AN ATTEMPT TO OBTAIN SPECIFIC PROTEIN ANTIGENS FROM LEPROUS SPLEENS[1]

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Attempts have been made from time to time to prepare, from bacteria believed to be the causative agent of leprosy, a product that could be employed as an antigen in the precipitin or complement fixation test with the serum of leprous patients, or a product analogous to tuberculin which might be used in the diagnosis of infection by tubercle bacilli. In general, the preparations made have been derived from two sources, either acid-fast bacilli isolated in pure culture from the nodules of leprous patients or the leprous nodules directly.

The literature on this subject has been reviewed recently by Wade (13), whose monograph summarizes the various papers on the subject, most of which are now chiefly of historical interest. The interesting lepromin reaction of Mitsuda has been investigated thoroughly by Rodriguez (6), among others, and his article may be consulted for details on that subject.

Among the most recent attempts to secure an antigenic preparation for the skin test was that of Henderson, Aronson and Long (3), who prepared purified proteins from two strains of acid-fast bacilli isolated from leprotic skin nodules, using the method employed by Seibert (10) in the preparation of a product from tubercle bacilli designated TPT. They made intracutaneous tests on 158 patients in the U. S. Marine Hospital, Carville, La., and on 263 nonleprous persons for control, and reported that the reactions were not specific enough for diagnostic use.

A much more elaborate study, involving more than five thousand intradermal tests, was made in a cooperative survey arranged by McKinley in the Philippines (4) in which were used TPT protein products prepared by Henderson from four strains of supposed *M. leprae*, one strain of supposed *M. leprae murium*, several strains of tubercle bacilli of human, bovine and avian types, and

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several strains of saprophytic acid-fast bacilli. The conclusion reached was that in none of the substances employed was there present a specific antigen for a diagnostic skin test for leprosy, and the suggestion was made that the supposed strains of *M. leprae* from which the antigens were prepared were not related specifically and etiologically to leprosy.

It is evident that results comparable to those obtained with tuberculin in people infected with tubercle bacilli, have not been obtained with any preparation used for the diagnosis of leprous infection. This fact has led many, if not most, leprologists to feel that, if it exists at all, sensitiveness in leprosy is of such low degree as to make a test of the tuberculin type impractical. There is certainly more than reasonable doubt as to the justification for using any of the extant cultures of so-called leprosy bacilli for this purpose. It would seem logical, however, to use the acid-fast bacilli that are present in leprous lesions if they could be obtained free from tissue materials, or even if a preparation mixed with such material could be obtained that contains a high concentration of specific substance from these bacilli. Such preparations might be suitable both for intracutaneous and serological tests.

During the course of the studies at Carville to which reference was made above, observation of a large spleen of a patient dying with leprosy suggested the possibility that a specific product could be obtained from this source. The spleen was teeming with acid-fast bacilli, and it appeared hopeful that suitable methods might be devised to obtain protein fractions from them. Experiments conducted by Seibert (8) indicate that the proteins of the tubercle bacillus react in high dilution, although not constantly, with the serum of tuberculous patients, and react regularly in the skin test on such patients. It was thought that thorough grinding of the leprous tissue by the best method available, followed by aqueous extraction and precipitation of proteins, might yield a product containing enough specific bacillary protein to react in one or the other of these tests with leprous patients.

In the spring of 1937 a large spleen from a patient with advanced nodular leprosy was obtained through the courtesy of Dr. Sam Black, of the Carville institution. According to instructions it had been minced and suspended in 0.5 percent phenolized distilled water immediately after the necropsy. Sections and smears showed numerous acid-fast bacilli. After several days of extraction at room temperature the tissue was separated from the supernatant fluid by centrifuging and filtration through paper. The extract was then refiltered through a Mandler candle, and concentrated by ultrafiltration against 11 percent collodion membranes, which permit the pas-

sage of fluid, crystalloids and small molecules of colloidal substances but not the large molecules of whole proteins. After concentration to a small volume the liquid residue was filtered through a sterile Seitz pad. This concentrated watery extract was designated extract "A."

