THE PRESENT STATUS OF DIAGNOSTIC SKIN TESTS IN LEPROSY[1]

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The possibility of a diagnostic skin test for leprosy has long interested investigators concerned with this disease. In another disease, the etiological agent of which belongs to the so-called acid-fast group of bacteria, as does *Mycobacterium leprae*, namely, tuberculosis, such a diagnostic skin test has been available for several decades. It has been applied with great practical benefit in the control of tuberculosis both in man and in lower animals. It is curious that no similar skin test has been developed for leprosy. Such a test would be a most important factor in bringing about the control of leprosy. If the presence of leprosy infection could be determined *before* actual signs or symptoms of the disease appear, the chances of preventing the clinical manifestations of the disease through proper treatment and preventive measures might be greatly enhanced. Also, the existing foci of the agent of infection might be lessened, and in time possibly eradicated. The disease could then be brought under control. This possibility looms large in its practical importance when one considers that there probably exist in the world today between three and five million cases of leprosy and, because of the special and unique social implications of this disease, the financial burden upon society is one of tremendous responsibility. The question takes on added importance when it is realized that progress in the control of leprosy has been very slow indeed through segregation and treatment programs. In many localities the disease continues to remain a perennial problem of about the same magnitude from year to year or even from decade to decade.

With this problem in mind, the author went to the Philippine

[1]This paper is based upon the report of a Special Committee appointed by the Director of Health of the Philippine Islands, at the request of the author, to investigate certain antigens for skin testing in leprosy. The committee was composed of Drs. Sulpicio Chiyuto, Cristobal Manalang, José N. Rodriguez, Casimiro Lara, Felix Velasco, Walfrido De Leon, H. Windsor Wade, and the author.

Islands early in 1937 at the instance of the American Leprosy Foundation. He requested Doctor Eugenio Hernando, Director of the Bureau of Health, to appoint a committee of leprosy experts to cooperate with him in testing a variety of antigens by skin test in groups of leprosy cases and in control groups in Manila, Culion, and Cebu. Doctor Hernando very graciously appointed a special committee for this purpose.[1] The author is greatly indebted to the members of the committee for their untiring efforts in the work which extended over a period of several months.

The antigens available were supplied through the courtesy of several investigators in the United States. From the laboratory of Doctor Esmond R. Long, the Henry Phipps Institute, Philadelphia, Pennsylvania, we had TPTs (tuberculin-protein-trichloracetic-acid-precipitated) prepared from various acid-fast bacteria for which Dr. Long and his colleagues are well known. The method for the preparation of these antigens has been described by Seibert and Munday (6). The group of antigens available to our committee included the TPTs of *M. tuberculosis hominis*, *M. tuberculosis avium*, *M. tuberculosis bovis*, *M. smegmatis*, *M. phlei*, *M. marinum* No. 2, and strains of supposed *M. leprae*, including those of Duval (non-chromogen), Daines, Karlinski, and a strain isolated from human leprosy at the Henry Phipps Institute, known as L-1. In addition, a TPT of a strain of supposed *M. leprae murium* was supplied by Doctor Long. From the laboratory of Doctor Rudolph J. Anderson, Yale University, various chemical fractions from a strain of supposed *M. leprae* were also supplied the committee for skin testing. These chemical fractions were prepared by Anderson (1) and his group from a strain of supposed *M. leprae* known as Hygienic Laboratory Strain No. 370. This organism was isolated from a case of leprosy (Apa case) in Honolulu, Hawaii, about 1909. The organism has been carried in the Mulford Biological Laboratories at Glenolden, Pennsylvania, since February 4, 1926, and is there known as Strain 1629. The organism grows well on a synthetic medium and is highly chromogenic. The chemical fractions prepared from this organism, according to the methods of Anderson, included the protein, polysaccharide, phosphatide, leprosin (wax), and leprosinic acid derived from the wax. All of these fractions were tested in both lepers and control groups. Finally, through the interest of Doctor Lawrence W. Smith, Philadelphia, Pennsylvania, an antigen was prepared by Doctor B. Gruskin from the fibrin of leper's blood which it was thought might contain a specific protein fraction.

