COMPLEMENT-FIXATION TECHNIQUE

II. THE TITRATION OF WASSERMANN ANTIGEN

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After material has been produced for use as an antigen in a complement fixation test it is necessary to find out if the finished product has any complement-fixing ability in the presence of a serum containing a suitable antibody. Moreover, the proposed antigen must not have any undue anti-complementary property, and the "gap" between its anticomplementary titre and its complement fixing titre must be wide enough to permit its use with safety in the test. Finally, the product should be titrated by the method of optimal proportions. It is therefore suggested that in the standardization of any proposed antigen the following three steps should be taken.

1. The antigen must be shown to have complement-fixing ability. This can easily be ascertained by using a standard dose of complement and adding to it an appropriate positive serum in the presence of the proposed antigen. After a suitable period of incubation the presence or absence of complement fixation is demonstrated by the addition of sensitized red blood cells.

The technique is as follows: into each of the appropriate tubes (set up in two rows) is put a dose of antigenic material. The doses are graded in "doubling up" dilutions. Starting from the left with neat material, the dilutions are carried up to 1 in 128. Into each tube of the back row is put a dose of known "positive" serum, and similarly into each tube of the front (control) row a dose of normal serum. All tubes now receive a dose of 3 minimum hæmolytic doses of complement, and are incubated for a suitable time and at a definite temperature, usually 1 hour at 37° C. At the end of this time a dose of sensitized red cells is added, the contents of the tubes are well mixed by shaking, and after half an hour of further incubation at 37° C. the result of the trial test is read. The front (control) row containing normal serum is scrutinized, and the tube containing the lowest dilution of antigenic material which shows complete hæmolysis is noted. Its opposite number in

the back row, containing a known positive serum, is then observed. If this tube shows partial or complete hæmolysis the proposed antigen is rejected as being deficient in complement-fixing property. If no hæmolysis is seen in this tube, the tube in the back row containing the highest dilution of antigen and showing no hæmolysis is noted, and thus a rough estimate of the value of the antigen can be made.

Two points should be noted. Firstly, it is suggested that if the antigen is to be efficient the ratio between the highest dilution showing no hæmolysis in the back row and the lowest dilution showing complete hæmolysis in the front row should be at least 8 to 1. Secondly, if the antigen satisfies the above conditions and shows no anticomplementary action, that is, all tubes in the front row of the trial test show complete hæmolysis, an attempt should be made to concentrate the reagent until an antigen is produced which, when used in an undiluted condition in the trial test, will show definite anticomplementary action in the presence of normal serum. By this means the particular antigen can be made into the most suitable condition for titration by optimal proportions. Thus any antigen has a zone of reaction starting at a point where it ceases to be anticomplementary and ending where it is too weak to have any complement-fixing property. In order to get a true picture of the value of an antigen it is essential to define this zone.

2. The anticomplementary action of the antigen must be determined (a) by itself, and (b) in the presence of a normal serum. In this way the protective action of normal serum (if any) against the anticomplementary action of the antigen itself may be determined. In the case of some antigens which have practically no anticomplementary action this of course is unnecessary, but there are some bacterial antigens, such as gonococcal antigen, in which this action is often well marked.

TABLE I
ANTICOMPLEMENTARY ACTION OF ANTIGENS PLUS NORMAL SERUM
Ox 15 Alcoholic Heart Extract Containing 0.4 per cent. Cholesterol

Cholesterolized heart extract mixtures with saline in the proportion of						Complement dilutions						
						1/10	1/20	1/30	1/40	1/50	1/60	1/70
1 to 20 of saline	..	..	..	..	..	—	—	—	—	—	+	+
1 to 40 „ „	..	..	..	..	..	—	—	—	—	—	+	+
1 to 80 „ „	..	..	..	..	..	—	—	—	—	—	+	+
1 to 160 „ „	..	..	..	..	..	—	—	—	—	—	+	+
1 to 320 „ „	..	..	..	..	..	—	—	—	—	—	+	+
1 to 640 „ „	..	..	..	..	..	—	—	—	—	—	+	+
1 to 1,280 „ „	..	..	..	..	..	—	—	—	—	—	+	+
Normal Serum Control	..	..	..	..	..	—	—	—	—	—	+	+

+ = no hæmolysis. ± = partial hæmolysis. ≡ = almost complete hæmolysis. — = complete hæmolysis.

3. Finally, the antigen must be titrated by the method of optimal proportions. These three steps must be taken before an antigen can be used at its appropriate dose or dilution in a complement-fixation test.

It is proposed in this article to describe a technique of standardizing the Wassermann antigen. This is based partly on the work of Griffith and Scott.* The method of standardizing other types of antigens will be described in a subsequent article.

