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# THE BACTERIOLOGY OF LEPROSY\* A REVIEW

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#### THE HISTORY OF LEPROSY

Leprosy is one of the oldest diseases known to man, a truly ancient problem. In this heritage it ranks with at least two other diseases, namely, syphilis and trachoma. Our records of it go back some 3,500 years (156) and references to it in the Bible extend over a period of some 1,500 years, from 1491 B.C. to 33 A.D.

\*This review in a somewhat more extended form was received before the unfortunate death of the author, but the editorial revision of it, made with his consent, was not seen by him.—Editor

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The first Biblical reference to leprosy is in the fourth chapter of Exodus, according to which God gave the disease to Moses, though only for a brief moment, making him the first recorded leper in the chronicles of the Book. In the thirteenth chapter of Leviticus is a detailed description of what was thought of at that time as leprosy, and in the following chapter appears the "law of the leper" that was given by the Lord to Moses. The methods of cleansing the leper of his disease are described, as well as rules for the destruction of leper houses and the disposal of unclean materials. In other Biblical references astonishing miracles are described in the form of cures or cleansing of the disease, or on the other hand in the almost instantaneous appearance of it. In some references there is evidence that, in those days, leprosy was regarded as a curse which affected the issues of man by heredity; for example, in II Kings 5:27 it is said that "the leprosy therefore of Naaman shall cleave unto thee, and unto thy seed for ever."

Down through the ages this story of a plague known as leprosy, which is given so much attention in Biblical history, has established in the minds of many peoples of the earth the idea that the disease is one to abhor; that it is a curse to man and his children; that it is associated with filth and poverty and all that is unclean and detestable. From this attitude arose profound social implications in the view that it was necessary to segregate its victims from their fellow men; that, in short, the leper is a thing to be avoided and to be removed from sight and thought. History depicts nothing more tragic than the leper.

A question that is frequently asked today is whether or not the plague called leprosy in the Bible could in reality have been the disease that has been recognized by that name in more recent times. Many authorities believe that it was not. As Garrison says, modern dermatologists contend that Biblical leprosy (zaraath) was in reality psoriasis, and that is probably correct in part; but it is also more than probable that other diseases too were confused with leprosy.

Turning to the less uncertain terrain of more modern records, there is indisputable evidence of the existence of this disease many centuries ago. As far back as the fourteenth century there were made records of such technical observations as the symptom of anesthesia. Even at that time it was suggested that leprosy is contagious, and manuscripts contain records of the civil status

of supposed lepers. Walsh (280) indicated in "The Popes and Science" that Emperor Frederick II, in 1224 A.D., issued an edict regarding the practice of medicine from which it appears that one of the duties of the physician of that time was to determine the diagnosis of leprosy, because of the civil status of such patients. The existence of leprosy, then, assisted the physician in establishing an important place for himself in society and with the State; and, too, its existence and wide prevalence assisted greatly the development of hospitals. We are told by historians that in the fifteenth century people having such diseases as leprosy, trachoma, anthrax, bubonic plague, and a few others were not permitted to enter cities or were isolated or driven from cities, and such persons were not allowed to handle food and drink for sale. By that time books had appeared with descriptions and illustrations of leprosy, some old and some modern for the time. By the middle of the sixteenth century leprosy, among other epidemic diseases, was abating in many parts of Europe, so that the old lazar houses were abolished in those parts, though it persisted in a few areas, especially in Norway and Sweden.

The beginning of modern dermatology is said to date from the unfinished work of Willan, "On Cutaneous Diseases" (1796-1808), which was completed by his pupil Bateman. In this notable work psoriasis, the Biblical "leprosy" of Gehazi and Naaman, was more clearly defined and differentiated than before. It was in about this period that the study of tropical medicine had its beginning, marked by the treatise by James Lind (1716-1794); interest had turned to the matter with British activities in India. From that time on we find more accurate records and descriptions of leprosy, though over one hundred years were to elapse before Hansen observed the bacillus with which his name is associated: Later came the science of bacteriology, within which field comes the etiology of leprosy, though as will be seen various theories on that matter other than the bacterial—including the fish theory and the scurvy theory—were advanced and held for many years.

In summarizing the history of leprosy, we may say that it is generally conceded that this disease was known to the ancient Chinese, Indians and Egyptians, but that Biblical "leprosy" was probably psoriasis. Real leprosy was probably introduced into Greece three or four hundred years B.C., and by the 7th century A.D. it was quite prevalent in Southern Europe. Manson-Bahr (140) states that it was introduced into England about 950 and that the last British leper died in the Shetland Islands in 1798.

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At present the disease is widely distributed in tropical and subtropical countries, though it also occurs to some extent in colder climates. Estimates of the number of lepers vary from one to four millions (160), and in our survey of the geography of disease, in 1935 (157), nearly one and one-half million cases were recorded. The disease is common in India, China, the Philippines and certain parts of Africa. It still lingers in several other localities, including some of the European countries, parts of the West Indies and South and Central America, and even in the United States. There can be no doubt that leprosy still remains an important public health problem, one which every country that has lepers within its borders must take into account. The continued study of its etiological agent and of methods of treatment and control are, therefore, most important.

There is perhaps no problem in medicine which has been the subject of more acrimonious debate than leprosy, particularly with regard to the question of its etiology. Differences of opinion that have existed have at times become almost polemic in character, leading to controversies that have been emotional, relegating scientific and intellectual considerations to secondary status, with consequent impeding of the progress of discovery. However, a careful examination of the literature on leprosy during the past sixty years of investigation reveals that some definite progress has been made. It is the purpose of the present review to trace the development of our knowledge of the bacteriology of the disease from the time of Hansen to the present day.

# EARLY PERIOD OF THE BACTERIOLOGY OF LEPROSY

The microorganism known as *Mycobacterium leprae* was first described by Hansen ( $^{91}$ ) in 1874. He had first noted rod-like organisms which he thought were bacilli, in the cells of freshly excised lepromata; but staining methods were not then available to him and it was not until 1880 ( $^{92}$ ,  $^{93}$ ) that he was able to apply such methods to the organism. This was early in the age of bacteriology, and the beginning of an entirely new line of investigation in the study of leprosy. In the latter papers referred to Hansen discussed the claims of Neisser and of Edlund, both of whom had visited his laboratory and later published their opinions that the organism of leprosy was a micrococcus (Edlund), and a bacillus (Neisser). Hansen stated his case for priority of discovery, pointing out that Edlund's observation of micrococci in the blood of lepers was unreliable. He reported a careful study in which he demon-

strated bacilli in scrapings from nodular lesions after staining with methyl violet, a method which had been suggested to him by Koch. A plate of diagramatic drawings illustrates the morphology of the organism, showing variations from typical rod-like structures to chains of coccoid forms, as well as the peculiar grouping of the organisms now spoken of as globi.

During the same year—1880—Hillairet and Gaucher (104) claimed to have demonstrated organisms from the blood of lepers, attempted cultivation having given a filamentous growth, but that most certainly was a contamination. Harris (99) described certain microscopic preparations of leprosy tissue but made no mention of finding bacteria in them, and the same is to be said of a similar study by Caley, Liveing, Duckworth and Powell (32). A word of caution was introduced at this time by Besnier (24) who writing on the contagiousness of leprosy stated that it was premature to accept Hansen's observations as proven fact.

