THE CULTIVATION AND MORPHOLOGICAL STUDY OF A PLEOMORPHIC ORGANISM FROM THE BLOOD OF LEPROSY PATIENTS

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INTRODUCTION

Although Hansen discovered the leprosy bacillus in 1873, "its indefinite subcultivation in artificial media has never been unequivocally demonstrated" (22). The nearest approach to continued cultivation is considered by some workers to be that of McKinley and Soule (16) and Soule and McKinley (19, 20), who reported in 1932, from Puerto Rico, the cultivation from leproma material growths of mucoid, nonchromogenic colonies of acid-fast bacilli on egg media under an atmosphere containing 40 per cent oxygen and 10 per cent carbon dioxide. Shortly afterward Soule (18) repeated this cultivation at Culion, in the Philippines, but McKinley (14) stated that "...there exists no absolute proof as yet that any investigator...has actually succeeded in cultivating *Mycobacterium leprae* in vitro," because Koch's postulates had not been fulfilled satisfactorily (15). These workers produced localized, transient nodules in the skin of monkeys. Among other recent workers, Chaussinand (6) especially claims to have produced more widespread infection of animals with material from human leprous lesions, but as it has recently been stated, "as yet no animal host has been found to be susceptible to *Mycobacterium leprae* of human origin" (5). Typical generalized and persistent infection has not been obtained with any culture obtained from leprosy patients.

The microorganisms which have been isolated from leprosy lesions have been of four types: (1) diphtheroid bacilli which were either nonacid-fast or only weakly acid-fast, (2) chromogenic acid-fast bacilli, (3) nonchromogenic acid-fast bacilli, and (4) actinomyces. Some of the isolated strains subsequently grew readily on ordinary media. Topley and Wilson are of the opinion that the slow-growing nonchromogenic acid-fast bacilli probably represent the true leprosy bacillus and that many of the strains labeled *M. leprae* are either saprophytes or other acid-fast pathogens. According to them,

"...we are faced with three possibilities: either (i) they are contaminating organisms that have nothing to do with the causation of leprosy; or (ii) they are different stages in the life history of the true leprosy bacillus; or (iii) they are organisms whose presence is in some way associated with that of the true leprosy bacillus, which has not yet been cultivated."

Soule and McKinley (21) have stated that the difference in types of growth obtained by various workers may one day be explained by dissociation studies of *M. leprae,* a view analogous to the second alternative mentioned above.

The present paper describes not only the isolation of a consistent type of pleomorphic microorganism from the blood of ten out of eleven persons afflicted with leprosy, but also the development of this microorganism from heretofore unrecognized nonacid-fast forms into acid-fast rods and granules similar to those found in smears from patients with Hansen's disease and commonly recognized as the bacillus of Hansen, and furthermore the ability of these cultures to produce ulcerative lesions in the skin and ears of white mice. The failure of previous workers to obtain satisfactory cultures of the leprosy bacillus appears to have been due not so much to lack of a sufficiently favorable medium, but rather to the fact that the various pleomorphic forms here described, if seen or grown, were not recognized as forms capable of developing into the typical acid-fast rods of the Hansen bacillus.

In 1947, the writer suggested a technique of inoculating aseptically-obtained citrated blood into ten-day-old embryonated hen's eggs as a possible means of cultivating an acid-fast organism observed by Dr. Virginia Wuerthele-Caspe in smears from scleroderma patients (23). In an experiment involving blood from these patients, Wuerthele-Caspe suggested including a few bloods from leprosy patients, and she obtained three such specimens.[1] After ten days of incubation at 37°C., acid-fast bacilli were readily found in smears obtained from nine blood-inoculated eggs, and eight of the embryos were hemorrhagic and failed to develop. However, since 10 per cent of smears from uninoculated control chick embryos showed the presence of a mycobacterium-like organism, the origin of the acid-fast organisms observed in the eggs inoculated with patients' blood was uncertain, and therefore work with this medium was discontinued.

[1] Two were supplied by Dr. George L. Fite of the U. S. Marine Hospital at Carville, La., and the third was obtained from the Kings County Hospital, Brooklyn, N. Y.

The successful use by me of a clear liquid medium, described below, for growing *M. tuberculosis* and other acid-fast pathogens from patients' blood led to an attempt to cultivate *M. leprae* in this medium directly from the blood of leprosy patients. Dr. Frank C. Combes [2] kindly supplied blood specimens from 9 patients with leprosy and performed skin tests later mentioned. Most of these patients had formerly been cared for at the U. S. Marine Hospital, Carville, La.

ISOLATION OF THE CULTURES

Whole citrated leprosy blood, 0.5 cc., was inoculated into 5 cc. of the medium referred to. Growths were obtained after five days to two weeks of incubation at 37°C. Subcultures from these tubes developed plainly visible, finely granulated clumps which settled to the bottom of the tubes in the form of a mat, while the medium above remained clear. More than 30 bloods from control persons, including 12 tuberculin-negative nurses and 14 blood-bank donors, were similarly inoculated; these gave either no growth or else readily identified cocci or diphtheroids. Slide preparations made from the leprosy blood cultures and subcultures stained by the Ziehl-Neelsen method showed the following forms:

(1) Clumps of nonacid-fast granules and larger spherical bodies embedded in a lighter blue amorphous material. These symplastic forms resembled similar forms observed previously in smears from leprosy lesions (3).

(2) Large disk or ring forms, the larger ones averaging several microns in diameter (one-third the size of red blood cells), and possessing well-defined rims. These large nonacid-fast bodies often contained small acid-fast granules and occasionally rods (Fig. 1).

(3) Occasional free acid-fast granules and short rods.

(4) Nonacid-fast diphtheroid rods, numerous in litmus milk cultures.