The minced tissue remaining after the extraction was then lyophilized (1) and the dehydrated tissue pulverized in the ball mill at minus 70°C. (5). After two and one-half hours of grinding the temperature in the ball mill was allowed to rise to that of the room, and the resultant fine powder was extracted over night with 0.5 percent phenolized distilled water. The extracted tissue was then removed by filtration and the filtrate refiltered through a sterile Seitz pad. This filtered extract was then divided into two portions, one designated "B", which was bottled and lyophilized, and the other "Ba", which was kept in the liquid state.

A normal human spleen was processed in the same manner and used as a control.

The preparations thus made from the leprous spleen were used as antigens to test for homologous precipitinogens in (*a*) sera from cases of human leprosy, and (*b*) rabbit antisera prepared by immunizing normal rabbits against trichloracetic acid precipitated protein (TPT) fractions obtained from four different strains of so-called *M. leprae* and other acid-fast bacteria. At the same time similar preparations from normal human spleens were tested against the same sera.

The results are summarized in Tables 1, 2, 3 and 4. It will be noted that no reactions took place between the antigens described and rabbit antisera employed. At the same time no reactions occurred between the preparations from normal human spleen and any of the sera from leprosy patients.[3] However, precipitinogens were demonstrated in approximately 50 percent of the thirty-one sera from leprous patients tested with preparations from the leprous spleen.[4] The simple watery extract and the products made by extraction of the finely ground tissue all seemed effective. This result encouraged further trial. It was hoped that improved methods might lead to preparations of greater antigenic power.

A second spleen, larger than the first, was obtained through the courtesy of Dr. Black in October, 1937. Smears and sections of this spleen showed numerous acid-fast bacilli. It was thought that the newer methods devised by Seibert (9) for the preparation of tuberculin protein might permit the preparation of a product from the second spleen as specific as that obtained from the first one but more potent.

[3]See footnotes of Tables 1, 2 and 3.

[4]Dr. M. B. Lara of the Culion Leper Colony, Philippines, kindly forwarded the serum samples used in this series of tests and others to be described later in this paper.

TABLE 1. *Precipitin reactions with extract "A" antigen.*[a]

Sera	Antigen dilutions									Controls		Kahn reaction
	1:2	1:20	1:200	1:400	1:800	1:1,600	1:2,000	1:4,000	1:8,000	Serum	Antigen	
Human leprosy serum # 723	3+	0	0	0	0	0	0	0	0	0	0	0
Human leprosy serum # 469	—	2+	1+	0	0	0	0	0	0	0	0	0
Human leprosy serum # 712	—	0	0	0	0	0	0	0	0	0	0	?
Human leprosy serum # 691	—	1+	0	0	0	0	0	0	0	0	0	+
Human leprosy serum # 580	—	0	0	0	0	0	0	0	0	0	0	+
Human leprosy serum #1169	—	2+	2+	1+	0	0	0	0	0	0	0	0
Human leprosy serum # 584	—	2+	1+	1+	0	0	0	0	0	0	0	+
Human leprosy serum # 649	—	0	0	0	0	0	0	0	0	0	0	0
Human leprosy serum # 642	—	0	0	0	0	0	0	0	0	0	0	±
Human leprosy serum # 636	—	0	0	0	0	0	0	0	0	0	0	±
Human normal serum # 1	0	0	0	0	0	0	0	0	0	0	0	0
Human normal serum # 2	0	0	0	0	0	0	0	0	0	0	0	0
Human normal serum # 3	0	0	0	0	0	0	0	0	0	0	0	+
Human normal serum # 4	0	0	0	0	0	0	0	0	0	0	0	+

[a] All sera of this series were negative when tested with a corresponding extract "A" prepared from normal human spleen.

TABLE 2. *Precipitin reactions with extract "B" antigen.*[a]

