INTERNATIONAL JOURNAL OF LEPROSY

VOLUME 32, NUMBER 2 APRIL-JUNE, 1964

IMMUNOLOGIC STUDIES WITH *MYCOBACTERIUM LEPRAE* AND AN ACID-FAST MYCOBACTERIUM CULTIVATED FROM HUMAN LEPROSY NODULES[1]

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Many investigators have studied the antigenic composition of mycobacteria and tried to learn if these bacteria have antigens in common. Most of the immunologic work reported relates to *M. tuberculosis* ([11]), since this organism is not only of great clinical and epidemiologic importance but also can be cultivated easily. Immunologic studies with *M. leprae* have been hampered by the nonavailability of *M. leprae* in quantities sufficient for immunologic investigations. Although several workers ([7]) have claimed cultivation "*in vitro*" of acid-fast mycobacteria from leprosy nodules, the role of the cultivated bacteria in the causation of leprosy is debatable. Koch's postulates have not been proved in the case of *M. leprae*.

In 1956 studies were undertaken by Ranadive and co-workers at the Indian Cancer Research Centre with the object of cultivating *M. leprae in vitro*. Bapat, Ranadive and Khanolkar ([1, 2]) were able to cultivate acid-fast mycobacteria isolated from human leprosy nodules in modified tissue culture fluid. They designated the bacillus isolated as the "ICRC bacillus" (Indian Cancer Research Centre bacillus). During the course of the last six or seven years Ranadive and her group have isolated and cultivated morphologically identical organisms from six cases of lepromatous leprosy, and the bacilli have been in continuous cultivation for more than five years ([12]). They have now found it possible to grow the bacilli on a serum-free medium.

It was of great interest to establish whether or not the ICRC bacilli cultivated *in vitro* were identical with *M. leprae*. Since injections of *M. leprae* into laboratory animals are not known to induce the disease, it seemed likely that the identity of the ICRC bacilli with *M. leprae* would be decided if it could be established that their antigenic composition was the same. With this object in view the work detailed

[1]Received for publication September 10, 1963.

below was undertaken. The results obtained with the immunologic tests employed have indicated a complete lack of antigenicity of the *M. leprae* in the human host.

MATERIALS AND METHODS

Lepromin, lepromatous nodules and sera from leprosy patients were obtained from the Acworth Leprosy Home in Bombay. Some lepromatous nodules were obtained also from Dr. V. Ekambaram, State Leprosy Officer, Tirukoilur, Madras, and Dr. K. Ramanujam, Government Silver Jubilee Children's Clinic, Saidapet, Madras. The ICRC bacilli used were those cultivated in the laboratory of the Applied Biology Group, as already described by Bapat *et al.* (1). Suspensions of *M. leprae* were collected from lepromatous nodules and concentrated by the method of Nerurkar and Khanolkar (9). Preparations of Bacille Calmette Guerin (BCG) were obtained from the BCG Vaccine Laboratory, Madras.

For the preliminary experiments, *M. leprae* and ICRC bacilli were washed with saline and also with other solvents as indicated later in this communication. To obtain antisera specific for ICRC bacilli and *M. leprae,* rabbits were immunized with the corresponding bacilli together with Freund's incomplete adjuvant. The animals received, in all, six weekly subcutaneous injections of 0.75 ml. each of a suspension containing about 8×10^9 of the respective bacilli/ml. in either intact or disrupted condition. A booster dose was given 15 days after the fifth injection. Seven days after the last injection the animals were bled and the serum was separated. The animals were reimmunized by the same procedure after a rest period of two months.

The Ouchterlony agar gel diffusion technic (4) was used to study the antigenic composition of *M. leprae* and ICRC bacilli. The hemagglutination tests of Boyden (3) and of Stavitsky (14) were used with slight modifications as already described (13).

For the hemagglutination test, sensitized sheep erythrocytes were prepared by maintaining at room temperature for 45 minutes 1 ml. of tannic acid-coated sheep erythrocytes with 1 ml. of the respective antigen and 4 ml. of phosphate buffer of pH 6.4 The erythrocyte-antigen suspension was then centrifuged. The sedimented erythrocytes were washed twice with diluted normal horse serum and resuspended in 1 ml. of the same serum and the hemagglutination test was set up as already described (14).

The passive cutaneous anaphylaxis (PCA) test as described by Ovary (10) also was used to detect nonprecipitating and skin-sensitizing antibodies.

Guinea-pigs weighing about 350 gm. each were used in these studies. The hair on the back was removed with an electric shaver and the skin surface cleansed by a depilatory. A volume of 0.1 ml. of serial dilutions of the test antiserum was injected intradermally at desired spots on the back of the guinea-pig. Two hours later the guinea-pig was challenged with an intracardiac injection of 1 ml. of the antigen mixed with a solution of 1 per cent Evans blue in the proportion 1:1. If an antigen-antibody reaction occurred, within about 2 hours, normally the dye localized at the place where the antiserum was injected. The area of localization of the dye was taken to be proportional to the intensity of the reaction.