Further subcultures in the liquid medium showed smaller numbers of the large ring forms in the stained preparations, and in still later subcultures they were no longer found. When the growth obtained in the liquid medium was transferred to egg-malachite-green slants, there developed a few round, semi-transparent, nonchromogenic colonies, and in some tubes a few

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[2] Professor of Dermatology at New York University College of Medicine, and Chief of the Dermatological Service at Bellevue Hospital. I am grateful for his sympathetic interest and helpful advice.

chromogenic colonies of an apricot orange color. Growth from slants was floated on to the surface of the liquid medium, where it grew extremely slowly and sparsely as small whitish islands. Subcultures made from these islands continued to grow very slowly and sparsely. Smears prepared from this surface growth stained by the Ziehl-Neelsen technique revealed plump, short, acid-fast rods and granules, some of which appeared to be within disintegrating nonacid-fast amorphous material.

In litmus milk cultures made from growth in Jackson's medium, slender nonacid-fast diphtheroid rods predominated. The medium became decolorized and coagulated after two weeks.

Subcultures of two of the original blood cultures have been carried on for more than two years. The cultures isolated from other patients at later dates were also transferable. With the new medium it was not necessary to add carbon dioxide to obtain growth, but in the presence of that gas the cultures grew well, even slightly better than unexposed cultures. However, subcultures made from the tubes exposed to carbon dioxide grew out well in the absence of added gas. Growth on the solid media tried was exceedingly sparse in comparison with that obtained in the liquid medium. With five strains studied on various solid media, including egg slants and Dubos' medium, two types of colonies were obtained: nonchromogenic, semitransparent colonies, and chromogenic colonies of an apricot color. These were small in size but could be easily seen with a hand lens.

Cultures obtained from the blood of two patients and grown for at least four generations in Jackson's liquid medium were centrifuged, washed with saline, and suspended in saline containing 0.5 per cent phenol. The suspension was placed in a 37°C. incubator for one week and then tested for sterility. When tests indicated that the cultures were no longer viable and were uncontaminated by other organisms, 0.1 cc. doses of the phenolized suspensions were injected into the skin of a leprosy patient who had previously reacted positively to lepromin obtained from Carville. Dr. Combes noted that both of the culture preparations gave rise to papular lesions approximately 2 mm. in diameter. This patient had previously shown completely negative reactions to phenolized cultures which had been heated to 100°C. in flowing steam (Arnold sterilizer). A second patient tested has likewise reacted to both unheated preparations; a 3-mm. papule developed after one week. A third patient with lepromatous type leprosy gave a negative reaction to the phenolized unheated culture.

DEVELOPMENTAL MORPHOLOGY

The object of the morphologic study was to determine whether or not the nonbacillary forms observed in both stained and unstained culture preparations were capable of giving rise to acid-fast rods. Changes in the morphology of these nonbacillary forms were repeatedly followed: (*a*) by successive stained preparations made at intervals from four series of cultures in the liquid medium, and (*b*) by observations on three series of fifteen unstained, living hanging-drop cultures. Several hanging-drop cultures were followed photographically (Figs. 1-10).

Series 1, Stained preparations.—Four series of five tubes each of liquid medium were inoculated with 0.2 cc. of a suspension of a recently isolated four-week-old culture. Smears of the inocula showed nonacid-fast amorphous "L" type clumps [3] containing granules, and also a number of globoidal large bodies some of which contained small granules. The tubes of the series inoculated with this culture were placed in the incubator at 37°C. At intervals of three or four days, one tube was shaken and a drop of the culture placed on each of two new clean glass slides. The preparations were allowed to dry without being disturbed, while protected from air and dust by a glass petri dish cover, and were stained by the Ziehl-Neelsen technique. Acid-fast rods and granules appeared in these smears in from nine to fifteen days. Some of the above-mentioned large bodies showed acid-fast or semiacid-fast rims and contained acid-fast granules and occasionally rods.

Series 2, Hanging-drop cultures.—From a thirteen-day-old culture of the above series, 0.5 cc. was pipetted into a tube of fresh liquid medium. This fresh subculture was incubated for two days at 37°C. to test for possible contaminants prior to being used as inoculum for the hanging-drop preparations. Fifteen hanging-drop preparations were then made from this pure culture, sealed with vaseline, examined under both low- and high-powered objectives, and then placed at incubator temperature. Each drop was reexamined every three days for at least two weeks. Three series of fifteen hanging drops each were followed in this way. Of the total of 45 preparations, 34 gave growths which extended throughout the drops; 5 showed considerably less growth; and 6 showed little or no change

[3] Also known as pleuro-pneumonia-like, zoogleal, symplastic or matrix forms.

either in numbers or form. Lack of development seemed to be associated with slight drying, probably due to imperfect sealing around the cover slip.

The following changes were repeatedly observed in these hanging-drop cultures (Figs. 1-10).

(1) An increase in the number of clearly defined granules within the symplastic clumps.

(2) Thinning of the greenish matrix material between the granules, the material on thinning becoming greyish.

(3) Breaking up of the clumps into smaller groups, and a marked increase in the number of tiny free granules, which often spread throughout the drop.

(4) Many free granules developed tail-like extensions or lengthened out into rods in a manner similar to that of developing tubercle bacilli as described by Kahn (10, 11).

(5) As was also noted in the stained preparations described above, many of the large ring-bodies which were present contained small granules. Occasionally ring forms were seen which appeared to break up at their rims into granules or short rods, thus permitting the liberation of the inner granules.

(6) Observations of the last series of fifteen hanging drops during the third week indicated that a number of the new rods were swelling up and forming new ring forms (Fig. 10).

