

STUDIES ON THE SEROLOGY OF LEPROSY
THE COMPLEMENT-FIXATION REACTION
BY A MODIFIED ANTIGEN

THE HANDAI METHOD (HONDA)

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INTRODUCTION

Eitner (1906) first reported on serum reactions in leprosy with an emulsion of the leprosy node as the antigen, and later (1909) Sugai in Japan made a similar study. Since then many workers have published on serum reactions with various antigens. Their reports, however, do not cover long-term observations, and subsequent verification of their findings is lacking.

Tamiya (19) presented a noteworthy paper on a refrigerator method of the Wassermann reaction, which has made possible differentiation between leprosy and syphilis by group hemolytic reactions.

Sakakibara (14-18) conducted complement-fixation studies on the specificity of lecithin and cephalin in leprosy and syphilis, finding that the latter has specificity for leprosy. The addition of cholesterol in a suitable amount accelerated the reactions, but it made them nonspecific when added in excess.

Ichihara (8, 9) reported that an antigen prepared from an acid-fast bacillus derived from a rat leprosy node showed specificity for leprosy when supplemented by cephalin.

Yoshinaga (20-25) emphasized the specificity of cephalin for leprosy serum.

Following the introduction of the cardiolipin method by Pangborn in 1941, Honda and Yoshino (4-7) used it for studying leprosy, the antigen containing cardiolipin, cholesterol and cephalin in a 1:15:20 ratio. Positive complement-fixation reactions were obtained in 30 of 51 lepromatous cases (75%), 2 of 3 tuberculoid cases (67%), and 7 of 28 neural cases (25%).

Later, Ogata and associates (10, 11) made agglutination reactions with an antigen ratio of 1:1 cardiolipin and lecithin, and obtained positive results in 95 per cent of lepromatous cases, 100 per cent of tuberculoid cases, and 80 per cent of neural cases, but only 15 per cent in syphilis. This experiment was investigated by Higuchi and Minami, who stated in a paper presented at the Leprosy Section of the 14th Japan Medical Congress (1955) that the 1:1 ratio had a considerable rate of positivity for leprosy, but was negative in many early (macular) cases.

At the same meeting the present authors reported that a cephalin antigen was far superior to that of Ogata as regards specificity and sensitivity with leprosy serum. We have made studies on the antigen ratio in complement-fixation tests since 1951, and the results are presented here.

BASIC EXPERIMENTS

Cephalin was successfully purified by Folch (1-3) into the three fractions, phosphatidyl-ethanolamine, diphospho-inositide, and phosphatidyl-serine. In the present experiment, these three substances were studied quantitatively in relation to cardiolipin and cholesterol for the purpose of obtaining an antigen of greater sensitivity and specificity than those previously employed.

Materials.—The *stock solutions* used in this work¹ were: each of the three cephalin fractions, 1 per cent in ether; cardiolipin, 0.2 per cent in alcohol; cholesterol, 1 per cent in alcohol; and 0.1 per cent kaolin suspension.

Procedure.—The complement-fixation method used was based on the antibody-reduction method. The primary reaction was allowed to take place in an ice-box for 1½ hours, the secondary reaction in an incubator at 37°C for 1 hour. The inactivated serum was diluted to 1:4 or more. The complement was obtained from 5 guinea-pigs the day before the tests; 2 MHD units determined in a preliminary measurement were used. Fresh, washed bovine blood cells were made into a 3 per cent sensitized suspension.