Sera	Antigen dilutions									Controls		Kahn reaction
	1:2	1:20	1:200	1:400	1:800	1:1,600	1:2,000	1:4,000	1:8,000	Serum	Antigen	
Human leprosy serum # 631	—	0	0	0	0	0	0	0	0	0	0	+
Human leprosy serum # 271	—	3+	1+	±	0	0	—	—	—	0	0	0
Human leprosy serum # 581	—	0	0	0	—	—	—	—	—	0	0	0
Human leprosy serum # 552	—	3+	2+	1+	1+	0	0	0	—	0	0	+
Human leprosy serum #1167	—	2+	1+	1+	±	0	0	—	—	0	0	±
Human leprosy serum # 746	—	0	0	0	0	0	0	0	0	0	0	+
Human leprosy serum # 706	—	2+	1+	1+	1+	0	0	0	0	0	0	+
Human leprosy serum # 721	—	0	0	0	0	0	0	0	0	0	0	0
Human leprosy serum #1173	—	0	0	0	0	0	0	0	0	0	0	0
Human leprosy serum #1067	—	3+	2+	±	0	—	—	—	—	0	0	±
Human leprosy serum # 650	—	0	0	0	0	0	0	0	0	0	0	0
Human leprosy serum #1171	—	0	0	0	0	0	—	—	—	0	0	?
Human leprosy serum # 653	—	0	0	0	0	—	—	—	—	0	0	+
Human leprosy serum # 987	—	2+	2+	1+	0	—	—	—	—	0	0	?
Human leprosy serum #1166	—	0	0	0	0	0	0	—	—	0	0	0
Human leprosy serum # 707	—	3+	2+	0	0	0	—	—	—	0	0	0
Human leprosy serum # 489	—	3+	2+	1+	1+	1+	0	0	0	0	0	
Human normal serum # 5	—	0	0	0	0	0	0	0	—	0	0	0
Human normal serum # 6	—	0	0	0	0	0	0	—	—	0	0	0
Human normal serum # 7	0	±	0	0	0	0	0	0	0	0	0	0
Human normal serum # 8	0	0	0	0	0	0	0	0	0	0	0	0
Human normal serum # 9	—	0	0	0	0	0	0	0	0	0	0	+
Human normal serum # 10	—	0	0	0	0	0	0	0	0	0	0	+
Human normal serum # 11	0	0	0	0	0	0	0	0	0	0	0	+
Human normal serum # 12	0	0	0	0	0	0	0	0	0	0	0	+

[a] All sera of this series were negative when tested with a corresponding extract "B" prepared from normal human spleen.

TABLE 3. *Precipitin reactions with extract "Ba" antigen.*[a]

Sera	Antigen dilutions									Controls	
	1:2	1:20	1:200	1:400	1:800	1:1,600	1:2,000	1:4,000	1:8,000	Serum	Antigen
Human leprosy serum #1010	—	2+	1+	0	0	0	0	0	0	0	0
Human leprosy serum # 598	—	0	0	0	0	—	—	—	—	0	0
Human leprosy serum # 467	—	2+	2+	0	0	0	—	—	—	0	0
Human leprosy serum # 708	—	0	0	0	0	0	—	—	—	0	0
Human normal serum # 13	—	0	0	0	0	0	0	0	—	0	0
Human normal serum # 14	—	0	0	0	0	0	0	0	—	0	0
Human normal serum, Kahn +	—	0	0	0	0	0	0	0	—	0	0
Human normal serum, Kahn +	—	0	0	0	0	0	0	0	—	0	0

[a] All sera of this series were negative when tested with a corresponding "Ba" prepared from normal human spleen.

TABLE 4. *Precipitin reaction with extract "A" antigen and rabbit antisera to proteins from various strains of acid-fast bacilli.*

Rabbit antisera immune to protein (TPT) from	Antigen dilutions									Controls	
	1:2	1:20	1:200	1:400	1:800	1:1,600	1:2,000	1:4,000	1:8,000	Serum	Antigen
Human tubercle bacillus (H37)	0	0	0	0	0	0	0	0	0	0	0
Leprosy bacillus (Daines)	0	0	0	0	0	0	0	0	0	0	0
Leprosy bacillus (Aronson & Henderson)	0	0	0	0	0	0	0	0	0	0	0
Leprosy bacillus (Levi-Kedrowski)	0	0	0	0	0	0	0	0	0	0	0
Leprosy bacillus (Duval nonchromogenic)	0	0	0	0	0	0	0	0	0	0	0
M. thamnopheous (Aronson)	0	0	0	0	0	0	0	0	0	0	0

In this case the watery extract and the minced spleen were lyophilized and ground four and one-half hours in the ball mill at minus 70°C. Since results with tubercle bacilli have shown the efficacy of extraction in a slightly alkaline medium, after the temperature in the ball mill had again reached that of the room the pulverized tissue was extracted with phosphate buffer at pH 7.2. Phenol, 0.25 percent, was added for preservation. The buffered extract was then precipitated with 10 percent trichloracetic acid, as in the preparation of tuberculin protein, and the precipitate was repeatedly washed with ether. The final yield was a light brown powder which, it was hoped, would prove to contain, in addition to protein of the human spleen, specific protein from the acid-fast bacilli which it had contained.