Since conditions for growth in a sealed hanging drop are not ideal even in the best of media, nor are they favorable in the blood stream, especially during treatment with drugs such as promin or diasone, it seems reasonable to interpret the large globoidal rimmed bodies observed in recently isolated cultures from the blood of these leprosy patients as cyst-like forms developed by the organism under highly unfavorable conditions and serving to preserve its reproductive units. They appear to be analogous to the "large bodies" observed and described by Dienes and his co-workers (7) for other microorganisms, including more recently the Salmonella group. The matrix forms consisting of bodies surrounded by amorphous material appear to correspond to pleuro-pneumonia-like or "L" forms.

This hypothesis—i.e., that the large globoidal bodies with their small inner granules develop in response to an unfavorable environment—receives additional support from a study made by me in 1947 (4) of stained preparations from skin biopsy specimens of 117 treated cases of leprosy from Carville, obtained through the kindness of Drs. Combes and Fite. These leprosy cases had been treated for considerable periods, some for several

years, with drugs such as promin, diasone, streptomycin, and chaulmoogra oil. These preparations were stained by Alexander-Jackson's triple stain, a Ziehl-Neelsen stain with a modified counterstain technic which demonstrates the presence of non-acid-fast mycobacterial forms (1). Of these stained preparations, 58 (49%) showed the presence of large globoid bodies of varying size, both free and within a lighter staining matrix; 55 (47%) showed symplastic zoogleal forms with smaller granules and free granules; 25 (21%) showed acid-fast rods; while 2 (1.7%) showed none of these forms.

Biopsy specimens obtained from Dr. C. B. Lara, of the Culion Leper Colony in the Philippines, were presented to me by Dr. Cristobal Manalang, of Manila, for study with the triple stain. These specimens were from skin lesions of children who, though in contact with leprous parents, had not as yet developed frank leprosy and were considered probable preclinical cases. The nonacid-fast and semiacid-fast nonbacillary forms observed in these preparations have their counterparts in the above-mentioned biopsy smears and cultures obtained from known cases of Hansen's disease.

Manalang has stated (13), "A specific agent, probably a virus, precedes the appearance of the acid-fast *M. leprae.*" Electron microscope photographs of some of the matrix forms growing in pure culture (Figs. 11-14) show granules of viral dimensions as well as larger bodies. Also, as stated above, observations on hanging drops of amorphous clumps of matrix material and large bodies, both of which contained small granules, strongly indicate that in the human body, as well as under laboratory conditions, rods sprout from small granular precursors. Whatever the form of the organism, as is the case with *M. tuberculosis* the small viable granule is invariably present and appears to be the basic living unit.

Tubercle bacilli H37Rv, in Dubos' medium to which varying sublethal amounts of streptomycin had been added, after incubation at 37°C. at first swelled up and lost their ability to resist decolorization by acid alcohol, as has been observed by others. When, however, such cultures were observed in hanging drops and followed for as long as three weeks, it was found that zoogleal clumps or "L" forms and large globoidal bodies developed. Spinal fluid obtained from a child with tuberculous meningitis, who was undergoing treatment with streptomycin, showed no acid-fast rods in smears but did reveal a number of nonacid-fast nonbacillary forms. Streptomycin-resistant acid-

fast bacilli appeared about a month later, followed shortly by death of the child. Resistant bacilli of strain H37Rv also grew out in Dubos' medium to which streptomycin had been added. The resistant organisms grew abundantly in the form of orthodox acid-fast rods.

However, the various studies reported here, including observations on inoculated animals, suggest that recrudescence in both tuberculosis and Hansen's disease may also occur when, through lowered resistance or too early discontinuance of treatment, the globoidal or large bodies developed in response to antibodies or drug treatment break up, and their inner small granules, if viable, develop into nests of rods or globi still surrounded by their outer capsule wall. Grigoraki (9) describes what he believes to be a sexual phase of *M. tuberculosis,* in the course of which the granules aggregate to form a mass from which there emerges a thick filament bearing large oval bodies. These large bodies become detached and eject "microgametes," some of which are ciliated, some not. The gametes unite to form "diplococci" which elongate to form rods, thus recommencing the "asexual" phase. Grigoraki believes that this cycle precedes recrudescence in tuberculosis.

INOCULATION OF MICE WITH CULTURES ISOLATED FROM PATIENTS

A lot of 16 white mice of the albino C strain was obtained. Of these, 8 were selected at random and inoculated intraperitoneally with these microorganisms and placed in one cage. The remaining 8 mice were placed in a second cage and received inoculations of the medium alone. After eight months (equivalent to about twenty years in the life of a man), four of the culture-inoculated mice exhibited alopecia, ulcerative skin lesions, and occasionally subcutaneous nodules (Fig. 15). Two mice showed ulcerative destruction of the external ear. Two others, sacrificed after two and four months, had not developed outward lesions. The control mice showed no illness of any sort. Their offspring also remained healthy.

Young animals born to the inoculated mice were apparently healthy at birth. They were separated from their mothers after six weeks. Six months later some of these F_1 mice developed alopecia and skin lesions similar to those of the inoculated mice. F_2 litters born to two F_1 mothers showed, on birth, striking red ulcerative lesions in which numerous acid-fast organisms were found in smears and sections. The organisms were also found in, and recovered from, embryo F_2 mice *in utero,* and also from

the placenta of their mother. The organisms were likewise recovered in cultures made from the inoculated mice and their offspring. They were found and effectively demonstrated by the Fite method in paraffin tissue sections.