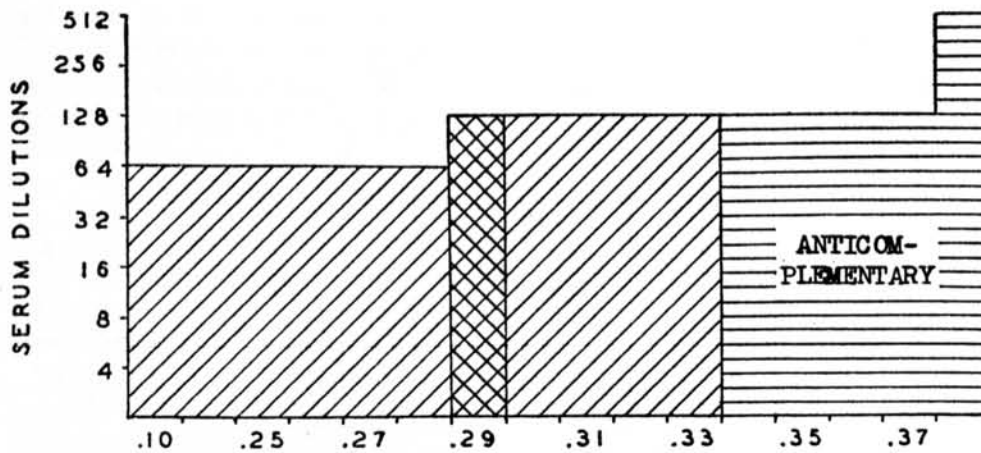
Cephalin fractions.—(1) Phosphatidyl-ethanolamine concentration: Units were made up from the stock solutions as follows: cardiolipin, 0.1 cc.; cholesterol, 0.1 cc.; diphospho-inositide, 0.1 cc.; and phosphatidyl-serine, 0.01 cc. The phosphatidyl-ethanolamine solution was added in quantities ranging from 0.1 cc. to 0.38 cc. To each of these units, absolute alcohol was added to make 1 cc., to which amount 19 cc. of 0.85 per cent saline solution and 5 cc. of the kaolin suspension were added. After standing for 30 minutes at room temperature, another 25 cc. of saline was added. Rapid mixing is required in preparing the diluted antigen solution.

Of this preparation, 0.5 cc. was added to 0.5 cc. aliquots of serially diluted serum, and then 2 MHD units of complement. These mixtures were shaken well and kept in an ice-box for 1½ hours. Next, as the secondary reaction, 0.5 cc. of the sensitized bovine-cell suspension was added and the reaction mixture was kept at 37°C for 1 hour for determination of hemolysis.

When more than 0.35 cc. of the phosphatidyl-ethanolamine solution was used, an anticomplementary action was observed. High sensitivity was maintained at 0.29-0.34 cc. (Text-fig. 1). The minimal amount of 0.29 cc. was selected for further use.

¹ Supplied by the Sumitomo Chemical Co. Ltd., Osaka Works.

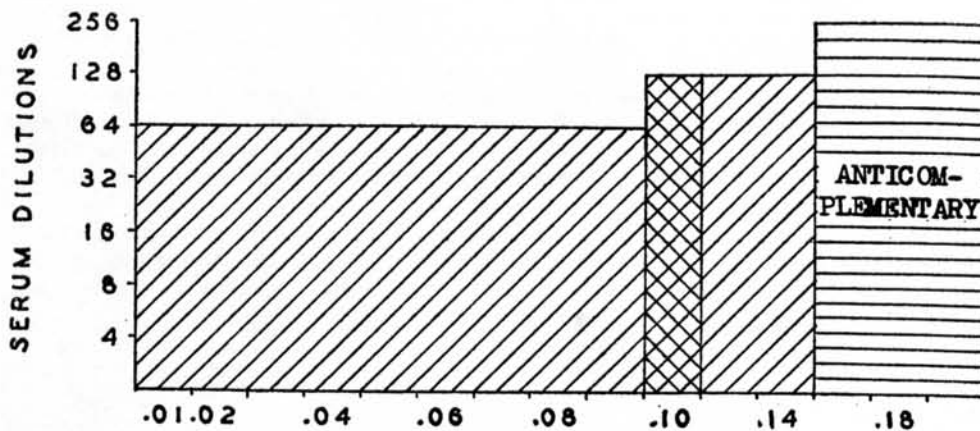
Pooled sera of lepromatous inpatients at the Kyoto University Hospital were employed.



TEXT-FIG. 1. Quantitative determinations of the phosphatidyl-ethanolamine fraction of cephalin with pooled serum of lepromatous cases, quantities of the 1 per cent stock solution as shown.

Other ingredients of each tube: cardiolipin (0.2%) 0.1 cc., cholesterol (1%) 0.1 cc., diphospho-inositide (1%) 0.1 cc., phosphatidyl-serine (1%) 0.01 cc.