Direct and indirect Coombs tests were used to detect incomplete antibodies in the sera of rabbits immunized with ICRC bacilli. The anti-rabbit globulin sera were obtained by immunizing fowls with rabbit gamma globulin.

The indirect Coombs test was used also to find out if possibly the sera of leprosy patients contained incomplete antibodies to ICRC bacilli. In these experiments the ICRC bacilli were used in the hope that incomplete antibodies, if present in leprosy sera, would become attached to the bacilli. Antisera to human gamma globulin were used in these experiments.

EXPERIMENTAL INVESTIGATION

Ouchterlony gel diffusion tests with antiserum to M. leprae *and ICRC bacilli.*—Immunologic studies were first carried out with anti-

serum to saline-washed intact *M. leprae* and ICRC bacilli, using the Ouchterlony gel diffusion test. Two to three days after the plates were set up a faint line appeared between the antiserum well and the three antigen wells. Later two more lines appeared in this region. All three precipitin lines were common to all the antigens used. In these experiments the serum of a patient with tuberculoid leprosy was used. It is believed that the positive lepromin reaction observed in tuberculoid cases indicates resistance to leprosy as manifested by the presence of antibodies to *M. leprae*. The lepromin reaction is considered by many as an antigen-antibody reaction ([6, 8]).

The results recorded in Figures 1 and 2 indicated that *M. leprae* and ICRC bacilli had antigens in common and that these were present also in the serum of a tuberculoid patient. These results could be interpreted as indicating (1) that the specific antigens of *M. leprae* were present in both the ICRC bacilli and the tuberculoid serum or (2) that the antigens common to the bacilli were those present in normal human serum without relation to the specific antigens of *M. leprae* or ICRC bacilli.

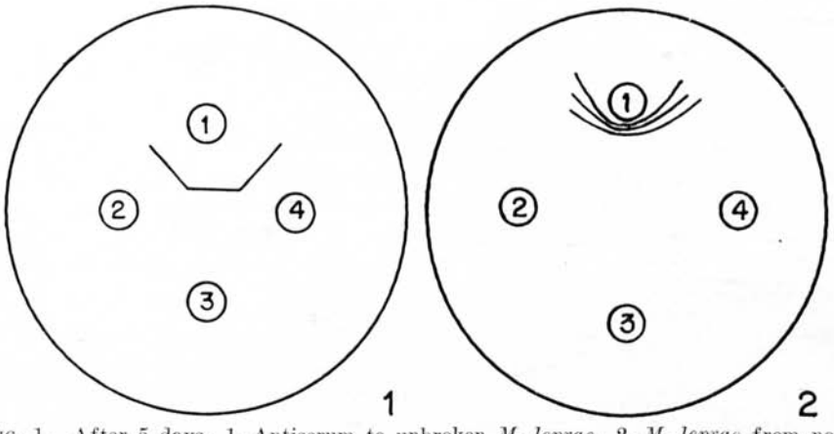

FIG. 1. After 5 days. 1. Antiserum to unbroken *M. leprae*. 2. *M. leprae* from nodules. 3. Leprosy patient's serum (tuberculoid type). 4. ICRC bacilli (pooled).

FIG. 2. Figure 1 after 10 days.

To test the second hypothesis normal human blood serum was used in the next few sets of experiments. It was surprising to note that normal human serum gave a larger number of precipitin lines with both the antiserum to *M. leprae* and the antiserum to the ICRC bacilli (Figs. 3 and 4). An Ouchterlony plate was set up also with antiserum to *M. leprae* in the central well and with two each of the six surrounding wells filled with serum of lepromatous patients, tuberculoid serum and normal human serum, respectively. Three days after filling the wells with antigens and antibodies, precipitin lines began to develop between the central well and the six peripheral wells. There were four lines of strong intensity, two of medium intensity, and one faint line. All these ultimately coalesced to give a common antigen-