The work of Hansen was, however, soon to be confirmed by various investigators. In 1881 Cornil (44), in a general discussion of the bacteriology of leprosy, described Hansen's organism. Cornil and Suchard (46) again confirmed the finding of the bacillus in the tissues; they suggested that its distribution between the tissue fibers constituted a barrier against its diffusion to the outside, and postulated that this accounted for the difficulty of contagion. Their colored illustrations leave little doubt that they were dealing with the true organism of leprosy. In an additional report Cornil (45) described leprous tissues from various organs (cutaneous nodule, lymph node, cornea, larynx, liver, testicle and cubital nerve), and stated that the microbe differs in size in different locations, it being five or six times as large in testicular tissue as in the skin.

These few early papers stimulated much interest in the question of the etiology of leprosy. For more than fifty years the organism observed by Hansen was to be a subject of debate, particularly with regard to its artificial cultivation, for it proved to be refractory to the ordinary methods of growing bacteria and, indeed, has seemed to be the ideal obligative parasite. Hopes of cultivating it *in vitro* have not, however, been entirely given up, and evidence is accumulating that it has finally been cultivated successfully upon artificial media. The original methods of study were, as has been seen, both subjective and objective, but they were more or less crude. As the science of bacteriology developed other methods were applied to this problem, though what is here

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considered the "early period" of the bacteriology of leprosy extended well into the 20th century; for reasons that will become evident that period is here considered as extending up to about 1918. That year did not bring any accepted solution of the problem of cultivation of M. leprae, but there were developments which stimulated further research in this field.

#### THE EARLIER METHODS OF STUDY

Gaucher, in 1881 (86), obtained some culture media and the use of an incubator from Bouchard and reported the growth of micrococci in the blood of lepers; these organisms, which occurred singly and in chains, were without doubt contaminants. Hansen's first attempts to cultivate his organisms had been unsuccessful, but in 1882 (95) he reported on cultivation work in which he had used gelatin and solidified blood serum, as suggested to him by Koch, and had obtained some long filaments composed of several bacilli. After five days, however, the bacilli were transformed to "grains" -no doubt meaning granular forms. He injected the ear of a monkey with his supposed culture, but after four months there remained no evidence of the injection; previously he had inoculated a monkey with leprous material, which was completely absorbed. In the same paper he reported finding no organisms in two specimens from the anesthetic form of the disease. In another one (94) he recorded negative results following the injection of rabbits and cats with leprosy material, and including a reference to similar results obtained by Kobner (123) in both monkeys and fish. At this time Hansen remarked—as is often done today—that leprosy is "cette maladie énigmatique."

In 1882 Thin (255) wrote that the bacilli are always to be found in cells as small as white blood cells and in lymphocytes and suggested that the disease might be spread through the lymph cells. He also mentioned the beaded appearance of the organisms; and for the first time, so far as we have been able to ascertain, he mentioned the fact that they retain fuchsin following the action of dilute nitric acid, which represented the introduction of a method still employed today in the study of this organism. In 1884 Patrick Manson (139) pointed out that the diagnosis of leprosy was still difficult and somewhat impractical. He suggested the method of squeezing the leprosy nodule to make it ischemic and then pricking it with a needle to obtain pure exudate, which was spread on cover-slips, dried and stained. He stated that he had found this method easy, expeditious, and a reliable way to diagnose leprosy.

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Simple staining methods had been introduced some years before, and these as well as new methods now became the subject of extended study by several histopathologists. Notable among them was Unna, who over a period of years was to contribute many reports dealing with staining and the histopathology of leprosy, culminating with an illustrated summary of his methods and findings (262). Steven (249) also presented a study of the skin of a typical case, concluding that leprosy is dependent upon the presence and development of a specific organic virus. Unna (263) described the peculiar clumping of the organisms, Hansen (96) applied the Gram stain to them, while Arning (7) searched for them in the neural type of the disease. This last author found that in putrid leprous tissues, or in the body of a leper who had been dead for three months, the bacilli were still to be found in great numbers. In 1884 he made the well-known inoculation experiment with a condemned criminal, to be mentioned again later.

A study of the morphology of M. leprae by Lutz (136) is of some interest at this time. He described and presented sketches of single and double club forms, coccoid forms and chains of these, and peculiar, rather large, round forms with rod-like tails and thread-like masses, all of which he believed represented various forms of the leprosy organism and illustrated its pleomorphism.

In 1888 Rake (198) reported on interesting attempts to cultivate the organism, in which he had employed various kinds of nutrient media, including serum from the blood and other sources. mixtures of serum with gelatin or agar, and ascitic fluid. For inoculum he used cutaneous nodules, femoral glands, pieces of viscera, nerve tissue and fluid from blisters and blood. Only from tissues (which gave plentiful degradation products, elements which over twenty years later were to be claimed to be essential for the cultivation of the bacillus) did he obtain any cultures. From such fragments of putrescent tissues he obtained smooth growths like drops of oil paint, canary-yellow, salmon-colored and white; common molds were also often present. These various growths included cocci, streptococci, large rods and small rods. He concluded that he had not succeeded in cultivating the leprosy bacillus. During the same year Bordoni-Uffreduzzi (26) reported the cultivation of a diphtheroid from leprosy postmortem tissues in peptone-glycerin-blood-serum. Inoculations of this organism into guinea pigs, rabbits and mice were all negative.

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At this time there were still those who doubted that leprosy is contagious, and failure to cultivate the organism and to induce the disease in lower animals did not serve to weaken that opinion. In 1889 Stallard (24) concluded that the spread of leprosy in the Sandwich Islands stood as absolute proof that the disease is contagious, but that it is only feebly so, less than tuberculosis. That was written nearly fifteen years after Hansen's discovery of the bacillus, and several years before the first International Leprosy Conference (Berlin, 1897).

Neisser (173, 174) continued a study of methods of staining the bacillus and suggested the use of rosanilin and pararosanilin. With regard to the coccoid forms of the organism, he raised the question whether they were of primary or secondary importance in the mechanism of infection. A report by Delépine (55), dealing with the different characters of the organism in different organs of the same patient, was interesting in that he demonstrated the bacillus in the sputum, bronchial nodules, the mucous membrane of the trachea and larynx, striped muscle, spleen, phalangeal joint and bone (found around the vessels in the Haversian spaces), intestinal ulcers and liver, as well as in the skin from various parts of the body. Thin (256) described tuberculosis complicating leprosy, and Campana (36) recorded secondary streptococcic infection. Stephan reported finding M. leprae in the blood stream of a case of the anesthetic type. Unna (265) pointed to the fatty substance in M. leprae and M. tuberculosis as differentiating them from other forms of bacteria.

During this period most investigators were concerned chiefly with demonstrating the presence of the organism in the tissues of lepers. The period definitely belonged to the pathologist, to whom is due much of the information about leprosy that was at hand early in the nineties of the past century.