The histopathology of the white mouse sections was not identical with that found in cases of human leprosy; rather, it resembled closely the type of response observed in a case of spontaneous "mouse leprosy" described by Krakower and Gonzalez (12) in a wild brown mouse in Puerto Rico. The wild mouse infection was transferred by them to albino laboratory mice and rats experimentally. The lesions of their Puerto Rican white mice, and also those of the white mice inoculated with my cultures of human origin, contained many polymorphonuclear cells as well as monocytes, whereas leprosy lesions in man contain but few polymorphonuclears. In mice the acid-fast organisms are not arranged in cigar-like bundles as in human leprosy. However, globus-like nests of acid-fast spherical forms and rods were seen in large numbers in the skin sections (Figs. 16-18). An inoculated mouse, sacrificed at the end of only four months, exhibited a histiocytic granuloma in the kidneys and ovary, and a few organisms were seen within the macrophages. In this animal most of the organisms were either nonacid-fast or semi-acid-fast.

IMMUNOLOGICAL TESTS

Preliminary serological tests have given the following results:

(1) *Agglutination tests.*—Clumping of the microorganisms was observed in the presence of five undiluted sera from leprosy patients, as shown by slide and test-tube experiments. No agglutination took place in the presence of undiluted pooled normal serum. The individual microorganisms, especially the globoidal forms, exhibited marked swelling in the presence of leprosy serum, reminiscent of the Neufeld "quellung" reaction with pneumococci.

(2) *Passive immunization.*—Tests for passive sensitization were performed with sera from the same five leprosy patients, on the skins of three normal white rabbits. Normal serum and antigen (a saline suspension of the microorganisms), and antigen alone, failed to give reactions, while the leprosy sera and antigen suspensions gave skin reactions in 24 to 48 hours. The redness and swelling, 1 to 2 cm. in diameter, persisted for about a week. The rabbits became sensitized to human serum, and if tested a second time gave marked swelling and necrosis at the site of intradermal inoculation with serum alone.

(3) *Skin tests of inoculated mice.*—Intracutaneous tests were performed on infected and control white mice with Carville lepromin to which leprosy patients had reacted satisfactorily. Although the thin skins of the mice were difficult to test, with care and dexterity good intracutaneous blebs were obtained.

Of 17 infected mice, 5 gave reactions in from 24 to 72 hours. The reaction consisted of a raised papule surrounded by a red area. One of the papules had a small point of necrosis in its center. None of the control mice reacted.

Of the 17 infected mice, 15 had been inoculated only five weeks prior to being skin tested. Of these 15 newly inoculated mice, none of which had as yet developed any sign of disease, 3 were positive reactors. Of the uninfected controls, 10 had received sterile medium five weeks previously, 2 had received sterile medium one year previously, while the remaining 6 had received no inoculations.

(4) *Skin tests of leprosy patients.*—As has been said, when two nonviable culture suspensions originating from different patients were inoculated into the skin of a patient with Hansen's disease who had previously reacted positively to lepromin, 2-mm. papular lesions were obtained with both of the strains. The second patient tested gave a 3-mm. papule after one week. (Figs. 19, 20.) As stated above a lepromatous case failed to react.

PREPARATION OF MEDIA

(A) *Liquid Base:*

Water	2,000	cc.
Healthy young beef lung [4]	2	lb.
Peptone (Difco's Bactopeptone)	20	gm.
Glucose	10	gm.
Glycerol	100	gm. (80 cc.)

Cut up the beef lung and boil it in water for 30 minutes. Filter through cotton or coarse paper into a flask containing the other ingredients, and heat again to dissolve. Adjust to pH 7.2. Autoclave for 15 minutes at 15 lb. pressure.

The above crude glycerol lung broth was used by von Szabocky in 1909 and found excellent for growing tubercle bacilli. Due, perhaps, to its marked turbidity, it has apparently not been widely used. However, it was found that this broth could be made crystal clear by ultrafiltration without losing its nutritive value. In its clarified state, this liquid medium is advantageous for research studies (*a*) because of its growth-stimulating properties, and (*b*) because the least amount of

[4] Pigs lung was found equally satisfactory and less expensive.

growth is clearly visible to the eye and cannot be confused with any inherent opacity of the uninoculated medium.

The process of ultrafiltration was carried out initially by Microbiological Associates, Inc., at Flemington, N. J. The ultrafiltrate was tubed in 5 cc. amounts into wide-mouthed, screw-top glass tubes (Kimble) and carefully tested for sterility before use. The ultrafilter employed was designed by Simms and Sanders(17). As described by Microbiological Associates, the medium was "passed through a collodion membrane having a porosity of 23 millimicrons, under a pressure of 425 mm. 5 per cent nitrogen and 95 per cent carbon dioxide."

Dr. J. Anderson, of the Bureau of Biological Research, Rutgers University, in a personal communication, states that the filtration of the crude broth through a Seitz pad renders the medium less satisfactory for growth, whereas passing it through sintered glass filter and then autoclaving yields a solution which, though not quite as clear as the ultrafiltered one described above, is "very satisfactory and is quickly prepared."

I have found it expedient to filter the medium as follows: first through very coarse paper, then through a No. 42 Whatman paper laid over a Buchner funnel coated with a layer of celite (infusorial earth—Johns Manville Filtercel), and finally through a Buchner-type funnel with fritted glass disc (Corning). After the third filtration the medium is tubed and autoclaved. The final pH should be neutral or slightly alkaline. Crude base may be stored, clarified at a later date, and reautoclaved without deleterious effect.

Various substances were added experimentally to the clear medium in order to favor the development of acid-fast forms: 0.1 cc. of a colloidal aqueous suspension containing 1 per cent lecithin [5] in water added to 5 cc. of the medium appeared to markedly favor acid-fastness; 0.1 cc. of globulin-free beef serum (Microbiological Associates) favored growth, and acid-fast rods developed in its presence, but they did not replace the other forms.

(B) *Solid Media:*

I have found that on adding the yolks of ten fresh eggs and 10 cc. of 2 per cent malachite green to 300 cc. of the crude liquid base, and then inspissating on two successive days, there results a solid medium which permits the growth of larger and more numerous colonies than have grown out on any of the other solid media used. Even on this solid medium, however, growth was not luxuriant.