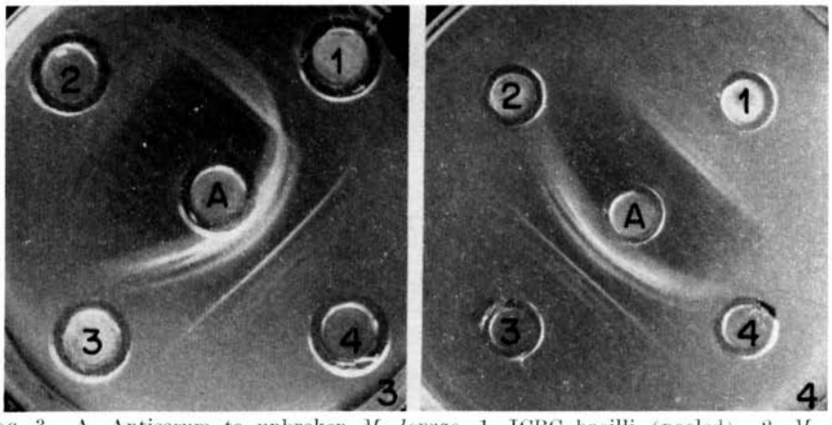

FIG. 3. A. Antiserum to unbroken *M. leprae*. 1. ICRC bacilli (pooled). 2. *M. leprae* from nodules. 3. BCG. 4. Leprosy patient's serum (tuberculoid type).

FIG. 4. A. Antiserum to unbroken ICRC bacilli. 1. ICRC bacilli (pooled). 2. BCG. 3. Leprosy patient's serum (tuberculoid type). 4. *M. leprae* from nodules.

antibody precipitin line around the central well. Similar results were obtained with the antiserum against ICRC bacilli.

These results indicate that the sera of leprosy patients, and normal sera as well, reacted with antisera to *M. leprae* and ICRC bacilli giving identical pictures. Evidently immunization with ICRC bacilli and *M. leprae* washed twice with saline gave rise to antibodies only to components of serum possibly adhering to the lipid coat of the bacilli. It was felt, therefore, that washing the bacilli with fat solvents like acetone and ether, and further digesting them with trypsin, would remove the lipid coat and also digest any tissue and blood protein sticking to the bacillary cell wall.

Accordingly both *M. leprae* and ICRC bacilli were washed with a 1:1 acetone-ether mixture. The bacilli were then supended in phosphate buffer of pH 7.6 and digested with trypsin. Rabbits were immunized with these bacilli. An Ouchterlony plate was set up with the antisera to the washed and trypsinized *M. leprae* and ICRC bacilli. In these plates BCG also was used since it is assumed by workers ([4, 5]) that *M. leprae* and BCG have some antigens in common. The results of the experiment revealed once again that washed and digested bacilli gave, with the antiserum, precipitin lines that were common also to normal human blood serum. BCG did not react with the antiserum. It was considered probable that the antigens specific to these bacilli were intracellular.

Accordingly attempts were made to extract the intracellular antigens of the ICRC bacilli and *M. leprae*. For this purpose the washed and trypsin-digested bacilli were disintegrated in a Mickle shaker in the presence of Ballotini beads. Whether the bacilli became disintegrated or not was determined by staining the bacillary material with acridine orange and viewing them under the fluorescent microscope. Electron micrographs of the bacilli (Figs 5 and 6), taken before and

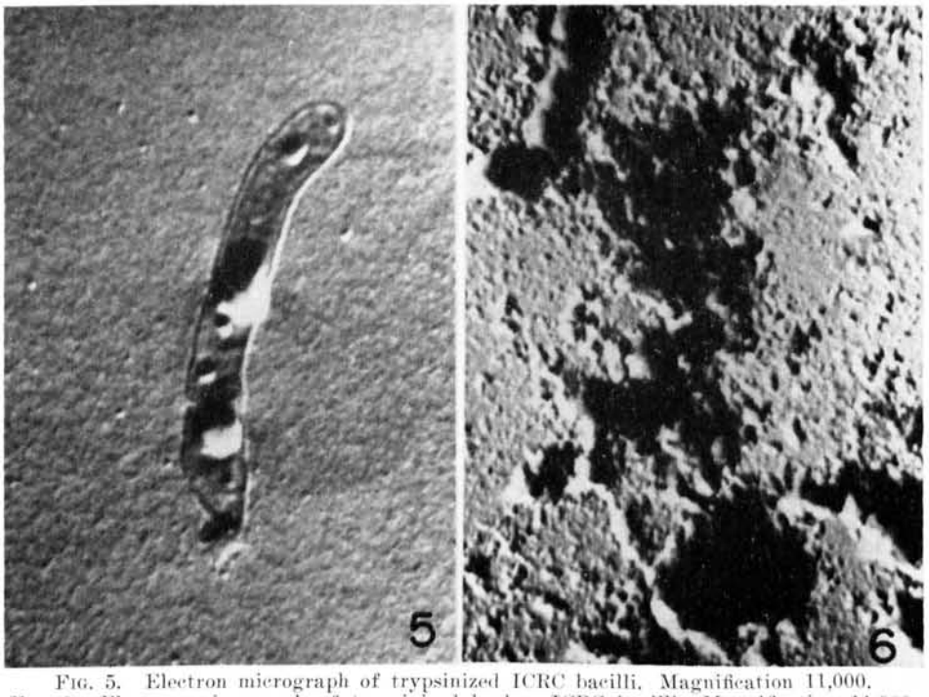

FIG. 5. Electron micrograph of trypsinized ICRC bacilli. Magnification 11,000.
FIG. 6. Electron micrograph of trypsinized broken ICRC bacilli. Magnification 11,000.

