A FURTHER NOTE UPON THE EXPERIMENTAL PRODUCTION OF LEPROSY IN THE MONKEY (MACACUS RHESUS), WITH A CRITICAL STUDY OF THE CULTURE EMPLOYED.*

By CHARLES W. DUVAL, M.D., and MAURICE COURET, M.D.

(From the Laboratories of Pathology and Bacteriology, Tulane University, New Orleans, Louisiana.)

Plates 40–45.

In May, 1911, one of us (Duval)[1] published briefly the results of some experiments upon a small series of monkeys in which lesions of leprosy followed the repeated injection of large quantities of acid-fast bacilli that had been cultivated from the specific lesions of human lepers. While the infection produced in the majority of the animals was localized at the site of inoculation, in two, leprous lesions developed in places remote from the injection site, and the animals showed many of the clinical symptoms common to the disease in man. However, even in the monkeys that developed disseminated lesions, complete recovery apparently occurred two or three months after the first appearance of symptoms; at least, all external evidence of the infection disappeared, and the animals seemed to be in perfect health. Although the external lesions subsequently disappeared, the experiment proved that this species of monkey could be infected with acid-fast bacilli derived from the lesions of human leprosy. Moreover, the identity of the artificially grown culture seemed established, for the experimental infection was in many essentials like leprosy in man.

Since these earlier experiments, we have discovered[2] that if the animal is sensitized with killed bacilli and given a second injection

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[1] *Jour. Exper. Med.*, 1911, xiii, 374.

[2] *Jour. Exper Med.*, 1911, xiii, 576.

of viable organisms after an interval of two or more weeks, local as well as disseminated lesions are more often induced. This is true for the monkey and for other animal species formerly regarded as refractory.

The purpose of this paper is (1) to report further upon the occurrence of leprosy in a monkey that developed the disease thirteen months after the first, and nine months after the last injection of a pure culture of *Bacillus lepræ,* the infection being identical in symptom-complex with that in man, and terminating fatally; and (2) to describe in detail the culture employed, together with critical remarks relative to other acid-resisting species with which the specific organism might be confused.

EXPERIMENTAL PART.

On October 17, 1910, a full-grown male monkey (*Macacus rhesus*) was inoculated hypodermically into the subcutaneous tissues of the right inguinal region with one cubic centimeter of a heavy, homogeneous suspension of a pure culture of acid-fast bacilli (approximately four million) which had been isolated one year previously from the human spleen at autopsy. 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 left inguinal region (approximately four billion bacilli being used). 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.

Two weeks after the second injection, a nodule the size of a split pea developed in the subcutaneous tissue at the site of the first inoculation and slowly increased in size from day to day, and in three weeks attained the size of a hazel-nut. The tumor mass ruptured spontaneously on the seventeenth day, discharging a grumous material which, on microscopic examination, was found to contain large numbers of acid-fast bacilli. Cultures from this material showed no pyogenic microörganisms. On November 1, the animal received, into the subcutaneous tissue of the right groin, a third

injection of the culture, which was followed in forty-eight hours by the appearance of a hard nodular mass, one centimeter in extent, that slowly continued to decrease in size. On November 16, the monkey was given subcutaneously a fourth injection of the culture (approximately four billion bacilli suspended in sterile normal salt solution). At this time, the animal, to all external appearances, was in perfect health.

On March 9, 1911, three months later, the animal showed the first signs of disseminated infection and presented, in part, the clinical picture of human leprosy (maculo-anesthetic type). On the right side of the face, a small, raised erythematous patch, approximately three centimeters in extent, appeared and gradually extended to the 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 serum showed the presence of acid-fast bacilli which resembled in size, shape, and arrangement the Hansen bacillus. The macular eruption persisted for two or three weeks, when it disappeared and the monkey for nine months afterwards showed no external evidence of the infection and appeared to be in robust health.

On November 2, 1911, eleven months after the last injection, it was noted that the tissues below the monkey's left eye were swollen, and the overlying skin was purplish red. This mass steadily enlarged and in ten days attained the size of a marble, which, in consequence, caused complete closure of the left eye (figure 9).

The mass involved the subcutaneous tissue of the whole lower eyelid and extended for 0.5 of a centimeter below the lower orbital ridge. The conjunctival sac and eyeball were not involved. On palpation, the mass showed distinct fluctuation, and, when incised, discharged three cubic centimeters of thick, golden yellow material. The abscess cavity was found to extend throughout the whole of the lower quadrant of the orbit. Microscopic examination of the pus showed innumerable slender, slightly curved and beaded acid-fast rods, which were, for the most part, arranged in groups of twos and threes within the protoplasm of polymorphonuclear leucocytes (figure 22).

The gross appearance of the tumor mass and the microscopic

picture of the content corresponded in every respect to the subcutaneous leprous abscess in the human subject. In this connection, it is worthy of mention that the pus from a human leprous abscess is composed almost entirely of polymorphonuclear cells and is sterile with respect to pyogenic organisms (Gurd).[3] Those not familiar with this phase of human leprosy would regard the abscess in either case as due to a secondary pyogenic infection because of the presence of such enormous numbers of pus cells; however, the absence of pyogenic organisms settles the question. Furthermore, there is abundant proof that the acid-fast bacilli in these abscesses are *Bacillus lepræ* and not some saprophytic species, since the latter never give rise to acute abscess formation and are readily cultivated. Besides, the absence of acid-fast growth on all media except that for the cultivation of *Bacillus lepræ* is further proof that the culture does not contain any of the saprophytic species.