Aside from the great practical importance of these investigations, there has also been a phase of considerable academic interest. The history of the bacteriology of leprosy is replete with many claims for the cultivation of the true germ of leprosy. This subject was exhaustively reviewed in a monograph by the author (4) in 1934 and it was then stated that "*there exists no absolute proof as yet* that any investigator during all of these years has actually succeeded in cultivating *Mycobacterium leprae in vitro.*" The chief difficulty in establishing specific etiological rank for any of these many strains of so-called or supposed *M. leprae* is the lack of an experimental laboratory animal for leprosy. Even man himself, supposedly the natural host, is disappointing in this regard, for attempts at the experimental transmission of leprosy to man have been very discouraging in that there is perhaps no generally accepted case of experimental human transmission of the disease on record (out of 145 human inoculations) with the possible exception of the Arning case in Honolulu, and this case is at least open to question. It has, therefore, been impossible to apply Koch's postulates to the question of etiology in leprosy.

However, it is generally believed that Hansen's bacillus is the cause of leprosy. The possibility of testing by skin test many antigens prepared from members of the acid-fast group of bacteria, some of which have been derived from leprosy cases, offered a possible new approach to the question of etiological specificity. Should any of these antigens appear specific for leprosy, it would, of course, be strong evidence that the microbe from which it came is of significance etiologically to the disease. And, fortunately, the study of these available antigens has wider implications than the names and sources would suggest. Recent investigations by Henderson (2) at the Henry Phipps Institute have shown close serological relationships existing between many of these acid-fast organisms. For example, the TPT of so-called *M. leprae* Daines is related serologically with the TPTs of such so-called leprosy strains as Clegg 1, Clegg HP1, L-1, L-3, Brinkerhoff 1, Brinkerhoff 2, *M. leprae murium*, Duval (chromogenic), Krause, Elly, Oto-Sato, Karlinski, Duval (non-chromogenic), and such other acid-fast organisms as *M. phlei*, *M. tuberculosis hominis* and *avium*, *M. marinum* No. 1; and indirectly with so-called *M. leprae* Levy-Kedrowsky (which is related serologically with Oto-Sato, Karlinski, Duval's non-chromogen, *M. phlei*, *M. tuberculosis avium* and *hominis*, and *M. marinum* No. 1). Again, there is a serological relationship between *M. tuberculosis hominis* and *bovis;* between *M. smegmatis* and *M. butyricum.* So that it is apparent

that when skin tests are made with such antigens as the TPT of the supposed *M. leprae* Daines, *M. leprae* Duval, *M. leprae* L-1, *M. leprae* Karlinski, or *M. leprae murium*, a very wide range of antigenic responses involving many other acid-fast organisms is being tested. The antigens fall into about seven groups with many interrelationships between these groups evidencing the fact that there are common antigenic components existing in many of the various strains which number about twenty-two in the group so far studied.

With these very practical and academic considerations before us, the committee undertook the skin testing work in leprosy cases and control groups with high hopes. In the beginning it was learned that some basic studies would have to be undertaken to determine proper dosage of the various antigens. For the TPTs of the *M. tuberculosis* group the dosage was already prescribed and based upon previous work of Long and his colleagues. It was decided, therefore, to begin with these doses for all the other antigens and to increase or decrease the dose as the studies seemed to warrant. The dose of each antigen employed is indicated in Table 1, with the corresponding degree of reaction in the patient. This subject is mentioned merely to emphasize that the dosage commonly employed with the TPT of *M. tuberculosis hominis*, for example, will not suffice for some of the other antigens. In the case of the polysaccharide of strain No. 370, for instance, it was found necessary to increase the amount of antigen many times, and in the case of the proteins from organisms other than the *M. tuberculosis* group it was found advisable to double the dose commonly used for this group.

