BACTERIOLOGICAL DIAGNOSIS OF LEPROSY BY MEANS OF BLOOD CULTURE[1]

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It is true that the bacillus of Armauer Hansen can be accepted as the cause of leprosy, but its cultivation has offered great difficulties. Acid-fast bacilli have repeatedly been cultivated from leprosy nodules, and these bacilli, naturally, were considered as the cause of the disease. One may recall the strains isolated by Duval, Williams, Nabaro. All of these grow excellently on our usual culture media, and most of them develop a yellow, some even a brilliant red, pigment. That these bacilli are the cause of leprosy is, as yet, not definitively decided, but there is much to be said against the assumption that in them we have to deal with the genuine leprosy bacillus.

In the first place, the excessive growth is against this view, because only a few investigators have succeeded in their cultivation, whereas the majority have had only negative results. In the second place, these cultivated strains are remarkably similar to other acid-fast bacilli which are to be found very extensively in free nature. Petri, Rabinowitsch-Kempner, Alfred Möller, Grasberger, Lombardo Pelegrini, Marpmann and other authors have grown such bacilli from milk, and especially from butter. These are distinguished from the genuine leprosy bacillus especially by their rapid growth on the common culture media, such as agar and glycerin-agar. Alfred Möller even found them on grass. Weber and Trautenheim found them on moss, Kaiser and Lovenstein in conduit water, Manteufel in earth, so that we can literally speak of the ubiquity of these bacilli.

It is, then, quite possible that these acid-fast bacilli may also vegetate in certain laboratories, or even on the skin of the human body, for they are closely related to the pseudo-diphtheria group of bacilli. These latter are especially common on the human skin, for which reason they have often been considered the cause of various diseases. We need recall only the Lustgarten-Welsch bacillus in syphilis, and the Neumann bacillus in carcinoma and pemphigus. We

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must be especially careful in dealing with this group. This is all the more essential when we remember that the smegma bacillus is not only found in the *smegma preputii,* but also in ear-wax (Gottstein) and generally on the skin. Hence it may be possible that in these so-called leprosy bacilli we are dealing with different forms of the smegma bacillus. However, I wish to say that I would not deny the possibility that this group may play a role in the etiology of leprosy.

In making cultures from the skin, there is naturally a chance of infection with such smegma bacilli. The circumstances are more favorable when we start with material that is certainly uncontaminated, such as the blood, for in it we do not have the organisms which live upon the skin and which cause mixed infections. I have been able to show in tuberculosis that the tubercle bacilli occur in the blood at a very early stage of the disease. Further, I have shown experimentally that two hours after subcutaneous injection the bacilli had already spread throughout the entire body. Later I performed experiments in which guinea pigs were injected intracutaneously on the ball of the large toe of the left hind foot with living tubercle bacilli. After twenty-four hours the foot was amputated, but this was always too late to prevent general infection.

Through my investigations on blood, in which more than nine thousand cases have been examined, it has been found that in the natural course of human tuberculosis the tubercle bacilli can be found very early in the blood by means of culturing—even when they have not yet been established in the organs. I take this opportunity to point out that in some human beings tuberculosis seems to have the character of a general infection without focalization.

During these investigations I isolated five strains of organisms which greatly resembled those described by Duval. The color of the colonies was somewhat deeper yellow—more reddish yellow. The individual bacilli seemed to be somewhat larger. Growth on my egg medium was extraordinarily luxurious, at first at incubator temperature, and then at room temperature. Doctor Wade saw these strains and was greatly interested to learn that such bacilli, resembling Duval's, had been obtained from nonleprous persons.

The question now arises what kinds of bacilli are found in the blood of lepers. In the first place it must be recognized that we may encounter the tubercle bacillus, having in mind the results of my