after the bacilli were ground, indicated that the bacilli were disintegrated by the treatment. Antisera were obtained from rabbits immunized with the acetone-washed and disintegrated ICRC bacilli and *M. leprae*. An Ouchterlony plate was set up with this antiserum as indicated in Figure 7. The antigens used were frozen and thawed ICRC bacilli, lepromin, broken ICRC bacilli, ether-acetone-washed and broken ICRC bacilli, normal human serum, "ICRCin" (prepared like lepromin, with ICRC bacilli as starting material), and, finally, a saline suspension of ether-acetone-washed ICRC bacilli. The results indicated that antiserum to ICRC bacilli reacted in such a way as to give a faint precipitin line with washed and broken ICRC bacilli and also with washed and broken *M. leprae,* and with lepromin. Since lepromatous nodules for isolation and concentration of *M. leprae* are not readily available, the antiserum to ether-acetone-washed and broken *M. leprae* was preserved for use in other immunologic experiments.

The experiment was repeated with slight modification. In this trial one of the two antiserum wells was filled with antiserum to ICRC bacilli absorbed by normal human blood serum. The absorption was carried out in order to remove from the antiserum all antibodies specific for normal human serum. On test with the Ouchterlony plate, as indicated in Figure 8, it was observed that the unabsorbed sera gave five precipitin lines with human serum, one medium line with lepromin and *M. leprae,* and one faint precipitin line with frozen and thawed ICRC bacilli. The unabsorbed antiserum did not give any antigen-

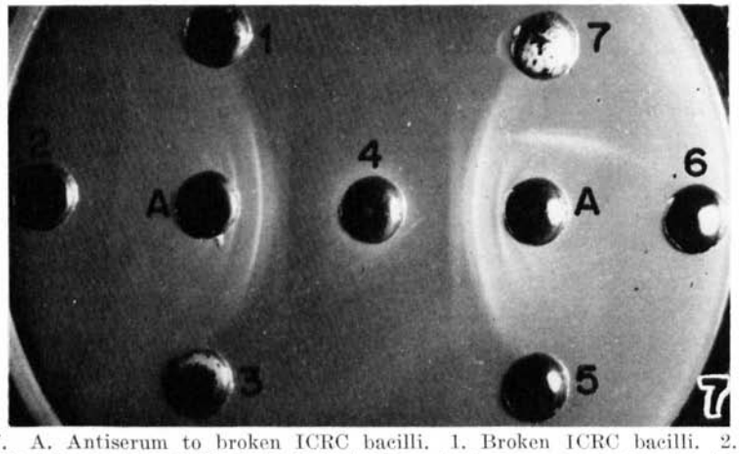

FIG. 7. A. Antiserum to broken ICRC bacilli. 1. Broken ICRC bacilli. 2. Lepromin (A.L.H.). 3. Freeze-thawed ICRC bacilli. 4. Normal human serum (HAS). 5. *M. leprae* from nodules. 6. ICRCin (T.M.H.) 1:10. 7. ICRC bacilli (pooled).

antibody precipitin line. These results indicated again that antisera even to ether-acetone-washed, trypsin-digested, and broken bacilli, contained antibodies specific only for components of human serum.

It seemed probable that neither *M. leprae* nor ICRC bacilli would give rise in the immunized rabbits to precipitating antibodies for the respective bacilli or for each other. Attempts were made therefore to determine if rabbit antisera would contain nonprecipitating skin-sensitizing or incomplete antibodies.

Hemagglutination tests with antiserum to M. leprae *and ICRC bacilli.*—The hemagglutination test of Boyden and Stavitsky was used. Besides helping to detect nonprecipitating antibodies, the test would indicate whether or not small amounts of antibodies were present in the antiserum. It was carried out as already described (9). The antisera to both *M. leprae* and ICRC bacilli proved able to agglutinate erythrocytes sensitized with either *M. leprae* or ICRC bacilli. On the basis of results of the Ouchterlony experiments, erythrocytes sensitized with normal human blood serum were used as a control in this

