THE BACTERIOLOGY OF LEPROSY

A REVIEW

By Earl B. McKinley, M.D.
From the Department of Bacteriology, Hygiene and Preventive Medicine
School of Medicine, The George Washington University
Washington, D. C.

(*Conclusion*)

THE NEWER KNOWLEDGE OF THE BACTERIOLOGY OF LEPROSY

In the preceding part of this review we discussed the work that had been done on the problem of the bacteriology of leprosy, and in related fields, up to about 1918. It was pointed out in a critical summary that it seemed necessary to admit that none of the micro-organisms that had been isolated from leprosy seemed to have been satisfactorily established as its true germ. We now pass to what we have designated as the period of our newer knowledge of the subject, 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.

NEWER METHODS OF STUDY

It is frequently said that the progress of science depends largely upon new ideas or hypotheses, and upon the technical methods required to test them critically. The leprosy bacillus was discovered in the infancy of the science of bacteriology; when Hansen first saw it, there were no staining methods for bacteria. As new bacteriological techniques were developed they were applied to the study of this infectious disease, as of others. Attempts to cultivate its germ have been responsible for several new bacteriological culture media, and without doubt the vast amount of work done on the staining of it has been helpful in other phases of the general field of bacteriology. Microchemical methods that were developed have been applied in the study of the chemistry of suspected leprosy germs, as of other bacteria.

Thus our knowledge of the etiological agent of leprosy has advanced with the progress of bacteriological technique. Unfortunately, however, even now the vexing technical problems which have confronted investigators in leprosy for the past sixty and more

years have not been entirely solved. Nevertheless, there is much hope that new methods, or at least new applications of methods, are now at hand which will eventually result in a generally acceptable solution of the problem of the cultivation of *M. leprae.*

In 1918 Wherry and Ervin (288) published a short note on the carbon dioxide requirements of *M. tuberculosis* which was later—around 1925—to become the subject of extensive studies by Novy, Roehm and Soule (180) and Novy and Soule (179), not only in connection with the germ of tuberculosis but with other organisms (see also Soule 232). These investigators laid down the technical fundamentals of the study of bacterial respiration and contributed much of what is known today regarding this interesting subject. As a matter of fact, however, the work of Hesse (103) on the gas exchange of the tubercle bacillus antedates that of Wherry by some twenty-five years, since Hesse, in 1893, analyzed the air present in two cultures of that organism over a period of 152 days. Moore and Williams (166) carried on similar studies with avian tuberculosis cultures in 1909. Later—in 1921—Corper, Gauss and Rensch (47) also determined CO_2 production in several cultures.

Though the study of Wherry and Ervin did not deal with an entirely new subject, the former is to be credited with employing for the first time, in 1930, the modified gaseous tension method in an attempt to cultivate *M. leprae;* that work will be referred to later in detail. It was with knowledge of that attempt, and with a background of the work of Novy and his colleagues regarding the basic laws of gaseous exchange on the part of certain bacteria, especially the tubercle bacillus, that in 1931 the writer with Soule undertook work with *M. leprae* and in 1932 (160, 234) described the cultivation of a germ thought by us to be that organism. This work will be described later.

Aside from the techniques which have been mentioned here, bacteriologists have developed no new methods of approach towards the solution of the problem of the etiology of leprosy. Let us see, however, what the years since 1918 have produced by way of further understanding of this problem.

RECENT ATTEMPTS TO CULTIVATE THE BACILLUS

The period 1919-1928.—At this time attempts were still being made to cultivate *M. leprae,* most investigators believing that that had not been done. Zironi (294) in 1920 published a short review of the cultivation work. Kohda (124) came to the conclusion that

Kedrowsky's organism is similar to the avian tubercle bacillus, that it possesses only weak pathogenicity, and that it is not specific to leprosy though it gives positive immunological reactions with leprous serum. Richad (206) stated, without proof, that the diphtheroid form of the leprosy bacillus may be its infective one.

In 1922 Walker (277) described four types of diphtheroids from leprosy tissues and concluded that the partly acid-fast one of Bordoni-Uffreduzzi and other authors can be cultivated more or less constantly from nasal and other open lesions of lepers, and also from nonulcerating lesions. This organism, he said, differs from other diphtheroids described in the literature in its size, its extreme pleomorphism, its partial acid-fastness, its fermentation reactions, and the peculiar colonies that it forms. Further, he stated that it is probably a cultural form of the pleomorphic and facultative acid-fast *M. smegmatis*. In the following year (278) he confirmed the cultivation of a chromogenic acid-fast organism from leprosy on the medium of Musgrave and Clegg, and stated that neither the amebae of Clegg nor the protein split products of Duval are necessary for the growth of these organisms. He asserted that chromogenic acid-fast organisms like Clegg's bacillus develop from Bordoni-Uffreduzzi's diphtheroid, in transplants from colonies of diphtheroids from smegma praeputii on Musgrave and Clegg's medium, in cultures from nonleprous nasal secretions, and from Hoffmann's diphtheroids isolated from such secretions. He concluded that Clegg's bacillus seems to be a developmental stage of the Bordoni-Uffreduzzi diphtheroid and that this organism seems to be identical or closely related to the pleomorphic and facultative acid-fast supposed to be *M. smegmatis*.

In 1925 Kondo (125) studied some fourteen different strains of so-called leprosy bacilli that had been cultivated by as many investigators. Cabral (30) reviewed the entire subject interestingly but added nothing new, and Mello and Cabral (164) published another short review. In 1927 de Souza Araujo (236) claimed to have cultivated the germ on a special liquid medium, upon which a shelf of solid medium rested on the shoulder of a potato tube; the leproma material to be cultured was placed on the shelf. He also reported the production of lesions in laboratory animals. In 1928 Kedrowsky (119) published his conclusions that the leprosy bacillus, like that of tuberculosis, may be both acid-fast and non-acid-fast, either in living tissue or on culture media; that only in occasional instances are pure cultures of acid-fasts obtained; and

that the bacillus of leprosy belongs to the group of actinomyces or streptothrix-like organisms. Souza-Araujo (238) also arrived at the last of these conclusions.

Regarding other phases of the leprosy problem, numerous papers were of course published during this decade. Among them was one by Pineda (193), who attempted to differentiate, morphologically and by staining, leprosy bacilli from other acid-fasts; by Solis and Wade (229) who, on the basis of findings in leprous children, showed that the belief that the nasal mucosa is one of the important sites of the primary lesion is erroneous, though on the other hand Wade and Solis (276) found this region to be an important residual site of bacillus-discharging lesions in recovering patients; by Pineda (194), who found bacilli in one or more of the deeper organs of all of 11 cases that had come to autopsy after having become bacteriologically negative under treatment, and also (195) found bacilli in 57 (or 53 percent) of 104 placentas examined. During this period Klingmüller (120) studied the granular forms of the bacillus, Franchini (78) reported a case of *M. leprae* septicemia, and Dumont (59) described a case of tuberculosis with accumulations of acid-fasts as in leprosy. Vedder (271) presented a discussion of the etiology of leprosy and described his experiments with reference to the possibility of transmitting the disease to human beings by insects. He employed mosquitoes, having demonstrated that those which fed on lepers had plenty of acid-fasts in their bodies when allowed to feed on his healthy subjects. By 1934 none of the three volunteers involved in this experiment had developed the disease.

Though it was published more recently, it is desired to mention a paper by Rodriguez, Mabalay and Tolentino (208) concerning Gram-positive organisms in leprosy lesions in which no acid-fast organisms are demonstrable. These authors believe that the organisms which they find to be Gram-positive but not acid-fast are in fact the organisms of leprosy, but that they are not merely degenerated forms since they are numerous in many cases of so-called "closed" or "incipient" cases of leprosy which have not undergone treatment. Here is a suggestion as to why, in some cases of leprosy, it may be impossible to demonstrate the acid-fast *M. leprae* in the lesions.

Another field of work that received much attention during these years was that of so-called rat leprosy. There has, of course, always been a question regarding its possible relation to the human disease. In 1922 Uchida (261) concluded that rats, while susceptible to other acid-fast bacterial diseases, are not susceptible to

human leprosy; he further showed that rat fleas carry many acid-fast organisms; and he isolated four strains of supposed rat leprosy bacilli, one of which produced pigment. At the same time Marchoux (144) expressed doubt that the rat disease bears any relation to the human leprosy. Mazza (153) stated that, when animals are injected with the bacilli of rat leprosy, they are taken up by the polymorphonuclear cells and distributed to the rest of the body. Sabrazes (216) also described how the macrophages in the liver take up these organisms, leaving the bile free of them. In 1928 Muir and Henderson (169) published a comprehensive study of this infection and offered further evidence that it bears no relation to the human disease. The B.C.G. vaccine induced no protective effects against it in their hands. They found that the human leprosy bacillus, when injected into rats, Chinese hamsters and Japanese dancing mice, gave completely negative results. While this subject of what we believe to be erroneously designated "rat leprosy" has no particular interest in this review, we quote these papers to illustrate the range of interest in the general subject of acid-fast bacterial infection and leprosy which has been manifested during the past several years.

The period 1929 to date.—In the period that remains to be dealt with there has been something of a revival of interest in the cultivation problem, and there have appeared a number of publications that are unusual as regards either the hypotheses presented or the technical methods employed and results obtained. At the same time, of course, numerous reports have dealt with work done along more or less usual lines; these will be looked at first.

In 1929 Shiga (225) reported some cultivation experiments which looked very encouraging, though we understand (Wade) that he became less certain that the organism that he cultivated was actually that one. Giordano (88) planted the blood of lepers on Hahn's medium and stated that he obtained cultures of the bacillus. It grew vigorously in subculture, he stated, producing acid-fast ramified (streptothrix) forms which he also found in the primary cultures. In 1930 Sonnenschein (230) reported cultivation of the bacillus on glycerol-egg and malachite green-egg media. Schlossmann (219) claimed similar results with Martin's bouillon. In the following year Marchoux, Markianos and Chorine (146) stated their belief that Shiga, Wherry and they themselves had alone succeeded in sustaining growth of *M. leprae* on artificial media. Vaudremer, Sézary and Brun (269) reported cultures from blood and lepromata, grown on potato impregnated with horse

serum and glycerol; but they were not quite certain of actual cultivation. Ota and Sato (182, 183) reported that they had obtained the bacillus on several different media, including Lowenstein's, Hahn's, Petroff's, Petragnani's, etc.; and they also cultivated *M. tuberculosis* from a leproma and a lymph gland of a leper. Pisacane (197) also reported that Hahn's medium was adaptable for the cultivation of the bacillus. In 1931 Henderson (102) described a pigmented culture grown from a leproma on ordinary media, once in 23 attempts; he did not claim that it was the leprosy bacillus. In the following year—1932—Peschkowsky and Malilin (192) reported growing the Hansen bacillus on glycerol-potato in 23 days. Eichbaum (77) attempted repetition of Shiga's cultivation work, with doubtful results. Denney and Eddy (56) studied the behavior of the leprosy bacillus and certain other acid-fast organisms in the presence of leukocytes and concluded that, of the fifty strains that they studied, only that of rat leprosy showed evidence of globus formation. The bacilli contained in "leper juice," when suspended with living leukocytes, did not evidence proliferation of either the free rods or the globi, but in pus obtained from leprous abscesses there was an increase in the number and size of the globi.

Turning back to 1929, in that year Walker (279) reviewed the subject of the etiology of leprosy and advanced certain new views of the problem. Among other things he stated that the acid-sensitive or partly acid-fast coccoid, diphtheroid, and actinomycoid organisms that have been cultivated repeatedly from leprosy are different stages in the life-cycle of the same organism, which is really an actinomyces. Considering this view and the biological characteristics of that group of organisms, and also the epidemiology of leprosy, he was led to investigate the possibility that the actinomyces of leprosy may originate from the soil. Fifty samples of the soil of Hawaii were examined and acid-fast organisms were found in 49 of them (98 percent). These organisms were all of the same species—facultatively acid-fast and extremely pleomorphic, developing cocci, diphtheroids, rods and filamentous forms. Walker further stated that these soil organisms and the ones isolated from leprosy are probably identical, and from this he concluded that leprosy is primarily an infection from the soil, probably of wounds, by this facultatively parasitic actinomyces. The great difficulty which has attended the cultivation of the germ from the tissues of lepers he would dispose of on the ground that most of the organisms in the tissues are dead. However, he did throw in this caution:

Actual proof of the identity of the actinomyces cultivable from leprosy with Hansen's bacterium in the tissues, like proof of the etiologic relation of Hansen's bacillus to leprosy, would depend upon the experimental reproduction of the disease in animals. Notwithstanding the absence of such proof, the evidence in support of both relations is convincing.