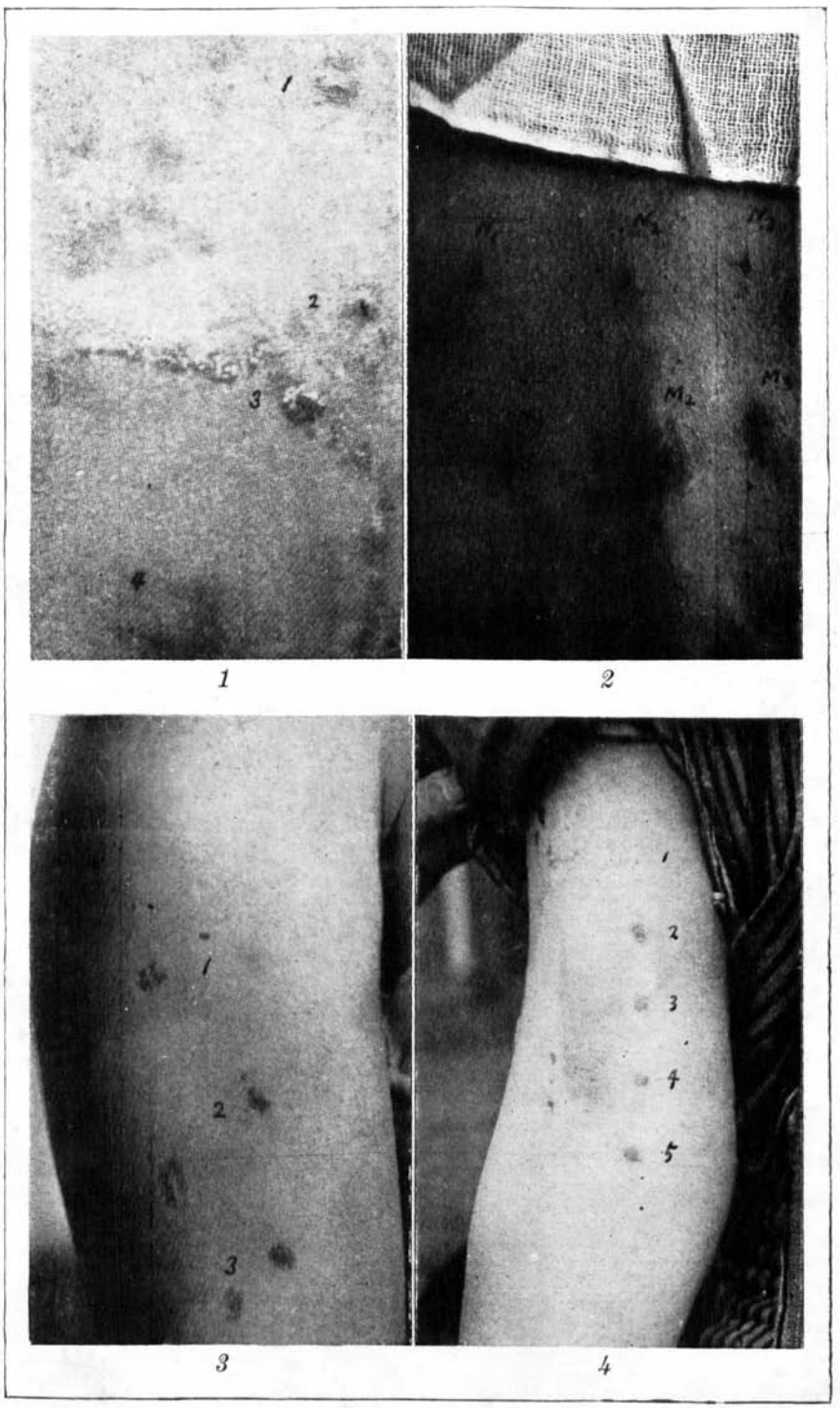


PLATE 1.

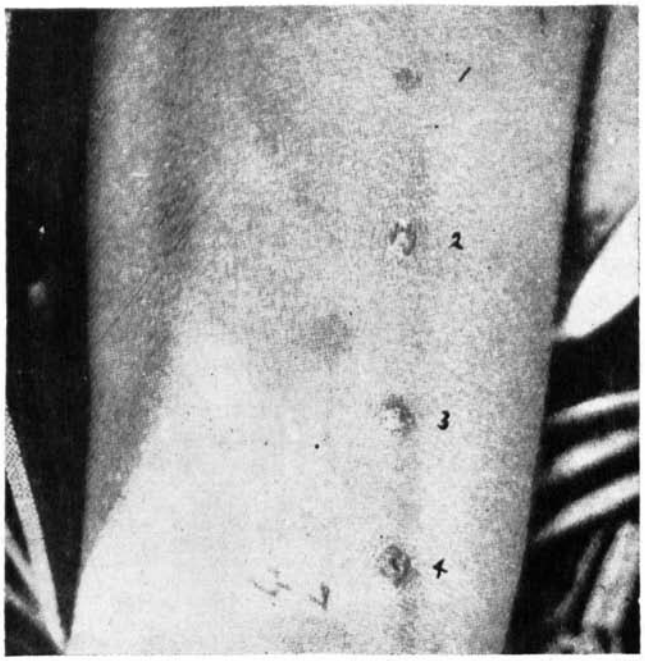
DESCRIPTION OF PLATE

PLATE 2.

FIG. 5. Patient T. F., nodular type. Reactions 13 days after injections of suspensions of acid-fast bacilli. No. 1, Mitsuda's vaccine (control), negative. No. 2, Duval's culture, ++ (0.6 centimeter diameter); No. 3, "Lepra 27" culture, + (0.6 centimeter diameter); No. 4, McCay's culture, +++ (0.6 centimeter diameter).