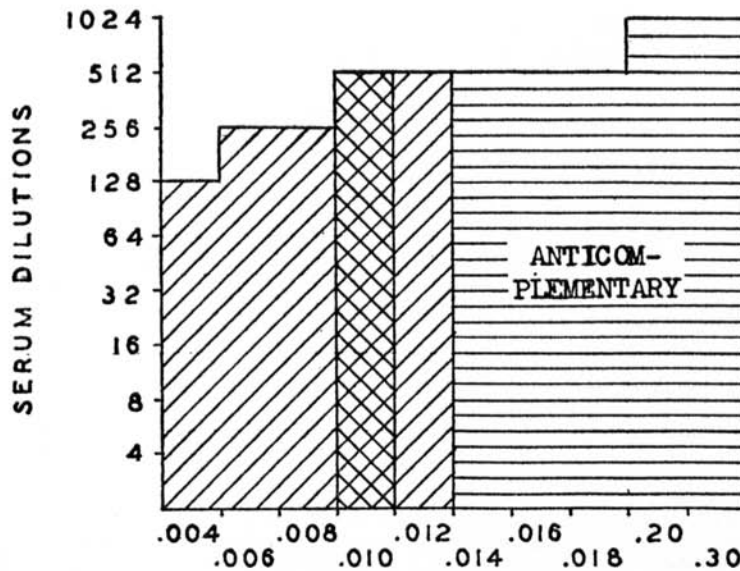
(2) Diphospho-inositide concentration: In this test the procedure and the several ingredients were as before, except that we used the selected dose of 0.29 cc. of the phosphatidyl-ethanolamine solution, and the quantities of the diphospho-inositide solution ranged from 0.02 to 0.2 cc. Anticomplementary action was observed with 0.16 cc. or more of the diphospho-inositide; the highest sensitivity was given by 0.1-0.14 cc. (Text-fig. 2). The minimal amount, 0.1 cc. was selected as the suitable dose.



TEXT-FIG. 2. Quantitative determination of the diphospho-inositide fraction of cephalin with pooled serum of lepromatous cases, quantities of the 1 per cent stock solution as shown.

Other ingredients: As stated in the legend of Text-fig. 1, except that 0.29 cc. of the phosphatidyl-ethanolamine solution was used.

(3) Phosphatidyl-serine concentration: Using the phosphatidyl-ethanolamine and diphospho-inositide solutions in the determined dosages, and other ingredients as before, units were made up containing the phosphatidyl-serine solution in quantities ranging from 0.004 to 0.03 cc. Anticomplementary action was observed with more than 0.014 cc.; the highest sensitivity was seen with 0.01-0.012 cc. (Text-fig. 3). The smallest quantity, 0.01 cc., was chosen as the optimal amount.



TEXT-FIG. 3. Quantitative determination of the phosphatidyl-serine fraction of cephalin with pooled serum of lepromatous cases, quantities of the 1 per cent stock solution as shown.

Other ingredients: As stated in the legend of Text-fig. 1 except 0.29 cc. of the phosphatidyl-ethanolamine and 0.1 cc. of the diphospho-inositide stock solutions.

The above results show that the most appropriate composition of the cephalin fractions is phosphatidyl-ethanolamine: diphospho-inositide: phosphatidyl-serine in a ratio of 29:10:1.

Antigenicity of individual cephalin fractions and related preparations.—In these experiments eight antigen preparations were used—the three single cephalin fractions, the cephalin mixture under various conditions, and a combination of the three substances, as listed below. The test serum was a pool of specimens from 5 leprosy cases of the lepromatous type.

(a) Phosphatidyl-ethanolamine (1%), 0.4 cc., added to 0.6 cc. of absolute alcohol. This antigen solution was diluted, and kaolin was added before use.

(b) Diphospho-inositide (1%), 0.4 cc., added to 0.6 cc. alcohol; used as in (a).

(c) Phosphatidyl-serine (1%), 0.4 cc., added to 0.6 cc. of alcohol; used as above.

(d) The three cephalin fractions in the proportions determined in the previous experiments (phosphatidyl-ethanolamine 0.29 cc., diphospho-inositide 0.1 cc., and phosphatidyl-serine 0.01 cc., total 0.4 cc.), with 0.4 cc. alcohol added. The solution was diluted and kaolin was added before use.