#### ATTEMPTS TO CULTIVATE HANSEN'S BACILLUS

The period 1890-1897.—In the preceding section mention has been made of some of the early attempts to cultivate M. leprae, including those of Hansen himself. In 1891 Kanthack and Barclay (112) reviewed previous work and reported their own experiments with culture media available to them (glycerine agar, employed in hanging drop and in Roux tubes), and concluded that they had met with little success. Ducrey (58), in 1892, claimed positive results in glycerin broth and in stab cultures of solid media in hermetically sealed tubes; he concluded that the organ-

ism is an anaerobe. In 1889 (33) and 1891 (34) Campana had reported unconvincingly on his cultivation work, but he now (35) reported his further efforts in which he used broth and peptone agar with 3 percent grape sugar. He claimed to have obtained in from seven to nine days, in the depths of the medium, growths of organisms which were slightly acid-fast and that contained acidfast granules. Byron (29) reported obtaining a pure culture of the organism, and a similar claim came from the laboratory of Rocca (207), but these results remained unconfirmed.

In the meantime the various theories regarding the etiology of leprosy were still being widely discussed. As early as 1889 these theories were reviewed by Abraham (1). He stated that Jonathan Hutchinson, the chief proponent of the fish theory, admitted that leprosy occurs in immigrants as well as in leper families, but that he still adhered to the belief that the consumption of fish, particularly of decomposed or salted fish, was responsible for the disease. Abraham also brought out the fact that many people who eat fish do not develop leprosy, recalling the observation of Thalozan, in Persia, that there was little leprosy in the lowlands of that country where fish is consumed, but much of it in the mountains of Kurdistan where the people do not eat fish. He mentioned Blanc's observations of 42 lepers from various countries who were living in the United States, and also mentioned Hansen's trip to America where in Wisconsin, Minnesota and the Dakotas records were found of 160 lepers, none of whose descendants had the disease. Seven prominent men who favored the contagion theory were listed by Abraham, and four who favored the noncontagion view, there being still men who held that the disease is hereditary. Rake, of Trinidad, according to Abraham, had had negative results of animal inoculations, favored neither theory, and believed that Arning had not conclusively demonstrated human transmission. He also said that Bangilli and Profeta, of Sicily, had reported negative human transmission experiments. He recalled the fact that in 1867 a questionnaire had been sent to 250 workers and that the vast majority did not believe in the contagion theory. The Royal College of Physicians at that time had paid little attention to the minority who favored contagion, but twenty years later, in 1887, a special committee of the College admitted the possibility of contagion.

A second paper of this period that is of great interest was a review by Unna (264) in which he stated that the core of the leprosy bacillus consists of a row of granules comparable to free cocci,

that these are surrounded by a capsule, and that a row of three, four, or eight of these granules in a capsule resembles a rosary (Lutz' "coccothrix"). Both Unna and Lutz suggested that M. leprae holds a position between cocci and bacilli. Unna also described, for the first time, an inner capsule surrounded by the outer one running tangentially over the cocci to give the picture of a rod. The bacillus, he stated, is covered by a glassy, mucous substance which holds many together as a clump or "zooglea"; there are no spores, and the nucleus consists of elements equivalent to these cocci; the unstained spaces are not signs of degeneration, but a feature of the normal development of the bacillus. Present-day advocates of theories regarding stages in the cyclogeny of bacterial cells would find comfort, no doubt, in these early views. Unna also commented on the nature of the so-called lepra cell, which at that time he believed did not consist of animal cell protoplasm but of vegetable mucus of bacterial origin. He stated that the gloea permeates the entire tissue in the direction of the lymph spaces between the fibers and cells of connective tissue and between cells of the prickle layer and the hair follicle, fills up the lymph spaces with sausage-shaped masses, leading to proliferation of connectivetissue cells quite apart from any process of inflammation. He concluded that only in acute eruptions is inflammation present. and that one-half or three-fourths of lepromata in substance consists of organisms.

The period 1897-1900 .- To return to the question of cultivation of M. leprae, up to the time of the Berlin conference (1897) and for the next three years after it, there were few claims to actual success. Only diphtheroids and possible anaerobes had been cultivated; no chromogenic organisms, acid-fast or nonacidfast, had been described with the exception of those obtained by Rake from putrescent tissues, and Rake himself did not consider them seriously as related to the disease. In 1897 Lévy (131) reported growing, upon glycerin agar with human blood serum, a diphtheroid that was nonpathogenic to rabbits, guinea pigs and mice. Similar diphtheroids were cultivated by Spronck (240), who obtained growths on glycerin-potato, blood-serum, and agar, and demonstrated specific agglutination with leper serum in rather high dilutions (1:1000); by Czaplewski (53), who got them from the nasal secretions and from an ulcerated nodule on sheep's blood-serum with glycerin, with negative results following inoculations into rabbits, guinea pigs and mice; and by Teich (254), who also cultivated them from nasal secretions.

Among the most interesting papers presented at the Berlin conference is one by Ashmead (9) who offered a theory concerning the development of leprosy as a disease, which he supposed to have had its origin in Central Africa. He postulated a bacillus which originally was no offender but which, whether suddenly or gradually, underwent "variation" and became virulent and began to ravage mankind. He held that after a disease like leprosy has affected a race for some time it loses its virulence, and suggested that varying degrees of inbreeding among a people favor immunity to the disease.

In these three years interest in the general subject of leprosy did not wane, as is illustrated by papers by Weber (281), who reported finding the bacilli in the sperm; by Calabrese (31), who found them in the urine of a leper with nephritis; by Riatti (205), who observed them in the spinal ganglia; and by Scange (217); who produced large accumulations of organisms by transplanting leprosy material into the brains of pigeons. In 1898 Babes (10) published a treatise on the leprosy bacillus and the pathologic anatomy of the disease (expanded two years later to a more comprehensive work). Later (11) he suggested that the bacillus elaborated a toxin and that some of the general and local symptoms of the disease. particularly those referable to the central nervous system, were due to such a toxin. Scholtz and Klingmüller (221) reported on their cultivation work, in which they used a considerable variety of media but isolated no germ which they felt they could call M. leprae. Also, they could not extract from leprosy bacilli any substance analogous to tuberculin. They concluded that the cultures of organisms that had been reported probably have nothing to do with leprosy.

The period 1900-1909.—In 1900 and 1901 came reports of the cultivation work of Kedrowsky (115), who described an organism which we must class as a diphtheroid, together with some of those previously reported. This organism, isolated from leprosy tissues in two cases and from an abscess, was grown on placenta extract agar but later subcultured on plain and glycerin agars. Young cultures were acid-fast, but as they became older they lost their acid-fastness except for their granules. Kedrowsky claimed to have recovered the organisms from rabbits several months after inoculation. This work left the problem about as before, but it served to keep interest and hope alive.

For the next few years, until 1903, little was reported in this line of investigation. In that year Kedrowsky (116) reaffirmed his belief that he had succeeded in cultivating the germ of leprosy. Karlinski (113) stated briefly that he had cultivated it from sera from three patients; later, in 1912, he was to report the cultivation of an acid-fast nonpigmented organism which he was able to keep alive for several months. Guarch (89) published a review that added nothing new to the picture.