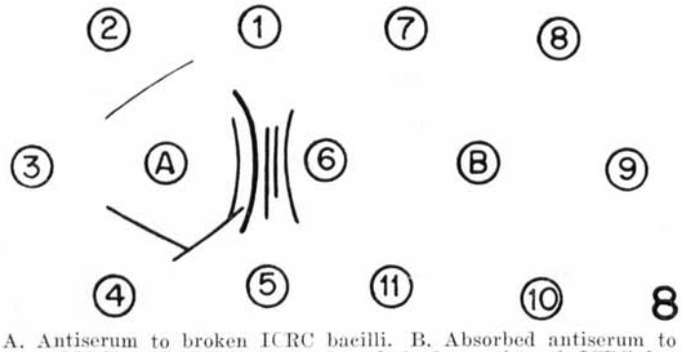

FIG. 8. A. Antiserum to broken ICRC bacilli. B. Absorbed antiserum to broken ICRC bacilli. 1 and 7. ICRCin (T.M.H.) 1:10. 2 and 6, freeze-thawed ICRC bacilli. 3 and 9. ICRC bacilli in saline (pooled). 4 and 10, *M. leprae* from nodules. 5 and 11, lepromin (A.L.H.) 1:10.

TABLE 1.—*Results of hemagglutination test with rabbit antiserum to normal human blood plasma.*

Dilution of antiserum	Degree of agglutination of sheep erythrocytes treated with each of the antigens		
	Broken ICRC bacilli	Broken *M. leprae*	Normal human serum (HAS)
Undiluted	+++	+++	++++
1:2	+++	+++	++++
1:4	+++	++	+++
1:8	++	++	+++
1:16	++	++	+++
1:32	+	+	++
1:64	+	+	++
1:128	+	+	++
1:256	+	±	++
1:512	±	±	+
1:1024	−	−	+
1:2048	−	−	+
1:4096	−	−	+

experiment. Surprisingly the erythrocytes sensitized with normal blood serum were agglutinated also by both antisera (Table 1). Furthermore, the antiserum to human blood serum was able to agglutinate erythrocytes sensitized with the suspension of ether-acetone-washed, trypsin-digested, and broken *M. leprae,* ICRC bacilli and normal human blood serum. The antisera to *M. leprae* and ICRC bacilli absorbed with normal human blood serum failed to agglutinate erythrocytes sensitized to any of the three antigens mentioned above.

Hemagglutination experiments were carried out also with sera of leprosy patients, in order to ascertain if the sera contained antibodies specific for *M. leprae.* Sera were obtained from 30 persons, including cases of both tuberculoid and lepromatous leprosy. Aliquots of erythrocytes sensitized with both trypsin-digested and broken *M. leprae,* trypsin-digested ICRC bacilli, and lepromin, were titrated with serial dilutions of the 30 sera. Sera of the leprosy patients did not agglutinate the erythrocytes sensitized with any of the substances used.

Passive cutaneous anaphylaxis test with the antiserum to ICRC bacilli.—Since the hemagglutination test indicated that antiserum to ICRC bacilli did not contain nonprecipitating antibodies specific for antigens of the bacilli, the passive cutaneous anaphylaxis (PCA) test in guinea-pigs was carried out. It was expected that the test would indicate whether the antisera had at least skin sensitizing antibodies specific for ICRC bacilli as well as *M. leprae.*

The PCA test was carried out as indicated above in the paragraphs on "Materials and Methods." For these experiments 12 guinea-pigs, divided into 3 groups with 4 animals in each, were used. The antisera to *M. leprae,* and to nontrypsinized broken ICRC bacilli and trypsinized broken bacilli, were used in dilutions up to 1:128. Guinea-pigs of

the first group were injected intradermally with seven dilutions of antiserum to trypsin-digested and broken ICRC bacilli. The second and third groups were injected with seven dilutions of the antiserum to nontrypsinized, broken ICRC bacilli, and the antisera to trypsinized and broken lepra bacilli, respectively. The antigens as indicated in Table 2 were mixed with 1 per cent Evans blue solution and injected into the guinea-pigs by the cardiac route. One of the guinea-pigs from the third group was injected with a suspension of trypsinized broken ICRC bacilli and the other with a suspension of nontrypsinized broken ICRC bacilli. The results were recorded after two hours, and localization of the dye at the site of antiserum injection was taken as a positive reaction. The area of the skin reaction showed the intensity of the antigen-antibody reaction. In all groups of animals positive reactions were obtained at the site of injection, as shown in Table 2. The results as observed in the table indicated that all the antisera used contained antibodies which reacted also with normal human blood serum. These results were confirmed by carrying out the reverse passive cutaneous anaphylaxis (RPCA) test according to Ovary ([10]), using the antisera to trypsinized broken and nontrypsinized broken ICRC bacilli and normal human serum. The human serum was diluted up to 1:128. A volume of 0.1 ml. of the undiluted serum and each of the seven dilutions was injected intradermally in the dorsal region of eight animals. Two each of the injected animals were challenged with intracardiac injections of antiserum to trypsinized broken ICRC bacilli and nontrypsinized broken ICRC bacilli before and after absorption with normal human blood serum. The results as indicated in Table 3 confirmed the fact that antisera to trypsinized and nontrypsinized ICRC bacilli contain antibodies reacting with normal blood serum.