(e) As in (d), ether being used in place of alcohol.

(f) As in (d), kaolin not added.

(g) The mixture of cephalin fractions used in (d), plus 0.1 cc. of each of the cardiolipin and cholesterol stock solutions, and 0.4 cc. alcohol. Dilution and additions of kaolin as before.

Results: The results of this experiment, given in Table 1, show that the individual fractions of cephalin possessed no antigenic ability except phosphatidyl-serine (c), which had a little.

TABLE 1.—Antigenicity of various antigens.^a

Antigen	Serum dilution									Antigen control	Serum control
	4	8	16	32	64	128	256	512	1024		
A. Ph.-ethanolamine (alcohol, kaolin)	3	3	3	3	3	3	3	3	3	3	3
B. Ph.-inositide (alcohol, kaolin)	3	3	3	3	3	3	3	3	3	3	3
C. Ph.-serine (alcohol, kaolin)	2	2	2	3	3	3	3	3	3	3	3
D. Cephalin mixture (alcohol, kaolin)	0	0	0	0	1	3	3	3	3	3	3
E. Cephalin mixture (ether, kaolin)	0	0	0	0	0	0	0	0	0	2	3
F. Cephalin mixture (alcohol)	3	3	3	3	3	3	3	3	3	3	3
G. Cephalin, cardio- lipin, cholesterol (alcohol, kaolin)	0	0	0	0	1	1	1	1	1	3	3

^a 0=no hemolysis, 1=25-40% hemolysis, 2=65% hemolysis, 3=80-100% hemolysis.

The mixture of cephalin with alcohol and kaolin (d) gave a positive result up to the 1:64 dilution; but when no alcohol was used (e) the antigen was anticomplementary, and when kaolin had not been added (f) antigenicity was absent.

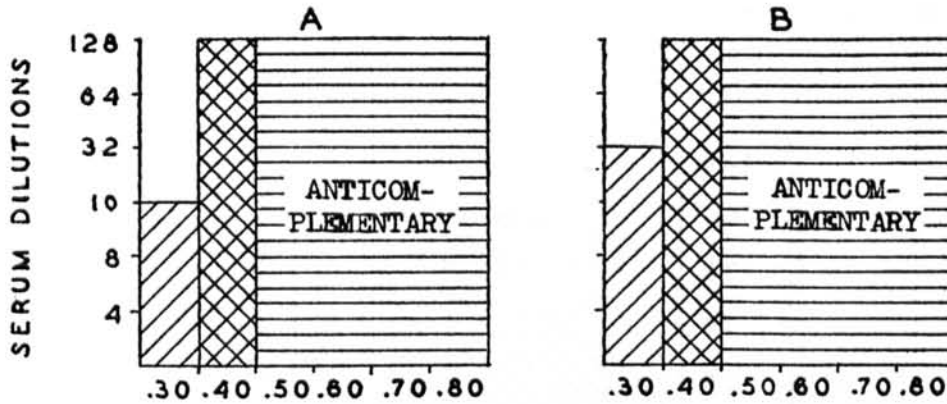
The complete antigen (g) containing cardiolipin and cholesterol, exhibited strong antigenic ability, with a positive reaction even at 1:1024 dilution. It seemed logical to continue the investigation on the basis of that combination.

Concentration of the cephalin fraction mixture.—Using a 1 per cent mixture of the cephalin fractions in the 29:10:1 ratio, an experiment was performed to ascertain the optimal dosage of that mixture with fixed amounts of cardiolipin and cholesterol. Each unit contained 0.1 cc. of each of the stock solutions of those substances, the amounts of the cephalin mixture ranging from 0.1 to 0.8 cc. Two lots were tested, one with and the other without kaolin added when the antigen was diluted.

In the lot with kaolin there was an anticomplement effect with more than 0.5 cc. of the cephalin. The strongest reactions were in the units up to the 1:128 serum dilution when 0.4 cc. cephalin was employed.

In the lot without kaolin, the anticomplement effect occurred, as before, with more than 0.5 cc. The strongest reaction was with the 0.4 cc. dose in the 1:64 serum dilution, but the sensitivity was less than when kaolin was added.