Recently, better growth was obtained on an agar medium.

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[5] Asolectin, a product prepared from soy lecithin, by Associated Concentrates, Woodside, Long Island, N. Y. is now used.

(C) *Agar:*

Water	1000	Liter
Beef extract	5	grams
Dextrose	10	grams
Yeast extract	1	gram
Gelysate	2	grams } Special peptones from Baltimore Biological Laboratories
Myosate	2	grams }
Asolectin (Associated Concentrates) made from commercial soy lecithin	40	ml of a 1% aqueous suspension
Agar	20	grams

Slants prepared in Kimble screw top glass tubes 105x25 mm. keep well.

DISCUSSION

The findings here recorded are consonant with the view that the clinical picture and pathology observed and reported here were due to the organism inoculated, and strongly suggest that this organism is Hansen's bacillus. If the pleomorphic microorganism described is the etiological agent of leprosy, support is given to the view of those who postulate the existence of some form or forms of *M. leprae* other than acid-fast rods alone.

It is hoped that the studies reported here will open the door to the problem of readily cultivating the organism of leprosy, and of recognizing the organism in all its various guises. The way is also pointed towards a more refined and specific agent for skin tests and serological studies.

SUMMARY

1. A pleomorphic microorganism has been isolated from blood specimens of ten of eleven leprosy patients.

2. The morphological changes of this organism from nonbacillary, nonacid-fast forms to acid-fast rods and granules similar to those observed and recognized in Hansen's disease are described.

3. Media are described for the isolation and continuous cultivation of this microorganism.

4. Infection and pathology in the white mouse following inoculation of the organism is reported, and also the reisolation of the infecting agent.

5. Transmission of the infection from culture-inoculated mice to their offspring has been shown. Transmission from F_1 to their young via the placenta is indicated by the findings.

ABSTRACTO EN ESPAÑOL

Usando medios de cultivo especiales y procedimientos meticulosos de tinción, ambos descritos en detalle en el texto del

artículo, la autora reporta el hallazgo de microorganismos pleomórficos en la sangre de 10 pacientes leprosos. Los microorganismos tienen propiedades variables, aveces son bacilares, otras cocoides, en ocasiones ácido-resistentes, y otras veces no-ácido resistentes. La autora hace incapíe en la semejanza con los hallazgos originales reportados per Hansen. Además, la autora fué capaz de inocular ratoncillos y producir lesiones en ellos, de las cuales recobró los microorganismos patógenos. También aporta evidencia de la transmisión transplacentaria de dichos microorganismos.

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DESCRIPTION OF PLATES

PLATE 7.

The photomicrographs in this plate and the next one were made from hanging drop cultures of organisms recently isolated from the blood of a leprosy patient. 1000X.

FIGS. 1-4. Ring-shaped large bodies and zoogleal clumps. Note granules budding from the rims of rings. Top ring in Fig. 1 is open.