At first, we were inclined to regard the abscess as tubercular, thinking that it might be similar to that in the human cases of tuberculosis which were reported by Theobald Smith,[4] in 1898, and by one of us (Duval),[5] in 1909. However, the failure to induce lesions in the guinea pig, or to obtain from the pus cultures of tubercle bacilli upon suitable media, ruled out this possibility.

One cubic centimeter of the pus from the infra-orbital abscess was emulsified in ten cubic centimeters of sterile water, and two guinea pigs, weighing 690 and 670 grams, respectively, were each inoculated subcutaneously and intraperitoneally with five cubic centimeters. These animals were perfectly normal when killed eighteen days after the inoculation. There was no evidence of tuberculosis either macroscopically or microscopically. Another portion of the pus was planted upon the ordinary laboratory media and special media (Dorset egg, human serum, neutral blood agar, and 1 per cent. alkaline fish agar) for the cultivation of tubercle and lepra bacilli. The ordinary media for the recovery of pyogenic bacteria remained sterile, and no growth of any kind appeared on the special media for the cultivation of the tubercle bacillus, though incubated at 37° C. for a period of six weeks.

[3] *Jour. Infect. Dis.*, 1911, viii, 39.
[4] *Jour. Boston Soc. Med. Sc.*, 1898, i, 309.
[5] *Jour. Exper. Med.*, 1909, xi, 403.

A good growth of acid-fast bacilli occurred in twelve days on the alkaline fish and neutral blood agars. The first generation showed a great variety of acid-fast rods, including many diplococcoid forms which retained intensely the carbolfuchsin stain after decolorization with Gabbet's solution for one minute; however, the majority of the organisms in a given microscopic field were long, slender, and beaded. In the third generation, all beaded forms had disappeared and the fields now showed nothing but deeply staining short forms (figure 4).

After incising the abscess on the face and expressing the pus, the wound partially healed, and, in consequence, there remained a wellmarked ectopia of the lower lid.

Simultaneously with the appearance of the subcutaneous abscess in the soft tissues below the monkey's left eye, there developed another subcutaneous mass over the external occipital protuberance. Two weeks after its appearance, this tumor was 2.5 centimeters in depth, and 4.5 centimeters in its greatest extent (figures 10 and 11). The mass was firmly adherent to the skin, which, over the highest part, was denuded of hair and greatly thickened. The tumor ruptured spontaneously three weeks after its appearance and discharged a serosanguineous material rich in slender, beaded acid-fast bacilli. After the escape of a moderate amount of the tumor content, there remained a hard central core that was firmly adherent to the calvarium. The skin in the immediate neighborhood, though not adherent to the mass, was greatly thickened, and showed, on microscopic sections, as did sections from the central core, a dense mosaic of proliferated cells of the epithelioid type interspersed with innumerable lepra cells, many of which contained the so-called globi (figure 21). Cultures upon a variety of media were likewise prepared from this material and guinea pigs were inoculated subcutaneously and intraperitoneally. The acid-fast bacilli grew only upon the special lepra medium, but were associated with a contaminating staphylococcus which had gained entrance because the mass had ulcerated at the time the material was removed for cultivation. The guinea pigs that had been inoculated were killed after two and three weeks, respectively, and at autopsy showed no evidence of tuberculosis either macroscopically or microscopically.

A few weeks after the appearance in the monkey of the lesions described above, several cutaneous leprous tubercles developed upon the lower part of the animal's body; one, measuring 0.5 of a centimeter on the inner side of the left leg, and eight others, varying in size from 0.5 of a centimeter to one centimeter in diameter, in the right groin. These nodules were purplish red in color, distinctly elevated, and firmer than the normal skin. Subsequently several of these areas became ulcerated. Examination of the bloody serum from these ulcers showed numbers of acid-fast bacilli of the characteristic morphology and arrangement of *Bacillus lepræ* in the human tissues.

An interesting and important discovery in this case was the finding of innumerable acid-fast bacilli in the nasal secretion, although at the time of the first examination there was no apparent gross, nasal pharyngeal lesion. It was noted one week later, however, that the left external nostril had become reddened and markedly ulcerated. The animal died on December 7, 1911, and was autopsied six hours after death.

AUTOPSY PROTOCOL.

The body is that of a moderately emaciated, large, male monkey (*Macacus rhesus*). The post-mortem rigidity is marked. With one exception the superficial lymph-nodes are not palpable. The palpable node is the size of a pea, and is situated in the left inguinal region just above Poupart's ligament.

On the top of the head, at a point corresponding to the occipito-parietal fissure, and 1 cm. to the right of the median line, is an indurated and ulcerated nodular mass, 3.5 cm. in diameter. The walls of this mass are formed above by the greatly thickened skin and underlying tissues, and beneath by both plates of the skull which, at this point, is eroded over an area 1 cm. in length by 0.7 cm. in width (figure 13). The floor of the nodule is formed by the thickened and roughened dura mater which is adherent to the calvarium at this point.

Below the left eye and involving the lower lid is an ulcerated area 1.5 cm. in diameter. This area extends back into the orbit involving the periosteum of the sphenoid and lacrimal bones, and extends through the lachrymal duct to the nose where it involves the turbinated bones and nasal septum. It terminates in a thickened, red, and indurated area at the left nasal orifice. The sphenoid and lacrimal bones are necrotic. The eyeball is not involved.

Upon the inner surface of the right thigh are two small ulcers measuring 0.2 and 0.3 cm. in diameter, and upon the abdominal wall over the right iliac fossa are eight similar ulcers measuring 2 to 6 cm., respectively. These ulcers are shallow, though their walls are thick, indurated, and considerably elevated from the surrounding skin. There is a brown irregular macule which measures

about 1.5 cm. in diameter at McBurney's point. The animal's hair is everywhere sparse and dull.