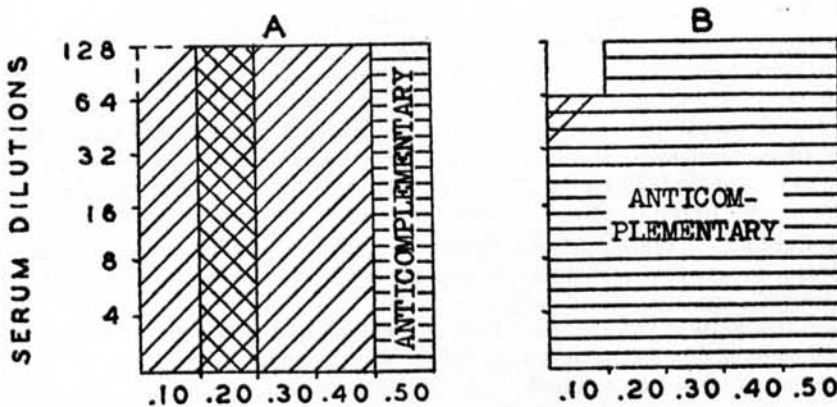
Thus, it was concluded that the antigen containing 0.4 cc. of the cephalin mixture in 1 cc. of the antigen solution, with kaolin added, is the most satisfactory one.



TEXT-FIG. 4. Effects of different doses of the cephalin stock solution, as shown, with pooled lepromatous serum. A. Kaolin added when the antigen was diluted. B. Kaolin not added. (With 0.1 and 0.2 cc. of the cephalin solution, not shown, the results were negative.)

Other ingredients: The cardiolipin and cholesterol stock solutions, 0.1 cc. each.

Cholesterol concentration.—For this determination each 1 cc. of the antigen solution containing 0.4 cc. of the cephalin mixture as determined in the previous tests, 0.1 cc. of the cardiolipin solution, and a range of 0.1-0.5 cc. of the cholesterol solution. As before, a lot with kaolin was compared with one without it. In the former lot anticomplementary action occurred with 0.5 cc. of the cholesterol, strong reactions with 0.2-0.4 cc. The minimal amount, 0.2 cc., was thus found to be appropriate. In the latter lot, the reactions were weaker, and there was always an anticomplement effect. This antigen was therefore considered unsuitable.

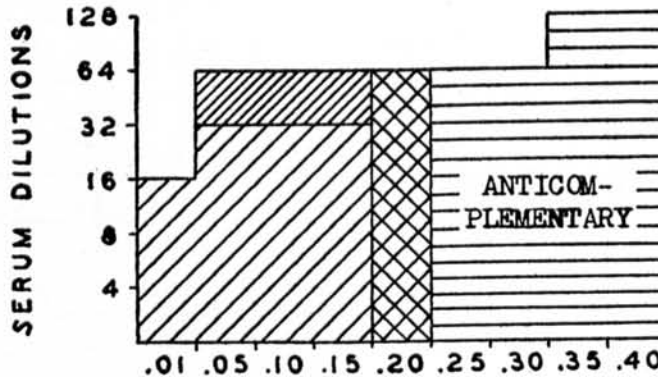


TEXT-FIG. 5. Effects of different doses of the cholesterol stock solution, as shown, with pooled lepromatous serum. A. Kaolin added when the antigen was diluted. B. Kaolin not added.

Other ingredients: The cardiolipin stock solution 0.1 cc., and the cephalin solution 0.4 cc.

Cardiolipin concentration.—In this determination the optimal quantities of the cephalin and cholesterol (0.4 cc. and 0.2 cc., resp.) were constant, while the cardio-

lipin for complement fixation ranged from 0.01 to 0.4 cc. Kaolin supplementation was used, because of the previous findings. It was found that when more than 0.35 cc. of cardioliipin was employed, anticomplement action developed. The strongest reaction occurred at 0.2-0.3 cc., and so the minimal amount, 0.2 cc., was adopted.



TEXT-FIG. 6. Effects of different doses of the cardioliipin stock solution, as shown, with pooled lepromatous serum.

Other ingredients: The cholesterol stock solution 0.2 cc., and the cephalin solution 0.4 cc.