In 1904 Rost (212), in Rangoon, reported that he had succeeded in growing the leprosy germ on a beef medium from which the salts and chlorine had been eliminated. The organism grew well in dialysed broth, and it produced a white or slightly yellow, stringy growth, particularly yellow on solid media when the salts were removed. He classified the acid-fast bacilli as of the "a-chloretic group," comprising *M. leprae*, *M. tuberculosis* and *B. lustgarten*. With his culture he prepared a "leprolin" (not to be confused with the test antigen of Mitsuda) by growing it in distilled beef extract for six weeks at 37°C., sterilizing the cultures, passing them through a Pasteur filter, and adding glycerin. In patients treated with this substance sensation returned to anesthetic areas and some nodules exhibited signs of breaking down. No animal inoculations with the culture were reported.

In the following year Weil (282) reported the cultivation of *M. leprae* on egg medium; and Klitin (121, 122) cultivated from excised nodules organisms that must be added to the group of diphtheroids already described, although this author stated that his cultures produced lesions in rabbits and guinea pigs and that it was possible to recover the organisms from these lesions. At about the same time Turner (259) reported failure to confirm Rost's findings; but he defended Rost, who had been receiving much criticism, and pointed out that he was not the first investigator to make a mistake with regard to the cultivation of this organism and that probably he would not be the last one to do so.

Up to this point (1905) no organism cultivated from leprous material had been generally accepted as the causal agent of the disease; the bacillus still remained apparently the classical obligative parasite. In the next four or five years no new observations on this matter were recorded, though as usual there were many papers that dealt with other phases of the leprosy problem. Among them was one by Hansen (97) on his views concerning the fish theory, in which he did not believe; by Wherry (284, 285) on rat leprosy and on acid-fast organisms from rat leprosy and human leprosy in flies (this author found the bacillus of rat leprosy in the rat louse); by Sugai (250) who, as did Wherry, studied the

agglutination of M. leprae in the sera of lepers, but who, unlike Wherry, found that it agglutinated in dilutions as high as 1:500, and with most types as high as 1:100, though control sera were entirely negative; by Gaucher (87), who also stated that serological tests with the leprosy bacillus were of value as an aid in diagnosis; by Lutati (135), who described the granular degeneration of the bacilli; by Recio (199), who discussed the mode of spread of leprosy and concluded that, given a predisposition to the disease, the chief avenues of entrance of the infectious agent were through the nasal mucous membranes and by way of the hands, especially in children; by von Düring (60), who commented on hereditary factors in leprosy, to which he gave scant importance; and by Boeck (25), who reported finding M. leprae in the feces, where he stated it might remain for a year or more. These papers give at least some idea of the lines of investigation which were in progress during this period.

The period 1909-1912.-This brings us into 1909, and on toward the end of what we have arbitrarily called the early period of the bacteriology of leprosy. In that year we find the work of Clegg (42, 43), in the Philippines, who reported the cultivation of M. leprae in symbiosis with the ameba, the theory being that in the lesions the bacillus obtains its nourishment from the products of the metabolism of the tissue cells and that the amebae would provide such substances in the cultures. Material containing amebae was spread upon the medium in plates and, if symbiotic bacteria (cholera vibrios or typhoid bacilli) were present, Clegg stated, the amebae grew in from two to ten days. Leprous spleen emulsion containing acid-fast bacilli was then added to this culture, and by serial subcultures multiplication of the bacilli was demonstrated. These acid-fast rods were short and plump, differing from the usual morphology of M. leprae, but Clegg rightly asserted that nothing was known with certainty about the morphology of this organism upon artificial medium. There was the usual question of whether or not the bacilli in these cultures were really multiplying, but in a subsequent communication Clegg stated that by heating the culture containing amebae, symbiotic bacteria and acid-fasts for 30 minutes at 60°C. a pure culture of M. leprae had been obtained. Thus isolated it grew on ordinary laboratory media. Inoculation into guinea-pigs, Clegg stated, resulted in the production of lesions macroscopically and microscopically similar to leprosy in human subjects.

In 1910 Currie, Brinckerhoff and Hollmann (51), having

employed Clegg's method, confirmed his work. The ameba-cholera-lepra cultures were carried through from three to ten generations. As will be seen, this organism of Clegg belongs with the group of acid-fast chromogenic bacilli such as several other investigators have isolated from leprosy.

In the meantime Teague (253) had made 5 percent glycerine extracts of leper nodules and spleen, and (for a control material) the skin of cholera patients, and had vaccinated 50 lepers and 50 nonleprous persons. For the most part the reactions were the same in both groups. Wooley (293) had previously used saline extracts as a sort of tuberculin.

In 1910, also, Duval (61) published the first report of his cultivation work, which had begun in the previous year for the purpose of confirming the work of Clegg and of Sugai—the latter of whom had reported success in infecting Japanese dancing mice with leprosy material. Duval also intended "to attempt further to grow the bacilli directly from the tissues without the aid of symbiotic organisms, and to prove by animal inoculation the identity of the culture." The organism once started, he said elsewhere (62), the amebae, or tryptophane, were no longer essential; the cultures grew well on any neutral or slightly alkaline medium. This organism of Duval belongs to the same group of acid-fast chromogens as does Clegg's bacillus.

In the following year Duval (63) stated that after the initial culture had been started "growth is luxuriant and reaches its maximum in forty-eight to sixty-four hours"; also that, like the tubercle bacillus, these cultures "require abundant oxygen." (Both of these statements. will be referred to later.) In 1912 Duval and Wellman (74) described a new method of obtaining the organism, based upon the use, either in a liquid medium or with glycerinated agar, of mammalian placental tissue extract such as was originally employed by Kedrowsky in 1901. With these media the growths were profuse—both the initial cultures and subsequent transplants, it was said. Some of the cultures were without pigment while others were distinctly chromogenic. Elsewhere (75) these authors published a critical study of these organisms, with a consideration of their etiological significance. The findings may be summarized as follows:

There were two kinds of acid-fast organisms in the 22 cultures that had been grown from 29 cases of leprosy. These were: (a) a chromogen essentially similar to that of Clegg (14 cases), and (b) a nonchromogen of very different character (8 cases). One strain of a nonacid-fast diphtheroid,

held to correspond to that of Kedrowsky, was also obtained. The opinion of Rost, Williams, Bayon and others that the leprosy bacillus is one of such pleomorphism that it can be a streptothrix, a diphtheroid and an acidfast bacillus was not shared.

The chromogenic organism started growth with difficulty, but subsequently grew readily and profusely upon ordinary media. It was pleomorphic and showed wide variations in its morphology, and also in its acidfastness. The moist-growing, nonchromogenic strain, on the other hand, grew only on special media, remained difficult to cultivate even after repeated subculturing, and did not become chromogenic. Tinctorially it resembled the tubercle bacillus, being always acid-fast; morphologically it resembled the diphtheria bacillus.

Inoculation experiments with both types were regarded as inconclusive. Serological tests indicated that the Clegg organism was not related to the ordinary acid-fast chromogenic saprophytes, and that the Duval organism (nonchromogen) was different from all other acid-fast bacilli. The role of Clegg's bacillus in the etiology of leprosy the authors held to be unsettled; they were "inclined to ascribe to it a minor if not a negligible part." That of Duval, on the other hand, was considered as deserving "more serious attention than any other organism so far cultivated from the human leprous lesion."

