AN INVESTIGATION INTO THE USE OF CARDIOLIPIN ANTIGENS*

III. CHOICE OF A FORMULA FOR CARDIOLIPIN WASSERMANN ANTIGEN FOR USE IN THE WHITECHAPEL WASSERMANN TECHNIQUE

BY

I. N. ORPWOOD PRICE

Whitechapel Clinic, London Hospital, and V.D. Reference Laboratory, Medical Research Council

The choice of a formula for the antigen is limited by four considerations :

(1) The anticomplementary action of the antigen.
(2) The complement-fixing properties of the antigen.
(3) The occurrence of floccules when the antigen is made in saline suspensions.
(4) The unit strength of the formula to be employed.

It has already been shown (Price, 1953a, b) that as far as the anticomplementary properties of the antigen are concerned, the ideal formula is cardiolipin 1, lecithin 1, cholesterol 10, the unit strength of the reagents being 0·05 per cent. (Table VI, page 17).

With regard to the complement-fixing properties of the antigen, the most sensitive antigen was found to be one in which the proportion of the reagents is cardiolipin 1, lecithin 1, cholesterol 10–6, (Tables X, XIII, and XV, pp. 79, 81, and 82).

Precipitates in Cardiolipin Antigenic Suspensions

In choosing the unit strength ratio figure for use in the make-up of a cardiolipin antigen, some attention should be given to the formation of macroscopic floccules in the saline suspensions employed as antigens. It can be shown that the proportion of lecithin in a cardiolipin antigen bears no relation to the production of this phenomenon. Thus five groups of antigens, each containing different amounts of cardiolipin (0·0075 to 0·06 per cent.) and constant amounts of cholesterol (0·5 per cent.), were examined after different amounts of lecithin had been added to the antigens of each group. No precipitates were obtained with any antigens of a group until the cardiolipin-cholesterol ratio exceeded 1 to 10, irrespective of the cardiolipin-lecithin ratios which ranged from 1–5 to 5–1. The production of these precipitates is related to the cardiolipin-cholesterol ratio of the antigen.

Table XVI is constructed from the results of the examination of ten groups of antigens, but the three groups shown are sufficient to demonstrate the point at issue. It can be seen that, as the gap between the ratio of the two ingredients increases, so do the precipitates appear with greater dilutions of saline.

TABLE XVI

PRECIPITATION TITRES OF CARDIOLIPIN ANTIGENS DUE TO EXCESS RATIO CHOLESTEROL CONTENT

Antigens			Cardiolipin-Cholesterol Ratio	Precipitation Titre
Cardiolipin (per cent.)	Lecithin (per cent.)	Cholesterol (per cent.)		
0·1	0·1	1	1–10	None at 1 in 10 or above
0·1	0·1	0·5	1–5	
0·1	0·1	0·25	1–2·5	
0·1	0·1	0·125	1–1·25	
0·05	0·1	1	1–20	1 in 10
0·05	0·1	0·5	1–10	None at 1 in 10 or above
0·05	0·1	0·25	1–5	
0·05	0·1	0·125	1–2·5	
0·025	0·1	1	1–40	1 in 40
0·025	0·1	0·5	1–20	1 in 10
0·025	0·1	0·25	1–10	None at 1 in 10 or above
0·025	0·1	0·125	1–5	

It should be pointed out that after a time (1 hr.) the precipitates agglutinate and can be easily separated by centrifugalization. If resuspended in saline they are antigenic when used in a complement-fixation test. Also it can be shown that the original antigen (when well shaken before use) is more sensitive than the supernatant fluid left after removal of the precipitate. It therefore seems that particulate antigens of this nature are to be avoided if possible.

Determination of Unit Ratio Strengths of Cardiolipin Antigen Mixtures

An attempt was made to demonstrate the unit strength of the formula cardiolipin 1, lecithin 1, cholesterol 10, which would constitute the most sensitive antigen.

* Received for publication September 3, 1952.

Thus antigens of five unit strengths from 0·0125 to 0·15 per cent. were titrated and tested. The make-up is shown in Table XVII.