Nonspecific reactions.—It was believed that the antigen employed in these experiments was highly sensitive with leprosy serum. To ascertain whether or not antigens of the same composition might give group hemolytic reactions with non-leprosy sera, three pooled sera from 10 hospitalized tuberculous cases, 5 cases of syphilis, and 5 normal subjects were tested. The syphilis cases selected had shown strong positive Wassermann reactions but had not returned to negative by Tamiya's method. The serum dilutions ranged from 4 to 128.

(1) Cephalin concentration: In the antigen used in this determination the doses of cardioliipin and cholesterol were constant (0.2 cc. of each per cc.), while the cephalin mixture was used in amounts ranging from 0.1 to 0.6 cc. Negative reactions were obtained with all three pooled sera, tuberculous, syphilitic and normal.

(2) Cholesterol concentration: In this instance the cephalin and cardioliipin components were constant (0.4 and 0.2 cc., resp.), and the cholesterol doses varied from 0.1 to 0.4 cc. Again the results were negative in all of the tests.

(3) Cardioliipin concentration: With the cephalin and cholesterol constant (0.4 and 0.2 cc., resp.), the cardioliipin ranged from 0.01 to 0.4 cc. Here, also, all of the results proved negative.

Thus the antigen which proved of optimal composition for pooled lepromatous sera, and closely similar antigens, failed to give nonspecific positive reactions with the three lots of pooled sera tested.

Summary.—(1) The most sensitive antigen contained 0.4 cc. of a 1 per cent mixture of the cephalin fractions, 0.2 cc. of 1 per cent cholesterol, and 0.2 cc. of 0.2 per cent cardioliipin, together with 0.2 cc. of absolute alcohol. To this mixture was added, rapidly, 19 cc. of 0.85 per cent saline (making a 1:20 solution), and then 5 cc. of 0.1 per cent kaolin. The mixture was allowed to stand for 30 minutes at room temperature, after which another 25 cc. of saline was added rapidly.

(2) The most suitable ratio of the stock solutions of the cephalin fractions was phosphatidyl-ethanolamine: diphospho-inositide: phosphatidyl-serine = 29:10:1. The ratio of the total ingredients was cardiolipin: cholesterol: cephalin mixture = 1:5:10.

(3) This antigen failed to give nonspecific reactions with pooled sera from tuberculous, syphilitic or normal subjects.

CLINICAL TESTS

With the antigen and technique arrived at in the basic experiments, complement-fixation tests were made with individual sera from leprosy cases of the different types, and with various nonleprosy sera. The clinical course was observed in some of the leprosy cases.

TESTS IN LEPROSY CASES AND CONTROL GROUPS

Materials and method.—A total of 110 leprosy sera, of types as shown in Table 2, were obtained from patients in two dermatology clinics and two leprosaria. Sera from 113 nonleprosy cases, as shown in Table 3, were obtained from two hospitals and one leprosarium. These subjects comprised cases of tuberculosis, syphilis, cancer and pregnancy, and normal individuals. The syphilis cases had not become negative by Tamiya's method.

The standard of positivity of the reaction adopted was more than 65 per cent hemolysis of the 3 per cent sensitized bovine-cell suspension with a 1:4 dilution of the serum tested.

Findings.—Leprosy sera: The 110 sera examined were from 65 lepromatous cases, 23 macular cases, and 22 neural cases. Positive results were obtained in 60 of the lepromatous cases (92.4%), 17 of the tuberculoid cases (73.9%), and 19 of the neural cases (86.4%). Combining the tuberculoid and neural cases, the positives amounted to 80 per cent (36 of 45 cases). In total, 96 of the 110 cases were positive (87.3%).

TABLE 2.—Complement-fixation reactions with 110 sera of leprosy patients.

Type	No. of cases	Positive reactions	
		Number	Per cent
Lepromatous	65	60	92.4
Tuberculoid	23	17	73.9
Neural	22	19	86.4
TOTAL	110	96	87.3

Nonleprosy sera: It would be ideal if the antigen used, which has a high activity in clinical application in leprosy, should show low reactivity with nonleprosy sera. As a matter of fact, completely negative results were obtained with the sera from the 19 tuberculous cases, 6 cancer cases,

and 40 healthy persons that were tested. Of the 38 syphilis sera, 3 (7.9%), and of the 10 pregnant cases, 2 (20%), gave nonspecific positive reactions. This rate in the pregnancy cases is relatively high, but it does not seem to be too significant clinically.