As all the PCA reactions were positive in the above experiment, it was inferred once again that the human blood serum contaminant present in the bacillary suspension was responsible for the positive reaction. In order to avoid the interference by blood serum, the test was repeated with antiserum to *M. leprae* and ICRC bacilli absorbed with normal blood serum. The experiment was carried out using both absorbed and unabsorbed antisera. Three groups of guinea-pigs, with four animals in each group, were employed. Each animal received three dilutions of the absorbed sera on one side of the back and three dilutions of the unabsorbed sera on the other side of the back. Antisera to (1) trypsinized broken ICRC bacilli, (2) nontrypsinized broken ICRC bacilli, and (3) broken *M. leprae* were used. Two animals in the third group were injected with identical dilutions of the antiserum to broken lepra bacilli and were given intracardiac injections of a suspension of trypsinized broken ICRC bacilli.

No reaction was observed at the spots where absorbed antiserum to nontrypsinized and trypsinized broken ICRC bacilli, and broken *M. leprae* was used (Table 2), but the reaction was positive on the side where the unabsorbed serum was injected.

TABLE 2.—*Results of passive cutaneous anaphylaxis test using antisera to* M. leprae, *ICRC bacilli and human blood serum with broken* M. leprae, *broken ICRC bacilli and blood serum as antigens.*

Antigens (injected by intracardiac route)	Rabbit antiserum to trypsinized and nontrypsinized (broken) ICRC bacilli								Absorbed rabbit antiserum to trypsinized and nontrypsinized (broken) ICRC bacilli							
	Reaction size in mm.								Reaction size in mm.							
	0	1:2	1:4	1:8	1:16	1:32	1:64	1:128	0	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Trypsinized (broken) ICRC bacilli	8	8	8	7	7	6	6	6	—	—	—	—	—	—	—	—
Nontrypsinized (broken) ICRC bacilli	8	8	7	7	7	6	6	6	—	—	—	—	—	—	—	—

Antigens (injected by intracardiac route)	Rabbit antiserum to broken *M. leprae* obtained from crushed nodules								Absorbed antiserum to broken *M. leprae* obtained from crushed nodules							
	Reaction size in mm.								Reaction size in mm.							
	0	1:2	1:4	1:8	1:16	1:32	1:64	1:128	0	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Trypsinized (broken) ICRC bacilli	6	6	5	5	5	4	4	4	—	—	—	—	—	—	—	—
Nontrypsinized (broken) ICRC bacilli	6	6	6	5	5	4	4	4	—	—	—	—	—	—	—	—

TABLE 3.—*Results of reverse passive cutaneous anaphylaxis test using normal human blood serum and antisera to ICRC bacilli (trypsinized broken and nontrypsinized broken)*

Antisera injected by intracardiac route	Normal human blood serum							
	Reaction size in mm.							
	0	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Antiserum to trypsinized, broken ICRC bacilli	8	7	7	7	6	6	5	5
Absorbed antiserum to trypsinized broken ICRC bacilli	—	—	—	—	—	—	—	—
Antiserum to nontrypsinized, broken ICRC bacilli	8	8	7	7	7	6	6	5
Absorbed antiserum to nontrypsinized broken ICRC bacilli	—	—	—	—	—	—	—	—

These experiments indicated that no skin-sensitizing antibodies specific for either *M. leprae* or ICRC bacilli were present in the sera tested.

Since precipitating, nonprecipitating and skin-sensitizing antibodies were not present in antisera either to *M. leprae* or ICRC bacilli, experiments were carried out to see if the sera contained incomplete antibodies. The presence of incomplete antibodies to ICRC bacilli in the sera of rabbits immunized with ICRC bacilli was tested by using fowl antiserum to rabbit globulin. The ICRC bacilli incubated with antiserum to ICRC bacilli were not agglutinated when the rabbit antiglobulin serum were added.

Experiments were carried out also to determine if sera of leprosy patients have incomplete antibodies. Again the ICRC bacilli were used, in the lack of *M. leprae* for these experiments. Bacilli incubated with the sera of leprosy patients failed to agglutinate when the Coombs serum (rabbit antiserum to human gamma globulin) was added to suspensions of the bacilli.

All the work detailed above indicated that rabbit antiserum to *M. leprae* and ICRC bacilli contained antibodies specific only to components of human blood serum. The sera of leprosy patients, also, failed to show the presence of antibodies specific for *M. leprae,* as revealed by all the tests carried out.