The peritoneal cavity is dry and the peritoneum is pale, moist, and glistening and shows no adhesions. The mesenteric and retroperitoneal lymph nodes are not palpable. The spleen and liver show no adhesions to neighboring structures, and the diaphragm is at its normal position. The appendix appears normal. A lymph node, the size of a pea, is found below the skin on the left side just above Poupart's ligament. On section, this node shows no gross lesions.

The pleural cavities are dry and the lungs appear normal. Both visceral and parietal pleuræ are smooth and moist. The lymph-nodes of the thoracic cavity are not palpable.

The pericardial cavity and heart are normal.

The spleen is not enlarged. It shows, however, two yellowish nodules deep beneath the capsule, which measure 6 cm. in diameter. One of these areas is situated on the internal surface of the organ, a short distance below the superior border, and the other is at the lower pole (figure 14). On section, they appear as fairly well circumscribed yellowish areas that gradually become diffused into the surrounding pulp. No other gross abnormalities are present.

The liver is not enlarged, and the capsule is smooth, though the lymphatics show prominently. Five golden yellow nodules, varying in size from 0.2 to 3 cm. in diameter, are found on the under surface of the right lobe (figures 15 and 16). These are slightly elevated above the surface and are somewhat softened. While they are, for the most part, spherical in shape, their outer borders do not end abruptly but rather infiltrate the surrounding tissues. On section, a thick golden yellow material is found to occupy the center of these nodules. The organ shows otherwise nothing remarkable. The gall bladder is negative.

The pancreas, stomach, and intestines are negative.

The kidneys, adrenals, bladder, and testes are slightly congested, but otherwise show no macroscopic change.

The organs of the neck are negative, except the nasopharynx which is red, swollen, and slightly eroded, the continuation of a similar condition in the nasal mucous membrane.

The Brain.—The dura mater is adherent to the calvarium at the point below the erosions of the skull described above. Here it is thickened and nodular, and its outer surface is eroded. It is not adherent below this lesion to the pia-arachnoid, which is clear and smooth throughout.

Numerous hemorrhagic extravasations are seen beneath the pia mater. These vary in size from 0.5 to 5 cm. in diameter (figure 12). The hemorrhages are largest and most numerous on the middle and frontal lobes and about the Sylvian fissure, though they are also numerous about the pons and cerebellum. They are noticeably absent over the occipital lobe, the upper border of which lies immediately below the lesion of the dura and calvarium.

The spinal cord, spinal ganglia, ulnar brachial, and sciatic nerves show no gross changes.

Anatomical Diagnosis.—Leprosy of skin (maculotubercular type); leprous nodules in liver and spleen; acute hemorrhagic leptomeningitis (leprous); ulcerated nasopharynx (leprous); abscess (infra-orbital).

Material from the nodules in the liver and spleen was removed under

aseptic precautions and transplanted to the following media: Dorset egg, containing 2 per cent. glycerin, neutral blood agar, 1 per cent. tryptophan fish agar, human and rabbit serum agars, and, in addition, cultures were prepared on the ordinary laboratory foodstuffs. All the media remained sterile as regards microörganisms other than an acid-fast species. The tryptophan and blood agar media showed a fine growth that was discernible to the naked eye, on the ninth day after incubation at 37° C., in the form of minute discrete pin point colonies. Stained preparations from these colonies showed solidly staining, short acid-fast bacilli, resembling in morphology the bovine tubercle bacillus. It is noteworthy that the slender, beaded rods of the material transplanted had changed completely under artificial cultivation. Furthermore, the same difference was noted with regard to the bacilli cultivated from the liver and spleen nodules. Although the organisms were morphologically and tinctorially indistinguishable in the tissue from the tubercle bacillus, their arrangement in dense clusters or packets associated with the so-called lepra cell served at once to differentiate them.

DESCRIPTION OF THE CULTURE.

The acid-fast organism used in this experiment was isolated in pure culture from the spleen of a fatal case of human leprosy which occurred at the Louisiana Leper Home in May, 1909. At the time the monkey was first inoculated, the culture had been under artificial cultivation for more than a year and, in consequence, was growing well on a variety of enriched media.

The bacilli in the initial transplant multiplied very slowly, and only after repeated subculturing upon a special medium over a period of three months was it possible to obtain a pure culture. No macroscopic growth was discernible upon any medium for several weeks, although, on microscopic examination, it was evident that multiplication had started, since the spherical masses or globi in the transferred tissue bits were several times their original size, and the organisms in many places covered whole fields of the microscope in close masses of slender, slightly curved beaded rods (figures 20 and 21). The morphology of the organism at this period did not differ from the Hansen bacillus of the tissues, with the exception, perhaps, that they were somewhat longer and thicker (figure 3). After the culture had accustomed itself to the conditions, *in vitro*, growth occurred more rapidly, and the individual bacilli appeared smaller and more diffusely scattered, where formerly they were in isolated packets or clumps.

All the earlier attempts to colonize the culture failed, although it grew readily upon a nutrient alkaline agar in association with ameba and *Bacillus typhosus;* however, after repeated subplating, separate colonies were obtained after five to six weeks' incubation at 32° C.

