TWO SEROLOGICAL STUDIES.

(1). The Syphilis Flocculation Test, with special reference to the Sachs-Georgi reaction.

(2). Fixation of Complement by B.Typhosus and Normal Guinea-pig's Serum: a contribution to the study of natural antibodies.

by

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C O N T E N T S.

THE SYPHILIS FLOCCULATION TEST

with special reference to

The SACHS-GEORGI reaction.

The application by Wassermann, Neisser and Bruck of the principle of the Bordet-Gengou complement-fixation phenomenon to the serological diagnosis of syphilis has led to the elaboration of a test which is now widely employed not only for diagnosis but also for the control of treatment of this disease. The Wassermann test, as it is called, has fulfilled its early promise and it occupies a unique position among present day serological methods, both on account of the universality of its use and the accepted reliability of its results. It is of comparatively little moment as affecting its clinical value that, from a theoretical standpoint, a positive Wassermann reaction cannot yet be adequately explained on the basis of a simple immunity reaction between an antigen derived from Treponema pallidum, a homologous antibody in a syphilitic patient's serum, and complement. The evidence which has accumulated in the course of the routine application of the test by many workers during the past twenty years is convincing, and fully warrants the conclusion that, in Britain, a positive reaction indicates syphilitic infection. It is of course essential for the validity of this conclusion that the positive result should have been obtained by a worker conversant with serological methods, and employ-ing adequate control of the particular modification of

the technique of the test which he may favour.

In the case of a negative reaction however a similar degree of certainty is unfortunately lacking and it is well known that in a proportion of cases of undoubted syphilis, especially in its later manifest--ations, serological confirmation of the clinical diagnosis cannot be obtained by the Wassermann test. This is particularly regrettable as it has led to many attempts to increase the sensitiveness of the test, and some of these have merely succeeded in favouring the occurrence of false positive results; thus bring--ing discredit on the reliability of the orthodox and tried methods. It cannot be emphasised too strongly that any modification of the established methods which permits the occasional occurrence of false positives should be condemned unequivocally and its use for diagnostic purposes in routine tests abandoned. If due attention were paid to this postulate it is probable that much confusion would be obviated, and that the literature would be spared the unnecessary burden of much with which it has been, and still is being, en--cumbered. Apart altogether from the inconclusiveness of a negative Wassermann reaction as a means of excluding syphilitic infection, the complexity of the test itself has stimulated endeavours to find some alternative

method which, while at least as accurate in its results, would be simpler in its application and, if possible, less prone to those fallacies which, in the Wassermann test, have to be guarded against by rigorous control of each of the participating reagents. Of special importance in this latter respect is the complement; a reagent which varies markedly both in deviability and haemolytic activity, two biological properties on which the Wassermann test depends and both of which are peculiarly unstable. A serological method for the detection of syphilitic infection which could be carried out without recourse to the use of complement has much therefore to commend it. That such might be practicable was noted very shortly after the original contribution of Wassermann and his co-workers when it was observed that, in certain circumstances, a precipitate occurred in mixtures of syphilitic antigen* (as used in the Wassermann test) and the serum of a syphilitic patient, no other reagents being present. Much work has since been undertaken with a view to determining the precise conditions under which such precipitum formation is favoured, and we are indebted principally to Meinicke and to Sachs and Georgi for a large amount of research

* The term "antigen" is retained throughout, for the sake of convenience. It denotes a saline dilution of cholesterolised heart-extract.

which has brought this flocculation test into a position of repute as a likely substitute for the more complex Wassermann reaction. Other workers ---- Vernes in France, Dreyer and Ward in Britain, and Kahn in America ---- have introduced various modifications in technique but, in each instance, the principle is essentially the same and the tests associated with these names all depend on the presence of flocculi (precipitate) in mixtures of a lipoid antigen with serum from a syphilitic patient, and the absence of such flocculi when normal, i.e. non-syphilitic, serum is used. It must be kept prominently in mind however that, although an alternative serological test for the detection of syphilitic infection is of great interest, it fails signally to be of practical importance so long as its technique is not very definitely simpler than that of the Wassermann reaction, and so long as its results are not at least as accurate and as easily interpretable. The Wassermann test has been thoroughly exploited and as its deficiences and difficulties are well recognised allowance can be made for them. Complex and little understood though its various biological reagents are, one is justified in claiming that as a routine test it has established itself firmly as a dependable standard, and that until a simpler and better standard is available

it must continue to hold first place; a position to which experience of its use entitles it. It is the object of this thesis to show that, in the present state of its evolution, the syphilis flocculation reaction falls considerably short of what is reasonably demanded of a substitute for the Wassermann test, and that although its technique is much simpler than that of the latter, and its results on the whole closely corresponding, yet as a practical method its routine use is associated with difficulties which tend to render its behaviour capricious, and which frequently make the result a matter of individual opinion rather than of undoubted fact.

When these studies were commenced, two methods of carrying out the flocculation test had commended themselves more than others, both on account of their simplicity and the favourable results which they were yielding in the hands of different workers. One of these, the Meinicke test, to which reference will be made later, had attracted considerable attention in German speaking countries while the other, the Sachs-Georgi test, had been utilised in Britain by Taniguchi and Yoshinare and also, in modified form, by Dreyer and Ward. As the Sachs-Georgi technique was even simpler than the Meinicke it was adopted as the standard for

the preliminary studies and if a description of the method is given here it will obviate the necessity for much repetition in the text,

Sachs-Georgi Test.

Apparatus: Glass beaker, for saline.
Graduated pipettes (1.0 and 0.1 c.c.) with mouthpiece and india-rubber tube.
Test-Tubes (3" x ½"), sterilised in a hot-air oven.
Racks for tubes.
Water-bath at 100 °C. for sterilising beaker and pipettes.
Water-bath at 55°C. for heating serum.
Incubator at 37°C.

Reagents: Saline. 0.85 per cent. solution of pure Sodium Chloride in distilled water; heated in Koch steriliser.
Absolute Alcohol.
Alcohol-Saline. A freshly prepared 1 in 6 dilution of absolute alcohol in saline.
Serum. Human serum which has been heated in the water-bath at 55°C for half-an-hour, some hours before use. **
Antigen. A 1 in 6 dilution with saline of a cholesterolised alcoholic-extract of human heart muscle. (The preparation of this extract is given in detail in appendix 5). The dilution is carried out in two stages:-
(a) 1 volume of the cholesterolised heart-extract is mixed rapidly with 1 volume of saline by pouring the saline into the extract and immediately pouring the mixture from one tube to the other several times. It is then allowed to stand at room temperature for 5 minutes, after which
(b) it is similarly rapidly diluted with an additional 4 volumes of saline. This final mixture is used in the tests after it has stood at room temperature for from 5 to 15 minutes.

** Blood is most conveniently obtained by vene-puncture. It is collected in a sterile test-tube, provided with a cotton wool plug or a rubber cork, and is allowed to coagulate. The clot is gently separated from the sides of the tube and the specimen is stored in a cool place until required for the test. The serum, which should be free from red blood corpuscles and from gross bacterial contamination, is then pipetted off and, if need be, centrifugalised.

Technique. Three tubes are used for each serum to be tested and the reagents are added in the amounts, and in the order, shown in the table. The pipettes are rinsed out with saline, at least three times, between the addition of different reagents.

In preparing the antigen, the pipette is rinsed with absolute alcohol both before and after the measurement of cholesterolised heart-extract.

Tube No.		1	2	control. 3.
Saline,	c.c.	0.9	0.9	0.9
Serum,	c.c.	0.05	0.1	0.1
Alcohol-Saline,	c.c.	nil	nil	0.5
Antigen,	c.c.	0.5	0.5	nil.

Tubes 1 and 2 thus contain Antigen + Serum, while Tube 3 which serves as a serum control contains no antigen. Alcohol however is present in the same concentration as in Tubes 1 and 2. The contents of each tube are mixed by gentle shaking after the addition of the serum and the antigen (or alcohol-saline), and the completed tests are placed in the incubator at 37°C. for from 18 to 20 hours.

Controls: (1). A known positively-reacting, and a known negatively-reacting serum, are included with each set of tests. In the case of the positive serum it is preferable, if possible, to use one which causes relatively feeble flocculation, i.e. a weakly-reacting serum.

(2). An antigen control is indispensable. It consists of a tube containing saline 0.9 c.c. + antigen 0.5 c.c. If at the end of the period of

incubation this tube shows any flocculation or granularity, the results of the main tests cannot be accepted.

Readings are made immediately on the withdrawal of the tubes from the incubator, and shaking of the tubes is to be avoided since it tends to break up and disperse the flocculi which may have formed, and thereby to render their detection less easy.

Each tube is examined separately and a suitable arrangement of lighting is desirable in order to facilitate the reading. This consists essentially of oblique illumination of the contents of the tube while being viewed against a dark background.

Fine flocculi just visible to the naked eye are recorded as:	+
Definite flocculi in a turbid fluid: ...	++
Flocculant sediment with supernatant fluid slightly turbid:	+++
Coarse flocculant sediment with clear supernatant fluid:	++++

The use of a hand lens is not as a rule desirable but it may be employed to confirm the presence of fine flocculi. The detection of these fine flocculi can be further simplified by causing them to circulate, by gentle agitation of the tube.

Slight turbidity in a tube (greater than that of the antigen control) is recorded as ±

If the antigen control is free from flocculi and if the control sera react normally, the presence of flocculi in

Tubes 1 or 2 (or in both) of a given test is accepted as a POSITIVE reaction, provided also that no flocculi are visible in Tube 3.

In practice it was found that, using this technique, two main difficulties were encountered: -

(a), difficulty in reading the finer degrees of flocculation and

(b), lack of uniformity in the behaviour of the same sample of syphilitic serum when tested on more than one occasion; e.g. very coarse flocculi (++++) in one test; hardly perceptible flocculi (+ or $\pm$)in another. The first of these is possibly dependent to some extent on personal experience but, even conceding that this may be an important factor, the difficulty is none the less very real and, when considered in conjunction with the second one referred to, it has to be appreciated that not infrequently the accuracy of a result hinges on the recognition of extremely fine flocculi in the test mixtures. Confirmation of this is to be found in the writings of most workers although but little stress is laid on its grave significance from a practical standpoint. It amounts indeed to an admission that a result which one observer will record as positive (i.e. flocculation), another or several others reading the same tubes will record as negative (i.e. no flocculation).

Sachs and Georgi orginally recommended the use of an agglutinoscope as an essential part of the technique of their test but in later publications they indicate that a hand lens will also serve the purpose. In the case of the modified Sachs-Georgi technique which is employed in France in "syphilimétrie", its originator, Vernes, is so insistent on the necessity for accurate recognition of the finer degrees of flocculation that he is not content to make readings in the usual way but has devised a somewhat complicated and expensive type of comparator whose use he regards as obligatory if the records, even of experienced workers, are to show reasonable correspondence. It is essential that the existence of this difficulty in reading results should not be minimised since it at once places the Sachs-Georgi reaction on quite a different footing from the Wassermann test. In the latter, there is no justification for any gross discrepancy between the readings of a given test by different observers. Haemolysis is a convincing and easily detected change and the reading of results consists simply in noting its presence or absence. There may be a difference of opinion in the interpretation of a result but there can be none in reading it. In the Sachs-Georgi reaction, on the other hand, the actual reading of a result is of itself often a matter of opinion, and individual opinion may of course be further involved

in interpreting this result (cf. Logan; Wang).

The second difficulty encountered in the use of the Sachs-Georgi technique, viz. the varying intensity of the flocculation induced by the same sample of syphilitic serum when tested on several occasions, merits very close attention. All degrees of this have been experienced by the author, but possibly the most striking example is the one which will now be cited in detail. The table shows the results obtained on three successive days when testing the same sample of syphilitic serum by the Sachs-Georgi technique.

				control.
	Tube No.	1.	2.	3.
9:10:22	antigen (A)	++++	++++	-
	antigen (B)	+++	+++	-
10:10:22	antigen (A)	±	-	-
	antigen (B)	-	-	-
11:10:22	antigen (A)	++++	++++	-
	antigen (B)	+++	+++	-

On each occasion two antigens are employed, viz. A4 and B2 (vide, appendix 6-8).

The serum is a mixture of several Wassermann positive sera from cases of clinical syphilis attending a Venereal

Diseases Treatment Centre, and it is not more than five days old at the time of the first Sachs-Georgi test. It has been stored in an ice-box and is kept there in the intervals between the tests. The portion used on 9:10:22 is heated at 55°C. for half-an-hour, twenty-four hours before testing; that for 10:10:22 at 54°C. for half-an-hour, six hours before testing; and that for 11:10:22 at 55°C. for half-an-hour, six hours before testing. The same sample of saline is used for all the tests. It is kept in a glass-stoppered bottle and its H-ion concentration, as determined by indicator dyes, is pH = 5.8. The same cholesterolised heart-extracts are used in the preparation of the antigens and the total volume of each antigen dilution prepared on each occasion is the same, viz. 6 c.c. The heart-extracts are stored in glass-stoppered bottles in the dark, at room tempera-ture.

All the glassware used in the tests is in regular use for Sachs-Georgi reactions and it is not used for any other purpose. It is cleaned by the author personally and is handled by him alone. The technique on each of the three days is identical, and there is no difference in the time occupied in completing the tests or in the incubation period allowed on the three occasions.

The behaviour of the antigen controls is uniformly normal,

as is also that of a negative control serum.

This instance provides an extreme example of the variations which occur in the Sachs-Georgi reaction but, although extreme, it is not the only one of its kind which has been encountered (cf. appendix 191-192).

It is specially noteworthy on account of the completeness of the control of each of the reagents since, on the three successive days, these were not merely similar, they were identical. Serum, saline, and cholesterolised heart-extracts were portions of exactly the same stock specimens and yet, on one of the days, flocculation failed to occur in the presence of the syphilitic serum. There can be no question of any abnormality on the part of either of the batches of cholesterolised heart-extract used in the preparation of the antigens. Each was an optimum antigen, as determined by the method detailed in appendix 6, and the stocks from which portions were withdrawn on the three occasions presently under review were in regular use during the succeeding six months, when their behaviour was as consistently satisfactory as that of any other Sachs-Georgi antigens with which I have worked. The saline (0.75 per cent. NaCl) differed in no way from what has been found to be

suitable for the purpose of the test and, on several days both before and after 10:10:22, the same sample was in use and normal flocculation occurred in the presence of syphilitic serum.

Its H-ion concentration was determined by indicator dyes on 2:10:22 and again on 11:10:22. On each occasion the finding was the same viz. pH = 5.8. It will be noted that there is a difference in the temperature at which the portions of serum were heated for the three tests, and also in the time interval prior to the test at which the heating was carried out. That these factors cannot have played any part in determining the failure of the test on 10:10:22 will be apparent from the work to be described later (vide, p.62), and accordingly they will not be discussed here. It would appear that the explanation of the absence of flocculation on one of the days can only be attributed to a lack of sensitiveness on the part of the antigens used on that occasion. The preparation of the Sachs-Georgi antigen involves the dilution of cholesterolised heart-extract with saline, with the immediate appearance of turbidity in the mixture, and since the intensity of this turbidity depends on the method by which the mixing of saline and extract is effected it is apparent that the physical state of the antigen can be influenced by the technique employed in

its preparation.

In the case of the Wassermann reaction it has been shown that the physical state of the antigen bears some relationship to its sensitiveness (vide, Browning and Mackenzie), and a similar relationship is implied in the case of the Sachs-Georgi reaction and its various modifications (Meinicke, Vernes, Sigma, Kahn) by the precautions which are taken to try and ensure uniformity in the method of dilution of the heart-extract, and thereby in the physical state of the final product.

In the course of the present studies however all attempts to demonstrate such a relationship have proved unsuccessful. By no minor variation of the standard method of dilution has it been possible to abolish, or even markedly to lessen, the sensitiveness of an antigen, and yet on many occasions dilutions prepared with customary care in the routine manner have proved much less sensitive than usual. Now and again such lack of sensitiveness has been absolute, as in the example just quoted, but it is not proposed to lay great stress on the danger of such complete failures. When these occur they are readily recognised on account of the absence of flocculation with the control syphilitic serum and the repetition of all the tests becomes obligatory. The greatest danger of false results is encountered when working with an antigen

which chances to be only feebly sensitive. Such an antigen may give fairly definite, or even very marked (++++), flocculation with some syphilitic sera, e.g. with the positive control, while with others the flocculation is so fine that it is liable to be overlooked even on very careful examination of the tubes. In my experience this difficulty is of much more frequent occurrence than perusal of the literature might suggest, and it cannot be gainsaid that it constitutes a serious disadvantage of the flocculation test. As a source of fallacy in results it has been encountered much too often to permit of its being passed over lightly, and it is unfortunately not a fallacy which can easily be guarded against by controls which are practicable in routine work.

In view of these two major difficulties which occur in the routine use of the Sachs-Georgi technique an experimental investigation of the various factors which might influence the reaction was undertaken in order to determine whether or not the difficulties could be overcome. The studies lend themselves to being grouped under four main headings:-

(A) SALINE ; (B) SERUM ; (C) ANTIGEN ; (D) METHOD OF TEST, and they are presented in this form in the following pages. It may be noted that, unless it is otherwise indicated, pooled samples of negatively-reacting and positively-reacting sera have been used in all the experimental work,

and that the latter were derived invariably from cases of clinical syphilis with a positive Wassermann reaction. Details of these pooled sera are given in appendix 10.

(A). SALINE.

Varied Concentration of Sodium Chloride. The effect of varying the concentration of Sodium Chloride was studied first as it seemed desirable to determine preliminarily whether the behaviour of normal and syphilitic serum in the Sachs-Georgi test was modified in any way by such a procedure. A stock 10 per cent. solution of pure NaCl in distilled water was prepared, filtered through paper, and stored at room temperature in a sterile, chemically clean, glass-stoppered bottle. From this stock solution, by appropriate dilution with distilled water, a series of salines of diminishing NaCl concentration were easily obtained as required for the tests. The various salines in the series from strongest to weakest were always made the one from the other, and not directly, except in the case of the first, from the stock 10 per cent. solution. Parallel Sachs-Georgi test with normal and with syphilitic serum were then carried out in which salines of differing Sodium Chloride concentrations were used, and so arranged that the only variant in corresponding tubes of the parallel tests was the amount of Sodium Chloride present. In each pair of tests (normal and syphilitic serum), the same saline was used both in the preparation of the antigen and for diluting the sera. The results showed that, within certain limits, the sodium chloride concentration may be

varied considerably without there being any definite effect on the specificity of the Sachs-Georgi reaction, or on the intensity of the flocculation which occurs in the presence of syphilitic serum.
These are tabulated in appendix 22-27 and they may be summarised as follows:-

When the saline contains 1 per cent. or more of sodium chloride there is an increasing tendency for some flocculation to occur in the presence of normal serum, and if the sodium chloride concentration exceeds 1.5 per cent. spontaneous flocculation of the antigen is frequently induced; such flocculation however not appearing until after incubation. It is of interest that this spontaneous flocculation of the antigen by the higher concentrations of sodium chloride is sometimes inhibited by normal serum, i.e. that flocculi appear in the antigen control although they are absent in the tubes containing antigen + normal serum. This however is not the rule and when it has been observed the effect has been limited since, with still greater concentrations of sodium chloride, flocculation occurs both in the antigen control and in the presence of the serum. A similar effect has been noted by Georgi, and also by Meinicke. It is apparent therefore that the concentration of sodium chloride in the saline used in the Sachs-Georgi test must not exceed 0.9 per cent.

if false positive results are to be avoided. At the lower limit of the range of sodium chloride concentrations which have been investigated the results indicate that the presence of the electrolyte is essential for the purpose of the test, as practically no flocculation occurs with syphilitic serum if a saline containing less than approximately 0.2 per cent. sodium chloride is used. The critical concentration of sodium chloride in this respect was found to be 0.18 per cent. and the flocculation obtained with a saline of this strength proved to be very nearly as good as in parallel tests in which larger amounts (0.75 per cent.) of the salt were present. While studying the effect of varied sodium chloride concentration a control was ordinarily included in which no added electrolyte was present, i.e. water was used as the "saline" both in the preparation of the antigen and for diluting the sera. When distilled water was employed flocculation never occurred with syphilitic serum, but when tap-water was used fine but undoubted flocculi occasionally made their appearance, and it seems probable that such positive results are to be attributed to the traces of electrolyte which tap-water usually contains. From these observations on the effect of varying the sodium chloride concentration it seems justifiable to conclude that discrepancies in the results

of Sachs-Georgi tests with the same syphilitic serum on different occasions cannot be due to slight differences in the sodium chloride content of the salines which may be used; some other factor must be responsible.

This conclusion is in keeping with what has been recorded by Münster, but Sachs and Georgi specifically recommend the use of 0.85 per cent. saline since it had been found by Georgi, Georgi and Lebenstein, and Brandt that with weaker NaCl solutions there was a tendency for positive reactions to be less intense. I have not been able to confirm these observations as to the prime importance of 0.85 per cent. concentration.

Over a considerable period, on several occasions, saline containing 0.75 per cent. sodium chloride has been employed in routine tests with individual sera, and the results proved fully as satisfactory as in parallel series in which 0.85 per cent. saline was used. Some syphilitic sera did flocculate less markedly with the weaker saline but, on the other hand, others flocculated more strongly. These minor variations in the intensity of flocculation were quite irregular in their type and it seems more reasonable to ascribe them to individual peculiarity on the part of certain sera than to the use of saline of a particular strength.

Use of Sodium Salts other than the Chloride. Since the presence of electrolyte had been shown to be essential it seemed desirable to study the effect of varying the nature of the electrolyte used, and to this end experiments were undertaken in which, on the one hand, the anion was varied and, on the other, the kation. The question of the possible effect of changes in H-ion concentration through using salts whose solutions varied in reaction was also borne in mind, and the H-ion concentration of the different electrolyte solutions was therefore determined by indicator dyes.

Differing Anions. The following thirty-two Sodium salts have been used and, in every instance, a series of parallel tests has been carried out in which the only variant was the concentration of the particular electrolyte.

Sodium acetate. 11a. 11b.
arsenate. 12, 13.
benzoate. 14, 15.
borate. 16, 17.
bromide. 18, 19.
chlorate. 20, 21.
chromate. 28, 29.
cinnamate. 30, 31.
citrate. 32, 33.
cresotinate (ortho-) 34, 35.
Sodium cresotinate (para-) 36, 37.
cyanide. 38, 39.
ferrocyanide. 40, 41.
formate. 42, 43.
hypophosphite. 44, 45, 46, 47.

(The numbers indicate the pages in the Appendix on which the experiments are shown.)

In general, a 1 per cent. solution of the chemically pure salt in distilled water has constituted the stock supply from which the weaker solutions have been prepared, and, in order to eliminate as far as possible differences due to individual peculiarity on the part of a particular batch of heart-extract, two different samples of cholesterolised heart-extract (each of which was an optimum antigen) have been utilised in these tests. It is to be noted further that the same two samples of cholesterolised heart-extract were used for all tests included in this section, and therefore that comparison of results obtained on different days, or with different electrolyte solutions, is permissible.

The syphilitic serum for these tests was always a pooled

specimen consisting of at least six sera each of which gave a strongly positive Wassermann reaction; the actual complement-fixing power of such pooled sera has been determined from time to time and it usually lay at upwards of 20 minimum hæmolytic doses of complement. The normal serum was similarly a pooled specimen, representing a mixture of not less than ten sera each of which reacted negatively in the Wassermann test, and none of which was derived from a known treated case of syphilis. The Sachs-Georgi technique was employed but, in place of the usual saline, the different electrolyte solutions were substituted in turn, i.e. the electrolyte solution was used both for the preparation of the antigen and as a diluent for the sera. A control with 0.75 per cent. Sodium Chloride solution as the saline was included with each batch of tests.

The results may be summarised briefly by stating that any of the sodium salts in the foregoing list, except such as yield strongly alkaline solutions, can be used instead of the chloride without marked effect on the sensitiveness of the Sachs-Georgi test or on the intensity of positive reactions. Salts which in 1 per cent. solution proved markedly alkaline (e.g. tribasic phosphate, cyanide, borate, sulphite, chromate, benzoate, molybdate) yielded antigens devoid of turbidity,

and usually devoid even of faint opalescence. With such antigens only slight flocculation, or no flocculation, occurred in the presence of syphilitic serum. The citrate, taurocholate, and phospho-tungstate solutions, although not alkaline in reaction, also proved unsuitable as substitutes for the chloride in the strengths in which they were tested. An exception was found in the case of Sodium Iodate which, although frankly alkaline in 1 per cent. solution, proved as suitable as sodium chloride, and this was confirmed by repetition of the experiment. Of the other sodium salts the only one which appeared to yield better positive reactions that Sodium chloride (without causing flocculation in the presence of normal serum) was the salicylate, but this superiority could not be confirmed on subsequent occasions, even when the same samples of electrolyte solutions were used. It seems certain that the coarser flocculation observed when this salt was first tested must have been due to some factor other than the nature of its anion.

Differing Kations. The possibility of replacing Sodium Chloride by neutral salts of other bases in the preparation of the saline used in the flocculation test was also investigated, and the results of experiments of this

kind are shown in appendix 82-89.

In the case of the monovalent kations Potassium and Ammonium (appendix 89), solutions of the chlorides proved as suitable for the purpose of the Sachs-Georgi test as a chemically equivalent solution of Sodium chloride, but when salts with divalent kations were substituted the test was rendered useless. The chlorides of Barium, Strontium, Calcium or Magnesium (appendix 86-88), even when used in concentrations as weak as N/75, all caused spontaneous flocculation of the antigen. With still weaker solutions of these salts the antigen control remained free from flocculi, but flocculation occurred with normal serum as markedly as with syphilitic, i.e. the results were non-specific, and it was not found possible to restore the specificity of the reaction by still greater reduction in the strength of these electrolyte solutions. The normal serum continued to show flocculation until a stage was reached at which the concentration of electrolyte was so weak that no flocculation occurred with any serum, normal or syphilitic. Magnesium sulphate (appendix 82-85) behaved similarly, while the chlorides of Mercury and Cadmium also proved unsuitable on account of their action in precipitating the serum proteins.

These results are in keeping with the observations recorded by Neukirch (1920) who found that N/7 solutions of the bromide, nitrate, acetate, or sulphate of Sodium and the chloride of Potassium could all be used instead of the corresponding sodium chloride solution in the Sachs-Georgi test. They also confirm his finding that Magnesium and Calcium chlorides are unsuitable, but although in his hands the use of Ammonium Sulphate led to non-specific flocculation it is to be noted that this effect is apparently not produced by all Ammonium salts since the chloride, used in the present work, proved a satisfactory substitute for the similar Sodium salt. The conclusion that the presence of electrolyte is essential for the purpose of the flocculation reaction is further borne out by Neukirch's failure to obtain flocculation in tests in which a solution of glucose was used as the "saline", and confirmation has been obtained of his finding that mixtures of Calcium chloride and sodium chloride offer no advantage over simple sodium chloride solution (cf. Witebsky). Various mixtures of these salts have been tried in the course of the present studies but, in all instances in which the controls behaved satisfactorily, the flocculation obtained in the presence of syphilitic serum was much poorer than in the parallel test in which plain sodium chloride solution

was used as the saline. This was specially noteworthy on the numerous occasions on which Ringer's solution was employed; the results were specific but they were invariably weak and therefore particularly difficult to read. The effect of sodium chloride in interfering with the precipitating action of calcium chloride, when mixtures of the two are used in the flocculation test, was noted by Neukirch and investigated in greater detail by Witebsky; it is of interest as affording an analogy to the earlier work of Neisser and Friedemann with mastic sol and trivalent salts.

H-ion concentration of saline.

In view of the possibility that differences in the H-ion concentration of the saline used in the Sachs-Georgi test might influence the course of flocculation, detailed attention has been devoted to this point. The pH values of every batch of saline, and of every electrolyte solution, which has been used has been determined by indicator dyes (bromo-cresol purple; phenol red; Universal Indicator (B.D.H.): phenolphthalein) and it has been found: -

(a), that different samples of saline (0.85% or 0.75% sodium chloride in distilled water) show but little variation in H-ion concentration, as determined by this method;

(b), that the H-ion concentration of saline stored in a glass-stoppered bottle, at room temperature, for from 3 to 21 days ordinarily remains unchanged;

(c), that electrolyte solutions with pH values as widely different as pH = 5.1 and pH = 9.0 may be perfectly suitable for the purpose of the Sachs-Georgi test.

There is thus no evidence that slight differences in H-ion concentration of the saline are of importance in relation to flocculation in the Sachs-Georgi test, and it has further been found that even on rendering the saline

sufficiently alkaline (by the addition of NaOH solution) to yield a definite red colour with phenolphthalein (pH = 9-10) no appreciable effect is produced in the test, either in respect of its specificity or the sensitiveness of the antigen.

The flocculation which occurs with a syphilitic serum is fully as coarse in the test in which the alkaline saline is used as in the parallel test with ordinary saline, and there is no flocculation with normal serum or in the antigen control. Such an observation is exemplified in appendix 204 and it seems sufficiently conclusive to dispense with the necessity for any further consideration of the rôle of slight difference in H-ion concentration of the saline in determining irregularity in the behaviour of the Sachs-Georgi test. Further reference to the question of H-ion concentration will be made later however in connection with the studies on the antigen.

Age of saline. Münster, and Sachs and Georgi, recommend that the saline should be as fresh as possible, while the former states that he regularly observed spontaneous flocculation of negative sera when old saline was used. Nothing similar has ever been noted in my experience, and saline which has been stored for several months and used at irregular intervals has proved consistently satisfactory for the purpose of the flocculation test.

Sterilisation of saline. In Glasgow it has not been found necessary to sterilise the saline for the Sachs-Georgi test. Samples, stored in a glass-stoppered bottle and used at irregular intervals, have remained uncontaminated by moulds or visible bacterial growth for as long as six months, and it has been immaterial whether the solvent was tap-water or distilled water.

Reduction of surface tension. Müller was of the opinion that the presence of certain chemical agents such as ether, acetone, chloroform, etc., favours flocculation as a result of the property of reducing surface tension, and it seemed worth while endeavouring to confirm his hypothesis. The results of the experiments are shown in appendix 98-103. Chloroform was used first of all and it was incorporated in the saline for the tests in concentrations ranging from saturation to 1 in 6,400. The strongest chloroform-saline was prepared by adding excess of chloroform to a portion of the stock 0.85% sodium chloride solution in a glass-stoppered bottle, and shaking repeatedly during the ensuing 48 hours in order to effect saturation. The weaker chloroform-salines were made from a 0.5% solution of chloroform in saline by appropriate dilution with plain saline. Parallel Sachs-Georgi tests were then carried out with normal and with syphilitic sera, so arranged that each

serum was tested: -

(a), using saline without added chloroform,

(b), using salines containing varying concentrations of chloroform.

The results (appendix 98-99) show that the flocculation in the tests with syphilitic serum is fully as coarse and complete in the tubes without added chloroform as in those in which it is present, while when saline saturated with chloroform was used the sensitiveness of the test was definitely diminished. This is exactly the reverse of what was to be expected if Müller's hypothesis is correct.

Ether (0.5% in saline) was used similarly, as shown in appendix 99, but it exerted no appreciable effect on the course of the flocculation. In the case of acetone (appendix 100-101), not only was this reagent used in saline in the same way as described for chloroform (i.e. both for the preparation of the antigen and for diluting the sera) but its possible effect was also studied when added wholly to the serum, the antigen being prepared with plain saline in the routine manner. Even when present in a concentration of 5% it proved to be quite without any effect in favouring flocculation. It is to be noted that for certain of these experiments the Sachs-Georgi technique was modified to the extent of testing

a larger number of serum dilutions than the two used in the standard method, and that this procedure is specially suitable for revealing any action possessed by the reagents under investigation (Mackie). The use of chloroform, ether, and acetone thus failed signally to yield any evidence in support of Müller's contention and it has been further disproved by the results obtained with bile salt (appendix 102), a reagent which reduces surface tension markedly. Solutions of sodium taurocholate when used instead of physiological saline in the Sachs-Georgi test caused inhibition of flocculation (appendix 81),and the same effect was apparent when the bile salt was used in conjunction with sodium chloride (appendix 102). Sera from cases of jaundice however have always been found to react normally. They flocculate only if the patient is a syphilitic, and such flocculation has never been observed to be better than that obtained with non-icteric syphilitic sera tested at the same time, either in respect of its intensity or the range of serum dilutions in which it occurs (e.g. serum 6, appendix 208). Such results are not altogether surprising if one bears in mind that although there is no accurate method for the quantitative estimation of bile salt in serum (Wells) it is probably present, even in cases of severe hepatogenous jaundice,

in amounts too small to influence the course of specific flocculation in the Sachs-Georgi test, e.g. 0. 1 gm. per litre (Gilbert, Chabrol and Bénard). It is noteworthy however that in the Meinicke test (M.R.), in the preliminary stage of which all sera flocculate, some icteric sera flocculate poorly although the majority behave normally (Meinicke; Joel). It may well be that the unsatisfactory flocculation obtained with certain of these sera, under the special conditions of the Meinicke reaction, is related to their bile salt concentration.

There can be little doubt that Müller's results must have been dependent on some factor other than the presence of chloroform, etc., and it may not be inappropriate to recall one's personal experience when sodium salicylate was used as the electrolyte. This salt gave better flocculation than sodium chloride on the first two occasions on which it was used in the Sachs-Georgi test, but its superiority could not be confirmed on repetition of the experiments in spite of the fact that the same sample of sodium salicylate was again employed. Moreover it must not be forgotten that alcohol is already present in the antigen used in the flocculation tests, and that its concentration is sufficient to minimise any surface tension reducing action of additional

reagents such as chloroform. Far from favouring flocculation, the use of these reagents has in my hands either proved to be without demonstrable effect on the course of the Sachs-Georgi reaction or else it has led to definite inhibition of flocculation. Diminution in the intensity of positive results is also associated with incubation of flocculation tests at temperatures higher than 37°C. (Mackie; Baumgärtel; Huber), and although this effect is not necessarily to be ascribed to the reduction in surface tension brought about by the increase in temperature it is permissible to observe that reduction of surface tension is bound to occur under these conditions and that flocculation is not favoured. The evidence which is available all goes to suggest that any action on the part of agents which have the property of reducing surface tension manifests itself not in the way of a favouring of flocculation but the reverse, and this is in keeping with what is known regarding the effect of such reagents on the stability of hydrophilic sols in general.(cf. Dognon). As a rule they tend to increase the stability of such systems and, although exceptions are known, it seems probable that in the case of the mixture of serum and lipoid antigen constituting the flocculation test their action is ordinarily the stabilising one, and that their presence therefore is likely to hinder rather than to help.

Formalin. During the present work it was found that a certain batch of heart-extract, prepared from human heart which had been fixed in 10% formol-saline and then washed thoroughly and dried prior to extraction with alcohol, failed completely as an antigen for the Sachs-Georgi test; no flocculation occurred with syphilitic sera. Since Kirdany (unpublished) had observed that the addition of small amounts of formalin interfered with specific flocculation in the Sigma reaction of Dreyer and Ward, it seemed possible that the presence of traces of this reagent which had not been removed by washing might be responsible for the failure of this extract as an antigen. Similar extracts prepared from dried heart which had not been subjected to preliminary fixation with formalin had already proved themselves suitable for use in the Sachs-Georgi test. Experiments were therefore undertaken to determine what concentration of formalin was sufficient to inhibit flocculation by syphilitic sera when tested with a standard optimum antigen (appendix 6), and in preliminary tests (appendix 90-91) it was found that the use of saline containing at least 1 part in 1,000 was necessary in order that a definite effect might be apparent. Accordingly for the subsequent work a 1 in 300 dilution of commercial formalin in saline (0.75% NaCl) was used as the stock solution, and from this a series of increasing dilutions

were prepared with plain saline, as required. Twenty-four syphilitic sera and five normal sera, all from individual cases, were examined and in every instance parallel tests were carried out using (a), saline without added formalin, and (b), saline containing formalin in varying concentration. The results are shown in appendix 92-97 and they afford a striking demonstration of the very marked inhibitory effect which formalin produces in the Sachs-Georgi reaction. When present in a concentration of 1 in 500, in the saline used for the test, it inhibited flocculation completely in the case of 12 out of the 24 syphilitic sera tested, while with 11 of the remainder it reduced the intensity of the flocculation from "++++" or "+++" to "+" or "±". In most instances a concentration of 1 in 1,000 was sufficient to interfere markedly with the intensity of positive reactions and the effect was more obvious in the tube containing the larger amount of the test serum (0.1 c.c.). Only one of the sera (No. 5, appendix 93) was exceptional in that its ability to flocculate proved relatively resistant to the inhibitory action of formalin, but it is noteworthy that even with this serum the effect of the formalin was evident and that it was greater in the presence of the larger serum volume. A similar zonal effect associated with the presence of formalin is seen with 12 of the other sera and it is specially well

exemplified in the case of No.4 (appendix 96) This observation is of considerable interest in view of the suggestion offered by Mackie and others that the explanation of the zone phenomenon, whereby certain syphilitic sera cause flocculation only when tested in dilutions higher than those ordinarily used in the Sachs-Georgi reaction (cf. appendix 185), is to be sought in the presence of an inhibitory agent in such sera. They have suggested that by dilution of these sera the concentration of the inhibiting agent is gradually reduced until a stage is reached at which it is insufficient to interfere with the flocculating property of the serum; specific precipitation therefore then ensues. In the case of the formalin effect however, such a hypothesis will only be satisfactory if one postulates further that the action of the formalin is indirect, affecting primarily some constituent of the serum in such a way as to render it inhibitory to flocculation; or, alternatively, becoming itself inhibitory as a result of such an interaction. With smaller serum volumes, or with lower formalin concentrations, there might thus be produced insufficient amounts of the inhibitory substance to abolish specific flocculation, although sufficient to lessen it more or less markedly, depending on the degree to which either the serum or the formalin is diluted. In any of the tests

under review, the concentration of formalin in the tube in which flocculation occurs is just as great as in the one in which it is inhibited, and as the volume of antigen and the total volume are also constant there can be little doubt that the inhibition is dependent on an action of the formalin on the serum. Whether the effect is to be interpreted as a direct or an indirect one is highly speculative, and in the meantime it would seem preferable simply to note that formalin consistently interferes with specific flocculation in the Sachs-Georgi test, and that in the majority of cases this interference manifests itself as a zone phenomenon. The possibility of change in the H-ion concentration of the saline sufficient to account for the absence of flocculation, and brought about by the addition of formalin, has been excluded; with several samples of commercial formalin which have been used, the H-ion concentration of a 1 in 500 dilution in saline, determined by indicator dyes, has been found to correspond with that of many batches of ordinary saline which have proved perfectly satisfactory for the purpose of the Sachs-Georgi test.

Summary of Section (A).

(1). The presence of electrolyte is essential for the purpose of the Sachs-Georgi test.

(2). The concentration of electrolyte (sodium chloride) may be varied within considerable limits without affecting the delicacy of the test, but if it exceeds 1% there is a tendency for non-specific results to occur. With concentrations of sodium chloride below 0.2% the intensity of specific flocculation diminishes rapidly.

(3). Slight variations in the sodium chloride concentration of different samples of saline (0.85% NaCl) cannot account for fluctuations in the sensitiveness of the Sachs-Georgi test.

(4). Various sodium salts may be substituted for the chloride, provided that they do not yield strongly alkaline solutions. The citrate, taurocholate, and phospho-tungstate, although not alkaline, are also unsuitable.

(5). The chlorides of Potassium and Ammonium may also be substituted for sodium chloride, but salts of divalent bases are useless for the purpose of the test.

(6). Variations in the H-ion concentration of different samples of saline (NaCl solution) are not of

(6). importance so far as the delicacy of the test is concerned.

(7). The addition of reagents which reduce surface tension, such as chloroform, acetone, or ether, exerts no favourable influence on the intensity of specific flocculation.

(8). Formalin, if present in the saline in a concentration of 1:500, inhibits specific flocculation.

(B). SERUM.

Age and storage. As a rule the sera which have been utilised in the course of the present work have been not more than seven days old when tested, and most of them have been used within 72 hours of withdrawal from the patients. Such samples, both individual sera and pooled specimens, have been kept in a cool place prior to examination and it has been customary in the case of individual sera (i.e. sera each of which is from a separate patient) to leave them in contact with the clot until the day of the test, when they are pipetted off and centrifugalised. The pooled specimens are stored as serum alone (free from clot), and they are centrifugalised at the time of the initial mixing of the sera and again, if need be, just before inactivation on the day of the test. Occasionally older samples of syphilitic serum have been used, but such sera have invariably been kept frozen hard in a refrigerator and their specific flocculating properties have been found to remain unchanged for at least three to four weeks (appendix 150; 153). In some cases a strongly positive Sachs-Georgi reaction (++++ in both tubes) has still been obtained with a pooled serum which had been kept frozen for as long as six

months, but this has not been the rule; such sera usually react weakly. No syphilitic serum however has yet been encountered whose positivity in the flocculation test diminished rapidly in the course of a few days after its withdrawal from the patient (cf. Münster, Mackie), and when any tendency for slight weakening of the reaction with increasing age of the serum has been observed it has generally been found that subsequent tests with the same serum, when still older, failed to confirm any deterioration in its flocculating power. Appendix 148 may be cited as an outstanding example. This pooled syphilitic serum gave a "++++ ++++" reaction when five days old (heated at 56°C., twenty-four hours before test; when eight days old (heated at 55°C., twenty-five hours before test) the reading was distinctly weaker, "+++ ++", but when twelve days old (heated at 55°C., twenty-four hours before test) a "++++ +++" reaction again resulted. Moreover, as will be referred to later, it failed to flocculate when tested on the sixth day (heated at 54°C.), but reacted "++++ ++++" on the eighth day under identical conditions of inactivation. It is obvious therefore that the varying intensity of these flocculation results cannot have been due to a progressive weakening of the reactivity of the serum, nor can it

have been dependent on the inactivation. The only explanation which appears reasonable is that the batches of antigen used for the different tests varied in sensitivity, in spite of the fact that they were all prepared in the same way from the same cholesterolised heart-extract and the same saline. It is very important that the occurrence of such fluctuations in the intensity of flocculation on re-testing the same serum should not be overlooked since it constitutes an ever present source of fallacy when endeavouring to assess the effect of any variation of the test conditions. Examples of similar fluctations with pooled syphilitic sera are shown in appendix 149, 151 and 152, and with individual syphilitic sera (102, 108, and 109) in appendix 136, 139, 140; from these it is apparent that the fluctuation is not always in the direction of a weaker result with the older serum. In half of the cases it is the reverse, and, as these experiments have not been specially selected for tabulation but are typical examples, there does not appear to be any justification even for suspecting that the flocculating power of a syphilitic serum tends to diminish within a few days of the blood being shed. Indeed it is difficult to understand why Münster elected to recommend that the test should be carried out

within three days of the withdrawal of the blood when he himself records a serum which gave a weakly positive reaction when first tested, but a strong positive when re-tested four days later.