FIG. 6. Patient O. H., nodular type. Reactions one month after injections of (V) Mitsuda's vaccine, negative, and (K) suspension of Kedrowsky's culture, ++.

FIG. 7. Patient T. K., neural type. Reactions one month after injections of Mitsuda's vaccine (V), and Kedrowsky suspension (K). Both +++.



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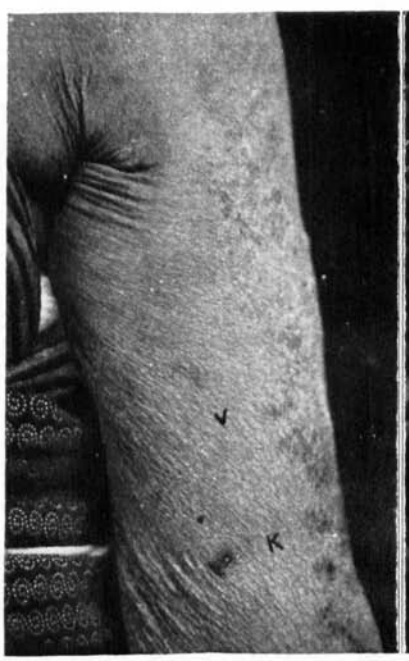

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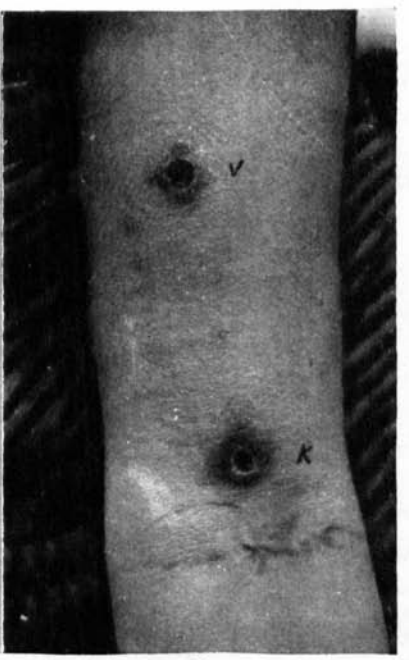

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PLATE 2.

experimentation on the cultivation of that organism from blood. It is very remarkable that tubercle bacilli are not only found in all known forms of tuberculosis, but also in 90 per cent of the cases of acute polyarthritis, and also in cases of primary chronic polyarthritis. Up to the present I have succeeded in getting pure cultures in 169 cases of polyarthritis. It is to be realized that these cases were examined weekly, and that in some of them it was possible to obtain a tubercle bacillus culture from their blood as many as eight times.

Still more interesting are the findings, obtained jointly with Prof. Kren, in lupus erythematoides. Here we were able to keep cases under observation for two years, and to take blood specimens from time to time. The results in this condition were really striking. In one case (Z.), we obtained bacilli from the blood seven times in the course of two years. It is of interest that in this case all therapy failed. Thirty-two per cent of the cases of lupus erythematoides were positive on a single examination, and 100 per cent positive on four examinations. Conrad, of the Dermatological Clinic, showed through blood culture the presence of tubercle bacilli in the blood stream in 6 out of 7 cases. Mathiessen, of the Vienna Skin Clinic, achieved similar results in this condition. With Kissmeyer, of the Copenhagen Finsen Institute, I investigated 48 cases, in which, however, only 3 positive results were obtained. The results of the tests are clearly related to the clinical conditions. Kren, who possesses the greatest amount of lupus erythematoides material, and who has studied such material the longest, concludes that a certain parallelism exists between the presence of tubercle bacilli and the clinical symptoms. So long as tubercle bacilli circulate in the blood, we can not speak of cure.

I would like also to refer to my work on tubercle bacilli in diseases of the central nervous system. In multiple sclerosis and retrobulbar neuritis, tubercle bacilli are found in the blood in a very large proportion of cases. The virulence of these strains was tested in guinea pigs, and they proved to be typical strains of the human type. The histological findings in retrobulbar neuritis also suggest that that condition and multiple sclerosis may have a tuberculous etiology. This disease of the optic nerve is recognized to-day, in 90 per cent of the cases, as an accompanying symptom of multiple sclerosis. Many authors, for many years, have made the casuistic statement that, in retrobulbar neuritis, tuberculosis can be demonstrated histologically in the region of the optic nerve (Igersheimer, Meller, Pilat, Urbanek,

Mariot, Lasky). Therefore, it may well be that the tubercle bacillus also plays a role in the genesis of this nerve disease.