The dose of each antigen having been determined, the skin tests were made by the intradermal method. Tuberculin syringes with No. 26 gauge needles, one-half inch in length, were employed. In each case 0.1 cc. of antigen was injected with care being taken to produce a raised wheal having the typical orange peel appearance. The antigen solution was prepared fresh each day before it was used and sterile physiological saline was employed as the diluent. In the beginning the reactions were watched carefully during the first hour or two after injection, then twenty-four hours later, and finally after forty-eight hours. Since nothing significant came from the early readings, or in most instances from the twenty-four hour observation, a routine was finally adopted to take readings and measurements forty-eight hours after injection, as is done with the tuberculin test. Measurements were made with vernier calipers (horizontal, vertical, and thickness) and carefully recorded on each chart. The reactions were graded 1- to 4-plus. Redness

was regarded as of less significance than swelling, though in each instance, if erythema was present, it was noted in the record. A reaction consisting of an area of swelling measuring from 5 to 10 mm. in average diameter was recorded as "1-plus"; a similar reaction measuring from 10 to 20 mm. in average diameter was recorded as "2-plus"; such a reaction exceeding 20 mm. in average diameter was recorded as "3-plus"; while a reaction exceeding 20 mm. in average diameter with central necrosis was given a "4-plus". Reactions measuring less than 5 mm. in average diameter were listed as doubtful or negative. If there was no swelling at the site of injection, the reaction was read as negative.

Since most of the antigens tested in our study were of the TPT variety, as prepared by the Henry Phipps Institute, it may be of interest to give here in brief the method of preparing these antigens. Doctor Seibert has contributed the following notes regarding the method:

The method is applicable to all of the proteins from any of the acid-fast strains of bacilli.

The bacilli are grown for eight weeks at 37.5°C. upon Long's synthetic medium. The bacilli are removed by filtration through china silk on a Buchner funnel and then through a Mandler filter. The clear yellow tuberculin filtrate, preserved with 0.5 percent phenol, is then concentrated by ultrafiltration on a 13 percent gun-cotton-glacial-acetic-acid membrane.

The membranes are impregnated by means of suction during one minute immersion of an alundum cup of medium porosity under a solution of gun-cotton. The gun-cotton solution is made by dissolving 13 gm. of dried gun-cotton, designated as "high temperature nitro-cellulose, 15-30° viscosity" in 100 cc. glacial acetic acid. After the membranes are washed free of acid, they are used for the ultrafiltration of the tuberculin filtrate.

When the entire tuberculin filtrate has been concentrated as much as possible by this ultrafiltration process, it is washed with 0.5 percent phenol solution by continued ultrafiltration until the filtrate is chloride- or iron-free, and then again concentrated to a very small volume. It is then filtered through the Seitz filter, and a pure solution of the tuberculin protein and polysaccharide results. The protein is precipitated from this pure colloidal solution in a final concentration of 10 percent trichloracetic acid and, after standing over night, the precipitate is centrifuged off and washed by centrifugation several times with 10 percent trichlora-

cetic acid until the wash is clear and colorless. The drained precipitate is partly dried *in vacuo* and then ground to a fine powder under large volumes of ether, which simultaneously dehydrates the product and removes the trichloracetic acid, leaving a fine cream-colored powder, TPT.

To prepare the stock solution of TPT, 50 mgm. of the powder is accurately weighed. About 1 cc. of sterile physiological saline[2] containing 0.5 percent phenol is added, and then 1/10 N sodium hydroxide is slowly dropped in from a burette while the suspension is being stirred. As soon as the protein is just completely in solution no more alkali is added. The solution is then carefully neutralized to litmus with 1/10 N hydrochloric acid and made to 5 cc. volume with phenolized saline. The solution is then filtered through paper into sterile containers. This solution is a 1 percent TPT solution and one cc. contains 10 mgm. of TPT.

From the 1 percent TPT solution the following dilutions are made for testing:

The stock solution is a 1 percent solution (1cc.=10 mgm. TPT). It is stable. Shake before using.

Solution A (1cc.=0.05 mgm. TPT)=*second dose strength solution.* Prepare by measuring 0.1cc. of stock (1 percent) solution very accurately with sterile technique and putting into 19.9 cc. sterile saline. Dose=0.1cc.=0.005 mgm.