In the case of normal serum, there has been only one instance of any departure from negativity in the Sachs-Georgi reaction associated with increasing age of the specimen. All other normal sera, of which more than sixty have been subjected to repeated tests, have yielded consistently negative results, irrespective of their age or the conditions under which they had been stored. The exceptional serum (appendix 107) was a pooled specimen, prepared as usual by mixing at least ten sera derived from patients free from clinical or serological stigmata of syphilis, and it showed no sign of bacterial contamination during the sixteen days that it was available for use. It was tested on eight occasions and the same batch of cholesterolised heart-extract was used in the preparation of the antigens for all the tests. When four, six, and seven days old, it caused no flocculation in any of the tubes of the extended form of test which was being used, but on the tenth day, and on four subsequent occasions, slight but distinct flocculi were present in one or more of the tubes containing the highest serum concentrations,

and these flocculi were not composed of bacteria. The serum controls and the antigen controls for these tests showed no flocculation. The reason for this abnormal behaviour is wholly obscure, but it is very unlikely that it was due to any factor outwith the serum itself, since other normal sera (both pooled and individual specimens) which were tested in parallel with it, on the days on which it flocculated, gave completely negative reactions. Such sera as flocculated on these occasions were either Wassermann positive also, or else they were from patients undergoing anti-syphilitic treatment at a Venereal Diseases Centre, i.e. patients with a definite history of syphilis. One can but record the observation and emphasise that it was quite exceptional.

So far as the age of the serum for use in the Sachs-Georgi reaction is concerned, the outcome of one's experience has been that, within fairly wide limits, this is not a factor of any decisive significance in relation to the delicacy of the test, but that one may occasionally encounter a serum whose behaviour in this respect is abnormal. One syphilitic serum has been observed (vide, p.59) which failed to flocculate when tested within a few hours of the withdrawal of the blood sample, although reacting positively when four

days old, and one presumably normal serum, which reacted negatively during the first week, showed slight flocculating power in all later tests. In order therefore to eliminate such possible sources of fallacy it is desirable to avoid carrying out flocculation tests with freshly shed specimens of blood or with specimens which are more than seven days old. Taniguchi and Yoshinare fixed a similar limit for the age of the serum (7 days), but they make no reference to results with recently drawn specimens.

Bacterial contamination. In the course of these studies when attention was being directed principally to the serum and the influence of changes in it on flocculation, the only factor which has been found to interfere in any way with the Sachs-Georgi test is the occurrence of bacterial contamination. This leads, not infrequently, to a haziness or definite turbidity of the serum which cannot readily be removed by centrifugalisation, and which introduces an additional difficulty in reading results. Bacterial growth has never been found to deprive a syphilitic serum of its specific flocculating power, but it has caused erroneous results in the case of normal serum owing to the appearance of a fine granularity in the tests which simulates specific flocculation (± to +), and which cannot readily be differentiated from such. It is noteworthy that in such cases the serum control tubes rarely show any granularity, thereby rendering this source of fallacy all the greater. Sera therefore which show bacterial contamination are not suitable for flocculation tests, although they may behave satisfactorily in the Wassermann reaction, and when specimens have to be kept for several days it is desirable to store them in a refrigerator in order to lessen the risk of this source of fallacy.

Lipaemia. Certain sera are met with from time to time which tend to separate when kept in a cool place, and to show a scum of lipoid nature which may at first sight suggest bacterial growth. Such sera however are in no way unsuitable for flocculation tests, and if they are thoroughly remixed prior to inactivation it has generally been found that the slight turbidity disappears at the temperature of the water-bath, or at the temperature of incubation of the tests. Even sera of grossly lipaemic type have presented no fallacy in the flocculation tests; the reading of results may be a little more difficult owing to the greater turbidity of the antigen-serum mixtures, but granularity or definite flocculation has not been observed except in the case of specimens from cases of clinical syphilis.

Haemolysis. Serum from a sample of blood in which considerable spontaneous haemolysis has occurred is commonly encountered, but at no time has any evidence been obtained that the presence of such dissolved haemoglobin interferes with the Sachs-Georgi test; syphilitic sera flocculate, while normal sera do not. Since it is recommended however not to use serum showing haemolysis for certain modifications of the syphilis flocculation test (e.g. Kahn test), the effect

of the addition of a haemoglobin solution to syphilitic, and to normal, serum has been investigated experimentally as follows:

The clot from a specimen of normal human blood, withdrawn twenty-four hours previously, is thoroughly broken up with a sterile glass rod, mixed with saline and centrifugalised at high speed. The supernatant fluid is pipetted off and the sediment is washed three times with sterile saline (0.75% NaCl solution). After the final washing, the last traces of saline are removed with a capillary pipette and 10 c.c. distilled water is added to the sediment in the centrifuge tube whose contents are then thoroughly mixed and allowed to stand at room temperature for ten minutes to permit of complete haemolysis. The tube is then centrifugalised until the deep red supernatant fluid is perfectly clear, and 5 c.c. of this is pipetted into a dry sterile tube and mixed with 5 c.c. of 1.5% NaCl solution. A considerable amount of precipitate appears in the mixture and this is removed by centrifugalisation, and the clear red supernatant fluid, representing a saturated solution of human haemoglobin in 0.75% saline, is pipetted off. This constitutes the haemoglobin solution for the experiment and it is used as follows with

four different sera, all of which are free from haemolysis:-

serum (1), POOLED syphilitic serum, 7 days old.

serum (2), Individual do. do., 3 days old.

serum (3), POOLED normal do.,12 days old.

serum (4), Individual do. do., 1 day old.

Serum (4) is from the specimen of blood from which the haemoglobin solution is prepared.

Parallel Sachs-Georgi tests are carried out in which each serum is tested with, and without, added haemoglobin and, for the latter, the haemoglobin solution is mixed with the serum prior to inactivation in order to reproduce as far as possible the conditions obtaining when testing a serum showing spontaneous haemolysis. As a control, a third test is made with each serum in which the serum is heated (inactivated) after dilution with saline instead of with haemoglobin solution. It will simplify the description of the three tests if they are represented thus:-

(a), serum heated at 55°C.

(b), serum diluted with an equal volume of haemoglobin solution and then heated at 55°C.

(c), serum diluted with an equal volume of saline and then heated at 55°C.

In order not to miss any effect which the addition of the haemoglobin might exert, the modified Sachs-Georgi

technique (appendix 9) is used, and seven tubes containing mixtures of serum and antigen are set up for every test instead of two. All tubes contain the same total volume, comprising 1.0 c.c. of a serum dilution and 0.5 c.c. of antigen, and an additional antigen control is included, as follows:-

saline ----------	0.8 c.c.
haemoglobin solution (heated at 55°C)	0.2 c.c.
antigen ----------	0.5 c.c.

The same antigen is used for the whole of the tests. The results of this experiment are shown in appendix 109-110 and it is clear that the addition of the haemoglobin to the various sera, both syphilitic and normal, was without the slightest effect on their Sachs-Georgi reactions. This observation serves to confirm numerous personal experiences with other sera showing various degrees of spontaneous haemolysis, and it warrants acceptance of the view expressed by Münster and others that such sera are not unsuitable for use in the flocculation test.

BILE PIGMENT. Sera from cases with jaundice have already been referred to (p.33), and the presence of bile pigments in serum has not been found to occasion any fallacy in the Sachs-Georgi test.

SERUM CONTROL. The inclusion of a serum control as an essential part of the Sachs-Georgi test has invariably been practised but only on one occasion was it found to serve any useful purpose (vide p.95). Had it not been for this solitary exception I would agree with Meinicke, Mackie, Kahn, and others, that the setting up of serum controls is a waste of time, but there can be no doubt that although sera which flocculate spontaneously must be extraordinarily rare it is essential to exclude this possible source of fallacy. Bacterial growth as a cause of turbidity in a serum control is referred to by several authors (eg. Rice) but I have not observed it in any of my tests; its occurrence is probably dependent to a large extent on local conditions. (cf. p.48).

INACTIVATION. In the great majority of the modifications of the syphilis flocculation test presently in use the patient's serum is employed in the inactivated state, i.e. after preliminary heating in a water-bath at 55°C. The only notable exceptions are two of Meinicke's reactions (Trübungsreaktion, "M.T.R", and Klärungsreaktion, "M.K.R") for which fresh, unheated, serum is used. Mackie, however, in the course of an experimental study of the flocculation test for syphilis, was led to conclude that the sensitiveness of the reaction as a serodiagnostic method was increased, without its specificity being in any way impaired, when fresh serum was used instead of serum which had been inactivated at 55°C He recommended further that the test should be carried out as soon as possible after withdrawal of the blood sample from the patient, since he found that the flocculating property of fresh (unheated) syphilitic serum diminished, the longer the interval between the shedding of the blood and the performance of the test. Mackie's work in this connection has been repeated in the present studies, but his results have not been confirmed; indeed, with the sera which have been examined in Glasgow, the results have been practically the reverse. Arrangements were made with the physicians in charge

of the Venereal Diseases Treatment Centre at the Western Infirmary whereby the specimens of blood for Wassermann test were delivered at the laboratory within one hour of withdrawal from the patients. These samples, which were received in batches, were centrifugalised as soon as they arrived and sufficient clear serum was pipetted off for the purpose of parall--el Sachs-Georgi tests; (a), with the fresh (unheated) serum, and (b), with the serum after inactivation. Mackie's method of employing a range of serum concentrations instead of the two which constitute the routine technique of the Sachs-Georgi test was followed, since in this way a more extended comparison of the results obtained with the inactivated serum and with the fresh serum is possible (appendix 9). In the first tube for such a test, 2.0 c.c. of a 1 in 5 dilution of fresh, or inactivated, serum is prepared; the remaining tubes each receive 1.0 c.c. saline. By withdrawing 1.0 c.c. of the contents of tube I, transferring it to tube II, mixing and transferring 1.0 c.c. of the mixture to tube III and so on in the series, a range of increasing serum dilutions is easily and accurately prepared. From the last tube in the series 1.0 c.c. is withdrawn and rejected, so that, when this stage of the setting up of the tests is completed, each

tube contains 1.0 c.c. of a mixture of serum and saline. All the sera were dealt with in this manner and there were two rows of tubes for every specimen; the one containing fresh (unheated) serum and the other inactivated serum. When all the serum dilutions had been prepared, sufficient antigen was made for the whole batch of tests and 0.5 c.c. was pipetted into every tube; this proportion being the same as in the standard Sachs-Georgi technique. Serum controls, consisting of serum (fresh, or inactivated) 0.2 c.c. + saline 0.8 c.c. + alcohol-saline 0.5 c.c., and an antigen control were always included as usual.

Using this method, it was found practicable to deal with as many as fifteen samples of blood at one time so that the completed tests could be placed in the incubator within three to five hours of the withdrawal of the specimens from the patients. A total of 110 sera, all derived from cases attending, or presenting themselves at, the Venereal Diseases Treatment Centre were subjected to this extended form of Sachs-Georgi test, and every one of these sera was tested both heated and unheated. The results are shown in detail in appendix 111 to appendix 127 where, for the sake of simplicity, the readings are tabulated consecutively, instead of in

the form of separate tables for each day's tests. This arrangement is permissible since there was uniformly satisfactory behaviour of all controls during the five weeks over which this part of the investigation extended. The same sample of cholesterolised heart-extract was used in the preparation of the antigens for all the tests, and the flocculation in the presence of the syphilitic sera, used as controls, was invariably of +++ or ++++ type in the tubes containing serum dilutions 1 in 10 and 1 in 20 (Standard Sachs-Georgi test). The Wassermann reactions of the sera were also tested, usually from 1 to 3 days later, and these are shown in the tables for the purpose of comparison. It may be noted that, when any gross discrepancy existed between the Wassermann and Sachs-Georgi results, both tests were repeated and the original findings were confirmed.

The results obtained in the series of parallel tests with heated and unheated sera may be summarised as follows:-

Sera showing NO flocculation in either test ------ 47.
Sera showing flocculation in one or both tests --- 63

110.

With the 63 positively reacting sera, flocculation occurred:-

Only with heated serum ----------34 cases.
Only with UNHEATED serum ----------nil.
With both heated and unheated serum ----------29 cases.

It is specially noteworthy that not one case was encountered in which flocculation occurred only with UNHEATED serum, and that approximately one half (54%) of the 63 positively reacting sera failed to show any flocculation when tested UNHEATED. Furthermore, a comparison of the intensity of the flocculation obtained with unheated serum as opposed to heated serum, in the case of the 29 sera which flocculated in both tests, is again unfavourable to the use of unheated serum:

flocculation equally good in both tests ----------6
flocculation slightly better with unheated serum ----2
flocculation better with heated serum ----------21

In continuance of the repetition of Mackie's work, ten positively reacting sera from the foregoing series were re-tested later, when they were from three to seven days old (appendix 128-140). The tests were again carried out in the extended form (appendix 9), and each serum was used both heated and unheated. Three sera (appendix 131, 133, 136) which had failed to flocculate in the unheated state at the first test failed again when re-tested; heated however, they/

flocculated as strongly as when similarly tested on the first occasion. The remaining seven sera had all reacted positively, both unheated and heated, when tested originally but, on re-testing, it was found that only four of them had retained any flocculating power in the unheated state, and that this was now weaker. After heating, they still flocculated as strongly as at first (appendix 129, 132, 137, 138).

Special interest attaches to the observations with serum 108 (appendix 139). This serum, whose Wassermann reaction was positive, had failed to cause any flocculation (heated or unheated) when tested within four hours of its withdrawal from the patient. It was re-tested four days later with the following result:-

Sachs-Georgi reaction (unheated serum), Negative.

Sachs-Georgi reaction (heated serum), Positive.

The possibility of an error having been made in carrying out either of the sets of tests was considered very carefully, but it seems as certain as it is possible to be in such matters that the safeguards in use were adequate to prevent an error in technique passing undetected. All the other sera tested at the same time gave results in keeping with their Wassermann reactions, and the same control sera were used on both occasions

and behaved equally well. It must be noted however that on the day of the second test the antigen control showed fine flocculation (+), and that it might be contended therefore that instability of the antigen on this occasion had rendered it more sensitive to traces of specific flocculating power possessed by this particular serum. That such was not the explanation can be stated with certainty, since a strong positive Sachs-Georgi reaction was obtained with the same serum (heated) two days later when spontaneous flocculation of the antigen did not occur. There seems little doubt, therefore, that one was dealing with a syphilitic serum which was unsuitable for the flocculation test until some time after the blood had been shed, and whose syphilitic nature would have been missed if the test had been carried out with the freshly drawn serum. It is not my desire to exaggerate the importance of such a source of fallacy in the flocculation test. The finding was quite exceptional and it is of interest chiefly in emphasising that sera are occasionally met with which are abnormal in their behaviour. One can but conclude that Mackie's sera which flocculated better when freshly drawn, and when unheated, come into a similar category, or that his results were dependent on the nature of the heart-

extract which he used and the manner in which he diluted it. No example of this peculiarity --- indeed no suggestion of it --- has been demonstrated in any of the 63 syphilitic sera specially investigated in the course of the present work, and the evidence has all pointed to the marked superiority of heated serum over unheated serum for the purpose of the Sachs-Georgi test. Furthermore, if cognisance is taken of the exceptional serum which failed to flocculate when recently drawn, but which reacted positively four and six days later, it would appear to be desirable to avoid carrying out flocculation tests with freshly shed specimens of blood in order to eliminate this possible source of fallacy. Watson subsequently confirmed that recently drawn samples of syphilitic serum, when used unheated, might fail to flocculate in the Sachs-Georgi test, and as he employed Mackie's technique in carrying out the tests (extended range of serum concentrations) his results, like those of Taniguchi and Yoshinare and those recorded above, are free from the objections raised against the original one-tube method. Georgi, Münster, Neukirch, and Sachs and Georgi, using the original method, have all recorded similar observations, and concluded that unheated serum was unsuitable for the

purpose of the Sachs-Georgi test.

Temperature of inactivation. Since no doubt can be entertained that it is essential for the satisfactory behaviour of the Sachs-Georgi test that inactivated serum should be used, the question of the optimum temperature for such inactivation falls to be considered. This may be approached in two ways; (a), observation of the behaviour of the same syphilitic serum divided into several portions which are heated at different temperatures, and which are then all tested in parallel, at the same time, with the same antigen, or (b), reviewing the results obtained with a considerable number of syphilitic sera heated at different temperatures and tested with different antigens in the course of the routine application of the flocculation test over a period of several months. Both of these methods have been utilised and in appendix 146 examples are shown of parallel tests in which the same pooled sample of syphilitic serum, heated at different temperatures, has been used. The results obtained are in keeping with those of other workers (cf. Georgi, Neukirch, Watson) and it was not deemed necessary to pursue this line of investigation further. At a tempera-

-ture of 53°C. there is a falling off in the intensity of the subsequent flocculation, while a similar weakening is evident at the upper limit of the temperature range studied (61°C.). As these extremes however are quite outwith the range of temperature variations likely to occur when a water-bath regulated by a thermostat is used, the effect of such inactivation temperatures on the course of the flocculation test is not of practical importance. In the present work a water-bath regulated at 55°C. to 56°C. has ordinarily been employed for the inactivation of the sera, and the same thermometer has been in use throughout. Any fluctuations in temperature, below 55°C. or above 56°C., have either been produced intentionally for experimental purposes, or they have been due to accidental damage to the thermostat, noted prior to the carrying out of the tests.

The analysis given in appendix 154-156 of the results of 102 tests with 33 different samples of pooled syphilitic serum lends support to the conclusion that minor variations in the temperature of inactivation can have but little effect on subsequent flocculation. The actual tests on which the analysis is based were spread over a period of eighteen months, in the course of which several

different batches of cholesterolised heart-extract were used as antigen. All of these were optimum antigens, and every one of the sera reacted strongly positive in the Wassermann test, and was derived solely from cases of clinical syphilis. For purposes of comparison, the flocculation results have been classified in four groups:-

GROUP.		SACHS-GEORGI READINGS.
I.	=	++++ in both tubes of test.
II.	=	++++ in one tube; +++ in the other.
III.	=	+++ in both tubes of test.
IV.	=	all other degrees of flocculation.

Groups I and II include all the strongly positive flocculation reactions, while the feeble reactions constitute Group IV. The results obtained with sera which had been inactivated at 54°C. or 57°C. are fully as satisfactory as those with sera inactivated at 55°-56°C. and, although the total number of tests in the former class is much smaller than in the latter, it may be permissible to note that the percentage of strongly positive reactions is practically the same in both (47% and 44%). In the case of the feeble reactions (Group IV) the analysis again fails to indicate any unfavourable

effect on flocculation of inactivation temperatures of 54°C. or 57°C. 34% of the tests with sera inactivated at these temperatures come into Group IV, while with sera inactivated at 55° - 56°C. 35% of the tests fall into the same category.

The opportunity may be taken here to emphasise that, in spite of the fact that one was working with pooled specimens of syphilitic serum, and that the tests were all carried out under optimum conditions as far as these are known, only 46 out of a total of 102 tests yielded strongly positive Sachs-Georgi reactions; further, that 36 others (Group IV) gave flocculation of the type which is difficult to read and which, in a proportion of the cases, was liable to be overlooked (+ or ±). Such results are unfortunately in complete agreement with my general experience of the behaviour of the Sachs-Georgi test and they compare very unfavourably with what can be reasonably expected of the Wassermann reaction, especially in view of the unequivocal nature of the sera which were used.

As has been mentioned previously in this section it is very difficult indeed to assess the effect on flocculation of any one factor, such as variation in the temperature of inactivation of the

serum, since other factors, only some of which are known, have probably been operative at the same time. An alteration in the intensity of flocculation associated with the use of a particular sample of serum is not necessarily to be ascribed to the special treatment to which that sample may have been subjected, and a repetition of the experiment may often yield an entirely different result. The case shown in appendix 148 may be recalled as it merits special emphasis in this connection.

On 10:10:22 this syphilitic serum which had been inactivated at 54°C., instead of at 55°-56°C., gave no flocculation in the test, yet on 12:10:22 a further portion of the same serum, under exactly similar conditions of inactivation and tested with the same cholesterolised heart-extract as antigen, gave a ++++ reaction. It is on account of the relative frequency with which such apparently contradictory results have been encountered in experimental work that one is disinclined to attach much importance to effects which are associated only occasionally with modifications of some particular physical factor, and that it seems preferable, as in the present instance, to focus attention on a broad analysis of the results obtained in an extended

series of reasonably comparable tests. So far as the question of the temperature of inactivation of the serum is concerned, my observations would appear to warrant the conclusion that any temperature between 54°C. and 57°C. is suitable, and that with the use of a water-bath regulated by a reliable thermostat such fluctuations in temperature as may occur are very unlikely to account for the irregularities in flocculation which prove so vexatious in the routine application of the Sachs-Georgi test.

Interval between inactivation of serum and test. No specific reference has been found in the work of Sachs and Georgi to the length of time which should be allowed to elapse between the inactivation of the serum and the carrying out of the test but Taniguchi and Yoshinare recommended that it should be several hours, and it is probable that they were influenced in this by Münster's observations and by Meinicke's statement that serum should never be used for his flocculation test immediately after inactivation. Meinicke believed that an interval was necessary to permit of stabilisation of the change induced in the serum by the heating, but he apparently based this conclusion on hypothetical considerations alone. In any case, so far as Taniguchi and Yoshinare are concerned, these workers were interested primarily in a comparison of the results of the Wassermann and Sachs-Georgi tests, and not in an experimental study of the latter, so the technique which they employed was determined almost wholly by the recommendations of the original contributors, and not by personal investigation of the various factors which might influence the test.

Very shortly after the commencement of the present studies, evidence began to accumulate

which suggested that better results, in the way of coarser flocculation, were frequently obtained when the tests were carried out with sera which had not been allowed to stand for the usual four to six hours after heating, and detailed attention was accordingly directed to this point. A series of samples of pooled syphilitic serum were used and each serum was divided into several portions which were heated for half-an-hour in the same water-bath, at varying intervals before the test. The different portions of each serum were then all tested at the same time, with the same antigen, and it was usually arranged that the completed tests were ready for incubation within half-an-hour of the inactivation of the last portion of serum. An example of such an experiment is given in appendix 142 and, in this instance, the increased intensity of flocculation associated with the use of the most recently inactivated portion of serum is particularly striking. It is comparatively rarely however that the variation in the results obtained with the different portions of a serum has been so well marked, and in many instances no appreciable difference has been demonstrable (e.g. appendix 143, 144). In others (e.g. appendix 141, 145) it has been so slight as to come within the limits

of what has been observed when duplicate parallel tests have been carried out with a serum, using the same antigen for both tests (e.g. appendix 105-106). None the less it is to be noted that, although the use of syphilitic serum immediately after inactivation has only very occasionally been associated with great increase in its flocculating power, no evidence has been obtained so far that a serum ever flocculates less strongly when tested immediately after inactivation than when tested after an interval of several hours. The outcome of one's experience in this respect has led to the conclusion that, in the Sachs-Georgi test, it is preferable to use sera as soon as possible after they have been inactivated, e.g. within half-an-hour, and that there is no necessity whatever to allow them to stand at room temperature for some time before use. The analysis of the results of 102 tests with pooled syphilitic sera, to which reference has already been made in connection with the temperature of inactivation, (appendix 154-156) is of value in confirming the validity of this conclusion, since it shows that not only was the highest proportion of strongly positive Sachs-Georgi reactions obtained with the sera which were tested within half-an-hour of inactivation, but also that with these sera the smallest proportion of feeble reactions occurred.

Normal sera were of course included in this experimental work and no tendency to non-specific flocculation was ever observed as a result of testing them immediately after inactivation. Meinicke's contention therefore, although it may be justified so far as his particular form of the flocculation test is concerned, is not of general applicability and it is of interest that Kahn (1930) has now come to the conclusion that for his test also "it is desirable to use the serum soon after heating, if possible, within 30 minutes". He recommends that, if it has to be kept for four hours or longer, it should be re-heated for ten minutes just before use.

Summary of Section (B).

(1). As a rule, the age of the serum is not of importance so far as the delicacy of the Sachs-Georgi test is concerned. Freshly shed serum is undesirable and it is preferable to use specimens which are not more than a week old.

(2). Sera which show bacterial contamination are unsuitable for use in the flocculation test.

(3). Lipaemic sera, bile-stained sera, or sera which show spontaneous haemolysis, occasion no fallacy in the test.

(4). Inactivation of the serum is essential, and no useful purpose is served by carrying out duplicate tests with fresh (unheated) serum.

(5). Sera should be tested as soon as possible after inactivation.

(6). Fluctuations in the intensity of specific flocculation are not dependent on slight variations in the temperature at which the sera may have been inactivated. In general, any temperature between 54°C. and 57°C. is suitable.

(C). ANTIGEN.

In considering the question of the part which may be played in the flocculation test by factors which affect primarily the antigen it is necessary as a preliminary to make it quite clear that human heart muscle has been used as the source of the alcoholic extract for all the work to which reference will be made in this section. Sachs and Georgi prepared their original extracts from beef-heart, guinea-pig's heart, or syphilitic liver, but in a later publication (1920) they stated that human heart is also suitable. Wodtke found human heart satisfactory and Taniguchi and Yoshinare used it exclusively in the series of comparative tests which they carried out. Their flocculation results are fully as good as those obtained by many other workers who used beef-heart antigens, and there is thus adequate precedent for employing human-heart extract in the Sachs-Georgi reaction without necessarily having to fear that it may be inferior to beef-heart extract for the purpose of the test. It does not react with heterophile antibody (Taniguchi) and it is therefore preferable to extracts made from guinea-pig's or horse's organs, a point of considerable importance /

although one which appears to have been overlooked by several workers. The false positive results, for example, obtained by Georgi (1918), both in complement-fixation and flocculation tests in which guinea-pig's heart-extract was used as antigen, are obviously explicable on the basis of heterophile antigen-antibody reactions; and the fact that sheep's blood corpuscles contain heterophile antigen, although this has not been demonstrated in sheep's organs, inclines one to regard the use of sheep's heart-extract as less desirable than that of similar extracts from frankly non-heterophile species (cf. Mackie). It may introduce the possibility of a similar source of fallacy. Human heart is used by Bruck; horse heart is preferred by Meinicke for the various tests which he has devised (except the recent "Klärungsreaktion"), and by Vernes for "syphilimétrie"; beef heart is used by Kahn, by Sachs and Witebsky ("citocholreaktion"), and now also by Meinicke ("M.K.R."); calf's heart by Dreyer and Ward (Sigma reaction), and by Dujarric de la Rivière. In the case of the last two, the preparation of the calf's heart-extract is carried out as described by Bordet and Ruelens but Meinicke, Vernes, Sachs and Witebsky, and Kahn detail special

methods for their antigens to which reference will be made later (vide, p.106).

The titration of heart-extract in order to determine the optimum dilution in which to use it, and the optimum amount of cholesterol which should be added, is an essential step in the Sachs-Georgi technique, but,since this procedure involves dilution of the alcoholic solutions with saline,it may be well in the first place to discuss what effect such dilution may have on the final product as used in the test. Sachs and Georgi have contended ever since they introduced their test that the method of dilution of the cholesterolised heart-extract with saline is of prime importance in relation to the sensitiveness of the mixture as an antigen, and they apparently base this assertion mainly on the previous work of Sachs and Rondoni in connection with the antigen for the Wassermann test (vide, Browning and Mackenzie). Gaehtgens, Hohn, and Freiwirth also express the opinion that the sensitiveness of the antigen is probably dependent on the state of its dispersion, and therefore on the manner in which the dilution of the extract with saline is carried out. Georgi (1918), it is true, published a single set of experiments which had as its aim the demonstration of the effect on flocculation of variation in the method of dilution of the antigen, but his results are not convincing as the slight differences which he tabulates are no greater than what may well occur

in any set of duplicate Sachs-Georgi tests. In the course of the present studies it was not long until doubts arose as to whether the importance of this factor had not been exaggerated, and considerable attention has therefore been devoted to trying to elicit information on this point.

Turbidity. The method of dilution recommended by Sachs and Georgi consists of pouring one volume of saline as quickly as possible into one volume of cholesterolised heart-extract, and immediately thereafter pouring the mixture from one tube to the other several times. Turbidity appears almost instantly and it increases quickly in intensity. The turbid emulsion is allowed to stand at room temperature for a short time and it is then further diluted with an additional four volumes of saline, the mixing being effected rapidly as in the first instance. The dilution prepared in this way constitutes the antigen used in the tests, but it is allowed to stand at room temperature for from five to fifteen minutes before being added to the serum-saline mixtures in the tubes. Except for experimental purposes this method has been rigorously adhered to in the present studies and, in the tables shown

in the appendix, any deviation from the standard technique is invariably indicated.

There can be no doubt that the turbidity of the routine Sachs-Georgi antigen is wholly dependent on the amount of saline added to the heart-extract at the primary dilution, and the time that is allowed to elapse before diluting further. If a series of tubes is arranged each containing 1.0 c.c. cholesterolised heart-extract and if these are diluted in turn with volumes of saline ranging from 0.1 c.c. to 2.0 c.c. (by increases of 0.1 c.c.), it is found that the addition of the smallest amounts of saline (0.1 and 0.2 c.c.) fails to cause any trace of turbidity in the mixtures, but that as the proportion of saline to extract increases they become progressively more and more opalescent and finally turbid. If the volume of saline is in excess of the volume of extract there is a gradual diminution in the intensity of the turbidity, and this is rendered much more obvious when the final dilution of the mixture to 1 in 6 has been carried out. The proportion of one volume of saline to one volume of extract generally falls within the zone in which maximum turbidity is produced and, as has been noted above, this turbidity

tends to increase in intensity the longer the mixture is allowed to stand at room temperature. If the second stage of the dilution is carried out immediately after the first, a less turbid antigen is obtained than if an interval of five minutes or longer is allowed between the dilutions. With certain batches of heart-extract it has been found that the proportion of one volume of saline to one volume of extract, in the primary dilution, may not yield the most turbid antigen which can be prepared. The extract for example which was used for the experiments shown in appendix 167-168 gave maximum turbidity by mixing 1.0 c.c. with 0.75 c.c. saline and subsequently adding 4.25 c.c. saline, to make the final dilution 1 in 6., but this is to be regarded as exceptional and the standard Sachs-Georgi technique nearly always yields the most turbid antigen which it is possible to prepare by carrying out the dilution in two stages. An even more turbid emulsion can be obtained by adding saline slowly, drop by drop, and shaking the mixture thoroughly all the time or, in the case of some extracts, by floating the extract on the saline and causing gradual mixing of the two by slow rotation of the tube. As will be seen however,

antigens made in either of these ways are often unsuitable for use in the Sachs-Georgi test.

The behaviour of a series of antigens of maximum turbidity, prepared in different ways with the same samples of cholesterolised heart-extract and saline, is exemplified in appendix 158, and 167--170 which show that, although all the antigens were homogeneous in appearance when they were ready for use, certain of them flocculated spontaneously during the period of incubation of the tests. Such antigens are of course unsatisfactory and it will be noted that they include those in which the dilution was effected slowly and some in which an interval of more than ten minutes was allowed between the first and second additions of saline. It is apparent therefore that the technique recommended by Sachs and Georgi aims at producing an antigen of maximum turbidity compatible with sufficient stability for the purpose of the test. The most turbid antigens are unsuitable because they tend to flocculate spontaneously, but it must be emphasised that this behaviour is not always uniform. On several occasions a slowly diluted antigen, or a rapidly diluted one which had been allowed to stand for

fifteen to thirty minutes between the first and second additions of saline, has proved sufficiently stable to remain homogeneous throughout the period of incubation of the tests. With such antigens however the element of uncertainty as to whether or not they will flocculate spontaneously is ever present, and they are undesirable for routine use on account of the relative frequency with which this occurs, thereby necessitating repetition of the whole batch of tests. In practice it has been found that the method of dilution originally prescribed by Sachs and Georgi is very reliable and that it is only on rare occasions that spontaneous flocculation of the antigen is observed. Unfortunately however it does occur sometimes, without apparent cause, and I cannot agree with Mackie (1921) that the inclusion of an antigen control is unnecessary or that it is ever justifiable to dispense with it.

Sensitiveness. The problem of the relationship which may exist between the turbidity of an antigen and its sensitiveness in the flocculation test is beset with peculiar difficulties, not the least of which is the fact already referred to that slight but definite differences in the intensity of flocculation are observed from time to time in duplicate tests in

which the same sample of syphilitic serum and the same sample of antigen are used. A series of such tests is shown in appendix 105-106 and it is to be noted that, when one speaks of the same sample of antigen being used in each pair of tests, one means that sufficient 1 in 6 saline dilution of the cholesterolised heart-extract was prepared to serve for both tests, and therefore that exactly the same reagent was pipetted into all tubes. Saline, inactivated serum, and antigen dilution', were all standing ready and duplicate tests were set up with them instead of one. When variations in the intensity of flocculation can occur in such circumstances it is not surprising that similar variations are frequently observed when a sample of serum is tested with two separately prepared dilutions of the same heart-extract with the same saline, and there can be little justification for assuming that such differences are necessarily due to undetected modification of the technique of dilution of one of the antigens (appendix 160, 161, 164, 165). Minor differences cannot be regarded as significant and, when one is studying the effect of varying the method of preparation of the antigen dilution deliberately, it is essential that a certain technique should usually be

associated with definitely poorer, or definitely better, results before it can be permissible to conclude that this particular technique has any influence on the sensitiveness of the antigen. With this in mind it has to be admitted that all attempts to prepare antigens of consistently diminished sensitiveness by some well defined departure from the standard technique of dilution have failed signally, except in the case of certain antigens which are wholly free from turbidity. The only success which has attended these efforts has been in the way of producing emulsions which flocculate spontaneously, i.e. which are hypersensitive, and which are therefore quite useless for test purposes. By no modification of the method of dilution of the cholesterolised heart-extract with saline have I ever succeeded in abolishing the sensitiveness of the resulting antigens, and I have therefore been quite unable to reproduce experimentally an antigen which, like the one cited at the beginning of this summary (p. 11), would fail to show gross flocculation in the presence of pooled syphilitic serum.

Initially the effect of pouring the extract into the saline at the primary dilution,

instead of vice versa, was investigated but it was found that this had no definite influence on sensitiveness. Sometimes an antigen prepared in this way gave slightly poorer flocculation with syphilitic serum than a standard antigen tested in parallel with it, but there was often no appreciable difference, and sometimes the result with the modified antigen proved the better of the two. Appendix 158 will serve to illustrate the usual findings. Similarly, as is exemplified in appendix 167-168, the effect of allowing a longer interval than five minutes between the first and second dilutions is ordinarily either insignificant or undetectable, unless indeed it leads to spontaneous flocculation of the antigen. The possibility of temperature variations being a factor of importance was next considered and antigens were prepared with saline at 0°C., at room temperature, warmed to 55°C., or heated to 100°C. Examples of such experiments are shown in appendix 159, 166, 169, 170, and it is clear that any differences in flocculation which occurred were either trivial or else they appeared in one set of tests but not in another. In the case of appendix 159, for example, the antigen prepared with saline which had been heated to 100°C. flocculated

comparatively poorly with the syphilitic serum used on that occasion, but this was quite an exceptional observation. As a rule such an antigen has been found to react just as well as the control prepared with saline at room temperature and, as is shown in appendix 166, it is often possible actually to boil the antigen without producing thereby the slightest impairment of its sensitiveness. Boiling the antigen, however, is one of the instances of a modification in technique which has occasionally been associated with the occurrence of spontaneous flocculation, and it would appear that stability or instability in this respect may vary with different samples of cholesterolised heart-extract. Some extracts indeed when diluted with saline are so relatively unstable that they precipitate at once on being brought to the boil, e.g. "citochol" extract (Sachs and Witebsky). Shaking the antigen vigorously in a shaking-machine, centrifugalising it for fifteen minutes at approximately 4,000 revolutions per minute, or allowing it to stand for from nine to twenty-four hours before use have also all proved ineffective in depriving it of sensitiveness or even in rendering it relatively insensitive.

Since the rôle of such factors in

influencing the sensitiveness of the antigen could thus be discounted, attention was directed specifically to the effect of varying the turbidity of the emulsion which is used in the test. In all these experiments the ultimate dilution of the heart-extract is 1 in 6, so that the various antigens are all of the same strength although differing in physical appearance, and it is to be noted also that the extracts are all optimum antigens (vide, appendix 6-8). It was mentioned previously, in connection with the dilution of cholesterolised heart-extract with saline, that great differences in turbidity can readily be produced by varying the proportions in which these are mixed in the initial stage, and that an antigen of maximum turbidity is obtained when the saline is added drop by drop to the extract, with constant shaking of the mixture. Minor differences in turbidity, when using the same proportions of saline and extract, are observed as a result of variation in the rate at which the two reagents are mixed, or in the time that is allowed to elapse before the second stage of the dilution is carried out. These slight differences are best detected by the method used in standardising the opacity of bacterial suspensions, viz. by comparing the blurring

effect on clearly printed type when it is viewed through tubes of uniform calibre containing the suspensions. It was found that, judged in this way, there is often a difference in the opacity of two samples of the same Sachs-Georgi antigen, depending on the total volume of the dilutions which have been prepared, and it is not improbable that this is related to slight variation in the rate at which thorough intermixture of the saline and heart-extract has been effected. Many observations have since been made with pairs of antigens differing only in their total volume, and several experiments of this kind are tabulated in appendix 160 to 165. From these it is apparent that any variation in the intensity of flocculation obtained with the different antigens is very slight, and that the result is sometimes in favour of the smaller volume but just as often the reverse. Moreover, similar variation occurs not infrequently with two antigens of the same total volume, e.g. appendix 160 and 161, in spite of the fact that no difference in opacity can be detected. Very turbid antigens, prepared either by the drop method of dilution or by floating the extract on the saline and mixing very slowly (appendix 158, 167, 169), have not

proved more sensitive than the definitely less turbid control antigen, and they have the great disadvantage of tending to flocculate spontaneously. At the other extreme, when one resorts to the use of antigens of feeble turbidity, the evidence which has been obtained is very conflicting. It was noted in the section in which variation in the concentration of the electrolyte was being considered that many syphilitic sera still showed ++++ flocculation in the presence of as little as 0.2% NaCl (a strength which yields an antigen of very slight turbidity), and that certain sodium salts which could be substituted for the chloride yielded antigens which were only faintly opalescent. It was apparent therefore that diminished turbidity of the antigen did not necessarily imply diminished sensitiveness, but that such an effect might possibly be associated with the use of a particular sample of antigen, or with a particular sample of serum. It must be emphasised however that one is disinclined to attach major importance to the part played by the serum in determing such results, in view of the fact that one was using pooled specimens from undoubted cases of syphilis with strongly positive Wassermann reactions. A very large number of such specimens

has now been tested, both by the standard Sachs-Georgi technique and by the extended quantitative method (appendix 9), and all the results have pointed to the remarkable uniformity of reacting power possessed by such specimens. In the quantitative test their behaviour is very consistent (vide, appendix 128-140, "Syphilitic serum"), and the zone phenomenon so frequently seen with individual sera is for practical purposes avoided completely.

Apart altogether however from the use of antigens of varied turbidity it has been found that antigens which are devoid of the slightest trace even of opalescence may still give well marked flocculation in the presence of syphilitic serum; sometimes fully as intense as with the turbid antigen used as control. As will be seen, such a result depends very largely on whether the dilution of the heart-extract is made with saline or with distilled water, and it is also influenced by the volume of serum used in the test. The relative lack of sensitiveness of an antigen made by diluting the extract rapidly, and all at once, with five volumes of saline is referred to by Mackie (1921), and on the occasions on which such an antigen has been used in the present work the results have invariably

confirmed his observation. Appendix 167-168 is a typical example of such an experiment, and it shows how unfavourably the flocculation obtained with the rapidly diluted antigen compares with that obtained in the control (standard technique); but it has to be noted that although the flocculation is poor it is easy to detect on account of the total absence of turbidity in the serum-antigen mixtures. A somewhat different result however is obtained in tests in which the antigen is prepared by diluting the heart-extract rapidly with five volumes of distilled water, instead of saline, and these experiments will therefore be considered in some detail.

Sugden and Williams (partly unpublished), as an extension of their work on the action of protective colloids, carried out some observations with pooled specimens of human serum and the antigen used in the Sachs-Georgi test. They had already found that no essential difference could be demonstrated between normal and syphilitic serum so far as its action in protecting arsenious sulphide sol from precipitation by electrolyte was concerned, and that the curve of precipitation and protection obtained in such experiments was strictly comparable with that in similar experiments in which gelatine had been used as protective colloid instead of serum. A very striking difference between the two sera was revealed however when Sachs-Georgi antigen replaced the arsenious sulphide sol. A zone of precipitation occurred with both sera, but the mixture of syphilitic serum and antigen showed greater sensitiveness to the precipitating action of the electrolyte than did the mixture of normal serum and antigen. They were inclined to think that this zone of precipitation betokened some specific interaction between serum and antigen, as it had apparently no homologue in their experiments with serum and arsenious sulphide sol, and they suggested tentatively that the difference

between syphilitic and normal serum was of a quantitative rather than a qualitative nature. Furthermore, for the purpose of experimental studies, they did not regard with any favour the practice of diluting cholesterolised heart-extract with saline (electrolyte solution), as such a procedure is calculated to yield sols of varying physical constitution and therefore possibly variable in their properties. This opinion is of considerable interest and importance coming from workers who were considering the question solely from a physico-chemical standpoint, and who were not conversant with the literature of the syphilis flocculation reaction or with the difficulties which are experienced in its routine use. They emphasise the necessity of varying all the reagents in such a system as that comprising the flocculation test, and the desirability of preparing the antigen by diluting the extract with distilled water, if one wishes to obtain some insight into the mechanism of the reaction.