TABLE 3.—Complement-fixation reaction with sera from nonleprosy cases and healthy persons.

Group	No. of cases	Positive reactions	
		Number	Per cent
Syphilis	38	3	7.9
Tuberculosis	19	0	0
Cancer	6	0	0
Pregnancy	10	2	20.0
Healthy	40	0	0
TOTAL	113	5	4.4

REPEATED TESTS IN LEPROSY CASES

The relationship between the clinical course in leprosy and the complement-fixation reaction has been studied in four cases, as a part of a long-term follow-up observation. The patients were first seen by us, and sera for the first tests were taken, on January 23, 1954. The second examinations were on June 12, the interval therefore 5½ months. The results of the repeated tests with serum dilutions up to 1:128 are shown in Table 4.

CASE 1. N. T., a 26-year-old woman, when first seen had diffuse lepromatous infiltration of the face, irregular discolored patches over the back, and small round macules on the upper arms and lower extremities on both sides. Histological examination revealed lepromatous changes. The Mitsuda reaction was negative. At the second examination, following administration of DDS (diaminodiphenyl sulfone), the infiltration and macules were found to be well absorbed. Mitsuda reaction again negative.

On both occasions the complement-fixation reaction was positive with the highest serum dilution used. There was no diminution of the reaction within this range during the interval.

CASE 2. K. M., a 58-year-old woman, when first seen had many small to moderate-sized nodules on the face and extremities. The ulnar and median nerves were thickened, and the fingers had the claw-hand appearance. The Mitsuda reaction was negative. At the second examination the nodules had been fairly well absorbed. Mitsuda reaction again negative.

Both reactions were positive with the highest serum dilution. There was little change in the reaction within this range, as shown in the table.

CASE 3. T. J., a 62-year-old male, when first seen had many small nodules on the face and extremities. The Mitsuda reaction was negative. Following administration of DDS, the nodules had been almost absorbed at the time of second examination; the patient was enjoying a favorable clinical course. Mitsuda reaction negative.

In the first test the reaction was positive with the 1:16 serum dilution. In the second test, although the patient was much improved clinically, the reaction was found to be stronger, the result positive with the 1:128 dilution.

CASE 4. S. K., a 27-year-old woman. Diffuse infiltrations on the face and forearms of 3 years' duration had improved under treatment. The skin showed atrophy. The Mitsuda reaction was negative.

TABLE 4.—Repeated tests at about six months intervals, in 4 leprosy cases.^a

Case	Date of test 1954	Dilution of serum						Serum control	Antigen control
		4	8	16	32	64	128		
1	Jan. 23	0	0	0	0	0	0	3	3
	June 12	0	0	0	0	0	0	3	3
2	Jan. 23	0	0	0	0	0	0	3	3
	June 12	0	0	0	0	0	1	3	3
3	Jan. 23	0	0	0	1	2	3	3	3
	June 12	0	0	0	0	1	1	3	3
4	Jan. 23	0	0	1	1	1	2	3	3
	June 12	0	0	0	0	1	1	3	3

^a 0=no hemolysis, 1=25-40% hemolysis, 2=65% hemolysis, 3=80-100% hemolysis.

The first test gave positive results to the 1:32 dilution. When tested again the reaction was stronger, positive to the 1:128 dilution.

Conclusion.—From the results of these repeated tests it is concluded that in a period of nearly 6 months there are few changes in the complement-fixation reaction despite clinical improvement.²

SUMMARY AND CONCLUSIONS

From the point of departure indicated in the introduction of this report, further studies have been carried out on cardiolipin, cholesterol and cephalin in an effort to obtain an antigen composition with high

² It is to be noted that Sister Hilary Ross (13, 14) found that 9 of 10 lepromatous cases showed decreased reactions with Kahn tests made after an interval of four years. In syphilis, decrease of positive reactions develops later than clinical improvement under antisyphilis treatment.

sensitivity with sera of various types of leprosy but low sensitivity with other sera.