By 1929 the science of bacteriology was well into the era of life-cycle hypotheses, which had been gaining force since the interpretation of the phenomenon of dissociation earlier in the decade, and Walker found it to harmonize more with his judgment to consider that all of the various forms of organisms which have been recovered in leprosy are etiologically significant than to consider them contaminants or secondary invaders, as we have suggested earlier in this treatise. In this view he may be correct; at least it is a hypothesis that is entitled to consideration, and one which doubtless has been stimulating. The view that most of the organisms in the lesions are dead is probably true, but that does not explain why those which are alive are not cultivable with reasonable ease and regularity. In our opinion Walker has given bacteriologists interested in leprosy something to think about, but we also feel that there is danger in such a hypothesis in that, if it is taken too seriously, it may divert thought and action from the more probable cause of this disease, which we feel most strongly is not related to Walker's soil organisms.

At about this same time there was introduced another idea concerning the etiology of leprosy, one somewhat related to the current hypotheses regarding life-cycles of bacteria. Markianos (148), working with rat leprosy, suggested that there is a filterable and invisible form of its causative organism which will pass through the pores of a Chamberland filter. He stated that the organism first develops into primary elements and these granules are the first visible stage of the germ; that the filterable elements produce the disease in rats in the same time as the bacillary forms; that they develop rapidly when inoculated into young rats; and that they possess an affinity for the ganglionic tissue. Some time later Vaudremer, Sézary and Brun (270) reported that they had succeeded in cultivating the bacillus of human leprosy from filterable forms of the organism. Cantacuzene and Longhin (39) also described a filterable phase of *M. leprae*. They stated that its first visible forms are granules which later become typical leprosy bacilli. Others have suggested similar views of the possibility that filterable forms are a part of the life-cycle of the germ of leprosy and play a part in the development of the disease, but we are hardly

willing to accept them very seriously as yet, particularly since certain workers have shown that actual bacillary forms may be found in filtrates, and in the light of our present knowledge regarding the probable nature of the true ultraviruses.

Investigators who have been interested in the so-called virus diseases have long been the unsoliciting heirs of many diseases which have no place in that group. It has become more or less a habit to place any disease for which the etiologic agent could not be determined among those due to filterable viruses. That has been an easy and simple way to dispose of vexing problems but it has been confusing, and only now are we beginning to see a little more order in the virus field and to arrive at a concept and classification of these agents. In 1932 McKinley (155) attempted to present a modern concept of the ultramicroscopic virus diseases and a classification; he devoted some attention to the confusion which has existed about true viruses and bacteria, and pointed out that these two groups of agents are probably unrelated and distinctly different from each other.

The special field in which the writer has worked with Soule and with Verder is one in which, we believe, there is at least a hope of a possible solution of this problem. Earlier in this review we mentioned the original work on the gaseous metabolism of bacteria, and the fundamentals established by Novy and his colleagues on this subject. We also mentioned the application of the principle of modified gaseous tension to the cultivation of the leprosy bacillus by Wherry (286, 287), work that has an important bearing on our own. It will now be considered in more detail.

Work of Wherry: This author recognized that "one must furnish suitable respiratory conditions as well as proper food when cultivating bacteria." He and Ervin had shown that carbon dioxide was essential for the growth of *M. tuberculosis*, and Rockwell had demonstrated the same fact for certain other bacteria; consequently, Wherry gave special attention to this fact when he attempted to cultivate Hansen's bacillus. With different cultures on his special medium the carbon dioxide was increased and oxygen left normal or reduced, while some cultures were made anaerobic, with CO_2 present. Proliferation was recognized only by smears; it was apparent in cultures from three cases, at the end of 4 to 6 weeks. To quote:

The microscopic, colony-like masses of acid-fasts increased in number for a few weeks and then the growth appeared to be stationary. Subculture of a loopful of material containing several dozen colonies into the same medium resulted in the appearance of a large number of subcolonies and isolated masses and scattered acid-fasts. Two of the primary cultures in the above medium were successfully subcultured in the same medium... The best growth was obtained in cultures which were kept first at partial

oxygen tension (little O_2 but CO_2 present) for a month after which the tubes were kept under O_2 and CO_2.

Wherry evidently employed an exceedingly small inoculum, since he used only a loopful of blood expressed from a leprous nodule, yet though his methods of producing the various gaseous tensions were rather crude he was still able to report proliferation of the bacilli and even multiplication in subculture. The material transplanted was only a loopful containing several dozen colonies, but there resulted a large number of subcolonies and isolated masses and scattered acid-fasts upon his special medium. It is interesting to note that the best growth was obtained in cultures which were kept first at partial oxygen tension (little O_2 but CO_2 present) for a month, after which the tubes were kept under tension with these gasses.

A year later Oliver, de Leon and de Roda (181) reported on their attempt to confirm Wherry's work. Using the same gaseous tension method of cultivation, they failed to observe any evidence of proliferation of the organisms, though bacilli survived for a considerable time, in one extreme instance for 158 days. This work has been commented on at some length by Soule and McKinley (235).

Work of McKinley with Soule: The Leonard Wood Memorial Conference on Leprosy (204), which was held in Manila in 1931, gave emphasis to the need that the entire subject of the pathogenesis of leprosy be reinvestigated, and the necessity for the utilization of the most modern methods of research for the purpose of cultivating its causative agent. Stimulated by this conference to a large extent, and impressed with the possibility that modification of the gaseous environment of the cultures might be the key to the solution of this difficult problem, we began to investigate the matter in Puerto Rico early in 1931. The fundamentals established by Novy and his colleagues on the subject of the gaseous metabolism of bacteria formed the basis for the approach which we made to this problem. The three reports of McKinley and Soule (160) and Soule and McKinley (234, 235) have been mentioned. In presenting the following discussion of our work we shall hope to be as self-critical as we have taken the liberty to be with the work of other investigators. Our effort in this paper has been to review dispassionately the whole question of the etiology of this disease and to present the facts as they seem to us to exist up to the moment of writing.

Technique used.—From among the inmates of the Puerto Rico leper colony we selected a group of nodular lepromatous-type cases that had

several early lesions. Well isolated nodules on the arms and ears were selected, the skin was thoroughly cleansed with soap and water followed by several applications of tincture of iodine, and with local anesthesia four nodules from three different patients were enucleated aseptically. These tissues were emulsified with physiologic saline and filtered through sterile glass wool. Microscopically the suspension was found to be rich in acid-fast organisms. Two drops of it were transferred to each tube of medium with sterile bulb pipets.

The inoculated tubes were divided into four series and, in Novy jars, by the procedure of Novy, Roehm and Soule, were subjected to atmospheres containing (*a*) 10 percent oxygen and 10 percent carbon dioxide; (*b*) 20 percent oxygen and 10 percent carbon dioxide; (*c*) 40 percent oxygen and 10 percent carbon dioxide; and (*d*) no oxygen and 10 percent carbon dioxide. Control tubes were incubated in the normal atmosphere. Incubation was at 37°C., as usual. (The use of free carbon dioxide resulted from the findings of Novy and Soule that a definite concentration of that gas is necessary to maintain the physicochemical equilibrium between the extracellular and the intracellular carbon dioxide, and the concentration employed was that which, from previous work with bacteria and protozoa, seemed to be the most favorable for the primary isolation of organisms. It will be noted that only the oxygen concentration was varied.)

Results.—At the end of four weeks the jars were opened, several tubes contaminated with an ordinary spore-forming aerobe were discarded, and, since there was no macroscopic evidence of growth in the other tubes, smears were prepared from several of them. Microscopically there was found evidence of proliferation in several tubes. The original atmospheres were restored and the jars incubated for another two weeks, after which all tubes were examined by inspection and microscopically.

Viewed by transmitted and reflected light, colonies when present were seen to be very small, averaging about 1 mm. in diameter, and heaped up, with a distinct mucoid appearance and a loose filamentous border. The tubes were recorded as (*a*) positive with colonies and well formed, solid-staining rods (46 tubes); (*b*) questionable, without colonies but with well formed, solid-staining rods that seemed to have proliferated; and (*c*) negative, without colonies and in smears only granular acid-fast bodies or highly granular rods. Excepting the aerobic spore-forming contaminant noted, no other organisms of bacillary, coccoid or actinomyces types were observed in any of these tubes or in the subsequent cultures.

No medium or gaseous environment had uniformly positive results, though certain media seemed to have a distinct advantage, one of them being hormone glycerol agar. The most favorable gas environment seemed to be 40 percent oxygen and 10 percent carbon dioxide, which was in accord with findings with the tubercle bacillus that increased oxygen tension was advantageous. It was a striking fact that no growth took place in any of the air controls, or in the tubes incubated under anaerobic conditions plus carbon dioxide—in which, it may be mentioned, granulation of the bacilli was most conspicuous.

Second generation.—The material in 16 of the positive tubes was suspended and used in inoculating monkeys. That in the remaining 30 tubes was transferred to fresh media, the growth in a tube of one medium

being transferred entire to a like tube. After incubation for four weeks only eleven tubes were found to have typical colonies. The 40 percent oxygen and 10 percent carbon dioxide mixture again seemed to be the most favorable atmosphere, but there was no apparent choice of medium.

Other generations.—The growths in the eleven positive tubes were again transferred to the same number of tubes of fresh media of the same kinds and reincubated as before for four weeks (third generation). Colonies appeared in ten of these tubes, but they were no larger than those in the primary isolations. Only five of the ten tubes inoculated in the next transfer (fourth generation) developed typical colonies. Thereafter in making transfers larger numbers of tubes were seeded than were used as the source of the inoculum.

A later report was made by Soule and McKinley (235) after the organisms had been carried through sixteen generations. The number of positive tubes per generation had become small—6 in the 13th, 5 in the 14th, 3 in the 15th and 2 in the 16th. The organism, obviously, had not become adopted to growth on artificial media; to the contrary it seemed to be gradually losing its ability to multiply at all under those conditions. Efforts to find a more satisfactory medium have been unsuccessful, but the culture is still living and forming definite colonies in 1938—seven years, and more than sixty generations, after it was isolated.

In the later report mentioned we also described serological tests with our culture. No evidence of specificity was obtained with the precipitin test. Agglutination occurred in dilutions of 1:20 to 1:50 in only four out of twelve leper sera, but complement fixation was positive in nine of them.

More recently Soule (233) has repeated this cultivation work in the Philippines under the auspices of the Leonard Wood Memorial. The technique used was in general the same as before, but in addition he set up controls with killed (autoclaved) inoculum. That was done to meet the really unfounded criticism that our apparent subcultures were due to continued mechanical transfer of material from the original inoculum—a thing that patently was impossible, if for no other reason than because so very little material (two drops of a filtered suspension) had been used. Soule obtained 12 positive cultures from the material of 20 ordinary nodules; that from 6 broken-down nodules gave 2 cultures; and from 16 specimens of lepra-reaction pus 11 cultures were obtained. The colonies and the organisms themselves were identical with those isolated in Puerto Rico, and no other kinds of growths were observed; no nonacid-fast diphtheroids and no chromogenic acid-fast organisms appeared.

Tissue culture work of McKinley and Verder, and of Soule: In 1933 McKinley and Verder (161) described the cultivation of *M. leprae* in minced chick, and also human, embryonic tissue in Tyrode's solution. Multiplication took place under ordinary atmospheric conditions, but more recent experience indicates that these cultures, also, do much better under special gaseous environment, for the pH of the medium can be much better controlled in that manner. Subsequent attempts to enrich the tissue-culture medium have failed to produce one which will ensure much better growth than we reported. Later (162) we described the transfer of the bacilli from these tissue cultures to solid media, in the special atmosphere. The appearance of minute microcolonies was interpreted as direct evidence of the multiplication of the organisms.

It may be noted that in the annual report of the Surgeon General of the United States Public Health Service for 1932 (252) there is a report of the use of chick embryo tissue-culture for the cultivation of *M. leprae* which states that "in three instances of the cultures of human material there has apparently been a proliferation of the acid-fast bacilli planted and a definite growth of a diphtheroid in from five to seven days after inoculation." All of these cultures were carried through several transplants, one of them through fifteen, and the acid-fasts in the last transplants seemed to be as numerous as in the original culture. These results coincide, at least in some respects, with those of McKinley and Verder.

Soule repeated and extended the observations of McKinley and Verder when in the Philippines (233). Besides the chick embryo suspensions he used Carrel's chick-embryo plasma cultures and Lewis' methods. In this work, also, autoclaved controls were set up to avoid the criticism that mechanical transfer might be confused with proliferation. Growths were obtained in most instances —22 out of 26 with the technique of McKinley and Verder, and all of 8 times with Carrell's method—and subcultures were carried on as far as time permitted. The organisms were as has been described, and there were no contaminations of any kind.