Solution B (1cc.=0.0002 mgm. TPT)=*first dose strength solution.* Prepare by measuring 0.1cc. of Solution A very accurately with sterile technique and putting into 24.9 cc. sterile saline. Dose =0.1cc.=0.00002 mgm.

Make fresh solutions the day they are to be used and discard the unused portion at the end of the day. For testing those who do not react to the first dose it will be necessary to make only Solution A.

Increased strengths of the antigens were prepared from the original stock solutions by making suitable dilutions as desired with physiological saline. Stock solutions of the various chemical fractions of Hygienic Laboratory Strain No. 370, as prepared by Anderson, were also made up according to the Seibert method and subsequent dilutions were made in a similar manner as indicated above. In the cases of leprosin (wax) and leprosinic acid, how-

[2]The sterile saline should be made from freshly distilled water, which has been distilled with the use of a Kjeldahl trap to eliminate the possibility of contamination of the tuberculin solution with pyrogenic products, as described by F. B. Seibert, American Jour. Physiol. **67** (1923) 90; and **71** (1925) 621.

ever, pure olive oil was chosen as the diluent. This was kindly prepared for us by the chemical department at Culion. Also, in a few tests with the polysaccharide, the antigen was dissolved in a 2 percent gum acacia solution in order to determine if the antigen might not be localized for a longer period at the site of injection. In these last two instances it became necessary, of course, to run suitable controls with the diluent materials themselves.

EXPERIMENTAL DATA

One will nctice in Table 1 that a total of 5,174 skin tests were performed in the ten groups of patients or individuals employed in the experiments. All reactions indicate 48-hour readings. This is at variance with the so-called leprolin test (5) which is of value in assisting in the separation of the neural cases from the cutaneous cases of the disease. In the leprolin test cutaneous cases are negative while the neural cases are positive. However, the reaction, in the form of an infiltrated area, does not appear until many days have passed following the injection of leprolin. The time is usually from eight to twenty-four days. Leprolin consists of ground nodular material, suspended in physiological saline, heated to 60°C for one hour, filtered and carbolized.

The committee made skin tests at several institutions in different parts of the Philippine Islands. At Welfareville, near Manila, the children of lepers were available. Also, suitable control groups of children in the training schools were obtained. These latter had never been in contact with leprosy in so far as their history is known. The children of lepers had been in contact with the disease at the Culion Leper Colony for six months to two years prior to their removal to Welfareville. At the San Lazaro Hospital in Manila, both early and advanced cases of leprosy were available for testing. Most of these cases were of Philippine origin but some had recently been returned to the Philippines from Hawaii.

At the Culion Leper Colony, both advanced cases of the disease and preparole cases were tested. In other words, these groups represented both bacteriologically positive and negative cases. In addition, the professional and technical staff members of the institution generously volunteered to be tested with several of the antigens. There were over 80 individuals in this group and Culion is possibly the one place in the world where such a group could be obtained. These individuals had been in contact with the disease for varying periods ranging from a few months to

many years. Later a smaller group of such contacts were also tested at Cebu, bringing the total for this group of contacts to approximately 100.

At the Eversley Childs Treatment Station, at Cebu, two main groups of patients were tested. One group of patients consisted of bacteriologically positive lepers who had recently been admitted to the station. This group represented patients much less advanced in the disease than either the Culion or Manila group of lepers. The second group consisted of bacteriologically negative cases awaiting parole. At Talisay, where comprehensive epidemiological studies are in progress, it was possible to obtain a group of more intimate contacts. Here family groups were available and our series consists of 109 individuals from 24 families having one or more members afflicted with leprosy.

The groups studied represent, therefore, various stages and gradations of the disease, as well as different degrees of contact. They also represent both bacteriologically positive and negative cases. Both sexes were included in the several groups. The ages ranged from young children to old adults. Control groups of both children and adults, with presumably no previous contact with leprosy, were also included. It may be said here that apparently age and sex had no bearing or relation to the type of response elicited with the antigens prepared from supposed strains of *M. leprae*. Our data concerning the tuberculin reaction in these groups will constitute a separate paper to be published later. The reactions with the tuberculosis antigens are given, however, in Table 1.