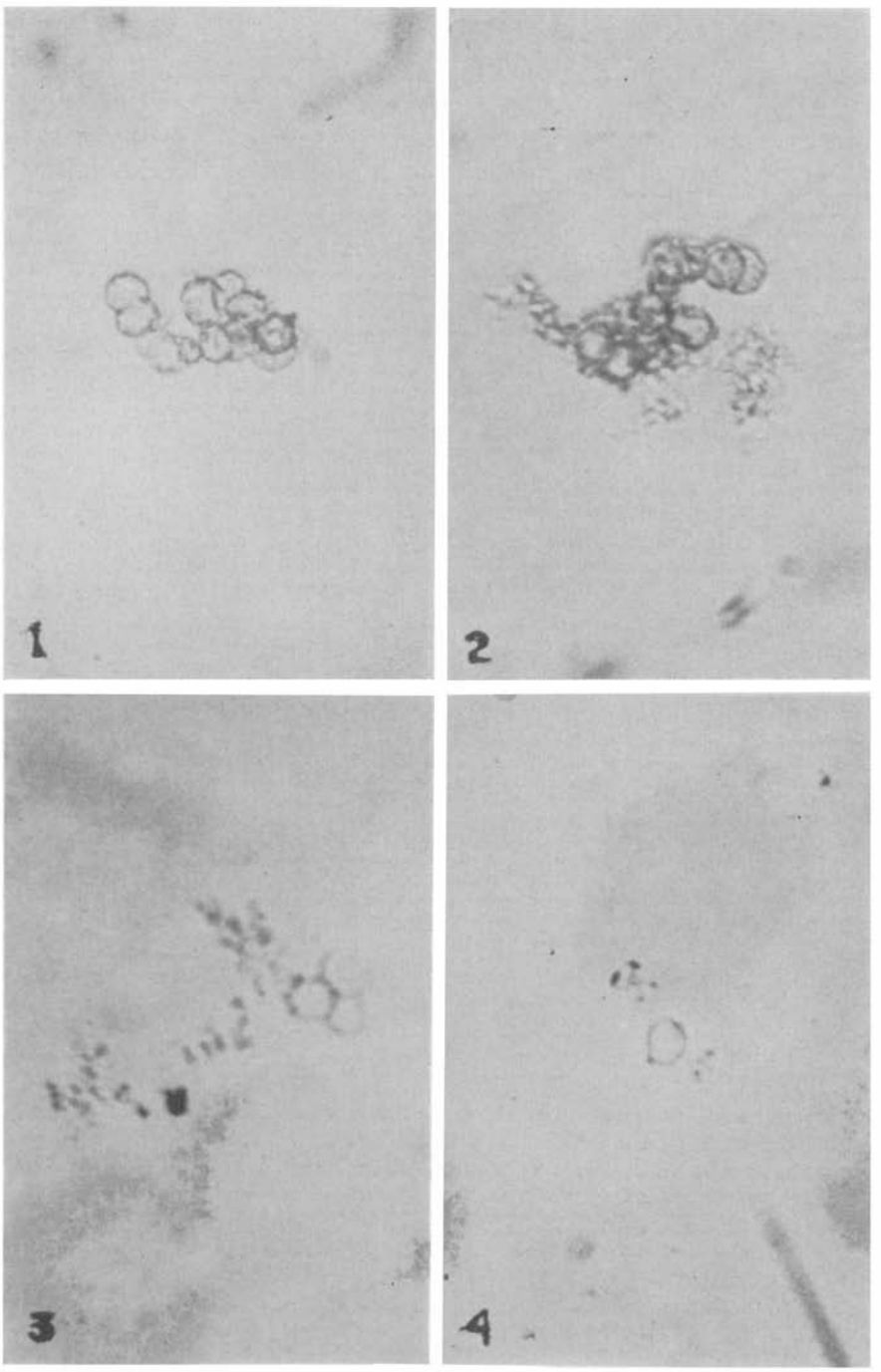


PLATE 7.

PLATE 8.

Photomicrographs of the same material as in Plate 1. 1000x.

FIG. 5. Clumps of granules, and large globoid body at the right.

FIG. 6. Same material as Fig. 5, after three days. The clumps have separated. There is an aperture in the rim of the large body, toward the right.

FIG. 7. Same material after eight days. Above and a little to the right of the center is a granule with a tail-like extension. A small clump at the lower left.

FIG. 8. Same material after ten days. Free granules and a group of young rods.

FIG. 9. Another hanging-drop culture after two weeks. Young rods.

FIG. 10. Same material as Fig. 9, after three weeks. New ring forms are developing.

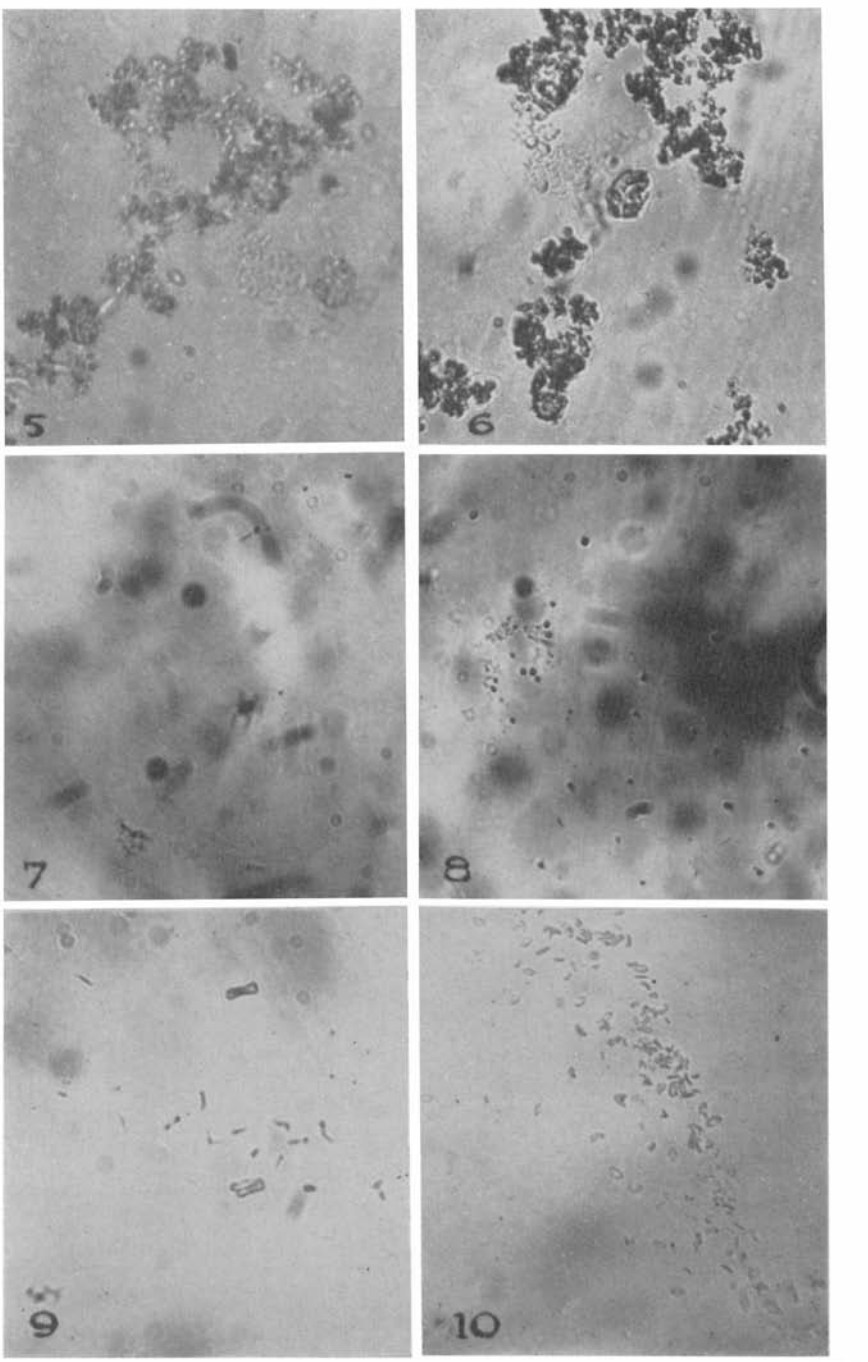


PLATE 8.

PLATE 9.