Summary: From this review of the special work just dealt with, it would seem that there is being assembled a considerable amount of data which gives encouragement concerning the problem of the cultivation of *M. leprae.* The least that can be said of this work is that it is encouraging. We trust, however, that we may continue to maintain the conservative point of view which we have so far tried to express in our publications regarding this question.

We are under no illusions regarding this question, which has been one of perennial controversy. We realize that Koch's postulates have not been entirely fulfilled. Nevertheless, we feel that in the organism isolated by Soule and the writer we have one that is quite distinct from those that have ordinarily been described as the leprosy bacillus. It is apparently one of extremely delicate constitution and most difficult to cultivate artificially, and so far we have found only two methods which will succeed. Perhaps this difficulty in cultivating and maintaining it is the main point in its favor; at least these characteristics seem to harmonize quite favorably with the history of the organism. The fact that it is a non-chromogenic bacillus is also in its favor, as is also the fact that—as will be seen shortly—suggestive lesions may be produced with it in experimental animals. It is felt that these findings should be tested thoroughly and rigidly by other investigators.

RECENT ANIMAL EXPERIMENTS

To return again to 1919, we find that during the following years there appeared many reports regarding the production of possibly leprotic lesions in laboratory animals. Bradley (28) described rather extensive nodular lesions in the *M. rhesus* monkey. Maucione (152) reported further experiments on the inoculation of the anterior chamber of the rabbit's eye; he obtained definite opacities in the cornea. Limousin (132) performed similar experiments, repeating his inoculations six months later and keeping his rabbits for 22 months before sacrificing them; at autopsy he found no lesions except in the lungs, where there were nodules rich in acid-fast bacilli which he believed to be leprosy organisms. Reenstierna (202) reported further experiments, claiming the production of leprosy lesions in monkeys 39 days after inoculation. Banciu (12) inoculated rabbits intravenously with tissue emulsion but apparently produced no lesions, nor did the sera fix complement. However, he injected other rabbits intravenously with the sera of lepers, finding that the sera of these animals possessed fixation properties for a few hours, but were entirely negative in 24 hours; in a dog the fixation properties remained for 48 hours. Mariani (147) injected both virulent and killed leprous material into man intradermally, but produced only various grades of reactions, without lesions. This was in 1925. In 1926 Reenstierna (203) again reported experimental lesions in monkeys, *M. rhesus* and *M. sinicus*. The following year Roffo (209) produced most interesting lesions in African monkeys and American *Cebus* monkeys. Franchini and

Cendali (81) studied the possibilities of infecting the white rat, but without convincing results. Muir, Henderson and Landeman (170) published a fine study of rat leprosy and pointed out that its organism is only related to the human species as avian and bovine tuberculosis are related to human tuberculosis.

In 1928 de Souza-Araujo (237) produced local cutaneous nodules in the white mouse which he believed typical of human leprosy, though later he did not feel so convinced. Franchini (79) in 1929 reported further experiments with monkeys, describing nodular lesions in *M. sinicus*. In 1930 Tisseuil (257) injected man intradermally with both *M. leprae* and *B. puliforme* and slow-developing abscesses followed, evidently due to the latter organism only. Franchini (80) described the last stages of experimental leprosy in a monkey which had been inoculated more than three years previously; the animal died in a state of general decline, with paralysis of its hind legs, and a few months previous to death a leprous nodule which contained many Hansen bacilli developed in the area of original inoculation. At about the same time Schobl, Pineda and Miyao (220) reported the results of experiments with the Philippine monkey, to which repeated subcutaneous injections of leprous material had been given over the eyebrow; they concluded that there is an allergic factor involved which assists in the production of lesions. In 1931 Borrel and Larrousse (27) infected rats with both leprosy and the cysticercus of *T. crassicola* and reported that there occurred a localization of leprosy in the livers of these animals.

Further papers on the experimental transmission of leprosy to animals were published by de Souza-Araujo (239); by Cantacuzene and Longhin (38), who claimed to have produced lesions in the white rat; and by Ota and Sato (184, 185), who described lesions in white rats four to five months after inoculation. In 1932 Pinoy and Fabiani (196) recorded failure to produce lesions following intraperitoneal injection of leprosy material in a splenectomized monkey. Finally, in the report of the Surgeon General of the United States Public Health Service for 1931 (251), there are described attempts to infect rats with both human and rat leprosy by dropping infective material into the nose. Both organisms apparently penetrated the nasal mucosa, for acid-fast organisms were later to be found in the cervical lymph nodes, in the lungs, and in the spleens of animals so treated. Granulomatous tissue changes were produced in kittens injected with similar materials.

Experiments of McKinley and Soule: Reference has already been made to the recent experiments of the writer and Soule (160, 234, 235). This work is summarized as follows:

Inoculations with nodule material.—Previous attempts had been made, without success, to inoculate monkeys, rabbits and guinea-pigs, using suspensions of leprosy nodules introduced by various routes, among them the intratesticular, intraperitoneal and intracerebral. The intradermal route in monkeys having been shown by recent investigators to be promising, we inoculated eight *M. rhesus* and five *C. olivaceus* monkeys in that manner over the eyebrow with a pooled mixture of the nodule-suspensions that had been used in the cultivation experiments that have been described. In all but one monkey granulomatous nodular lesions developed in 18 to 20 days. The nodules were firm and red, without tendency to soften, though one ulcerated (injury?) a week later.

Several nodules were removed, parts of them being used for sectioning and the rest for culturing. Smears showed numerous acid-fast bacilli. Sections showed definite granulomas consisting of nodular accumulations of large mononuclears with infiltrations of lymphocytes and clumps of polymorphonuclears. Cells with foamy cytoplasm were not present. Multinucleated giant cells were sometimes found. Acid-fast organisms and granules were present.

An emulsion of a part of one nodule, containing relatively few acid-fast bacilli, was inoculated in the same way into three more monkeys but in none of them did lesions develop. In connection with this apparent resistance to infection it is to be said that the inoculation of bacilli was not massive, as in the first lot of animals. The negative results at least show that the mere injection of ground-up nodular tissue does not suffice to produce nodules like those in the first animals.

Inoculations with culture organisms.—After growths were obtained in cultures, as described, ten *M. rhesus* and seven *C. olivaceus* monkeys were inoculated intradermally over the eyebrow with 0.5 cc. of a pooled suspension of bacilli from several cultures, and guinea pigs were also inoculated intraperitoneally to rule out *M. tuberculosis*. In five monkeys of each species there developed, in from one to two weeks, firm, hard and somewhat reddish nodules that varied in size up to 1 cm. in diameter and tended to regress rapidly after the third or fourth week. There was no evidence of secondary infection or suppuration. Smears from one lesion showed numerous acid-fast bacilli, with some mononuclear and polymorphonuclear cells; sections showed granulomatous changes, with marked infiltration of large mononuclears, polymorphonuclear cells and lymphocytes, marked edema, and occasional multinucleated giant cells; no foamy cells. No acid-fast organisms could be demonstrated; and though an occasional one been seen in other similar lesions they have on the whole been remarkable for their scarcity.

Later (235), when the organism had been under artificial cultivation for more than a year and was, if anything, decreasing in vigor of growth rather than increasing, it was found that cultures of the ninth, tenth and eleventh generations failed to produce lesions; they did not even give rise to any unusual local reaction. At the same time guinea pigs and several varieties of mice were inoculated with this culture material. Six and eight

weeks later the animals appeared normal and upon autopsy no evidence of infection was found, and no bacilli in the tissues.

Though the lesions produced in these monkeys had to be considered early ones, we believed that they constituted definite evidence of experimental transmission of the infection, especially since in control animals inoculated with various substances no lesions developed. However, the monkey apparently possesses considerable natural immunity to this infection, for such experimental lesions are not progressive and usually the infection is aborted within a few weeks. The reports of other investigators on the production of granulomatous nodular lesions in monkeys following similar inoculations with human leprosy material seem to be well founded; apparently one may with a great deal of regularity infect certain monkeys with such material by intradermal inoculation. Considering their work as a whole—the cultivation of the organism described, and the production of lesions in monkeys with both suspensions of human leprous materials and the first generation of their cultures, Soule and McKinley expressed their belief that the experiments indicate a step forward in the fulfillment of Koch's postulates for the causative agent in the disease of leprosy. It is to be borne in mind, however, that the suggestive lesions produced healed spontaneously, and also that similar lesions may be produced with heat-killed germs from leprosy tissue (lepromin), so that not too much significance can be attached to these lesions in monkeys.

The problem in leprosy is, of course, to produce in animals characteristic, progressive lesions as seen in man. As has been pointed out earlier in this review, no one can predict what human leprosy in an experimental animal should really be, but if it were possible to produce in animals progressive lesions which would not tend to heal spontaneously, we would at least have something more than we have at present, something that would doubtless be viewed with much more seriousness than the temporary lesions which have been produced so far.

With this thought in mind, McKinley and Verder (163) have recently attempted a somewhat new approach to the problem of producing more progressive lesions in laboratory animals. Considering the chemistry of the acid-fast group, it occurred to us that it might facilitate infection if we supplied the animal's tissues with an abundance of lipoid material, along the lines suggested by Nègre (172) in his work on tuberculosis. We therefore extracted lipoid material from the various tissues of the guinea pig with an acetone and water mixture, distilled off the acetone and sterilized the lipoids. Guinea pigs were then injected subcutaneously in one groin with 5 cc. doses of this lipoid suspension, and *M. leprae* was introduced

subcutaneously in the opposite one. This procedure resulted in some very marked ulcerative lesions in which acid-fast bacilli could be found, and these lesions in some instances have been very progressive.

In summing up the work done by the author with Soule and with Verder on the cultivation of *M. leprae* and the production of experimental lesions of leprosy in laboratory animals, we feel constrained to lean far over on the conservative side of both questions. We feel that in this work we have perhaps the most promising advances yet reported, that there is much evidence to support the view that we have actually cultivated *M. leprae*, and that we have produced lesions in laboratory animals which are at least encouraging, though we realize that Koch's postulates have not been definitely and positively fulfilled. We are hopeful that other investigators will attempt seriously and carefully to confirm our results, feeling that the time has come when investigators should lay aside bias and prejudice concerning this question and make every effort to check honestly and rigidly any line of investigation which offers any encouragement whatsoever for the solution of this problem. Hasty studies and careless conclusions, whether concerning our work or that of anyone else, are to be deplored. It is quite probable that many investigators in the past have been at least partly right in the conclusions they have drawn from their work, and perhaps we too are only partly right in our study of this problem, but it usually requires the contributions of many investigators to establish fully the essence of proof of a complicated problem such as that of the etiological agent of leprosy.

CHEMISTRY OF "*MYCOBACTERIUM LEPRAE*"

Consideration of the problem of the chemistry of the germ of leprosy has been left to the end of this review because it seemed essential to present the background of the work on its cultivation in order to interpret that which has been reported on its chemistry. Manifestly the chemist has had only two types of material to work with, material from leprosy tissues and cultures supposed to be of the bacillus. Regarding the former, nodular lesions of the lepromatous type contain great numbers of bacilli—abundant for a lesion, but not abundant as compared with numbers of organisms obtainable from ordinary bacterial cultures, and not free from tissue elements. As for the latter, any culture of supposed *M. leprae* that may be chosen for study is open to such question concerning its true nature that no chemical study which has been made with such cultures can be regarded as definitely one of the real germ of leprosy.

That is to say, there is no proof that any of the many organisms claimed to be *M. leprae* is actually that organism, and it is probable that most of them are not.

The earlier investigations.—Considering first the early period specified in this review, we find that in those several decades after Hansen's discovery of the bacillus very little work was done relative to the question of its chemistry. The first clues in that connection were discovered in the study of its staining reactions. Certain early papers, as those of Unna (265), led to the early recognition of the fact that the organisms of leprosy and tuberculosis are similar in their content of fatty substances, and that they differ from most other organisms in this respect. The acid-fast nature of these two organisms, which depends upon that peculiarity, was also known fairly early after their discovery. In 1907 Deycke and Reschad (57) described "nastin," a neutral fat extracted from a streptothrix grown from leprous material. Originally they had obtained good results in treating leprosy with the streptothrix vaccine, but later they concluded that these results were due to the fat content of the organism.

In 1910 Unna (267) made further investigation of the fat content of *M. leprae* by staining reactions after treatment with various reagents—alcohol, acetone, chloroform and different concentrations of alkali. He also (266) suggested a method of staining to determine whether the organisms were living or dead; if they stained yellow or brown with safranin and were acid-resistant, they were considered dead.

