

COMPARATIVE STUDIES OF THE CARDIOLIPIN ANTIGENS WITH THE REGULAR ANTIGENS IN THE KOLMER COMPLEMENT FIXATION AND THE KAHN PRECIPITATION TESTS IN LEPROSY¹

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The isolation of the phospholipin called cardiolipin from beef heart muscles by Pangborn (15, 16, 17) in 1941, and its use with purified lecithin as an antigen in the serodiagnosis of syphilis, has been followed by many reports of its adaptability to the various types of present-day serologic methods (7, 8, 9, 18). Giordano *et al.* (4) state in substance that the cardiolipin antigens give results of maximum sensitivity and of much greater specificity in tests for syphilitic reagin than do the antigen extracts used in the Eagle, Hinton, Kahn, and Mazzini tests. Kline (9) believes that, because of their superior quality, the cardiolipin-lecithin-cholesterol antigens should soon replace those now used in tests for syphilis, and that although it is not completely specific for syphilitic reagin the cardiolipin antigen may well serve as a base for the development of a single standard test for syphilis worthy of universal adoption.

It has long been known that leprosy sera contains an antibody capable of reacting positively with antigens commonly used in tests for syphilis (1, 2, 3, 6). It has been our experience in conducting the various serodiagnostic tests for syphilis that in leprosy patients the greatest percentage of positive reactors is found in those with the lepromatous type, while in patients with the neural type the percentage is small. Our experience is in keeping with those of other workers (1, 6). Naturally, the problem of the "biologic" nonspecific positive reaction is of much interest with any new antigen.

It was believed that the results obtained in syphilis with the cardiolipin antigens were of sufficient significance to warrant a comparative study of these antigens in leprosy patients. Before attempting this study, Dr. J. F. Mahoney, Medical Director of

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the Venereal Disease Research Laboratory at Staten Island, kindly offered to run the cardiolipin antigens with the regular antigens in the Kolmer complement fixation test and the Kahn precipitation test in parallel with this laboratory, in order to check our technique. Sera from 76 patients, presumably non-syphilitic, were thus checked. The report submitted by that laboratory is presented in Table 1. Due to a misunderstanding, the cardiolipin antigens in the Kolmer and Kahn tests were not run in parallel on all of the sera. Of the 76 sera, 37 were checked by the Kolmer technique, utilizing the regular and the cardiolipin antigens, and 39 were checked by the Kahn regular antigen in parallel with the Kahn cardiolipin antigen.

TABLE 1.—Results of testing 76 serums (37 with Kolmer regular and Kolmer cardiolipin antigens, and 39 with Kahn regular and Kahn cardiolipin antigen) from patients with established diagnosis of leprosy.¹

Result	Kolmer		Kahn	
	Regular antigen	Cardiolipin antigen	Regular antigen	Cardiolipin antigen
Positive	6 cases	20 cases	22 cases	16 cases
Doubtful	3 cases	4 cases	11 cases	8 cases
Negative	27 cases	12 cases	6 cases	15 cases
Anticomplimentary	1 case	1 case		

¹ From the Venereal Disease Research Laboratory, Staten Island, New York, November 1948 to February 1949.

Of the 39 cases in which the Kahn tests were run, 6 were less positive with the cardiolipin antigen than with the regular one, 3 were less doubtful, and the number of negatives was 15 against 6. Of the 37 cases in which the Kolmer tests were run, 16 showed a greater positivity with the cardiolipin antigen than with the regular one, and the number of negatives was 12 against 27.

Selection was made of 260 cases representing the various stages of progression of the disease, the lepromatous type predominating, all free from syphilis as far as we were able to ascertain. Of these cases, 225 had had repeated tests over a period of several years, and all had been found serologically positive with one or more of the various tests used (Kolmer, Kahn, and Mazzini). Fewer positive and doubtful reactions were obtained with the Mazzini flocculation slide test than with either the Kolmer or the Kahn test. There were 111 positive

reactions and 36 doubtful ones with the Mazzini test, 169 positive and 23 doubtful with the Kolmer test, and 214 positive with the Kahn test. The number of combinations of positivity are shown in Table 2. The remaining 35 cases of the 260 (30 of lepromatous type and 5 neural) had given consistently negative results with all of the tests used.