Attempts were made also to find out if tissue-bound antibodies could be demonstrated in guinea-pigs sensitized with *M. leprae* and ICRC bacilli. The experiments carried out with uteri of guinea-pigs sensitized with washed and broken bacilli, have revealed that tissue-bound antibodies also are directed toward components of human blood

serum. The details of these and other experiments carried out with ICRC bacilli grown on a serum-free medium will be reported elsewhere.

DISCUSSION

The results of the Ouchterlony gel diffusion tests, carried out with unbroken *M. leprae* and ICRC bacilli, indicated that when used with incomplete Freund's adjuvant they were capable of producing antibodies in immunized rabbits. It was observed that antisera to *M. leprae* and ICRC bacilli gave three precipitin lines not only with the bacillary suspensions but also with the sera of lepromatous and tuberculoid patients, as well as normal human sera, giving the same number of precipitin lines, which coalesced, indicating identity. These results indicated that antisera obtained by immunizing rabbits with saline-washed *M. leprae* and ICRC bacilli gave rise to antibodies only to components of normal human blood serum. At this stage it seemed possible that rabbits immunized with either of the bacilli developed antibodies to the components of blood serum sticking to the respective bacilli. In the case of *M. leprae* these components would be acquired from the serum present in the human tissue; in the case of ICRC bacilli, these components evidently would be from the serum present in the tissue-culture-modified fluid. The Ouchterlony gel diffusion experiments, carried out with ether-acetone-washed and trypsin-digested *M. leprae* and ICRC bacilli, also revealed that the precipitating antibodies in antisera to both types of bacilli were specific only for normal human blood serum.

The hemagglutination test carried out with the antisera to both saline-washed and ether-acetone-washed and trypsin-digested bacilli also indicated that the sera tested did not contain nonprecipitating antibodies specific for either *M. leprae* or ICRC bacilli. The hemagglutination tests carried out with antisera absorbed with normal blood serum did not agglutinate cells sensitized either with the *M. leprae,* the ICRC bacilli, or the normal and leprosy sera used in the experiments.

The results noted above indicated that washing the bacilli with saline or ether-acetone mixture, or further digesting them with trypsin, did not remove the serum layer sticking to the bacillary cell wall.

Yanagisawa ([15]) observed that trypsin-digested leprosy bacilli were able to give a better skin reaction in sensitized guinea-pigs. He concluded that trypsin digested the nonspecific tissue proteins adhering to the bacilli. The results reported here, however, indicate that trypsin-digested bacilli could not give rise to antibodies for antigens of the bacilli. It was expected that disintegration of the bacilli in the Mickle shaker would release specific antigens that might be intracellular. Antiserum to washed trypsin-digested and disintegrated ICRC bacilli again showed antibodies reacting not only with either of the antigens used for immunization but also with human blood serum.

The passive cutaneous anaphylaxis test using antiserum indicated that the antisera to *M. leprae* and ICRC bacilli did not contain skin-

sensitizing antibodies specific for the respective organisms. Skin-sensitizing antibodies which were formed reacted with normal human serum. Absorption of the antiserum with normal human serum completely removed the ability of the antiserum to give a positive PCA reaction with either *M. leprae* and ICRC bacilli or with normal human serum.

According to Chaussinand (5) immunization with BCG acts as a prophylactic against leprosy. Burrell and Rheins (4) reported the presence of antibodies in leprosy sera which were observed to give precipitin lines with both lepromin and BCG. The results of Ouchterlony experiments reported here have shown, however, that antisera to both *M. leprae* and ICRC bacilli failed to react with intact BCG.

Experiments were carried out also to study the presence of tissue-bound antibodies in antisera to *M. leprae* and ICRC bacilli, by means of the Schultz-Dale technic. This work, conducted in collaboration with K. G. Anantanarayan of the Haffkine Institute, Bombay, has indicated that the tissue-bound antibodies in the immunized guinea-pigs also were found specific against antigens common to human blood serum. Further, ICRC bacilli cultivated on Löwenstein-Jensen medium showed the presence of antigens common to blood serum, although the medium did not contain such antigens. Details of these experiments and others in progress will be reported elsewhere.

The results thus far discussed indicate that immunization with *M. leprae* and ICRC bacilli gives rise in rabbits and guinea-pigs to antibodies reacting only with components of human blood serum. Antigens specific for the bacilli could not be detected by the use of various immunologic technics employed in the detection of precipitating, nonprecipitating, skin-sensitizing, tissue-bound or incomplete antibodies. Both *M. leprae* and ICRC bacilli gave identical results in the immunologic experiments carried out.