In 1911 Much (167) pointed out that *M. tuberculosis* contains albuminous substances, neutral fats in combination with fatty alcohol, and a mixture of fatty acids and lipoids, and he stated that *M. leprae* contains the same substances, but probably not the poisonous substance which is present in the tubercle bacillus. That there is a relationship between these organisms he found from an experiment in which tubercle-immunized goats were injected with leprosy bacillus, with the result that extensive leprous alterations developed. During the same year Gurd and Denis (90) studied the chemistry of Duval's strain of supposed *M. leprae* and concluded that the protein portion of this organism represents practically the whole of the toxic element.

More recent investigations.—Naturally there were many limitations imposed upon investigators of the chemistry of *M. leprae* in the period discussed. Even had there been an acceptable

culture of the organism, the chemical methods of microanalysis at that time were extremely limited. It has of course been known for decades that the acid-fast bacteria contain a waxy or fatty substance not common to other species, and there have been many papers dealing with this subject and with studies of the relation of this substance to the staining of *M. leprae*. However, no very serious work was accomplished on the chemistry of the acid-fast organisms until the last two decades or less. Reference can be made to only a few of the studies made since 1923 on the chemistry of that group in general or of the leprosy bacillus in particular.

The use of oils in the treatment of leprosy raised the question as to how they acted, and in 1923 Rogers (210) spoke of "defatting" the organism of leprosy by the injection of chaulmoogrates and morrhuate. Paldrock (186), continuing his interest in the staining of *M. leprae*, in 1926 studied it by various staining methods after treating it with ether, and his findings emphasized the fatty substance contained in it. In later studies of many strains of supposed leprosy bacilli (1927) he found that they all behaved identically under microchemical analysis, all containing free nucleic acid, bound nucleic acid (as nuclear protein), karyonic acid (in the granules), free lipoid (in the membranes and granules), and lipoproteins (in the granules). In 1929 Schlossmann (218) reported briefly on the antigenic lipoids of several strains of *M. leprae* and *M. tuberculosis*. Markianos (151) stated that a defatted antigen of so-called rat leprosy did not prevent, but tended to retard, the development of that disease, and that it acts favorably in the treatment of both rat and human leprosy. Wells, DeWitt and Long (283), in 1923, reviewed the chemistry of the tubercle and leprosy bacilli, and Long and Campbell (134) stated that the latter had 9.7 percent of total lipids which had a saponification number of 188 and contained 27.2 percent of unsaponifiable matter.

Regarding the well-known work of Adams and his colleagues, in 1932 Stanley and Adams (242) studied the surface tension of 120 acids, and in the same year Stanley, Croleman, Greer, Sacks and Adams (243) reported on the bactericidal action of certain synthetic organic acids towards several acid-fast organisms, including eight strains of so-called *M. leprae*. They concluded that when the molecules of these aliphatic acids possess a certain combination of physical properties, then bactericidal action towards the various acid-fast organisms appears. One factor is the molecular weight, with maximum action appearing ordinarily in molecules containing

15 to 18 carbon atoms; ability to form soapy solutions in aqueous solutions of sodium salts seems important in effective acids; also, effective acids seem to be good depressants of surface tension.

In 1932, also, Uyei and Anderson (268), and Anderson and Uyei (5), described their work on the chemistry of an acid-fast organism isolated from a case of leprosy in Honolulu in 1909, the Hygienic Laboratory No. 370. Its chemical analysis (3,000 cultures) showed phosphatide 100.5 gm., acetone-soluble fat 289.5 gm., chloroform-soluble wax 444.8 gm., total lipoids 834.6 gm., polysaccharide 41.2 gm., dry bacillary residue 3,389.8 gm., and dry bacterial matter, per culture, 1.488 gm. They state that the figure for lipoids represents only those portions that can be extracted by alcohol-ether and by chloroform at room temperature. These authors have also stated that the phosphatide found had proved to be similar in composition to the phosphatides isolated from other acid-fast bacteria, but with certain differences; for example, it is exceedingly stable and cannot be hydrolyzed with dilute aqueous acid. The solid saturated fatty acid is not homogeneous but consists of palmitic acid with a slight admixture of a new fatty acid with high molecular weight which they could not identify. Two unsaturated fatty acids were found which on catalytic reduction were converted into palmitic and stearic acids. There was also a small amount of wax-like substance in the ether-soluble constituents. When the phosphatide is saponified with dilute alcoholic potassium hydroxide, only the fatty acids and glycerophosphoric acid are split off, while the polysaccharide complex is left intact. This polysaccharide when hydrolyzed yields about two parts of mannose, one of inosite, and one of a reducing hexose which is probably invert sugar or fructose. Some further studies on the polysaccharide have been reported recently by Newman and Anderson (175).

Besides the difficulties in attempting chemical studies of *M. leprae* that have been pointed out, there is still another one which may cause further embarrassment for the chemist, as well as for the bacteriologist. Recently we have been hearing something about possible variants in the acid-fast group. Dissociation as a phenomenon has been well established with other bacterial species, and investigators have looked for evidences of this phenomenon among the acid-fasts. Furthermore, it would not be surprising if variants were found in this group, as among most other bacterial forms. In 1929 Zolkevitsch (296) reported that with radium emanations she was able to produce bizarre forms from Kedrowsky's bacillus; enor-

mous threads and branched forms were produced quite commonly. Later Kahn and Schwarzkopf (111) reported variants in cultures of *M. tuberculosis* and of the bacillus of rat leprosy. As methods of producing changes they employed animal passage, rapid transfers on glycerol broth containing normal inactivated rabbit serum, and aging of cultures in the incubator. The leprosy "strain 368" with which they worked mutated spontaneously in each direction, to R and S, on plates of Petroff's egg medium.

In 1932 Reed and Gardiner (200) studied the S and R types of a strain of so-called *M. leprae* and concluded that they may be differentiated by acid agglutination. Also, electrophoretic potential determinations indicated a similar type difference. They state that the iso-electric point of the S type is at pH 1.2 and of the R type at pH 2.2. Acid agglutination occurred at about the same electrophoretic potential, namely at about 18.2 millivolts.

SKIN TESTS WITH VARIOUS ANTIGENS

Recently the writer has done work in the Philippines in connection with the testing, by means of intradermal reactions to antigenic fractions, of the relationships of various organisms that have been isolated from leprosy cases. One phase of this work was the initiation of large-scale primary cultivation of the organism described by us, for the purpose of collecting enough bacterial material to permit the separation of its antigenic components. This work, described by McKinley and de Leon (159) and still under way, will be referred to again.

The other phase of the work, the findings of which have been reported recently (158), was the testing, in patients, of several antigenic substances derived from various acid-fast bacteria. Anderson at Yale, and Long in Philadelphia, with their associates, had prepared numerous substances of that nature. Anderson, working with supposed *M. leprae* Hygienic Laboratory No. 370, had prepared a protein, a polysaccharide, a phosphatide, a wax (leprosin) and leprosinic acid (derived from the wax). Long had prepared proteins (tuberculin-protein, trichloracetic-acid-precipitated) from many acid-fast bacteria, including *M. tuberculosis* (hominis, bovis and avium), *M. smegmatis*, *M. phlei*, *M. marinum* (fish origin), *M. murium*, and the supposed *M. leprae* strains of Duval, Daines and Karlinski, and one strain isolated at his own institution.

These various antigens were used in intradermal skin tests in: (1) children of lepers who had been removed from contact with

their leprous parents after from six months to two years; (2) early segregated (bacteriologically positive) cases; (3) advanced cases in segregation; (4) previously positive cases that had become negative and were awaiting parole; (5) relatives of lepers—the so-called family groups; (6) physicians, nurses, technicians, assistants who had worked in contact with lepers—professional group; and, finally, (7) a control group of boys and girls in a disciplinary training school who so far as known had never had any contact with leprosy. From the results of more than 5,000 skin tests it was concluded that none of the antigens employed is specific for leprosy, and that consequently these tests gave no evidence that any of the microbes from which they had been prepared has an etiological relationship to the disease.

These experiments are of wider interest when one realizes that Henderson (101) has described the serological interrelationships existing between many of the bacteria included in our study and to several others (Lévy-Kedrowsky, Clegg 1, Clegg HP1, L-3, Brinckerhoff 1 and 2, Krause, Eddy, Ota-Sato—all strains of supposed *M. leprae*). Therefore, our skin tests have wider implications than the individual antigens tested would suggest.

This work has emphasized what has already been said in this review, namely, that most of these supposed strains of *M. leprae* which have been cultivated from leprosy cases are probably not strains of Hansen's bacillus at all. This is further emphasized by the mass culture work with de Leon that has been referred to. In nearly 10,000 cultures that have been prepared at the time of writing, not once has there been cultivated an acid-fast bacterium like the various supposed leprosy cultures mentioned above. Following the most careful technique in removing the lepromas used for culture purposes and in preparing the cultures, we simply do not meet with the other organisms which have been so often described by previous investigators. As for the one described by Soule with the author, in our experience so far something less than ten percent of our cultures have been positive. This would seem to indicate, as was previously suspected, that most of the organisms in leprosy lesions are dead. The fact that only a few colonies are produced on each culture slant would further suggest that the number of living organisms found is extremely limited. This may account for the many failures in culture work, and this recent experience coincides with our earliest observations made several years ago. It is our hope to report later on the results of skin tests with antigens prepared from this culture.

SUMMARY

In this review of the bacteriology of leprosy we have attempted to present the subject with the background of a rather comprehensive literature which has accumulated during the past sixty-five years, since Hansen first described the germ in leprous tissues. It is, of course, no easy task to select from the literature the most important contributions bearing upon such a matter, particularly one that has been the subject of so much controversy.

In tracing the scientific history of this disease we have seen that many claims and counterclaims have been made for and against many of the organisms which have been isolated from lepers. Too often in investigations of this problem deductions have not always rested upon facts alone; as in other fields of work prejudice and bias have to a large extent colored the discussions. This was, perhaps, only a human reaction to a very human problem and to be expected, but the writer feels that this state of mind has not contributed much towards the solution of the problem.

Considering all of the facts it must be said that there does not exist today *any absolute proof* that any investigator has actually succeeded in the artificial cultivation of the leprosy bacillus. We are aware that there are investigators who will not agree with this statement, who probably feel that organisms cultivated by them from the tissues of lepers represent the true *M. leprae*. We can appreciate this point of view. Yet the author with his colleagues, who have also secured cultures which they feel are probably of that organism, are of the opinion that that statement is the only fair one that can be made at this time. Nevertheless, we feel definitely that our organism has more in its favor than any other one which has been submitted as *M. leprae*, though it is grown only with great difficulty and is very sparse in growth. Obviously further advances as regards cultural methods are required. Meanwhile, no doubt, other investigators will be critical of the rather feeble results which we are able to obtain, but we hope that at least serious efforts will be made to confirm our findings up to this point.

As for animal experimentation, we feel again that the only fair statement which can be made at the present time is that no investigator has yet succeeded in producing in any experimental animal the counterpart of human leprosy as it is known in man. Naturally, in this statement we include our own attempts in this direction, though we feel that we have perhaps gone somewhat farther than others in establishing our organism as *M. leprae* through animal

experimentation. There is hope that new approaches to this problem may eventually lead to success in producing progressive lesions of the disease in lower animals. If this can be accomplished in a satisfactory manner, then there is also hope of eventually determining beyond doubt whether or not a given culture, suspected of being the leprosy bacillus, is really that organism.

Regarding the chemistry of *M. leprae*, it must again be pointed out that until an authentic culture of this organism can be obtained it is useless to pretend that we know anything definite regarding its composition except by analogy with what is known concerning other acid-fast bacteria. It is quite probable that all of the members of the acid-fast group have many chemical characteristics in common, but the chemical study of *M. leprae* up to the present time cannot be regarded as enjoying the same status as that of *M. tuberculosis*.

What, then, is the etiological agent of leprosy? Investigators the world over still believe that the Hansen bacillus fills that role. There seems to be no good reason to question the status of that organism, which is so constantly associated with lesions of this disease. Its final cultivation on artificial media will one day be accepted, another chapter in the study of this disease will be brought to a close, and new vistas of study will be opened.

REFERENCES

(1) ABRAHAM, P. S. The etiology of leprosy; a criticism of some current views. Practitioner **43** (1889) 153-160.

(2) ABRAHAM, P. S. Note on some early attempts at the cultivation of the bacillus of leprosy. Trans. Soc. Trop. Med. & Hyg. **5** (1911-12) 181-183.

(3) ALDERSON, H. E. Artificial cultivation of the lepra bacillus in Hawaii. California State Jour. Med. **9** (1911) 109.

(4) ALFONSECA, J. D. DE J. Les voies d'émission du bacille de la lèpre. Paris, Jouve & Cie, 1915, No. 51, 62 pp.