Ordinary crude cephalin was fractionated by Folch's method into phosphatidyl-ethanolamine, diphospho-inositide, and phosphatidyl-serine, and these were combined in various ratios. In order to obtain the optimal mixture-ratio of cardiolipin, cholesterol and cephalin, two were kept constant and the other changed serially in quantity. The optimal ratio of the three cephalin fractions (in the order stated above) for high sensitivity with pooled sera from lepromatous cases was found to be 29:10:1. The process was repeated with this cephalin mixture and varying quantities of cholesterol and cardiolipin. The least reactive amounts of these various substances were selected as optimal amounts, as prevention of the development of nonspecific reactions was desired.

The optimal combination proved to be 0.2 cc. of 0.2 per cent cardiolipin, 0.2 cc. of 1 per cent cholesterol, and 0.4 cc. of 1 per cent cephalin fractions (0.29 cc. of phosphatidyl-ethanolamine, 0.1 cc. of diphospho-inositide, and 0.01 cc. of phosphatidyl-serine), plus 0.2 cc. of absolute alcohol. The cardiolipin: cholesterol: cephalin ratio of this antigen is 1:5:10.

The addition of an adequate amount of kaolin when diluting the antigen increased the sensitivity and decreased the anticomplementary action.

Preliminary tests of the complement-fixation reaction with this antigen for nonspecificity showed that pooled sera of tuberculous, syphilitic and normal cases gave no reactions.

In the clinical application of this test to individual sera of leprosy patients, 60 of 65 (92.4%) from lepromatous cases were positive, 17 of 23 (73.9%) from tuberculoid cases, and 19 of 22 (86.4%) from neural cases, totalling 96 positives out of 110 cases (87.3%).

Of nonleprosy sera, 19 from tuberculous cases, 6 from cancer cases, and 40 from normal persons all were negative, and only 3 of 38 syphilitic cases (7.9%) and 2 of 10 pregnancy cases (20%) gave nonspecific positives.

Four lepromatous cases were tested twice with an interval of nearly 6 months between tests. None of them showed material decrease of positivity in the second test despite improvement of the clinical symptoms; in fact, in two of them there was some increase of intensity. It would seem that leprosy is not likely to show serological improvement in so short a time.

It is believed that the cardiolipin-cholesterol-cephalin antigens are suitable for various clinical uses, as they possess high antigenicity in lepromatous, tuberculoid and neural leprosy but give few group hemolytic reactions with sera of tuberculosis, syphilis and cancer and normal persons with the exception of pregnancy.

This reaction has been called the Handai method (Honda).

RESUMEN

Después de repasar previa labor realizada con cefalina en antígenos usados para la prueba de fijación del complemento en la lepra, los A. A. describen con todo detalle su elaboración de un antígeno que contiene colessterina, cardiolipina y una mezcla de fracciones de cefalina, con adición de caolín, que muestra mucha especificidad para los sueros leprosos.

Las fracciones de cefalina son las de Folch: fosfatidilo-etanolamina, difosfo-inositida y fosfatidilo-serina. En experimentos encaminados a averiguar las mejores proporciones de estas fracciones que deben usarse en el elemento cefalínico del antígeno total, se determinó que deberían ser 29:10:1, respectivamente, de soluciones al 1 por ciento en éter.

Con esa mezcla de cefalina (1 por ciento en éter) y con cardiolipina (0.2 por ciento en alcohol) y colessterina (1 por ciento en alcohol), se descubrió en forma semejante que la mejor proporción de las tres soluciones básicas era 10:5:1, respectivamente.

Los ensayos en 110 sueros de leprosos arrojaron altos porcentajes positivos en las tres formas de la enfermedad (el más bajo en los casos tuberculoideos, pero de 74 por ciento en ellos), llegando a 87.3 por ciento el total de positivos. Los resultados fueron negativos en todos los sueros procedentes de 40 personas sanas, 19 casos tuberculosos y 6 casos cancerosos; de 38 sueros de sífilíticos 3 (7.9 por ciento) fueron positivos y lo mismo 2 de 10 (20 por ciento) sueros de gestantes.

Pruebas repetidas al cabo de unos seis meses en 4 casos de lepra mejorando con la sulfonoterapia indicaron que no es probable que haya en tan poco tiempo mayor disminución de la intensidad de la reacción.

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