During this same period several other reports on cultivation work appeared. In 1910 Kedrowsky (117, 118) made a supplementary report, as did also Campana (37) and Küster (127). There was nothing new in these reports. Twort (260) described the use of Dorset's medium to which ground-up M. leprae had been added; with it he obtained a slow growth of a nonchromogenic acid-fast bacillus which later grew faster, but subcultures on ordinary laboratory media were negative.

Serra (222) reported an organism which he cultivated from three cases out of seven, on Campana's medium combined with sterile organs of guinea pigs. Subcultures were obtained in a few days in the depths of tubes of glucose agar, this again suggesting that the leprosy organism was anaerobic. Animal experiments were negative.

MacLeod (138) published an interesting and comprehensive review of the state of knowledge of leprosy, in which he said that no medium had been found on which the leprosy bacillus would grow invariably. Darling (54) quoted Hansen as stating (in 1903) that the leprosy bacillus must be regarded as an obligative parasite which can thrive only in the tissues of man, and he accepted this statement as the last word on the subject. It may be noted that Bayon (15) called attention to the fact that Sir Patrick Manson was the first to attempt to cultivate the germ of leprosy—he had sealed the serum of a leper in a little glass tube, placed it inside an egg and put the egg under a hen to hatch.

The work of Williams in this period was of considerable interest at the time. In 1910 (289) he reported growing a streptothrix and a bacillus in ordinary nutrient broth. The first was nonacid-fast, the latter acid-fast; both caused general and local reactions when injected into lepers but neither was infective for laboratory animals. In the following year (290) he stated that both of the two bacterial forms that he had isolated-a nonacidfast diphtheroid in addition to the acid-fast bacillus-were phases of the streptothrix. The diphtheroid he made acid-fast by cultivating it with the ameba. He quoted Unna regarding the large and varied series of forms that the leprosy organism may present, and concluded that the causative agent of this disease is a pleomorphic streptothrix. Liston and Williams (133) also reported the isolation of a streptothrix from the spleen of a leper. It, they said, resembled the organisms described by Deycke and by Rost, and exhibited many variations in growth, staining, morphology, etc. Then Williams (290) attempted to classify the organisms found in their work, these being a nonacid-fast diphtheroid, a nonacid-fast streptothrix in mycelial form, and an acid-fast streptothrix in both mycelial and bacillary forms. At about the same time Rost (213) reported cures of leprosy by the use of streptothrix vaccines.

Bayon (14) obtained a diphtheroid from leprous material, using placental-extract agar or horse-serum-nutrose agar plus 2 percent of ground-up smegma bacilli. This diphtheroid, he said, acquired acid-fast properties on being injected into mice or rats, or when cultivated upon Dorset's egg medium. Later (15) he reported some interesting new work in which he had been able to produce nodules and lesions in rabbits by injecting the smegma bacillus and such other acid-fast germs as human and avian strains of M. tuberculosis, as well as killed acid-fast organisms. He had been unable to produce anything similar to leprosy with Duval's organism. He favored the view that the causative germ of leprosy is a streptothrix, and stated (16) that Kedrowsky's organism was like those described by Hansen; and that he himself had also isolated a similar germ. Human and rat leprosy Bayon considered to be identical or closely allied diseases; he pointed out that Dean had reported that the rat leprosy germ is agglutinated by the serum from human lepers. In the following year (19) he reported a comparative study of the leprosy cultures of Clegg,

Duval, Rost, Williams and Kedrowsky, from which he had concluded that only Kedrowsky's organism, and those similar to his own, produced leprous lesions in animals; he believed that the other ones mentioned were saprophytic.

During the same year—1912—Duval and Harris (73) and Duval (65, 66) published further papers on the status of the bacteriology of leprosy. Currie, Clegg and Hollmann (52) reviewed the literature on the subject and stated their belief in the organism of Clegg. Smith and Rivas (228) employed trypsinized culture media and reported six successful transplants of M. leprae.

Working with rat leprosy, Zinsser and Carey (295) tried to cultivate its germ in tissue cultures. Using the spleen tissue and serum of young rats, they found that the organisms grew and multiplied in some of the cultures, but apparently never outside of the tissue cells. Hollmann (105) also worked with rat leprosy and reported success in the cultivation of its organism with Clegg's method, and further stated that acid-fasts were demonstrated in the tissues of white rats following inoculation of his cultures. Guinea pigs were negative.

A further survey of the literature of this period gives additional evidence of the great interest which existed in the leprosy problem. Much (167) reported that the sera of lepers reacted with other acid-fast organisms as well as with M. leprae; Duval. Gurd and Hopkins (72) presented further studies dealing with immunity in leprosy; Abraham (2) published an additional review of the cultivation work; Alderson (3) reviewed the work of Brinckerhoff, Currie and Hollmann in Hawaii (already commented on above); Hansen (98) discussed again the question of heredity in leprosy; Sorel (231) studied the analogy between rat and human leprosy and concluded that in each the infection may remain latent and never produce symptoms of the disease; Leboeuf (130) reported finding enormous numbers of the bacilli in Musca domestica; Lagane (129) added another report on the presence of acid-fast bacilli in the urine of lepers; and Kritschewsky and Bierger (126), on the basis of the Bordet-Gengou reaction, brought evidence to favor Kedrowsky's culture, in contrast with that of Duval.

The period 1913-1917.—Of special interest were the papers by Fraser and Fletcher (83) and Fraser (82). These investigators, taking special care to prevent contamination, removed leprous nodules from 32 lepers and made 373 inoculations on various media, including placental agar. Although their material was swarming with acid-fast organisms there was not a single instance in which they were satisfied that any growth or multiplication of the organisms took place. They believed that previous workers who depended upon microscopical evidence of growth must have failed to observe the bacterial richness of the material employed for inoculation, and they doubted the possibility of saying, in a case where no macroscopic growth is apparent, that an increase of organisms has occurred. "Inconsistency and pleomorphism," Fraser stated, "are the outstanding features of the recent publications on the subject of leprosy." Two years later Fraser and Fletcher (84) emphasized forcibly the need of careful excision of leprous tissue to be used for cultivation purposes. They reflected the skin from over the nodules to be removed and excised them free from contamination-a thing which, they pointed out, is not simple to do. In their earlier work they had grown diphtheroids in one or two tubes out of twenty-which organisms they regarded as contaminants-but in their later work such organisms did not appear. There was no evidence, in their opinion, in favor of Kedrowsky's bacillus as the true germ of leprosy.<sup>1</sup>

Reenstierna (201) in 1913 isolated organisms from leprosy tissue which he considered to be the same as Kedrowsky's and with which he attempted to infect animals; this work will be referred to again later. Further papers came from Duval in 1914-15 (68, 69), in which he stated that the real organism of leprosy is a bacillus, and an acid-fast one. He pointed out that Kedrowsky, in fifteen years, had been able to isolate from only two cases the organism which he, Kedrowsky, regarded as the germ of leprosy, and that Bayon, who believed that he had cultivated the same organism, had only recovered it once. Kendall, Day and Walker (114) studied the metabolism of several of the acid-fast organisms, and concluded that M. leprae differs in this matter from the grass and smegma bacilli. Wolbach and Honeij (292) considered the diphtheroid bacilli in relation to the leprosy problem, and in particular with regard to the presence of such organisms in normal and pathological tissues. They also presented (291) an excellent critical review of the bacteriology of both human and rat leprosy. Much of what they said in 1914 regarding this problem remains true today, as will be seen.