It is noteworthy that if the transplanted human leprous tissue had contained some common saprophytic acid-fast species in addition to the Hansen bacillus, no difficulty would have been experienced in its cultivation, at least it would not have taken months to accomplish its isolation. It is well known that the ordinary saprophytes, which might be confused with *Bacillus leprœ* because of their similarity in morphological and tinctorial properties, grow readily upon almost any laboratory medium. Furthermore, the source of this culture (spleen) makes it improbable that it is an extraneous acid-resisting microörganism, such as the bacillus of timothy hay, etc., even granting that some of the so-called "confusing group" may accidentally occur at times in the exposed leprous ulcer.

In general, the lepra culture retained for many generations the morphology of the Hansen bacilli in the tissues. After the twentieth or thirtieth generation on artificial medium, which corresponded to the time when artificial growth activity was permanently established, the bacilli showed a decided change in size and shape and in their manner of arrangement. The rods now assumed a diplobacillary form that resembled the young cultures of the bovine tubercle bacillus. The difference was so marked that, unless the transformation had been followed from day to day, one would doubt whether the resulting growth of small, deeply staining rods was the progeny of the original beaded forms. We have repeatedly observed that this same culture, when passed through a susceptible animal, again assumes the characteristic slender, beaded appearance of the Hansen bacillus of the tissues. Although this transformation indicates that the culture is rapidly growing and has become accustomed to saprophytic conditions, it is no criterion that the organism is incapable of producing leprosy, as the experiment reported herein proves.

The change in morphology and manner of division of the bacilli

in culture is easily followed by a routine microscopic study. Here a careful observation of stained preparations or of the organism observed in hanging drop, shows that a massing of the chromatin substance takes place in the individual bacilli to form one or more swollen ovoid bodies indicating amitotic division. This massing of the chromatin becomes more and more definite until one or two well formed, deeply staining, oval bodies appear in the parent cell. During the early stage of this transformation only a few of the beaded types show this alteration; however, from day to day, more and more bacilli undergo the change, and subsequently the culture consists entirely of single and diplobacillary forms (figures 6 and 8).

In the stained preparations, the contrast in density of coloring between the old, slender, slightly curved rods and their enclosed, as well as released progeny is so striking that there is no mistaking the relation of the two or the manner of reproduction during this period. Furthermore, there is hardly a possibility, for those experienced in the initial cultivation of *Bacillus lepræ* from the tissues, to take the two forms as distinct species, or to assume that the long variety is the true Hansen bacillus which failed to multiply and, in consequence, became lost or killed by the other species.

The manner of division for *Bacillus lepræ* may occur in two other ways; namely, by transverse fission which gives rise to rods arranged in pairs or short chains of two or more pairs, and by longitudinal cleavage which results in close packets of bacilli arranged in parallel rows. Often in these packets it is common to note that a short, plump, solidly staining rod will alternate in regular sequence with a thin faintly staining bacillus (figures 5 and 7).

There are apparently a number of factors that influence the manner of division of *Bacillus lepræ,* among which may be mentioned the reaction of the medium, the kind of artificial foodstuff, the oxygen supply, and the species of animal in which the bacilli are harbored. Whereas in cold-blooded animals the bacilli seem to divide by transverse cleavage, in warm-blooded animals they multiply, as a rule, by amitotic division of the chromatin, though at times transverse and longitudinal division undoubtedly occur.

Aside from the fact that the diplococcoid form of *Bacillus lepræ*

is capable of producing leprous lesions and will again revert in the animal body to the basic type (slender and beaded), there are distinct biological differences that serve to separate the culture from all known saprophytic acid-fast species. We wish to state, in further support of the specificity of the culture, that in each instance where we have attempted the cultivation of the Hansen bacillus, the resulting growths have corresponded in every particular. The probability of always recovering the same saprophyte from internal as well as external lesions in every case of leprosy from which we have cultivated an acid-fast bacillus seems extremely remote. It is true that some strains of *Bacillus lepræ* are recovered in pure culture more readily than others, but in the majority of instances, the specific organism is recovered with great difficulty, and only after making subcultures on special medium. Again, in a few cases, it is almost impossible to obtain *Bacillus lepræ* in pure culture, though growing well in symbiosis with cultures of typhoid, etc.

While it is almost impossible to confuse the initial culture of *Bacillus lepræ* with any of the common acid-fast saprophytes, old cultures of *Bacillus lepræ, i. e.,* cultures that have become accustomed to artificial growth conditions and are multiplying readily upon a variety of media, may possibly be confused with the bacillus of timothy hay, because they both produce a somewhat similar pigment (figures 1 and 2). Even with regard to this property, a comparative study shows distinct and constant differences for the two species. On the other hand, there is no chance of mistaking *Bacillus lepræ* for any of the other known acid-resisting saprophytes, such as Moeller's grass bacilli and Rabinowitch's cultures from butter and milk.

Pure cultures of *Bacillus lepræ*, when grown upon solid medium, more especially on a 1 per cent. alkaline fish agar, always give a moist, cadmium colored growth that is glistening, easily detached, and does not spread from the needle track; while the bacillus of timothy hay, the only species with which *Bacillus lepræ* might be confused, invariably produces a dull, dry, brick red growth that spreads rapidly over the whole surface of the medium. On the most favorable medium, it requires three to five days for the most

rapidly growing cultures of *Bacillus leprœ* to show visible growth; while the bacillus of timothy hay, under the same conditions, gives a well marked growth in twenty-four hours. Cultures of *Bacillus leprœ* though growing luxuriantly on a variety of special media will, on ordinary nutrient agar either fail to grow or, at most, give a very faint growth; but the bacillus of timothy hay grows profusely on this medium. Again, *Bacillus leprœ* is a facultative aerobe, while all strains of the bacillus of timothy hay with which we have worked are obligate aerobes. *Bacillus leprœ* gives readily a homogeneous bacterial cloud when suspended in a fluid medium, and the bacillus of timothy hay, like the tubercle bacillus, is emulsified with great difficulty, and often not at all. On glycerin bouillon, prepared after the method of Theobald Smith for the differentiation of tubercle bacilli, the end reaction for *Bacillus leprœ* is neutral or slightly alkaline, while the bacillus of timothy hay produces no change in the reaction of the medium. Finally, the various serum tests (complement fixation, agglutination, reaction, etc.) are specific and serve to differentiate sharply the lepra culture from any of the known saprophytic acid-resisting group.