As in the previous experiment a normal human spleen was treated in the same manner and the resulting product was used as a control material.

Precipitin and complement fixation tests were carried out with thirty-two sera from cases of leprosy. Precipitin tests were made in antigen dilutions ranging from 1:2 to 1:8000. In no case did a positive precipitin reaction occur. Unfortunately, presumably because of their age, the sera were all anticomplementary and complement fixation tests could not be made.

Naturally this disappointing result led to the question if the preparation from the leprous spleen contained any substance at

all that was not present in the corresponding preparation from the normal spleen. This question could best be answered by serological experiments based on the technic of precipitinogen absorption. Accordingly a series of rabbits were immunized to the leprosy spleen proteins and another series to the protein prepared from normal spleens. In each case potent antisera were obtained. Each reacted homologously with their respective antigens in antigen-dilutions of 1:100,000 (Table 5), and each also reacted heterologously in the same dilution with the other antigen.

TABLE 5. *Precipitin reactions with rabbit antisera and leprous and normal spleen antigens.*

Rabbit antiserum immune to	Leprous spleen antigen. dilutions										Controls	
	1:200	1:500	1:1,000	1:2,000	1:4,000	1:10,000	1:20,000	1:40,000	1:100,000	1:200,000	Serum	Antigen
Extract "A" of leprous spleen #1	3+	3+	3+	2+	2+	2+	2+	1+	1+	0	0	0
Phosphate buffer extract of leprous spleen #2	4+	3+	3+	2+	2+	2+	2+	1+	1+	0	0	0
Extract "A" of normal spleen #1	3+	2+	3+	2+	2+	2+	2+	1+	1+	0	0	0
Phosphate buffer extract of normal spleen #2	3+	3+	3+	2+	2+	2+	2+	1+	1+	0	0	0
	Normal spleen antigen											
Extract "A" of leprous spleen #1	3+	3+	3+	2+	2+	1+	2+	1+	1+	0	0	0
Phosphate buffer extract of leprous spleen #2	3+	3+	3+	3+	2+	2+	2+	1+	1+	0	0	0
Extract "A" of normal spleen #1	4+	3+	3+	2+	2+	2+	2+	2+	1+	0	0	0
Phosphate buffer extract of normal spleen #2	3+	3+	3+	2+	2+	2+	1+	1+	1+	0	0	0

In order to determine if the leprous spleen preparation contained a protein additional to that contained in the normal spleen protein preparation, absorption experiments were carried out as follows: The antiserum to the leprous spleen protein preparation was allowed to react with the normal spleen protein antigen for 12 hours at 37°C. in the antigen-antiserum concentration of the equivalence point, viz., one part of antigen to 35,000 parts of antiserum. The resulting precipitate was removed by centrifuging, and leprous spleen antigen was added to the supernatant antiserum. No further precipitation occurred, a fact apparently indicating the absence of specific bacillary protein in the leprosy spleen antigen preparation (Table 6).

Since, however, the substances concerned in the serological

tests and the allergic skin reaction are different, the latter being of haptene character, it was thought that an antigen that would react in the skin test might be present. It is known that tuberculoprotein below a certain molecular size is active in the skin test, but not in serological tests with tuberculous patients (Seibert [11]). Accordingly, arrangements were made for a skin testing program on groups of lepers and nonlepers. A cooperative enterprise was planned with Dr. E. B. McKinley, but this was abruptly ended by the disaster to the "Hawaiian Clipper" on which he was travelling to the Philippines. Later the projected survey was made possible through the cooperation of Dr. H. W. Wade, of Culion, to whom the materials prepared from the last named spleen preparation were sent.[5]