(5) ANDERSON, R. J. AND UYEI, N. The chemistry of the lipids of tubercle bacilli. XXVII. The composition of the phosphatide fraction of the Bacillus leprae. Jour. Biol. Chem. **97** (1932) 617-637.

(6) ARAGAS, H. DO B. Consideraçõoes sobre o papel do mosquito na transmissão da lepra. Conf. Soc. Sud-americano de Hig., 1916. Buenos Aires, 1917, vol. 1, pp. 125-136.

(7) ARNING, E. Ueber das Vorkommen des Bacillus leprae bei Lepra anaesthetica sive nervorum. Virch. Arch. **97** (1884) 170-171.

(8) ARNING, E. Eine Lepra-Impfung beim Menschen und Demonstration einer Sammlung von Lepraabgüssen. Verhandl. d. deutsch. dermat. Gesellschaft. Wien **1** (1889) 9-25.

(9) ASHMEAD, A. S. Descent and variation of the bacillus. Mitth. u. Verhandl. d. internat. Lepra Conf. Berlin, 1897, vol. 1, pp. 10-13.

(10) Babes, V. Untersuchungen ueber den Lepra-bacillus und ueber die Histologie der Lepra. Berlin, Karger, 1898; Die Lepra. Wien, Alfred Hölder, 1901.

(11) Babes, V. Sur les toxines de la lèpre. Cong. Internat. de Méd. Paris, 1900, pp. 195-199.

(12) Banciu, A. Sur la survivance de la réagine lépreuse injectée chez les animaux. Compt. rend. Soc. Biol. **92** (1925) 529.

(13) Banciu, A. (Sur les propriétés acquises par le sérum des lapins inoculés avec un produit lépreux.) Compt. rend. Soc. Biol. **92** (1925) 531.

(14) Bayon, H. Demonstrations of specimens relating to the culture of the leprosy bacillus. British Med. Jour. **2** (1911) 1269-1272.

(15) Bayon, H. Demonstration of acid-fást germs cultivated from cases of leprosy. Trans. Soc. Trop. Med. & Hyg. **5** (1911-12) 103-105.

(16) Bayon, H. The culture and identification of the germ of leprosy and the relationship of the human disease to rat leprosy. Trans. Soc. Trop. Med. & Hyg. **5** (1911-12) 158-167.

(17) Bayon, H. Die Zuechtung und Identifikation des Leprabacillus und die Beziehungen des menschlichen Aussatzes zur Rattenlepra. Berliner klin. Wochen. **49** (1912) 768.

(18) Bayon, H. On the transmission of leprosy to animals by direct inoculation. British Med. Jour. **1** (1912) 424-426.

(19) Bayon, H. A comparative experimental study of the leprosy cultures of Clegg, Duval, Kedrowsky, Rost and Williams. British Med. Jour. **2** (1912) 1191-1194.

(20) Bayon, H. A critical review of recent experimental leprosy research. South African Med. Rec. **11** (1913) 201-222.

(21) Bayon, H. Demonstration of culture of leprosy "bacillus." Trans. Internat. Cong. Med., London, 1913. 1914, sec. 4, pt. 2, p. 135.

(22) Bayon, H. Leprosy; a perspective of the results of experimental study of the disease. Ann. Trop. Med. & Parasit. **9** (1915) 1-90.

(23) Bayon, H. The artificial cultivation of Hansen's bacillus. Ann. Trop. Med. & Parasit. **9** (1915) 535-538.

(24) Besnier, E. La lèpre est-elle contagieuse? La bactérie de la lèpre. Gaz. hebd. de Med. **18** (1880) 114-116.

(25) Boeck, C. Om forekomsten af Leprabaciller i faeces hos knudede Spedalske, disse bacillers kilde og senere skjaebne. Norsk. Mag. f. Laeg. **8** (1910) 1011-1018; Ueber das Herstammen und das weitere Schicksal der mit den Faeces entleerten Leprabazillen. Dermat. Stud., Hamburg & Leipzig. **20** (1910) 436-441.

(26) Bordoni-Uffreduzzi, G. La coltivazione del bacillo della lebbra. Arch. per Sci. med. **12** (1888) 53-68.

(27) Borrel, A. and Larrousse, F. Localisation lépreuse dans le foie du rat par le cysticerque du *Taenia crassicola*. Compt. rend. Soc. Biol. **105** (1931) 822-823.

(28) Bradley, B. Preliminary note on the apparent transmission of leprosy to a macaque monkey. Med. Jour. Australia **2** (1919) 414-416.

(29) Byron, J. M. Pure cultures of lepra bacillus. Research Loomis Lab., Univ. City of New York **2** (1892) 87-90.

(30) CABRAL, J. Estado actual da sciencia sobre a bacteriologia da lepra, com investigacões pessoais sobre as culturas do bacillo de Hansen. Arq. Indo-Portug. med. e Hist. nat. **3** (1926) 107-143.

(31) CALABRESE, A. Sulla presenza di bacilli di Hansen nelle urine di un leproso affetto da nefrite. Lavori Cong. Med. int., 1898-99, vol. 9, pp. 497-499.

(32) CALEY, W., LIVEING, R., DUCKWORTH, D. AND POWELL, R. D. Trans. Clin. Soc. Lond. **13** (1880) 1-10.

(33) CAMPANA, R. Tentativi ripetuti, ma senza risultato positivo, nella cultura del bacillo leproso. Riforma med. **5** (1889) 1454; 1460.

(34) CAMPANA, R. Un bacillo simile al bacillo leproso sviluppatosi in tentative di coltura di tessuti con lepra tubercolare. Riforma med. **7** (1891) 159.

(35) CAMPANA, R. Altre particolarita di un bacillo simile al bacillo leproso sviluppatosi in tentativi di coltura di lepra tubercolare. Riforma med. **8** (1892) 796; 808.

(36) CAMPANA, R. Streptococchi settici insieme a bacilli della lepra in un caso di nefrite leprosa. Bull. e Atti R. Accad. med. Roma **20** (1894) 55-56.

(37) CAMPANA, R. Ueber die Kultur des Leprabacillus und die Ubertragung der Lepra auf Tiere. Zeitschr. f. Hyg. u. Infekt. **67** (1910) 361-372.

(38) CANTACUZÈNE, J. AND LONGHIN, S. Transmission expérimentale de la lèpre humaine au rat blanc. Compt. rend. Acad. Sci. **195** (1932) 533-535.

(39) CANTACUZÈNE, J. AND LONGHIN, S. De l'existence d'un ultra-virus chez le bacille de la lèpre humaine. Compt. rend. Soc. Biol. **109** (1932) 1003-1004.

(40) CHIRIVINO, V. Sul trapianto di noduli leprosi nella camera anteriore dell' occhio del coniglio. Gazz. internaz. Med. **15** (1912) 121; 150.

(41) CHIYUTO, S. Early leprotic changes in children and their bearing on the transmission and evolution of the disease. Month. Bull. Bur. Health (Manila) **13** (1933) 5-48.

(42) CLEGG, M. T. Some experiments on the cultivation of Bacillus leprae. Philippine Jour. Sci. **4** (1909) 77-79.

(43) CLEGG, M. T. The cultivation of the leprosy bacillus. Philippine Jour. Sci. **4** (1909) 403-414.

(44) CORNIL, V. Note sur le siège des bactéries dans la lèpre et sur les lésions des organes dans cette maladie. Bull. Acad. Méd. **10** (1881) 1308-1310.

(45) CORNIL, V. Note sur le siège des bactéries dans la lèpre et sur les lésions des organes dans cette maladie. Union méd. **32** (1881) 1045-1057.

(46) CORNIL, V. AND SUCHARD. Note sur le siège des parasites de la lèpre. Ann. Dermat. et Syph. **2** (1881) 653-657.

(47) CORPER, H. J., GAUSS, H. AND RENSCH, O. B. Studies on influence of carbon dioxide on resistance to tuberculosis; effect of carbon dioxide on tubercle bacillus. American Rev. Tuberc. **5** (1921) 562-587.

(48) COURET, M. The behavior of Bacillus leprae in cold-blooded animals. Jour. Exper. Med. **13** (1911) 576-589.

(49) Crow, G. B. Acid-fast bacilli in the circulating blood of lepers. United States Navy Med. Bull. **6** (1912) 25-34.

(50) Cumston, C. G. Modes of transmission of Bacillus leprae. New York Med. Jour. **108** (1918) 992-994.

(51) Currie, D. H., Brinckerhoff, W. R. and Hollmann, H. T. On the cultivation of bacillus of leprosy by the method of Clegg. Publ. Health Rep. **25** (1910) 1173-1175.

(52) Currie, D. H., Clegg, M. T. and Hollmann, H. T. Cultivation of the bacillus of leprosy. Lepra **13** (1912) 71-86.

(53) Czaplewski. Ueber einen aus einem Leprafalle gezüchteten alkohol und säurefesten Bacillus aus der Tuberkelbacillengruppe. Centralbl. f. Bakt. **23** (1898) 97; 189-194.

(54) Darling, S. T. Observations on the factors concerned in the cultivation of leprosy bacilli and on the development of a therapy. Proc. Canal Zone Med. Assn., 1911, pt. 1, pp. 122-132.

(55) Delépine, S. Demonstration on a case of tubercular leprosy to show the different characters presented by the Bacillus leprae growing in the various organs of the same patient. Trans. VII Internat. Cong. Hyg. & Dermog., 1891-92. Vol. 2, pp. 235-238.

(56) Denney, O. E. and Eddy, B. E. Comments on *in vitro* behavior of lepra and certain other acid-fast organisms in presence of leukocytes. Arch. Dermat. & Syph. **27** (1933) 794-806.

(57) Deycke Pasha and Reschad Bey. A bacterial fat as immunizing substance in leprosy; its theoretic significance and practical application. Therapist **17** (1907) 49; 67; 79.

(58) Ducrey, A. Tentativi di coltura del bacillo della lepra con risultato positivo. Gior. italiano Mal. ven. **27** (1892) 76-99.

(59) Dumont, J. Les formes Hanséniennes du bacille tuberculeux. Paris méd. **2** (1928) 355-356.

(60) Düring, E. V. Zur Frage de Heredität der Lepra. Lepra **11** (1910) 47-56.

(61) Duval, C. W. The cultivation of the leprosy bacillus and the experimental production of leprosy in the Japanese dancing mouse. Jour. Exper. Med. **12** (1910) 649-665.

(62) Duval, C. W. Notes on the biology of B. leprae. New Orleans Med. & Surg. Jour. **63** (1910-11) 549-559.

(63) Duval, C. W. The cultivation of the leprosy bacillus from the human tissues with special reference to the amino-acids as culture media. Jour. Exper. Med. **13** (1911) 365-373.

(64) Duval, C. W. The experimental production of leprosy in the monkey (Macacus rhesus). Jour. Exper. Med. **13** (1911) 374-379.

(65) Duval, C. W. The present status of the bacteriology of human leprosy. Science **36** (1912) 281-285.

(66) Duval, C. W. A critical review of the bacteriology of human leprosy. (With special reference to the chromogenic and non-chromogenic acid-fast strains.) Trans. XV Internat. Cong. Hyg. & Demog., Washington, 1913, vol. 2, parts 1-2, pp. 52-56.

(67) Duval, C. W. The organisms isolated from the lesions of human leprosy. British Med. Jour. **2** (1912) 1189.

(68) Duval, C. W. Pertinent remarks upon the bacteriology and pathology

of leprosy. New Orleans Med. & Surg. Jour. **67** (1914-15) 1009-1015.

(69) DUVAL, C. W. The behavior of the Hansen bacillus in vitro. American Jour. Trop. Dis. **2** (1914-15) 185-190.

(70) DUVAL, C. W. AND COURET, M. A further note upon the experimental production of leprosy in the monkey (Macacus rhesus) with a critical study of the culture employed. Jour. Exper. Med. **15** (1912) 292-306.

(71) DUVAL, C. W. AND GURD, F. B. Experimental immunity with reference to the bacillus of leprosy. Part I. A study of the factors determining infection. Jour. Exper. Med. **14** (1911) 181-195.

(72) DUVAL, C. W., GURD, F. B. AND HOPKINS, R. Experimental immunity with reference to the leprosy bacillus. Part II. A study of the factors determining the cure of individuals infected by the Bacillus leprae. Jour. Infect. Dis. **9** (1911) 350-365.

(73) DUVAL, C. W. AND HARRIS, W. H. Further studies upon the leprosy bacillus. Its cultivation and differentiation from other acid-fast species. Jour. Med. Res. **28** (1913) 165-198.

(74) DUVAL, C. W. AND WELLMAN, C. A new and efficient method for cultivating Bacillus leprae from the tissue; with observations on the different strains of acid-fast bacilli found in leprous lesions. Jour. American Med. Assoc. **58** (1912) 1427.