SUMMARY AND DISCUSSION.

Fatal leprosy, with all its clinical and pathological manifestations in man, may be experimentally induced in the monkey (*Macacus rhesus*) with a pure culture of the acid-fast bacillus cultivated by one of us (Duval) from a leprous lesion in man.

To produce the disease experimentally, it seems necessary to give the animal repeated injections of large numbers of leprosy bacilli at given intervals for a period of months.

That the infection is more likely to follow where sensitization is first established is definitely proven by the specific experiments that we have carried out upon a variety of laboratory animals. The first injection, we assume, sensitizes the animal and may consist of either killed or viable lepra bacilli.

The necessity of first sensitizing the monkey and then giving repeated doses of viable organisms over a long period might explain the relative infrequency of the disease in man; at least, it offers an

explanation of the fact that man rarely, if ever, contracts leprosy, although intimately associated for an indefinite period with those afflicted with the disease.

The leprous lesions in the monkey are histologically indistinguishable from those in man and do not essentially resemble the specific lesion of tuberculosis, blastomycosis, or the lesions experimentally produced with saprophytic acid-fast species, since the appearance of large lepra cells and the arrangement of the bacilli in dense packets within these cells to form the so-called globi is a constant and characteristic feature for the experimental as well as the human lesion (figures 17, 18, and 19).

The production of leprosy in the monkey proves conclusively that the acid-fast bacillus cultivated by one of us (Duval) from the human lesion is the Hansen bacillus and not some extraneous saprophyte, and that it is the etiological factor in human leprosy.

In our experience, it has been extremely difficult to produce, in the lower animals, more than a transient localized lesion with human leprous material rich in the specific bacilli, unless the animal is first sensitized, when lesions histologically identical with those produced by pure cultures are easily induced. Therefore, it is natural to expect that cultures of *Bacillus lepræ* which are many generations removed from the parent stem are less likely to infect, unless given in larger doses on the ground of loss in virulence.

When experimental leprous lesions occur in the internal organs, they are more often found in the liver and spleen, while the experimental lesions occasionally produced in the lower animals with some of the saprophyte species, such as the bacillus of timothy hay, Moeller's grass bacilli, etc., rarely, if ever, occur in these organs (Abbott and Gildersleeve).[6] These authors did not find lesions in the liver and spleen in a single instance after inoculating forty-five rabbits intravenously with large doses of the "confusing group." Furthermore, the cell picture and the appearance and arrangement of these bacilli in the lesions in no way resemble experimental leprosy (Hölscher).[7]

It is no indication that a given culture is not the Hansen bacillus

[6] *Tr. Assn. Am. Phys.*, 1902, xvii, 37.

[7] *München. med. Wchnschr.*, 1901, xlviii, 1483.

because the individual organisms differ in size and shape from those in the tissues, since it is a well known fact that marked variations in morphology are common for many bacterial species under natural and artificial conditions. One of us (Couret)[8] has already pointed out that there is a wide variation in morphology for *Bacillus lepræ* under different environments. The experimental work serves not only to emphasize this fact, but is proof that a transformation from the slender beaded rods of the tissues to solidly staining diplococcoid forms of culture does occur for *Bacillus lepræ;* and, conversely, that the coccoid forms of culture may again assume the slender beaded appearance by passage through warm-blooded animals.

EXPLANATION OF PLATES.

PLATE 40.

FIG. 1. A pure culture of *B. lepræ* grown on 1 per cent. alkaline fish agar, showing the maximum growth after six weeks' incubation at 32° C. Note the color (cadmium) and heavy corrugated appearance of the growth. This culture was isolated from the spleen of a fatal case of human leprosy in November, 1909, and caused fatal leprosy in the monkey here reported.

FIG. 2. The same culture (*Bacillus lepræ* I) showing the amount of surface growth on glycerine bouillon that occurs in three to six weeks after the flasks are surface seeded. Note the wrinkled character of the growth and its similarity, except in color, to the tubercle cultures.

PLATE 41.

FIGS. 3, 4, and 6. Microphotographs of the same culture (*Bacillus lepræ* I) stained with carbolfuchsin and treated with 30 per cent. nitric acid followed by 95 per cent. alcohol. Note the variation in morphology. In 3, the individual rods are long, slender, and distinctly beaded, and arranged in clusters or clumps; in 4, they are solidly stained and arranged in pairs and short chains; in 6, the rods are solidly stained and appear as diplococcoid or lanceolated pairs.

FIGS. 5, 7, and 8. Drawings showing the wide variation in morphology that may occur for the same culture of *Bacillus lepræ* under different cultural environments.

PLATE 42.

FIG. 9, 10, and 11. A *Macacus rhesus* monkey showing the experimental leprous lesions about the head and face. Note in figure 9 the partial closure of the left eye with ulcers and nodules in the regions involving the face, bridge of nose, and the superciliary ridge. Figure 10 shows a large ulcerated nodule. 5 cm. in diameter, on the occiput. Figure 11 is a profile view of the occipital mass.