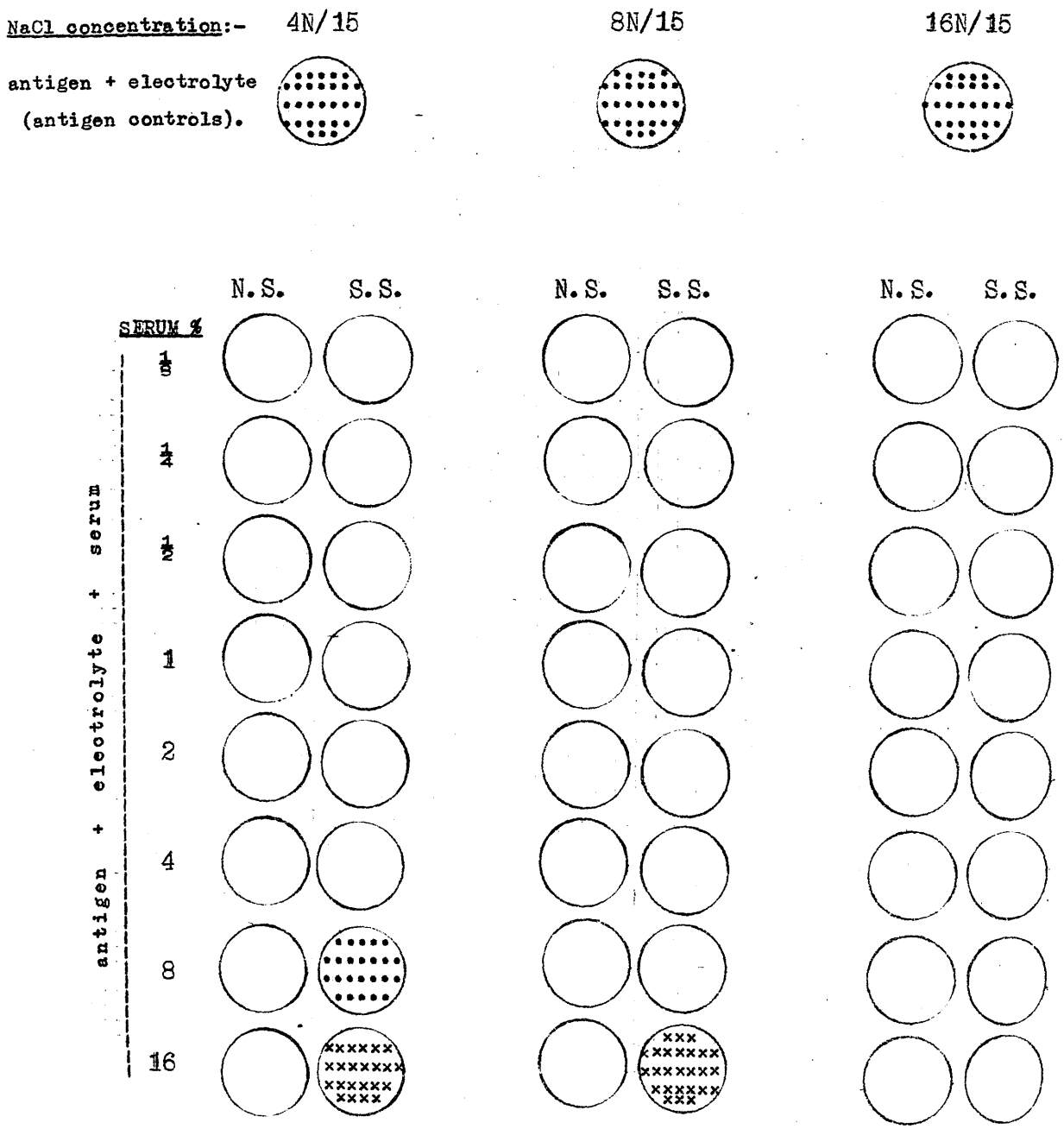
In view of these suggestions, a series of experiments has been carried out in which a 1 in 6 dilution of cholesterolised heart-extract with distilled water has served as the antigen,

and in which the concentrations both of electrolyte and serum have been varied in parallel tests. Sodium chloride was used as the electrolyte and a solution of 8N/5 strength (the symbol "N" is used with its customary chemical significance) served as the stock from which higher dilutions could be prepared. The serum for the experiments was inactivated as usual, and parallel series of dilutions of each serum were prepared with electrolyte solutions of diminishing strength, so that each tube might contain 1.0 c.c. of a serum-electrolyte mixture. Finally 0.5 c.c. of the antigen was added to every tube, its contents were mixed, and the tests were incubated at 37°C. for twenty-four hours or longer. The mixtures are free from any trace of opalescence or turbidity and they remain so unless precipitation occurs. Readings are therefore easy to make. Preliminarily it was necessary to determine the upper limit of electrolyte concentration beyond which flocculation is induced in the antigen control (antigen + electrolyte solution), and the limits of the zone in which precipitation occurs in the presence of serum; having fixed these points, it was possible to use a more restricted form of experiment for subsequent tests, and so to examine

Figure I.

(to face p. 93).

Protective Effect of Normal or Syphilitic Serum.



No change occurs in the mixtures shown unmarked.

several sera at one time. The initial experiment is shown in appendix 171-173 and it was found that turbidity appeared in the antigen control if the NaCl concentration was 4N/15 or higher, and that both normal and syphilitic serum caused precipitation in the presence of concentrations of electrolyte at or below N/60. It will be noted that the presence of serum (normal or syphilitic) interferes with the precipitation of the antigen by the higher electrolyte concentrations (16N/15 to 4N/15); the protecting effect is evident with all concentrations of normal serum (1/8% - 16%) but, in the case of syphilitic serum, it is less complete (1/8% - 4%).

This protective action of normal serum has already been referred to in connection with previous work (e.g. p.19) and it appears to be a very constant phenomenon. It is clear that, within certain limits, syphilitic serum exercises a similar action since, in the present instance, it not only protected completely when in concentrations of 1/8% to 4%, but also protected partially in concentrations of 8% and 16%. No opalescence or turbidity was observed in the tubes containing 16N/15 NaCl and 8% or 16% of serum although it was

present in the corresponding antigen control. A complete duplicate set of tests was also carried out with the same two sera in which a 1 in 6 dilution of absolute alcohol with distilled water was used instead of antigen, thus constituting serum controls, but no turbidity or precipitation appeared in any of these tubes. For the later experiments therefore, in which individual specimens of normal and syphilitic serum were used instead of pooled samples, the electrolyte concentrations were restricted to the range 2N/15 to N/120, and serum controls were omitted except in the case of the highest serum concentrations.

The results of seventeen such tests (six normal sera and eleven syphilitic sera) are shown in appendix 176-184, and from these it is evident that flocculation occurs with normal serum when the concentration of electrolyte is N/30 or less, provided that the serum concentration does not exceed 8%. No change occurs in any of the tubes outwith these limits. It is to be noted further that the flocculation is definitely zonal. With electrolyte concentrations of N/30 and N/60 the bulkiest precipitates occur when the serum concentration is from 1/2% to 1%, while, with

larger or smaller proportions of serum, precipitation becomes progressively less copious and finally ceases. When N/120 electrolyte is used, the zone of precipitation is broader but it still shows a distinct tendency to reach a maximum with increasing concentration of the serum, and then to tail off as the serum percentage continues to rise. Four of the six normal sera gave no flocculation until the electrolyte concentration fell below N/30, and with the two which did flocculate at this point the slight change was apparent in two or three tubes only. The result to which one would draw specific attention is that, provided the electrolyte concentration was N/30 or higher, none of the normal sera caused any flocculation when present in concentrations of over 2%, and that with a serum concentration of 16% no flocculation occurred in the presence of any of electrolyte solutions which were used. This will be referred to again later.

Appendix 174-175 is the record of an experiment with a specimen of normal serum which is the only example of its kind that I have ever encountered. Flocculation occurred in the serum controls and, had they not been included, the result would have been very misleading. It is

obvious however that this serum does not constitute an exception to what has just been stated with regard to the flocculation obtained with normal sera in general; the upper zone of precipitation which occurred in the serum-antigen mixtures can be discounted in view of the similar zone of precipitation in the corresponding serum controls. The serum, whose Wassermann reaction was negative, was twelve days old when tested and had been stored in the refrigerator. It was deeply bile stained, but free from any trace of turbidity, and when retested three days later it again flocculated in the same serum controls.

In the case of each of the syphilitic sera a zone of flocculation is present which corresponds very closely indeed with the zone shown by normal serum, in that precipitation occurs with low concentrations of serum when the electrolyte concentration falls to N/30 or less. Just as with normal serum, this precipitation reaches a maximum with serum concentrations of from 1/2% to 1%, and beyond this it tends to fall off. The outstanding feature however of the results obtained with syphilitic sera is the occurrence of precipitation in a zone which has

no counterpart in the tests in which normal serum is used, and which is characterised by the appearance of turbidity or sedimentation in all tubes containing 8% or 16% of serum, especially in those in which the electrolyte concentration is highest. This is common to all syphilitic sera which have been examined, but individual variations occur both in respect of the intensity of the flocculation and the limits of serum and electrolyte concentrations with which it appears. Serum 4 for example (appendix 177), when present in a concentration of 1% and upwards, caused flocculation with 2N/15 NaCl, but the majority of the sera failed to show flocculation with this strength of electrolyte until their concentration reached 4% or over. Syphilitic serum thus differs from normal serum in that it causes precipitation in the presence of higher electrolyte concentrations and that, with low electrolyte concentrations, it still causes precipitation outwith the zone in which this occurs with normal serum. It will be noted that with these low electrolyte concentrations the upper end of the zone of precipitation common to both syphilitic and normal serum is overlapped by the zone peculiar to syphilitic serum, thereby leading to the appearance of two points of maximum sedimentation; the one when the serum concentration

is from 1/2% to 1% (common to both sera), and the other when it reaches 16% (peculiar to syphilitic serum). This is definitely apparent in the case of 8 of the 11 individual samples of syphilitic serum, as well as with the pooled specimen, and it suggests very strongly that the zone of precipitation associated with low concentrations of serum and electrolyte is dependent on a property possessed by all sera, and that syphilitic serum may differ qualitatively rather than quantitatively from normal serum; it causes precipitation in a zone of high serum and high electrolyte concentration in which normal serum is ineffective and which is essentially discontinuous with the zone of precipitation common to both sera (cf. Brandt). Overlapping of the two zones may mask the gap between them to a greater or less extent but the results afford no evidence that the upper zone is a direct extension of the lower. If such tests are examined after 12-15 hours incubation it has been found that the two zones are sometimes then quite separate, and that it is only after longer incubation that opalescence or precipitation makes its appearance in the intervening tubes.

These experiments have also proved instructive in confirming what had been observed previously in connection with the instability of the antigen in the presence of higher concentrations of sodium chloride. Reference has been made to the fact that spontaneous flocculation of the antigen occurs frequently in the standard Sachs-Georgi test when a saline containing more than 1.5% NaCl is used, and, in the present instance, opalescence appeared in the antigen control tube containing 4N/15 NaCl (1.56%), although with lower concentrations of electrolyte these controls remained perfectly clear.

There can be little doubt that an antigen prepared in the routine manner by diluting cholesterolised heart-extract with saline (0.85% NaCl) behaves differently in the presence of most syphilitic sera from a similar antigen prepared with distilled water; the former has consistently been found to show well-marked flocculation with as little as 0.006 c.c. of a pooled serum, whereas with the latter it has been exceptional to find flocculation with less than 0.06 c.c. (4%). In this respect the saline-antigen is the more sensitive but it has to be noted that, although it

undoubtedly reacts with smaller volumes of syphilitic serum than the water-antigen, it sometimes reacts very poorly with the larger of the two serum volumes ordinarily used in the Sachs-Georgi test (zone phenomenon), and on at least one occasion a syphilitic serum has been encountered which failed to flocculate at all unless very small volumes were used (appendix 185). This serum was re-tested on two subsequent occasions with precisely the same result so that there is no question of the zone phenomenon having been due to some chance factor which affected one particular batch of tests. The tendency for certain syphilitic sera to flocculate poorly when tested in relatively high concentration is not uncommon, and a 1 in 5 dilution (0.2 c.c.) cannot be regarded as the optimum proportion for a one tube test. This has been my usual experience and it has not been specially associated with the use of any one batch of heart-extract but has occurred with all the antigens which I have prepared. Furthermore the same phenomenon was observed by Wodtke and by Taniguchi and Yoshinare, both of whom concluded that it was advisable to use at least two serum volumes in the routine test. Such results do not appear

to be dependent in any way on the use of human heart as a basis for the antigen, as workers who have used beef-heart (e.g. Krishnan), or sheep's heart (e.g. Mackie), have recorded the occurrence of a similar zone phenomenon in their tests. It is somewhat surprising therefore that the technique of the test, as still prescribed by Sachs and Georgi, should be restricted to the use of a single serum volume (0.2 c.c.) since this seems likely to increase the number of poor flocculation results without, so far as I have been able to judge, ever enabling one to detect a syphilitic serum which would otherwise have been missed. In the limited number of tests which have been carried out with an antigen diluted with water, and adjusting the electrolyte concentration if need be by using a stronger saline for diluting the serum (e.g. appendix 186-188), no suggestion of a zone phenomenon of this type has been observed but, on the other hand, this method does not hold out any hope of yielding more uniformly good results with syphilitic serum (flocculation of "++++" type) than the standard method. In the tables which are shown (appendix 176 - appendix 182) there is just as great variation in the intensity of individual reactions as what occurs when the

standard technique is used, and, although the precipitation is relatively easy to detect on account of the absence of any trace of turbidity from the initial mixtures, critical examination of every tube independently is still imperative. Moreover, a test of this type involves the use of a comparatively large amount of serum and, as such, it is less desirable than one for which a smaller volume suffices. The precipitation too is slower in its appearance and an incubation period of 36 hours is required for optimum results.

In the Sachs-Georgi test therefore it has been found that the sensitiveness of the antigen, so far at least as its capacity for reacting with small amounts of serum is concerned, can be modified with certainty by diluting the heart-extract with distilled water instead of with saline, and that an antigen prepared by diluting the extract rapidly and all at once with saline is less sensitive than a corresponding antigen made by carrying out the dilution in two stages. None of the modifications of the two stage method of dilution however has ever succeeded in providing an antigen of consistently diminished sensitiveness, and no satisfactory explanation has therefore been obtained

of the varying sensitiveness of antigens prepared in the routine way, on different days, from the same samples of cholesterolised heart-extract and saline. There is strong reason none the less for suspecting that such variations are most likely to be related to slight differences in the physical state of the antigens, and it seems certain that their physical state is very largely dependent on factors which are operative at the moment when the saline is added to the extract. Once this dilution has been effected, the resulting antigen may be subjected to a variety of relatively extreme physical shocks (e.g. boiling, shaking, etc.,)without producing any definite modification of its sensitiveness, as gauged in the flocculation test, and yet, as has been shown, twin samples of antigen, prepared at the same time with the same extract and the same saline, may differ demonstrably in their capacity for reacting with the same syphilitic serum. Some evidence of the influence which factors associated with the act of dilution may exert on the physical state of the antigen is obtained if one prepares a series of antigens from the same cholesterolised heart-extract and saline, but using 1.5% NaCl instead of 0.85%, and if one takes all possible

care to ensure that every one of the antigens is quantitatively the same as any of the others, and that the method of dilution is uniform throughout the whole series. In such experiments it has been found that some of the batches of antigen may flocculate spontaneously, after incubation at 37°C., while others do not; in one instance two out of six flocculated and, in another, one out of four. It is thus possible to demonstrate conclusively that gross variations do occur in the physical state of antigens prepared apparently identically and subjected to identical conditions subsequently, although the nature of the factors which determine the differences remains unknown. It seems not unreasonable to assume that minor degrees of variation in physical state may also be possible which are insufficient to lead to observable physical change, but which may yet be detectable in a biological test such as the flocculation reaction. In the example which has been quoted, a saline of higher sodium chloride concentration than usual was employed in order deliberately to increase the probability of precipitating the antigen, but it may be recalled that the routine antigen itself, diluted with physiological saline, sometimes flocculates spontaneously

in spite of customary care in its preparation; evidence of variation in the physical state of the antigen is thus occasionally available even when the standard technique of the test is strictly adhered to.

It must be emphasised that the Sachs-Georgi antigen is not alone in proving somewhat fickle in its sensitiveness, but that all the other methods which have been devised for carrying out the syphilis flocculation reaction are associated with similar uncertainty as to how any one sample of antigen dilution will behave as compared with another, and that the authors by whose names the various methods are designated are all of one mind as to the existence of a relationship between physical state and sensitiveness. Satisfactory proof of such a relationship however is lacking. Unfortunately, too, their unanimity of opinion extends no further, and there is complete absence of agreement both as to the particular physical state which characterises an antigen of optimum sensitiveness and the method best suited for preparing such an antigen at will. Meinicke in his earlier work ("M.R.", "M.D.R.") stresses the importance of using distilled water for the primary dilution

of the heart-extract, in order that the resulting turbidity of the mixture may occur gradually, as he believed that in this way greater uniformity in the physical state of different samples of dilution could be obtained. When the dilution is made with saline, turbidity appears instantly and, as its intensity is dependent on the rapidity with which mixing is effected, it is difficult to control. At this period he was apparently unduly influenced by the results of some experiments in relation to an analogous flocculation test for glanders, in which he had obtained definite evidence that the sensitiveness of his glanders antigen was intimately related to its turbidity. He admits that in the syphilis flocculation test he had failed to find evidence of any similar relationship and in his more recent tests ("M.T.R.", "M.K.R.") he has departed altogether from the water method of dilution and now uses hypertonic salines (3% and 3.5%). Vernes attaches so much importance to the physical state of the antigen employed in his test that an electrically driven mechanical mixer and a standardised dropping pipette are deemed essential for the process of dilution of the heart-extract. Double distilled water serves as the diluent, and

into this the extract is dropped at a constant rate while a glass propeller, rotating at a constant speed, keeps the mixture in motion. Dreyer and Ward similarly make use of a slowly diluted antigen for their Sigma reaction, but in this case 0.9% NaCl solution is the diluent and it is dropped into the extract at a constant rate from a height of 36 centimetres. These authors claim that antigen dilutions prepared on different days, and strictly in accordance with their instructions, are identical in physical state and sensitiveness. Of this however no proof is offered, and other workers have undoubtedly observed fluctuations in the intensity of the flocculation obtained in repeated Sigma tests with the same syphilitic serum (e.g. Jones, Norel), although they have sought to ascribe them to factors other than varying sensitiveness of the antigen. In the case of the more recent tests devised by Kahn and Sachs and Witebsky, the antigen employed is not an emulsion or sol but a suspension of a relatively coarse precipitate and it is therefore totally different from antigens of the Sachs-Georgi type. None the less it is emphasised in these instances also that the method of diluting the heart-extract with saline, in order to produce the precipitate, is of prime importance

to the sensitiveness of the antigens and that sensitiveness depends on the size of the particles constituting the precipitate, i.e. on physical state. These tests will be referred to later, but it may be stated here that in my hands the Kahn and "citochol" antigens have proved no more satisfactory than the Sachs-Georgi antigen so far as uniformity in their sensitiveness is concerned.

The difficulty which is experienced in preparing antigens of uniform sensitiveness from the same sample of heart-extract has an interesting parallel in the work of Sugden & Williams on protective colloids. They found that for the purpose of their experiments it was necessary to carry out the whole of their tests at one time, with the same solution of gelatine, as gelatine solutions prepared on different days, although of the same strength and made in the same way from the same sample of gelatine, varied in their protecting power when tested with the same stable arsenious sulphide sol and the same electrolyte solution. The antigen for the syphilis flocculation reaction is thus not unique in its /

peculiarity of varying in its properties from day to day, and it is specially noteworthy that an analogous hydrophilic sol like gelatine has been found to show somewhat similar variability, under relatively simple physico-chemical conditions. The tests carried out by Sugden & Williams are at least uncomplicated by biological factors such as are operative in a serological system in which the possibility exists for a specific antigen-antibody reaction.

OPTIMUM ANTIGEN. Sachs and Georgi specifically indicate the need for titrating every batch of heart-extract in order to determine the degree to which it should be diluted with alcohol, and the proportion of cholesterol which should then be added, so that a cholesterolised extract of optimum sensitiveness may be prepared. Their observations had led them to conclude that different samples of heart-extract vary in their lipoid content, both quantitatively and qualitatively, and that for the purpose of the flocculation test the relative proportions of lipoid and cholesterol present in the antigen are of greater moment than their absolute amounts.

The technique of the titration which they prescribed, the complete details of which are given in appendix 6-8, involves testing a known syphilitic and a known normal serum with twelve different antigens, all made from the same sample of heart-extract but varying in lipoid and cholesterol content, and selecting the particular antigen which gives the coarsest flocculation with syphilitic serum while showing no flocculation with normal serum or in the antigen control. It is advisable to choose the two best antigens, as judged in this way, and subsequently to test them in parallel, on several occasions, with a series of known normal and known syphilitic sera before finally deciding which of the two is on the whole the more satisfactory. The stock heart-extract is then diluted in bulk with alcohol and cholesterolised so as to yield the mixture which has thus been found to constitute the optimum antigen, and such cholesterolised heart-extract should be stored in a stoppered bottle and kept at room temperature. It merely requires dilution with saline in the routine manner in order to provide the antigen used for the flocculation test, and it retains its properties unchanged apparently

indefinitely.

An example of a titration of optimum antigen is shown in appendix 189-195 and it is evident from the preliminary test (appendix 189) that the antigens designated A4 and B2 were incomparably superior to all the others. Accordingly these two were used in parallel on four subsequent occasions, in a series of tests with 49 individual sera, and the results, which are summarised in appendix 195, are shown in detail in appendix 190-194. Briefly stated, the flocculation results with the A4 antigen agreed with those of the Wassermann test in 34 cases and disagreed in 15, while with the B2 antigen they agreed in 35 cases and disagreed in 14. The relatively large number of disagreements is readily accounted for since, on one of the days, both antigens were practically devoid of sensitiveness (appendix 191-192), thus affording another extreme example of antigenic inconsistency of the type which was considered in detail at the beginning of the summary (p. 11) and which, as has already been discussed in the present section, it has not proved possible to reproduce experimentally. No cause for the lack

of sensitiveness of the antigens on this particular day could be discovered and it is specially remarkable that two separately prepared dilutions of different samples of cholesterolised heart-extract (A4 and B2) should both have shown the same peculiarity in the same batch of tests. Moreover it was the same two extracts which were in use when the previously recorded failure occurred (p.11), and on that occasion also the lack of sensitiveness made itself apparent not only with the routine antigens but also with the experimental antigens in which sodium hypophosphite or sodium sulphocyanide replaced sodium chloride. These observations almost compel one to seek the explanation of the phenomenon in a factor or factors which affected the tests as a whole on these days, and not merely the antigens, but all efforts to trace such a factor have proved futile. Temperature of inactivation of the sera, temperature of incubation of the tests, temperature of the laboratory in which the tests are set up, cleanliness of glassware, purity of the atmosphere of the laboratory and of the incubator (freedom from chemical fumes, etc.), reaction of the saline,

time occupied in setting up the tests, duration of incubation, shaking of racks, have all been investigated; even factors such as barometric pressure, time of day, and season of year have been considered but all have had to be dismissed. No modification of these factors such as is possible under ordinary test conditions has ever succeeded in causing syphilitic serum to fail to flocculate, and it has invariably proved equally impossible to prepare an antigen devoid of sensitiveness by any modification of the two stage method of diluting cholesterolised heart-extract. In spite of this, one has experienced these chance instances of total failure of the flocculation test on several occasions, and with extracts other than the two which are presently under consideration. Although they are instructive, in so far as they constitute extreme examples of the fluctuations in sensitiveness which the Sachs-Georgi antigen has been found to exhibit from day to day, they are disappointing in that they have afforded no clue whatever as to the underlying cause.

This series of tests with 49 sera merely confirmed the preliminary observation that

antigens A4 and B2 were much alike in their behaviour, and little help was obtained towards deciding which was the better, although it was noted that flocculation with B2 was on the whole less coarse than with A4 (the more turbid antigen). Accordingly, both antigens were employed in the whole of the experimental work in which different sodium salts were being used as the electrolyte, and these results appear in the tables in section A of the appendix (in the horizontal columns marked "control"). A4 proved to be the antigen which consistently gave the coarsest flocculation with syphilitic serum, while showing none with normal serum or in the antigen control, and it was finally selected therefore as the optimum antigen from this particular batch of heart-extract. It will be obvious from the detailed description of this example of a titration of optimum antigen that the final decision can only be made after a fairly extensive trial of two or more lipoid-cholesterol mixtures, selected from the larger series in the preliminary test. It is not improbable that the recommendation of Sachs and Georgi, that at least two antigens should always be employed in routine tests, has been determined by the relative difficulty

of ever being sure that any one cholesterolised extract is necessarily the optimum for all syphilitic sera. Appendix 196 is another example of a preliminary titration, but in this case the only mixture which gave satisfactory flocculation was B2. In the usual series of subsequent tests it proved itself to be the optimum antigen from this batch of heart-extract, thus corresponding with the original antigens used by Sachs and Georgi who found that, as a rule, the B2 mixture contained the optimal proportions of lipoid and cholesterol. This however has not been my experience and, with the extracts which I have prepared, one or other of the "A" series of mixtures has generally been the most satisfactory.

It may be noted that pooled samples of serum have invariably been used for these preliminary titrations, in order to avoid as far as possible the variability in reacting power which is often associated with individual sera, but that, even taking this precaution, one is not entirely successful in eliminating such differences. This is borne out by the examples shown in appendix 197-200 in which a series of

optimum antigen titrations of two batches of heart-extract are carried out with several samples of pooled syphilitic serum. For appendix 197, parallel titrations of a heart-extract were set up at one time with three specimens of pooled syphilitic serum, and the results show that although serum 3 flocculated comparatively poorly it reacted best with the A4 mixture, as also did serum 1. Serum 2 however gave the best flocculation with antigens B3 and B4 and it was accordingly re-tested on two subsequent occasions (appendix 198), when the superiority of these two antigens over all the others was confirmed. Antigen A4, which proved as a result of long experience to be the optimum antigen from this batch of heart-extract, was thus not the best antigen for this particular syphilitic serum, although admittedly it did not fail to detect it, and if this serum had been used alone in the preliminary test the A4 antigen, which took third place, would not have been selected at all for further investigation. The "C" series of mixtures do not as a rule yield such coarse flocculi as the others, even when the results have to be recorded by the same number of plus signs, and for this reason they

are purposely omitted from consideration. The second group of tests, with another batch of heart-extract, is shown in appendix 199-200 and, while exemplifying the same point, they illustrate yet another type of result which is sometimes obtained when titrating an optimum antigen. On the first occasion on which this extract was tested, ten out of the twelve antigens prepared from it seemed to be equally good, but on re-titrating twice later, with the same serum, differences made their appearance and the "A" series of mixtures proved themselves better antigens than the other two. The extract was then re-titrated with three different samples of pooled serum (appendix 200), the tests being carried out in parallel, and it was found that the "A" series was still the best with sera nos. 1 and 3, but not with no. 2. The preliminary titration of this extract was not therefore particularly helpful, and for the subsequent tests with individual sera considerable difficulty was experienced in deciding which antigens to use. A1, A2, and B3 were finally selected for further investigation and, of these, A2 gave the most uniformly satisfactory results in an extended series of observations. Just as in the preceding

instance, it is to be noted that, if by chance serum no. 2 had alone been used in the preliminary titration, the A2 mixture, which took fourth place, would not have merited further consideration; indeed that the mixtures of the "B" series were all either as good as, or better than, the corresponding "A" series mixtures.

These experiences emphasise that the titration of optimum antigen is a procedure which demands a great deal of time and care if it is to be carried out in a manner which is at all likely to be satisfactory, and that, even then, it is not calculated to ensure that the antigen ultimately selected is the optimum antigen for all syphilitic sera. Particularly must it be noted that, although all the sera used in the above experiments were pooled specimens, considerable variations in their reacting powers with different antigens were none the less demonstrable. Similar variations are even more prominent with individual specimens of serum, and their use in the preliminary titration was found to make the choice of the two best antigens so difficult that it had to be abandoned. The routine therefore which has been adopted is to use pooled samples of serum for the

initial titration, and to repeat this titration two or three times so as to increase the probability of choosing the two antigens which are really the best. These two are then tested out in parallel with a series of fifty or more individual sera, and the final decision is based on the results obtained in these tests. There is one further point in connection with the preliminary titration which has always to be borne in mind, namely that, as twelve separate saline dilutions of cholesterolised heart-extracts have to be made, there is always the possibility that apparent inferiority of one or more of the antigens in any one test is dependent on a relative lack of sensitiveness of the particular saline dilution which is used on that occasion, and not on an inherent lack of sensitiveness on the part of the lipoid-cholesterol mixture from which it has been prepared. Variations in sensitiveness of this type have already been considered in detail and they appear to furnish the most likely explanation of a result such as was obtained with antigen B3 in the second test shown in appendix 199. This source of fallacy, taken in conjunction with the possible variability in reacting power

of different samples of pooled serum, makes it imperative that the primary selection of the two best antigens should not be made on the strength of a single preliminary titration, but that this form of test, tedious though it is, should be repeated several times.

Heart Extract. The method of preparation of the heart-extract originally recommended by Sachs and Georgi (appendix 5) has stood the test of time and experience, and, so far, no radical change has been suggested by any of the workers who have given their attention specially to the question of the antigen. The proportion of 5 parts of alcohol, by volume, to 1 part of moist heart, by weight, has been confirmed by Rosenberg to be the least which will yield a suitable extract, and he found that such an extract did not differ essentially in its antigenic properties from one prepared by extracting the same weight of heart with ten volumes of alcohol. Vermast and Krishnan advise standardising the extract so that it may contain a definite weight of extractives, or a definite weight of extractives + cholesterol, per unit volume, but the proportions which they select as optimal differ by as much as 100 per cent. and neither of them makes any reference

to Sachs and Georgi's method of titrating optimum antigen. In a general way they come to the conclusion that different extracts vary in their lipoid content, and so require adjustment, by appropriate dilution with alcohol and addition of cholesterol, in order to render them best suited for test purposes, but as this is precisely what Sachs and Georgi emphasised originally it would not appear that there is anything essentially new in the recommendations of these workers. For the more recent modifications of the flocculation test, the preparation of the antigen from moist heart has been superseded by the use of dried and powdered heart, which is extracted preliminarily with ether in order that an alcoholic extract suitable for test purposes may be obtained. This is a return to the method originally used by Meinicke who found that the extract of moist human heart for the Sachs-Georgi test could be replaced by an extract of the same heart, dried, provided that the dried product was subjected first of all to treatment with ether. He believes that the water present in fresh heart dilutes the alcohol used in extraction sufficiently to prevent its acting as a solvent for fatty substances which can interfere with flocculation, and that, when dried

heart is used, these substances, unless removed by preliminary extraction with ether, pass into solution and so render the extract unsuitable for the test. I have confirmed Meinicke's observation that direct alcoholic extracts of dried human heart do not yield suitable antigens for the Sachs-Georgi test but that excellent antigens can be obtained if the dried heart is first of all extracted with ether. An example of the preliminary titration of such an extract is shown in appendix 201-202 and, at first sight, it seemed much superior to the average samples of Sachs-Georgi extract with which I had at that time had experience. Not only were the flocculi very coarse but complete sedimentation was obtained when syphilitic serum was tested with any of the antigens of the "A" or "B" series, as well as with two of the four in the "C" series. Subsequent investigation made it clear that such a result was not the usual one, with this particular sample of extract, and that it showed just as great variation in its capacity for reacting with different syphilitic sera, both pooled and individual specimens, as a Sachs-Georgi extract which had been prepared in the orthodox manner from a portion of the same heart in the

fresh, undried, state. Its behaviour in the first test was thus analogous to that of the Sachs-Georgi extract shown in appendix 199. Flocculation always tended to be somewhat coarser with the antigen from dried heart than with the corresponding Sachs-Georgi antigen (cf. Sachs), but this slight advantage was insufficient to compensate for the extra time and trouble involved in drying the heart and treating with ether, as preliminaries to extraction with alcohol. Indeed one is of the opinion that if such relatively complex procedures should prove necessary in order to obtain an optimum antigen for the flocculation test, especially when one remembers that different samples of extract vary in constitution as well as in reacting properties, no matter how they may be prepared (Georgi; Meinicke; Stilling; Sachs, Klopstock & Ohashi; Kahn; Krishman), the test becomes fully as complicated as the Wassermann reaction and therefore restricted in its possible usefulness. The variability shown by extracts prepared from dried heart is well exemplified in the case of the Kahn test to which reference will be made later (p.151) Preliminary extraction of dried heart with acetone has also been tried, on the analogy of the antigen

used in the Sigma reaction, but it has not proved satisfactory for the purpose of the Sachs-Georgi test. An example of such an extract is shown in appendix 203 and, in subsequent tests, it was found to yield even poorer flocculation when individual, instead of pooled, specimens of serum were used. It is of interest to note that this extract was prepared from the same sample of dried heart as the Meinicke extract shown in appendix 202, and to compare the flocculation results obtained. The extract of acetone-insoluble lipoids yielded the best antigens in the "C" series of dilutions, while this same series included the poorest antigens from the Meinicke extract. This finding would appear to be related to differences in the selective solvent actions of acetone and ether, whereby the former fails to remove certain constituents which are unfavourable to flocculation as completely as the latter. It has already been stated that a suitable antigen for the Sachs-Georgi test was not obtained on direct extraction of this dried human heart with alcohol, no matter how highly the extract was subsequently diluted, but it is obvious that preliminary extraction with either acetone or ether left a residue from which an

alcoholic extract of some value for test purposes could be prepared. The extract from the acetone residue required greater dilution to make it suitable for use than did the Meinicke type of extract, although both extracts were prepared from the same proportions of dried heart and alcohol. If Meinicke's explanation (p.121) is correct, then ether is a more efficient solvent than acetone for the constituents of dried heart which interfere with flocculation; but it tends to remove the specific flocculating substance as well, since the alcoholic extract of the ether-residue showed weakening of its reacting power, on dilution, more quickly than the corresponding extract of the acetone-residue ("C" series dilutions). These experiments on varying the method of preparation of the heart-extract did not afford any indication of the possibility of improving the Sachs-Georgi test along such lines, and there seems little doubt that the simple procedure recommended originally by Sachs and Georgi is as satisfactory as any (cf. Rosenberg). A poor extract has occasionally resulted but the great majority have proved suitable, provided that the initial titration of optimum antigen was properly carried out.

One is convinced that adherence to simplicity of technique as far as possible, rejecting a poor extract should this be obtained, is preferable to resorting to complex procedures which, at their best, are restricted in their usefulness and which fail to eliminate the major difficulty, variability in the intensity of flocculation produced by different syphilitic sera when tested with the same antigen. These remarks refer specifically to the Sachs-Georgi test but they are also applicable to other modifications of the syphilis flocculation reaction, for the special conditions of which special antigens are sometimes an essential feature.

Cholesterol etc. The addition of cholesterol to extracts used in the flocculation test is due to the work of Sachs and Georgi who found that, as was already well known in the case of the Wassermann reaction, this reagent had the property of intensifying the results and thereby in increasing the specificity of the test. Their original observation in this respect has been fully confirmed by other workers and, as was shown by Georgi, there is an optimum amount of cholesterol for any particular

sample of heart-extract. This also has been confirmed by all workers who use cholesterolised-extracts except Kahn who, so far, still adds a fixed proportion to his antigen, and those who favour the Sigma reaction (Bordet and Ruelens antigen). In the case of the latter however, Norel found that the sensitiveness and the specificity of this test were influenced favourably by increasing the amount of cholesterol in the antigen. Meinicke has all along avoided the use of cholesterol for the various tests which he has devised, and he attains the desired degree of sensitiveness in the different antigens by employing higher concentrations of electrolyte or by the addition of tolu balsam, either alone or supplemented with benzoic acid. Similar resinous substances have found favour for the same purpose with other workers, and gum benzoin has been used by Dujarric de la Rivière and by Sachs, Klopstock and Ohashi; gamboge by Dujarric de la Rivière; Peruvian balsam by Hartmann; while Voge has sought to base a serological test for syphilis on the differential precipitating action of normal

and syphilitic serum on a simple benzoin sol. The Vernes reaction (syphilimétrie) is exceptional among tests which are nowadays practised on a large scale in that the antigen contains no added cholesterol or resin. The antigen used by Bruck is also prepared from a simple heart-extract, while Kiss recommends the use of a plain lecithin solution.

Keeping Properties. The extracts used in the flocculation test are all characterised by considerable stability. In the case of the Sachs-Georgi reaction, I have found that a cholesterolised heart-extract which had been stored at room temperature for as long as three years was still as satisfactory in its behaviour as when it was freshly prepared. A small amount of sediment tends to separate out on standing but this may be removed by filtration as often as necessary, without any apparent effect on the sensitiveness of the antigen. Similar observations in the case of the Kahn antigen are recorded by Kendrick and Jenks, while Meinicke's antigens and those for the recent citochol reaction have been placed on the market as commercial products. Presumably therefore they have also proved themselves stable in their properties.

REACTION. In Section A it was mentioned that it had been found possible to incorporate dilute NaOH solution in the saline used in the Sachs-Georgi test, in amounts sufficient to bring its pH to approximately 9.0, without any appreciable effect on the intensity of the flocculation obtained with syphilitic sera. The record of the experiment is shown in appendix 204, but, as it seemed desirable to obtain more accurate information as to any action which changes in H-ion concentration might exert, another experiment was carried out in which the extended form of test (appendix 9) was used, and in which one of the salines was made sufficiently alkaline to yield a full red colour with phenolphthalein (appendix 205). It is obvious from this that alteration of the H-ion concentration of the saline over a considerable range of pH values was without the slightest effect on subsequent flocculation, either in respect of its intensity or the minimum amount of serum by which it was induced. The controls (duplicate tests with normal serum, antigen controls, and serum controls) all behaved satisfactorily. In connection with this experiment not only was the reaction of the different salines known but

the H-ion concentration of the antigens prepared with these salines was also determined (indicator dyes), and it was found that the antigen made with the saline whose pH was 10.5+ failed to give any trace of pink colouration on the addition of phenolphthalein solution. The reaction of the antigen was therefore much less alkaline than that of the saline used in its preparation (pH value of saline = 10.5+; pH value of antigen = 8.5-), and this change was found to be due to definite acidity on the part of the cholesterolised heart-extract, acidity which was determined partly by the alcohol present and partly by substances in solution. The stock supply of absolute alcohol, such as had been used in preparing the heart-extract, was found to require the addition of 0.08 c.c. N/1 NaOH per 100.c.c. in order to cause the slightest change in bromo-cresol purple as indicator, i.e. to bring its reaction to pH = 5.6 approximately, while similar adjustment of the reaction of the cholesterolised heart-extract necessitated the addition of 0.19 c.c. N/1 NaOH per 100.0 c.c. It seemed desirable

therefore to repeat the experiment and to carry out the tests with a sample of cholesterolised heart-extract whose reaction had been adjusted to pH = 5.6, the so-called iso-electric point of serum. It must be noted that although pH 5.5 - 5.6 is the most constant iso-electric point of human serum, at least two others are known (Dognon). A new batch of heart-extract was used, whose optimum antigen was A4, and the reaction of a portion of the crude extract was adjusted to pH = 5.6, as described above, while to the remainder no alkali was added. The optimum antigen of these two extracts (normal and adjusted) was then titrated in parallel tests on two occasions, using the same sera on both days and using absolute alcohol whose pH had been adjusted to 5.6 for diluting the "adjusted" extract. The series of dilutions (A, B. C) of the "normal" extract were made with stock absolute alcohol to which no alkali had been added, while the same cholesterol solution and the same saline (pH = 5.6) were used for the whole of the tests. The results of these parallel titrations are shown in appendix 206-207 and it will be noted that, although the same reagents were used on both days (sera, saline, extracts,

and alcohols), there are considerable differences in the intensity of flocculation obtained in corresponding tubes. Such findings have already been considered in detail and it has been emphasised that they constitute a serious source of trouble in the flocculation test. A4 proved to be the optimum antigen both with the "normal" and with the "adjusted" extract, and the flocculation obtained with the latter was on the whole better than with the former (a convenient method of determining which antigen is the best is to add together the plus signs which represent the results on different days; thus A4 "normal" = 10 and A4 "adjusted" = 16). The superiority of the "adjusted" extract was more apparent than real for, in a series of subsequent tests with individual sera (appendix 208), it failed to justify any claim to special merit as the results with the "normal" A4 antigen were as good in every way as those with the "adjusted" A4. It did not appear therefore that the natural acidity of cholesterolised extract or commercial alcohol was of itself a probable cause of poor flocculation results, since variations in the

intensity of flocculation occurred even when using extract, alcohol, and saline whose reactions had been adjusted to the iso-electric point of serum, and it was apparent that there could be no objection to using "adjusted" extract and alcohol in the preparation of antigens for a repetition of the experiment with saline of varied H-ion concentration. Accordingly another series of tests of the type shown in appendix 205 was carried out, but on this occasion the pH of the cholesterolised heart-extract (A4) was adjusted to 5.6 by the addition of 0.2 c.c. N/1 NaOH per 100.0 c.c., and the reactions of the different salines ranged from pH = 5.8 to pH = 10.0. As a result, the antigens which were prepared with these reagents gave pH values of 6.0 to 9.0 approximately, as determined by Universal Indicator (British Drug Houses), but it was found that these differences in reaction were abolished as soon as the antigens were mixed with the serum dilutions in the tubes. This is readily explicable in view of the well known buffer action of serum, on account of which it requires the addition of 50 times as much NaOH

to bring its pH to 8.2, or 327 times as much acid to bring its pH to 4.0, as does an equal volume of water (Dognon). The difference in the pH values of the completed tests in which the antigens of pH = 6.0 and pH = 9.0 had been used was of the order of 7.2 as opposed to 7.4 and the intensity of flocculation in all tests with syphilitic serum was the same, thus confirming what had been observed in the two previous experiments. There can be no doubt that variations in the H-ion concentration of the reagents for the Sachs-Georgi test, such as may occur for example with the use of different samples of saline, are not of the slightest importance so far as the delicacy of the test is concerned. Much greater variations than are ever likely to occur in the ordinary course of such serological work have repeatedly proved to be without any effect on flocculation, and, even when an antigen with a pH value of 9.0 was used, the result with syphilitic serum was as strongly positive as with the normal antigen tested in parallel. Neukirch records a similar conclusion and its applicability to the Meinicke reaction (M.D.R.) is borne out by the work of

Bauer & Nyiri; to the Kahn reaction by that of Jennett and of Hernandez; and to syphilis flocculation tests in general by the experiments of Wolf and Rideal.