In the same year-1914-Johnston (109) reported 28 cultiva-

<sup>1</sup>These studies of Fraser and Fletcher are very significant in relation to the work of the writer with Soule, as will be pointed out in more detail later on.

tions of organisms from leprous material. Of these 20 were diphtheroids, 5 were other rods, and 3 were streptothrices. Animal inoculations, except in the case of one guinea pig, were negative. This author had become convinced that M. leprae represents only an acid-fast, bacillary stage in the life cycle of a markedly pleomorphic streptothrix. Later (110) he classified the various forms of M. leprae as (1) the classical type; (2) a fragmentary or degenerative type, including those with either coarse or fine granules; (3) the solid type, including long and short forms; and (4) the nocardial or streptothrix type.

Also in 1914 there appeared an interesting report by McCoy (154) of cultivation experiments involving material from 83 cases. One specimen came from a case of the anesthetic type, but the cultures were negative; 18 cases were "mixed," and three positive cultures were obtained from them; from 64 nodular cases eight positive cultures were obtained. Of these 11 strains, 9 showed various shades of yellow and grew freely, while the other 2 never showed more than the slightest suggestion of yellow and grew very slowly on plain or glycerin agar, though growth was luxuriant on glucose agar. None of the organisms was pathogenic for laboratory animals. Certain ones of them showed variations as regards acid-fastness in different environments. McCoy found that it was useless to make transfers of these cultures unless coccoid forms of the organism were present, which he felt were the first evidence of growth; when the organisms were in clumps, no matter what their number, there was no certainty that growth would take place. In spite of frequent transplantings, many of the inoculated tubes would not develop any growth, and though the cultures thrived for four or five generations they then disappeared.

Rost (214) in 1914 restated his belief in the leprosy streptothrix. In 1915 Fraser and Fletcher (85) presented further arguments to support their conclusion that the acid-fast bacillus of Kedrowsky was not *M. leprae.* Bayon (23) described bacillary deposits obtained in rabbits with organisms from leprosy nodules and with Kedrowsky's cultures. He stated that it is impossible to expect skin lesions in the experimental animal; that only discrete deposits of organisms should be expected in the organs; that hundreds of negative observations should not invalidate the proof positive of a single successful inoculation in an animal; and he again (22) stated his belief that Kedrowsky's culture was the true germ of the disease. At this time Stanziale (248) reported obtaining, on ordinary kinds of media, heavy cultures

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that from the illustrations which accompany the paper may have been diphtheroids. In the following year Harris and Lanford (100) published work with the agglutination reaction, using a number of acid-fast organisms with sera from human cases of leprosy and from experimental animals. They concluded that:

Until some further refinement in these procedures is devised, but little reliability can be placed upon this type of test as a means of identification of any culture isolated from the lesion of leprosy as the bacillus of Hansen.

During the period just dealt with there was, as usual, much activity in connection with other phases of the leprosy problem. There was evidenced considerable interest concerning the question of the presence of the bacillus in the blood stream. Marchoux (141) stated that it is found in the blood macrophages and exceptionally in the polymorphonuclear cells; Crow (49) found it in the circulating blood in about 80 percent of cases; Honeij (107) demonstrated it in the blood and noted that this observation warrants the assumption that insect transmission of the disease is a possibility; Alfonseca (4) reported finding it in a placenta, as well as in various secretions; Iyengar (108) found it in 7 out of 40 cases examined; and Hollmann (106) found it in the bloods of 6 out of 22 cases-in two instances only a single organism was found, but in a nodular case with lepra fever many acid-fasts were demonstrable in the blood though they could not be found after subsidence of the reaction.

Papers on the relation of rat and human leprosy were published by Bayon (17) and Marchoux (143), on insect transmission by Verteuil and Verteuil (274), who concluded that blood-sucking insects transmit the disease; by Aragas (6), who considered the possibility of mosquito transmission, and by Cumston (50), who discussed the various modes of spread of the infection, including that by way of insects, particularly *Demodex* as suggested by Borrel. Merian (165) published an interesting paper on the appearance of the bacillus in a cowpox pustule following vaccination.

# ANIMAL EXPERIMENTATION

From the time of the Hansen's discovery of the leprosy bacillus, through the several decades up to 1917, there were many attempts to infect experimental animals with leprosy by inoculating them either with human leprous materials containing large numbers of acid-fast organisms, presumably M. leprae, or with supposed cultures of the organism. Here we will review most of the important work reported during this period, so far as possible separately as regards the nature of the inoculum used.

Human inoculations.-It should be mentioned that many attempts (some 145, according to reports) have been made to inoculate man himself with human leprous material, but the results have not been satisfactory. There is perhaps no case on record of deliberate experimental human transmission which can be accepted as a proven fact, with the possible exception of that of Arning. In 1886 there appeared an editorial report (76) which described his inoculation of the arm of a condemned Hawaiian Fourteen months after the material had been introcriminal. duced, bacilli were found at the site of inoculation. There were no constitutional symptoms of the disease at that time, but subsequently the man became leprous (8). It has remained a moot question whether he was infected by the inoculation, as Vedder (271) believes is the case, or was infected naturally from leprous contacts.

Animal inoculations with human leprous material.—The attempts to transmit leprosy to lower animals began very early after Hansen's discovery of the bacillus. Indeed, Hansen himself was one of the first to make this attempt; in 1882 he reported (94, 95) negative results of inoculations of rabbits, cats and monkeys, and called attention to the negative results of Köbner (123) with monkeys and fish. Neisser's early animal experiments were also negative in so far as "real leprosy" is concerned, although suspicious nodules were produced in both dogs and rabbits. These early reports evidently did not stimulate much effort along this particular line of investigation, and one finds only scattered references to experiments of this kind during the next twenty years, which were mostly negative.

In 1905 Nicolle (176) described experimental lesions in bonnet monkeys inoculated in several places with saline emulsions of leprous tissue containing bacilli. The inoculations were made by scarification (temporal-frontal region), friction (conjunctiva and nasal mucous membrane), and subcutaneously (over left eye and in eyebrow). Four days after the inoculations no signs of the injections remained, but on the sixty-second day a subcutaneous nodule appeared and three days later it had extended markedly and the skin was adherent. Removed, unchanged, a week later, sections of this lesion showed numerous lymphocytes, mononuclear leucocytes, a trace of caseation, and M. leprae, two or more in cells. Nicolle believed the lesion to be a true leprosy one.

In 1908 Marchoux and Bourret (143) reported "negative" or "doubtful" lesions in chimpanzees following inoculation of leprous material. Two years later Stanziale (244) described the production of an experimental lesion of leprosy by injecting leprosy material into the anterior chamber of the eye of a rabbit.