[8] *Jour. Exper. Med.*, 1911, xiii, 576.

FIGS. 12 and 13. The brain and calvarium of the same monkey, showing multiple diffuse hemorrhagic areas in the pia-arachnoid, and erosion through the skull caused by the leprous process shown above in figures 10 and 11.

PLATE 43.

FIG. 14. A complete section of the monkey's spleen. Note the two leprous nodules, one at either pole. These areas are similar in color and consistence to those in the liver.

FIGS. 15 and 16. Two views of the monkey's liver, in which there are five sharply defined leprous nodules, four of them situated in the inferior lobe. These nodules are deeply seated, soft, and of a golden yellow color.

PLATE 44.

FIGS. 17, 18, and 19. Microphotographs of experimental leprous lesions produced with pure cultures of *B. lepræ* I. Note in figure 19 the dense mosaic of epithelioid cells interspersed with the so-called lepra cells. Figure 18 shows leprous tubercles in the peri-neural tissue and lymphoid cell infiltration between the nerve fibres. Figure 17 shows the early microscopic lesion which is composed of lymphoid, plasma, and epithelioid cells.

PLATE 45.

FIGS. 20 and 21. Microphotograph of stained smear preparations (carbol-fuchsin and Gabbett's solution) from one of the golden yellow nodules in the liver. Note the enormous masses or globi of acid-fast bacilli, which is a striking feature in the experimental, as well as human lesion.

FIG. 22. Pus from the leprous abscess of a monkey, showing polymorphonuclear cells that are phagocytic for *Bacillus lepræ*. Note the striking resemblance of the bacilli to *Bacillus tuberculosis*.

FIG. 1.

FIG. 2.