Examination of Table 1 shows no clear-cut diagnostic skin test for leprosy with any of the antigens studied. The children of Culion lepers gave 58, 60, and 51 positive reactions with the TPTs of supposed strains of *M. leprae* of Karlinski, Henry Phipps Institute L-1, and Daines, respectively. They gave 48, 46, and 49 negative reactions for these antigens in the same order. Advanced cases of leprosy at Culion gave 54, 69, 72 positive reactions with the TPTs of these antigens, with 41, 26, and 23 negatives in the same order. Early cases of leprosy in Cebu gave 8 and 14 positive reactions for the TPTs of Karlinski and Duval, with 25 and 17 negative reactions. Advanced cases of leprosy at the San Lazaro Hospital in Manila gave 20, 32, 29, and 6 positive reactions with the TPTs of Karlinski, L-1, Daines and Duval, respectively, and 80, 68, 71, and 4 negative reactions for these antigens in the same order. The preparole cases at Culion gave 69, 72, and 73 positive reactions

with the TPTs of Karlinski, L-1, and Daines, respectively, with 23, 20, and 19 negative reactions in the same order. However, the preparole cases at Cebu gave 29 and 53 positive reactions with the TPTs of Karlinski and Duval, with 71 and 47 negative reactions, somewhat the reverse of results with similar antigens employed in a comparable group at Culion. It is believed that, since the Culion patients have been at the colony for many years longer than have the cases at Cebu, the infection with tuberculosis is much heavier and more widely disseminated. This seems to be borne out by the results of the tuberculin tests, since the preparole cases at Culion gave 86 positives with TPT *M. tuberculosis* and only 6 negatives, and the preparole cases at Cebu gave 78 positives with this antigen with 22 negatives. The groups constituted 92 and 100 patients respectively and are therefore comparable.

The family contact group at Talisay, Cebu, gave 54 positive reactions with the TPT of Karlinski and 55 negative reactions. The physician-nurse-technician groups added together for both Culion and Cebu gave 69, 65, 76, and 17 positive reactions with the TPTs of Karlinski, L-1, Daines, and Duval, respectively, and 15, 19, 8 and 0 negative reactions with these antigens in the same order. The bulk of these tests were made at Culion, however, and again the question of tuberculosis infection would have to be seriously considered, since we know there exists close relationships between many of these antigens of the acid-fast group of bacteria. When one considers the control group of boys and girls at Welfareville, it becomes apparent that little significance is to be attached to the relatively small differences we have seen in the reactions to the TPTs of several of the supposed strains of *M. leprae*. This group gave 66, 70, and 69 positive reactions with the TPTs of Karlinski, L-1, and Daines, and 43, 39, and 31 negative reactions with these antigens in the same order. These results are quite comparable with the reactions obtained in the advanced leper series at Culion. The reactions of this control group with the TPT of *M. tuberculosis* resulted in 99 positives and 11 negatives, while the Culion group of advanced cases showed 66 positives and 29 negatives with this antigen. In this connection it is well to note also that the advanced leper group at San Lazaro Hospital, in Manila, gave 85 positive and 15 negative reactions with the TPT of *M. tuberculosis*. The early cases at Cebu gave 39 positive and 38 negative reactions with this antigen. The children of lepers, the family contacts at Talisay, Cebu, and the professional group at Culion gave 97, 70, and 44 positive reactions with the TPT of *M. tuberculosis*, and 24, 39,

and 38 negative reactions, respectively. It is also interesting to note that the family contact group at Talisay gave 55 positive reactions with the TPT of *M. tuberculosis bovis* with 54 negative reactions.

The TPT of a supposed strain of *M. leprae murium* was tested in the series of advanced lepers at Culion, in the preparole cases at Culion, and in the professional-technical group at Culion, with 68, 75, and 74 positive reactions, respectively, and 27, 17, and 10 negative reactions.