(75) DUVAL, C. W. AND WELLMAN, C. A critical study of the organisms cultivated from the lesions of human leprosy, with a consideration of their aetiological significance. Jour. Cutan. Dis. **30** (1912) 397-409; *also* Jour. Infect. Dis. **11** (1912) 116-139.

(76) [EDITORIAL] The inoculation of a condemned criminal. Med. Rec. **29** (1886) 449.

(77) EICHBAUM, F. Erfahrungen und Beobachtungen an Säurefesten Stäbchen. Zeitschr. f. Immunitäts. **74** (1932) 31-55.

(78) FRANCHINI, G. Setticemia da bacilli di Hansen in un caso di lebbra nodulare. Arch. italiano Sci. med. colon. **9** (1928) 643-646.

(79) FRANCHINI, G. Reproduccion experimental de la lepra en el mono. Prensa méd. argentina **15** (1929) 1423-1424.

(80) FRANCHINI, G. Sulla riproduzione sperimentale della lebbra nella Scimmia. Arch. italiano Sci. med. colon. **11** (1930) 1-4.

(81) FRANCHINI, G. AND CENDALI, G. Riproduzione sperimentale della lepra nella scimmia e nel ratto bianco. Arch. italiano Sci. med. colon. **8** (1927) 3-6.

(82) FRASER, H. The cultivation of the bacillus of leprosy. Jour. Trop. Med. **16** (1913) 164.

(83) FRASER, H. AND FLETCHER, W. The bacillus leprae; has it been cultivated? Lancet **2** (1913) 918-921.

(84) FRASER, H. AND FLETCHER, W. The cultivation of the leprosy bacillus. Ann. Trop. Med. & Parasit. **9** (1915) 381-382.

(85) FRASER, H. AND FLETCHER, W. Leprosy and Kedrowsky's bacillus. Lancet **2** (1915) 13-16.

(86) GAUCHER, E. Culture des bactéries de la lèpre. Compt. rend. Soc. Biol. **3** (1881) 201-202.

(87) GAUCHER, E. Etiologie et bactériologie de la lèpre. Jour. Méd. int. **13** (1909) 1-4.

(88) GIORDANO, M. Ricerche culturali in casi de lebbra. Arch. italiano Sci. med. colon. **10** (1929) 8-13.

(89) GUARCH, F. S. Estudios sobre el cultivo del bacilus de la lepra y desarrollo de la enfermedad en el animal. Ann. San. mil. **5** (1903) 681; 819.

(90) GURD, F. B. AND DENIS, W. The biochemistry of Bacillus leprae. Jour. Exper. Med. **14** (1911) 606-611.

(91) HANSEN, G. A. Spedalskhedens Arsager. Norsk. Mag. f. Laegevidensk. **9** (1874) 1-88.

(92) HANSEN, G. A. Bacillus leprae. Nord. med. Ark. **12** (1880) 1-10.

(93) HANSEN, G. A. The bacillus of leprosy. Quart. Jour. Micro. Sci. **20** (1880) 92-102.

(94) HANSEN, G. A. Études sur la bactérie de la lèpre. Arch. de Biol. **3** (1882) 659-667.

(95) HANSEN, G. A. Culture du bacillus de la lèpre. Nord. med. Ark. **14** (1882) 11-12.

(96) HANSEN, G. A. Die Lage der Leprabacillen. Virch. Arch. **103** (1886) 388-392.

(97) HANSEN, G. A. Zu Hutchinsons Fischtheorie. Lepra **7** (1907) 27.

(98) HANSEN, G. A. Heredity of leprosy. Lepra **12** (1911-12) 147-152.

(99) HARRIS, V. D. Report on certain microscopic specimens of various tissues obtained from a case of true leprosy. Trans. Clin. Soc. (London) **13** (1880) 11.

(100) HARRIS, W. AND LANFORD, J. A. The agglutination reaction with sera derived from human cases of leprosy and from the experimental animal upon various members of the acid-fast group. Jour. Med. Res. **34** (1916) 157-167.

(101) HENDERSON. H. J. A serological comparison of the proteins of various strains of leprosy bacilli and other acid-fast bacteria. Internat. Jour. Lep. **5** (1937) 267-275.

(102) HENDERSON, J. M. Preliminary observations on acid-fast organisms isolated from human lesions. Indian Jour. Med. Res. **19** (1931) 145-153.

(103) HESSE, W. Ueber die gasförmigen Stoffwechselproducte beim Wachsthum der Bakterien. Zeits. f. Hyg. Infekt. **15** (1893) 17-37.

(104) HILLAIRET AND GAUCHER, E. Note sur le parasitisme de la lèpre. Compt. rend. Soc. Biol. **2** (1880) 386-388.

(105) HOLLMANN, H. T. The cultivation of an acid-fast bacillus from a rat suffering with rat leprosy. A preliminary report.) Publ. Health Rep. **27** (1912) 69.

(106) HOLLMANN, H. T. B. leprae in the circulating blood of lepers. Pub. Health Bull. No. 75, 1916, pp. 15-19.

(107) HONEIJ, J. A. Leprosy; the presence of acid-fast bacillus in the circulating blood and excretions. Jour. Infect. Dis. **17** (1915) 376-387.

(108) IYENGAR, K. R. K. A note on the presence of acid-fast bacilli in the blood of lepers. Indian Jour. Med. Res. **7** (1919-20) 235-237.

(109) JOHNSTON, J. A. A contribution to the bacteriology of leprosy. Philippine Jour. Sci., Section B, Trop. Med. **9** (1914) 227-232.

(110) JOHNSTON, J. A. The varying morphology of Bacillus leprae and the

routine microscopic examination of nasal mucus in lepers. Philippine Jour. Sci. **12** (1918) 115-119.

(111) KAHN, M. C. AND SCHWARZKOPF, H. Single cell dissociation of acid-fast bacteria. Mycobacterium of avian tuberculosis; mycobacterium of "rat leprosy." Jour. Bact. **25** (1933) 157-199.

(112) KANTHACK, A. A. AND BARCLAY, A. Beitrag zur Cultur des Bacillus leprae. Virch. Arch. **125** (1891) 398-404.

(113) KARLINSKI, J. Zur Bacteriologie der Lepra. Allg. med. Centr. **72** (1903) 965.

(114) KENDALL, A. J., DAY, A. A. AND WALKER, A. W. The metabolism of "lepra bacillus," grass bacillus, and smegma bacillus in plain, dextrose, mannite, and glycerin broths; studies in acid-fast bacteria. V. Jour. Infect. Dis. **15** (1914) 439-442.

(115) KEDROWSKY, W. J. Artificial cultures of the leprosy bacillus. Russ. Arch. Pathol., Klin. Med. i Bakteriol. **10** (1900) 449-467.

(116) KEDROWSKY, W. J. Experimentele Erfahrungen über Lepraimpfungen bei Tieren. Centralbl. f. Bakt. **35** (1903) 368-374.

(117) KEDROWSKY, W. J. Ueber Kulturen von Leprabazillen und deren Verimpfung mit Erfolg. Verhandl. Gesellsch. deutsch. Naturf. u. Aertze **82** (1911) 424-426.

(118) KEDROWSKY, W. J. Experimentelle Untersuchungen über Lepraimpfungen bei Tieren, zur Bakteriologie und pathologischen Anatomie der Lepra. Zeitschr. Hyg. u. Infekt. **66** (1910) 1-97.

(119) KEDROWSKY, W. J. The microbiology of leprosy bacillus. Jour. Trop. Med. **31** (1928) 17-21.

(120) KLINGMÜLLER, V. Ueber die granuläre Form des Lepraerregers. Med. Rev. **44** (1927) 649-653.

(121) KLITIN, I. I. [Microbiology of the bacilli of leprosy and their action on animals.] Voyenno Med. Jour. **3** (1905) 231; 447.

(122) KLITIN, I. I. [Artificial cultures of leprosy bacilli and their action on animals.] Russk. Vrach. **4** (1905) 336-343.

(123) KÖBNER, H. Uebertragungsversuche von Lepra auf Thiere. Virch. Arch. **88** (1882) 282.

(124) KOHDA, K. On the cultivation of Bacillus leprae. Kitasato Arch. Exper. Med. **4** (1920-21) 141-158.

(125) KONDO, S. Der Verwendungstoffwechsel der sogenannten Leprabacillen. Zeitschr. Hyg. u. Infekt. **104** (1925) 714-728.

(126) KRITSCHEWSKY, J. AND BIERGER, O. Zur Frage über das Verhältnis des Bacillus leprae Hansen zu einigen bei Lepre gezüchteten Mikroörganismen. Zeit. Hyg. u. Infekt. **73** (1912-13) 509-560.

(127) KÜSTER-FREIBURG, E. Eine einfache Methode der Leprabazillenanreichenrung. Verhandl. Gesellsch. deutsch. Naturf. u. Aertze **82** (1911) 406.

(128) KYRLE, J. Beitrage zur Frage der Lepra-Ueberimpfung auf Affen. Frankfurt. Zeits. f. Path. **19** (1916) 172-197.

(129) LAGANE, L. Bacillurie provoquée dans la lèpre. Bull. Soc. Path. exot. **5** (1912) 784-786.

(130) LEBOEUF, A. Dissémination du bacille de Hansen par le mouche domestique. Bull. Soc. Path. exot. **5** (1912) 860-868.

(131) LÉVY, E. Ein neues aus einem Fall von Lepra gezüchtetes Bacterium

aus der Klasse der Tuberkelbacillen; Studien über diese Klasse. Arch. f. Hyg. **30** (1897) 168-183.

(132) Limousin, H. Inoculation de la lèpre humaine du lapin. Compt. rend. Acad. Sci. **178** (1924) 599.

(133) Liston, W. G. and Williams, T. S. B. A streptothrix isolated from the spleen of a leper. Calcutta, 1912, Gov. Print. 8 pp.

(134) Long, E. R. and Campbell. L. K. Lipin content of acid-fast bacilli. American Rev. Tuberc. **6** (1922-23) 636-641.

(135) Lutati, C. V. Sulle granulazioni degenerative del bacillo della lepra nelle ghiandole sudorifere. Gazz. Med. italiano **50** (1909) 421-423.

(136) Lutz, A. Zur Morphologie des Mikroörganismus der Lepra. Derm. Stud. (Hamburg) **1** (1886) 77-100.

(137) Machow, D. Zur Frage über Kedrowskis Leprakultur. Centralbl. f. Bakt. **67** (1912-13) 434-446.

(138) MacLeod, J. M. H. A brief survey on the present state of our knowledge of the bacteriology and pathological anatomy of leprosy. Lepra **11** (1910) 309-320.

(139) Manson, P. The demonstration of the Bacillus leprae. Lancet **2** (1884) 342.

(140) Manson-Bahr, P. H. Manson's Tropical Diseases. New York, Wm. Wood & Co., 1926, p. 443.

(141) Marchoux, E. Les migrations du bacille de la lèpre. Lepra **11** (1910) 57-60.

(142) Marchoux, E. Culture d'un bacille acido-résistant provenant du mucus nasal des lépreux. Bull. Soc. Path. exot. **4** (1911) 89-91.

(143) Marchoux, E. The problem of leprosy. British Med. Jour. **2** (1912) 1191.

(144) Marchoux, E. Is rat leprosy transmissible to man? Bull. Acad. Méd. **87** (1922) 545-547.

(145) Marchoux, E. and Bourret, G. Essai d'inoculation de la lèpre au chimpanze. Bull. Soc. Path. exot. **1** (1908) 416-420.

(146) Marchoux, E., Markianos, J. and Chorine, V. Le bacille de la lèpre a-t-il été obtenu en cultures artificielles? Compt. rend. Soc. Biol. **106** (1931) 1191-1193.

(147) Mariani, G. Nouve osservazioni sulle reazioni provocate sperimentalmente con materiale lebbroso nell'uomo. Gior. italiano Dermat. e Sif. **66** (1925) 402-426.

(148) Markianos, J. Le développement du virus filtrant avant sa transformation en bacille de la lèpre des rats. Bull. Soc. Path. exot. **22** (1929) 537.

(149) Markianos, J. L'ultra-virus de la lèpre des rats. Bull. Soc. Path. exot. **22** (1929) 896.

(150) Markianos, J. Développement rapide du virus filtrant de la lèpre des rats après inoculation aux jeunes rats. Bull. Soc. Path. exot. **22** (1929) 758.

(151) Markianos, J. Recherches sur l'action préventive, sur la lèpre des rats, de l'antigene de bacilles dégraissés. Bull. Soc. Path. exot. **23** (1930) 149-150; **23** (1930) 150-152.

(152) Maucione, L. Risultati delle inoculazioni di frammenti e di emulsione di lepromi umani nella camera anteriore e nella cornea del coniglio. Arch. Ottal. **31** (1924) 385-408.