Summary of Section (C).

(1). The simple method of preparing heart-extract recommended by Sachs and Georgi is as satisfactory as any.

(2). The titration of every batch of heart-extract is essential, and the selection of the optimum antigen necessitates a prolonged series of observations.

(3). Cholesterolised heart-extract retains its reacting properties unchanged for years.

(4). No definite relationship between the turbidity of the antigen and its sensitiveness in the test has been demonstrable.

(5). Variations in the sensitiveness of different batches of the same antigen are of frequent occurrence, but the cause of such variations is obscure.

(6). It has not proved possible to abolish the sensitiveness of an antigen by any modification of the method of its preparation.

(7). The H-ion concentration of the antigen used in the flocculation test is unimportant.

(D). Method of Test.

It is a tribute to the care and thoroughness with which Sachs and Georgi must have carried out the preliminary work in connection with their test that the method has undergone only two slight modifications since it was first introduced in 1918. Originally, 1.0 c.c. of a 1/ in 10 dilution of inactivated serum in saline was mixed with 0.5 c.c. of antigen, the mixture was incubated at 37°C. for two hours and then left at room temperature over night (24 hours). Several workers, including Sachs and Georgi themselves, found that with this method there was a tendency for non-specific results (positive reactions with normal sera) to occur, especially in cold weather, but that this could be avoided if the tests were kept in the incubator overnight instead of at room temperature. Accordingly they adopted incubation of the tests at 37°C. for 18 to 20 hours as the standard technique (1919). Subsequently (1921) they reported that better results were obtained if 1.0 c.c. of a 1 in 5 dilution of serum was used as the test volume instead of 1.0 c.c. of a 1 in 10 dilution, and since then their method has

been as follows:-

1.0 c.c. of 1 in 5 dilution of inactivated serum in saline +

0.5 c.c. antigen. Mix and incubate at 37°C. for 18-20 hours.

The mode of preparation of the antigen and the use of serum and antigen controls remain the same as originally.

It will be noted that only one tube is used for each test and that it contains 0.2 c.c. of serum. Sachs and Georgi have adhered to this technique in spite of the fact already referred to that many syphilitic sera show comparatively poor flocculation when 0.2 c.c. is used, or even when 0.1 c.c. is used, although they flocculate well if the test volume is 0.05 c.c. There is no evidence either that a syphilitic serum is ever likely to be missed by omitting the 0.2 c.c. volume. For these reasons the standard technique adopted for the present work (appendix 1) employs two tubes for each test, one containing 0.1 c.c. of serum and the other 0.05 c.c. (cf. Wodtke; Taniguchi and Yoshinare), but it is otherwise the same as that of Sachs and Georgi.

The value of incubation in favouring the specificity of the reaction has received full confirmation at the hands of different workers, and the literature includes several studies devoted specially to the influence of temperature on the course of flocculation. The earliest of these is by Meinicke who found that 37°C, is the optimum temperature for flocculation with syphilitic sera, while Neukirch was able to show that, in parallel tests carried out at 37°C., at room temperature, and at 0°C., there is a progressive weakening in the intensity of specific flocculation with lowering of the temperature of incubation. A similar weakening has also been observed if the tests are kept at 40° - 45°C. (Münster; Sachs; Baumgärtel; Huber) and the optimum temperature for the Sachs-Georgi reaction is given as 36.5° - 38° C. (Sachs). It was noted by Baumgärtel and others that, if the tests are examined after two hours at 37°C., certain normal sera may show flocculi which disappear after longer incubation (reversible flocculation). As an outcome of this observation he studied the effect of temperature on mixtures of normal serum and Sachs-Georgi antigen, and found that almost all human sera

will flocculate provided a suitable temperature is used; some flocculate at 15°C., others at 5°C., while others fail to flocculate unless the temperature is maintained at 0°C. The non-specific flocculi disappear when the mixtures are warmed to 37°C. Baumgärtel prepared flocculi of this type on a large scale and he found that they could be used to differentiate between normal and syphilitic serum, since the latter rendered them resistant to subsequent warming, while the former had no such effect. This observation was confirmed by Huber and it provides an analogy to what occurs in the Kahn or citochol tests for which a precipitate from a cholesterolised extract is used as the antigen. In these tests, the lipoid precipitate persists in the presence of syphilitic serum but it is readily dispersed, or even dissolved, when it is mixed with normal serum.

In view of these results, care has always been taken in the course of the present work to ensure that the incubator (37°C.) in which the tests were placed overnight was not subject to changes of temperature of more than 1°C. It can be stated with certainty that the fluctuations in the intensity of flocculation which have been

observed so frequently when testing the same sample of serum on more than one occasion cannot have been dependent in any way on the temperature of incubation. There has been ample opportunity for checking the efficiency of the thermostat and on many occasions the incubator has been opened, frequently and carelessly, while tests were inside, in the hope that such deliberate interferences with the constancy of the temperature might influence positive reactions, but no such effect has ever been observed. Moreover, since Sachs and Georgi indicate that specific flocculation is weaker in tests which are incubated overnight than in tests carried out by the original method (2 hours at 37°C., followed by 18 hours at room temperature), attention has been paid to the possibility of differences in the duration of the incubation period being a cause of variation in the results of repeated tests. There is no evidence however that tests incubated for 20 hours and tests incubated for 24 hours show any uniform variation in the intensity of flocculation. Strongly positive reactions can often be detected after three or four hours at 37°C. (cf. Sachs; Baumgärtel;

Mackie), but I have never been able to convince myself that any of these become weaker on longer incubation, and my usual experience has been quite the reverse. Many positive reactions fail to show definitely until the tests have been incubated for about twelve hours and they increase gradually in intensity up to the time for reading the results (18-20 hours). At this stage a difference of 2-4 hours in the total duration of incubation has not been associated with appreciable variation in the intensity of flocculation in parallel tests. Variation in the size of the tubes used for duplicate tests has also been considered as a possible cause for such fluctuations, but this also can be ruled out of count and, contrary to what might have been expected, smaller tubes (3" x $\frac{1}{4}$") were not found to be of any advantage in making the reading of weak results easier. Shaking of the tests, which is an essential part of the technique of the Kahn reaction and which is referred to by Watson as being valuable in the Sachs-Georgi test, has failed consistently in my hands to have any effect whatever on the intensity of specific flocculation. Not only have tests been shaken by hand, or in a

mechanical shaker, immediately after the addition of the antigen, but they have also been subjected to prolonged vibration during incubation, as a result of the proximity of a high speed centrifuge. None of these procedures has ever led to any effect on flocculation, as judged by comparison with duplicate tests placed in another incubator and kept perfectly still. Differences in the time taken in setting up parallel tests, and differences in the time such tests are allowed to remain at room temperature before being put in the incubator, have both been investigated as possible causes of variation in results but these factors can be eliminated. The only modification of technique which definitely interferes with the delicacy of the test is the use of an incubation temperature higher than 40°C. and, while it has been found that 45°C. (water-Bath) prevents flocculation of the more weakly-reacting sera, it has proved necessary to go still higher in order to abolish strongly positive results. At 55°C. flocculation has not been observed with any serum.

The question of the specificity of the Sachs-Georgi reaction has not come within the scope of this study which has been devoted primarily

to the problem of the variability of the test and to the difficulty experienced in reading so many of the results. Sachs and Georgi in their original communication reported 94.94% of agreements (614 positive; 2016 negative) between the results of their test and those of the Wasserman reaction in a series of 2,770 sera, and comparable findings have since been recorded by many other workers who have used the test for diagnostic purposes. It is well known however that the agreement is not so close in the case of sera from syphilitic patients under treatment or from patients with latent syphilis (Meyeringh; Taniguchi and Yoshinare), and opportunity was taken to carry out a short series of comparative tests with sera submitted from a Venereal Diseases Treatment Centre in order to confirm that the reagents in use were at least as satisfactory as those of other workers. The results are tabulated in appendix 112, and, as the percentage figures for agreements and discrepancies tallied with those of Taniguchi and Yoshinare in a similar, but larger, series, there seemed to be no reason for suspecting that one's experience of the

behaviour of the test might be exceptional. In general it may be said that, when used for diagnostic purposes, most positive results are cleanly cut and therefore easy to read, but that with cases of syphilis undergoing treatment they are frequently of the type which demands great care in reading in order to ensure that weak reactions may not be overlooked.

No attempt has been made to vary the relative amounts of serum dilution and antigen recommended by Sachs and Georgi, as other workers (Münster; Mackie) have confirmed that these represent the optimum proportions suited to the other conditions of the test, and as there are at least two important considerations which deter one from departing from a technique which has already proved itself reliable. In the first place, although it has been shown that specific flocculi are composed almost entirely of lipoid material derived from the antigen (Niederhoff; Taniguchi; Sachs and Sahlmann), and that the reacting property of syphilitic serum is associated with its globulin fraction (Mackie; Sahlmann), the mechanism of the flocculation reaction remains unknown and, in

the present state of knowledge, physical chemistry does not appear to be in a position to offer help towards furnishing an explanation. The hypotheses put forward by such workers as Meinicke,who attributes specific flocculation to a disturbance of the normal affinities of serum-antigen mixtures for Na- and Cl- ions (Kochsalzgleichgewichtsumlagerung), or Sachs, who envisages an alteration in the state of dispersion of the globulins of syphilitic serum (Globulinveränderung; Dispersitätsvergröberung), are as unenlightening as they are imaginative, and in neither instance does it seem that any experimental evidence in support of these speculations can be adduced by their authors, or any satisfactory analogy found in the realm of colloid chemistry. Meantime therefore one is more inclined to concentrate attention on one or other of the reputable methods already in existence, and to try and define as accurately as possible the factors which may definitely influence flocculation, either favourably or unfavourably, than to increase the complexity of the problem by introducing further modifications. In the second place, it is by no means improbable, as is contended by Meinicke,/

that all methods of carrying out the syphilis flocculation reaction are essentially the same in nature, and that any alteration in technique affecting one of the participating reagents invariably necessitates alteration of the conditions of the test as a whole, if optimum results are still to be obtained. As he himself has no fewer than five modifications to his credit, all carried out in different ways but with the same type of antigen, and all resulting in a differentiation between normal and syphilitic serum by the presence or absence of flocculation, he has done much to substantiate this view. It is of special interest, too, in relation to the argument of this thesis, that each of these modifications has been devised in order to overcome the difficulty experienced in reading the finer degrees of flocculation. The "third modification" (M.D.R.) was presented as an improvement on the preceding "water-method" and "salt-method". The "opacification test" (M.T.R.), as originally introduced, was to make the reading of results a matter of ease, thereby implying that difficulties had existed with the earlier methods; but it was found later that the addition of

benzoic acid facilitates matters still further by intensifying positive results. Now (1929) the "clearing reaction" (M.K.R.) has made its début and Meinicke claims that it eliminates the difficulties associated with reading the results of the M.T.R., difficulties which had not hitherto been admitted, and which if really overcome will ensure that this latest test will prove more acceptable than its predecessors.

A great variety of methods is already available for carrying out the syphilis flocculation reaction and concerning these an extensive literature has come into being. It has been collected and incorporated in a single comprehensive review by Klopstock, who traces the history of the evolution of the test and who summarises the details of the different techniques; but it is apparent that many of these methods are of interest rather than utility, since they are either less delicate than the Wassermann reaction or else they are so easily influenced by extraneous factors as to be unreliable in their results. So far as tests which have found favour on a large scale are concerned, there are only four, besides the Sachs-Georgi reaction, which are sufficiently

simple in technique and satisfactory in their behaviour to merit serious consideration as possible substitutes for the Wasserman reaction, viz. Meinicke's "third modification", Meinicke's "opacification test", the Vernes test, and the Kahn test.

Meinicke Tests. The Meinicke reactions have been widely used in German-speaking countries and the "third modification" gives results which parallel closely those of the Sachs-Georgi test (Meyeringh). As has already been mentioned however, the "third modification" has now been superseded by the "opacification test" and Meinicke himself does not deny that the change was determined partly at least by the difficulties in reading the finer degrees of flocculation in the earlier method. Unfortunately neither the M.D.R. nor the M.T.R. lends itself to experimental study as the details of the preparation of the antigens for these tests have not been published, and the manufacture of suitable extracts is entrusted to a German commercial firm, Reports by German workers leave no room for doubt that different samples of these extracts vary both in sensitiveness and in specificity (Klopstock and Dölter; Klopstock and Hilpert), and the fact that

Meinicke has now introduced a "clearing reaction" (M.K.R.), which is stated to overcome the difficulties in reading the results of the M.T.R., would appear to justify the conclusion that variation in the intensity of flocculation (or turbidity) produced by different syphilitic sera is still a troublesome factor in these tests, just as it is in the case of the Sachs-Georgi reaction. Furthermore, at the recent Laboratory Conference on the Serodiagnosis of Syphilis held at Copenhagen (League of Nations Reports) the M.T.R., carried out by Meinicke himself, gave more apparently false positive results than any of the other flocculation methods which were used (p. 166 , infra). It cannot therefore be regarded as a possible substitute for the Wassermann test, and indeed no such claim is made for it by Meinicke.

Vernes test. The Vernes reaction is peculiar in that its practice seems to be confined to the "Institut prophylactique" in Paris, where it is carried out on a very large scale under the personal supervision of Vernes. Like the Sigma reaction of Dreyer and Ward it is a quantitative test by means of which it is claimed that the effect of anti-syphilitic treatment can be studied

and controlled, but the results which Vernes obtained with his method at the Copenhagen Conference were so poor as to make one wonder if they are really a fair example of its usual behaviour. Not only were doubtful results with non-syphilitic sera very common, but many syphilitic sera reacted negatively although they gave strong positive reactions with all other methods. Apart altogether from the questions of specificity and sensitiveness, the Vernes technique is unlikely to gain favour on account of the expense of the elaborate apparatus which is necessary and which has already been referred to (p.[10]106). The special antigen too, obtainable from Paris, is another source of difficulty and I have only found one reference to its use by anyone other than Vernes himself.

Kahn Test. The Kahn test differs from all previous modifications of the syphilis flocculation reaction in that the results are available within half-an-hour, and it is not surprising therefore that it has attracted considerable attention. The essential difference between its technique and that of the earlier methods lies in the avoidance of dilution of the reagents, thus permitting them to interact

in a concentration higher than has hitherto been used. Credit is due to Kahn for his discovery that the precipitate obtained on adding a relatively small proportion of saline to a suitable cholesterolised heart-extract could be employed for the rapid differentiation of syphilitic serum from normal serum, and for having defined optimum conditions for a test of this type. As long ago as 1919 Meinicke had noted that normal serum dissolved such a precipitate, but, in the form of test which he used, he had failed to find any difference in the action of syphilitic serum; Baumgärtel, on the other hand, was able to identify normal and syphilitic sera by means of non-specific flocculi (p. 140) but such a method was too laborious to be of practical use. It is in this feature alone that there is anything novel in the Kahn test and the remainder of its technique is but an application of principles already established by Meinicke or by Sachs and Georgi. The extract which is used is prepared from dried beef-heart after preliminary extraction with ether (cf. Meinicke), and cholesterol is added as recommended by Sachs and Georgi. Kahn's original papers describe the preparation of his antigen as a relatively simple

procedure which had been found to yield products of uniform sensitiveness, but he has abandoned this mistaken belief and insists that very careful preliminary titration and adjustment of different samples of extract are necessary if they are to behave satisfactorily in the test. Suitable dilution with alcohol, and appropriate cholesterolisation, are now essential steps in the preparation of "Standard Antigen", and he has thus returned to the principle laid down both by Meinicke and by Sachs and Georgi that different extracts require dilution in varying degree if the optimum antigen is to be obtained. The titration and adjustment of Kahn standard antigen is a very complicated procedure and it is now emphasised that, unless the antigen is purchased from an authorised distributor, no reliance can be placed on its suitability for test purposes. It is fortunate that the variability of different samples of such antigens is at last recognised by Kahn, for his earlier writings are characterised by a certain dogmatism which, besides being undesirable, was soon found by other workers to be unwarranted. Isabolinski and Podwalnaja report having had to make many attempts before they finally succeeded

in preparing an antigen which would behave satisfactorily and, in my hands, it has not yet been possible to obtain one which ensures complete absence of fine flocculi from the antigen control, in spite of the fact that I have adhered strictly to the technique prescribed by Kahn and used the "Bacto" dried beef-heart which he specially recommends. It is regrettable too, in view of later work, that the claim should ever have been made (1923) that the test is so adjusted that nearly all syphilitic sera give ++++ reactions, and that + and ++ reactions are practically eliminated. Now (1930), in spite of all the care which has been devoted to perfecting the test and to standardising the antigen, Kahn has to classify the varying degrees of flocculation under no fewer than five headings:-

"Definitely visible particles".
"Less clearly cut particles".
"Finer particles".
"Still finer particles".
"Extremely fine particles".

That difficulty is experienced in reading the results of the Kahn test is admitted by all who

use it, except apparently by Kahn himself, and even those who are most favourably disposed towards it express dissatisfaction with the numerous weak reactions which occur, and the trouble which their reading entails. (Owen; Giordano; Becker; Hopkins and Rockstraw; Johnson; Leiboff; Figueira and Trincao; Kolmer; Cookson and Brown). Meinicke also, who praises the simplicity of the test, finds it very difficult to read and, if one may judge from his other writings, this opinion is unlikely to be a prejudiced one. Moreover, Figueira and Trincao, in their series of tests, were working with an antigen provided by Kahn himself; thus there cannot be any question of their unfavourable results being dependent on the use of an unsatisfactory sample of cholesterolised-extract. Such findings are in complete agreement with my own limited experience of the test. The strong positive results are easily recognised on the most casual examination of the tubes, but it is quite the reverse with the weaker reactions for the detection of which each tube must be withdrawn from the rack, in turn, and examined under favourable conditions of illumination. Herein lies a disconcerting feature of the test, for I have found it

necessary to avoid examining the tubes too critically, otherwise extremely fine, but none the less perfectly definite, flocculi are visible in the antigen control and in some of the tests with sera which are certainly not syphilitic. All normal sera do not show these fine flocculi, and this is probably to be attributed to individual differences in protecting power such as have already been referred to in the case of the Sachs-Georgi reaction, and such as have been noted by Meinicke in connection with his tests. It is apparent that this observation is not wholly dependent on a personal factor since it is laid down by Kahn (1930) that if a hand lens is used to facilitate reading results it must be of low magnification (2x or 3x), in order to ensure "clear cut negative reactions, with entire freedom from visible particles", and in his original paper (1923) he refers to shaking as dispersing "the weak, non-specific precipitates which occasionally form in negative serums". Demanche also states that the use of a lens may introduce a source of fallacy, and Becker records the observation of a very fine precipitate in the antigen controls which might be confused with a

positive reaction. This is undoubtedly a dangerous disadvantage in the Kahn test since experience has to be relied upon to enable one to know which flocculi to disregard and which to accept as specific, a fact to which the report of the Copenhagen conference bears witness. If the results obtained by Boas and by Kahn are compared (p.166,infra) it will be seen that, although Kahn returned no false positive,and only five doubtful,reactions in the series of tests with normal sera, Boas, using the standard Kahn technique and testing the same samples of serum on the same days as Kahn, returned three false positives and nine doubtful reactions. On the other hand, he reported 181 negative results to Kahn's 161 in the tests with sera from patients with a history of syphilis, thus causing their results in this series to differ by 4%. If these discrepancies were not due to differences in opinion as to the significance of some of the finer degrees of flocculation, they must be attributed either to varying sensitiveness of the antigens or else to the difficulty inherently associated with reading weak flocculation results.

Personally I find the Kahn test more difficult to read than the Sachs-Georgi, for its flocculi are less coarse, and weak reactions are often only slightly more granular than the antigen control, or the control in which normal serum is used. The recent "citochol" reaction (Sachs and Witebsky) which is analogous in every way to the Kahn test has confirmed this impression. A great deal of time is spent in examining and re-examining doubtful tubes, under varying conditions of lighting, and I repeatedly find myself quite unable to decide whether the result should be recorded as fine flocculation or not. So far as the Kahn test is concerned, comparatively little work appears to have been done in the way of re-testing the same sample of serum on several occasions, but Meinicke (Kahn test) has observed fluctuation in the intensity of flocculation in such circumstances and similar fluctuations are shown in the report of the Copenhagen conference, although here the total number of sera which were re-tested was small.

The outstanding merit of the Kahn test is its rapidity and this is naturally a very attractive feature, but there is not the slightest

doubt that it shares the disadvantages of its predecessors in that only a proportion of the results are easy to read (++ or greater); that zone phenomena are encountered (Kahn; Hopkins and Rockstraw); that some degree of spontaneous flocculation occurs not infrequently in the antigen control; and that the sensitiveness of the antigen is subject to variations, the cause of which is obscure and which cannot therefore be entirely eliminated. In specificity it compares unfavourably with the Sachs-Georgi reaction, and this is probably dependent partly on the difficulty in differentiating between the finer degrees of specific flocculation and the fine granularity sometimes shown by the controls, and partly on the sacrifice of specificity to speed. For the latter, a highly unstable antigen or, as it is generally expressed, a very sensitive antigen has to be used, thus tending to make the test so delicate that the limit of safety for certain sera is occasionally exceeded. For this reason Sachs is definitely of the opinion that the slower flocculation methods are to be preferred, and, although his "citochol" reaction (a rapid one) has been found to possess a high

degree of specificity (Kliewe and Engelhardt), he still (1929) regards a positive Sachs-Georgi result as a more reliable indication of syphilitic infection than a similar finding with the rapid test. Non-specific results with the Kahn test are recorded by several workers (Boas; Sheplar; Lyons and MacNeal; Kolmer; Figueira and Trincao) and it is not surprising that it has generally been found to be somewhat more sensitive than many of the other flocculation methods. The table on page 166. will serve as an example, but it must be noted that the overwhelming superiority of the Kahn test, as compared with the Wassermann reaction (Harrison's method), shown by these results is due in very large part to the nature of the sera which were used. It is known that the flocculation method gives a higher proportion of positives than the Wassermann test in the case of syphilitic patients who have been treated (Taniguchi and Yoshinare), but, as it is only recently that both tests are beginning to be applied as a routine in the control of treatment, the significance of such positive results is still undecided. Of the 502 sera tested at Copenhagen no fewer tham 332 were

from treated cases of syphilis (305 tertiary or latent) and 43 from cases of untreated latent syphilis and, according to Sachs who was present at the conference, the diagnosis of syphilis in a proportion of the "latent" cases was based solely on the history, and presumably also on the serological finding. There was no clinical evidence of the disease. The results of Wassermann and flocculation tests with such sera are bound to disclose a larger number of discrepancies than what occur in the course of their application in routine diagnostic work, and it is somewhat surprising that the figures for the Sachs-Georgi reaction show it to have been less sensitive than the Wassermann. The correspondence in the results of the Kahn and Wassermann reactions is ordinarily very close, and in a recently published series of parallel tests with 1,000 sera they were found to agree in 98.4% of the cases (Cookson & Brown). Standard Kahn antigen, obtained from an authorised source of supply, was used and the Wassermann tests were carried out by Harrison's method, as at the Copenhagen conference. The Kahn test has been considered in some detail on account of the fact/

that Kahn himself continues to advocate it as a satisfactory substitute for the Wassermann reaction, a view which is contrary to that of every other author whom I have consulted. It has been given a trial on an extensive scale in America where, in addition, it has benefited from a publicity which is not readily available for other methods; and one cannot but feel that the early enthusiasm evoked by its deceptive simplicity, and the novelty of its speed, was allowed to obscure the imperfections and difficulties which it shares in common with all the other modifications of the syphilis flocculation reaction. These are gradually being recognised and, during the past four years, I have been unable to trace a single reference to the Kahn test, with the exception of Kahn's own publications, in which the difficulty of reading the weaker reactions has not been a matter for adverse comment.

Experience of the use of the flocculation methods has thus revealed their technical limitations, and especially has it become evident that flocculation, as an indicator of a reaction between syphilitic serum

and a lipoid antigen, is characterised by an uncertainty of behaviour which can prove very troublesome, and which is dependent on factors whose nature is not yet understood. The technique of the Wassermann reaction is certainly less simple than that of the flocculation test but its various reagents are amenable to some measure of control, and the haemolytic system, as an indicator, ensures that all results --- positive, weak-positive, doubtful, or negative --- are rendered obvious by convincing and easily appreciable differences in the test mixtures. If for no other reason than this, it is still preferable to the flocculation test whose outstanding disadvantages are the difficulty in detecting any but the strongly positive reactions and the impossibility of controlling the sensitiveness of the antigen. In the case of the Sachs-Georgi reaction, to which the special studies presented in this thesis were directed, all attempts to overcome the variable behaviour of an optimum antigen, when tested with the same pooled syphilitic serum on different occasions, have proved unavailing and, in spite of careful attention to every factor likely to

influence flocculation, the results have sometimes been sharp and easily recognised, at other times so feeble as almost to pass undetected. There is however another circumstance which renders impracticable the substitution of a flocculation test for the Wassermann reaction. Meinicke and Sachs have long recognised that not only are positive flocculation results sometimes obtained with syphilitic sera which react negatively in the Wassermann reaction, but that the converse is also observed, and it seems possible that such anomalies in the reacting power of certain sera may be dependent on the association of flocculating property and complement-fixing property with different fractions of serum globulin (Mackie; Mackie & Watson). In some instances however a zone phenomenon, such as is shown in appendix 185, may account for a negative flocculation result. Similar disagreements are recorded in the case of the Kahn test (Hopkins & Rockstraw; Sheplar, Lyons & MacNeal; Figueira & Trincao), and the generalisation is thus warranted that neither the flocculation test alone nor the Wasserman reaction alone will detect all syphilitic sera; the two tests are

mutually complementary. Sometimes their results show complete agreement, sometimes they are totally at variance; but often the significance of a weakly positive or doubtful reaction in the one receives confirmation from a frankly positive reaction in the other. This conclusion, which is in keeping with the resolutions adopted at the laboratory conference in Copenhagen, makes any further comparison of the individual merits of the two methods unnecessary as neither of them is wholly satisfactory as an independent standard for diagnostic purposes. The floccu-lation test, on account of its simplicity and in spite of its difficulties, has no lack of enthusiastic advocates, but their preference cannot reasonably be permitted to outweigh the considered opinion of the two workers, Meinicke and Sachs, to whom the method owes its origin, and both of whom express the con-viction that, although it is a valuable addition to the existing methods for the serological diagnosis of syphilis, it can only be of real service, in any of its modifications, when used in conjunction with the Wassermann reaction.

T_A_B_L_E.

(Compiled from the Report of the Second Laboratory Conference on the Serodiagnosis of Syphilis. Copenhagen. 1928).

Test:-	Wa.R.	S-G	MTR.	SIGMA	KAHN (Boas)	KAHN (Kahn)
502 syphilitic sera.						
Positive.	210	208	246	257	294	305
Doubtful.	78	23	38	76	27	33
Negative.	214	253	218	169	181	161
total tested =	502	484	502	502	502	499
435 non-syphilitic sera.						
Positive.	0	0	9	6	3	0
Doubtful.	12	1	13	35	6	5
Negative.	423	430	413	393	426	429
total tested =	435	431	435	434	435	434

The 502 syphilitic sera include:-

27 from cases of TREATED primary or secondary syphilis.
50 .. .. tertiary syphilis.
255 .. .. latent syphilis.
43 .. .. .. latent syphilis (untreated).

Wa.R. = Wassermann reaction. S-G = Sachs-Georgi reaction.
MTR. = Meinicke's opacification test. SIGMA = Sigma reaction.
KAHN = Kahn test, carried out by (a), Boas and (b), Kahn.

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FIXATION of COMPLEMENT by B. TYPHOSUS

and

NORMAL GUINEA-PIG'S SERUM.

A contribution to the study of natural antibodies.

During the course of experimental work on the antigenic properties of certain products of B. typhosus (Eberthella typhi) the desirability arose of testing the sera of the immunised animals for a complement-fixing antibody to this organism, but difficulty was encountered in carrying out such tests, as the bacillary suspension by itself was invariably found to cause zonal fixation of the haemolytic complement of normal guinea-pig's serum. In the literature bearing on the subject, the only record of observation of a similar phenomenon which has been traced is in the work of Ritz and Sachs (1917), confirmed later by Kondo (1923). These workers, studying the inactivation of complement by various agents, noted that, within certain limits, a suspension of B. prodigiosus (Serratia marcescens) showed greater anti-complementary effect in the presence of large doses of complement than in the presence of smaller doses; a paradoxical result which they ascribed to alterations in the state of dispersion of the globulin of the complement-containing serum. The phenomenon does not appear to have been observed with B. typhosus, and the present study embodies

the results of an investigation of the conditions under which it occurs and offers an explanation as to its cause..

The phenomenon.

The strain of B. typhosus ("R11") regularly employed in connection with the work is a laboratory stock strain which has been maintained during the past sixteen years. It was obtained originally from one of the local hospitals for infectious diseases, and it conforms culturally and serologically to the criteria for B. typhosus.

For the demonstration of the phenomenon an 18 to 24 hours' growth of B. typhosus on casein-digest-agar is emulsified in sterile 0.85 per cent. solution of NaCl in distilled water, by flooding the agar surface with the saline and rocking, or rotating, the container gently so that the fluid may wash off the growth. The bacillary emulsion is decanted into a tall narrow cylinder in which it is allowed to stand undisturbed for two hours, so that any coarser particles may settle, after which the upper two-thirds are pipetted off and the remainder rejected. A preliminary test is carried out in order to determine the greatest concentration of this suspension which, in the

(to follow p.174)

TABLE 1.**

Zonal fixation of guinea-pig's complement by B. typhosus.

Complement	M.H.D.	2	4	6	9	12	16	20	25	30	40	50
	c.c.	0.015	0.03	0.045	0.0675	0.09	0.12	0.15	0.1875	0.225	0.3	0.375
Typhoid suspension 0.5 c.c.		C	ftr	0	0	0	0	0	0	tr	vmk	jc

Complement=pooled sera of three guinea-pigs. M.H.D.=0.0075 c.c.

Typhoid suspension = suspension in sterile saline of *B. typhosus* (24 hours' growth on casein-digest agar). The suspension is unheated, and it has been tested preliminarily as detailed on p.175. It is not haemolytic for ox blood cells.

**Unless otherwise indicated, the following details are applicable to all tables shown in the text:-

The mixtures of typhoid suspension and complement are incubated at 37°C. for 1½ hours prior to addition of the haemolytic system; thereafter 37°C. for 1½ hours.

Readings are made when tests are withdrawn from incubator, and are checked after tubes have stood at room temperature overnight.

Haemolytic system = 3 per cent. suspension in saline of thrice-washed ox blood sediment, sensitised with 5 M.H.D. of rabbit versus ox haemolysin. Test volume = 0.5 c.c.

Saline = 0.85 per cent. solution of $NaCl$ in distilled water.

Rigorous controls have been used for each set of tests, but only the more important of such controls are shown.

Degrees of lysis are indicated thus:-

0 = no lysis. ftr = faint trace. tr = trace

d = distinct. mk = marked. vmk = very marked.

ac = almost complete. jc = just complete. C = complete lysis.

presence of 2 M.H.D. of complement, will be devoid of anticomplementary action. To this end, a series of dilutions of the suspension is prepared with saline (e.g. 1 in 2; 1 in 3; 1 in 4; 1 in 6; etc.) and a constant volume (0.5 c.c.) of each of these is tested with 2 M.H.D. of guinea-pig's complement. The mixtures of bacilli and complement are incubated at 37°C. for one-and-a-half-hours, after which the haemolytic system is added. A further incubation of one-and-a-quarter hours is given and the readings are then made. The strongest suspension permitting complete lysis of the test corpuscles is selected as suitable for the main test, and this is carried out as shown in Table 1 which serves as a characteristic example of the phenomenon under consideration.

Since results of the type shown in Table 1 were consistently obtained it was apparent that, without modification of the technique usually employed, complement-fixation tests with this strain of B. typhosus and the sera of immunised animals would be valueless if guinea-pig's serum were used as the source of complement. Attention was directed therefore to investigating the factors underlying the phenomenon and in the following

(to follow p.175)

TABLE 11.

Variation in complement-fixing power of different strains of B. typhosus.

Complement (M.H.D.)	2	4	7	10	15	20	30	40	50
(c.c.)	0.015	0.03	0.0525	0.075	0.1125	0.15	0.225	0.3	0.375
Strain *Lab* . .	C	ac	mk	ftr	vmk	C	C	C	C
Strain *R.A.M.C.* .	C	jc	mk	ac	C	C	C	C	C
Strain *Edinburgh* .	jc	vmk	d	0	mk	C	C	C	C
Strain *Rll*** . .	ac	mk	0	0	0	0	0	ftr	C
Strain *Rawlings* §.	C	C	C	C	C	C	C	C	C

Complement = pooled sera of two guinea-pigs. M.H.D. = 0.0075 c.c.

Bacillary suspensions prepared from 24 hours' growths of each of the strains of *B. typhosus* on casein-digest agar. The suspensions are of approximately the same degree of turbidity and they have been heated in a water-bath at 55°C. for one hour. Test volume of each = 0.5 c.c.

None of the bacillary suspensions is haemolytic for ox blood cells.

**This is the strain used regularly in the present work.

§ One of the two strains of *B. typhosus* with which zonal fixation of complement has not been demonstrated even when very turbid, unheated, suspensions are used.

pages a summary of the results which have been forthcoming will be presented.

Variations in the complement-fixing power of different strains of B. typhosus.

Twenty-one strains of B. typhosus have been examined and, with two exceptions, they have all been found to cause zonal fixation of guinea-pig's complement (as shown in Table 1) provided that a suspension of suitable density is used, and that the test is carried out with an adequate range of complement dosages. Different strains, however, vary considerably in their ability to fix complement in this way and, with four of those examined, a zone was not demonstrated at the first test although in later tests it was found to occur. With some strains the zone is very short and it may occur only when very turbid suspensions are employed; while with others, an extended zone is shown even with suspensions containing a few hundred million bacilli per c.c. In Table II, which exemplifies such variations in complement-fixing power, the suspensions of the different strains are of approximately the same degree of turbidity (1500 to 2000 million bacilli per c.c.) and the tests are all carried out at the same time, with the same complement.

(to follow p.176)

TABLE 111.

Complement-fixation by typhoid bacilli (autoclaved and washed).

Complement c.c.	0.02	0.03	0.04	0.06	0.08	0.12	0.16	0.2	0.25
Bacillary suspension c.c.	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
(A) Using Fresh Complement	C	C	0	0	0	0	0	tr	C
(B) Using "treated complement"	jc	C	C	C	C	C	C	C	C

The total volume in each tube is made up to 0.7 c.c. with saline.

M.H.D. of complement (fresh or "treated") = 0.0125 c.c.

Bacillary suspension is not haemolytic for ox blood cells. Density of suspension = 1500 million per c.c.

"Treated complement" is not haemolytic for ox blood cells.

"Treated complement" is prepared with charcoal (vide, p. 201).

The density of the suspensions is estimated by means of "Standard Opacity Tubes" (Burroughs Wellcome & Co.)

One of the strains in the series was recently isolated; the others are laboratory stock strains obtained from a variety of sources; while the two which, even on repeated testing, failed to cause zonal fixation of complement are the "Rawlings"and "Lister (786)" strains from the National Collection of Type Cultures. They differ from the classical type of B. typhosus, and from all the other strains used, in that they do not ferment maltose (the "Difco" product), but they are agglutinable up to the titre of an anti-typhoid serum developed by immunising with a different strain (e.g. "R11"). I have learned recently that the two strains are really the same; "Lister (786)" was originally a sub-culture from "Rawlings" but it has been maintained separately ever since for a special purpose.

Living Bacilli or killed.

The "R11" strain causes zonal fixation of complement not only when used in the form of a suspension of living bacilli but also with suspensions which have

been heated at 55°C. for one and a half hours (water-bath), and with suspensions of bacilli which have been autoclaved at 115°C. to 120°C. for twenty minutes and subsequently washed repeatedly with sterile saline. Table III (A) which is an example of a test carried out with such a suspension of autoclaved and washed bacilli shows the extent of the zone of complement-fixation which occurs under these conditions. These observations refer to freshly prepared suspensions, but suspensions of killed bacilli which have been stored in a cool place for several days have also proved suitable for the demonstration of the phenomenon.

The "Rawlings" and "Lister" strains have been tested repeatedly, using living suspensions containing from 500 to 20,000 million bacilli per c.c., and with a range of complement dosages extending from 2 to 30 M.H.D., but zonal complement-fixation has not been demonstrated with either of them. They have proved quite exceptional in this respect and the only anticomplementary effect which they have shown has been in the presence of minimal complement dosages (2 to 6 M.H.D.), when very dense suspensions are used.

Of the eighteen other strains in the series which has been examined, fourteen have been tested only in the form of suspensions of living bacilli, while suspensions heated at 55°C. have been used in the case of the remaining four.

"Rough" forms of B. typhosus.

In view of the work of Arkwright (1921) and others on "rough" and "smooth" variants of B. typhosus, the suggestion was offered that the exceptional behaviour of the strains "Rawlings" and "Lister" might be related to their being "rough" forms. This suggestion has been followed up but it is apparent that such is not the explanation. A typical "rough" form, kindly provided by Dr. Arkwright, behaves quite differently in its sensitiveness to NaCl from either the "Rawlings" or "Lister" strains. These two remain in homogeneous suspension even in the presence of 10 per cent. of NaCl, while the "rough" form shows rapid, and practically complete, sedimentation in the presence of 0.9 per cent. of the salt. Moreover, the colonies on agar of the "Rawlings" and "Lister" strains are devoid of the characteristic features shown by those of the "rough" form; also the latter ferments maltose readily. Zonal complement-

fixation has not been demonstrable however with Dr. Arkwright's "rough" strain, whether used living or killed.

Density of suspension.

With a strain with which the incidence of the zone of complement-fixation occurs in the presence of low complement dosages (2 to 6 M.H.D.), the commencement of the zone may be masked by the anticomplementary action common to bacterial suspensions in general, unless a suspension of suitable density is used. With too dense a suspension of such an organism the result would appear to indicate that its direct anticomplementary effect was very great (leading to the destruction of 20 or more M.H.D. of complement), whereas in reality it represents the summation of two demonstrably distinct actions on the complement; (a) direct (common to most bacterial suspensions), and (b) the zonal fixation which, as the present work will show, is the result of an indirect action. It is usually desirable, therefore, to carry out a preliminary titration (as detailed on p. 175) in order to determine the greatest concentration of bacilli in unit volume of the suspension which, after incubation

with 2 M.H.D. of complement, will permit of complete lysis of the test corpuscles. On diluting the original suspension to the concentration thus determined, zonal fixation of complement will be evident on testing with an adequate series of suitably graded complement dosages, while similar, but increasingly shorter, zones of fixation will usually be obtained with still greater dilutions of the original suspension (cf. Table IV).*

* following p.185.

The culture medium.

As a general rule, casein-digest-agar has been used as the culture medium but no relationship seems to exist between the nature of the medium on which the organisms are grown and the ability to cause zonal fixation of complement. Only certain organisms grown on this agar behave like B. typhosus ("R11"), and B. typhosus grown on other media (e.g. solidified blood-serum) retains its complement-fixing power (zonal). Further, as has been noted above, typhoid bacilli which have been autoclaved and subsequently washed repeatedly with saline have proved suitable for the demonstration of the phenomenon. It has been found also that if the operation of inoculating a tube of casein-digest-agar, but using a sterile

platinum needle, is carried out and the tube incubated at 37°C. for 18 to 24 hours, the washings obtained by treating this control tube with saline (in exactly the same way as in the preparation of bacterial suspension) are practically devoid of anticomplementary effect. Unit volume (0.5 c.c.) of such washings may show fixation of, as a maximum, 3 M.H.D. of complement but no indication of any action on complement, in the presence of higher dosages, has been observed on any of the occasions on which this control has been used. Mackie (unpublished), who has recently had the opportunity of using the "R11" strain in similar experiments, was unable to confirm my finding that suspensions of the killed (autoclaved) bacilli could still cause zonal complement-fixation. On seeking an explanation of this unexpected observation it was apparent that the nature of the culture medium on which the organisms had been grown must have been the factor responsible; Mackie used a meat-extract-agar while, for my experiments, casein-digest-agar had served as the routine medium. The work with living and killed suspensions was therefore repeated and it has yielded a most interesting result. Fresh (unheated)

suspensions of the "R11" strain, whether grown on meat-extract-agar or casein-digest-agar, cause zonal fixation of complement equally well; but it is only the suspension from the casein-digest-agar which retains this property after heating at 115° - 120°C. (autoclave). Killed bacilli from meat-extract-agar cultures have now been tested on four occasions, using suspensions of 15,000 - 20,000 million organisms per c.c., but no suggestion of zonal complement-fixation has been demonstrable; any anticomplementary effect shown by these suspensions was slight (4-6 M.H.D.). Portions of the same cultures, however, tested as fresh (unheated) suspensions (2,000 million per c.c.) and with the same specimens of complement, yielded a typical zone in every instance. On the other hand, autoclaved suspensions of bacilli grown on casein-digest-agar have never yet failed to fix complement zonally, even when the density of the suspension used was as low as 1,500 -- 2,000 million per c.c. (seven experiments). It would thus appear that the living bacilli possess the property of causing zonal fixation of complement irrespective of the nature of the medium on which they have been grown, but that this property is

sometimes thermostable and sometimes relatively thermolabile; the difference being determined by the use of different culture media. Mackie has now confirmed this finding, which promises to provide an interesting field for further investigation, but, as it is outwith the scope of the present work, it has not meantime been followed up.

Age of Culture.

In practically all cases, cultures of 18 to 24 hours' growth at 37°C., have been used, but, so far as the "R11" strain is concerned, the age of the culture is not of importance since zonal complement-fixation has been demonstrated with cultures at least a month old.

The complement.

As a routine, the guinea-pigs have been bled on the evening prior to the test, the blood stored in a cool place overnight and the serum pipetted off and centrifugalised next morning. In several instances the complement has been obtained by killing the animals immediately before the tests, defibrinating the blood and recovering the serum by centrifugalisation.

