

THE EXPERIMENTAL PRODUCTION OF LEPROSY IN THE MONKEY (MACACUS RHESUS).*

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Many investigators have attempted to reproduce leprosy of man in the lower animals under natural and various artificial conditions, but aside from a slight local reaction that has occasionally developed at the site of inoculation the reported results have been invariably negative. Certainly it cannot be claimed that the type of the disease as we see it in man has been produced under any conditions in the lower animals. Recently Clegg,¹ Sugai,² and the author,³ among others, have shown that leprosy lesions occur in the guinea pig and Japanese and white mice following intraperitoneal or subcutaneous injections of cultures of leprosy bacilli or the infected human tissues, but in no instance has the clinical picture of human leprosy manifested itself in these animals.

Nicolle⁴ claims to have produced by subcutaneous inoculation of bits of leprosy tissue typical leprosy nodules in the monkey. However, he does not state that in these animals there developed leprosy with its symptom-complex as we see it in man.

The failure to occasion more than a localized infection in the experimental animal with emulsions of leprosy bacilli obtained from bits of human tissue may be attributed in part, possibly, to the fact that in the tissues the bacilli are too few in number and for the most part are non-viable. It sometimes happens in experimental tuberculosis that we fail to infect the susceptible guinea pig with human tuberculous material, and this is explained by the scarcity

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¹ *Philippine Jour. Sci.*, 1909, iv, 403.

² *Lepra*, 1909, viii, 203.

³ *Jour. Exper. Med.*, 1910, xii, 649; *Arch. Int. Med.*, 1911, vii (in press).

⁴ *Semaine méd.*, 1905, xxv, 116.

of bacilli in the tissue employed for inoculation and by the circumstances that many of them are avirulent or dead. Failure is even more to be expected with experimental leprosy since the animals used are certainly less susceptible to leprosy than to tuberculosis.

Prior to the cultivation of *Bacillus lepræ* outside of the animal body, it was only possible to work with the infected human tissue; and judging from the small number of bacilli that multiply when this material is transferred to a suitable artificial culture medium, it may be asserted that in leprosy more than in tuberculosis relatively fewer organisms are living; in my opinion this explains the failure of former workers to transmit leprosy to the monkey by direct implantation of human leprosy tissue. On the other hand, when pure cultures are available and large quantities of viable leprosy bacilli are injected at given intervals, typical leprosy of the tubercular type may be reproduced in the *Macacus rhesus* monkey.

In this paper I wish to give briefly the results of some experiments upon monkeys in which disseminated leprosy has followed the repeated injection of large quantities of leprosy cultures. The cultures used were grown on slanted glycerinated blood agar for forty-eight hours and the growth washed down in five cubic centimeters of normal saline solution and thoroughly emulsified, and the suspension so prepared was employed as the injection material. Three strains of *Bacillus lepræ* were utilized in the experiments, one of which has been under cultivation for about a year and is now more than twenty generations removed from the parent stem, and the others have been growing upon artificial media six and nine months respectively.

EXPERIMENTAL PART.

Experiment I.—On October 17, 1910, a full-grown monkey (*Macacus rhesus*) was inoculated hypodermatically into the subcutaneous tissues of the right inguinal region with one cubic centimeter of a heavy suspension of leprosy bacilli. Twenty-four hours after the injection the monkey appeared perfectly well and showed no signs of inflammation at the site of inoculation. Eight days later (October 25) the animal was given a second injection of the

culture into the subcutaneous tissues of the right breast. At this time there was still no evidence of either localized or general infection resulting from the first injection. The monkey was apparently in perfect health. One week after the second inoculation, or eighteen days after the first, a nodule the size of a split pea developed in the subcutis over the right groin at the site of the first inoculation, which slowly increased in size from day to day and in three weeks attained the size of a pigeon's egg. At this time a small nodule also appeared at the site of the second inoculation. On November 1 the animal received a third injection of the culture into the subcutaneous tissue over the right groin which was followed in forty-eight hours by the appearance of a hard nodular mass that has slowly continued to increase in size. The skin about this nodule showed numerous smaller indurated areas. The animal received a fourth injection of the culture into the subcutaneous tissue of the left breast on November 16. In three days a purplish red tumor approximately two centimeters in diameter developed and two weeks later the overlying skin became firmly adherent, thin, and shiny. This tumor mass ruptured on the seventeenth day discharging a grumous material which on microscopic examination contained large numbers of leproid cells filled with acid-fast bacilli. Cultures prepared from this material remained sterile with respect to pyogenic microorganisms; but *Bacillus lepræ* was recovered in pure culture upon special artificial media.

One of the nodules in the groin that seemed to fluctuate on pressure and was similar in other respects to the nodule in the breast that opened spontaneously, was incised and found to consist of a homogeneous, semigelatinous material showing no evidence of tissue necrosis. In color and consistence the cut surface of the mass resembled an incised lymph gland. Surrounding the nodule was a narrow hemorrhagic zone which was sharply defined and did not extend into the tumor mass.