TABLE 2.—*Combinations of positive reactions in 225 cases found serologically positive with regular antigens over a period of several years.*

Combination	Number of Cases
Kolmer, Kahn, and Mazzini positive	103
Kolmer and Kahn positive, Mazzini negative	29
Kolmer positive, Kahn and Mazzini negative	11
Kolmer and Kahn positive, Mazzini doubtful	26
Kahn positive, Kolmer and Mazzini negative	33
Kahn positive, Kolmer and Mazzini doubtful	10
Kahn positive, Kolmer doubtful, Mazzini negative	5
Kahn positive, Kolmer doubtful, Mazzini positive	8
Total	225

In the present study all analyses were completed within 3 days after the collection of the blood. The antigens used were supplied through the courtesy of Dr. Mahoney of the V. D. R. Laboratory, Staten Island. The sheep cells were obtained from our own sheep. The techniques of all authors were carried out without modification. Sera from 25 known cases of syphilis (not residents of Carville) were run in parallel with the sera of the patients.

The results obtained in the 225 cases which had given positive serological reactions in the past are presented in Table 3. The 35 cases which had been consistently negative were all found to be negative with the cardiolipin antigens of Kolmer and Kahn, and they are not included in the table. The sera from the 25 known cases of syphilis all gave positive results with all of the antigens used; they, too, are not included in the table.

Of the 225 cases dealt with, 101 were found to be positive and 54 negative with all four of the antigens used. Among the remaining 70 cases there was more or less discrepancy. It is apparent that fewer positive results were obtained with the Kahn cardiolipin antigens than with his regular one; the latter

TABLE 3.—Results of tests of 225 serums with regular and cardiolipin antigens in the Kolmer complement fixations and the Kahn precipitation tests.

Result	Kolmer		Kahn	
	Regular antigen	Cardiolipin antigen	Regular antigen	Cardiolipin antigen
Positive	101 cases	151 cases	159 cases	112 cases
Doubtful	6 cases	6 cases	12 cases	11 cases
Negative	116 cases	68 cases	54 cases	102 cases

gave 159 positives against 112 with the former. In no instance did we find a positive Kahn reaction using the cardiolipin antigen and a negative reaction using the regular one. In the Kolmer tests the reverse was found, namely, 151 positives with the cardiolipin antigen and 101 with regular one.

The lesser number of positive Kolmer and Kahn reactions with the regular antigen, noted in the 225 cases checked with the cardiolipin antigens (Table 3) as against the figures in Table 2, is regarded as a result of the intensive sulfone (promin and diasone) treatment of our patients. Serological work now in progress in this laboratory indicates that changes in the serological titers parallel closely the changes in the clinical manifestations of leprosy. With improvement a positively reacting patient tends to become less strongly positive, and he may become negative. The results of this work will be the basis of a future report.

Kahn (8), referring to work with the cardiolipin antigens, states:

With regard to the relationship between cardiolipin and Kahn antigens in the performance of the standard Kahn test, it is believed that in the present state of limited knowledge of the clinical value of cardiolipin antigen, it would be best to use it side by side with the Kahn antigen. In performing the Kahn test, Kahn antigen with its relatively unknown lipid content and cardiolipin antigen with its known and highly purified lipids, should, in combination, make a desirable antigenic team. It is naturally assumed that, because cardiolipin antigen is highly purified, it is superior to the older, crude alcoholic extracts. Such assumption in biology does not, however, always hold true.

Kahn also states in substance, in another article (14), that cardiolipin antigen in the standard Kahn test, in the present state of knowledge of this antigen, is employed in his laboratory only supplementarily to the Kahn antigen. Also (8), that cardiolipin antigen represents an outstanding advance in the ser-

ology of syphilis and that every effort should be made to employ it, especially in clinical studies and in official evaluation studies of tests for syphilis, so that its value and limitations will become fully established.

SUMMARY

During a period of eight months we have employed the cardiolipin-lecithin-cholesterol antigens of Kolmer and Kahn in parallel with their regular antigens on 260 patients. Of these, 225 had had repeated serological tests over a period of several years and all had been found to be positive in one or more of the various tests used (Kolmer, Kahn and Mazzini), the Mazzini test showing the least number of positive and doubtful reactions.

Analysis of the results obtained in these 260 patients indicates greater sensitivity with cardiolipin antigen in the Kolmer complement fixation test.

The Kahn cardiolipin antigen was found to be superior to the regular Kahn antigen.

The sera of presumably nonsyphilitic leprosy patients exhibit a tendency towards false positive reactions when tested with the cardiolipin antigens in the Kolmer complement fixation and the Kahn precipitation tests, but to a lesser degree in the Kahn test than in that of Kolmer.

Sera from 35 patients who had been consistently negative serologically over a period of several years gave negative results with the cardiolipin antigens employed.

Sera from 25 nonleprosy patients known to have syphilis gave positive results with both of the cardiolipin antigens employed, as well as with both of the regular antigens.

Our results indicate that comparative Kahn tests using cardiolipin and regular antigens warrant further study in leprosy patients over a period of time, to determine if the reactions obtained can be consistently reproduced.

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