SUMMARY

Immunologic investigations were carried out to determine the antigenic composition of *M. leprae* and compare it with that of acid-fast mycobacteria isolated from lepromatous nodules and cultivated *in vitro* (ICRC bacilli). Antisera specific for *M. leprae* and ICRC bacilli were obtained from rabbits immunized with the respective bacilli. The bacilli were washed with a mixture of equal parts of ether and acetone and digested with trypsin in order to remove the serum and tissue proteins sticking to the bacillary cell wall. The bacilli were further disintegrated, so that the intracellular antigens would be available for the immunologic reactions.

The results of the investigations have indicated that immunization with *M. leprae* and ICRC bacilli gives rise in rabbits and guinea-pigs to antibodies reacting only with components of human blood serum. Antigens specific for the bacilli alone could not be detected by using the various immunologic technics employed for detection of precipitat-

ing, nonprecipitating, skin sensitizing, tissue-bound or incomplete antibodies. *M. leprae* and the ICRC bacilli gave identical results in the immunologic experiments.

RESUMEN

Fueron realizadas investigaciones inmunológicas para determinar la composición antigénica del *M. leprae* y compararla con aquellos de la micobacteria acido-resistente aislada de nódulos lepromatosos y cultivados *in vitro* (bacilos ICRC). Fueron obtenidos antisueros específicos para el *M. leprae* y bacilos ICRC de conejos inmunizados con el respectivo bacilo. Los bacilos fueron lavados con una mezcla de partes iguales de éter y acetona y digeridos en tripsina, con el objeto de remover las proteínas del suero y tejidos adheridos a la pared celular bacilar. Los bacilos fueron luego desintegrados, de tal modo que los antígenos intracelulares estuvieran disponsibles para las reacciones inmunológicas.

Los resultados de las investigaciones han indicado que la inmunización con el *M. leprae* y el bacilo ICRC produce en conejos y cobayos anticuerpos que reaccionan solo con los componentes de suero sanguineo humano. No se pudieron descubrir antígenos específicos para el bacilo solo usando varias técnicas inmunologicas empleadas para la detección de anticuerpos precipitantes, no precipitantes, sensibilizantes de la piel, complejos tisulares o incompletos. El *M. leprae* y el bacilo ICRC dieron idénticos resultados en los experimentos inmunológicos.

RESUMÉ

Des études immunologiques ont été menées en vue de déterminer la constitution antigénique de *M. leprae* et de la comparer à celle d'autres mycobactéries acido-résistantes isolées de nodules lépromateux et cultivées *in vitro* (bacille ICRC). Des anti-séra spécifiques pour *M. leprae* et pour le bacille ICRC ont été obtenus chez des lapins immunisés avec les bacilles correspondants. Les bacilles ont été lavés dans un mélange éther-acétone à parties égales et digérés par la trypsine pour débarrasser la membrane cellulaire des bacilles du sérum et des protéines tissulaires qui y adhérent. Les bacilles ont alors été désintégrés, afin d'en libére les antigènes cellulaires destinés à servir aux réactions immunologiques.

Les résultats de ces études out indiqué que l'imunisation avec *M. leprae* et avec les bacilles ICRC provoque chez les lapins et les cobayes le développement d'anticorps qui réagissent seulement avec les constituents du sérum sanguin humain. Par le recours à diverses techniques immunologiques utilisées pour la détection des anticorps, que ceux-ci donnent lieu à des réactions de précipitation ou non, sensibilisent la peau, qu'ils soient liés aux tissus ou incomplets, il n'a pas été possible de mettre en évidence des antigènes spécifiques pour les bacilles seulement. *M. leprae* et les bacilles ICRC donnent des résultats identiques au point de vue de l'experimentation immunologique.

Acknowledgements.—The authors are grateful to Dr. N. Figueredo, Retired Superintendent, Acworth Leprosy Home, Dr. U. Maruthi Rao, former Assistant Director, Medical Services, Madras, Dr. K. Ramanujam, Medical Officer, Government Silver Jubilee Children's Clinic, Saidapet, and Dr. V. Ekambaram for making available the leprosy material employed in these studies, and to Dr. K. S. Ranganathan, Retired Director, B.C.G. Vaccine Laboratory, Madras, for making available the BCG.

The authors thank the Atlas Powder Company, Wilmington, Delaware, U.S.A., for the gift of Arlacel used in the preparation of the incomplete adjuvant. It is a pleasure to acknowledge the help of Miss Katy K. Sadri during the earlier part of the work, and of our colleagues in the Applied Biology Group of the Indian Cancer Research Centre for assistance in culturing and maintaining the bacilli, and of Dr. Satyavati Sirsat for help in making the electron micrographs.

It is a pleasure to acknowledge the help of Dr. Kamal J. Ranadive, Acting Director, Indian Cancer Research Centre for giving all the help needed. Without it the work reported here would not have been possible.

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