Either of these methods of preparation yields a serum whose haemolytic complement is fixed zonally by a suitable typhoid suspension, and this property is retained, for a week or two, at least, when the serum is preserved frozen hard in the refrigerator. In order to eliminate the possibility of the phenomenon being associated with a particular breed of guinea-pig, or with local conditions, animals have been procured from sources of supply other than the usual, but in the course of an extensive series of tests no animal has yet been found, whatever its age, weight, or origin, whose complement has not been fixed in the manner indicated by the strain of B. typhosus ordinarily employed. Animals specially obtained from London and elsewhere were killed within twenty-four hours of their arrival at the laboratory and their sera used as complement without delay.

The amount of complement fixed.

Early observations suggested that not only the commencement, but also the upper limit, of the zone of complement-fixation was related to the turbidity of the bacillary suspension employed, and an example of experiments undertaken to

(to follow p.185)

TABLE IV

Effect of dilution of typhoid suspension on amount of complement fixed.

Dilutions of typhoid suspension	Complement M.H.D.	1½	2	4	6	9	12	16	20	25	30	40	50	60	70	80
1 in 2		C	C	0	0	0	0	0	0	0	0	0	tr	d	mk	vmk
1 " 4		C	C	ftr	0	0	0	0	0	0	tr	vmk	jc	...	...	...
1 " 8		C	C	C	0	0	0	0	tr	vmk	jc	C	C	...	...	...
1 " 12		C	C	C	C	0	0	tr	mk	jc	C	C	C	...	...	...
1 " 16		C	C	C	C	ftr	ftr	mk	C	C	C	C	C	...	...	...
1 " 20		C	C	C	C	0	tr	ac	C	C	C	C	C	...	...	...

M.H.D. of complement = 0.0075.c.c.

Bacillary suspensions are unheated. Test volume of each = 0.5 c.c.

furnish further information on this point is given in Table IV.

For this experiment, a dense suspension of the "R11" strain was prepared and from this a series of increasing dilutions with saline were made, each of which was tested at the same time with an extensive range of doses of the same guinea-pig's serum. As the total volume in corresponding tubes of the series is constant, it is apparent that the amount of complement fixed is dependent on the number of bacilli present. Attention may again be directed to the fact that the density of the typhoid suspension affects the incidence of the fixation zone; with the strongest suspension this begins in the presence of 0.03 c.c. of complement while, with the most dilute, more than 0.045 c.c. of the serum must be present before complement-fixation is manifest. Owing to the very large amount of complement fixed by these suspensions, and to their nature, it is a matter of great difficulty to obtain precise information as to the actual quantitative relationship between the number of bacilli used and the number of complement doses fixed but, in the course of a

(to follow p.186)

TABLE V.

Effect on complement-fixation of variation of the total volume in which serum and bacilli interact.

Complement {M.H.D.	2	4	6	8	10
Complement {c.c.	0.015	0.03	0.045	0.06	0.075
Bacillary suspension c.c.	0.25	0.25	0.25	0.25	0.25
Without added saline . .	0	0	0	0	0
With saline added to 0.5 c.c.	ac	0	0	0	0
" " " " 0.9 c.c.	C	C	0	0	0
" " " " 1.2 c.c.	C	C	jc	tr	ftr

M.H.D. of complement (in total volume 0.5 c.c) = 0.0075 c.c.
Bacillary suspension is unheated.

series of experiments of the same type as figured in Table IV., the general trend of the results has pointed to a remarkably close parallelism betweem them. The effect of varying the total volume, while keeping the number of organisms constant, has also been studied and it has been found that on increasing the volume in which the bacilli and guinea-pig's serum are permitted to interact, the commencement of the zone of complement-fixation does not appear until somewhat higher complement dosages are present. An example is given in Table V.

Relation to agglutinins in guinea-pig's serum.
On many of the occasions on which the phenomenon was being studied the guinea-pig's serum used as complement has been tested for the presence of agglutinins for B. typhosus. In such tests both Standard Agglutinable Culture (Oxford) of the organism and suspensions of the "R11" strain were employed, and incubation of the tests was carried out at temperatures of 55°C. (water-bath) and 37°C. (incubator). When 55°C. is the temperature of incubation, agglutination has never been observed with concentrations of guinea-pig's serum ranging from 1 in 4 to 1 in 200 but, in tests

incubated at 37°C., for twenty-four hours, there was occasionally some agglutination of the "R11" strain in the presence of serum concentrations of 1 in 2 and 1 in 4. At 37°C. however no agglutination of Standard Culture was obtained. Agglutinins, when present, were therefore minimal in amount and usually thermo-labile. Confirmation of the thermo-lability of such traces of agglutinating power has been obtained on several occasions by carrying out parallel tests with the guinea-pig's serum (a) fresh, and (b) after heating for half an hour in a water-bath at 55°C. It must be emphasised that the presence of demonstrable traces of agglutinins for B. typhosus is not essential for zonal fixation of haemolytic complement. Guinea-pig's serum has been met with which, even in a concentration of 1 in 2, proved devoid of agglutinating power for the "R11" strain (tested at 37°C.) but which showed fixation of upwards of 50 M.H.D. of its haemolytic complement by 0.5 c.c. of the actual typhoid suspension used in the agglutination test.

The treatment of complement with B. typhosus.

As it seemed possible that the phenomenon might be associated with the presence in the complement-containing serum of a natural antibody to B. typhosus an experiment was undertaken to determine

(to follow p.188)

TABLE VI

"Treated complement."

Complement	M.H.D.	2	4	6	9	12	15	20	25	30	40	50
	c.c.	0.01	0.02	0.03	0.045	0.06	0.075	0.1	0.125	0.15	0.2	0.25
Bacillary suspension c.c.		0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Using Fresh Complement		jc	vmk	tr	0	0	0	0	0	0	tr	vmk
Using "Treated complement"		C	C	C	C	C	C	C	...	C	C	...

M.H.D. of complement (fresh or "treated") = 0.005 c.c.

"Treated complement" is not haemolytic for ox blood cells.

Bacillary suspension heated at 55°C. for one hour prior to use.

the effect of treating the guinea-pig's serum at 0°C. with the organisms, as a preliminary to its use in the usual way. This was tried on the analogy of the deprivation of guinea-pig's serum of its natural lysin for ox blood cells by treating it at 0°C. with ox blood sediment and, as is shown in Table VI, such treatment succeeded in yielding a product, practically unaltered in its haemolytic activity, whose haemolytic complement was not fixed by B. typhosus alone.

Preparation of "treated complement".

A measured volume of concentrated suspension of B. typhosus in saline (heated or unheated) is centrifugalised and the supernatant fluid pipetted off. The sediment is chilled by placing the tube in chopped ice for five minutes after which a volume of guinea-pig's serum, similarly chilled, is added, equivalent to 8 M.H.D. of complement for each c.c. of diluted suspension represented by the bacillary sediment; e.g. if the concentrated bacillary suspension required to be diluted to 1 in 6 (determined by preliminary tests as detailed on p.175) in order to be suitable for demonstrating the zone phenomenon, then the sediment from 15 c.c.

(to follow p.189)

TABLE VII

Examples of the effect of "treatment" on H.H.D. of complement.

Haemolytic system c.c.	0.5	0.5	0.5	0.5
Complement c.c.	0.0025	0.005	0.0075	0.01
Complement A (fresh	ftr	C	C	C
Complement A ("treated"	ftr	ac	C	C
Complement B (fresh	...	tr	...	ac
Complement B ("treated"	...	ftr	...	ac
Complement C (fresh	ftr	jc	C	C
Complement C ("treated"	ftr	vmk	C	C
Complement D (fresh	...	ac	...	C
Complement D ("treated"	...	mk	...	C

Tests incubated for one hour at 37°C.

"Treated complement" is not haemolytic for ox blood cells.

Complements A, B, C and D are specimens used on different occasions.

represents 90 c.c. of diluted suspension and will, therefore, be used to treat 8 x 90 M.H.D. of guinea-pig's serum. The bacilli are mixed with the serum by stirring with a glass rod and the mixture is allowed to stand in chopped ice for one hour. Thereafter it is centrifugalised at high speed for fifteen minutes; the supernatant fluid is pipetted off and re-centrifugalised; any sediment in the tube is rejected and the clear serum constitutes "treated complement". Table VI serves as a further example of the extent of the zone of complement-fixation which occurs with B. typhosus and fresh guinea-pig's serum (6 to 40 M.H.D.) and it shows the total absence of any complement-fixation when "treated complement" is substituted for the fresh serum. The same result has now been obtained on many occasions. "Treatment" of the serum deprives it of the power to show fixation of its haemolytic complement in the presence of B. typhosus and, as is exemplified in Table VII, such "treatment" rarely causes more than an insignificant increase in the haemolytic dose.

(to follow p.190)

TABLE VIII

Example of imperfect "treatment" of complement.

complement (M.H.D.)	1	2	4	7	10	15	20	30	40	50
complement (c.c.)	0.01	0.02	0.04	0.07	0.1	0.15	0.2	0.3	0.4	0.5
Bacillary suspension c.c.	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Using fresh complement	C	C	0	0	0	0	0	0	0	ftr
Using "treated" complement"	vmk	C	C	C	C	C	vmk	ftr	0	ftr

Bacillary suspension in unheated.

M.H.D. of complement (fresh or "treated") = 0.01 c.c. "Treated complement" is not haemolytic for ox blood cells. It is prepared with bacilli previously heated at 55°C. for 1 hour.

From these initial observations it was apparent that the destruction of the haemolytic complement of guinea-pig's serum which occurred in the presence of B. typhosus under the conditions of the test, could not be due to a direct effect of the bacilli on complement, but that it was rendered possible by the interaction of some additional factor present in the serum. "Treatment" has not invariably succeeded in abolishing completely the zone of complement-fixation which normally occurs with a suspension of B. typhosus and fresh complement. Occasionally a zone has persisted even after "treatment" of the serum, but such a zone has always been very much shorter than the one resulting from the use of the fresh (untreated) serum. It begins only when relatively large complement dosages are employed but its upper limit may coincide with that of the zone shown by the fresh serum. An example is given in Table VIII and as a general rule, it has seemed justifiable to attribute such results to inefficient "treatment" with too small an amount of bacillary sediment in proportion to the volume of serum.

"Treated bacilli".

Since "treatment" of guinea-pig's serum in the

manner described above was the means of removing some factor normally present, it appeared desirable to examine the bacilli used in the process in order to determine whether or not they had been altered in any way as a result of their contact with the serum. The bacillary sediment recovered after "treatment" of complement was washed with sterile saline and finally suspended in saline so as to yield an emulsion of the same density as that ordinarily used in the tests. For example, a measured volume of a strong suspension of B. typhosus in saline is centrifugalised and the sediment utilised for "treating" a portion of complement. With the remainder of the strong suspension a preliminary titration is carried out to determine how much dilution is necessary so that zonal fixation of complement may be optimal (vide ,p. 175), and it is diluted accordingly, e.g. to 1 in 8. When the bacilli which have been in contact with the complement at 0^{o}C. are recovered, they are, after washing, suspended in a volume of saline eight times that of the measured volume of strong suspension from which they were originally obtained. In this way two suspensions of B. typhosus of equal density are prepared, the one consisting of

(to follow p.192)

TABLE IX

Sensitisation of typhoid bacilli by contact with guinea-pig's serum at o°C.

"Treated complement"	M.H.D.	1	2	3	4½	6	8	10	15
	c.c.	0.015	0.03	0.045	0.0675	0.09	0.12	0.15	0.225
Bacillary suspension c.c.		0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Using suspension of normal bacilli		vmk	jc	C	C	C	C	...	...
Using suspension of "treated" bacilli.		0	0	0	0	0	ftr	mk	C

"Treated" bacilli have been in contact with guinea-pig's serum at 0°C for one hour.

Normal bacilli have been kept at 0°C for one hour and have been centrifugalised in parallel with "treated" bacilli.

Suspensions of normal and "treated" bacilli are of equal densities.

Preliminarily the bacilli were heated at 55°C for two hours in water-bath.

M.H.D. of "treated complement" = 0.0158.c. It is not haemolytic for ox blood cells.

Note.- "Treated complement" showed no zone phenomenon with the suspension of normal bacilli when tested in a full range of doses (1 to 40), thus showing that the treatment had been adequate. The upper limit of the zone with fresh complement, used as a control, was at 35 to 40 M.H.D.

The bacillary suspensions are not haemolytic for ox blood cells.

normal bacilli and the other of bacilli which have been in contact with the serum for an hour. These two suspensions are now tested in parallel with a range of increasing doses of "treated complement", as shown in the experiment given in Table IX.

Bacilli which have been used to "treat" complement thus differ from the normal bacilli in that they can fix "treated complement", i.e. they behave like bacilli sensitised with a corresponding antibody. This finding has been confirmed and the conclusion is therefore warranted that normal guinea-pig's serum contains a factor which, in conferring on typhoid bacilli the power to fix complement, resembles a specific antibody.

The action of guinea-pig's serum deprived of haemolytic complement.

For the removal of haemolytic complement from the serum heated stromata of blood cells (ox or sheep) fully sensitised with the homologous immune body were used. A 12 per cent suspension in saline of thrice-washed sheep blood sediment is heated for several hours in a water-bath at 55°C. The sediment is recovered by centrifugalisation and washed with saline as often as may be necessary to ensure that the supernatant fluid is clear and

colourless. This washed sediment is suspended in saline and sensitised with ten times the M.H.D. of the homologous immune body,* of which 2 c.c. are required per 100 c.c. of the original suspension. Ox blood sediment sensitised with rabbit v. ox immune body has been used similarly. After standing for some hours (usually overnight) in a cool place the sediment is recovered by prolonged centrifugalisation, and, after removal of the supernatant fluid as completely as possible, it is mixed with an equal volume of complement and the mixture incubated at 37°C., for from two to three hours. At the end of this time the mixture is centrifugalised and the clear supernatant fluid is pipetted off. This product consisting of guinea-pig's serum deprived of its haemolytic complement will be referred to as "absorbed serum". On the numerous occasions on which it has been prepared it has consistently been found to be devoid of haemolytic complementing activity in dosages representing 100 times the M.H.D. of the fresh serum and, by itself, it showed no anticomplementary effect in the largest amounts used in the tests.

* Haemolytic serum for sheep's corpuscles (Burroughs Wellcome & Co.).

(to follow p.194)

TABLE X

B. typhosus + *"absorbed serum"* + *"treated complement."*

Complement	M.H.D.	1	2	3	4	6	10	15	20	30	nil
	c.c.	0.005	0.01	0.015	0.02	0.03	0.05	0.075	0.1	0.15	nil
Bacillary suspension + native complement		C	vmk	ftr	0	0	0	0	ftr	C	0
Bacillary suspension + "treated complement"		tr	C	C	C	C	C	C	C	C	...
Bacillary suspension + "absorbed serum" + "treated complement"	"absorbed serum" 0.005 c.c.	jc	C	jc	mk	ftr	ftr	tr	...	C	...
	"absorbed serum" 0.01 c.c.	C	jc	ftr	ftr	0	0	tr	...	ac	...
	"absorbed serum" 0.015 c.c.	ac	ftr	0	0	0	0	0	...	ac	...
	"absorbed serum" 0.02 c.c.	d	0	0	0	0	0	0	...	vmk	...
	"absorbed serum" 0.04 c.c.	0	0	0	0	0	0	0	...	...	....
Saline 0.5 c.c. + "absorbed serum" 0.04 c.c. + "treated complement"		C	C	C	C	C	C	C	...	C	....

Test volume of bacillary suspension = 0.5 c.c. Suspension is unheated.

"Treated complement" is prepared with coal dust (*vide* p.198); it is not haemolytic for ox blood cells.

Haemolytic system = 3 per cent. suspension of thrice-washed ox blood sediment sensitised with 5 M.H.D. of immune body from rabbit.

"Absorbed serum" (prepared with heated stromata of sheep's blood cells) 0.5 c.c. + haemolytic system = no lysis.

Native complement, "treated complement" and "absorbed serum" are all from the same sample of pooled guinea-pig's serum.

By means of the procedures which have been detailed, guinea-pig's serum can be prepared as (a) "treated complement" and (b) "absorbed serum", and these fractions, along with the original native serum, are available for testing with a suspension of typhoid bacilli as follows:-

(1) Bacilli + native serum (increasing doses).

(2) Bacilli + "treated complement" (increasing doses).

(3) Bacilli + "absorbed serum" + "treated complement" (increasing doses).

The tests are carried out in parallel, using the same typhoid suspension throughout and "treated complement" "absorbed serum" and native serum (complement) which are portions of the same specimen of pooled guinea-pig's serum. Table X is a characteristic example of the results obtained in such an experiment, and it shows that "treated complement", which is not fixed by typhoid bacilli alone or by the "absorbed serum" alone, is fixed by mixtures of these two. Moreover, when small amounts of "absorbed serum" are used, the complement fixation may be zonal. In the presence of larger amounts, the result is precisely that of fixation of complement by a mixture of an antigen with its homologous antibody, and it would appear

(to follow p.195)

TABLE XI

Complement	M.H.D.	1	2	3	4	6	8	10	14	18	nil
	c.c.	0.01	0.02	0.03	0.04	0.06	0.08	0.1	0.14	0.18	nil
Bacillary suspension, c.c.		0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Using native complement		C	C	C	vmk	d	ftr	0	0	0	0
Using incubated complement		jc	C	C	C	C	C	C	C	C	...

M.H.D. of complement (native or incubated) = 0.01 c.c.

Native and incubated complement are portions of the same sample of pooled guinea - pig's serum.

Incubated complement has been kept at 37°C for two hours prior to use in the test. Native complement has stood at room temperature for the same time.

Bacillary suspension is unheated. Density = 1000 million bacilli per c.c.

from these experiments also that the phenomenon under consideration must be attributed to the presence in normal guinea-pig's serum of a natural antibody to B. typhosus. Additional evidence in support of this conclusion is afforded by the fact that typhoid bacilli which have been in contact with "absorbed serum" at 0°C. behave, after washing with saline, like sensitised bacilli, i.e. they fix "treated complement".

Investigation of the thermo-stability of this natural antibody has yielded very varied results. "Absorbed serum" sometimes retains its specific anticomplementary power (i.e. in the presence of B. typhosus) after heating in a water-bath at 55°C. for half an hour and sometimes loses it. Further, it has been found that some samples of guinea-pig's serum (native) will lose the antibody if incubated for a few hours at 37°C. and this may explain certain failures to prepare an "absorbed serum" which would be active in sensitising typhoid bacilli. Table XI is an example of such a serum, in which no loss in haemolytic activity was brought about by the incubation although the natural antibody for B. typhosus disappeared.

Effect of "treating" complement with agents other than B. typhosus.

The method originally employed for depriving guinea-pig's serum of its sensitising factor for B. typhosus (viz. "treatment" of the serum at 0°C. with the bacilli) is troublesome and well-nigh impracticable when a considerable quantity of "treated complement" is required. Even when Kolle flasks of 12 cm. diameter are used for the cultures the growth obtained from several of these is sufficient for the "treatment" of a limited volume only of complement and, as was found repeatedly, in-complete deprivation of the serum of its sensitising factor frequently attended the attempts to obtain 4 c.c. or more of "treated complement". For the work in connection with the action of "absorbed serum" it was necessary to have available a much larger volume of "treated complement" than 4 c.c. and an endeavour was made to find an alternative mode of preparation.

It had been observed that "treatment" of guinea-pig's serum with a strain of B. typhosus which habitually showed only a short zone of complement-fixation, even when very dense suspensions were used, was just as effective in removing the

(to follow p.197)

TABLE XII

"Treatment" of complement with B. coli communis *instead of* B. typhosus.

Complement c.c.	0.01	0.02	0.03	0.04	0.06	0.08	0.1	0.14	0.18	nil
Bacillary suspension.	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Using native complement	c	c	vmk	d	ftr	ftr	tr	ac	C	0
Using complement "treated" with *B. typhosus*	ac	C	C	C	C	C	C	C	C	...
Using complement "treated" with *B. coli communis*	ac	C	C	C	C	C	C	C	C	...

Bacillary suspension = unheated suspension of *B. typhosus*.

M.H.D. of native complement = 0.0075 c.c.

M.H.D. of complement "treated" with *B. typhosus* = 0.01 c.c.

M.H.D. of complement "treated" with *B. coli communis* = 0.01 c.c.

Native complement and the two types of "treated" complement" are all from the same sample of pooled guinea-pig's serum.

The bacilli used in "treating" complement have been heated for two hours at 55° C.

The strain of *B. coli communis* used above has failed, on repeated tests, to cause zonal fixation of complement. Anticomplementary effect is shown only in the presence of 2 to 3 M.H.D.

B. coli communis is not haemolytic for ox blood cells.

sensitising factor as was "treatment" with the "R11" strain, and that "treatment" with an organism (B. coli communis) with which zonal complement-fixation had not been demonstrable was similarly effective (Table XII). Any anticomplementary effect of this strain of B. coli communis was slight and it was shown only in the presence of low complement dosages (2 to 3).

It was decided therefore to "treat" guinea-pig's serum with an adsorbing agent which was non-bacterial. Kieselguhr was inapplicable in view of the fact that it removes haemolytic complement and attention was directed primarily to coal dust. (A purified product prepared by Sutcliffe & Co., was used.)

> 1 grm. of coal dust is mixed thoroughly with 2 c.c. of 0.85 per cent. solution of NaCl in distilled water, and 2 c.c. of fresh guinea-pig's serum are then added. The contents of the tube are stirred with a glass rod and the mixture is allowed to stand at room temperature for two hours. (If the process is one of simple adsorption, two hours is probably longer than is necessary. In

connection with this work, however, ample time is usually available for "treatment" while other reagents are being prepared.) The stirring is repeated frequently during the course of the two hours' period, at the end of which the mixture is centrifugalised and the supernatant fluid pipetted off. If it is not perfectly clear it is centrifugalised a second time, and any sediment is rejected.

It was found that the M.H.D. of complement "treated" in this way was the same as that of a portion of the fresh serum which had been similarly diluted with saline, similarly stirred during the course of two hours at room temperature and similarly centrifugalised. Parallel tests were therefore made (as in the analogous experiment shown in Table VI) with typhoid suspension and (a) fresh serum, (b) serum "treated" with coal dust, and the results showed that coal dust had proved effective in removing the sensitising factor for B. typhosus. Serum "treated" in this way has been very largely used in the experimental work recorded above and on all occasions it has behaved in precisely the same manner as serum "treated" with bacillary sediment.

It has been noted previously that "treatment" with bacillary sediment sometimes failed

(to follow p.199)

TABLE XIII

Example of imperfect "treatment" of complement (coal dust).

Complement	M.H.D.	1	2	3	4	6	8	10	15	20	nil
	c.c.	0.01	0.02	0.03	0.04	0.06	0.08	0.1	0.15	0.2	nil
Bacillary suspension c.c.		0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Using native complement		jc	0	0	0	0	0	0	0	0	0
Using "treated" complement		tr	C	C	C	C	C	C	mk	ftr	...

Bacillary suspension = unheated suspension of *B. typhosus.*

"Treated complement" prepared with coal dust.

to remove the sensitising factor completely (vide p.191) and similar failures have occasionally been encountered in the case of serum "treated" with coal dust, even when the "treatment" given could reasonably have been expected to be adequate. Table XIII is an example of an experiment in which this occurred but it does not appear that failures of this kind are to be attributed to relative inefficiency of coal dust for the purpose required. "Treated complement" prepared by adsorbing the serum with coal dust has now been used very extensively and experience of its use fully warrants the conclusion that this mode of preparation is as reliable as "treatment" with chilled typhoid bacilli. It has the great advantage that it dispenses with the necessity of having to arrange for very large amounts of bacterial growth, and of maintaining a temperature of $0^{o}C.$ during the course of "treatment." Further, it permits of the preparation of considerable volumes of "treated complement" easily, and with precise knowledge as to the degree to which the original serum has been diluted in the process.

Commercial <u>powdered glass</u> has also been employed with success as a substitute for typhoid bacilli in "treating" complement, but its use was not favoured on account of the difficulty of freeing it

from traces of alkali. Moreover, owing to the relatively greater weight of its particles, it settles out of suspension very speedily and its use necessitates almost constant stirring of the serum during the course of "treatment". For depriving guinea-pig's serum of its sensitising factor for B. typhosus, however, it is as efficient as either bacilli or coal dust.

Willow charcoal (Bragg's medicinal) has recently been used instead of coal dust (following the technique detailed on p.198) and, on all occasions on which it has been employed, "treatment" has succeeded in depriving guinea-pig's serum completely of its natural antibody to B. typhosus; the dose of haemolytic complement has not been affected by "treatment", and a shorter period of centrifugalisation is required to remove the charcoal from the serum at the end of the process. Experience with willow charcoal as the adsorbing agent is, as yet, limited but it is proving itself even more satisfactory than coal dust for the purpose required. The hydrogen-ion concentrations (Universal Indicator (British Drug Houses)) of "treated" and fresh serum are apparently so nearly the same that no gross difference has been detected, and it is worthy of note that "treatment"

of complement with coal dust or charcoal is without effect on its deviability, as estimated quantitatively in the Wassermann reaction.

Organisms other than B. typhosus.

Different strains of B. typhosus vary in their property of causing zonal fixation of guinea-pig's complement; in two strains of this organism, as well as in a laboratory strain of B. coli communis, the property appeared to be entirely lacking. A number of allied organisms have also been tested, but the results have varied and, with different strains of the same organism, the uniformity of action shown by the great majority of the strains of B. typhosus has not been demonstrated. Five strains of B. paratyphosus (A, B, or C) yielded typical zones, but four others failed so to do even when very turbid suspensions were used. With organisms of the Gaertner-Aertrycke group, and with paracolon bacilli, similar irregularity in behaviour was found, while a second strain of B. coli communis gave a zone, thus differing from the laboratory strain of this organism. It would appear, therefore, that many coliform bacilli react with guinea-pig's complement like B. typhosus, but with greater differences in individual behaviour.

(to follow p.202)

TABLE XIV

Zonal complement-fixation by anthracoid bacillus.

Complement (M.H.D.)	2	3	4½	6	8	10	12	15	nil
Complement (c.c.)	0.02	0.03	0.045	0.06	0.08	0.1	0.12	0.15	nil
Bacillary suspension c.c.	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Using native complement	C	vmk	0	0	0	0	0	mk	0
Using "treated complement"	C	C	C	C	jc	jc	vmk	...	...

Bacillary suspension = anthracoid bacilli, autoclaved and thrice washed with sterile saline. The washings when tested with complement are found to be devoid of any anticomplementary effect.

Density of suspension corresponds with tube 8 in the standard opacity series.

"Treated complement" (12 M.H.D.) is not haemolytic for sheep's cells.

"Treated complement" prepared with coal dust.

Native complement and "treated complement" are from the same sample of guinea-pig's serum.

The total volume in each tube is constant, viz. 0.7 c.c.

Haemolytic system = sensitised suspension of sheep's cells.

(to follow p.202)

TABLE XV

Zonal complement-fixation by Staphylococcus aureus.

Complement c.c.	0.005	0.01	0.015	0.02	0.025	0.03	0.04	0.05	0.06	0.08	0.1	nil
Staphylococcus suspension c.c.	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Using native complement	jc	C	ftr	0	0	0	0	0	0	ftr	ac	0
Using "treated complement"	mk	jc	C	C	C	C	C	C	C	C	C	...

Staphylococcus suspension = 1000 million per c.c. Unheated.

M.H.D. of complement (native or "treated") = 0.0075 c.c.

"Treated complement" prepared with charcoal.

Haemolytic system = sensitised suspension of sheep's cells.

"Treated complement" is not haemolytic for sheep's cells.

The total volume in each tube is constant, viz. 0.6 c.c.

An anthracoid bacillus, of Bacillus vulgatus type, has also been examined. Cultures of this organism grown in casein-digest-broth for 18 to 24 hours are autoclaved at 120°C. for half an hour and the killed bacilli are recovered by centrifugalisation. After washing five times with sterile saline they are re-suspended in saline and tested in the usual way. Zonal complement-fixation occurs and it is as striking a phenomenon as with the "R11" strain of B. typhosus. Table XIV shows the extent of the zone of complement-fixation when fresh guinea-pig's serum is employed and the marked diminution of the zone when the serum "treated" imperfectly with coal dust is substituted.

A recently isolated strain of Staphylococcus aurens has yielded the interesting result shown in Table XV. This organism causes fixation of very large amounts of complement, but the zonal nature of the fixation is only demonstrable when 1 M.H.D. is included in the range of complement doses used. If tested according to the method ordinarily employed in studying the anticomplementary action of bacteria or other agents (i.e. using multiples of the M.H.D. of complement), the existence

(to follow p.203)

TABLE XVI

Use of rabbit's complement.

Complement M.H.D.	2	4	6	9	12	nil
Bacillary suspension c.c.	0.5	0.5	0.5	0.5	0.5	0.5
Using guinea-pig's complement	jc	mk	0	0	0	0
Using rabbit's complement (A)	0	0	C	C	C	...
Using rabbit's complement (B)	0	0	ftr	C	...	...

The suspension of *B. typhosus* has been heated at 55°C.

M.H.D. of guinea-pig's complement = 0.005 c.c.

M.H.D. of rabbit's complement (A) = 0.09 c.c.

M.H.D. of rabbit's complement (B) = 0.1 c.c.

of a zone would tend to be missed and the suspension would appear simply to be very anticomplementary. As with B. typhosus and the anthracoid bacillus, "treatment" of the guinea-pig's serum deprives it of its property of showing complement-fixation with this staphylococcus.

The effect of serum other than guinea-pig's.

Fresh normal rabbit's serum has been used as complement and tested, in a range of doses, with a suspension of B. typhosus known to be suitable for the demonstration of zonal fixation of guinea-pig's complement. The result obtained is shown in Table XVI and it would appear probable that the zone phenomenon does not occur when rabbit's complement is used. There are however certain difficulties associated with carrying out a satisfactory test of this type, the most obvious being that the comparatively high M.H.D. of rabbit's complement precludes the practicability of employing the same extensive range of doses as, on the analogy of guinea-pig's complement, may be necessary for the demonstration of the phenomenon. The question has, however, been approached from another standpoint and the effect of the addition of small amounts of fresh normal rabbit's serum to mixtures of typhoid suspension

(to follow p.204)

TABLE XVII

Effect of addition of fresh rabbit's serum to mixtures of B. typhosus *and "treated complement."*

Complement	M.H.D.	1	2	3	4	6	8	10	nil
	c.c.	0.01	0.02	0.03	0.04	0.06	0.08	0.1	nil
Bacillary suspension c.c.		0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
(1) Using native complement		jc	0	0	0	0	0	0	0
(2) Using "treated complement"		tr	C	C	C	C	C	C	...
(3) Same as (2) but with addition of "absorbed serum" 0.025 c.c.		0	0	0	0	0	0	0	...
(4) Same as (2) but with addition of fresh rabbit's serum 0.05 c.c.		0	0	...	0	0	0	...	...
Control.-Same as (3) but with saline instead of bacillary suspension.		C	C	C	C	C	C	C	...
Control.-Same as (4) but with saline instead of bacillary suspension.		C	C	...	C	C	C	...	...

The suspension of *B. typhosus* is unheated.

"Treated complement" prepared with coal dust.

M.H.D. of rabbit's complement = 0.075 c.c.

"Absorbed serum" 0.5 c.c. + haemolytic system = no lysis.

and "treated complement" (guinea-pig's) has been studied. An example of such an experiment is given in Table XVII and it shows that the rabbit's serum which was used played a rôle exactly similar to that of the "absorbed" fraction of guinea-pig's serum, i.e. it conferred on a typhoid suspension the ability to fix "treated complement".

It must be emphasised, however, that such a result is not obtained with every specimen of rabbit's serum and, although the finding exemplified in Table XVII has been confirmed, a failure to confirm it has also to be recorded. Thus in a similar test (with the same strain of B. typhosus) the addition of a similar volume of fresh normal rabbit's serum was without effect; no complement-fixation occurred. In view of such observations it seems justifiable to conclude that the phenomenon of zonal fixation of guinea-pig's complement by B. typhosus, and by certain other organisms, is not explicable on the basis of a simple effect of, or on, serum in virtue of its being serum. Were it so, there should not be gross difference in the action of fresh specimens of serum from healthy normal individuals of the same species (rabbit), when added to mixtures of the bacilli and "treated complement".

(to follow p.205)

TABLE XVIII

Inhibition of zonal fixation of complement by heated human serum.

Complement	M.H.D.	2	4	6	9	12	2	4	nil
	c.c.	0.01	0.02	0.03	0.045	0.06	0.01	0.02	nil
Bacillary suspension		0.5	0.5	0.5	0.5	0.5	nil	nil	0.5
Without human serum.		C	C	mk	0	0	...	...	0
With addition of heated human serum 0.05 c.c.		ac	C	C	C	C	C	C	...

The suspension of *B. typhosus* has been heated at 55°C.

The human serum has been heated for half an hour at 55°C. It contains 40 standard agglutinin units (Dreyer) per c.c. for *B. typhosus*. It is not haemolytic for ox blood cells.

Complement = fresh guinea-pig's serum.

The effect of the addition of serum which has been heated at 55°C. for half an hour to mixtures of typhoid suspension and fresh guinea-pig's complement has also been studied; for this purpose both rabbit's and human serum have been used. The results are extraordinarily varied and difficult to interpret. The addition of 0.05 c.c. of certain normal inactivated sera (rabbit's or human) leads to an extension of the zone of complement-fixation into the lower range of complement dosages, thus yielding a result which would be interpreted as "positive" in the type of complement-fixation test usually carried out to detect antibody to B. typhosus. In other cases, similar additions of inactivated serum (rabbit's or human) actually diminish the extent of the zone, and when such diminution has been observed the effect has been shown by the absence of any complement-fixation in the comparatively limited range of complement dosages used (2 to 12). One of these sera (shown in Table XVIII has been tested on many occasions and its action in this way has been consistent, in spite of the fact that it contained over 40 standard agglutinin units (Dreyer) per c.c. for B. typhosus. Examples of each of these effects of the addition

of inactivated serum have been observed in the same series of tests.

The sera, other than guinea-pigs', which have been used both fresh and after heating at 55°C. have thus exhibited wide differences in their effect on typhoid bacilli, and it seems probable that some of these differences are related to irregularity in the distribution of the natural antibody in different species, while others are probably due to varying thermo-stability of the antibody. With guinea-pig's serum there has been striking uniformity in the findings, in the course of observations extending over more than five years, and the haemolytic complement of such serum has never failed to be fixed zonally by a suitable suspension of the usual strain of B. typhosus.

Discussion.

In the literature of previous work on complement-fixation tests with B. typhosus the absence of any reference to the phenomenon here described is apparently to be attributed mainly to the employment by the various authors of a single, arbitrarily arranged, complement dosage. Such a method, although based on the analogy of the original work of Wassermann in connection with the serological diagnosis of syphilis, is open to the objections recognised by Muir and Martin (1906) in their use of varied doses of complement and emphasised by Browning and Mackenzie (1924). Further, as the recent work of Sugden and Williams (1926) on colloid protective phenomena has shown, it is essential that in such tests each of the participating reagents must be varied if erroneous conclusions are to be avoided.

Dean (1910 and 1911) has drawn attention to the necessity of varying both antigen and antibody when carrying out complement-fixation tests with B. typhosus, and he has shown that such a method is a very delicate and specific means of differentiating closely allied bacteria. He uses however a fixed complement dosage (0.05 c.c. fresh

guinea-pig's serum acting in a total test volume of 1.5 c.c.) and as antigen he employs a watery extract of the bacilli instead of a suspension of the organisms in saline. Either of these departures from the technique followed in the present work may account for the absence of spontaneous fixation of complement by the antigen alone; the strain of B. typhosus used may also have been of the type less suited for eliciting the phenomenon although proving a good antigen for detecting immune antibody. In a later communication (1912) Dean amplifies his previous work and takes cognisance of the desirability of varying the test volume of complement as well as that of the antigen and immune serum. The antigen used however is a watery extract of bacilli and the maximum complement dosage tested is 0.2 c.c. of guinea-pig's serum acting in a total test volume of 1.5 c.c. From the present work it is evident that for the occurrence of spontaneous complement-fixation the concentration of guinea-pig's serum is of more importance than its absolute volume or haemolytic dosage (vide Tables IV and V), and this observation taken in conjunction with the nature of the antigen used by Dean would appear to offer adequate explanation of the absence of the phenomenon in his results. His insistence on the necessity for

titrating diminishing amounts of antibody with diminishing amounts of antigen, if traces of the latter are to be detected, is supported by findings of the type exemplified in Table IV. Here there is a striking close parallel to his results with mixtures of typhoid antigen, antiserum and complement, although in the experiment shown in Table IV no antiserum has been added and the only variants are typhoid bacilli and complement.

Parker (1923) has confirmed Dean's findings, but used the "Rawlings" strain of B. typhosus which has consistently failed to cause zonal fixation of guinea-pig's complement, Takenomata (1924), in a lengthy communication on non-specific complement-fixation by a variety of "pseudo-antigens", among which he includes bacterial cultures, restricts himself to the use of a single complement dosage (0.1 c.c. of a 1 in 6 dilution of guinea-pig's serum, acting in a total test volume of 0.55c.c.). Certain of his conclusions are therefore likely to be founded on incompletely controlled observations, but it is of interest that he noted considerable variability in the action of inactivated rabbit's serum in leading to non-specific fixation of guinea-pig's complement by bacterial suspensions or extracts.

Further, he indirectly confirms the findings of the present work that such complement-fixation does not occur at 0°C., and that heating of rabbit's serum may diminish or destroy its ability to lead to fixation of the arbitrary complement dosage in the presence of bacterial suspension.

Ritz and Sachs (1917), amplifying their earlier communication (1911), record the observation of zonal fixation of guinea-pig's complement by suspensions of B. prodigiosus and Staphylococcus, and they conclude that this is analogous to the inactivation of complement which occurs in salt-free media or in the presence of cobra-venom. Friedberger and Putter (1920) failed to demonstrate the phenomenon with B. prodigiosus, but Kondo (1923) was able to confirm the findings of Ritz and Sachs. These workers recognise that the action of the bacilli on complement is indirect, but they reject the idea of the presence of an antibody and explain the result on the basis of primary globulin alterations in the guinea-pig's serum which are initiated by the bacilli and which lead to inactivation of the complement. As it seems probable that a common basis should suffice to explain the zonal fixation of complement brought about by bacteria in general (e.g. B. prodigiosus, coliform bacilli, anthracoid bacilli,

staphylococci) the arguments which they adduce in support of their hypothesis merit careful consideration, all the more so as certain of the observations in the present work render its acceptance difficult for B. typhosus. They lay stress on the fact that by the use of weak acid or alkali, and without thereby damaging the complement, they are able to deprive guinea-pig's serum of its capacity for showing zonal complement-fixation in the presence of B. prodigiosus, and in this they see good reason for rejecting the antibody hypothesis in favour of that of primary globulin alteration. The absence of complement-fixation at 0°C. tends further to confirm them in their point of view, although the justification for such conclusions would not appear to be beyond question. Alterations in the state of dispersion of the globulin constitute only one of the possible results of the addition of weak acids or alkalis to serum, and Ritz and Sachs admit that there is unfortunately no way of proving chemically that such alterations occur. Further, the possibility of removing an antibody from serum, without damage to the complement function, is well known, e.g. haemolysins (natural or immune). In the present work it has been shown that by bringing

with fresh guinea-pig's serum at 0°C. a similar change is induced in the serum to that obtained by Ritz and Sachs by the use of acid or alkali - the complement remains practically undamaged but it can no longer be fixed by a suspension of typhoid bacilli; the chilling of the serum to 0°C. does not of itself effect the change; the presence of the organisms is essential. What appears to be of prime importance, however, is the fact that not only is the serum altered by its contact with the bacilli, but the bacilli themselves undergo changes as a result of which their subsequent behaviour resembles that of organisms which have been sensitised with the corresponding antibody. This finding has been repeatedly confirmed both by the use of native serum and "absorbed serum" (vide pp.193 and 196), and at no time have results been obtained which tended in any way to throw doubt on the validity of the conclusion that treatment of guinea-pig's serum with bacilli, coal dust, charcoal, or powdered glass, actually removes some definite factor from it. For the sake of simplicity the process may be likened to adsorption and the results would thus indicate that many agents, bacterial or physical, can adsorb the factor in normal guinea-pig's serum on the presence of which zonal fixation

of complement by B. typhosus depends, but that only on certain organisms does its adsorption confer the ability to fix complement. It might of course be suggested that the factor which is removed by treatment is in reality a precipitate, resulting from gross changes in the state of dispersion of the proteins of the serum, but such a hypothesis is quite untenable. If the fixation of complement were dependent on precipitum formation one would be obliged to admit that some strains of B. typhosus had the power of causing such precipitation, others actually of preventing it. Moreover, Ritz and Sachs expressly state that the globulin alterations to which they attribute the phenomenon are not associated with precipitum formation, although they are prepared to concede that the physical factors which determine the alteration may be such as might cause precipitation under certain conditions.

The question as to which of the so-called fractions or components of haemolytic complement are involved in the inactivation brought about by B. typhosus and other organisms has not been studied in the present work. Ritz and Sachs however attach importance to their conclusion that cobra-venom and B. prodigiosus both affect the same component, since

complement which has been rendered inactive by either of these agents can be re-activated by the addition of guinea-pig's serum which has been heated at 55°C. (third component). They postulate therefore that cobra-venom and B. prodigiosus act on complement in the same way and that, as the inactivation of complement by venom has been explained in previous communications by themselves and others on the basis of globulin alterations, such is likely also to be the explanation of the action of B. prodigiosus. The results obtained by Coca (1914) in similar work on the inactivation of complement by cobra-venom must also be taken into account. He confirmed that complement which had been rendered inactive by cobra-venom, by yeast, or by sarcinae, could be re-activated by the addition of third component (native serum heated at 55°C.), but he found also that venom-inactivated serum could be re-activated by yeast-inactivated serum. It appeared therefore that the actions of venom and yeast on third component were not identical. Further, the study of the quantitative relationships of yeast-inactivated and venom-inactivated sera to third component yielded results which indicated radical differences in the behaviour of one guinea-

pig's serum as compared with that of another. Gordon, Whitehead and Wormall (1926) have recently reported a fourth component of complement which is associated with the albumin-fraction of serum (thereby differing from third component), and they find that mixtures of (1) serum deprived of third component (e.g. by yeast or by heating at 55°C.) and (2) serum deprived of fourth component show restoration of the complement function. These observations may offer an alternative explanation of the result obtained by Coca with mixtures of yeast-inactivated and venom-inactivated serum, and they raise doubt as to the justification for the conclusions of Ritz and Sachs that loss of third component is implied when an inactive serum can be re-activated by serum heated at 55°C., or that primary globulin alterations are necessarily implied in the inactivation of complement. Further, although with certain immune haemolysins it has been found that the so-called mid-piece fraction of complement is primarily involved in the haemolysis of the sensitised cells (and thereby in the inactivation of complement), it does not appear that this affords adequate grounds for assuming that the inactivation of complement by other antigen-antibody combinations

is necessarily brought about in the same way. The probability of differences in the action of natural as opposed to immune antibodies must also be borne in mind, especially with regard to the effect on complement of antigen-antibody combinations, e.g. the differing affinities for complement conferred on an antigen by natural as compared with immune haemolysin (Bordet,1927).