TABLE 6. *Precipitin reactions after absorption.*

Rabbit sera after treatment with leprous antigen	Leprous spleen antigen, dilutions										Controls	
	1:20	1:200	1:500	1:1,000	1:2,000	1:4,000	1:10,000	1:20,000	1:40,000	1:100,000	Serum	Antigen
Antiserum to protein from leprous spleen #1	±	0	0	0	0	0	0	0	—	—	0	0
Antiserum to protein from leprous spleen #2	0	0	0	0	0	0	0	0	—	—	0	0
Antiserum to protein from normal spleen #1	0	0	0	0	0	0	0	0	—	—	0	0
Antiserum to protein from normal spleen #2	0	0	0	0	0	0	0	0	—	—	0	0
Rabbit sera after treatment with normal spleen antigen	Normal spleen antigen											
Antiserum to protein from leprous spleen #1	±	0	0	0	0	0	0	0	—	—	0	0
Antiserum to protein from leprous spleen #2	0	0	0	0	0	0	0	0	—	—	0	0
Antiserum to protein from normal spleen #1	0	0	0	0	0	0	0	0	—	—	0	0
Antiserum to protein from normal spleen #2	0	0	0	0	0	0	0	0	—	—	0	0

The material supplied to this committee consisted of three preparations, as follows:

A. Protein from normal human spleen, 5 mgm. per cc.

B. Protein from a human leprous spleen (No. 2), prepared by trichloracetic acid precipitation of the concentrated buffered extract as in the preparation of the tuberculin product TPT, 5 mgm. per cc.

[5] These tests were carried out on authorization of Dr. E. D. Aguilar, Director of Health of the Commonwealth of the Philippines, by the same committee which had worked with Dr. McKinley on the previous occasion, composed of Drs. S. Chiyuto, chairman, C. B. Lara, W. de Leon, C. Manalang, J. N. Rodriguez, F. Velasco and H. W. Wade. The report of this committee appears as the preceding article in this issue.

C. Protein from a human leprous spleen (No. 2), prepared through simple concentration by ultrafiltration without trichloracetic acid precipitation, as in the preparation of the tuberculin product TPU (Seibert [1]), 5 mgm. per cc.

As will be noted in the report by the committee, "range-finding" tests carried out at the San Lazaro Hospital led to the selection of a dose of 0.1 cc. of a 1:10 dilution of these products. Readings were made after 24 and 48 hours. At the close of the tests it was apparent that no indication of a specific reaction was found with either of the two antigens B and C. Their behavior in the skin of lepers and nonleprous persons was not significantly different from that of the control preparation A. It is noteworthy that general reactions occurred in a few patients, but they were approximately as frequent and as strong after injection of the control normal spleen preparation as with the leprous spleen products.

This result indicated the unlikelihood of successful preparation of suitable antigenic material by the type of method used. However, while arrangements for the skin tests referred to were being made a third spleen was received from Dr. Black, in December, 1938. Smears and sections showed numerous acid-fast bacilli, although not as many as in the previous spleens. Since steady improvements had been made in the preparation of antigenic materials from the tubercle bacillus, it seemed wise to take advantage of possession of this final material rich in bacilli. This spleen was treated initially in the same way as the first, and the further preparation was carried out by methods with which it was hoped very little or no denaturization of the protein would occur, as in the present preparation of the purified protein derivative of tuberculin. The emulsified watery extract and spleen tissue were divided into two fractions, called 3A and 3B.

Fraction 3A was prepared by a method analogous to that used in making tuberculin, by mixing six liters of the emulsion with an equal amount of Long's synthetic medium of twice the normal concentration. The mixture was adjusted to pH 7.1 and heated in the Arnold sterilizer for three hours at 97°C. The tissue was removed from the extract by filtration, dried in the chryochem apparatus (2), and pulverized in the ball mill at minus 70°C. for four and one-half hours. The ground mass was then brought to room temperature and extracted overnight with 0.5 percent phenolized distilled water. This extract was filtered through a Seitz pad to remove tissue and bacterial bodies, 100 cc. of glycerine per liter was added, and the glycerinated extract was concentrated over a steam bath to one-fifth of the original volume. This concentrate was ultrafiltered against 11 percent collodion membranes, vialed and "chryochemed."

Fraction 3B was made from six liters of the leprosy spleen emulsion by filtering to remove the tissue, which was dried in the chryochem ap-

paratus and ground in the ball mill at minus 70°C. for four and one-half hours. The pulverized mass was then brought to room temperature and extracted over night with 0.5 percent phenolized distilled water. This extract was combined with the original watery extract and then filtered, first through paper and then a Seitz pad, and concentrated by ultrafiltration against collodion membranes. The concentrate was washed with distilled water to remove the phenol, filtered through a Seitz pad and "chryochemed."