TABLE XVII

KEY TO ANTIGEN USED FOR THE DETERMINATION OF THE UNIT RATIO STRENGTH OF THE MOST SENSITIVE CARDIOLIPIN ANTIGEN (FORMULA 1 : 1 : 10)

Antigen	Percentage Contents			Optimum Titre of Antigen	Anticomplementary Titre in absence of Normal Serum
	Cardiolipin	Lecithin	Cholesterol		
1	0·15	0·15	1·5	1/640	1/40
2	0·075	0·075	0·75	1/480	1/40
3	0·05	0·05	0·5	1/240	None
4	0·025	0·025	0·25	1/120	None
5	0·0125	0·0125	0·125	1/70	None

Thirty-two sera were now tested in parallel, using these antigens at their optimal titres. The results are shown in Table XVIII.

It is remarkable that whichever of these antigens is used the titres of the sera tested were, within experimental limits, identical. Thus, providing that the ratio of the contents of the antigens is kept constant, it is of little consequence, so far as sensitivity is concerned, which is used, provided it is employed at its optimal titre. Two things should be remembered :

(1) That if the unit strength of the antigen is 0·075 per cent. or above, the antigen exhibits anticomplementary properties when in a suspension of 1 in 40 of saline (Table XVII, above). Even if the optimal titres of such antigens are well above the range of their anticomplementary titres, ordinary prudence would counsel one to avoid using these antigens unless they could be shown to be more sensitive than those made up at a lower unit strength, *e.g.* 0·05 per cent.

(2) On the other hand, antigens of this type work equally well over a wide range of unit strengths below 0·075 per cent., and the optimum titre depends upon the saline dilution mixture factor. The unit strength at which sensitivity becomes lower is approximately 0·004 per cent., and at 0·0008 per cent. these mixtures cease to be antigenic.

Thus it seemed that for technical ease of production, supply, and use a unit strength of 0·05 per cent. should be chosen, and the formula of cardiolipin antigen for use in the Whitechapel Wassermann technique has been defined as cardiolipin 0·05 per cent., lecithin 0·05 per cent., cholesterol 0·5 per cent.*

It may be said that whilst the conclusions already reached are true as far as the reagents tested are concerned, it does not necessarily follow that other preparations of such reagents, when mixed and tested, would yield similar results.

With this in mind, it was decided to carry out two experiments : one to determine the effects of using antigens made up of different brands of cardiolipin in combination with the same batches of lecithin and cholesterol, and the other to test antigens derived from the same batches of cardiolipin and cholesterol, but using different brands of lecithin.

TABLE XVIII

SENSITIVITY OF CARDIOLIPIN ANTIGEN (FORMULA 1 : 1 : 10) AS AFFECTED BY THE UNIT STRENGTH

Serum No.	Quantitative P.P.R. Results	Cardiolipin Antigens with Serum Titre Recording				
		1	2	3	4	5
1	Negative	16	16	16	16	16
2	Negative	16	16	16	16	16
3	2	32	32	32	32	32
4	1	32	32	32	32	32
5	2	32	32	32	32	32
6	Negative	8—	8—	8—	8—	8—
7	4	16	16	16	16	16
8	Negative	4	4	4	4	4
9	4	16	16	16	16	16
10	Negative	16	16	16	16	16
11	4	16	16	16	16	16
12	4	16	16	16	16	16
13	2	8	8	8	8	8
14	1	16	16	16	16	16
15	2	16—	16—	16—	16—	16—
16	1	8—	8—	8—	8—	8—
17	1	16	16	16	16	16
18	1	4—	4—	4—	4—	8—
19	Negative	16—	16—	16—	16—	16—
20	Negative	4—	4—	4—	4—	4—
21	Negative	16	16	16	16	16
22	Negative	16—	16—	16—	16—	16—
23	Negative	8	8	8	8	8
24	2	16	16	16	16	16
25	4	32	32	32	32	32
26	2	16	16	16	16	16
27	2	32—	32—	32—	32—	32—
28	2	32—	32—	32—	32—	32—
29	4	64—	64—	64—	64—	64—
30	1	16—	16—	16—	16—	16—
31	4	64	64—	64—	64	64
32	4	16—	16—	16—	16—	16—

* All the foregoing work was performed with the following reagents
(1) Cardiolipin, Burroughs and Wellcome, Batch 122050.
(2) Lecithin, Burroughs and Wellcome, Batch 123050.
(3) Cholesterol, V.D.R.L., Batch 22/3/51.