For these reasons one is meantime disinclined to attach decisive significance to the behaviour of the so-called fractions or components of haemolytic complement in the presence of the various agents which lead to inactivation of the complement function. It may be that further research will necessitate a readjustment of the present conception of complement-fixation in general, and possibly also of the nature of antibodies, both natural and immune, but it would appear desirable to recognise that these "components" which have been described in relation to complement action are as yet far from being clearly defined or properly understood. It may be well therefore not to stress their importance unduly, but to regard conclusions based on their behaviour as of the nature of interesting speculations rather than as arguments in support

of, or against, a particular hypothesis.

The results which have been obtained with the "R11" strain of B. typhosus can be reasonably explained on the assumption that normal guinea-pig's serum contains a factor which plays the rôle ordinarily ascribed to an antibody, and until it can be shown definitely that in some of its characters this factor differs fundamentally from known natural antibodies it seems unnecessary to seek to explain it on any other basis. It has to be noted, however, that in applying the term antibody to this factor in normal guinea-pig's serum, emphasis is being laid on the distinction between natural antibodies and immune-antibodies (antibodies produced as a result of active immunisation). Comparatively little work has as yet been devoted to the former group; but the behaviour of the natural haemagglutinins and haemolysins has been found to differ in certain respects, e.g. specificity and thermo-stability, from that of the corresponding immune-antibodies. It seems justifiable, therefore, to refer to the factor in normal guinea-pig's serum on which zonal fixation of complement by B. typhosus depends as a natural antibody. It resembles an immune antibody in that - (a) it unites with the bacilli at 0°C.

without leading to fixation of complement, and (b) bacilli which have thus been in contact with guinea-pig's serum at 0°C. behave like bacilli which have been sensitised with the homologous immune antibody, i.e. they fix "treated complement". That there are features in its behaviour which are difficult to explain on the antibody hypothesis is admitted freely (e.g. the zone phenomenon, and the relatively great resistance to the effect of heat possessed by the receptors of certain strains), but in the present state of knowledge it would not appear that the interpretation of these is rendered any less difficult by the alternative hypothesis to which attention has already been directed. The question of the zone-phenomenon is of special interest and it has not been neglected, but detailed reference to the observations which have been made in connection with this difficult problem is meantime omitted, since the results are as yet incomplete.

The natural antibody, as has been shown, is quite distinct from complement, since it can be removed from the serum without material increase in the haemolytic dose, and since it has been possible to deprive serum of its haemolytic

complement without removal of the antibody. In its property of sensitising some organisms (e.g. B. typhosus, Staphylococcus), while failing to sensitise others, it shows a certain degree of specificity, but of greater interest possibly is its comparative lack of specificity. It can be removed from the serum, partially or completely, by a variety of agents, bacterial or otherwise, as well as by those organisms which it sensitises, and the great variability which it shows in relation to its resistance to the effect of heat (being sometimes more labile than complement) further differentiates it from the group of specific immune bodies. These properties find analogies in what has been described in connection with certain other natural antibodies, e.g. by Landsteiner and Reich (1908) for natural haemagglutinins and by Thiele and Embleton (1914) in their work on the genesis of immune haemolysins. The former workers record the removal of natural haemagglutinin from rabbit's serum by casein and the thermo-lability of this antibody, while the latter, although they regard the natural haemolysin as a modification of complement, emphasise the gradual transition from thermo-lability to thermo-stability which it undergoes in the course of development.

Some of the results obtained in the present work tend to throw considerable doubt on the reliability of complement-fixation tests in which bacterial suspensions are used as antigens (haptenes) and normal guinea-pig's serum as complement. Only two strains of B. typhosus out of twenty-one (in reality 1 out of 20; since "Rawlings" and "Lister" (786) are the same) have been encountered which appear to be free from a source of fallacy in such tests, and it is of interest that these strains differ also from the others in their fermentative powers. Reference has been made, further, to the extraordinarily varied effects produced by the presence in such tests of small amounts of serum from different species (rabbit and human). It appears that inhibition of the spontaneous fixation of guinea-pig's complement by typhoid bacilli sometimes occurs as a result of the addition of certain sera which may, or may not, contain immune antibodies to B. typhosus. In other instances, similar additions of normal sera (heated at 55°C.) lead to increased fixation of complement although the added serum is not of itself anticomplementary. More detailed investigation of these points is

required, but they serve still further to emphasise the recognised complexity of the factors involved in such biological reactions, and even to enhance the necessity for the most rigid control of each of the participating systems.

Summary.

(1) Guinea-pig's serum shows zonal fixation of its haemolytic complement in the presence of a suspension in saline of the majority of strains of B. typhosus.

(2) Different strains of B. typhosus vary in their ability to cause zonal fixation of guinea-pig's complement. Two out of twenty-one strains examined have failed to do so.

(3) The phenomenon is explicable on the basis of the presence in normal guinea-pig's serum of a natural antibody. This resembles immune antibody in its ability to sensitise the organisms, so that they can then fix complement, but it differs from immune antibody in its thermo-lability and in its comparative lack of specificity.

(4) This antibody varies greatly in its sensitiveness to heat and it is sometimes more labile than complement.

(5) B. typhosus unites with the antibody at 0°C., usually thereby becoming sensitised, but no fixation of complement occurs at 0°C.

(6) The antibody can be removed from guinea-pig's serum not only by organisms with which it reacts specifically (leading to complement-fixation) but also by other organisms, and by agents such as coal dust, charcoal, or powdered glass.

(7) Haemolytic complement can be removed from guinea-pig's serum without destruction of the antibody.

(8) Certain other organisms (coliform bacilli, anthracoid bacilli and staphylococci), also cause zonal fixation of guinea-pig's complement.

(9) The addition of heated normal serum (rabbit's or human) to mixtures of typhoid bacilli and guinea-pig's complement sometimes inhibits, sometimes increases, the zone of complement-fixation.

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Sachs-Georgi Test.

STANDARD TECHNIQUE.

Saline. 0.85% solution of pure sodium chloride in distilled water; heated in Koch steriliser.

Alcohol-saline. A freshly prepared 1 in 6 dilution of absolute alcohol in saline.

Serum. Human serum, free from blood cells, which has been heated in a water-bath at 55° - 56°C. for half-an-hour, several hours before the test.

Antigen. A 1 in 6 dilution with saline of a cholesterolised alcoholic-extract of human heart muscle (appendix 5). The dilution is carried out in two stages:-

(a), 1 volume of the cholesterolised heart-extract is mixed rapidly with 1 volume of saline, by pouring the saline into the extract and immediately pouring the mixture from one tube to the other several times. It is then allowed to stand at room temperature for five minutes, after which

(b), it is similarly rapidly diluted with an additional 4 volumes of saline. This final mixture is used in the test after it has stood at room temperature for from five to fifteen minutes.

Three tubes (3" x $\frac{1}{2}$") are used for each serum to be tested and the reagents/

are added in the amounts, and in the order, shown in the table. Measuring pipettes are rinsed out with saline, at least three times, between the addition of different reagents In preparing the antigen, the pipette is rinsed with absolute alcohol both before and after the measurement of cholesterolised heart-extract.

tube no.		1.	2.	3.
Saline,	c.c.	0.9	0.9	0.9
Serum,	c.c.	0.05	0.1	0.1
Alcohol-saline,	c.c.	nil	nil	0.5
Antigen,	c.c.	0.5	0.5	nil

Tubes 1 and 2 thus contain antigen + serum, while Tube 3 which serves as a serum control contains no antigen. Alcohol however is present in the same concentration as in Tubes 1 and 2. The contents of each tube are mixed by gentle shaking after the addition of the serum and the antigen (or alcohol-saline), and the completed tests are placed in an incubator at 37°C. for from eighteen to twenty hours.

Controls. (1). A known positively reacting, and a known negatively reacting, serum is included with each set of tests. In the case of the positive serum it is preferable to use one which causes relatively feeble flocculation, i.e. a weakly reacting serum.

(2). An antigen control is indispensable. It consists of a tube with saline 0.9 c.c. + antigen 0.5 c.c.

Readings are made immediately after the withdrawal of the tubes from the incubator and shaking is to be avoided as it tends to break up and disperse the flocculi which may have formed, thereby rendering their detection less easy. Each tube is examined separately and a suitable arrangement of lighting is desirable in order to facilitate the reading. This consists essentially of oblique illumination of the contents of the tube while being viewed against a dark background.

If the antigen control is free from flocculi and if the control sera(positive and negative) react normally, the presence of flocculi in Tubes 1 or 2 (or in both) of a given test is accepted as a POSITIVE reaction, provided also that no flocculi are visible in Tube 3.

The use of a hand lens is not as a rule necessary but it may be employed to confirm the presence of fine flocculi. The detection of fine flocculi can also be simplified by causing them to circulate, by gentle agitation of the tube.

Appendix 4.

++++ = coarse flocculant sediment with clear supernatant fluid.

+++ = flocculant sediment; supernatant fluid slightly turbid.

++ = definite flocculi suspended in a turbid fluid.

\+ = very fine flocculi just visible to the naked eye.

± = turbidity greater than that of the antigen control, but no definite flocculi visible.

\- = negative result. Opalescence as in antigen control.

(The above is based on the description by Taniguchi and Yoshinare).

Appendix 5.

PREPARATION of HEART EXTRACT.

Human heart is freed from surface fat, minced finely, and weighed.

It is then ground thoroughly in a clean mortar with well washed silver sand which has been dried and sterilised.

Absolute alcohol is added in the proportion of 5.0 c.c. for every gramme of minced heart and the mixture is transferred to a clean, dry, stoppered, bottle which is kept at room temperature for 48 hours, and shaken occasionally.

At the end of this period the mixture is filtered through paper and the filtrate constitutes Heart Extract.
It is customary to store this in a well stoppered bottle and to keep it in the dark at room temperature.
A varying amount of deposit tends to appear in the extract within a day or two of its preparation and this should be removed by re-filtration as often as may be necessary.

Heart extract prepared in this way forms the basis of the antigen for the Sachs-Georgi test but before it is ready for use it requires:-

(1), dilution with absolute alcohol.

(2), addition of cholesterol.

The precise amounts of alcohol and cholesterol most suitable for any given sample of heart extract are determined by titration, and the method employed is detailed under "Optimum Antigen" (Appendix 6).

OPTIMUM ANTIGEN.

(For preparation of heart-extract, see Appendix 5).

The optimum antigen which can be prepared from any given sample of heart-extract is determined by carrying out parallel Sachs-Georgi tests with a known normal and a known syphilitic serum, and using twelve different antigens all of which have the extract as their basis but which vary in their lipoid and cholesterol concentrations. (Details of the preparation of these antigens are given below The tests are all carried out at the same time and, when the results are read, the antigen is noted which yields the coarsest flocculi with the syphilitic serum while showing no flocculation with the normal serum or in the antigen control. This is the Optimum Antigen.

It is generally preferable however to select two of the antigens which are definitely superior to the other ten (i.e. which show coarser flocculi in the presence of syphilitic serum but none in the antigen control or in the presence of normal serum), and to test these two in parallel on several subsequent occasions with a series of normal and syphilitic sera, in order that the one which consistently gives the more satisfactory results may finally be chosen.

The original sample of heart-extract is then diluted in bulk with absolute alcohol and cholesterolised so as to yield the mixture which has thus been selected as the optimum antigen, and such cholesterolised extract has been found to retain its properties unimpaired for many months when stored in a stoppered bottle, in the dark, at room temperature. For use in the Sachs-Georgi test it merely requires dilution with saline according to the routine technique (see Appendix 1).

(Continued).

The following are the details of the preparation of the antigens for optimum antigen titration:-

"A", "B", and "C" are clean dry test-tubes into which the volumes of heart-extract and absolute alcohol shown are measured with a graduated pipette:

		"A".	"B"	"C".
heart-extract	c.c.	2.2	1.5	1.1
absolute alcohol	c.c.	2.2	3.0	3.3

Three dilutions of the heart-extract (lipoid) are thus obtained, viz. extract: alcohol = 1:1, 1:2, 1:3. The contents of each tube are mixed by gentle shaking, or by sucking up and expelling the mixture several times with the pipette, and then1.0 c.c. from tube "C" is pipetted into each of four clean dry test-tubes (3" x ½"). These are designated C1, C2, C3, and C4. Similarly four tubes (B1, B2, B3, and B4) each receive 1.0 c.c. from tube "B"; and four tubes (A1, A2, A3, and A4) 1.0 c.c. from tube "A". The measurements are made in this order so that the same pipette may be used without rinsing and without risk of increasing the concentration of lipoid by passing from a stronger solution to a weaker. A 1.0 per cent. solution of pure cholesterol in absolute alcohol is now added in the following amounts:

to tubes A1, B1, and C1 -------- 0.03 c.c. per tube

to tubes A2, B2, and C2 -------- 0.045 c.c. " "

to tubes A3, B3, and C3 -------- 0.06 c.c. " "

to tubes A4, B4, and C4 -------- 0.075 c.c. " "

The contents of each tube are mixed thoroughly and then a volume of the mixture, equal to the volume of cholesterol solution added, is withdrawn and rejected. The total volume remaining in every tube is thus 1.0 c.c. and the series represents varied dilutions of the original heart-extract, (lipoid) mixed with varied amounts of cholesterol. These twelve cholesterolised heart-extract dilutions are now diluted with saline (0.85 per cent. NaCl solution) according to the routine Sachs-Georgi technique so as to yield twelve antigens

(Continued).

(A1, A2, etc.) for use in the test.

In practice it is convenient to have a rack which holds 36 tubes in three rows of 12. The front row is occupied by the twelve tubes A1 to C4, in this order from left to right; 1.0 c.c. saline is pipetted into each tube of the middle row, and 4.0 c.c. saline into each tube of the back row. The contents of the first tubes in the front and middle rows are mixed by pouring the saline rapidly into the cholesterolised heart-extract and transferring the mixture quickly from one tube to the other several times. The tube with the mixture is returned to the front row and the empty tube is laid aside. Then the second tubes in the front and middle rows are dealt with similarly, and so on in the series to the twelfth pair. Ordinarily about two minutes suffice to complete this stage of the mixing and a further period of three minutes is allowed to elapse before commencing the final dilutions. These are made in turn by pouring the saline rapidly from a back row tube into the corresponding tube in the front row, beginning with A1, and transferring the mixture quickly from one tube to the other several times. After the mixing of the contents of the twelfth pair of tubes has been completed the antigens are allowed to stand for ten minutes before being used in the tests.

Example: If as a result of titration, as described above, A4 is selected as the optimum antigen, the stock cholesterolised extract is prepared thus:

Heart-extract ----------	100 c.c.
Absolute alcohol ----------	100 c.c.
Cholesterol solution (1.0 per cent.)	15 c.c.

i.e. heart-extract 1 part + absolute alcohol 1 part, with the addition of 0.075 c.c. of cholesterol solution per c.c. of the mixture.

Appendix 9.

Extended Form of Sachs-Georgi Test.

(after Mackie)

This differs from the Standard Technique (appendix 1) in one respect only, viz. the use of a series of increasing serum dilutions in each test instead of the two which constitute the standard method.

The serum dilutions are prepared as follows:-

A row of test-tubes (3" x ½") is set out in a rack and, into the first, one pipettes 0.4 c.c. serum and 1.6 c.c. saline, thus making a 1 in 5 dilution of the serum. The remaining tubes in the row each receive 1.0 c.c. saline. By withdrawing 1.0 c.c. of the contents of tube I, transferring it to tube II, mixing and transferring 1.0 c.c. of the mixture to tube III and so on in the series, a range of increasing serum dilutions is easily and accurately prepared (1 in 5; 1 in 10; 1 in 20; etc. From the last tube in the series 1.0 c.c. is withdrawn and rejected so that, when this stage is reached, each tube contains 1.0 c.c. of a mixture of serum and saline.

The number of tubes in the series may be varied as required and the Standard Sachs-Georgi test is represented by the serum dilutions 1 in 20 and 1 in 10. (cf. tubes 1 and 2, appendix 2.) The setting up of the tests is completed as in the Standard Technique by the addition of 0.5 c.c. antigen to each tube.

Appendix 10.

Section (A).

SALINE.

Unless otherwise indicated, the Standard Sachs-Georgi Technique (appendix 1) is employed for all tests shown in this section.

The various electrolyte solutions are substituted for the saline (0,85% NaCl), and they are therefore used both for diluting the sera and for the preparation of the antigens.

Chemically pure salts are used in the preparation of the stock electrolyte solutions (generally of 1.0% strength) and the various dilutions shown in the tables are made from these with distilled water.

The sera are POOLED specimens and their Wassermann reactions are known. "Normal" serum is a mixture of ten or more sera from cases which are free from clinical or serological evidence of syphilis (serum from a known treated case of syphilis is never included). "Syphilitic" serum is a mixture of six or more sera whose individual Wassermann reactions are strongly positive.

"Individual" specimens of serum (i.e. sera each of which is from a separate patient) are used for one or two experiments, but in all such cases a note to this effect appears in the table.

Appendix 11.

The same batches of cholesterolised heart-extract (A4 and B2) are used in the preparation of the antigens for the whole of the work in connection with the effect of different electrolytes.

Serum controls are always set up, but reference to these is omitted unless where flocculation occurs.

Electrolyte.

SODIUM ACETATE.

SALINE. Aqueous solution of Sodium Acetate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., five hours before use.

ANTIGEN. A4. Readings made after 19 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control
serum c.c.	0.05	0.1	0.05	0.1	nil
sodium acetate.					
0.2 per cent.	-	-	±	+	-
0.4 per cent.	-	-	+	++	-
0.6 per cent.	-	-	±	++	-
0.8 per cent.	-	-	+	+++	-
1.0 per cent.	-	-	±	++	-
Control.					
0.75 per cent.NaCl	-	-	++	+++	-

pH of 1.0 per cent. sodium acetate solution = 7.2

Appendix 11b.

Electrolyte.

SODIUM ACETATE.

SALINE. Aqueous solution of Sodium Acetate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., five hours before use.

ANTIGEN. B2. Readings made after 19 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control
serum c.c.	0.05	0.1	0.05	0.1	nil
sodium acetate.					
0.2 per cent.	-	-	±	+	-
0.4 per cent.	-	-	-	++	-
0.6 per cent.	-	-	+	++	-
0.8 per cent.	-	-	++	+++	-
1.0 per cent.	-	-	+	++	-
Control.					
0.75 per cent NaCl	-	-	++	++	-

pH of 1.0 per cent. sodium acetate solution = 7.2.

Appendix 12.

Electrolyte.

SODIUM ARSENATE.

SALINE. Aqueous solution of Sodium Arsenate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., seven hours before use.

ANTIGEN. A4. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
sodium arsenate					
0.2 per cent.	-	-	-	-	-
0.4 per cent.	-	-	-	+	-
0.6 per cent.	-	-	+	++	-
0.8 per cent.	-	-	+	++	-
1.0 per cent.	-	-	+	+++	-
Control.					
0.75 per cent.NaCl	-	-	++	+++	-

pH of 1.0 per cent. sodium arsenate solution = 7.2

Appendix 13.

Electrolyte.

SODIUM ARSENATE

SALINE. Aqueous solution of Sodium Arsenate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., seven hours before use.

ANTIGEN. B2. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
sodium arsenate.					
0.2 per cent.	-	-	-	+	-
0.4 per cent.	-	-	-	+	-
0.6 per cent.	-	-	+	++	-
0.8 per cent.	-	-	+	++	-
1.0 per cent.	-	-	+	++	-
Control					
0.75 per cent. NaCl	-	-	+	++	-

pH of 1.0 per cent. sodium arsenate solution = 7.2

Appendix 14.

Electrolyte.

SODIUM BENZOATE.

SALINE. Aqueous solution of Sodium Benzoate, various concentrations.

SERA. Normal and Syphilitic sera, heated for one hour at 56°C, five hours before use.

ANTIGEN. A4. Readings made after 18 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
sodium benzoate.					
0.2 per cent.	-	-	-	-	-
0.4 per cent.	-	-	-	-	-
0.6 per cent.	-	-	-	+ -	-
0.8 per cent.	-	-	-	+ -	-
1.0 per cent.	-	-	-	+ -	-
Control.					
0.75 per cent.NaCl	-	-	++	++	-

pH of 1.0 per cent sodium benzoate solution = 10 (approx)

Electrolyte.

SODIUM BENZOATE.

SALINE. Aqueous solution of Sodium Benzoate various concentrations.

SERA. Normal and Syphilitic sera, heated for one hour at 56°C., five hours before use.

ANTIGEN. B2. Readings made after 18 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
sodium benzoate					
0.2 per cent.	-	-	-	-	-
0.4 per cent.	-	-	-	±	-
0.6 per cent.	-	-	-	+	-
0.8 per cent.	-	-	-	±	-
1.0 per cent.	-	-	-	±	-
Control					
0.75 per cent.NaCl	-	-	+	++	-

pH of 1.0 per cent sodium benzoate = 10 (approx).

Appendix 16.

Electrolyte.

SODIUM BORATE.

SALINE. Aqueous solution of Sodium Borate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty nine hours before use.

ANTIGEN. B2. Readings made after 20 hours at 37°C.

	NORMAL serum		SYPHILITIC serum		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
sodium borate.					
0.2 per cent.	-	+	-	±	-
0.4 per cent.	-	-	-	-	-
0.6 per cent.	-	-	-	-	-
0.8 per cent.	-	-	-	-	-
1.0 per cent.	-	-	-	-	-
Control					
0.75 per cent.NaCl	-	-	+++	+++	-

pH of 1.0 per cent sodium borate solution = 10.5 +

Appendix 17.

Electrolyte.

SODIUM BORATE.

SALINE. Aqueous solution of Sodium Borate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty nine hours before use.

ANTIGEN. A4. Readings made after 20 hours at 37°C.

serum c.c.	NORMAL serum. 0.05	0.1	SYPHILITIC serum. 0.05	0.1	ANTIGEN control. nil.
sodium borate.					
0.2 per cent.	-	-	-	+	-
0.4 per cent.	-	-	-	-	-
0.6 per cent.	-	-	-	-	-
0.8 per cent.	-	-	-	-	-
1.0 per cent.	-	-	-	-	-
Control					
0.75 per cent.NaCl	-	-	+++	++++	-

pH of 1.0 per cent. sodium borate solution = 10.5 +

Appendix 18.

Electrolyte.

SODIUM BROMIDE.

SALINE. Aqueous solution of Sodium Bromide, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty two hours before use.

ANTIGEN. A4. Readings made after 20 hours at 37°C.

	NORMAL serum		SYPHILITIC serum		ANTIGEN control
serum c.c.	0.05	0.1	0.05	0.1	nil
sodium bromide					
0.2 per cent.	-	-	-	-	-
0.4 per cent.	-	-	++	+++	-
0.6 per cent.	-	-	+++	+++	-
0.8 per cent.	-	-	+++	+++	-
1.0 per cent.	-	-	+++	+++	-
Control					
0.75 per cent.NaCl	-	-	++	+++	-

pH of 1.0 per cent. sodium bromide solution = 6.0

Appendix 19.

Electrolyte.

SODIUM BROMIDE.

SALINE. Aqueous solution of Sodium Bromide, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty two hours before use.

ANTIGEN. B2. Readings made after 20 hours at 37°C.

	NORMAL serum		SYPHILITIC serum		ANTIGEN control
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium bromide					
0.2 per cent.	-	-	-	±	-
0.4 per cent.	-	-	+	+++	-
0.6 per cent.	-	-	++	+++	-
0.8 per cent.	-	-	++	++	-
1.0 per cent.	-	-	++	++	-
Control					
0.75 per cent.NaCl	-	-	+	++	-

pH of 1.0 per cent. sodium bromide solution = 6.0

Electrolyte.

SODIUM CHLORATE.

SALINE. Aqueous solution of Sodium Chlorate, various concentrations.

SERA. Normal and syphilitic sera, heated for one hour at 56°C., eight hours before use.

ANTIGEN. A4. Readings made after 18 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
sodium chlorate.					
0.2 per cent.	-	-	-	±	-
0.4 per cent.	-	-	+	++	-
0.6 per cent.	-	-	+	++	-
0.8 per cent.	-	-	+++	+++	-
1.0 per cent.	-	-	+++	+++	-
Control					
0.75 per cent. NaCl.	-	-	++	++	-

pH of 1.0 per cent. sodium chlorate solution = 5.8

Appendix 21.

Electrolyte.

SODIUM CHLORATE.

SALINE. Aqueous solution of Sodium Chlorate, various concentrations.

SERA. Normal and syphilitic sera, heated for one hour at 56°C., eight hours before use.

ANTIGEN. B2. Readings made after 18 hours at 37°C.

	NORMAL serum		SYPHILITIC serum		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
sodium chlorate.					
0.2 per cent.	-	-	-	±	-
0.4 per cent.	-	-	+	++	-
0.6 per cent.	-	-	+	++	-
0.8 per cent.	-	-	+	++	-
1.0 per cent.	-	-	++	++	-
Control.					
0.75 per cent NaCl	-	-	+	++	-

pH of 1.0 per cent. sodium chlorate solution = 5.8

Appendix 22.

Electrolyte.

SODIUM CHLORIDE

SALINE. Aqueous solution of Sodium Chloride, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 55°C., immediately before use.

ANTIGEN. B2. Readings made after 18 hours at 37°C.

	NORMAL serum		SYPHILITIC serum		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
sodium chloride					
0.85 per cent.	+	++	+	++	-
1.5 per cent.	+++	+++	++	+++	-
2.5 per cent.	+++	+++	+++	+++	+
3.5 per cent.	+++	+++	++	+++	++
4.5 per cent.	++++	++++	++++	++++	+++
5.5 per cent.	+	++	+++	+++	++

pH of 0.85 per cent. sodium chloride solution = 5.8

Electrolyte.

SODIUM CHLORIDE.

SALINE. Aqueous solution of Sodium Chloride, various concentrations.

SERA. Normal and syphilitic sera, heated for half -an-hour at 56°C., five hours before use.

ANTIGEN. A 4. Readings made after 18 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium chloride					
nil(Tap water)	-	-	+-	++	-
0.85 per cent.	-	-	++++	++++	-
1.5 per cent.	++	++	++++	++++	-
2.5 per cent.	++	++	+++	++++	+-
3.5 per cent.	++	++	++++	++++	++
4.5 per cent.	++	++	+++	+++	++
5.5 per cent.	++	++	+++	+++	++

pH of 0.85 per cent. sodium chloride solution = 5.8

Appendix 24.

Electrolyte.

SODIUM CHLORIDE.

SALINE. Aqueous solution of sodium chloride, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 57°C., five hours before use.

ANTIGEN. A 4. Readings made after 18 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
sodium chloride					
nil (Tap water)	-	-	-	++	-
0.1 per cent.	-	-	++++	++++	-
0.25 per cent.	-	-	+++	++++	-
0.5 per cent.	-	-	++++	++++	-
0.75 per cent.	-	-	++++	++++	-
1.0 per cent.	+	+	+++	+++	-
1.25 per cent.	+++	+++	+++	+++	±

Appendix 25.

Electrolyte.

SODIUM CHLORIDE.

SALINE. Aqueous solution of Sodium Chloride, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 57°C., two hours before use.

ANTIGEN.B 2. Readings made after 19 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium chloride.					
nil (Tap water)	-	-	-	-	-
0.1 per cent.	-	-	-	+	-
0.25 per cent.	-	-	+++	+++	-
0.5 per cent.	-	-	+++	+++	-
0.75 per cent.	-	-	+++	+++	-
1.0 per cent.	-	-	++++	++++	-
1.25 per cent.	+	+	+++	+++	-

Appendix 26.

Electrolyte.

SODIUM CHLORIDE.

SALINE. Aqueous solution of Sodium Chloride, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty six hours before use.

ANTIGEN. A 4. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium chloride					
0.026 per cent.	-	-	-	-	-
0.05 per cent.	-	-	-	-	-
0.07 per cent.	-	-	-	-	-
0.1 per cent.	-	-	-	+	-
0.2 per cent.	-	-	+++	++++	-
0.75 per cent.	-	-	++	++++	-

pH of 0.2 per cent. sodium chloride solution = 6.0

Appendix 27.

Electrolyte.

SODIUM CHLORIDE.

SALINE. Aqueous solution of Sodium Chloride, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., five hours before use.

ANTIGEN. A4. Readings made after 19 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
sodium chloride					
0.1 per cent	-	-	-	+	-
0.12 per cent.	-	-	-	+	-
0.14 per cent.	-	-	±	+++	-
0.16 per cent.	-	-	+	+++	-
0.18 per cent.	-	-	++	+++	-
0.2 per cent.	-	-	++	+++	-
0.75 per cent.	-	-	+++	++++	-

Electrolyte.

SODIUM CHROMATE

SALINE. Aqueous solution of Sodium Chromate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., thirty three hours before use.

ANTIGEN. A4. Readings made after 18 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
sodium chromate.					
0.2per cent.	-	-	-	++	-
0.4 per cent.	-	-	-	++	-
0.6 per cent.	-	-	-	+	-
0.8 per cent.	-	-	-	+	-
1.0 per cent.	-	-	-	+	-
Control.					
0.75 per cent.NaCl	-	-	++	+++	-

pH of 1.0 per cent. sodium chromate solution = 9.5 (approx)

Appendix 29.

Electrolyte.

SODIUM CHROMATE.

SALINE. Aqueous solution of Sodium Chromate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., thirty three hours before use.

ANTIGEN. B2. Readings made after 18 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium chromate					
0.2 per cent.	-	-	-	+	-
0.4 per cent.	-	-	-	+	-
0.6 per cent.	-	-	-	+	-
0.8 per cent.	-	-	-	+	-
1.0 per cent.	-	-	-	±	-
Control					
0.75 per cent. NaCl	-	-	++	++	-

pH of 1.0 per cent. sodium chromate solution = 9.5 (approx).

Appendix 30.

Electrolyte.

SODIUM CINNAMATE.

SALINE. Aqueous solution of Sodium Cinnamate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty four hours before use.

ANTIGEN.A4. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
sodium cinnamate.					
0.2 per cent.	-	-	-	++++	-
0.4 per cent.	-	-	+	++++	-
0.6 per cent.	-	-	++++	++++	-
0.8 per cent.	-	-	++++	++++	-
1.0 per cent.	-	-	+++	++++	-
Control					
0.75 per cent.NaCl	-	-	++++	++++	-

pH of 1.0 per cent.sodium cinnamate solution =7.2

Electrolyte.

SODIUM CINNAMATE.

SALINE. Aqueous solution of Sodium Cinnamate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty four hours before use.

ANTIGEN. B2. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
sodium cinnamate					
0.2 per cent.	-	-	-	+++	-
0.4 per cent.	-	-	+++	+++	-
0.6 per cent.	-	-	++++	++++	-
0.8 per cent.	-	-	+++	++++	-
1.0 per cent.	-	-	++++	++++	-
Control					
0.7% per cent. NaCl	-	-	+++	+++	-

pH of 1.0 per cent. sodium cinnamate solution = 7.2

Appendix 32.

Electrolyte.

SODIUM CITRATE.

SALINE. Aqueous solution of Sodium Citrate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour, at 56°C., nine hours before use.

ANTIGEN. A4. Readings made after 18 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
sodium citrate.					
0.2 per cent.	-	-	-	-	-
0.4 per cent.	-	-	-	±	-
0.6 per cent.	-	-	-	+	-
0.8 per cent.	-	-	-	++	-
1.0 per cent.	-	-	±	++	-
Control.					
0.75 per cent.NaCl	-	-	++	+++	-

pH of 1.0 per cent. sodium citrate solution = 7.3

Electrolyte.

SODIUM CITRATE.

SALINE. Aqueous solution of Sodium Citrate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., nine hours before use.

ANTIGEN. B2. Readings made after 18 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium citrate.					
0.2 per cent.	-	-	-	-	-
0.4 per cent.	-	-	-	±	-
0.6 per cent.	±	-	-	+	-
0.8 per cent.	-	-	±	+	-
1.0 per cent.	-	-	±	++	-
Control					
0.75 per cent.NaCl	-	-	++	+++	-

pH of 1.0 per cent.sodium citrate solution = 7.3

Electrolyte.

SODIUM ortho-CRESOTINATE.

SALINE. Aqueous solution of Sodium ortho-Cresotinate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty three hours before use.

ANTIGEN. A4. Readings made after 20 hours at 37°C.

	NORMAL serum		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
sodium ortho-cresotinate.					
0.2 per cent.	-	-	-	+	-
0.4 per cent.	-	-	++	+++	-
0.6 per cent.	-	-	++++	++++	-
0.8 per cent.	-	-	++++	++++	-
1.0 per cent.	-	-	++++	++++	-
Control.					
0.75 per cent.NaCl	-	-	++	+++	-

pH of 1.0 per cent. sodium ortho-cresotinate solution = 5.1

Appendix 35.

Electrolyte.

SODIUM ortho-CRESOTINATE.

SALINE. Aqueous solution of Sodium ortho-Cresotinate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty three hours before use.

ANTIGEN. B2. Readings made after 20 hours at 37°C.

	NORMAL serum		SYPHILITIC serum.		ANTIGEN control
serum c.c.	0.05	0.1	0.05	0.1	nil
sodium ortho-cresotinate.					
0.2 per cent.	-	-	-	++	-
0.4 per cent.	-	-	++	+++	-
0.6 per cent.	-	-	+++	+++	-
0.8 per cent.	-	-	++	++	-
1.0 per cent	-	-	++	++	-
Control.					
0.75 per cent.NaCl	-	-	++	++	-

pH of 1.0 per cent.sodium ortho-cresotinate solution = 5.1

Electrolyte.

SODIUM para-CRESOTINATE.

SALINE. Aqueous solution of Sodium para-Crssotinate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty two hours before use.

ANTIGEN. A4. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
sodium para-cresotinate.					
0.2 per cent.	±	-	-	-	-
0.4 per cent.	-	-	-	±	-
0.6 per cent.	-	-	-	+++	-
0.8 per cent.	-	-	++++	++++	-
1.0 per cent.	-	-	+++	+++	-
Control.					
0.75 per cent.NaCl	-	-	++	+++	-

pH of 1.0 per cent.sodium para-cresotinate solution = 6.(

Electrolyte.

SODIUM para-CRESOTINATE.

SALINE. Aqueous solution of Sodium para-Cresotinate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty two hours before use.

ANTIGEN. B2. Readings made after twenty hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
sodium para-cresotinate.					
0.2 per cent.	-	-	-	-	-
0.4 per cent.	-	-	-	±	-
0.6 per cent.	-	-	+++	+++	-
0.8 per cent.	-	-	+++	+++	-
1.0 per cent.	-	-	++	+++	-
Control.					
0.75 per cent. NaCl	-	-	+	++	-

pH of 1.0 per cent. sodium para-cresotinate solution = 6.0

Appendix 38.

Electrolyte.

SODIUM CYANIDE.

SALINE. Aqueous solution of Sodium Cyanide, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty six hours before use.

ANTIGEN. A 4. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium cyanide.					
0.2 per cent.	-	-	-	-	-
0.4 per cent.	-	-	-	-	-
0.6 per cent.	-	-	-	-	-
0.8 per cent.	-	-	-	-	-
1.0 per cent.	-	-	-	-	-
Control.					
0.75 per cent.NaCl.	-	-	++	+++	-

pH of 1.0 per cent.sodium cyanide solution = 10.5 +

Electrolyte.

SODIUM CYANIDE.

SALINE. Aqueous solution of Sodium Cyanide, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty six hours before use.

ANTIGEN. B 2. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium cyanide.					
0.2 per cent.	-	-	-	-	-
0.4 per cent.	-	-	-	-	-
0.6 per cent.	-	-	-	-	-
0.8 per cent.	-	-	-	-	-
1.0 per cent.	-	-	-	-	-
Control.					
0.75 per cent.NaCl.	-	-	+	++	-

pH of 1.0 per cent.sodium cyanide solution = 10.5 +

Electrolyte.

SODIUM FERROCYANIDE.

SALINE. Aqueous solution of Sodium Ferrocyanide, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., six hours before use.

ANTIGEN. A 4. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium ferrocyanide.					
0.2 per cent.	-	-	-	++	-
0.4 per cent.	-	-	+	+++	-
0.6 per cent.	-	-	+	+++	-
0.8 per cent.	-	-	+++	+++	-
1.0 per cent.	-	-	+++	+++	-
Control.					
0.75 per cent.NaCl	-	-	++++	++++	-

pH of 1.0 per cent.sodium ferrocyanide solution = 7.1

Electrolyte.

SODIUM FERROCYANIDE.

SALINE. Aqueous solution of Sodium Ferrocyanide, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., six hours before use.

ANTIGEN. B2. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
sodium ferrocyanide.					
0.2 per cent.	-	-	-	++	-
0.4 per cent.	-	-	+	+++	-
0.6 per cent.	-	-	+	++++	-
0.8 per cent.	-	-	+	++++	-
1.0 per cent.	-	-	++	++++	-
Control					
0.75 per cent.NaCl	-	-	+++	+++	-

pH of 1.0 per cent.sodium ferrocyanide solution = 7.1

Electrolyte.

SODIUM FORMATE.

SALINE. Aqueous solution of Sodium Formate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 57°C., six hours before use.

ANTIGEN. A 4. Readings made after 18 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control,
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium formate.					
0.2 per cent.	-	-	++	++++	-
0.4 per cent.	-	-	++++	++++	-
0.6 per cent.	-	-	++++	++++	-
0.8 per cent.	-	-	+++	++++	-
1.0 per cent.	-	-	+++	++++	-
Control.					
0.75 per cent. NaCl.	-	-	++++	++++	-

pH of 1.0 per cent. sodium formate solution = 6.8

Electrolyte.

SODIUM FORMATE.

SALINE. Aqueous solution of Sodium Formate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 57°C., six hours before use.

ANTIGEN. B 2. Readings made after 18 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium formate.					
0.2 per cent.	-	-	+	++	-
0.4 per cent.	-	-	+++	+++	-
0.6 per cent.	-	-	+++	+++	-
0.8 per cent.	-	-	+++	+++	-
1.0 per cent.	-	-	++	++	-
Control.					
0.75 per cent. NaCl	-	-	+	++	-

pH of 1.0 per cent. sodium formate solution = 6.8

Appendix 44.

Electrolyte.

SODIUM HYPOPHOSPHITE.

SALINE. Aqueous solution of Sodium Hypophosphite, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 54°C., six hours before use.

ANTIGEN. A 4. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium hypophosphite.					
0.2 per cent.	-	-	±	+	-
0.4 per cent.	-	-	++	++	-
0.6 per cent.	-	-	++	++	-
0.8 per cent.	-	-	++	±	-
1.0 per cent.	-	-	++	±	-
Control.					
0.75 per cent.NaCl.	-	-	±	-	-

pH of 1.0 per cent.sodium hypophosphite solution = 7.2

Electrolyte.

SODIUM HYPOPHOSPHITE.

SALINE. Aqueous solution of Sodium Hypophosphite, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 54°C., six hours before use.

ANTIGEN. B 2. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium hypophosphite					
0.2 per cent.	-	-	+	+	-
0.4 per cent.	-	-	+	+	-
0.6 per cent.	-	-	+	+	-
0.8 per cent.	-	-	+	-	-
1.0 per cent.	-	-	+	-	-
Control.					
0.75 per cent.NaCl	-	-	-	-	-

pH of 1.0 per cent.sodium hypophosphite solution = 7.2.

Electrolyte.

SODIUM HYPOPHOSPHITE.

SALINE. Aqueous solution of Sodium Hypophosphite, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 54.5 °C., twenty four hours before use.

ANTIGEN. A 4. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium hypophosphite.					
0.2 per cent.	-	-	++	++++	-
0.4 per cent.	-	-	++++	++++	-
0.6 per cent.	-	-	++++	++++	-
0.8 per cent.	-	-	+++	++++	-
1.0 per cent.	-	-	+++	++++	-
Control.					
0.75 per cent.NaCl.	-	-	++++	++++	-

pH of 1.0 per cent.sodium hypophosphite solution = 6.8

Electrolyte.

SODIUM HYPOPHOSPHITE.

SALINE. Aqueous solution of Sodium Hypophosphite, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 54.5 °C., twenty four hours before use.

ANTIGEN. B 2. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium hypophosphite.					
0.2 per cent.	-	-	++	+++	-
0.4 per cent.	-	-	+++	+++	-
0.6 per cent.	-	-	+++	+++	-
0.8 per cent.	-	-	++	+++	-
1.0 per cent.	-	-	++	+++	-
Control.					
0.75 per cent.NaCl.	-	-	++	++	-

pH of 1.0 per cent.sodium hypophosphite solution = 6.8

Electrolyte.

SODIUM HYPOSULPHITE.

SALINE. Aqueous solution of Sodium Hyposulphite, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty four hours before use.

ANTIGEN. A 4. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium hyposulphite.					
0.2 per cent.	-	-	-	++	-
0.4 per cent.	-	-	++	+++	-
0.6 per cent.	-	-	++++	++++	-
0.8 per cent.	-	-	++++	++++	-
1.0 per cent.	-	-	++++	++++	-
Control.					
0.75 per cent.NaCl	-	-	+++	+++	-

pH of 1.0 per cent. sodium hyposulphite solution = 5.8

Appendix 49.

Electrolyte.

SODIUM HYPOSULPHITE.

SALINE. Aqueous solution of Sodium Hyposulphite, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty four hours before use.

ANTIGEN. B 2. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium hyposulphite.					
0.2 per cent.	-	-	-	++	-
0.4 per cent.	-	-	++	++	-
0.6 per cent.	-	-	++	+++	-
0.8 per cent.	-	-	+++	+++	-
1.0 per cent.	-	-	+++	+++	-
Control.					
0.75 per cent.NaCl	-	-	+	+	-

pH of 1.0 per cent.sodium hyposulphite solution = 5.8

Electrolyte.