Another normal human spleen was prepared in the same manner as this third spleen and used for control.

As in the case of the second leprosy spleen, precipitin reactions were run with serum from leprous patients. Again the presence of precipitinogens was not demonstrated in any case. A final small series of skin tests, however, is contemplated.

For study by the absorption technique a series of rabbits were immunized to these new leprous spleen proteins (fractions 3A and 3B) and another series at the same time to the fractions prepared from the normal spleen. In each case potent antisera were obtained, reacting homologously with their respective antigens in antigen dilutions of 1:100,000. In this case each serum reacted heterologously to the other antigen in antigen dilutions of 1:40,000. As can readily be seen in Table 7, normal spleen protein reacted strongly both with antiserum to normal spleen fractions 3A and 3B, and antiserum to leprosy spleen fractions 3A and 3B, and the leprosy spleen protein reacted strongly with both antiserum to normal spleen fractions 3A and 3B, and antiserum to leprosy spleen fractions 3A and 3B.

TABLE 7. *Precipitin reactions with rabbit antiserum and spleen fractions "BA" and "3B".*

Rabbit antisera immune to	Antigen	Antigen dilution											Controls	
		1:50	1:100	1:200	1:500	1:1,000	1:2,000	1:4,000	1:10,000	1:20,000	1:40,000	1:100,000	Serum	Antigen
Leprous spleen fraction 3A	Leprous spleen fraction 3A	3+	3+	2+	2+	2+	1+	1+	1+	1+	1+	1+	0	0
Normal spleen fraction 3A	Leprous spleen fraction 3A	3+	2+	—	2+	2+	2+	2+	1+	1+	±	0	0	0
Leprous spleen fraction 3B	Normal spleen fraction 3B	3+	3+	2+	2+	2+	2+	2+	1+	1+	±	0	0	0
Normal spleen fraction 3B	Normal spleen fraction 3B	2+	3+	3+	2+	2+	2+	2+	2+	2+	1+	±	0	0

It was decided to absorb the leprosy spleen protein with normal spleen antiserum and see if, after absorption, any reacting substance remained which would react with leprosy spleen antiserum. The results, shown in Table 8, indicated that the ab-

sorption was complete. Therefore, obviously, no specific protein could be detected in the leprosy spleen by serological methods.

As a final check, electrophorectic studies of the normal and leprous spleen preparations, 3A and 3B, as well as of fractions prepared similarly from extracts of the L1 strain of leprosy bacillus (designated L1-TPU for the unheated preparation) were undertaken to determine if any substance peculiar to leprosy spleen tissue could be detected by this method. The Tiselius electrophoresis apparatus (12) was used for this purpose, and constant conditions were maintained for all of the experiments. The current was kept constant at 3 milliamperes, all solutions were made in phosphate buffer of pH 8.0 and ionic strength $\mu = 0.02$, and comparable solutions were of equal concentration. The number of black bands appearing on the photographic screen indicated the number of different components present. It was noteworthy that the L1-TPU was a very complex solution, exhibiting seven components.

TABLE 8. *Precipitin reactions after absorption, spleen fractions "3A."*

Antigen	Sera	Antigen dilutions											Controls	
		1:50	1:100	1:200	1:500	1:1,000	1:2,000	1:5,000	1:10,000	1:20,000	1:59,999	1:100,000	Serum	Antigen
Leprous spleen fraction 3A	Leprous spleen fraction 3A	3+	4+	3+	3+	2+	3+	2+	2+	2+	1+	1+	0	0
Leprous spleen fraction 3A with normal spleen fraction 3A	Leprous spleen fraction 3A	±	0	0	0	0	0	0	0	0	0	0	0	0

The mobilities recorded in Table 9 are for the components ascending toward the anode. It must be understood that in complex mixtures of this nature these values may not be very accurate, especially in the ascending column of solution. However, in a survey study such as this one, determinations of the number of components and their relative strengths are of greater significance.