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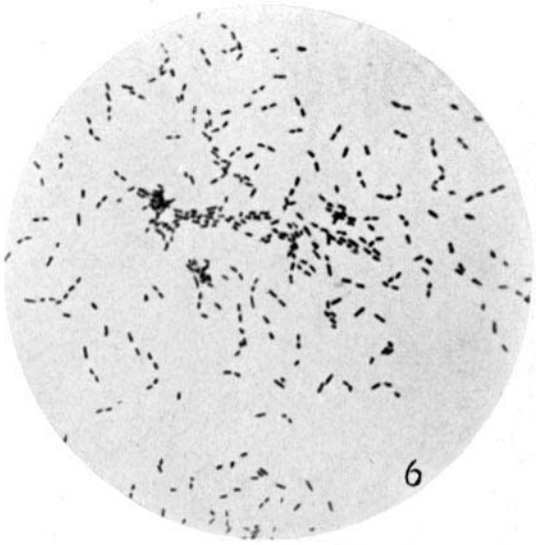
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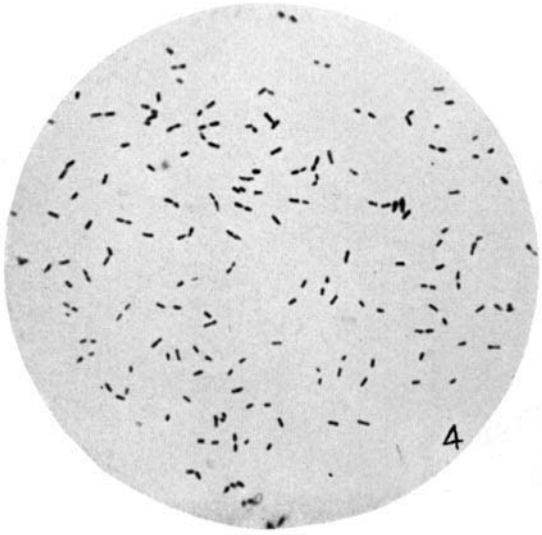
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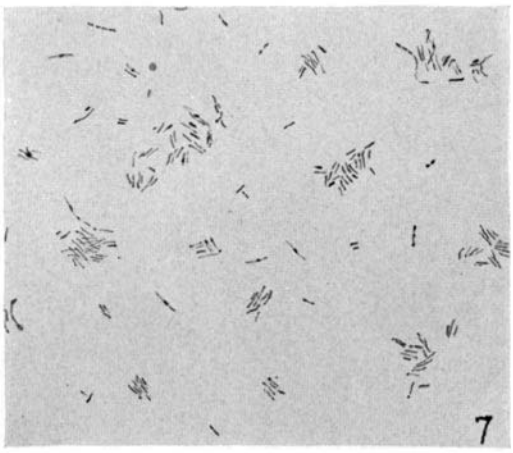
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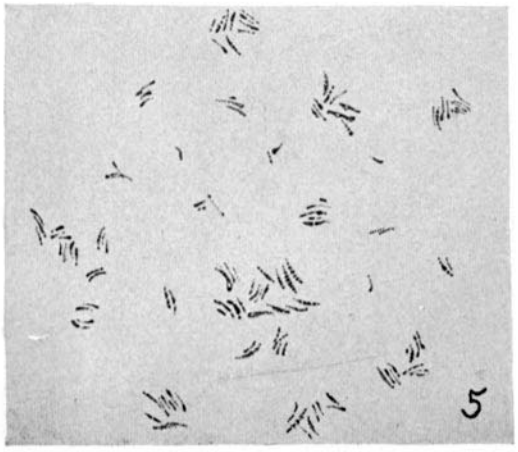
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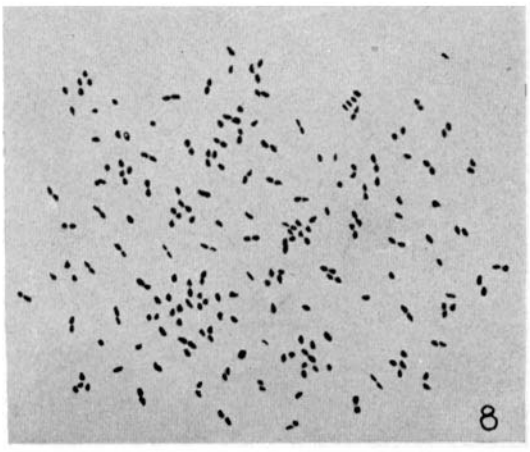
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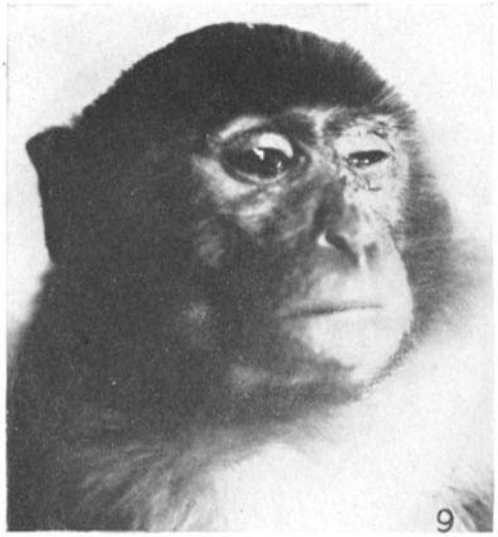
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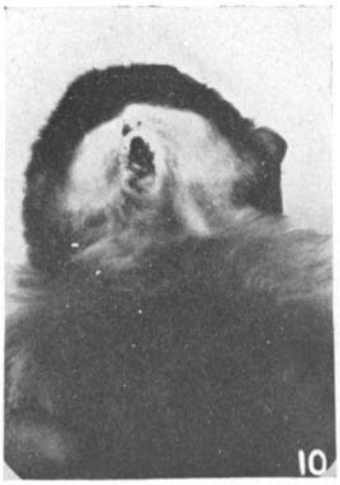
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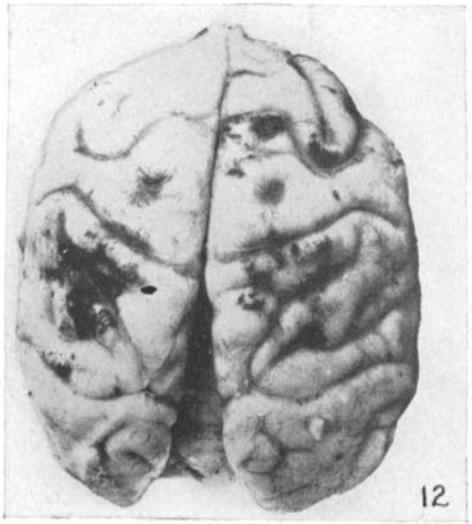