The TPTs of certain known saprophitic acid-fast bacteria were also tested in some of our groups. The TPT of *M. smegmatis* was tested in the children of lepers, in the advanced cases of leprosy at San Lazaro Hospital, and in the control group of boys and girls at the training school at Welfareville. These groups gave 36, 26, and 57 positive reactions, respectively, and 64, 74, and 43 negative reactions in the same order. The results are not regarded as significant although the control group showed relatively fewer negative reactions. The TPT of *M. phlei* was tested in the advanced cases of leprosy at Culion, in the preparole cases at Culion, and in the physician-nurse-technician group at this colony. These groups gave 54, 69, and 71 positive reactions, respectively, and 41, 23, and 13 negative reactions in the same order. In this case the control group (somewhat different from the control group at Welfareville since the professional-technician group at Culion may be regarded as contacts) showed a higher number of positive reactions. The TPT of so-called *M. marinum* No. 2 was tested only in the control group of boys and girls of the training school at Welfareville. This group gave 8 positive and 1 negative reaction, hardly significant except that, in view of the high percentage of positive reactions in nonleprosy contacts, the committee did not feel it worth while to make further tests with this antigen.

The Gruskin antigen, prepared from the fibrin of the blood of lepers, was tested in only two groups, namely the control boys and girls group at Welfareville and in the advanced leprosy cases at the San Lazaro Hospital in Manila. These groups gave 0 and 0 positive reactions, respectively, and 9 and 10 negative reactions. The tests were relatively few and opportunity did not present itself to the committee to extend the series further at this time. Since there were no positive reactions in either group, the antigen did not appear to be significant.

We have left the discussion of our results with the various chem-

ical fractions of Hygienic Laboratory Strain 370 until the last. In the first place, our supply of some of these antigens was extremely limited and consequently some of the series are not large. However, the committee feels that, for the most part, these antigens were tested in a sufficient number of cases to determine their value, with the possible exception in the case of one antigen, namely leprosinic acid, which will be commented upon later.

The protein, polysaccharide, and phosphatide of strain 370 were tested in the children of lepers at Welfareville, in the control group of boys and girls at this institution, and in the advanced lepers at San Lazaro Hospital. Adding the reactions together without respect to dosage, these groups gave 10, 2, and 4 positive reactions, with 11, 8, and 26 negatives for the protein; 12, 5, and 0 positive reactions, with 62, 62, and 10 negative reactions, respectively, for the polysaccharide; and 3, 8, and 1 positive reactions, with 13, 2, and 29 negatives, respectively, for the phosphatide. In addition, the polysaccharide of strain 370 was tested in the group of early cases of leprosy at Cebu, with only 3 positive reactions and with 58 negative reactions. It would seem that without question the polysaccharide fraction of the supposed *M. leprae*, Hygienic Laboratory Strain 370, can be ruled out as without significance, since of a total of 212 tests performed with this antigen only 20 were positive and 192 were negative with a large preponderance in all groups, whether cases of leprosy or controls, in the negative column. The protein fraction also appears to be without significance. In the group of advanced lepers, 26 reactions were negative and only 4 were positive. Nothing of further interest concerning this antigen appears in the relatively few tests performed in the children of lepers and control groups, and further study of the protein fraction did not appear to be indicated. The three groups tested with the phosphatide fraction of strain 370 (see above) gave 12 positive reactions and 44 negative reactions. Here again the antigen seems to be without significance based upon the 29 negative and only 1 positive reactions found in the advanced leper group at the San Lazaro Hospital. The numbers tested with the phosphatide in the other two groups are small indeed but the results appear to be without significance. It is to be noted that the degree of the reaction is only 1- or 2-plus wherever it is positive, that is in 12 instances of a total of 56 tests.

We come finally to a consideration of the leprosin and leprosinic acid fraction of strain 370. The leprosin fraction was tested in the children of lepers group at Welfareville, in the control group

of boys and girls at this institution, and in the group of advanced cases of leprosy at San Lazaro Hospital. The leprosinic acid fraction was also tested in these three groups, but in addition this fraction was tested in the family group of contacts at Talisay, Cebu. The results with the leprosin fraction for the three groups mentioned above show 6, 3, and 0 positive reactions, respectively, with 24, 27, and 30 negative reactions in the same order. The results seem hardly significant except for 100 percent negatives in the leprosy cases in contrast to small percentages of positives in the other two groups. This may, however, lend some support to the possible significance of the results obtained with the leprosinic acid fraction which we will now consider.