(153) MAZZA, S. Fagocitosis de los bacilos de Stefansky en el peritones de ratas y cobayos. Prensa méd. argentina **11** (1924-25) 879-881.

(154) MCCOY, G. W. The cultivation of acid-fast bacilli from lepers by the use of symbiotic organisms. Publ. Health Bull. No. 66, 1914 pp. 11-14.

(155) MCKINLEY, E. B. A concept of the ultramicroscopic virus diseases and a classification. Science **76** (1932) 449-454.

(156) MCKINLEY, E. B. The etiology of leprosy. Medicine **13** (1934) 377-504.

(157) MCKINLEY, E. B. A Geography of Diseases. Washington, D. C. The George Washington Univ. Press. 1935.

(158) MCKINLEY, E. B. The present status of diagnostic skin tests in leprosy. Internat. Jour. Lep. **6** (1938) 33-46.

(159) MCKINLEY, E. B. AND DE LEON, W. Mass cultivation of *Mycobacterium leprae*. Internat. Jour. Lep. **5** (1937) 259-266.

(160) MCKINLEY, E. B. AND SOULE, M. H. Studies on leprosy; experimental lesions in monkeys and cultivation of bacillus leprae. Jour. American Med. Assoc. **98** (1932) 361-367.

(161) MCKINLEY, E. B. AND VERDER, E. Cultivation of *Mycobacterium leprae*. Proc. Soc. Exper. Biol. & Med. **30** (1933) 659-661.

(162) MCKINLEY, E. B. AND VERDER, E. Further studies on the cultivation of *Mycobacterium leprae*. Proc. Soc. Exper. Biol. & Med. **31** (1933) 295-296.

(163) MCKINLEY, E. B. AND VERDER, E. Unpublished data.

(164) MELLO, F. DE, AND CABRAL, J. Essais de culture du bacille de Hansen. Bull. Soc. Path. exot. **19** (1926) 771-774.

(165) MERIAN, L. Positiver Leprabazillenbenfund im Inhalte einer Kuhpockenpustel bei einem an Lepra tuberosa leidenden Patienten. Centralbl. Inn. Med. **33** (1912) 989-995.

(166) MOORE AND WILLIAMS. Cited by NOVY, F. G. AND SOULE, M. H. Jour. Infect. Dis. **36** (1925) 168-232.

(167) MUCH, H. Serological and experimental studies on leprosy. Trans. Soc. Trop. Med. & Hyg. **5** (1911-12) 175-180.

(168) MUIR, E. (WITH BULLOCH, W.) Leprosy. *In:* A System of Bacteriology in Relation to Medicine. London, 1930, His Majesty's Stationery Office, vol. 5, pp. 345-382.

(169) MUIR, E. AND HENDERSON, J. M. Rat leprosy. A record of experimental work carried on at School of Tropical Medicine and Hygiene, Calcutta, between Oct. 1925 and Aug. 1927. Indian Jour. Med. Res. **15** (1928) 807-817.

(170) MUIR, E., HENDERSON, J. M. AND LANDEMAN, E. Tumour-like growths caused by intraperitoneal inoculation of *B. leprae murium*. Indian Jour. Med. Res. **15** (1927) 15-19.

(171) NAKANO, H. Experimentelle Untersuchungen über die Infektionsmöglichkeit von japanischen Hausratten, Kaninchen und Meerschweinchen mit Lepramaterial. Arch. Dermat. u. Syph. **113** (1912) 781-798.

(172) NÈGRE, L. Action des lipides des organes du cobaye sur l'évolution de la tuberculose expérimentale de cet animal. Compt. rend. Soc. Biol. **112** (1933) 1301.

(173) Neisser, A. Ueber die tinctoriellen Verhältnisse der Leprabacillen; Erwiderung an P. G. Unna. Fortsch. d. Med. **7** (1889) 816-820.

(174) Neiser, A. Ueber die Structur der Lepra- und Tuberkelbacillen mit specieller Berücksichtigung der Rosanilin und Pararosanilin-farbstoffe, und über Leprazellen. Verhandl. d. deutsch. dermat. Gesellsch. **I** (1889) 29-56.

(175) Newman, M. S. and Anderson, R. J. Ueber die Polysaccharide der Leprabazillen. Hoppe-Seyler's Zeits. f. physiol. Chemie **220** (1933) 1-4.

(176) Nicolle, C. Reproduction expérimentale de la lèpre chez le singe. Caducée (Paris) **5** (1905) 61.

(177) Nicolle, C. and Blaizot, L. Reproduction expérimentale de la lèpre chez les singes inférieurs. Compt. rend. Soc. Biol. **69** (1910) 231-233.

(178) Nicolle, C. and Blaizot, L. Essais de reproduction de la lèpre chez le chimpanze et les singes inférieurs. Compt. rend. Soc. Biol. **70** (1911) 991-993.

(179) Novy, F. G. and Soule, M. H. Microbic respiration. II. Respiration of the tubercle bacillus. Jour. Infect. Dis. **36** (1925) 168-232.

(180) Novy, F. G., Roehm, H. R. and Soule, M. H. Microbic respiration. I. The compensation manometer and other means for the study of microbic respiration. Jour. Infect. Dis. **36** (1925) 109-167.

(181) Oliver, W. W., de Leon, W. and de Roda, A. P. The attempted cultivation of *Mycobacterium leprae*. Philippine Jour. Sci. **46** (1931) 611-625.

(182) Ota, M. and Sato, S. Cultures de deux variétés d'un bacille acido-résistant à partir du sang et du léprome de lépreux. Compt. rend. Soc. Biol. **107** (1931) 1062-1063.

(183) Ota, M. and Sato, S. Culture du bacille tuberculeux à partir d'un léprome typique et d'un ganglion lymphatique de lépreux. Compt. rend. Soc. Biol. **107** (1931) 1064-1065.

(184) Ota, M. and Sato, S. Réproduction de la lèpre chez les animaux par l'inoculation de cultures du M. leprae. Compt. rend. Soc. Biol. **109** (1932) 29-32.

(185) Ota, M. and Sato, S. Réproduction de la lèpre chez les rats blancs par l'inoculation de lépromes en émulsion. Compt. rend. Soc. Biol. **109** (1932) 75-77.

(186) Paldrock, A. [Chemistry of causal agent of leprosy.] Dermat. Wochenschr. **81** (1925) 1431-1433.

(187) Paldrock, A. [Chemistry of Bacillus leprae.] Dermat. Wochenschr. **82** (1926) 801-804.

(188) Paldrock, A. Zur chemie des Lepraerregers. Dermat. Wochenschr. **84** (1927) 289-292.

(189) Paldrock, A. Das mikrochemische Verhalten der Lepraerreger und der Tuberkelbazillen. Dermat. Wochenschr. **84** (1927) 487-491.

(190) Paldrock, A. On the chemistry of the leprosy organism. American Jour. Trop. Med. **7** (1927) 405-408.

(191) Park, W. H. and Williams, A. Pathogenic Microöganisms. Lea and Febiger, Phila., 1933, 10th ed.

(192) Peschkowsky, G. W. and Malilin, I. M. Ueber Prof. Kuleschas

Methode der Züchtung von Reinkulturen des Leprabazillus. Centralbl. f. Bakt. **126** (1932) 137-145.

(193) PINEDA, E. V. Differentiation of *Mycobacterium leprae* from the more common acid-fast bacilli. Jour. Philippine Islands Med. Assoc. **4** (1924) 373-378.

(194) PINEDA, E. V. Studies on the negative leper. I. The persistence of *Mycobacterium leprae* in the negative leper. Jour. Philippine Islands Med. Assoc. **7** (1927) 109-111; *also* Ibid **8** (1928) 65-67.

(195) PINEDA, E. V. The presence of *Mycobacterium leprae* in the placenta and umbilical cord. Jour. Philippine Islands Med. Assoc. **8** (1928) 67-70.

(196) PINOY, E. P. AND FABIANI, G. Essai négatif d'inoculation de la lèpre chez un singe splénectomisé. Compt. rend. Soc. Biol. **110** (1932) 489-490.

(197) PISACANE, C. Tentativi de cultura del bacillo de Hansen col terreno di Hahn. Boll. Soc. italiano Biol. sper. **6** (1931) 912-915.

(198) RAKE, B. Report on cultivation experiments with the Bacillus leprae. British Med. Jour. **2** (1888) 215-220.

(199) RECIO, A. Sobre la etiologia de la lepra. Sanid. y. Benefic. (Habana) **2** (1909) 384-389.

(200) REED, G. B. AND GARDINER, B. G. Studies in the variability of tubercle bacilli. V. Acid agglutination and electrophoretic potential in M. leprae. Canadian Jour. Res. **6** (1932) 622-631.

(201) REENSTIERNA, J. Ueber die Kultivierbarkeit und morphologie des Lepraerregers und die Uebertragung der Lepra auf Affen. Arch. Dermat. u. Syph. **116** (1913) 480-554.

(202) REESTIERNA, J. Réproduction expérimentale de la lèpre chez les singes inférieurs. Compt. rend. Acad. Sci. **181** (1925) 828-830.

(203) REENSTIERNA, J. Réproduction expérimentale de la lèpre chez les singes inférieurs. Ann. Inst. Pasteur **40** (1926) 78-88.

(204) Report of the Leonard Wood Memorial Conference on Leprosy. Philippine Jour. Sci. **44** (1931) 449-480.

(205) RIATTI, C. Effetti del bacillo leproso sui ganglii spinali dei conigli, in rapporto coll'azione irritante, che possono ridestare su di essi alcuni agenti chimici. Clin. dermosifilopat. r. Univ. di Roma, 1898, pp. 121-149.

(206) RICHAD, R. Ueber das Lepravirus. Beitr. Klin. Tuberk. **46** (1920-21) 392-394.

(207) ROCCA, C. Culture del bacillo leproso ovute da un'altra inferma di lepra tubercolare. Bull. Soc. Lancisiana Osped. di Roma **18** (1898) 3.

(208) RODRIGUEZ, J., MABALAY, E. AND TOLENTINO, J. C. Gram positive forms of *Mycobacterium leprae* from leprotic lesions bacteriologically negative for acid-fast organisms. Philippine Jour. Sci. **51** (1933) 617-629.

(209) ROFFO, A. H. Sobre la transmission de la lepra a los monos inferiores. Bol. Inst. Med. exper. para Estud. y Trat. Cancer **4** (1927) 64-91.

(210) ROGERS, L. Note on "defatting" of acid-fast bacilli of leprosy and tubercle by injections of chaulmoogrates and morrhuates. British Med. Jour. **2** (1923) 11.

(211) Rogers, L. and Muir, E. Leprosy. Wm. Wood & Co., New York, 1925.

(212) Rost, E. R. The cultivation of the Bacillus leprae. Indian Med. Gaz. **39** (1904) 167-169.

(213) Rost, E. R. A clinical report on the treatment of leprosy by the use of a vaccine prepared from cultivations of the leprosy streptothrix. Indian Med. Gaz. **46** (1911) 249-253.

(214) Rost, E. R. On the leprosy bacillus and allied bacilli. Trans. Internat. Cong., Lond., 1914, sect. 4, pt. 2, pp. 111-118.

(215) Russell, W. A case of leprosy by inoculation. South African Med. Rec. **8** (1910) 208.

(216) Sabrazès, J. Léprose massive de la rate et du foie; pas de bacilles de Hansen dans la bile. Compt. rend. Soc. Biol. **93** (1925) 1526-1528.

(217) Scanga, A. Gli effetti de tentativi con trapiantazioni de lepra sul cervello de alcuni ammali. Clin. dermosifilopat. r. Univ. di Roma, 1896-98, pp. 93-120.

(218) Schlossmann, K. Vergleichende Studien über Lipoidstoffe der Leprabazillen, Tuberkelbazillen und der tierischen organe. Zeits. f. Immunitats. **62** (1929) 447-463.

(219) Schlossmann, K. Cultivation of leprosy bacilli. Centralbl. f. Bakt. **115** (1930) 474-480.

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

(221) Scholtz, W. and Klingmüller, V. Ueber Züchtungs-Versuche des Leprabacillus und über sogenannte "Leprin." Lepra **1** (1900) 93-103.

(222) Serra, A. Contributo allo studio del bacillo di Hansen (riferisse il socio Mazza). Gior. italiano Mal. ven. **51** (1910) 312-330.

(223) Serra, A. Inoculation de culture du bacille de Hansen dans l'oeil du lapin. Lepra **12** (1911) 1-14.

(224) Serra, A. Dernières recherches sur l'inoculation de matériel lépreux dans l'oeil du lapin. Lepra **13** (1912) 237-247.