Further, tubercle bacilli are also found in 30 per cent of cases of dementia præcox. Up to the present 320 cases have been examined, for the blood specimens from which I am indebted to the Vienna Institutes, to Prof. Taussig of Prague, Dr. Wollander of Louvain (Belgium), and Prof. Sagl of Saxony. However, it must be said that in dementia præcox tuberculosis is a frequent cause of death, and that, therefore, tuberculosis may be a concomitant disease.

In leprosy this complication also exists, for tuberculosis is strikingly widespread among leprous persons. We also know that leprosy especially affects the nerve substance, as is shown in the excellent book on neural leprosy by Professor Lie, of Bergen, Norway. It would not be surprising if in chorea, which is recognized as a sequel of articular rheumatism, in 90 per cent of the cases, tubercle bacilli were found in the blood. It is, therefore, not impossible that in leprosy also the bacilli may be found in the blood by means of culturing.

For this purpose it would be necessary to employ a special technic in taking blood specimens. The best apparatus for this purpose is the automatic Haemaut Saug-Ampulle, which contains 3 cubic centimeters of a 10 per cent sodium citrate solution. In this apparatus it is possible to keep the blood in a sterile condition. Blood specimens from lepers sent in it from Madras, India, and specimens from tuberculous patients in San Francisco, have been received in sterile condition, though they were thirty days on the way. The culturing procedure requires great care. The preparation of the medium especially is difficult; it seems impossible to make it successfully in laboratories that are not especially well equipped.

PREPARATION OF THE MEDIUM

Eggs are first placed for a half hour in a hot, 5 per cent soft soap-soda solution, and then rinsed in running water. They are then allowed to lie for twenty minutes in a 1 per cent sublimate solution, and are then given a final rinsing.

ASPARAGIN SOLUTION

Potassium phosphate, K_2HPO_4	1 gm.
Sodium citrate	1 gm.
Magnesium sulphate	1 gm.
Asparagin (Merck)	3 gm.
Glycerin	60 cc.
Distilled water	1000 cc.

To every 150 cubic centimeters of this asparagin solution are added 15 cubic centimeters commercial milk, 12 gm. potato flour, and 24 cubic centimeters glycerin. This milky liquid is then sterilized for two hours in flowing steam. Four of the eggs prepared as above described are then added, and one more yolk. Glass beads are added, and after the mixture is shaken thoroughly, 5 cubic centimeters of a sterile 2 per cent solution of Congo red or malachite green. In preparing larger quantities, twenty eggs are utilized for every 600 cubic centimeters asparagin solution. The mixture is filtered through sterile gauze and poured into test tubes, from 5 to 6 centimeters in each tube. It is coagulated and sterilized by being kept in steam at 80° for two hours on two successive days. For sterility control, keep in the incubator overnight.

This medium is characterized by the fact that instead of peptone it contains asparagin as the source of nitrogen. In my earlier experiments I found that peptone hinders the cultivation of the tubercle bacillus from infectious material. The high glycerin content, 7 to 8 per cent, is intended to keep the medium soft as long as possible. The fresher the medium, the better the results.

If the blood is taken sterile, which is guaranteed by this Saug-Ampulle, then the procedure is relatively simple. The blood is first well centrifuged, the serum pipetted off, the red blood corpuscles dissolved by adding sterile distilled water and treated with this until the hemoglobin has been entirely removed. The sediment is then transferred to the medium by means of a sterile pipette. After inoculation the tubes are sealed and placed in the incubator in a horizontal position, so as to secure the largest possible surface for growth.

It is naturally very important to begin with a sterile material, that is, material containing the least possible number of foreign germs. Skin ulcers harbor a large variety of bacteria, making it extremely difficult to decide which of the cultures obtained from them is to be considered the pathogen. If the blood or the infectious material is not already in a sterile condition, an attempt must be made to kill off the contaminating bacteria by means of weak disinfectants. My finding that tubercle bacilli can withstand 20 per cent sulphuric acid for fifteen minutes without their vitality being injured has been strikingly confirmed in their pure cultivation from infected material, whether sputum, pus, urine, or skin tissue. This method, however, cannot be applied in leprosy without modification, because leprosy bacilli cannot withstand such high acid concentrations. Their waxy covering is not as resistant as that of the tubercle bacilli. After preliminary experiments I have come to the conclusion that the wax

of the leprosy bacilli has a much lower melting point than has that of the tubercle bacilli. For this reason we cannot use sulphuric acid concentrations greater than 5 per cent. Here, too, the blood must first be completely freed of hemoglobin whenever it is to be exposed to the action of sulphuric acid. It would by all means be better for the solution of this difficult problem always to work with sterile material. I therefore take the liberty of suggesting that blood specimens be sent me in the Saug-Ampulles mentioned above. I shall be very glad to place at the disposal of the senders the results of my investigations, as well as the cultures obtained.