Electron microscope photographs taken by Dr. James Hillier, R. C. A. Laboratories, Princeton, N. J., of organisms recently isolated from the blood of a leprosy patient. 15,500X (1 μ = 3 cm.) before reduction (reduction nearly 50 per cent).

FIG. 11. Large ring-shaped body in zoogleal clump; diameter 2 microns.

FIG. 12. Cluster of smaller spherical bodies; size 0.25 to 0.5 microns.

FIG. 13. Globoid bodies from colony on egg slant; diameter 1.5 microns.

FIG. 14. Submicroscopic granule forms, 20 to 70 millimicrons in diameter, in matrix from colony on egg slant.

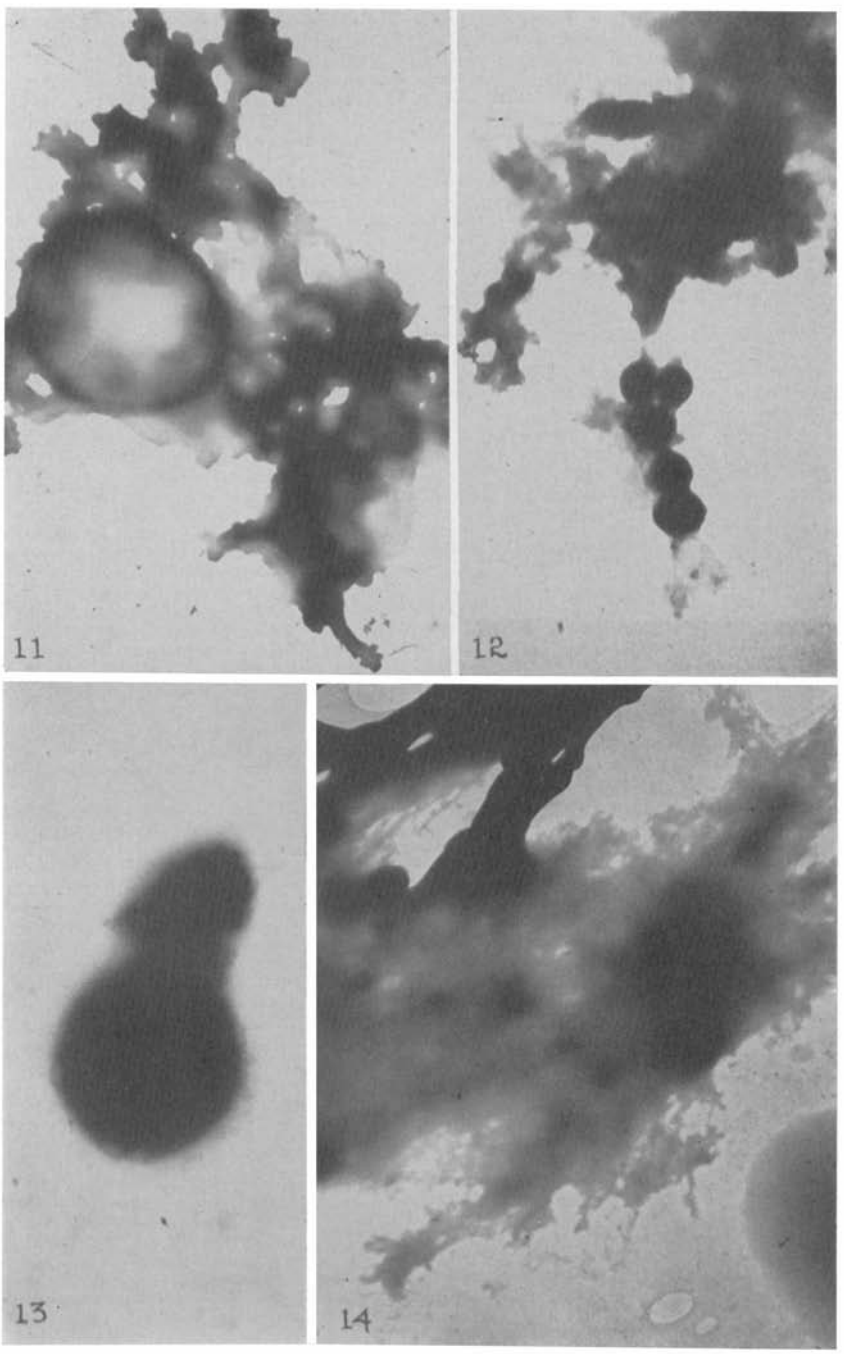


PLATE 9.

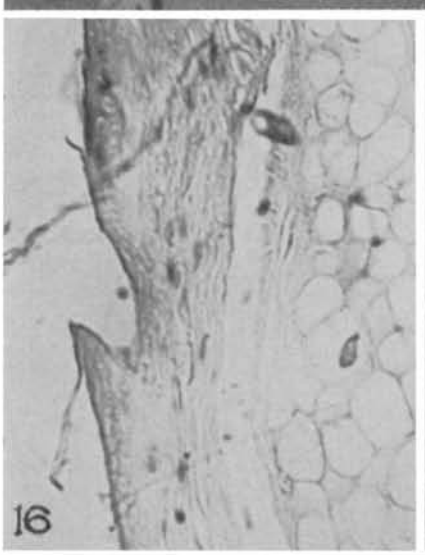
PLATE 10.

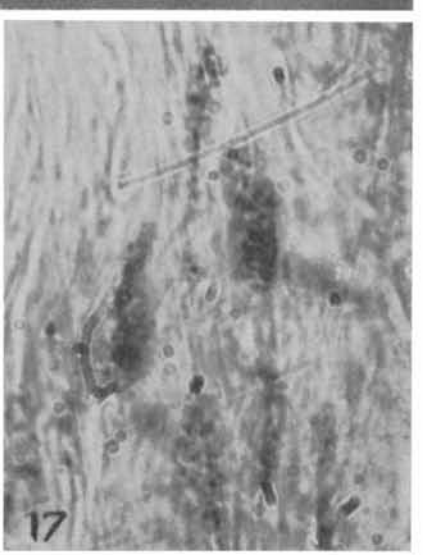
FIG. 15. Inoculated mouse with ulcerative skin lesions.