(225) Shiga, K. Studien über die Kultur der Leprabazillen. Centralbl. f. Bakt. **114** (1929) 511.

(226) Shiga, K. Studien über die Kultur der Leprabacillen. Iste Mitteilung. Acta Med. **12** (1929) 72-80.

(227) Shiga, K. Die Culture und die Kolonienbildung der Leprabacillen. Jour. Chosen Med. Assoc. **19** (1929) 541-543.

(228) Smith, A. J. and Rivas, D. The culture and biological characteristics of lepra bacillus. New Orleans Med. & Surg. Jour. **65** (1912-13) 265-275.

(229) Solis, F. and Wade, H. W. Bacteriological findings in children of lepers, with special reference to nasal lesions. Jour. Philippine Islands Med. Assoc. **5** (1925) 365-369.

(230) Sonnenschein, C. Kultur von Leprabakterien auf Glyzerinei- und Malachitgrünei-Nährboden. Centralbl. f. Bakt. **117** (1930) 284-286.

(231) Sorel, F. Recherches du bacille de Hansen dans les ganglions de per-

sonnes saines vivant dans l'entourage des lépreux. Bull. Soc. Path. exot. **5** (1912) 698-702.

(232) SOULE, M. H. Microbic respiration. III. Respiration of Trypanosoma lewisi and Leishmania tropica. Jour. Infect. Dis. **36** (1925) 246-308.

(233) SOULE, M. H. Cultivation of *Mycobacterium leprae*. Proc. Soc. Exper. Biol. & Med. **31** (1934) 1197; also personal communication.

(234) SOULE, M. H. AND MCKINLEY, E. B. Cultivation of B. leprae with experimental lesions in monkeys. American Jour. Trop. Med. **12** (1932) 1-36; *reprinted in* Internat. Jour. Lep. **1** (1933) 53-80.

(235) SOULE, M. H. AND MCKINLEY, E. B. Further studies on experimental leprosy and cultivation of *Mycobacterium leprae*. American Jour. Trop. Med. **12** (1932) 441-452.

(236) DE SOUZA-ARAUJO, H. C. Cultura do bacillo de lepra. Brazil-med. **1** (1927) 199.

(237) DE SOUZA-ARAUJO, H. C. Sur la transmission de la lèpre humaine a la souris blanche. Compt. rend. Soc. Biol. **99** (1928) 1337-1339; *also* Transmissão de lepra humana ao camondongo branco. Sciencia med. **6** (1928) 373-377.

(238) DE SOUZA-ARAUJO, H. C. Essais de culture du *Mycobacterium leprae* (*Coccothrix leprae* Lutz, 1886). Isolement à partir d'un léprome d'un actinomyces, *Actinomyces lepromatis* n. sp. Compt. rend. Soc. Biol. **100** (1929) 937-939.

(239) DE SOUZA-ARAUJO, H. C. Experimental leprosy. Trans. Roy. Soc. Trop. Med. & Hyg. **24** (1931) 577-598.

(240) SPRONCK, C. H. H. Le culture du bacille de Hansen et le sérodiagnostic de la lèpre. Sémaine med. (Paris) **18** (1898) 393-395; *also* De cultuur van den basil van Hansen en de serodiagnostiek van lepra. Nederl. Tijdschr. v. Geneesk. **34** (1898) 522-531.

(241) STALLARD, J. H. The leprosy bacillus. British Med. Jour. **2** (1889) 1388.

(242) STANLEY, W. M. AND ADAMS, R. Surface tension studies on 120 acids. Jour. American Chem. Soc. **54** (1932) 1548.

(243) STANLEY, W. M., COLEMAN, C. H., GREER, C. M., SACKS, J. AND ADAMS, R. Bacteriological action of certain synthetic organic acids towards M. leprae and other acid-fast bacteria. Jour. Pharm. & Exper. Therap. **45** (1932) 121-162.

(244) STANZIALE, R. Inoculazioni di materiale leproso nella camera anteriore dei conigli. Gior. italiano Mal. ven. **45** (1910) 702-737.

(245) STANZIALE, R. Sulla transmissione sperimentale della lepra; una importantissima comunicazione. Gior. internaz. Sc. med. **33** (1911) 555-563.

(246) STANZIALE, R. Richerche sulle inoculazioni di materiale leproso nella camera anteriore dell'occhio nei conigli. Atti r. Accad. Med. Chir. di Napoli **65** (1911) 51-58.

(247) STANZIALE, R. Isolamento colturale di un bacille acidoresistente del occhio leproso di coniglio. Atti. r. Accad. Med. Chir. di Napoli 67 (1914) 189-193.

(248) STANZIALE, R. Neue Untersuchungen über die experimentellen leprösen Läsiones des Kaninchenauges. Centralbl. f. Bakt. **75** (1915) 498-507.

(249) STEVEN, J. L. The leprosy bacillus. British Med. Jour. **2** (1885) 94-96.

(250) SUGAI, T. Ueber die Agglutination der Leprabazillen durch das Serum von Leprakranken. Dermat. Zeitschr. **16** (1909) 141-144.

(251) SURGEON GENERAL, United States Public Health Service, Annual Report for 1931. Washington, D. C., p. 35.

(252) SURGEON General, United States Public Health Service, Annual Report for 1932. Washington, D. C., p. 30.

(253) TEAGUE, O. The cutaneous reaction in leprosy. Preliminary report. Philippine Jour. Sci. **4** (1909) 323-327.

(254) TEICH, M. Beiträge zur Kultur des Lepra bacillus. Centralbl. f. Bakt. **25** (1899) 756-761.

(255) THIN, G. On the bacillus of leprosy. Proc. Roy. Med. & Chir. Soc. **I** (1882-5) 146-149.

(256) THIN, G. Présentation de préparations du bacille de la lèpre. Bull. Soc. française Dermat. et Syph. **4** (1893) 251.

(257) TISSEUIL, J. Réaction, chez l'homme, du tissu cellulaire sous-cutané et du derme, aux bacilles lépreux tués par la chaleur. Bull. Soc. Path. exot. **23** (1930) 20-23.

(258) TRUFFI, Innesto di tessuto leproso nella camera anteriore dell'occhio del coniglio. Gior. italiano Mal. ven. **46** (1912) 96-107.

(259) TURNER, G. The cultivation of the Bacillus leprae. Lancet **2** (1905) 1138.

(260) TWORT, F. W. A method for isolating and growing the lepra bacillus of man (preliminary note). Proc. Roy. Soc. **83** (1910) 156-158.

(261) UCHIDA, M. Studies on rat leprosy. Japan Med. World **2** (1922) 4-7.

(262) UNNA, P. G. Heilung eines Falles von Lepra tuberosa. Zur Färbung der Leprabacillen. Zur Histologie der leprösen Hant. Monats, f. Prakt. Derm. (1885) 33-77.

(263) UNNA, P. G. Wo liegen die Leprabacillen? Deutsche med. Wochenscher. **12** (1886) 123-118.

(264) UNNA, P. G. On the microorganisms of leprosy. Dublin Jour. Med. Sci. **89** (1890) 112-118.

(265) UNNA, P. G. Der Fettgehalt der Lepra- und Tuberkelbacillen. Deutsche Med. Ztg. **17** (1896) 1037-1047.

(266) UNNA, P. G. Est-il possible de mettre en évidence la mort des bacilles de la lèpre dans l'organisme humain? Ann. Dermat. et Syph. **I** (1910) 481-486.

(267) UNNA, P. G. Zur Mikrochemie des Leprabazillus. Dermat. Stud. **21** (1910) 283-299.

(268) UYEI, N. AND ANDERSON, R. J. The chemistry of the lipoids or tubercle bacilli. XXVI. Separation of the lipoid fractions from the leprosy bacillus. Jour. Biol. Chem. **94** (1932) 653-660.

(269) VAUDREMER, A., SÉZARY, A. AND BRUN, C. Essais de cultures du bacille de Hansen du sang et des lépromes. Compt. rend. Soc. Biol. **106** (1931) 1225-1228.

(270) VAUDREMER A., SÉZARY A. AND BRUN C. Culture de germes provenant de lépromes et de rate lépreuse filtrée. Compt. rend. Soc. Biol. **109** (1932) 624-626.

(271) VEDDER, E. B. A discussion of the etiology of leprosy, with especial reference to the possibility of the transference of leprosy by insects and the experimental inoculation of three men. Philippine Jour. Sci. 37 (1928) 215-243.

(272) VEROTTI, G. Resultati ottenuti delle inoculazioni intraperitoneali di emulsione di leproma nei conigli. Gior. internaz. Sci. med. 35 (1913) 481-489.

(273) VEROTTI, G. Inoculazione di emulsione di leproma in una scimmia (Cercopithecus), a produzione di un nodulo primario e di noduli secondari lepromatosi. Atti r. Accad. Med. Chir. di Napoli 67 (1914) 175-185.

(274) VERTEUIL, F. A. AND VERTEUIL, F. L. Bacillus leprae in the nasal mucous membrane. Canadian Med. Assoc. Jour. 2 (1913) 8-14.

(275) VIGNOLO-LUTATI, C. Contributo di ricerche sperimentali sulla lepra. Morgagni 57 (1915) 334-342.

(276) WADE, H. W. AND SOLIS, F. Studies on negative lepers. II. The importance of nasal lesions. Jour. Philippine Islands Med. Assoc. 7 (1927) 111-114.

(277) WALKER, E. L. Bacteriology of leprosy; diphtheroid in leprosy. American Jour. Trop. Med. 2 (1922) 293-303.

(278) WALKER, E. L. Contributions to bacteriology of leprosy; chromogenic acid-fast bacillus of Clegg. American Jour. Trop. Med. 3 (1923) 417-424.

(279) WALKER, E. L. Some new aspects of the etiology and endemiology of leprosy. Jour. Prev. Med. 3 (1929) 167-195.

(280) WALSH, J. J. The Popes and Science. (Translation.) New York, Fordham Univ. Press, 1908, pp. 420-423.

(281) WEBER, K. Werden die Leprabacillen von einem Leprakranken ausgeschieden, und auf welche Weise verlassen sie den Körper? J. B. Hirschfeld, Leipzig, 1897.

(282) WEIL, E. Essais de culture du bacille lépreux. Ann. Inst. Pasteur 19 (1905) 793-803; Bull. Soc. française Dermat. et Syph. 17 (1906) 170.

(283) WELLS, H. G., DEWITT, L. M. AND LONG, E. R. The chemistry of tuberculosis. Baltimore, 1923, p. 110.

(284) WHERRY, W. B. Notes on rat leprosy and on the fate of human and rat lepra bacilli in flies. Publ. Health Rep. 23 (1908) 1481-1487.

(285) WHERRY, W. B. I. Experiments on vaccination against rat leprosy. II. On the extraction of rat lepra bacilli from watery emulsions by means of chloroform. III. Rat lepra bacilli in the rat louse. Jour. Infect. Dis. 6 (1909) 630-633.

(286) WHERRY, W. B. Cultivation of acid-fast bacillus from leprosy. Philippine Jour. Sci. 43 (1930) 577-581.

(287) WHERRY, W. B. Note on the culivation of an acid-fast bacillus from leprosy. Jour. Infect. Dis. 46 (1930) 263-266.

(288) WHERRY, W. B. AND ERVIN, D. M. The necessity of carbon dioxide for the growth of B. tuberculosis. Jour. Infect. Dis. 22 (1918) 194-197.

(289) WILLIAMS, T. S. B. The cultivation of leprosy bacillus. Lepra 12 (1910) 131-138.

(290) WILLIAMS, T. S. B. A lecture on leprosy; a new view of its bacteriology and treatment. British Med. Jour. **2** (1911) 1582-1585; *also* Indian Med. Gaz. **46** (1911) 1-8.

(291) WOLBACH, S. B. AND HONEIJ, J. A. A critical review of the bacteriology of human and rat leprosy. Jour. Med. Res. **29** (1914) 367-423.

(292) WOLBACH, S. B. AND HONEIJ, J. A. The diphtheroid bacillus from leprosy lesions. Jour. Med. Res. **29** (1914) 1-8.

(293) WOOLEY, P. G. The bacteriotherapy of leprosy. Proc. Soc. Exper. Biol. & Med. **4** (1907) 121-122.

(294) ZIRONI, A. Sulla coltura del bacillo di Hansen. Boll. d. Ist. sieroterap. milanese **I** (1920) 381-395.

(295) ZINSSER, H. AND CAREY, E. G. A contribution to the study of rat leprosy. Jour. American Med. Assoc. **58** (1912) 692-695.

(296) ZOLKEVITSCH, A. Morphologic and biochemical changes of Bactrium leprae Kedrowski, under "influence of radium emanations." Centralbl. f. Bakt. **113** (1929) 67-71.