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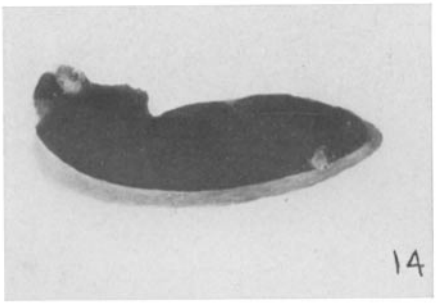
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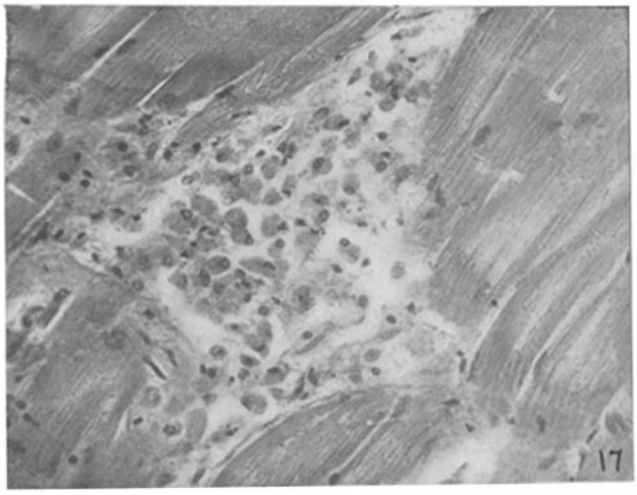
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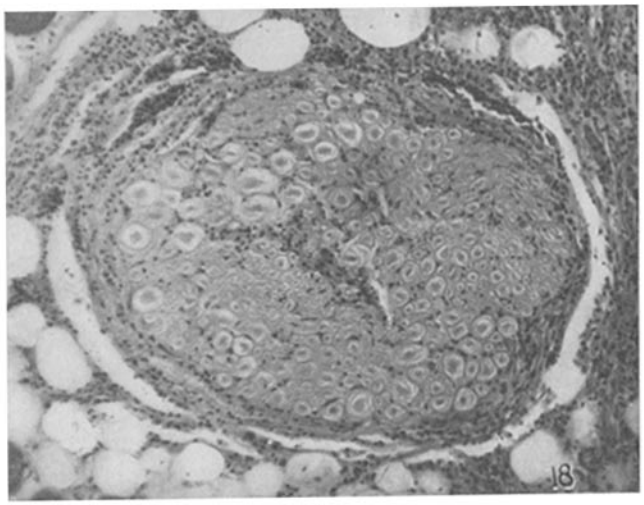
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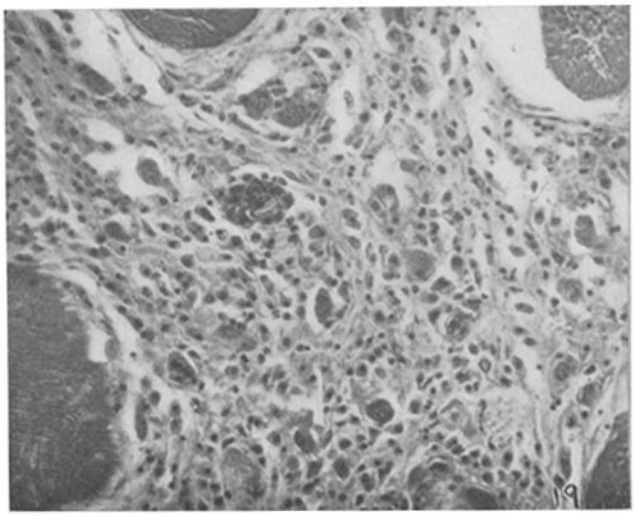
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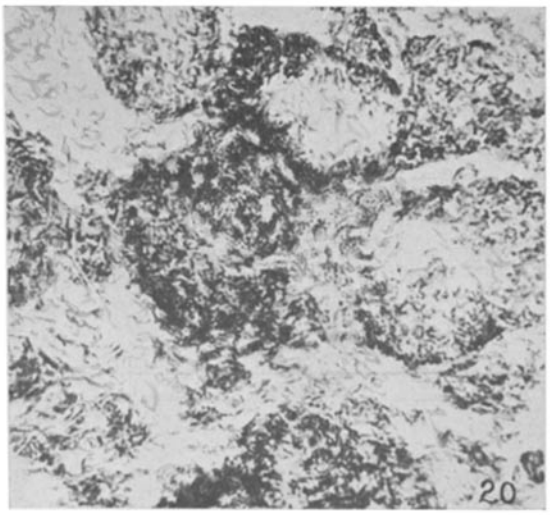
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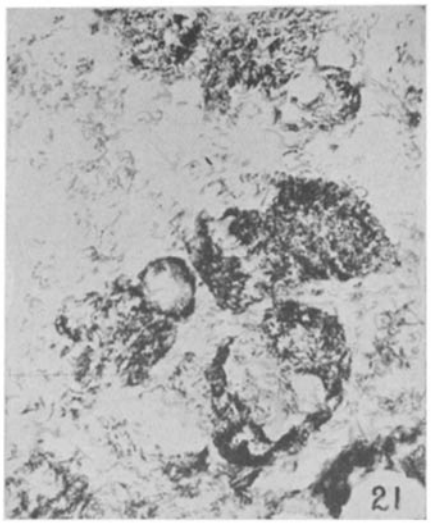
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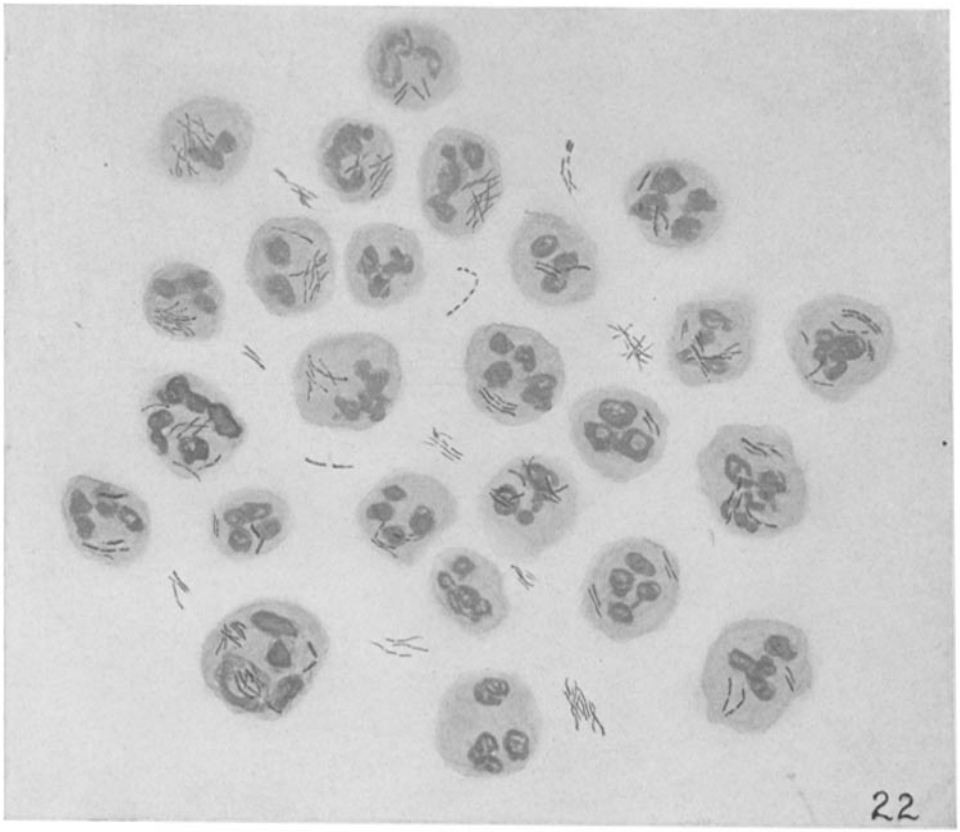
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