The outstanding facts revealed by the data in Table 9 are as follows: (*a*) certain components were found in both the unheated normal and leprosy spleen extracts (3B) which were not found in bacillary extracts (L1-TPU) and which may be tissue proteins; (*b*) in the fractions 3A which had been heated the number of components was reduced from six or seven to two or three; undoubtedly

the coagulable proteins had been removed; (*c*) an extra slower and weak component (A′′) split off from the A component in the bacillary (L1-TPU) extract, as well as in both heated and unheated leprosy spleen extracts (3A and 3B), but not in either heated or unheated normal spleen extracts (3A and 3B).

TABLE 9. *Electrophoretic mobilities in cm² volt⁻¹ sec⁻¹.*

Component	L1-TPU	Unheated extracts 3B		Heated extracts 3A	
		Normal spleen	Leprous spleen	Normal spleen	Leprous spleen
A	−12.9(s)	−14.7(s)	−11.4(s)	−12.71(s)	−16.1(s)
A′	−15.3	−16.8(w)	−15.9(m)		−
A′′	−10.6(w)		−10.9		−10.7(w)
B	− 6.5(s)	−12.5(w)	−10.0(s)		
B′	−10.0				
B′′	−10.6				
C		−12.4(w)	− 9.1		
C′		− 1.6	− 7.7		
D		− 1.5	− 2.2		
E	+ 1.9	+ 1.0	+ 1.2(s)	0	0
Concentration	1.69%	1.54%	1.63%	0.65%	0.65%

(s) = a strong component; (m) = a moderately strong component; (w) = a weak component.

This last fact may perhaps be significant. It suggests that chemical methods might be successful in detecting a trace of a substance where serological methods have failed in discovering differences between the normal and diseased spleens. Obviously, however, the additional element responsible for the extra band could be the result of chemical changes in the splenic tissue rather than of a substance derived directly from the acid-fast bacilli present. This problem is being investigated. It seems apparent, however, that leprosy spleens, although exceedingly rich in acid-fast bacilli, cannot be made to yield specific protein reacting in either the usual serological tests or in the skin test, by the best methods available for grinding and extraction.

SUMMARY

The Seibert techniques for the preparation of specific proteins of the tubercle bacillus were applied to spleens rich in acid-fast bacilli, from leprosy patients, in the hope that there could be obtained specific proteins of the microorganism of leprosy which could be used in serological or skin tests for the diagnosis of leprosy.

Preparations were made from three such spleens. In the case of the first one the organ was ground, the minced tissue was extracted with water, and the aqueous extract was concentrated by ultrafiltration. A corresponding preparation was made from a

normal spleen. The concentrated extract from the leprosy spleen reacted with 14 of 31 leprosy sera in the precipitin test, but in none of 14 sera from healthy persons or 2 from Kahn-positive patients in Philadelphia. The preparation from normal spleen did not react with any of the sera. The preparation from the leprous spleen, which appeared to act as an antigen with the 14 leprosy sera, did not react with rabbit antiserum to proteins from various strains of supposed *M. leprae*.

In the case of the second spleen, what was believed to be a superior method of grinding, extraction and protein purification was used. Extraction was carried out with faintly alkaline buffered phosphate solution, more thorough grinding was employed, and the trichloracetic acid method was used to precipitate the proteins. The refined proteins thus obtained were used as antigens in the precipitin test with the sera of 32 leprous patients, but in no case did a positive reaction occur. It proved impossible in this case to demonstrate the presence of protein other than that derived from the spleen used, for after rabbit antiserum immune to the leprosy spleen protein was saturated with a corresponding protein from a normal spleen, the addition of leprosy spleen protein caused no further precipitate.

It was hoped, however, that a protein comparable to tuberculin protein, reactive in the skin test, might be present. A cooperative investigation of this question was carried out by a committee in Manila, the report of which appears in the preceding article. No evidence for the presence of an antigen reacting specifically in the skin of lepers was obtained.

Preparations were made from a third leprous spleen by methods believed to combine any advantages of the methods employed in making preparations from the first two. In this case, also, no reaction could be obtained between any of several protein preparations made and the sera of leprous patients, nor could a specific protein be detected in the leprosy spleen preparations by the serological absorption technique. However, examination by physicochemical methods in the Tiselius electrophoresis apparatus revealed the presence of some substance not found in preparations from normal spleen. This substance is being further investigated.

It seems clear that the methods used do not result in the preparation from leprous spleens of antigens suitable for precipitin or skin tests for the diagnosis of leprosy.

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