We have listed above the four groups in which the leprosinic acid fraction was tested. Since we may have something of significance with this antigen, we will give the number of positive and negative reactions after the name of each group:

GROUP A.	Children of lepers.....	27 positive	3 negative
GROUP B.	Controls, Training School.....	30 positive	0 negative
GROUP C.	Advanced cases, S. L. Hosp. ...	9 positive	21 negative
GROUP D.	Family groups, contacts	34 positive	75 negative

In addition to the above, three tests were performed with a small quantity of remaining material of the leprosinic acid fraction in the early leprosy group at Cebu, with 1 positive and 2 negative reactions. They are too few to have any significance and it was unfortunate that our supply of antigen did not permit a larger series of tests with this group.

What is the significance, if any, of our results with the leprosin and leprosinic acid fractions of the supposed strain of *M. leprae*, No. 370? The rather high incidence of negative reactions with the leprosin fraction in individuals having little or no leprosy infection (24 negatives out of 30 tests, and 27 negatives out of 30 tests in the two groups at Welfareville) and the total absence of positives in advanced cases of leprosy (30 negatives out of 30 tests at San Lazaro Hospital) seem to indicate little or no significance for this substance. In the case of leprosinic acid, however, the situation appears to be reversed in that we have a high percentage of positive reactions in the children of lepers and in the control group of boys and girls at Welfareville, while the advanced cases of leprosy give only about 30 percent of positives as contrasted with roughly 75 percent and 100 percent positives for the other two groups. It is, of course, difficult to know what the significance of the reactions may be in the family group contacts of Talisay, since we do not know whether

they are infected with leprosy or not. On the whole it seems impossible to draw any conclusions from these tests with leprosin and leprosinic acid, except to point out that in these experiments there is a faint clue that the lipoid fractions of these acid-fast bacteria may be the important material to study in future experiments. Of course there is also other important evidence for this theory which is well known to investigators in this field. Certainly the studies reported in this paper indicate that the other components of these acid-fast bacteria appear to have little or no significance when tested intradermally in suitable groups of leprosy cases and control series. The results would suggest that the organisms from which these substances have been obtained are not the specific germs of leprosy.

In 1932 McKinley and Soule (3) reported the successful cultivation of an organism from leprosy tissue which is apparently different from any germ previously described for this disease. This organism grows only in an atmosphere of oxygen and carbon dioxide when first isolated. It has now been under cultivation for about six years and has been carried through over sixty subculture generations. It grows meagerly and today, after six years, it grows in the same sparse manner with only a few small colonies to each culture. With a large incubator in which a gaseous tension may be produced, and having a capacity of 2880 cultures, attempts are now being made to produce cultures in large numbers so that antigens for future skin tests in cases of leprosy and suitable controls may be performed. Possibly such antigens may be specific and will give us the long-sought-for diagnostic skin test for this disease.

CONCLUSIONS

Over five thousand intradermal skin tests have been performed with antigens prepared from various acid-fast bacteria, some of which have been isolated from cases of leprosy. The antigens have included the TPT (protein) of many of these organisms; the protein, polysaccharide, phosphatide, leprosin (wax), and leprosinic acid from one strain of acid-fast isolated from a case of leprosy; and a protein prepared from the fibrin of the blood from leprosy cases. Cases of leprosy in various stages of the disease, individuals who have been in contact with the disease, suitable control individuals having had no contact with leprosy, individuals of both sexes and varying ages, cases of both neural and cutaneous leprosy predominating, and cases bacteriologically positive and negative have been given intradermal tests with these antigens.

The study indicates that in none of the antigens studied have

we found a specific antigen for a diagnostic skin test for leprosy. The work also suggests that the supposed strains of *M. leprae* from which several of the antigens were prepared are not related specifically and etiologically to the disease.

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