Titration of Wassermann Antigen

Stage 1.—Wassermann antigens as used in this country are usually made by the addition of saline to alcoholic ox heart muscle extracts sensitized with cholesterol. In my experience I have never found any such antigen which fails to exhibit some complement-fixing ability in the presence of known syphilitic serum. Thus in the case of Wassermann antigen the first step of the titration technique may be omitted, but before passing on it is well to consider the sensitizing action of the cholesterol. This action was investigated many years ago by numerous workers, and the amounts of cholesterol added to the alcoholic heart extract was shown to be most effective at a strength of about 0.4 per cent. During the last few months this question has been re-examined and similar results have been obtained. From experiments starting with the neat alcoholic heart extract and adding increasing amounts of cholesterol it can be shown that there is a zone of increasing sensitization up to 0.4 per cent. This is maintained, but not increased, until about three times that amount of cholesterol has been added. On the addition of greater amounts of cholesterol the sensitivity gradually falls.

These conclusions were reached by comparing the results obtained on titrating a known syphilitic serum with Wassermann antigen sensitized by increasing amounts of cholesterol. The serum titres recorded with the varying fortified Wassermann antigens were compared, thus giving the optimal zone of sensitization.

Stage 2. Estimation of Anticomplementary Action of Antigen plus a normal Serum.—This is carried out by taking the alcoholic extract, to which has been added 0.4 per cent. cholesterol, and from this mixture making a series of antigen suspensions with increasing amounts of saline. In this way from one cholesterolized alcoholic extract of heart extract a series of antigens is made (Table I).

The amounts of saline added ranged between 20 and 1,280 ml. of saline to 1 ml. of cholesterolized heart extract. These were then added to increasing dilutions of complement ranging from 1 in 10 to 1 in 70, each tube also containing a standard amount of normal serum. In this way the anticomplementary action of the antigen plus the serum was titrated, and it will be seen that the complement titre in the case of the 1 in 20 suspension was 1 in 50 whilst all the other antigens gave a reading of 1 in 55. From these complement titres the necessary diagnostic doses for use in the final standardization by optimal proportions are calculated in the same manner as was described in a previous communication on complement titration (Orpwood Price, 1949).

In the above example the complement dose for the cholesterolized heart extract suspension 1 in 20 was a complement dilution of 1 in 40, whilst for all the other suspensions it proved to be a dilution of 1 in 44. Having thus demonstrated the necessary diagnostic dose of complement for each antigen prepared, the titration of the cholesterolized heart extract by means of optimal proportions is the next step.

* See Reports on Public Health and Medical Subjects No. 1, 1920, H.M.S.O.

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TABLE II

OPTIMAL PROPORTION TITRATION OF ANTIGEN

Ox 15 Alcoholic Heart Extract Containing 0.4 per cent. Cholesterol

Cholesterolized heart extract mixtures with saline in the proportion of	Comple-ment dose	Serum dilutions							Antigen control	Antigen + normal serum control
		1/10	1/20	1/30	1/40	1/50	1/60	1/70		
1 in 20 of saline ..	1-40	+	±	—	—	—	—	—	—	—
1 in 40 „ „ ..	1-44	+	+	±	—	—	—	—	—	—
1 in 80 „ „ ..	1-44	+	+	±	—	—	—	—	—	—
1 in 160 „ „ ..	1-44	+	+	±	±	—	—	—	—	—
1 in 320 „ „ ..	1-44	+	+	±	±	±	—	—	—	—
1 in 640 „ „ ..	1-44	+	+	±	±	±	—	—	—	—
1 in 1,280 „ „ ..	1-44	±	±	—	—	—	—	—	—	—
Syphilitic serum control ..	1-44	<div style="display: flex; align-items: center;"> <div style="margin-right: 10px;">1/5</div> <div style="margin-right: 10px;">1/10</div> <div style="margin-right: 10px;">1/20</div> <div style="margin-right: 10px;">1/50</div> <div style="margin-right: 10px;">1/100</div> <div style="margin-right: 10px;">1/200</div> <div style="margin-right: 10px;">1/400</div> </div> } Fractions indicate serum dilutions								
Normal serum control ..	1-44									

+ = no hæmolysis. ± = partial hæmolysis. ± = almost complete hæmolysis. — = complete hæmolysis.

Stage 3. Determination by Optimal Proportions of Antigen Suspension Suitable for use in the Test.—The method of performing this titration is to take the antigen suspensions already made and add them to suitable serum dilutions of a known syphilitic serum, the range of dilutions employed depending on its titre. To each antigen row is added the complement dose as determined by the previous complement titration (Table II).

It will be seen from this that the serum used was of moderate strength, and it is suggested for ease of working that a serum of a Wassermann titre 1 in 40 or thereabouts be used. Suitable controls as shown in Table II are necessary, and it will be seen that the antigen suspensions giving the best results lie in the zone of antigen suspensions 1 in 160 to 1 in 640. Using these suspensions it will be seen that one is likely to get the most sensitive reactions in this zone, and in order to make this technique a

working proposition it is suggested that one is entitled to take the midpoint of this zone (1 in 160 to 1 in 640) as being the most suitable suspension to use in the test. In this instance the suspension should be one part of the cholesterolized (0.4 per cent.) heart extract to 400 parts of normal saline.