In 1910 Nicolle and Blaizot (177) published a second report on the production of lesions in monkeys (Macacus sinicus). Lesions had appeared in two animals in 62 and 68 days, with bacilli demonstrable on the 56th and 37th days. They inoculated one of these animals again before the first lesion disappeared, and 13 days later there was a cold abscess which persisted for 100 days. A third inoculation resulted in a lesion which persisted for 63 days. In the second animal they produced lesions which persisted for different periods of time ranging from 29 to 150 days. It appeared that repeated inoculations resulted in a diminution of the incubation time and in lesions which persisted for increasing periods of time. Microscopically the lesions appeared like those of human leprosy, with M. leprae present both inside and outside of the cells. A year later the same authors (178) reported on further work in which chimpanzees were used as well as lower monkeys.

In the same year-1911-Marchoux (142) produced local abscesses under the skin of grey and white rats with nasal mucus from lepers, and the abscess material was found to contain acid-resistant bacilli which was cultivated, but he was not sure that he was actually dealing with M. leprae. Various other communications on this general subject appeared, including further ones by Stanziale (245, 247) on experimental lesions in the anterior chamber of the rabbit's eye, in which there was multiplication of the bacillus; one by Serra (224) again describing experimental lesions in the rabbit's eye; by Truffi (258) on the same subject; by Nakano (171), describing experimental lesions of leprosy in the Japanese house rat; by Chirivino (40) who also reported the production of nodules in the eye; and two by Verrotti (272, 273), who produced primary and secondary lepromatous nodules by intraperitoneal injection of leprosy material into rabbits, and secondary nodules in monkeys. Bayon (18, 20) reviewed the subject of direct inoculation of animals and concluded that the most suitable ones are the rabbit, rat, or mouse.

In 1916 Kyrle (128) introduced leprosy tissue into three *rhesus* monkeys and obtained nodular lesions which appeared in 18 to 22 days (similar, in point of incubation time at least, to those that McKinley and Soule (160) obtained many years later). About this time another paper on the production of leprosy lesions in the eye of the rabbit was published by Lutati (275).

Animal inoculations with material from cultures.—Among the very early papers dealing with this subject are those of Bordoni-Uffreduzzi (26), Lévy (131) and Czaplewski (53), all of whom employed supposed cultures of M. leprae in the inoculation of guinea pigs, rabbits and mice, with negative results. In 1900 Kedrowsky (115) employed his culture and reported that in rabbits he was able to produce granulomas containing acid-fast bacilli eight months following inoculation. Klitin (121, 122), in 1905, inoculated guinea pigs and rabbits with cultures of the diphtheroids that he had grown and reported the successful production of lesions, with subsequent recovery of short granular organisms, but these reports are not very convincing.

In 1909 Clegg (42, 43) reported the occurrence of lesions in guinea pigs following the injection of his cultures, which lesions he thought were quite similar to those of human leprosy, but they were not convincing enough to be judged positive, as subsequent events proved. Shortly afterward Serra (222) reported attempts to produce lesions in various animals with cultures of anaerobic bacilli obtained from leprosy material, but his results were quite negative.

In 1911 Bayon (14) injected his acid-resisting diphtheroid into mice and rats and recovered it later from their glands. He also reported (15) the production of lesions in rabbits following the introduction of cultures of *B. smegmatis* around the sciatic nerve. He found that such lesions could also be produced with *M. tuberculosis*, both human and avian, and later discovered that they could be produced with killed cultures of acid-fast organisms.

In 1911 Duval (64) reported the results of inoculations of his cultured organism into Macacus rhesus. Among other things he believed that the lesions produced were clinically similar to those of leprosy, and that lesions developed far from the site of inoculation, indicating spread of the infection from one side to another; he concluded that leprosy is reproducible in the monkey. This author's inoculation of the Japanese dancing mice has been alluded to elsewhere. In a further paper Duval and Gurd (71) stated that "few, if any, of the ordinary laboratory and domestic animals are immune against infection by Bacillus leprae"; the goat, horse, guinea pig and many cold-blooded animals (Couret) had been found susceptible to invasion by the organism used. Couret (48), having worked with such animals as tadpoles, frogs, snakes and turtles, and in addition gold-fish (Carassius auratus), spots (Leiostomus xanthurus) and a few mullets and croakers, reported that the leprosy bacillus "survives and multiplies in cold-blooded animals,

at least at room temperature in a warm climate..." The following year Duval and Couret (70) wrote that:

The production of leprosy in the monkey proves conclusively that the acidfast 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.

Meanwhile further papers dealing with this very interesting question were contributed by Serra (223), on lesions produced in the rabbit's eye with his culture; by Bayon (19), who stated that only Kedrowsky's organism is capable of producing lesions in animals and that those of Duval and Rost, which behaved culturally like saprophytes, do not cause leprous lesions; by Hollmann (105), who demonstrated organisms in the tissues of rats following inoculation with Clegg's culture; by Machow (137), who stated that Kedrowsky's culture is not pathogenic, or only slightly so, for mice; and by Reenstierna (201).

In 1914 Johnston ( $^{109}$ ) reported negative results in all but one of the guinea pigs inoculated with a streptothrix which he had isolated from the spleens of lepers in the Philippines. One animal showed an enlarged liver, with nodules from which the organism was recovered. At this time McCoy ( $^{154}$ ) reported his cultures, none of which was pathogenic for any of the laboratory animals.

In the same year Bayon (21), in another review of the question of animal transmission of leprosy, said that after inoculating over 400 animals he had concluded that such animals are rarely positive, but when positive their lesions are "identical with those met with in nerve organs of lepers." Later (23) he described "bacillary deposits" produced in rabbits, and stated frankly that he felt that skin lesions of leprosy in animals could not be expected, but that "partial and incomplete interpretation of hundreds of negative observations cannot invalidate the proof positive of a single successful inoculation." A later review (22) added nothing to the matter.

From these many reports of attempts to transmit leprosy to lower animals, it is readily apparent that down through the years there was a distinct divergence of opinion as to the susceptibility to infection with leprosy of any of the animals experimented with. We are left with a feeling of grave doubt that any of the investigators actually produced anything like real leprosy in any of the animals with which they experimented. None of those who worked with material from leprous patients made any very positive claim to have reproduced the disease in animals. But as we have seen, some of those who worked with cultures were quite positive and dogmatic in their claims. The matter was controversial for some decades, with several investigators endeavoring to substantiate their claims for the cultivation of the true Hansen bacillus on the ground of experimental production of leprosy in the laboratory animal with those cultures. However, up to 1918, the end of the period so far considered, no claim of experimental production of the disease in a lower animal was generally accepted.

> CRITICAL EVALUATION OF KNOWLEDGE OF THE BACTERIOLOGY OF LEPROSY AT THE END OF THE EARLY PERIOD

From the foregoing little is to be seen to warrant a feeling of optimism that up to 1918 there existed much satisfactory evidence for a single critical experiment upon which to base the establishment of the etiological agent of leprosy, as judged by Koch's postulates. The period covered so far is of more than four decades since Hansen first described the bacillus with which his name is connected. In many ways it is amazing that after so much time, and after the efforts of so many investigators, the matter remains in so unsatisfactory a state. So many claims have been made for success in the cultivation of the actual organism of leprosy, and for the transmission of the disease to animals, that a conservative person is tempted to view all such claims with something more than the proverbial grain of salt. Indeed, as late as 1925, Rogers and Muir (211), in their chapter on the etiology of this disease, stated:

Although more than fifty years have passed since the discovery of the lepra bacillus by Hansen, we have no certain proof that this organism has ever been cultivated *in vitro*. No other organism has ever resisted the efforts of bacteriologists so long.

