lepraemurium *into turtles.*—Six experimental inoculations of turtles were made. In the first 3 of these the remaining animals were sacrificed at 50, 100, and 120 days. Negative results were recorded. In the fourth group, comprised of 40 turtles, 12 animals survived and were sacrificed at 154 days. Some of these showed extensive lesions of the liver, which, at the time, were thought to be progressing. Transfers from these animals, carried only 35 days, yielded no result. In a 5th experiment, with both live and killed organisms, no differences were seen up to 4 months, but at 5 and 6 months a few animals inoculated with live bacilli showed larger lesions of the liver with many more bacilli. In a 6th experiment, in which animals received a heavy dose, bacilli dropped off in numbers after 30 days, but in one animal surviving 226 days the liver appeared so heavily infected that it seemed a possible cause of death. All the trans-

fers from this group were made at 45 days or earlier, and yielded no result.

In addition to the above named animals, 18 older (native) turtles were inoculated. Seven of these sacrificed at less than 100 days showed no evidences of progression of the infection. In the 11 animals examined at 5 to 6 months numerous lesions were seen, often tuberculoid in character, but usually with less than impressive numbers of bacilli. In a few of these the tuberculoid lesions were very large but encapsulated. In retrospect it seems possible to explain them on a basis of immune responses to bacillary products, rather than to increased numbers of bacilli.

The persistence of *M. lepraemurium* in turtles continues to be astonishing, as though all organisms survived. But reference to the comparisons with animals receiving boiled bacilli leaves the impression that reproduction of *M. lepraemurium* may have taken place only in a few animals, and then after a long period of time. There is at least a 4 month lag phase, and an experimental period of 12 to 18 months would be required to substantiate infection of turtles with *M. lepraemurium*.

Alligators and fish.—Five alligators inoculated with *M. lepraemurium*, and 5 with *M. leprae*, have yielded nothing, not even identifiable sites of inoculation after many months. A few alligators with lepraemurium inoculation are still alive, 20 months later. Some 20 bream, 8 catfish, and 20 Japanese mouth-breeders, have shown no lesions, although living *M. lepraemurium* were recovered (via mouse inoculation) after 6 weeks from one fish.

Other mycobacterial inoculations.—*M. tuberculosis bovis, M. spp. BCG, M. smegmatis,* and *M. phlei* were inoculated into 48 turtles and 80 *Anolis* lizards, in approximately equal distributions, in a dose of 0.05 mgm. per animal. No lesions were produced at the site of inoculation in any animals, and only rare small groups of organisms were found, in peritoneal tags, in the animals inoculated with the bovine tubercle bacilli. These organisms all disappeared rapidly, in contrast with the persistence of *M. leprae*, and, even more strikingly, *M. lepraemurium*.

COMMENT

An investigation of this kind is not a wholly scientific undertaking, often being conditioned by availability of animals and uncertain survival. It is complicated by the occurrence of types of pathologic processes in reptiles that are quite different from those in mammals. For example, granulomas in the lungs of turtles are extruded into alveolar-bronchial spaces, eventually sloughing away. An animal with most of the inoculum carried to the lungs may rid itself of the bulk of it by this process, as has been observed histologically many times. Mycobacteria inoculated intraperitoneally are taken up by phagocytes

which gather themselves together in a few large nodular masses adherent to peritoneal surfaces. Almost an entire intraperitoneal inoculum may be accumulated within a few grossly obvious small nodules, giving a false impression of an active or progressing lesion.

It is possible to encounter unrelated mycobacterial species in work such as this. One group of transfer turtles all died of an overwhelming massive mycobacterial infection within a few days, the cause not suspected immediately. A rapidly growing saprophyte was cultivated from the fish tank, following observation of mycobacteria in the epidermis of several fish.

The important feature of this work lies in the elimination of reptiles as a possible home for *M. leprae*. Two species, the *Anolis* lizard and the young painted turtle, fail to support the organism. This is particularly well shown when compared with results with *M. lepraemurium*, which persists morphologically in reptiles for many months. *M. leprae* persists better than the nonpathogens *M. smegmatis, M. phlei* and *BCG*, and better than *M. tuberculosis bovis*. Yet it is clear that, even though it may prefer a temperature lower than that of the human body, the reptilian atmosphere is unfavorable.

The toxicity of fresh suspensions of turtle tissues for other turtles of the same species is puzzling. The recipients show flaccidity of the extremities, and then of the neck, and may be mistaken for dead. Many

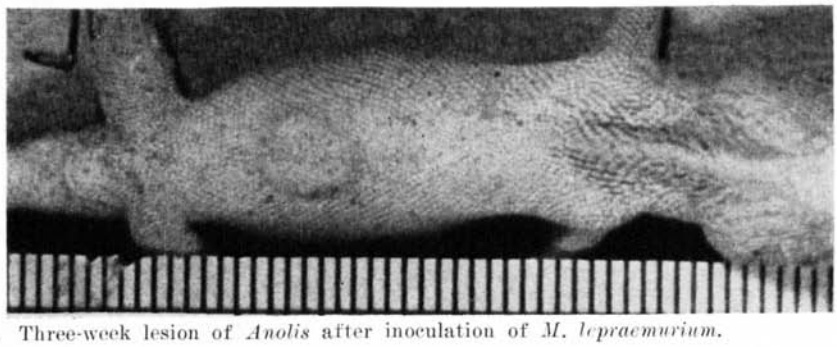

FIG. 1. Three-week lesion of *Anolis* after inoculation of *M. lepraemurium*.