Forty-six days after the first injection the monkey showed typical signs of disseminated infection and presented the clinical picture of human leprosy of the tubercular type of the disease. On the right side of the face a small raised erythematous patch approximately

three centimeters in extent appeared and gradually extended over the bridge of the nose, right eye, and forehead. The appearance of the patch was identical with the erythema noted in human leprosy during certain periods of the disease. This area was incised and the expressed bloody serum examined and numbers of acid-fast bacilli were found. Five days later, although the redness of the cheek and forehead had faded, the skin was left somewhat thickened and distinctly pigmented and was, therefore, in striking contrast with the uninvolved part of the face. The skin over the superciliary ridges also became reddish pink and roughened; while between the eye-brows there appeared several small elevated hard nodular masses.

The large nodules in the groin and chest have lost all sensation to pain as determined by the fact that the animal permits free incision without flinching, but evinces unmistakable signs of pain as soon as the knife extends into the surrounding normal skin. About the nodules, for a radius of two to three centimeters, the skin is distinctly hypersensitive, the slightest touch causing the animal to flinch.

Experiment II.—On November 4, a female *Macacus rhesus* monkey was given subcutaneously into the chest one cubic centimeter of an emulsion of the necrotic material obtained from one of the nodules of monkey 1. This material contained a great number of bacilli, but judging from the amount of growth that resulted in isolated patches over a thickly seeded artificial medium only a few of the bacilli were viable or capable of multiplication. Two weeks after the injection a small hard flattened nodule could be palpated which gradually subsided and in ten days to two weeks had completely disappeared. At this time the monkey was given a second injection subcutaneously into the left groin using the entire growth from a four day old slant culture. Five days later the animal showed at the site of the second inoculation a purplish red nodule approximately one centimeter in diameter, which at present is steadily increasing in size. Already there are analgesia and hypersensitiveness associated with the lesion. There is, however, no evidence as yet of superficial lesions elsewhere on the body. The animal received a third injection of the culture on November 18 into

the loose subcutaneous tissues of the right vulva. Forty-eight hours after the inoculation the vulva was swollen to three times its normal size and presented a deep purplish red color. On November 24 a macular eruption appeared on the chest in the mid-sternal region which persisted for three to four days when it disappeared, leaving the skin roughened and of a dusky gray discoloration. As in the first monkey, acid-fast bacilli were demonstrated in the bloody serum obtained from the incised macular lesion.

In the attempt to infect by way of the circulation, a third monkey, an adult male, was given intravenously into the left femoral vein a heavy suspension of leprosy bacilli. The animal received in the same manner a second injection one week after the first, using twice the amount of culture previously employed. At the present writing there is no evidence of cutaneous lesions or signs of infection elsewhere in the body. However, it is too early to expect skin lesions, judging from the time it requires to produce them in monkeys that have received subcutaneous injections.

The skin lesions are primarily exudative in character, and the exudation is soon followed by proliferation of the fixed cells in the area involved. The sections from the excised nodules experimentally produced are indistinguishable microscopically from those of human cases. The earliest observed changes in the tissues consist of a collection of lymphoid and plasma cells, the latter predominating, while the older or more advanced nodules showed great numbers of the so-called lepra and epithelioid cells intermingled in a loose matrix of young connective tissue. Where the nodule develops rapidly the central part sometimes undergoes a form of liquefaction necrosis. Associated with the lesion is always a marked dilatation of the bordering capillaries which gives to the overlying skin its dark purplish red color. The smaller vessels in the immediate neighborhood show a perivascular infiltration with lymphoid and plasma cells; while the nerves in and about the nodules are often more or less completely surrounded and pressed upon by proliferated cells of epithelioid type. The nerve fibers in the older nodules show evidence of degeneration which apparently is the result of pressure of the surrounding cells rather than of invasion of the nerve sheaths by the bacilli.

The lepra bacilli are chiefly contained within cells, very few being extra-cellular. Even in specimens obtained from the center of broken down nodules extra-cellular organisms are only exceptionally found.

Whether repeated inoculations of large numbers of viable lepra bacilli are necessary to cause generalized infection in the monkey is a question as yet undetermined. Considering the fact that the monkey is not highly susceptible to the disease under ordinary conditions, it is reasonable to assume that until the bacilli become parasitic in the new host only a few of them will survive in the first transfers. However, those that live and multiply in the monkey may in the course of time become permanently parasitic so that subsequent passages through other individuals of the species may be followed by more profound results.

The production of leprosy in the monkey is of far greater importance than the successful inoculation of smaller animals, as it offers a better opportunity to study the experimental disease and to work out problems that have a bearing on the pathology of the human disease. The successful transmission of clinical leprosy to the monkey establishes beyond doubt the leprosy nature of the acid-fast bacilli cultivated upon artificial media from human leprosy tissue, and proves conclusively that some strains, at least, retain their infectivity under conditions of artificial growth for more than a year. The experiments also bring out the necessity of first cultivating the bacilli on an artificial medium in order to obtain large numbers of viable organisms with which to accomplish the experimental production of the disease.

The analgesia of the leprosy nodules with which are associated hypersensitive zones in the monkey happened to be a marked feature of the cutaneous lesions of leprosy in man. This fact and that of the occurrence of erythematous patches, thickening and pigmentation of the skin in areas far removed from the inoculated site in the monkey, seem to prove conclusively the spread of the infection from one side to another, and to justify the statement that leprosy is reproducible in the monkey, in which animal many of the clinical and pathological phenomena common also to the human subject may be advantageously studied.