TABLE XIX

KEY TO CARDIOLIPIN ANTIGENS (1 : 1 : 10) FORMULA CONTAINING DIFFERENT BRANDS OF CARDIOLIPIN

Antigen	Antigen Contents			Optimum Titre of Antigen	Anticomplementary Titre in absence of Normal Serum	Flocculation Titre
	Cardiolipin	Lecithin	Cholesterol			
8	BW122C50	BW123C50B	V.D.R.L. 23-2-51	1/360	None	None
9	BW122C50B	BW123C50B	V.D.R.L. 23-2-51	1/180	None	None
10	" Baltimore "	BW123C50B	V.D.R.L. 23-2-51	1/600	None	None
11	" New York "	BW123C50B	V.D.R.L. 23-2-51	1/360	None	None

The total of cardiolipin batches tested was limited by the number available at the time of the experiment. Since then, others have been tested by the same methods and found to give similar results.

One point of interest is the variation in the titres of the different antigens.

TABLE XX

SENSITIVITY OF CARDIOLIPIN ANTIGENS CONTAINING DIFFERENT BRANDS OF CARDIOLIPIN BUT THE SAME BATCHES OF LECITHIN AND CHOLESTEROL

Serum No.	Cardiolipin Antigens with Serum Titre Recordings			
	8	9	10	11
1	32	32	32	32
2	64	64	64	64
3	16—	16—	16—	16—
4	32	32	32	32
5	64	64	64	64
6	16—	16—	16—	16—
7	16—	16	16—	16—
8	64—	64—	64—	64—
9	64—	64—	64—	64—
10	16	16	16	16
11	32	32	32	32
12	16	16—	16—	16—
13	64—	64—	64—	64—
14	32	32	32—	32
15	32	32	32	32
16	16—	16—	16—	16—
17	8—	8—	8—	8—
18	16—	16	16—	16—
19	32—	16	16	32—
20	32—	32—	32—	32—
21	32	32	32	32
22	32—	32—	16	32—
23	64—	64—	64—	64
24	32	32	32	32

The set-up of the first experiment is depicted in Tables XIX and XX.

Table XX shows a surprising unanimity in results as expressed by the serum titres obtained by the use of the four antigens. It is apparent that all four samples of cardiolipin work at a uniform level of sensitivity if the antigens containing them are used at their optimum titres.

The second experiment is depicted in Tables XXI and XXII.

TABLE XXI

SENSITIVITY OF CARDIOLIPIN ANTIGENS CONTAINING DIFFERENT BRANDS OF LECITHIN BUT THE SAME BATCHES OF CARDIOLIPIN AND CHOLESTEROL

Serum No.	Serum Antigens with Serum Titre Recordings							
	1	2	3	4	5	6	7	8
1	64—	64—	64—	64—	64—	64—	64—	64—
2	32	32—	32—	32—	16—	32—	32—	32—
3	8—	8—	8—	8—	4—	8—	8—	8—
4	32	32	32	32	32—	32	32	32
5	64—	64—	64—	64—	32—	64—	64—	64—
6	32—	32—	32—	32—	16—	32—	32—	32—
7	64	64	64	64	32	64	64	64
8	16—	16—	16—	16—	8—	16—	16—	16—
9	4	4	4	4	Negative	4	4	4
10	16	16	16	16	8	16	16	16
11	16—	16—	16—	16—	8—	16—	16—	16—
12	32	32	32	32	16	32	32	32
13	16	16	16	16	16—	16	16	16
14	64	64	64	64	32	64	64	64
15	16	16	16	16	16—	16	16	16
16	32—	32—	32—	32—	16	32—	32—	32—
17	64—	64—	64—	64—	32—	64—	64—	64—
18	16	16	16	16	16—	16	16	16
19	32—	32—	32—	32—	16—	32—	32—	32—
20	8	8	8	8	4	8	8	8
21	32	32	32	32	16	32	32	32
22	4	4	4	4	Negative	4	4	4
23	32—	32—	32—	32—	16	32—	32—	32—
24	32	32	32	32	16	32	32	32