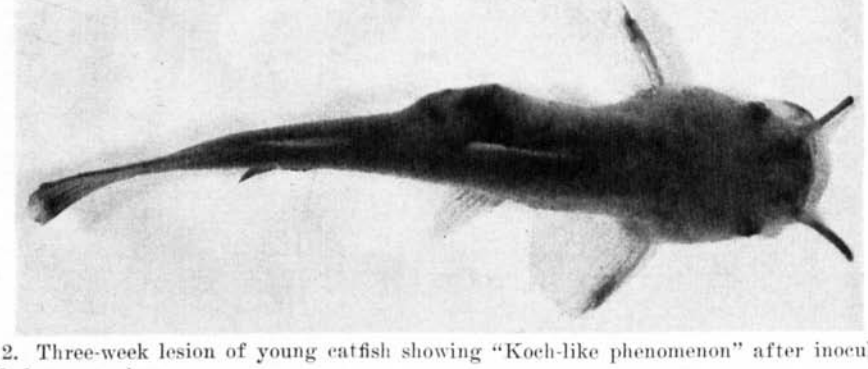

FIG. 2. Three-week lesion of young catfish showing "Koch-like phenomenon" after inoculation of *M. lepraemurium*.

recover, but many also die 24 to 72 hours after the inoculation.

A commonly observed feature both in lizards and in fish (Figs. 1, 2) is the development of something like a Koch phenomenon 3 to 4 weeks after inoculation with *M. lepraemurium*. The sites of inoculation can look most impressive at this time, but histologically the lesions show only much edema, with no increase in bacilli. Cutaneous hypersensitization can and does occur. In fish with scales the reaction may be obscured, but in catfish it is striking against the dark skin, and on subsiding it sometimes leaves a macule with a hyperpigmented border.

Clark and Shepard ([1]) observed growth of *M. marinum* in *Anolis* lizards to be negligible when the animals were kept at 10°C, moderate at 20°, and marked at 30°. Their animals were not fed, survived 2 to 23 days, and microdrop counts showed increase in numbers of bacteria. Most of their other observations are recorded from smears of tissues rated as to numbers of bacilli. This last was done routinely in the present work, also, but the smears were found unreliable (as compared with sections) in estimating extent of infection. Smears usually showed many *more* bacilli than were found in sections. *Persistence* of *M. marinum* in some of their animals, rather than growth, is suggested by their findings in some species, such as garter snakes, but nothing like the extracellular growth of masses of *M. marinum* in *Anolis* was observed in the present case of the leprosy bacilli.

SUMMARY

In order to determine possible infectivity of leprosy bacilli, both human and murine, for reptiles, more than 1400 reptiles were inoculated, principally small lizards (*Anolis*) and turtles. *M. leprae* persisted in trace numbers during experimental periods of 4 to 5 months, without suggestion of progression at any time, judged from histologic examinations. *M. lepraemurium*, on the other hand, persisted in some animals most astonishingly, as though all inoculated organisms survived, for periods of 4 to 6 months. Inoculations with heat-killed controls of *M. lepraemurium* showed that these, too, persisted extremely well. No changes developed in a few alligators and fish inoculated, and the results indicate that it is improbable that any reproduction of these microorganisms took place. Four strains of mycobacteria, *M. tuberculosis bovis, M. spp. BCG, M. phlei,* and *M. smegmatis,* all disappeared from the tissues of heavily inoculated animals even more rapidly than *M. leprae.*

RESUMEN

Con el objeto de determinar las posibilidades infectantes del bacilo leproso, tanto humano como murino, fueron inoculados más de 1400 reptiles, principalmente pequeños lagartos (*Anolis*) y tortugas. El *M. leprae* persistió en cantidades señalables durante el período experimental de 4 a 5 meses, sin sugestión de progresión en ningun momento, juzgados por los examenes histologicos. *M. lepraemurium,* por otro lado, persistió

asombrosamente en algunos animales, como si todos los organismos inoculados sobrevivieron por períodos de 4 a 6 meses. Las inoculaciones con controles de *M. lepraemurium* mueros al calor, mostraron que estos también persistieron extremadamente bien. No hubieron cambios en los pocos caimanes y peces inoculados, y los resultados indicaron que es improbable de que hubiera habido ninguna reproducción de estos microorganismos. Cuatro razas de micobacterias, *M. tuberculosis bovis, M. spp. BCG, M. phlei,* y *M. smegmatis,* desparecieron todos de los tejidos de animales inoculados fuertemente, aun mas rapidamente que el *M. leprae.*

RÉSUMÉ

Dans le but de déterminer un éventuel pouvoir infectieux des bacilles de la lèpre, tant ceux de la lèpre humaine que ceux de la lèpre murine, pour les reptiles, plus de 1400 reptiles ont été inoculés. Il s'agissait principalement de petits lézards (*Anolis*) et de tortues. *M. leprae* a persisté en très petit nombre durant des périodes experimentales qui se sont étendues sur 4 à 5 mois. Pour autant qu'on puisse en juger d'après les examens histologiques, il n'y a pas eu multiplication. D'autre part, *M. lepraemurium* a persisté chez quelques animaux d'une façon réellement surprenante, comme si tous les organismes inoculés avaient survéeu durant 4 à 6 mois. Des inoculations de contrôle menées avec *M. lepraemurium* tué par la chaleur one montré que celui-ci persistait de même fort bien. Aucun changement n'a été noté chez les quelques alligators et chez les poissons inoculés, et d'après ces résultats il semble improbable que ces microorganismes se soient reproduits. Quatre souches de mycobactéries, à savoir *M. tuberculosis bovis, M. spp. BCG, M. phlei* et *M. smegmatis,* ont toutes disparu des tissus chez des animaux inoculés à dose massive, et ce plus rapidement même qu'il n'avait été noté avec *M. leprae.*

REFERENCE

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