FIG. 16. Section of skin showing nests of acid-fast organisms, low power.

FIG. 17. Same section as Fig. 16, showing acid-fast rods and granules as seen with the oil-immersion objective.

FIG. 18. Section of skin of a 3-day-old mouse born with red ulcerative lesions, showing nests of acid-fast organisms. With the oil-immersion objective the individual organisms appeared mostly spherical.





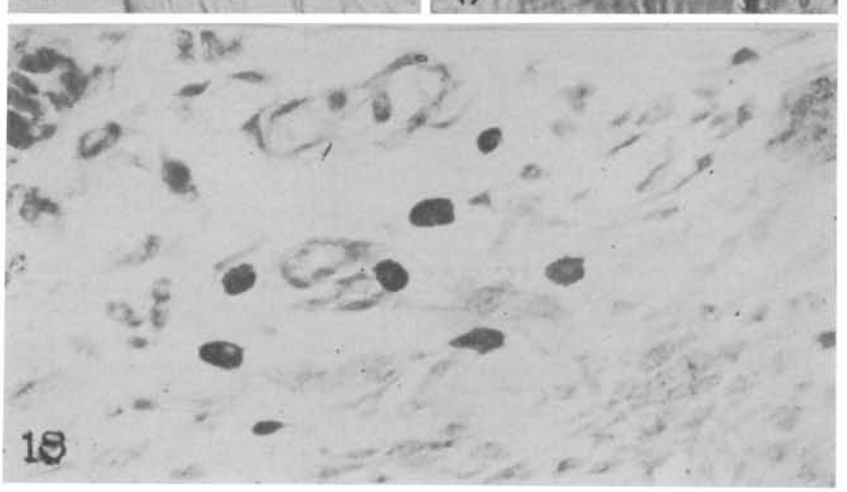


PLATE 10.

PLATE 11.

Skin reactions, obtained on patient S (tuberculin negative), to Carville lepromin and to suspensions of pure cultures of the microorganism isolated from the blood of patients with Hansen's disease.

FIG. 19. (a) Slight infiltration and pigmentation over area of 5 mm. (Carville lepromin).

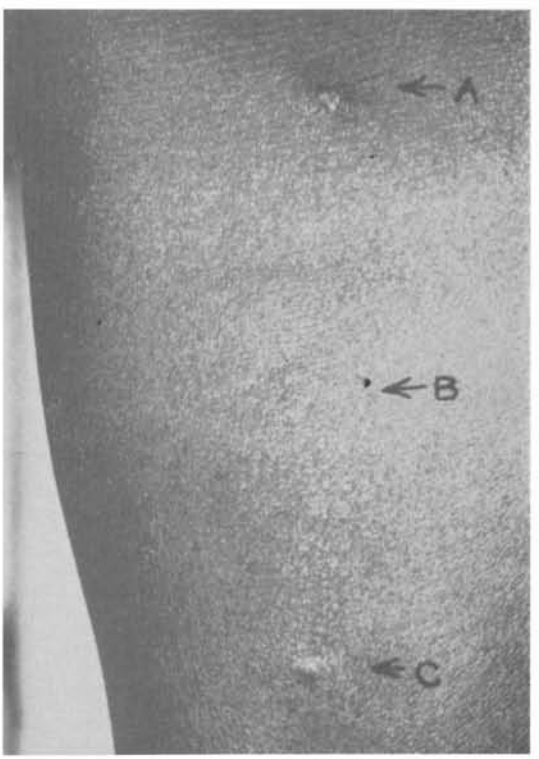
(b) Slight elevation and erythema 3 mm. in diameter (H. culture).

(c) Definite elevated papule 4 mm. in diameter with slight surrounding erythema 1 mm. in diameter. The papule has a scale which is attached centrally and free on periphery. (O culture).

FIG. 20. Duplicate tests on same patient.

(a) O culture—Depigmented macule 3 mm. in diameter, superficial scale attached peripherally as collarette.

(b) Carville lepromin—blood crust 3 mm. in diameter and surrounding superficial scale, 6 mm. in diameter.



19 →

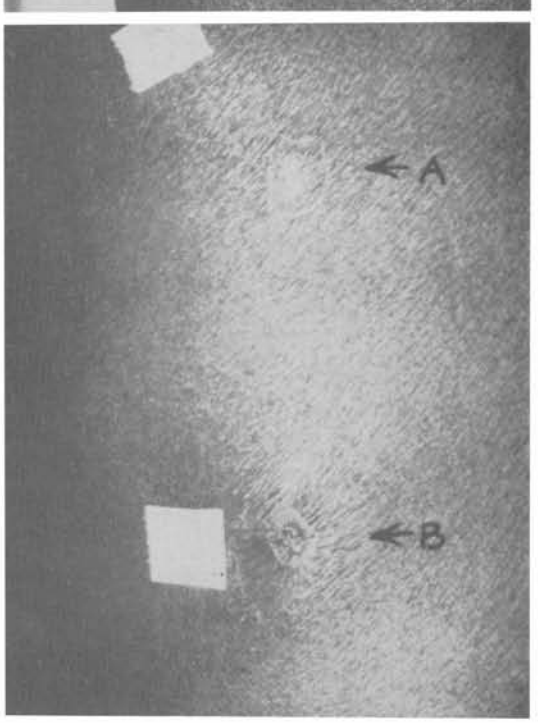


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PLATE 11.