It will be noted from Table XXII (p. 178) that, as in the case of the antigens made from different preparations of cardiolipin, there are considerable differences in the titre values of the antigens as determined by the method of optimum proportions.

Table XXI (above) is of great interest because it shows that (within experimental limits) identical results are obtained by the use of eight antigens made up from the same batch of cardiolipin and

TABLE XXII

KEY TO CARDIOLIPIN ANTIGENS (1 : 1 : 10) FORMULA CONTAINING DIFFERENT BRANDS OF LECITHIN

Antigens	Antigen Contents			Optimum Titre of Antigen	Anticomplementary Titre in absence of Normal Serum	Flocculation Titre
	Lecithin	Cardiolipin	Cholesterol			
1	New York, Egg Yolk A, 1951	Batch, Burroughs and Wellcome, 122C50B	V.D. Reference Laboratory, 22-3-51	1/360	None	None
2	New York, Egg Yolk B, 1951	Batch, Burroughs and Wellcome, 122C50B	V.D. Reference Laboratory, 22-3-51	1/200	None	None
3	New York, Beef Heart A, 1951	Batch, Burroughs and Wellcome, 122C50B	V.D. Reference Laboratory, 22-3-51	1/200	None	None
4	New York, Beef Heart B, 1951	Batch, Burroughs and Wellcome, 122C50B	V.D. Reference Laboratory, 22-3-51	1/340	None	None
5	Baltimore, Beef Heart, 1950	Batch, Burroughs and Wellcome, 122C50B	V.D. Reference Laboratory, 22-3-51	1/100	None	None
6	New York, Beef Heart Batch 80, 1950	Batch, Burroughs and Wellcome, 122C50B	V.D. Reference Laboratory, 22-3-51	1/360	None	None
7	Burroughs and Wellcome, Beef Heart 123C50B, 1951	Batch, Burroughs and Wellcome, 122C50B	V.D. Reference Laboratory, 22-3-51	1/200	None	None
8	Burroughs and Wellcome, Beef Heart 123C50, 1950	Batch, Burroughs and Wellcome, 122C50B	V.D. Reference Laboratory, 22-3-51	1/360	None	None

cholesterol but containing different brands of lecithin.

The other antigen (Antigen 5) gave somewhat weaker results with all the sera except No. 1. The low titre of this antigen (1 in 100) suggests that it is weakly reactive, and because the other components of the antigen have been found to be serologically satisfactory (see Antigen 9, Tables XIX and XX, page 177), the weakness may be due to this particular preparation of lecithin.* It should be noted that two of the preparations of lecithin used were derived from egg yolk, whereas the rest were isolated from beef heart muscle. No serological difference between these two types of lecithin was detected.

From the results of these two experiments it can be inferred that, provided the serological activities of different preparations of cardiolipin and lecithin are up to a reasonable standard, these components of cardiolipin Wassermann antigen are interchangeable, and if the antigens so made up are used at the optimum titres, they will react at the same level of sensitivity. This method of standardizing the sensitivity level of Wassermann antigens has already been shown to operate in the case of ox heart Wassermann antigens (Price and Wilkinson, 1951).

Furthermore, these experiments demonstrate a method by which a serologically weakly reacting component of a cardiolipin Wassermann antigen can be detected.

* This particular preparation was made in 1950 (exact date not specified) which might account for its loss of power.

REFERENCES

Price, I. N. Orpwood (1953a). *British Journal of Venereal Diseases*, **29**, 12.

———(1953b). *Ibid.*, **29**, 78.

———, and Wilkinson, A. E. (1951), **27**, 191.