SODIUM IODATE.

SALINE. Aqueous solution of Sodium Iodate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty nine hours before use.

ANTIGEN. A 4. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium iodate.					
0.2 per cent.	-	-	±	+++	-
0.4 per cent.	-	-	+++	++++	-
0.6 per cent.	-	-	++++	++++	-
0.8 per cent.	-	-	++++	++++	-
1.0 per cent.	-	-	++++	++++	-
Control.					
0.75 per cent.NaCl	-	-	+++	++++	-

pH pf 1.0 per cent.sodium iodate solution = 9.5 (approx)

Electrolyte.

SODIUM IODATE.

SALINE. Aqueous solution of Sodium Iodate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty nine hours before use.

ANTIGEN. B 2. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium iodate.					
0.2 per cent.	-	-	+	+++	-
0.4 per cent.	-	-	++	++++	-
0.6 per cent.	-	-	++++	++++	-
0.8 per cent.	-	-	++++	++++	-
1.0 per cent.	-	-	++++	++++	-
Control.					
0.75 per cent. NaCl.	-	-	+++	+++	-

pH of 1.0 per cent. sodium iodate solution = 9.5 (approx)

Appendix 52.

Electrolyte.

SODIUM IODATE.

SALINE. Aqueous solution of Sodium Iodate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 55°C., immediately before use.

ANTIGEN. A4. Readings made after 24 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium iodate.					
0.2 per cent.	±	±	++	+++	-
0.4 per cent.	-	±	++	+++	-
0.8 per cent.	-	-	+	++++	-
1.0 per cent.	-	-	+	++++	-
Control.					
0.85 per cent.NaCl.	-	-	+++	++++	-

pH of 1.0 per cent.sodium iodate solution = 10 (approx).

Electrolyte.

SODIUM IODIDE.

SALINE. Aqueous solution of Sodium Iodide, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty two hours before use.

ANTIGEN. A 4. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium iodide.					
0.2 per cent.	-	-	-	-	-
0.4 per cent.	-	-	-	++	-
0.6 per cent.	-	-	++	+++	-
0.8 per cent.	-	-	+++	+++	-
1.0 per cent.	-	-	+++	+++	-
Control.					
0.75 per cent. NaCl.		-	++	+++	-

pH of 1.0 per cent. sodium iodide solution = 5.6

Electrolyte.

SODIUM IODIDE.

SALINE. Aqueous solution of Sodium Iodide, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty two hours before use.

ANTIGEN. B 2. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium iodide.					
0.2 per cent.	-	-	-	-	-
0.4 per cent.	-	-	-	+	-
0.6 per cent.	-	-	++	++	-
0.8 per cent.	-	-	++	++	-
1.0 per cent.	-	-	++	++	-
Control.					
0.75 per cent.NaCl-		-	+	++	-

pH of 1.0 per cent. sodium iodide solution = 5.6

Appendix 55.

Electrolyte.

SODIUM MOLYBDATE.

SALINE. Aqueous solution of Sodium Molybdate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty six hours before use.

ANTIGEN. A 4. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium molybdate.					
0.2 per cent.	-	-	-	-	-
0.4 per cent.	-	-	-	-	-
0.6 per cent.	-	-	-	+	-
0.8 per cent.	-	-	-	+	-
1.0 per cent.	-	-	-	+	-
Control.					
0.75 per cent. NaCl	-	-	++	+++	-

pH of 1.0 per cent. sodium molybdate solution = 10.5 +

Electrolyte.

SODIUM MOLYBDATE.

SALINE. Aqueous solution of Sodium Molybdate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty six hours before use.

ANTIGEN. B 2. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
sodium molybdate.					
0.2 per cent.	-	-	-	-	-
0.4 per cent.	-	-	-	+	-
0.6 per cent.	-	-	±	+	-
0.8 per cent.	-	-	±	+	-
1.0 per cent.	-	-	-	+	-
Control.					
0.75 per cent. NaCl	-	-	+	++	-

pH of 1.0 per cent. sodium molybdate solution = 10.5 +

Electrolyte.

SODIUM NITRATE.

SALINE. Aqueous solution of Sodium Nitrate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 57°C., four hours before use.

ANTIGEN. A 4. Readings made after 22 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium nitrate.					
0.2 per cent.	±	+	-	+	-
0.4 per cent.	-	-	++	+++	-
0.6 per cent.	-	-	++	++++	-
0.8 per cent.	-	-	++	++++	-
1.0 per cent.	-	-	++	++++	-
Control.					
0.75 per cent. NaCl	-	-	+++	++++	-

pH of 1.0 per cent. sodium nitrate solution = 6.2

Electrolyte.

SODIUM NITRATE.

SALINE. Aqueous solution of Sodium Nitrate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 57°C., four hours before use.

ANTIGEN. B 2. Readings made after 22 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium nitrate.					
0.2 per cent.	+	++	-	+	-
0.4 per cent.	-	-	+	++	-
0.6 per cent.	-	-	++	+++	-
0.8 per cent.	-	-	++	+++	-
1.0 per cent.	-	-	++	++	-
Control.					
0.75 per cent.NaCl.	-	-	++	++++	-

pH of 1.0 per cent. sodium nitrate solution = 6.2

Electrolyte.

SODIUM NITROPRUSSIDE.

SALINE. Aqueous solution of Sodium Nitroprusside, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty four hours before use.

ANTIGEN. A 4. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
sodium nitroprusside.					
0.2 per cent.	-	-	-	+	-
0.4 per cent.	-	-	+	++	-
0.6 per cent.	-	-	++	+++	-
0.8 per cent.	-	-	+	+++	-
1.0 per cent.	-	-	+++	++++	-
Control.					
0.75 per cent.NaCl	-	-	+++	+++	-

pH of 1.0 per cent. sodium nitroprusside solution = 5.1

Electrolyte.

SODIUM NITROPRUSSIDE.

SALINE. Aqueous solution of Sodium Nitroprusside, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty four hours before use.

ANTIGEN. B 2. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium nitroprusside.					
0.2 per cent.	-	-	-	+	-
0.4 per cent.	-	-	+	++	-
0.6 per cent.	-	-	++	++	-
0.8 per cent.	-	-	++	++	-
1.0 per cent.	-	-	+++	+++	-
Control.					
0.75 per cent.NaCl.	-	-	+	+	-

pH of 1.0 per cent. sodium nitroprusside solution = 5.1

Electrolyte.

SODIUM OXALATE.

SALINE. Aqueous solution of Sodium Oxalate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty five hours before use.

ANTIGEN. A4. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
sodium oxalate					
0.2 per cent.	±	±	+	+++	±
0.4 per cent.	-	±	+++	+++	±
0.6 per cent.	-	±	+++	+++	±
0.8 per cent.	±	±	++	+++	±
1.0 per cent.	±	±	+++	+++	±
Control.					
0.75 per cent. NaCl	-	-	+++	+++	-

pH of 1.0 per cent. sodium oxalate solution = 7.3

NOTE. Doubtful flocculation (±) occurs in all serum control tubes containing sodium oxalate.

Electrolyte.

SODIUM OXALATE.

SALINE. Aqueous solution of Sodium Oxalate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty five hours before use.

ANTIGEN. B 2. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
sodium oxalate.					
0.2 per cent.	±	±	++	++	-
0.4 per cent.	±	±	+++	+++	±
0.6 per cent.	±	±	+++	+++	±
0.8 per cent.	±	±	+++	+++	±
1.0 per cent.	±	±	+++	+++	±
Control.					
0.75 per cent. NaCl	-	-	++	++	-

pH of 1.0 per cent. sodium oxalate solution = 7.3

NOTE. Doubtful flocculation (±) occurs in all serum control tubes containing sodium oxalate.

Electrolyte.

SODIUM PHOSPHATE (Monobasic)

SALINE. Aqueous solution of Sodium Phosphate (Monobasic) various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 57°C., four hours before use.

ANTIGEN. A 4. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium phosphate (monobasic)					
0.2 per cent.*	++++	+	++++	++	-
0.4 per cent.*	++++	-	++++	++	-
0.6 per cent.	+++	-	+++	++	-
0.8 per cent.	±	-	+	++	-
1.0 per cent.	-	-	-	-	-
Control.					
0.75 per cent.NaCl	-	-	+++	+++	-

pH of 1.0 per cent.sodium phosphate (monobasic) solution = 4.0 (approx.

* NOTE. Flocculation occurs in the serum control tubes of these tests.

Electrolyte.

SODIUM PHOSPHATE (monobasic)

SALINE. Aqueous solution of Sodium Phosphate (monobasic), various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 57°C., ten hours before use.

ANTIGEN. B 2. Readings made after 18 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium phosphate (monobasic)					
0.2 per cent. *	++++	+	++++	++++	-
0.4 per cent.	++++	-	++++	++++	-
0.6 per cent.	+	-	++++	+	-
0.8 per cent.	+	-	++	+	-
1.0 per cent.	+	-	+	+	-
Control.					
0.75 per cent.NaCl.	-	-	++	+++	-

pH of 1.0 per cent.Sodium phosphate (monobasic) solution = 4.0 (approx)

* NOTE. Flocculation (++++) occurs in the serum control tubes of these tests.

Electrolyte.

SODIUM PHOSPHATE (dibasic)

SALINE. Aqueous solution of Sodium Phosphate (dibasic), various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 57°C., four hours before use.

ANTIGEN. A 4. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium phosphate (dibasic)					
0.2 per cent.	-	-	-	-	-
0.4 per cent.	-	-	-	-	-
0.6 per cent.	-	-	-	-	-
0.8 per cent.	-	-	-	++	-
1.0 per cent.	-	-	+-	++	-
Control.					
0.75 per cent.NaCl.	-	-	+++	+++	-

pH of 1.0 per cent.sodium phosphate (dibasic) solution = 9.0 (approx)

Electrolyte.

SODIUM PHOSPHATE (tribasic).

SALINE. Aqueous solution of Sodium Phosphate (tribasic), various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 57°C., four hours before use.

ANTIGEN. A 4. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium phosphate (tribasic)					
0.2 per cent.	-	-	-	-	-
0.4 per cent.	-	-	-	-	-
0.6 per cent.	-	-	-	-	-
0.8 per cent.	-	-	-	-	-
1.0 per cent.	-	-	-	-	-
Control.					
0.75 per cent.NaCl.	-		+++	+++	-

pH of 1.0 per cent.sodium phosphate (tribasic) solution = 10.5 +

Electrolyte.

SODIUM PHOSPHOTUNGSTATE.

SALINE. Aqueous solution of Sodium Phosphotungstate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty two hours before use.

ANTIGEN. A 4. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium phosphotungstate.					
0.2 per cent.*	-	±	-	±	-
0.4 per cent.	-	-	-	-	-
0.6 per cent.	-	-	-	-	-
0.8 per cent.	-	-	-	-	-
1.0 per cent.	-	-	-	-	-
Control.					
0.75 per cent.NaCl.	-	-	+++	+++	-

pH of 1.0 per cent.sodium phosphotungstate solution =6.9

*NOTE. Doubtful flocculation (±) occurs in the serum control tubes of these tests.

Appendix 68.

Electrolyte.

SODIUM PHOSPHOTUNGSTATE.

SALINE. Aqueous solution of Sodium Phosphotungstate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty two hours before use.

ANTIGEN. B 2. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium phosphotungstate.					
0.2 per cent.*	-	±	-	±	-
0.4 per cent.	-	-	-	±	-
0.6 per cent.	-	-	-	-	-
0.8 per cent.	-	-	-	-	-
1.0 per cent.	-	-	-	-	-
Control.					
0.75 per cent.NaCl.	-	-	++	++	-

pH of 1.0 per cent.sodium phosphotungstate solution = 6.9

*NOTE. Doubtful flocculation (±) occurs in the serum control tubes of these tests.

Electrolyte.

SODIUM SALICYLATE.

SALINE. Aqueous solution of Sodium Salicylate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty three hours before use.

ANTIGEN. A 4. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium salicylate.					
0.2 per cent.	-	-	++	++++	-
0.4 per cent.	-	-	++++	++++	-
0.6 per cent.	-	-	+++	+++	-
0.8 per cent.	-	-	++++	++++	-
1.0 per cent.	-	-	++++	++++	-
Control.					
0.75 per cent.NaCl.	-	-	++++	++++	-

pH of 1.0 per cent.sodium salicylate solution = 6.5

Electrolyte.

SODIUM SALICYLATE.

SALINE. Aqueous solution of Sodium Salicylate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty three hours before use.

ANTIGEN.B 2. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium salicylate.					
0.2 per cent.	-	-	++++	++++	-
0.4 per cent.	-	-	++++	++++	-
0.6 per cent.	-	-	+++	+++	-
0.8 per cent.	-	-	++++	++++	-
1.0 per cent.	-	-	++++	++++	-
Control.					
0.75 per cent.NaCl	-	-	+++	+++	-

pH of 1.0 per cent.sodium salicylate solution = 6.5

Electrolyte.

SODIUM SULPHATE.

SALINE. Aqueous solution of Sodium Sulphate, various concentrations.

SERA. Normal and syphilitic sera heated for half-an-hour at 57°C., five hours before use.

ANTIGEN. A 4. Readings made after 19 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium sulphate.					
0.2 per cent.	-	-	-	++	-
0.4 per cent.	-	-	++	+++	-
0.6 per cent.	-	-	++++	++++	-
0.8 per cent.	-	-	++++	++++	-
1.0 per cent.	-	-	++++	++++	-
Control.					
0.75 per cent. NaCl.	-	-	++++	++++	-

pH of 1.0 per cent. sodium sulphate solution = 5.8

Electrolyte.

SODIUM SULPHATE.

SALINE. Aqueous solution of Sodium Sulphate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 57°C., nine hours before use.

ANTIGEN.B 2. Readings made after 18 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c,c.	0.05	0.1	0.05	0.1	nil.
sodium sulphate.					
0.2 per cent.	-	-	-	+	-
0.4 per cent.	-	-	+++	+++	-
0.6 per cent.	-	-	+++	+++	-
0.8 per cent.	-	-	+++	++++	-
1.0 per cent.	-	-	++++	++++	-
Control.					
0.75 per cent.NaCl.	-	-	++	+++	-

pH of 1.0 per cent.sodium sulphate solution = 5.8

Electrolyte.

SODIUM SULPHITE.

SALINE. Aqueous solution of Sodium Sulphite, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty four hours before use.

ANTIGEN. A 4. Readings made after 21 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium sulphite.					
0.2 per cent.	-	-	-	-	-
0.4 per cent.	-	-	-	-	-
0.6 per cent.	-	-	-	-	-
0.8 per cent.	-	-	-	-	-
1.0 per cent.	-	-	-	-	-
Control.					
0.75 per cent.NaCl.	-	-	++++	++++	-

pH of 1.0 per cent.sodium sulphite solution = 9.5 (approx).

Electrolyte.

SODIUM SULPHITE.

SALINE. Aqueous solution of Sodium Sulphite, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., twenty four hours before use.

ANTIGEN. B 2. Readings made after 21 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium sulphite.					
0.2 per cent.	-	-	-	-	-
0.4 per cent.	-	-	-	-	-
0.6 per cent.	-	-	-	-	-
0.8 per cent.	-	-	-	-	-
1.0 per cent.	-	-	-	-	-
Control.					
0.75 per cent.NaCl.	-	-	+++	+++	-

pH of 1.0 per cent. sodium sulphite solution = 9.5 (approx).

Appendix 75.

Electrolyte.

SODIUM SULPHOCYANIDE.

SALINE. Aqueous solution of Sodium Sulphocyanide, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 54°C., six hours before use.

ANTIGEN. A 4. Readings made after 20 hours at 37°C.

serum c.c.	NORMAL serum. 0.05	0.1	SYPHILITIC serum. 0.05	0.1	ANTIGEN control nil.
sodium sulphocyanide.					
0.2 per cent.	-	-	-	±	-
0.4 per cent.	-	-	+	+	-
0.6 per cent.	-	-	+	±	-
0.8 per cent.	-	-	±	±	-
1.0 per cent.	-	-	±	±	-
Control.					
0.75 per cent.NaCl.	-	-	±	-	-

pH of 1.0 per cent.sodium sulphocyanide solution = 6.0

Electrolyte.

SODIUM SULPHOCYANIDE.

SALINE. Aqueous solution of Sodium Sulphocyanide, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 54°C., six hours before use.

ANTIGEN. B 2. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium sulphocyanide.					
0.2 per cent.	-	-	-	±	-
0.4 per cent.	-	-	+	+	-
0.6 per cent.	-	-	+	+	-
0.8 per cent.	-	-	+	+	-
1.0 per cent.	-	-	+	+	-
Control.					
0.75 per cent.NaCl.	-	-	-	-	-

pH of 1.0 per cent.sodium sulphocyanide solution = 6.0

Electrolyte.

SODIUM SULPHOCYANIDE.

SALINE. Aqueous solution of Sodium Sulphocyanide, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 55°C., twenty five hours before use.

ANTIGEN. A 4. Readings made after 19 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium sulphocyanide.					
0.2 per cent.	-	-	-	++	-
0.4 per cent.	-	-	++++	++++	-
0.6 per cent.	-	-	++++	++++	-
0.8 per cent.	-	-	++++	++++	-
1.0 per cent.	-	-	++++	++++	-
Control.					
0.75 per cent.NaCl.	-	-	+++	+++	-

pH of 1.0 per cent.sodium sulphocyanide solution = 6.0

Electrolyte.

SODIUM SULPHOCYANIDE.

SALINE. Aqueous solution of Sodium Sulphocyanide, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 55°C., twenty five hours before use.

ANTIGEN. B 2. Readings made after 19 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium sulphocyanide.					
0.2 per cent.	-	-	±	++++	-
0.4 per cent.	-	-	++++	++++	-
0.6 per cent.	-	-	+++	+++	-
0.8 per cent.	-	-	+++	+++	-
1.0 per cent.	-	-	+++	+++	-
Control.					
0.75 per cent.NaCl.	-	-	+	+	-

pH of 1.0 per cent.sodium sulphocyanide solution =6.0

Electrolyte.

SODIUM TARTRATE.

SALINE. Aqueous solution of Sodium Tartrate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., ten hours before use.

ANTIGEN. A 4. Readings made after 18 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium tartrate.					
0.2 per cent.	-	-	-	-	-
0.4 per cent.	-	-	-	+ -	-
0.6 per cent.	-	-	+ -	++	-
0.8 per cent.	-	-	+	++	-
1.0 per cent.	-	-	+	++	-
Control.					
0.75 per cent.NaCl.	-	-	++	++	-

pH of 1.0 per cent.sodium tartrate solution = 6.8

Electrolyte.

SODIUM TARTRATE.

SALINE. Aqueous solution of Sodium Tartrate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., ten hours before use.

ANTIGEN. B 2. Readings made after 18 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium tartrate.					
0.2 per cent.	-	-	-	-	-
0.4 per cent.	-	-	-	+-	-
0.6 per cent.	-	-	+-	+	-
0.8 per cent.	-	-	+-	+	-
1.0 per cent.	-	-	+	++	-
Control.					
0.75 per cent.NaCl.	-	-	+	++	-

pH of 1.0 per cent.sodium tartrate solution = 6.8

Electrolyte.

SODIUM TAUROCHOLATE.

SALINE. Aqueous solution of Sodium Taurocholate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 57°C., five hours before use.

ANTIGEN. A 4. Readings made after 19 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
sodium taurocholate.					
0.2 per cent.	++++	++++	++++	+++	-
0.4 per cent.	±	++++	++++	++++	-
0.6 per cent.	++++	++++	++++	++++	-
0.8 per cent.	++++	++++	++++	++++	-
1.0 per cent.	+	+	±	++	-
Control.					
0.75 per cent.NaCl.	-	-	++++	++++	-

pH of 1.0 per cent.sodium taurocholate solution = 7.2

Note. Flocculation (+ to ++++) occurs in all serum control tubes containing Sodium Taurocholate.

Electrolyte.

MAGNESIUM SULPHATE.

SALINE. Aqueous solution of Magnesium Sulphate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 55°C., twenty five hours before use.

ANTIGEN. A 4. Readings made after 19 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
magnesium sulphate.					
0.2 per cent.	++	+	+	±	-
0.4 per cent.*	++++	++++	+	±	+
0.6 per cent.*	++++	+++	++++	+++	++
0.8 per cent.*	++++	++++	++++	+++	++
1.0 per cent.*	++++	+++	++++	+++	++
Control.					
0.75 per cent. NaCl.	-	-	+++	+++	-

pH of 1.0 per cent. magnesium sulphate solution = 6.2

* Note. Flocculation (+) occurs in serum control tubes of these tests.

Electrolyte.

MAGNESIUM SULPHATE.

SALINE. Aqueous solution of Magnesium Sulphate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 55°C., twenty five hours before use.

ANTIGEN. B 2. Readings made after 19 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
magnesium sulphate.					
0.2 per cent.	++	+	±	-	-
0.4 per cent.*	++	++	±	-	±
0.6 per cent.*	++++	++++	++	++	++
0.8 per cent.*	++++	++++	+++	+++	++
1.0 per cent.*	++++	+++	++++	+++	+
Control.					
0.75 per cent.NaCl.	-	-	+	+	-

pH of 1.0 per cent.magnesium sulphate solution = 6.2

* Note. Flocculation (+ to ++) occurs in the serum control tubes of these tests.

Electrolyte.

MAGNESIUM SULPHATE.

SALINE. Aqueous solution of Magnesium Sulphate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 55°C., twenty four hours before use.

ANTIGEN. A 4. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
magnesium sulphate.					
0.05 per cent.	-	-	±	±	-
0.075 per cent.	-	-	±	±	-
0.1 per cent.	-	-	-	±	-
0.15 per cent.	-	-	-	-	-
0.2 per cent.	++	++	±	±	±
Control.					
0.75 per cent.NaCl.	-	-	++++	+++	-

pH of 0.2 per cent.magnesium sulphate solution = 5.6

Electrolyte.

MAGNESIUM SULPHATE.

SALINE. Aqueous solution of Magnesium Sulphate, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 55°C., twenty four hours before use.

ANTIGEN. B 2. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
magnesium sulphate.					
0.05 per cent.	-	-	±	-	-
0.075 per cent.	-	-	-	-	-
0.1 per cent.	-	-	-	-	-
0.15 per cent.	-	-	-	-	-
0.2 per cent.	++	++	-	-	±
Control.					
0.75 per cent.NaCl.	-	-	++	+	-

pH of 0.2 per cent.magnesium sulphate solution = 5.6

Electrolyte.

CALCIUM CHLORIDE.

SALINE. Aqueous solution of Calcium Chloride, various concentrations.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56.5°C., five hours before use.

ANTIGEN. A 4. Readings made after 24 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
calcium chloride.					
0.2 per cent.	++	++	+	-	++
0.4 per cent.	++	++	+	-	+++
0.6 per cent.	+++	+++	+	-	+++
0.8 per cent.	+++	+++	++	+	+++
1.0 per cent.	+++	+++	+++	++	+++
Control.					
0.75 per cent.NaCl.	-	-	++++	++++	-

pH of 1.0 per cent.calcium chloride solution = 6.4

Electrolyte.

CALCIUM CHLORIDE and MAGNESIUM CHLORIDE.

SALINE. N/50, N/75, and N/100 solutions of Calcium or Magnesium Chloride.
N/10 Sodium Chloride is used as control.

SERA. Normal and syphilitic sera, heated for half-an-hour at 55°C., immediately before use.

ANTIGEN. A 3. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
calcium chloride.					
N/50	++	++	++	+	++
N/75	++	+	+	±	±
N/100	+	+	+	±	-
magnesium chloride.					
N/50	++++	+++	+	+	++
N/75	++	++	+	+	±
N/100	+	++	+	+	-
Control.					
N/10 NaCl.	-	-	++++	+++	-

Note. N/50, N/75 etc., are used with their customary chemical significance.

Electrolyte.

BARIUM CHLORIDE and STRONTIUM CHLORIDE.

SALINE. N/50, N/75, and N/100 solutions of Barium or Strontium Chloride.
N/10 Sodium Chloride is used as control.

SERA. Normal and syphilitic sera, heated for half-an-hour at 55°C., immediately before use.

ANTIGEN. A 3. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
barium chloride.					
N/50	+	++	+	+	++
N/75	+	++	+	+	±
N/100	+	++	++	+	-
strontium chloride.					
N/50	±	+	+	+	±
N/75	++	++	++	+	-
N/100	++	++	++	+	-
Control.					
N/10.NaCl.	-	-	++++	+++	-

Note. N/50, N/75 etc., are used with their customary chemical significance.

Electrolyte.

MONOVALENT KATIONS.

SALINE. Decinormal (N/10) solution of Sodium, Potassium, or Ammonium Chloride.

SERA. Normal and syphilitic sera, heated for half-an-hour at 55°C., one hour before use.

ANTIGEN. A 4. Readings made after 20 hours at 37°C.

Technique. Appendix 9.

	SYPHILITIC SERUM serum dilution				ANTIGEN control.
	1 in 10	20	40	80	nil.
N/10 Sodium Chloride.	++++	++++	++	±	-
N/10 Potassium Chloride.	++++	++++	++	+	-
N/10 Ammonium Chloride.	++++	++++	++	+	-

pH of all three electrolyte solutions = 5.6 (approx).

Note. The normal serum was tested in the same range of concentrations as the syphilitic serum, and showed no flocculation in any of the tubes.

FORMALIN.

SALINE. 0.8 per cent, aqueous solution of Sodium Chloride with, and without, the addition of Formalin.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., six hours before use.

ANTIGEN. A 4. Readings made after 19 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
Formalin.*					
Nil.	-	-	+++	++++	-
1 in 1,000	-	-	++	++	-
1 in 2,000	-	-	+++	++++	-
1 in 4,000	-	-	++++	++++	-
1 in 8,000	-	-	+++	++++	-
1 in 16,000	-	-	++++	++++	-
1 in 32,000	-	-	++++	++++	-

* The figures indicate the concentration of formalin in the saline, not in the completed tests.

FORMALIN.

SALINE. 0.8 per cent. aqueous solution of Sodium Chloride with, and without, the addition of Formalin.

SERA. Normal and syphilitic sera, heated for half-an-hour at 57°C., five hours before use.

ANTIGEN. A 4. Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
Formalin.*					
Nil.	-	-	++++	++++	-
1 in 50	-	-	-	-	-
1 in 100	-	-	-	-	-
1 in 200	-	-	-	-	-
1 in 400	-	-	-	-	-
1 in 800	-	-	+	+	-
1 in 1,600	-	-	+++	+++	-

* The figures indicate the concentration of formalin in the saline, not in the completed tests.

FORMALIN.

SALINE. 0.75 per cent. aqueous solution of Sodium Chloride, with, and without, the addition of Formalin.

SERA. Two normal (Nos. 10 and 11) and nine syphilitic sera (Nos. 1 to 9) from individual cases, heated for half-an-hour at 55°C., seventeen hours before use.

ANTIGEN. A 4. Readings made after 22 hours at 37°C.

	Serum 1.		Serum 2.		Serum 3.	
serum c.c.	0.05	0.1	0.05	0.1	0.05	0.1
Formalin.*						
Nil.	+	++	+++	+++	++++	++++
1 : 300	-	-	-	-	-	-
1 : 400	-	-	-	-	-	-
1 : 500	-	-	-	-	+ -	-
1 : 600	-	-	+ -	-	+ -	-
1 : 700	+ -	-	+ -	-	+	-
1 : 800	-	-	+ -	+ -	++	-
1 : 1,000	+ -	+ -	+	+ -	+++	+ -

* The figures indicate the concentration of formalin in the saline, not in the completed tests.

(Continued)

FORMALIN.

serum c.c.	Serum 4. 0.05	Serum 4. 0.1	Serum 5. 0.05	Serum 5. 0.1	Serum 6. 0.05	Serum 6. 0.1
Formalin.						
Nil	++++	++++	++++	++++	++++	++++
1 : 300	-	-	++	-	-	-
1 : 400	+-	-	++	-	-	-
1 : 500	+	-	+++	+	-	-
1 : 600	+	+	+++	+	-	-
1 : 700	+	+	+++	+	+-	-
1 : 800	++	+	+++	+	+	-
1 : 1,000	+++	+++	+++	++	++	-

(Continued)

FORMALIN.

serum c.c.	Serum 7. 0.05	Serum 7. 0.1	Serum 8. 0.05	Serum 8. 0.1	Serum 9. 0.05	Serum 9. 0.1
Formalin.						
Nil.	++++	++++	++++	++++	++++	++++
1 : 300	±	-	-	-	-	-
1 : 400	±	-	-	-	-	-
1 : 500	+	+	-	-	+	-
1 : 600	+	+	-	-	+	-
1 : 700	++	+	-	-	++	-
1 : 800	++	++	±	±	+++	±
1 : 1,000	+++	++	+	±	+++	±

(Continued)

FORMALIN.

serum c.c.	serum 10. 0.05	serum 10. 0.1	serum 11. 0.05	serum 11. 0.1	pH of saline.
Formalin.					
Nil.	-	-	-	-	6.0
1 : 300	-	-	-	-	5.6
1 : 400	-	-	-	-	5.6
1 : 500	-	-	-	-	5.6
1 : 600	-	-	-	-	5.6
1 : 700	-	-	-	-	5.8
1 : 800	-	-	-	-	5.8
1 : 1,000	-	-	-	-	5.8

No flocculation occurs in any of the antigen controls

FORMALIN.

SALINE. (a) 0.75 per cent. aqueous solution of NaCl;
(b) the same + Formalin 1 : 500.

SERA. One normal and three syphilitic sera (from individual cases) heated for half-an-hour at 55°C., sixteen hours before use.

ANTIGEN. A 4. Readings made after 22 hours at 37°C.

Technique. Appendix 9.

		serum dilution					
	1 in	10	20	40	80	160	320
Serum.	Saline.						
1.	(a)	-	-	-	-	-	-
	(b)	-	-	-	-	-	-
2.	(a)	+++	+++	++	+	-	-
	(b)	+	+	±	±	-	-
3.	(a)	++	++	+	±	-	-
		-	±	-	-	-	-
4.	(a)	++++	++++	++++	+++	++	+
	(b)	-	+	++	++	+	-

Antigen controls show no flocculation.

FORMALIN.

SALINE. (a) 0.75 per cent. aqueous solution of NaCl;
(b) the same + Formalin 1 : 500.

SERA. Two normal and twelve syphilitic sera (from individual cases) heated for half-an-hour at 55°C., eighteen hours before use.

ANTIGEN. A 4. Readings made after 22 hours at 37°C.

	Saline (a) (pH = 6.2)		Saline (b) (pH = 5.8)	
serum c.c.	0.05	0.1	0.05	0.1
serum 1.	++++	++++	+	±
2.	++++	++++	-	-
3.	+++	+++	±	-
4.	+++	++++	-	-
5.	++++	++++	-	-
6.	+++	++++	-	-
7.	++++	++++	-	-
8.	++++	++++	±	-
9.	+++	+++	-	-
10.	+++	++++	-	-
11.	++++	++++	±	-
12.	++	++	-	-
13.	-	-	-	-
14	-	-	-	-

Antigen controls shows no flocculation.

CHLOROFORM.

SALINE. 0.85 per cent. aqueous solution of Sodium Chloride, with, and without the addition of Chloroform.

SERA. Normal and syphilitic sera, heated for half-an-hour at 57°C., six hours before use.

ANTIGEN. A 4. Readings made after 18 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
Chloroform. *					
Nil.	-	-	+++	++++	-
1 : 200	-	-	+++	++++	-
1 : 400	-	-	++++	++++	-
1 : 800	-	-	++++	++++	-
1 : 1,600	-	-	++++	++++	-
1 : 3,200	-	-	++++	++++	-
1 : 6,400	-	-	++++	++++	-

* The figures indicate the concentration of chloroform in the saline, not in the completed tests.

CHLOROFORM AND ETHER.

SERA. Normal and syphilitic sera, heated for half-an-hour at 55°C., immediately before use.

SALINE. (a) 0.85 per cent aqueous solution of Sodium Chloride.
(b) the same saturated with chloroform.
(c) the same + 0.5 per cent. anaesthetic ether.

TECHNIQUE. Parallel Sachs-Georgi tests in each of which one of the above salines is used throughout (i.e. for diluting the sera and for the preparation of the antigen - A 4). Appendix 9.

Readings made after 20 hours at 37°C.

	SYPHILITIC serum serum dilution				ANTIGEN control.
1 in	10	20	40	80	nil.
Saline.					
(a)	++	++	++	++	-
(b)	++	+	+	±	-
(c)	++	++	++	++	-

Note. No flocculation occurs in any of the tubes of the duplicate tests with Normal serum.

ACETONE.

SERA. Normal and syphilitic sera, heated for half-an-hour at 55°C., immediately before use.

SALINE. (a) 0.85 per cent. aqueous solution of sodium chloride.
(b) the same + 1.25 per dent.acetone.
(c) the same + 2.5 per cent.acetone.
(d) the same + 5.0 per cent.acetone.

TECHNIQUE. Appendix 9. Parallel Sachs-Georgi tests in each of which one of the above salines is used throughout (i.e. for diluting the sera and for the preparation of antigen - A 4.)

Readings made after 20 hours at 37°C.

	SYPHILITIC serum. serum dilution.				ANTIGEN control.
1 in	10	20	40	80	nil.
Saline.					
(a)	++	++	++	++	-
(b)	++	++	++	++	-
(c)	++	++	+++	++	-
(d)	+	++	+++	++	-

Note. No flocculation occurs in any of the tubes of the duplicate tests with Normal serum.

ACETONE.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., five hours before use.

SALINE. 0.85 per cent. solution of Sodium Chloride in distilled water.

TECHNIQUE. A series of four dilutions of each serum is prepared with saline such that 0.5 c.c. contains respectively:- 0.1 c.c., 0.05 c.c., 0.025 c.c. or 0.0125 c.c. of serum, and four rows of tubes are set up, each row containing the above range of serum volumes.
To each tube is then added:
In first row:- 0.5 c.c. saline
In second row:- 0.5 c.c. of a 1 in 50 dilution of acetone in saline.
In third row:- 0.5 c.c. of a 1 in 250 dilution of acetone in saline.
In fourth row:- 0.5 c.c. of a 1 in 1250 dilution of acetone in saline.

The mixtures are allowed to stand at room temperature for half-an-hour after which 0.5 c.c. antigen (C4, prepared by dilution with saline) is added to every tube.

Readings made after 20 hours at 37°C.

	SYPHILITIC SERUM.				ANTIGEN control.
serum c.c.	0.1	0.05	0.025	0.0125	nil.
Acetone.*					
Nil.	+++	+++	+++	+	-
1 : 150	+++	+++	+++	+	-
1 : 750	+++	+++	+++	+	-
1 : 3750	+++	+++	+++	+	-

Note. No flocculation occurs in any tube of the duplicate test in which Normal serum is used.
*The figures indicate the concentration of acetone in the completed test (total volume = 1.5 c.c.)

BILE SALT.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., five hours before use.

SALINE. 0.85 per cent. solution of Sodium Chloride in distilled water.

TECHNIQUE. A series of four dilutions of each serum is prepared with saline such that 0.5 c.c. contains respectively:-
0.1 c.c. 0.05 c.c., 0.025 c.c. or 0.0125 c.c. of serum.
Four rows of tubes are set up, each row containing the above range of serum volumes, and then to each tube is added:-

In first row:- 0.5 c.c. saline.
In second row:- 0.5 c.c. of a 1 in 50 solution of sodium taurocholate in saline.
In third row:- 0.5 c.c. of a 1 in 250 solution of sodium taurocholate in saline.
In fourth row:- 0.5 c.c. of a 1 in 1250 solution of sodium taurocholate in saline.

The mixtures are allowed to stand at room temperature for half-an-hour after which 0.5 c.c. antigen (C 4, prepared by dilution with saline) is added to every tube.

Readings made after 20 hours at 37°C.

(Continued)

serum c.c.	Syphilitic serum. 0.1	0.05	0.025	0.0125	ANTIGEN control. nil.
Bile-Salt.*					
Nil.	+++	+++	+++	+	-
1 in 150	++	+	-	-	-
1 in 750	+	-	-	-	-
1 in 3750	+++	+++	-	-	-

Note. No flocculation occurs in any of the tubes of the duplicate test with normal serum. Doubtful flocculation (±) occurs in the serum control tubes containing 1 in 150 and 1 in 750 bile salt.

* The figures indicate the concentration of bile-salt in the completed test (total volume = 1.5 c.c.)

Section (B).

SERUM.

For Standard Sachs-Georgi Technique, see appendix 1 .
For Modified Technique, see appendix 9 .

All sera used in this Section are pooled specimens unless it is otherwise indicated. For details of "syphilitic serum" and "normal serum", see appendix 10. Serum controls are invariably set up but they are not shown in the tables as flocculation was never observed in any of them.

All antigens used in this Section are optimum antigens whose suitability for the Sachs-Georgi test had been confirmed.
Antigen controls are omitted from some of the larger tables in order to economise space. A footnote is inserted in such instances if any flocculation occurred in this control.

Flocculation results are recorded as detailed in appen-
-dix 4 .

Duplicate Tests.

SERA. Pooled syphilitic sera (heated). Duplicate parallel tests with each specimen.

ANTIGEN. In the case of sera B1 to B6, the same antigen is used throughout and the twelve tests are placed in the incubator together.
With each of the other sera a separate antigen is used for each pair of tests (main and duplicate).

TECHNIQUE. Standard Sachs-Georgi.

	Main test.		Duplicate test.	
serum c.c.	0.05	0.1	0.05	0.1
Serum.				
B1	++++	+++	++++	++++
B2	+++	+++	+++	+++
B3	+++	+++	++++	++
B4	++++	+++	++++	++++
B5	++++	++++	+++	+++
B6	++++	++++	++++	++++
B7	+++	+++	+++	+++
B8	++	++	+++	++
B9	+++	+++	++	++
B10	++	++	+++	+++

Serum.

SERA. Pooled syphilitic sera (heated). Duplicate parallel tests with each specimen.

ANTIGEN. A different antigen is used for each pair of tests (main and duplicate), but the two tests with each serum are carried out with precisely the same antigen, i.e. sufficient antigen is prepared to serve for both tests.

TECHNIQUE. Standard Sachs-Georgi (appendix 1). Readings are made after 20-24 hours at 37°C. The six sera were tested on different days but the results are shown in one table for the sake of simplicity. The controls behaved satisfactorily on all occasions.

	Main test.		Duplicate test.	
serum c.c.	0.05	0.1	0.05	0.1
Serum.				
1.	++	+++	++++	++++
2.	++++	++++	+++	+++
3.	++++	++++	++	++
4.	+	++	++++	+++
5.	++	++	+++	+++
6.	+++	++	++++	++

Repeated Tests.

SERUM. Pooled normal serum tested on eight occasions in an extended range of concentrations, during a period of sixteen days.

The serum is four days old at the time of the first test. It is stored in a cool place (not frozen) and shows no evidence of bacterial contamination, remaining perfectly clear till the last.

Separate portions are inactivated for the different tests.
Temperature of inactivation:- 55°C for test on 27.2.23
56.5°C for all other tests.

ANTIGEN. A 4. The same batch of cholesterolised heart-extract is used in preparing the antigens for all the tests.

TECHNIQUE. Appendix 9.

	serum dilution				
Date of Test.	1 in 5	10	20	40	80
20.2.23	-	-	-	-	-
22.2.23	-	-	-	-	-
23.2.23	-	-	-	-	-
26.2.23	+	-	-	-	-
27.2.23	+	+	±	-	-
28.2.23	+	±	±	-	-
6.3.23	+	+	-	-	-
7.3.23	+	+	-	-	-

Controls. The antigen control and the serum control show no flocculation in any of the tests.
On the days on which this serum shows flocculation other normal sera react negatively (no flocculation).

Serum.

Effect of the Presence of Haemoglobin in Serum.

HAEMOGLOBIN SOLUTION. A fresh specimen of blood from a healthy subject is centrifugalised; the serum is pipetted off and the coagulum left in the centrifuge tube is broken up thoroughly with a glass rod. The tube is filled with saline and centrifugalised; the supernatant fluid is withdrawn and the tube refilled with saline and its contents mixed. This washing of the sediment to remove traces of serum is repeated twice (three times in all) and, when the saline from the last washing has been pipetted off, 10 c.c. of distilled water is added to the washed sediment and the tube is shaken vigorously to mix its contents. After being allowed to stand at room temperature for ten minutes to permit haemolysis to occur, the tube is centrifugalised once more until any sediment is thrown down. 5 c.c. of the clear supernatant fluid is now mixed with 5 c.c. of a 1.5% solution of sodium chloride in distilled water, and the mixture is centrifugalised to remove the brown precipitate which forms. The clear deep red supernatant fluid, representing a saturated solution of haemoglobin in 0.75% saline, is pipetted off and used as detailed below.

ANTIGEN. A 4.

(Continued)

Serum

(Haemoglobin).

SERA. (1), pooled syphilitic serum.
(2), individual syphilitic serum.
(3), pooled normal serum.
(4), individual normal serum.
(5), individual syphilitic serum, shown in Appendix 185, and now re-tested.

Serum (4) is from the sample of blood from which the haemoglobin solution is prepared.

All the sera, except serum (5), are free from any trace of haemoglobin colouring.

TECHNIQUE. Three tests are carried out with each serum, except serum (5), using:-

(a), the serum inactivated without previous dilution.
(b), the serum mixed with an equal volume of saline prior to inactivation.
(c), the serum mixed with an equal volume of haemoglobin solution prior to inactivation.

The three portions of each serum are inactivated (half-an-hour at 55°C.) at the same time, in the same water-bath, immediately before use. After inactivation, a series of increasing dilutions of each portion of each serum is made with saline such that the total volume in each tube is 1 c.c. (In preparing these dilutions allowance is made for the previous dilution to which portions (b) and (c) of the sera have been subjected). 0.5 c.c. of antigen is then added to every tube and the tests are incubated for 18

hours/ (Continued)

Serum.

at 37°C. The tests are therefore standard Sachs-Georgi tests (using an extended range of serum concentrations) with and without the presence of dissolved haemoglobin.