Stage 4. Determination of Antigen Dilution with Normal Saline.—Having determined the suspension of cholesterolized heart extract in saline which is likely to give the most sensitive reactions, the final titration is undertaken in order to demonstrate what dilution of this suspension with normal saline should be used in the test proper.

The determination of the suitable dilution by optimal proportions depicted in Table III is similar to that shown in Table II with the exception that antigen dilutions with saline are made from the antigen suspensions of 1 in 400. It will be seen

TABLE III

OPTIMAL PROPORTION TITRATION OF ANTIGEN SUSPENSION

Ox 15 Alcoholic Heart Extract Containing 0.4 per cent. Cholesterol

Antigen suspension 1-400 saline	Serum dilutions						
	N	1/2	1/4	1/8	1/16	1/32	1/64
Neat	+	+	+	+	+	+	+
1-2	+	+	+	+	±	—	—
1-4	+	+	+	±	—	—	—
1-8	±	—	—	—	—	—	—
1-16	—	—	—	—	—	—	—
1-32	—	—	—	—	—	—	—

For the sake of brevity the complement doses and usual controls have been omitted. The former are estimated as described in stage 2.

that this suspension when used neat will give the best results.

As a matter of interest it may be pointed out that as a general rule the stronger the antigen suspension the greater the need for dilution with saline.

The use of saline as a diluent in the serum dilutions does not affect the validity of the above technique, since it can be shown that if normal serum is used as a diluent the effect is relative in as much as all the serum titres recorded are reduced proportionately.

It should be noted that in all the above titrations the reactions were carried out under the same conditions as are employed in the routine Harrison-Wyler Wassermann technique. The alcoholic heart extract used was made by extracting 1 g. of wet heart

muscle with 9 ml. of absolute alcohol for five days at room temperature and twenty-four hours at 4° C.

The contents of all the tubes when the titrations are completed contain 1/5 volume of serum, 1 volume of saline, 1 volume of complement, 1 volume of antigen, and 1 volume of sensitized red blood cells. The exception to this rule occurs with the serum and antigen control tubes. In the former the absence of antigen and in the latter the absence of serum is made up with an equivalent amount of normal saline. The unit volume referred to in the above description is 0.11 ml.

REFERENCE

Price, I. N. O. (1949). *British Journal of Venereal Diseases*, 25, 157.

BOOK REVIEW

Everyday Sex Problems. By Norman Haire, Ch.M., M.B. 1948. London: Frederick Muller Ltd. Pp. 268. 10s. 6d.

This book is a collection of short articles on various sex matters which have previously appeared in an Australian family magazine. On account of the susceptibilities of his public, the author was unable to write as frankly as he would have wished, and he now states that his views are much less conservative than would appear from the articles.

While we agree with his views that sex education is urgently needed, many will doubt whether this book offers an acceptable contribution to that end. It claims to be an attempt to educate the public gradually; it covers a wide field of sexology, but seldom follows any subject to a constructive conclusion. Often it recommends the reader to seek the advice of a competent authority, but omits to indicate where this can be found.

The old hypocrisy of sex is gradually being abandoned, but in its place most people believe that sex education should at this stage show a modest degree of restraint. By teaching physiological function and removing the mystery and secrecy of sex, we could achieve some progress; as a result we would pave the way to a recognition of the complex psychological reactions which arise from sexual maladjustments. The articles in this book tend to exceed these requirements. The introduction goes much further and vitiates any value the book might otherwise possess. It suggests a liberty and freedom in sex matters which is unacceptable.

Because sexual maturity at puberty precedes economic maturity in our civilization, the author feels that we must bridge the gap by attempting actively to lessen sexual hunger and misery. To do this he considers we may need to depart from the "accepted" sex code, "to which the vast majority of people do nothing more than lip service." Far from lessening sexual hunger and misery this would appear to be an aperitif to licence.

The author states: "Increasing numbers of men and women are no longer convinced that 'virtue' is the only virtue, and indeed many are beginning seriously to question whether sexual abstinence, *as such*, is really a virtue at all." He indirectly advocates trial marriage and points out that we should not buy a motor car or a house merely on outward appearance alone; but that we should want to look inside, try the performance of the car, or rent the house for a preliminary period with perhaps the option to buy later.

Such statements in a book intended as a guide to sex knowledge for the immature are a serious threat to morality, and few of us would consider allowing our children to read this work as an introduction to sex knowledge.

The title of the book is subtly misleading. It falsely implies a common preoccupation with sex and while the more timid may be relieved to find that others have experienced problems similar to their own, it approaches this most difficult problem in the wrong atmosphere and cannot be recommended.

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