Five years after this, in 1930, Muir (168) stated in another review of this subject:

In spite of very extended efforts by bacteriologists in all countries, it appears to be still the fact that the bacillus has not been successfully cultivated *in vitro*, nor has the disease been conveyed experimentally to animals or even to man.

Again, in 1932, Soule and McKinley (234), carefully reviewing the literature and attempting to evaluate conservatively their own investigation in this field, stated:

Since the discovery by Hansen in 1874 of small rods lying within the "lepra cells," this organism has been generally accepted as the cause of the affection, and yet of the many reports on the cultivation of Hansen's bacillus obtained from typical lesions of the disease there is none that has been accepted as establishing proof of the actual cultivation in vitro of B. leprae.

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We have, then, up to a very recent date, a situation regarding the etiology of leprosy which is, to say the least, most baffling, and a vast literature characterized by claims and counterclaims none or few of which have been finally accepted. It seems, therefore, desirable to look back over the terrain and attempt to determine, if possible, what of the mass of data which has been reported can be established as authentic fact.

Most of the various forms of organisms which have been isolated from leprosy tissue had already been described before 1918. These include diphtheroids, chromogenic acid-fast bacilli, nonchromogenic acid-fasts, anaerobic bacilli and actinomyces. It is quite apparent that all of these different organisms cannot possibly be the causal one of leprosy, though positive claims have been made for each of these forms, in most instances repeatedly. These claims in the case of several authors extend to success in the production of experimental lesions. It is rather amazing with what frequency such claims have been made, and in many instances upon what totally inadequate experimental grounds, when we consider that no investigator has ever yet succeeded in producing leprosy in any experimental animal, or in man, with absolute certainty. This statement is made advisedly, though it must be admitted that nobody knows what human leprosy in an experimental animal should look like, or of what precisely it should consist. If we expect to reproduce the precise picture of leprosy as we know it in man, then possibly the disease may never be established in laboratory animals. If, however, we are willing to accept as infection the production of local progressive and destructive lesions as a result of inoculation with leprosy material, then there is still hope of success-indeed more than mere hope, as will be pointed out later.

The conclusions arrived at in 1914 by Wolbach and Honeij (291), in their critical review of the bacteriology of leprosy which has been mentioned (291), are so true today that a part of them are quoted here:

To draw conclusions from a review of this sort is very difficult. Indeed, any conclusions must necessarily be speculative, for a new technic and a few clear-cut facts can completely change the whole aspect of the subject. It is advisable, however, to discuss the facts accumulated with the hope of defining more clearly the problems still to be solved in the bacteriology of leprosy. First of all, we must conclude that at least two, the diphtheroid and pigmented acid-fast, and possibly all four varieties of the bacilli are commonly found in leprosy tissue. The diphtheroid organisms have been found in all parts of the world; the pigmented acid-fast have been found

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most often in the Philippines and Louisiana, independently and by competent bacteriologists. The non-pigmented acid-fast anaerobic bacilli perhaps have not been found often enough to have special importance. The prevailing opinions as to the nature of the leprosy bacillus, however, force us to regard the few isolations of acid-fast, non-pigmented aerobic cultures as of extreme importance....

In connection with the pigmented acid-fast bacilli it must be insisted that the very carefully recorded experiments of Clegg and his associates and Duval and his associates admit of no other conclusions than that these organisms also come from leprosy tissue. Whatever their significance may be, the nature of the organisms, their free growth at ordinary temperatures and upon ordinary media, do not accord with our ideas of a parasite so highly specialized as the leprosy bacillus must be. It is difficult to understand why these cultures are so difficult to obtain in the first generation and so easy to maintain afterwards.

It is our considered opinion that this statement is sound. It is interesting to note that the circumstances which over twenty years ago made these authors regard the few isolations of nonpigmented, acid-fast, aerobic cultures as of extreme importance, still cause these organisms to be regarded as of unusual importance. Regarding the pigmented acid-fast bacilli which both Clegg and Duval isolated, Wolbach and Honeij felt that they did not accord with their ideas of a parasite so highly specialized as the leprosy bacillus must be. As late as 1930 neither Clegg's nor Duval's organism was accepted as the true germ of leprosy.

The diptheroids are even easier to dispose of, as Wolbach and Honeij pointed out. While there have been some few references to anaerobic bacterial forms, they have perhaps never been considered seriously as related to leprosy. During the past several decades we have learned something concerning bacterial tissue flora. We know, for example, that there are bacillary forms which are commonly found in lymph nodes, the only inference being that such organisms from time to time gain access to the body through the blood and lymph and are, for the most part, of such low virulence that they produce no actual infection, and are only discovered when such tissues are examined for them. If this is true of closed tissues-and the evidence is most positive-then what may we expect in the way of contaminating and saprophytic organisms in open lesions? It is now common knowledge that organisms such as diphtheroids are frequently found upon normal mucous membranes, on ulcerated surfaces and in skin lesions of all sorts. We are, therefore, forced to conclude that much of the early work on the bacteriology of leprosy is confused because many workers were unwittingly working with

contaminated materials, and because, to some extent at least, hope was paramount to good judgment when experimental data was evaluated.

We do not insist that all workers were concerned with contaminated materials, but in many instances it is the only explanation for the bizarre results reported. We may recall again that in 1915 Fraser and Fletcher (84) emphasized the importance of removing leprosy tissue for cultivation work with strict aseptic technic; when that was done they were unable to cultivate any organisms whatsoever from such tissue. We regard the experiments of these investigators as of decided significance in the light of our own with Soule, to be described in the next section.

So far in this criticism we have considered only the diphtheroids, the pigmented acid-fasts, and the anaerobes. What of the nonpigmented acid-fasts? These, as Park and Williams (191) have recently stated, constitute the modern conception of what the true leprosy organism should really be. That also appears to have been the general feeling in the matter twenty years ago. The nonpigmented acid-fasts of Weil, Karlinski, Marchoux, Twort, and Duval and Wellman—leaving aside Kedrowsky's acid-fast diphtheroid—were all nonpathogenic for animals. The organism isolated by Duval and Wellman (75) they themselves admitted was not conclusively proved the cause of leprosy. The organisms isolated by McCoy (154) should also be mentioned, particularly the two almost colorless strains, but it is noteworthy that they grew luxuriantly on glucose media and were not pathogenic to animals.

After having considered the most important types of organisms which were described up to 1918, it seems necessary to admit that none of these organisms was established beyond question as the *true leprosy germ*. We therefore pass to what is here designated as the period of our newer knowledge of the bacteriology of leprosy, with apologies for determining in an arbitrary fashion where one period should leave off and another begin, or indeed that two periods should be recognized.

(To be concluded)