CONTROLS. Additional antigen and serum control tubes are used in which the maximum amount of haemoglobin solution present in the main tests is incorporated. No flocculation occurs in any of the controls.

	serum dilutions.						
1 in	5	10	20	40	80	160	320
SERUM.							
(1)a.	+++	++++	++++	++++	+++	++	±
(1)b.	+++	++++	++++	+++	+++	++	-
(1)c.	+++	++++	++++	++++	+++	++	-
(2)a.	++++	++++	++++	++++	+++	+	-
(2)b.	+++	++++	++++	++++	++	-	-
(2)c.	+++	++++	++++	+++	++	±	-
(3)a. (3)b. (3)c.	-	-	-	-	-	-	-
(4)a. (4)b. (4)c.	-	-	-	-	-	-	-
(5).	-	-	-	-	±	+	++++

The tubes with serum dilutions 1 in 20 and 1 in 10 constitute the standard Sachs-Georgi test.

Summary of results of parallel Sachs-Georgi tests with Heated and Unheated serum, from 110 cases attending a venereal diseases treatment centre.

(Appendix 113 - Appendix 127.)

Sera showing no flocculation in either test 47
Sera showing flocculation in one or both tests 63

Occurrence of flocculation: -

(1)	only with Heated serum	34
(2)	only with Unheated serum	nil
(3)	with both heated and unheated serum	29
		63

Comparison of the intensity of flocculation with heated and unheated serum in the case of the 29 sera which reacted positively in both tests: -

(a)	flocculation equally good in both tests	6
(b)	flocculation better with Unheated serum	2 *
(c)	flocculation better with heated serum	21
		29

* Nos. 104 and 105. (Appendix 126 - 127).

Comparison of the results of Wassermann and Sachs-Georgi tests with 110 sera from cases attending a venereal diseases treatment centre.

(A) Concordant results in 89 cases (81 per cent.)

Wass.R.	+	S-G.R.	+	46 cases
Wass.R.	±	S-G.R.	±	2 cases.
Wass.R.	-	S-G.R.	-	41 cases.

(B) Results disagreed in 21 cases (19 per cent.)

Wass.R.	+	S-G.R.	±	2 cases.
Wass.R.	+	S-G.R.	-	3 cases.
Wass.R.	±	S-G.R.	+	3 cases.
Wass.R.	±	S-G.R.	-	4 cases.
Wass.R.	-	S-G.R.	±	6 cases.
Wass.R.	-	S-G.R.	+	3 cases.

\+ = positive or weak positive.
± = doubtful.
\- = negative.

Unheated Serum

HEATED and UNHEATED serum - parallel tests.

SERA. 110 individual sera from patients attending a Venereal Diseases Treatment Centre. Every serum is tested both HEATED and UNHEATED within five hours of the blood being shed. (The number of hours which elapse between the withdrawal of the blood sample and the placing of the completed tests in the incubator is shown in the column "Age", and the temperature of inactivation is shown in column "Temp").

The Wassermann reaction of each serum is shown under "Wass. R". (Pos = positive; W. Pos = weak positive; ? = suspicious; Neg = negative).

ANTIGEN. As the tests are carried out in groups, on different days, many samples of Antigen are employed. These are ALL prepared from the same stock cholesterolised heart-extract (A4) and they behave satisfactorily on each occasion with known normal and known syphilitic serum.

TECHNIQUE. Appendix 9 .

(H = test with HEATED serum; U = test with UNHEATED serum).

Unheated serum.

					serum dilution.				
			1 in		5	10	20	40	80
Serum.	Wass.R.	Age.	Temp.						
1.	Pos	5	55°	H	++++	++++	++++	+++	+
				U	-	-	±	+	±
2.	Neg	5	55°	H	-	-	-	-	-
				U	-	-	-	-	-
3.	Neg	4	55°	H	+	-	-	-	-
				U	-	-	-	-	-
4.	Neg	4	55°	H	-	-	-	-	-
				U	-	-	-	-	-
5.	W.Pos	4	55°	H	++	++	+	-	-
				U	+	-	-	-	-
6.	Neg	4	55°	H	-	-	-	-	-
				U	-	-	-	-	-
7.	Neg	4	55°	H	-	-	-	-	-
				U	-	-	-	-	-
8.	Pos	4	55o	H	++++	++++	+++	++	+
				U	-	-	-	-	-

(Continued)

Unheated serum.

HEATED and UNHEATED serum - parallel tests.

					serum dilution.				
		1 in			5	10	20	40	80
Serum	Wass.R.	Age.	Temp.						
9.	Pos	4	55°	H	++++	++++	++++	++++	+++
				U	-	-	-	-	++
10.	Neg	4	55°	H	-	-	-	-	-
				U	-	-	-	-	-
11.	Neg	4	55°	H	-	-	-	-	-
				U	-	-	-	-	-
12.	Pos	4	56°	H	-	-	-	-	-
				U	-	-	-	-	-
13.	Neg	4	56°	H	-	-	-	-	-
				U	-	-	-	-	-
14.	Neg	4	56°	H	-	-	-	-	-
				U	-	-	-	-	-
15.	Pos	4	56°	H	++	++	+	±	-
				U	-	-	-	-	-
16.	Neg	4	56°	H	-	-	-	-	-
				U	-	-	-	-	-

(Continued)

Unheated serum.

HEATED and UNHEATED serum - parallel tests.

Serum	Wass.R.	Age.	Temp.		1 in serum dilution. 5	10	20	40	80
17.	Neg	4	56°	H	-	-	-	-	-
				U	-	-	-	-	-
18.	Pos	4	56°	H	+++	+++	+++	+	±
				U	-	-	-	-	-
19.	Neg	4	56°	H	-	-	-	-	-
				U"	-	-	-	-	-
20.	Neg	4	56°	H	-	-	-	-	-
				U	-	-	-	-	-
21.	W.Pos	4	56°	H	++	++	+	±	-
				U	-	-	-	-	-
22.	Pos	4	56°	H	+++	+++	++	+	-
				U	-	-	-	-	-
23.	Neg	4	56°	H	-	-	-	-	-
				U	-	-	-	-	-
24.	?	4	56°	H	-	-	-	-	-
				U	-	-	-	-	-

(Continued)

Unheated serum

HEATED and UNHEATED serum - parallel tests.

					serum dilution. 1 in 5	10	20	40	80
Serum.	Wass.R.	Age	Temp.						
25.	W.Pos	4	56°	H	++	++	+	-	-
				U	-	-	-	-	-
26.	?	4	56°	H	++	++	++	+	-
				U	-	-	-	-	-
27.	Neg	4	56°	H	-	-	-	-	-
				U	-	-	-	-	-
28.	Neg	4	56°	H	-	-	-	-	-
				U	-	-	-	-	-
29.	Pos	4	56°	H	++++	++++	+++	+++	+++
				U	++	+	+	++	++
30.	Pos	4	56°	H	+++	+++	+++	++	-
				U	-	-	-	-	-
31.	Neg	4	56°	H	-	-	-	-	-
				U	-	-	-	-	-
32.	Pos	4½	55°	H	+++	+++	+++	++	++
				U	-	-	-	-	-

(Continued)

Unheated serum.

HEATED and UNHEATED serum - parallel tests.

Serum.	Wass.R.	Age	Temp.		1 in serum dilution. 5	10	20	40	80
33.	Pos	4½	55°	H	+++	+++	+++	++	-
				U	-	-	-	-	-
34.	?	4½	55°	H	++	+	-	-	-
				U	-	-	-	-	-
35.	Pos	4½	55°	H	++++	++++	++++	+++	+++
				U	+-	+	++	++	++
36.	Neg	4½	55°	H	-	-	-	-	-
				U	-	-	-	-	-
37.	Neg	4½	55°	H	-	-	-	-	-
				U	-	-	-	-	-
38.	Pos	4½	55°	H	-	-	-	-	-
				U	-	-	-	-	-
39.	Pos	4½	55°	H	++	++	++	+	-
				U	-	-	-	-	-
40.	?	4½	55°	H	-	-	-	-	-
				U	-	-	-	-	-

(Continued)

Unheated serum.

HEATED and UNHEATED serum - parallel tests.

					serum dilution.				
	1 in				5	10	20	40	80
Serum.	Wass.R.	Age.	Temp.						
41.	Neg	4½	55°	H	-	-	-	-	-
				U	-	-	-	-	-
42.	Neg	4½	55°	H	-	-	-	-	-
				U	-	-	-	-	-
43.	Neg	4½	55°	H	-	-	-	-	-
				U	-	-	-	-	-
44.	Neg	4½	55°	H	-	-	-	-	-
				U	-	-	-	-	-
45.	Pos	4½	55°	H	++	++	++	++	-
				U	-	-	-	-	-
46.	W.Pos	4½	55°	H	++	++	+	-	-
				U	+	-	-	-	-
47.	Neg	4½	55°	H	-	-	-	-	-
				U	-	-	-	-	-
48.	Neg	4	56°	H	-	-	-	-	-
				U	-	-	-	-	-

(Continued)

Unheated serum.

HEATED and UNHEATED serum - parallel tests.

Serum.	Wass.R.	Age.	Temp.		1 in serum dilution. 5	10	20	40	80
49.	Pos	4	56°	H	+++	+++	++	+	-
				U	-	-	-	-	-
50.	Pos	4	56°	H	++++	++++	+++	+++	+
				U	-	-	-	±	-
51.	?	4	56°	H	+++	++	±	-	-
				U	-	-	-	-	-
52.	Neg	4	56°	H	-	-	-	-	-
				U	-	-	-	-	-
53.	Pos	4	56°	H	++	++	++	+	-
				U	-	-	-	-	-
54.	Neg	4½	56·5°	H	-	-	-	-	-
				U	-	-	-	-	-
55.	Pos	4½	56·5°	H	++	++	+	-	-
				U	-	-	-	-	-
56.	Pos	4½	56·5°	H	+++	++++	++++	++++	++++
				U	-	+	+	++	++

(continued)

Unheated serum.

HEATED and UNHEATED serum - parallel tests.

Serum.	Wass.R.	Age.	Temp.		serum dilution. 1 in 5	10	20	40	80
57.	Neg	4½	56.5°	H	-	-	-	-	-
				U	-	-	-	-	-
58.	Pos	4½	56.5°	H	++++	+++	+++	++	+
				U	++++	++	++	-	-
59.	Neg	4½	56.5°	H	-	-	-	-	-
				U	-	-	-	-	-
60.	Pos	4½	56.5°	H	++++	++++	++++	++++	++
				U	-	-	-	-	+
61.	Pos	4½	56.5°	H	++	+	-	-	-
				U	-	-	-	-	-
62.	Pos	4½	56.5°	H	+++	++++	++++	++++	++++
				U	++	+++	++++	++++	++++
63.	Pos	4½	56.5°	H	++	++	+	-	-
				U	-	-	-	-	-
64.	Neg	4½	56.5°	H	-	-	-	-	-
				U	-	-	-	-	-

(Continued)

Unheated serum.

HEATED and UNHEATED serum - parallel tests.

					serum dilution.				
	1 in				5	10	20	40	80
Serum.	Wass.R.	Age.	Temp.						
65	Neg	4½	56.5°	H	-	-	-	-	-
				U	-	-	-	-	-
66.	Neg	4½	56.5°	H	-	-	-	-	-
				U	-	-	-	-	-
67.	Neg	4½	56.5°	H	-	-	-	-	-
				U	-	-	-	-	-
68.	Neg	4½	56.5°	H	-	-	-	-	-
				U	-	-	-	-	-
69.	Neg	4½	56.5°	H	-	-	-	-	-
				U	-	-	-	-	-
70.	W.Pos	4½	56.5°	H	++++	++++	+++	++	±
				U	-	-	-	-	-
71.	Pos	4	56.5°	H	++++	++++	++++	+++	±
				U	±	-	-	-	-
72.	Neg	4	57°	H	++	+	±	-	-
				U	-	-	-	-	-

(continued)

Unheated serum.

HEATED and UNHEATED serum - parallel tests.

					serum dilution.				
	1 in				5	10	20	40	80
Serum.	Wass.R.	Age.	Temp.						
73.	Pos	4	57°	H	++++	++++	++++	+++	++
				U	±	±	+	++	+
74.	Neg	4	57°	H	-	-	-	-	-
				U	-	-	-	-	-
75.	?	4	57°	H	-	-	-	-	-
				U	-	-	-	-	-
76.	Pos	4	57°	H	+++	+++	++	++	-
				U	-	-	-	-	-
77.	?	4	57°	H	++++	+++	++	++	-
				U	-	-	-	-	-
78.	W.Pos	4½	56°	H	++++	+++	++	+	-
				U	-	-	-	-	-
79.	Neg	4½	56°	H	-	-	-	-	-
				U	-	-	-	-	-
80.	Pos	4½	56°	H	++++	++++	++++	+++	++
				U	-	-	-	-	-

(Continued)

Unheated serum.

HEATED and UNHEATED serum - parallel tests.

					serum dilution.				
		1 in			5	10	20	40	80
Serum.	Wass.R.	Age.	Temp.						
81.	Neg	4½	56°	H	+++	+++	++	+	-
				U	-	-	-	-	-
82.	?	4½	56°	H	+	±	-	-	-
				U	-	-	-	-	-
83.	Neg	4	56°	H	+++	+++	+	±	-
				U	-	-	-	-	-
84.	Neg	4	56°	H	-	-	-	-	-
				U	-	-	-	-	-
85.	Pos	4	56°	H	++++	++++	++++	+++	++
				U	+	-	±	++	+
86.	Neg	4	56°	H	+	±	-	-	-
				U	±	-	-	-	-
87.	Neg	4	56°	H	+++	++	+	±	-
				U	±	-	-	-	-
88.	Neg	3	56°	H	+	+	±	-	-
				U	+	+	+	±	-

(Continued)

Unheated serum.

HEATED and UNHEATED serum - parallel tests.

Serum.	Wass.R.	Age.	Temp.		1 in serum dilution. 5	10	20	40	80
89.	Pos	3	56°	H	++++	++++	++++	++++	++
				U	++	+	±	-	-
90.	Neg	3	56°	H	-	-	-	-	-
				U	±	-	-	-	-
91.	Pos	3	56°	H	++++	++++	++++	+++	+
				U	+++	+++	++	+	-
92.	Pos	3	56°	H	++++	++++	++++	++++	++
				U	+	+	+	-	-
93.	Neg	3	56°	H	±	-	-	-	-
				U	±	-	-	-	-
94.	Pos	4	56°	H	±	±	+	-	-
				U	+	±	±	-	-
95.	Pos	4	56°	H	+	+	+	+	-
				U	+	+	±	±	-
96.	Neg	4	56°	H	++	+	±	-	-
				U	±	±	-	-	-

(Continued)

Unheated serum.

HEATED and UNHEATED serum - parallel tests.

					serum dilution.				
		1 in			5	10	20	40	80
Serum.	Wass.R.	Age.	Temp.						
97.	Neg	4	56°	H	+	-	-	-	-
				U	-	-	-	-	-
98.	Pos	4	56°	H	++++	++++	+++	+++	++
				U	+++	++	+	+	+-
99.	?	4	56°	H	++++	++++	++++	+++	+
				U	+	+	+-	-	-
100.	Neg	4	56°	H	++	+	+-	-	-
				U	-	-	-	-	-
101.	Pos	4	56°	H	++++	++++	++++	++++	++
				U	+	+	+-	-	-
102.	W.Pos	4	56°	H	+++	+++	++	+	+
				U	++	-	-	+-	-
103.	Pos	4	56°	H	+++	+++	++++	++++	++++
				U	++++	++++	++++	++++	++++
104.	Pos	4	56°	H	+-	++	++++	++++	++++
				U	++++	++++	++++	++++	++++

(Continued)

Unheated serum.

HEATED and UNHEATED serum - parallel tests.

					serum dilution.				
		1 in			5	10	20	40	80
Serum.	Wass.R.	Age.	Temp.						
105.	Pos	4	56°	H	+	++	++++	++++	++++
				U	++++	++++	++++	++++	++++
106.	Pos	4	56°	H	+++	+++	+++	++	++
				U	+-	+	+	+	+
107.	Pos	4	55°	H	+++	+++	++++	++++	+++
				U	++++	++++	++++	++++	+++
108.	W.Pos	4	55°	H	+-	+-	-	-	-
				U	+	+-	-	-	-
109.	Pos	4	55°	H	+++	+++	+++	++	+
				U	+	+	+	+	+-
110.	Neg	4	55°	H	-	-	-	-	-
				U	+	+-	-	-	-

UNHEATED SERUM.

Serum 12 is from a case of clinical syphilis (Wassermann reaction, positive). The serum is 4 hours old when tested on 20/2/23.

The same pooled sera (syphilitic and normal) are used on both dates, as controls.

Temperature of inactivation = 56°C.

Antigen. A 4. Technique: Appendix 9.

	1 in	serum dilution. 5	10	20	40	80
Syphilitic serum (heated)	20.2.23	+	+++	+++	+++	++
	26.2.23	+++	++++	++++	+++	++
Normal serum (heated)	20.2.23	-	-	-	-	-
	26.2.23	+	-	-	-	-
Serum 12 (heated)	20.2.23	-	-	-	-	-
	26.2.23	+	-	-	-	-
Serum 12 (unheated)	20.2.23	-	-	-	-	-
	26.2.23	-	-	-	-	-

UNHEATED SERUM.

Serum 56 is 4½ hours old when tested on 6/3/23.

The same pooled sera (syphilitic and normal) are used on both dates, as controls.

Temperature of inactivation = 56.5°C.

Antigen = A 4. Technique. Appendix 9.

		serum dilution.				
	1 in	5	10	20	40	80
Syphilitic serum (heated)	6.3.23	+++	++++	++++	++++	++
	9.3.23	+++	++++	++++	++++	+++
Normal serum (heated)	6.3.23	-	-	-	-	-
	9.3.23	+	-	-	-	-
Serum 56 (heated)	6.3.23	+++	++++	++++	++++	++++
	9.3.23	++++	++++	++++	++++	+++
Serum 56 (Unheated)	6.3.23	-	±	+	++	++
	9.3.23	-	-	-	±	++

UNHEATED SERUM.

Serum 58 is 4½ hours old when tested on 6/3/23.

The same pooled sera (syphilitic and normal) are used on both dates, as controls.

Temperature of inactivation = 56.5°C.

Antigem = A 4. Technique. Appendix 9 .

	1 in	serum dilution. 5	10	20	40	80
Syphilitic serum (heated)	6.3.23	+++	++++	++++	++++	++
	13.3.23	+++	++++	++++	++++	+++
Normal serum (heated)	6.3.23	-	-	-	-	-
	13.3.23	+-	-	-	-	-
Serum 58 (heated)	6.3.23	++++	+++	+++	++	+
	13.3.23	++++	++++	+++	++	+
Serum 58 (Unheated)	6.3.23	++++	++	++	-	-
	13.3.23	-	-	-	-	-

UNHEATED SERUM.

Serum 60 is 4½ hours old when tested on 6/3/23.

The same pooled sera (syphilitic and normal) are used on both dates, as controls.

Temperature of inactivation = 56.5°C.

Antigen = A 4. Technique. Appendix 9.

	1 in	serum dilution. 5	10	20	40	80
Syphilitic serum (heated)	6.3.23	+++	++++	++++	++++	++
	9.3.23	+++	++++	++++	++++	+++
Normal serum (heated)	6.3.23	-	-	-	-	-
	9.3.23	+	-	-	-	-
Serum 60 (heated)	6.3.23	++++	++++	++++	++++	++
	9.3.23	++++	++++	++++	++++	++
Serum 60 (unheated)	6.3.23	-	-	-	-	+
	9.3.23	-	-	-	-	+

UNHEATED SERUM.

Serum 62 is 4½ hours old when tested on 6/3/23.

The same pooled sera (syphilitic and normal) are used on both dates, as controls.

Temperature of inactivation = 56.5°C.

Antigen = A 4. Technique. Appendix 9 .

	1 in	serum dilution. 5	10	20	40	80
Syphilitic serum (heated)	6.3.23	+++	++++	++++	++++	++
	9.3.23	+++	++++	++++	++++	+++
Normal serum (heated)	6.3.23	-	-	-	-	-
	9.3.23	+	-	-	-	-
Serum 62 (heated)	6.3.23	+++	++++	++++	++++	++++
	9.3.23	+++	++++	++++	++++	++++
Serum 62 (unheated)	6.3.23	++	+++	++++	++++	++++
	9.3.23	-	++	++++	++++	+++

Appendix 133.

UNHEATED SERUM.

Serum 77 is 4 hours old when tested on 8/3/23.

The same pooled sera (syphilitic and normal) are used on both dates, as controls.

Temperature of inactivation = 57°C.

Antigen = A 4. Technique. Appendix 9.

		serum dilution.				
	1 in	5	10	20	40	80
Syphilitic serum (heated)	8.3.23	+++	++++	++++	++++	+++
	13.3.23	+++	++++	++++	++++	+++
Normal serum (heated)	8.3.23	+	±	-	-	-
	13.3.23	±	-	-	-	-
Serum 77 (heated)	8.3.23	++++	+++	++	++	-
	13.3.23	+++	+++	++	+	-
Serum 77 (unheated)	8.3.23	-	-	-	-	-
	13.3.23	-	-	-	-	-

UNHEATED SERUM.

Serum 82 is 4½ hours old when tested on 9/3/23. (Treated case of syphilis; Wassermann reaction, suspicious).

The same pooled sera (syphilitic and normal) are used on both dates as controls.

Temperature of inactivation = 56°C.

Antigen = A 4. Technique. Appendix 9.

	1 in	serum dilution. 5	10	20	40	80
Syphilitic serum (heated)	9.3.23	+++	+++	++++	++++	+++
	13.3.23	+++	++++	++++	++++	+++
Normal serum (heated)	9.3.23	+	±	-	-	-
	13.3.23	±	-	-	-	-
Serum 82 (heated)	9.3.23	+	±	-	-	-
	13.3.23	+	±	±	-	-
Serum 82 (unheated)	9.3.23	-	-	-	-	-
	13.3.23	-	-	-	-	-

Appendix 135.

UNHEATED SERUM.

Serum 98 is 4 hours old when tested on 20/3/23.

The same pooled sera (syphilitic and normal) are used on both dates, as controls.

Temperature of inactivation = 56°C.

Antigen = A 4. Technique. Appendix 9.

		serum dilution.				
	1 in	5	10	20	40	80
Syphilitic serum (heated)	20.3.23	++++	++++	++++	++++	++++
	26.3.23	++++	++++	++++	++++	+++
Normal serum (heated)	20.3.23	-	-	-	-	-
	26.3.23	+	+	+	+	+
Serum 98 (heated)	20.3.23	++++	++++	+++	+++	++
	26.3.23	+++	+++	++++	++++	+++
Serum 98 (unheated)	20.3.23	+++	++	+	+	±
	26.3.23	+	+	+	+	+

Note. Flocculation (+) occurred in the antigen control on 26/3/23.

UNHEATED SERUM.

Serum 102 is 4 hours old when tested on 20/3/23.

The same pooled sera (syphilitic and normal) are used on both dates, as controls.

Temperature of inactivation = 56°C.

Antigen = A 4. Technique. Appendix 9.

	1 in	serum dilution. 5	10	20	40	80
Syphilitic serum (heated)	20.3.23	++++	++++	++++	++++	++++
	26.3.23	++++	++++	++++	++++	+++
Normal serum (heated)	20.3.23	-	-	-	-	-
	26.3.23	+	+	±	+	+
Serum 102 (heated)	20.3.23	+++	+++	++	+	+
	26.3.23	++++	++++	++++	+++	++
Serum 102 (unheated)	20.3.23	++	-	-	±	-
	26.3.23	+	+	+	+	+

Note.

Flocculation (+) occurred in the antigen control on 26/3/23.

UNHEATED SERUM.

Serum 103 is 4 hours old when tested on 20/3/23.

The same pooled sera (syphilitic and normal) are used on both dates, as controls.

Temperature of inactivation = 56°C.

Antigen A.4. Technique. Appendix 9.

1 in		serum dilution. 5	10	20	40	80
Syphilitic serum (heated)	20.3.23.	++++	++++	++++	++++	++++
	26.3.23	++++	++++	++++	++++	+++
Normal serum (heated)	20.3.23	-	-	-	-	-
	26.3.23	+	+	+	+	+
Serum 103 (heated)	20.3.23	+++	+++	++++	++++	++++
	26.3.23	+++	++++	++++	++++	+++
Serum 103 (unheated)	20.3.23	++++	++++	++++	++++	++++
	26.3.23	+++	+++	++	+	+

Note. Flocculation (+) occurred in the antigen control on 26/3/23.

Appendix 138.

UNHEATED SERUM.

Serum 107 is 4 hours old when tested on 22/3/23.

The same pooled sera (syphilitic and normal) are used on both dates, as controls.

Temperature of inactivation = 55°C.

Antigen = A 4. Technique. Appendix 9 .

		serum dilution.				
	1 in	5	10	20	40	80
Syphilitic serum (heated)	22.3.23	+++	+++	++++	++++	+++
	26.3.23	++++	++++	++++	++++	+++
Normal serum (heated)	22.3.23	±	-	-	-	-
	26.3.23	+	+	+	+	+
Serum 107 (heated)	22.3.23	+++	+++	++++	++++	+++
	26.3.23	+++	++++	++++	++++	+++
Serum 107 (unheated)	22.3.23	++++	++++	++++	++++	+++
	26.3.23	++	++	++	++	+++

Note.

Flocculation (+) occurred in the antigen control on 26/3/23.

Unheated Serum.

Serum 108 is 4 hours old when tested in 22/3/23 (Treated case of syphilis; Wassermann reaction, weak positive).

The same pooled sera (syphilitic and normal) are used as controls on both dates.

Temperature of inactivation = 55°C.

Antigen = A 4. Technique. Appendix 9 .

	1 in	serum dilution. 5	10	20	40	80
Syphilitic serum (heated)	22.3.23	+++	+++	++++	++++	+++
	26.3.23	++++	++++	++++	++++	+++
Normal serum (heated)	22.3.23	±	-	-	-	-
	26.3.23	+	+	+	+	+
Serum 108 (heated)	22.3.23	±	±	-	-	-
	26.3.23	++++	++++	++++	+++	++
Serum 108 (unheated)	22.3.23	+	±	-	-	-
	26.3.23	+	+	+	+	+

Note.

Flocculation (+) occurred in the antigen control on 26/3/23.

Unheated Serum.

Serum 109 is 4 hours old when tested on 22/3/23.

The same pooled sera (syphilitic and normal) are used as controls on both dates.

Temperature of inactivation = 55°C.

Antigen = A 4. Technique. Appendix 9 .

		serum dilution.				
	1 in	5	10	20	40	80
Syphilitic serum (heated)	22.3.23	+++	+++	++++	++++	+++
	26.3.23	++++	++++	++++	++++	+++
Normal serum (heated)	22.3.23	±	-	-	-	-
	26.3.23	+	+	+	+	+
Serum 109 (heated)	22.3.23	+++	+++	+++	++	+
	26.3.23	++++	++++	++++	++++	+++
Serum 109 (unheated)	22.3.23	+	+	+	+	±
	26.3.23	+	+	+	+	+

Note.

Flocculation (+) occurred in the antigen control on 26/3/23.

INACTIVATION.

SERUM. Syphilitic serum heated for half-an-hour at 55°C as under:-

Portion (1), 72 hours before use.
Portion (2), 48 hours before use.
Portion (3), 24 hours before use.
Portion (4), 7 hours before use.
Portion (5), immediately before use.

The five portions of the serum are all tested at the one time with the same antigen.

ANTIGEN A 4. Technique:- Standard Sachs-Georgi.

serum c.c.	0.05	0.1	ANTIGEN control nil.
Portion (1)	++++	++++	-
Portion (2)	+++	+++	-
Portion (3)	+++	+++	-
Portion (4)	++++	++++	-
Portion (5)	++++	++++	-

A normal serum, heated for half-an-hour at 55°C., 24 hours before use, is used as control.
No flocculation occurs.

INACTIVATION.

SERA. Normal and syphilitic sera, heated for half-an-hour at 55°C., as under:-

Portion (1), 28 hours before use.
Portion (2), 4 hours before use.
Portion (3), immediately before use.

The three portions of each serum are all tested at the one time with the same antigen.

ANTIGEN A 4. Technique:- Standard Sachs-Georgi.

	NORMAL serum		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
Portion (1)	+	+	+	+	-
Portion (2)	+	+	+	++	
Portion (3)	-	-	++++	++++	

Note. Flocculation occurs with the normal serum in two of the tests.

INACTIVATION.

SERA. Normal serum heated for half-an-hour at 56°C. immediately before use.

Syphilitic serum:- (a), heated for half-an-hour at 56°C. immediately before use.

(b), heated for half-an-hour at 56°C. five days before use, and kept at room temperature in the interval.

TECHNIQUE. (Appendix 9 .) A series of dilutions of each serum is prepared with saline such that the total volume in each tube is 1.0 c.c. When these are ready, 0.5 c.c. of the same antigen (A4) is added to every tube and the completed tests are incubated at 37°C. for 20 hours.

The tests are therefore standard Sachs-Georgi tests in which an extended range of serum volumes is used. (The tubes with serum dilutions 1 in 20 and 1 in 10 constitute the standard test).

	serum dilution						
1 in	5	10	20	40	80	160	320
Serum.							
normal.	-	-	-	-	-	-	-
syphilitic (a).	++++	++++	++++	++++	++++	+++	++
syphilitic (b).	++++	++++	++++	++++	++++	+++	++

Note. The serum dilutions shown are based on the volume in the tubes prior to the addition of antigen, thus there is 0.2 c.c. serum in the tube "1 in 5".

INACTIVATION.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., as under:-

Portion (1), immediately before use.
Portion (2), 58 hours before use and kept at room temperature.
Portion (3), 58 hours before use and kept frozen.

The three portions of each serum are all tested at the one time with the same antigen.

ANTIGEN. A 4. Technique:- Standard Sachs-Georgi.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
Portion (1)	-	-	++++	++++	-
Portion (2)	-	-	++++	++++	
Portion (3)	-	-	++++	++++	

INACTIVATION.

SERA. Normal and syphilitic sera, heated for half-an-hour at 57°., as under:-

Portion (1), 50 hours before use.
Portion (2), 30 hours before use.
Portion (3), 4 hours before use.

The three portions of each serum are all tested at the one time with the same antigen.

ANTIGEN. A 4. Technique:- Standard Sachs-Georgi.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil.
Portion (1)	-	-	+	++	-
Portion (2)	-	-	++	+++	
Portion (3)	-	-	+++	++++	

INACTIVATION.

SERA. Normal and syphilitic sera, heated for half-an-hour at the undermentioned temperatures, five hours before use.

Portion (1) 50° - 51°C.
Portion (2) 52° - 53°C.
Portion (3) 56° - 57°C.
Portion (4) 60° - 61°C.

The four portions of each serum are all tested at the one time with the same antigen.

ANTIGEN. A 4. Technique:- Standard Sachs-Georgi.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
Portion (1)	-	-	±	+	-
Portion (2)	-	-	±	+	
Portion (3)	-	-	++	+++	
Portion (4)	-	-	+	+	

INACTIVATION.

SERUM. Pooled syphilitic serum. The same sample is used throughout but separate portions are inactivated for the different tests.

INACTIVATION. Half-an-hour at temperatures indicated.

TECHNIQUE. Standard Sachs-Georgi. The same cholesterolised heart-extract is used for all the tests.

Date.	Age of Serum.	Inactivation. Temperature.	Before Test.	Serum c.c. 0.05	0.1
17.10.22	7 days	55.5°	74 hours	++++	++++
do.	do.	55.5°	50 hours	+++	+++
do.	do.	54.5°	26 hours	++++	++
do.	do.	54.5°	8 hours	++++	++++
do.	do.	54.5°	½ hour	+++	+++
do.	do.	54.5°	74 hours	++++	++++

INACTIVATION.

SERUM. Pooled syphilitic serum. The same sample is used throughout but separate portions are inactivated for the different tests.

INACTIVATION. Half-an-hour at temperatures indicated.

TECHNIQUE. Standard Sachs-Georgi. The same cholesterolised heart-extract is used for all the tests.

Date.	Age of Serum.	Inactivation. Temperature	Before Test.	Serum c.c. 0.05	0.1
9.10.22	5 days	56°	24 hours	++++	++++
10.10.22	6 days	54°	6 hours	±	-
11.10.22	7 days	55°	6 hours	++++	++++
12.10.22	8 days	54°	24 hours	++++	++++
12.10.22	8 days	54°	6 hours	++++	++++
12.10.22	8 days	52.5°	3 hours	+++	++
12.10.22	8 days	55°	25 hours	+++	++
16.10.22	12 days	55°	24 hours	++++	+++

INACTIVATION.

SERUM. Pooled syphilitic serum. The same sample is used throughout, but separate portions are inactivated for the different tests.

INACTIVATION. Half-an-hour at temperatures indicated.

TECHNIQUE. Standard Sachs-Georgi. The same cholesterolised heart-extract is used for all the tests.

Date.	Age of Serum.	Inactivation. Temperature	Before Test	Serum c.c. 0.05	0.1
7.5.22	4 days	57°	2 hours	+++	+++
8.5.22	5 days	57°	5 hours	+++	+++
9.5.22	6 days	57°	9½ hours	++	+++
10.5.22	7 days	57°	4 hours	+++	+++
12.5.22	9 days	57°	10½ hours	++	+++
13.5.22	10 days	57°	4½ hours	++	++++
13.5.22	10 days	57°	30 hours	++	+++
15.5.22	12 days	56°	5 hours	++	++++

INACTIVATION.

SERUM. Pooled syphilitic serum. The same sample is used throughout, but separate portions are inactivated for the different tests.

INACTIVATION. Half-an-hour at temperatures indicated.

TECHNIQUE. Standard Sachs-Georgi. The same cholesterolised heart-extract is used for all the tests.

Date.	Age of Serum.	Inactivation. Temperature	Before Test.	Serum c.c. 0.05	0.1
20.2.23 *	6 days	56°	10 hours	+++	+++
22.2.23	8 days	56°	½ hour	++++	++++
22.2.23 *	8 days	56°	58 hours	++++	++++
23.2.23 *	9 days	56°	76 hours	+++	+++
26.2.23	12 days	56°	½ hour	++++	++++
27.2.23	13 days	55°	½ hour	++++	++++
28.2.23	14 days	56°	½ hour	++++	++++
6.3.23	20 days	56°	½ hour	++++	++++
7.3.23	21 days	56°	½ hour	++++	++++
8.3.23	22 days	57°	½ hour	++++	+++
9.3.23	23 days	56°	½ hour	++++	++++
13.3.23	27 days	56°	6 hours	++++	++++
13.3.23	27 days	56°	9 hours	+++	+++
15.3.33	29 days	56°	½ hour	+++	++

* These tests are carried out with the same portion of serum (inactivated on 20.2.23).

INACTIVATION.

SERA. Pooled syphilitic sera. The same serum is used in each group of tests, but separate portions are inactivated for tests on different days.

INACTIVATION. Half-an-hour at temperatures indicated.

TECHNIQUE. Standard Sachs-Georgi. The same cholesterolised heart-extract is used for each group of tests.

Date.	Age of Serum.	Inactivation. Temp.	Before test.	Serum c.c. 0.05	0.1	
15.3.22	4 days	55°	28 hours	+	+	A
15.3.22	4 days	55°	4 hours	+	++	
15.3.22	4 days	55°	½ hour	++++	++++	
16.5.22	7 days	56°	10 hours	++	++	B
16.5.22	7 days	56°	9 hours	++	+++	
16.5.22	7 days	56°	10 hours	+	++	
18.5.22	9 days	56°	5 hours	+	++	
18.5.22	9 days	56°	8 hours	++	++	
20.9.22	11 days	56°	23 hours	++++	++++	C
21.9.22	12 days	56°	24 hours	++++	++++	
22.9.22	13 days	56°	23 hours	++	+++	

INACTIVATION.

SERA. Pooled syphilitic sera. The same serum is used in each group of tests, but separate portions are inactivated for tests on different days.

INACTIVATION. Half-an-hour at temperatures indicated.

TECHNIQUE. Standard Sachs-Georgi. The same cholesterolised heart-extract is used for each group of tests.

Date.	Age of Serum.	Inactivation. Temp.	Before Test.	Serum c.c. 0.05	0.1	
25.9.22	6 days	56°	24 hours	+++	+++	D
26.9.22	7 days	56°	25 hours	+++	+++	D
1.10.22	12 days	56°	26 hours	++	+++	D
2.10.22	13 days	56°	22 hours	+++	+++	D
9.11.22	9 days	51°	5 hours	+ -	+	E
9.11.22	9 days	53°	5 hours	+ -	+	E
9.11.22	9 days	57°	3 hours	++	+++	E
9.11.22	9 days	61°	5 hours	+	+	E
8.1.23	6 days	56°	5 hours	++++	++++	F
11.1.23	9 days	56°	26 hours	++	+++	F

INACTIVATION.

SERA. Pooled syphilitic sera. The same serum is used in each group of tests, but separate portions are inactivated for tests on different days.

INACTIVATION. Half-an-hour at temperatures indicated.

TECHNIQUE. Standard Sachs-Georgi. The same cholesterolised heart-extract is used for each group of tests.

Date.	Age of Serum.	Inactivation. Temp.	Before Test.	Serum c.c. 0.05	0.1	
5.2.23	6 days	55°	7 hours	++++	+++	G
8.2.23	9 days	54°	2 hours	+	++	G
14.2.23	15 days	55°	96 hours	+++	+++	G
16.3.23	26 days	56°	½ hour	++++	++++	H
20.3.23	30 days	56°	½ hour	++++	++++	H
20.3.23	30 days	56°	120 hours	++++	++++	H
22.3.23	32 days	55°	½ hour	++++	+++	H
25.3.23	35 days	56°	½ hour	++++	++++	H

Summary of 102 TESTS with SYPHILITIC SERA.

The results are classified in four groups:-

Group 1 = ++++ in both tubes of test.
Group 11 = ++++ in one tube of test (+++ or less in the other).
Group 111 = +++ in both tubes of test.
Group 1V = all other degrees of flocculation.

SERA. 33 different samples of pooled syphilitic serum, all of which give a strongly positive Wassermann reaction. The temperature of inactivation and the interval between inactivation and completion of tests are shown.

ANTIGEN. As the tests are spread over a period of 18 months, several different batches of antigen are used. All of these were optimum antigens in routine use for Sachs-Georgi tests, and all had proved themselves satisfactory.

(Continued)

INACTIVATION.

temperature.	interval.	Group 1	Group 11	Group 111	Group 1V
54°C.	½ hour	1	-	1	-
	2-5 hours	-	-	-	1
	6-10hours	2	-	-	1
	24 hours	1	1	-	-
55°C.	½ hour	4	2	4	2
	2-5 hours	-	-	2	3
	6-10hours	2	1	1	3
	24 hours	2	3	1	3
56°C.	½ hour	11	2	1	3
	2-5 hours	2	1	1	3
	6-10hours	2	-	2	5
	24 hours	3	-	4	6
57°C.	½ hour	1	1	-	1
	2-5 hours	1	-	3	2
	6-10hours	2	1	-	2
	24 hours	-	-	-	1

(Continued)

SUMMARY.

Inactivation temperature.	Number of Tests.	Flocculation. Groups I and II.	Group IV.
54°C	8	5	2
55°C.	33	14	11
56°C.	46	21	17
57°C.	15	6	6
	102	46	36
Interval between inactivation and completion of test.			
½ hour.	34	22	6
2-5 hours	19	4	9
6-10hours.	24	10	11
24 hours.	25	10	10
	102	46	36

Section (C).

ANTIGEN.

For preparation of HEART-EXTRACT, see appendix 5.

For details of titration of optimum antigen, see appendix 6-8.

For Standard Sachs-Georgi Technique, see appendix 1.

The explanation of the terms "normal serum", "syphilitic serum", "pooled specimen", and "individual specimen" is given in appendix 10.

Reference to serum and antigen controls is omitted from many of the tables in this section in order to economise space. These controls were invariably set up and a note is always appended if they showed any flocculation. Where no note appears, these controls showed no change.

Antigen.

METHOD OF DILUTION with SALINE.

SERA. Two NORMAL and two SYPHILITIC sera, heated for half-an-hour at 55°C., seven hours before use.

ANTIGEN. Three antigens are used, each of which is prepared from the same stock cholesterolised heart-extract (A4) by dilution with the same sample of saline. The method of dilution varies:

- antigen (a); standard technique (see appendix 1).
- antigen (b); in the first dilution with saline the extract is poured rapidly into the saline instead of vice versa. The standard technique is followed thereafter.
- antigen (c); in the first dilution with saline the extract is floated on the saline and mixing is effected slowly, by gentle rotation of the tube. Standard technique is followed thereafter.

Readings made after 20 hours at 37°C.

serum c.c.	antigen (a). 0.05	antigen (a). 0.1	antigen (b). 0.05	antigen (b). 0.1	antigen (c). 0.05	antigen (c). 0.1
Serum.						
Normal (1).	-	-	-	-	+	++
Normal (2).	-	-	-	-	+	+
Syphilitic (1).	++++	+++	++++	+++	+++	+++
Syphilitic (2).	+++	++	+++	++	++	++
antigen control	-		-		+	

Antigen.

METHOD of DILUTION with SALINE.

SERA. Normal and syphilitic sera, heated for half-an-hour at 55°C., three hours before use.

ANTIGEN. Three antigens are used, each of which is prepared from the same stock cholesterolised heart-extract (A4) by dilution with the same sample of saline. The standard technique is followed in making these dilutions (see Appendix 1) but the temperature of the saline varies:

antigen (a); saline at room temperature.
antigen (b); saline at 55°C.
antigen (c); saline at 100°C.

Saline at room temperature is used for diluting the sera in the tubes for the whole of the tests.

Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
antigen (a).	-	-	+++	+++	-
antigen (b).	-	-	+++	+++	-
antigen (c).	-	-	++	+	-

Antigen.

SERA. Normal and syphilitic sera, heated for half-an-hour at 55°C., three hours before use.

ANTIGEN. Four separate batches are prepared from the same stock cholesterolised heart-extract (A4) by dilution with the same sample of saline. They are all made at the same time and by the same method (Standard Technique; see Appendix 1) and they differ only in volume:

antigen (a); total volume = 6 c.c.
antigen (b); total volume = 6 c.c.
antigen (c); total volume = 6 c.c.
antigen (d); total volume = 90 c.c.

Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
antigen (a).	-	-	+++	+++	-
antigen (b).	-	-	++	++	-
antigen (c).	-	-	+++	+++	-
antigen (d).	-	-	++	++	-

Antigen.

SERA. Normal and syphilitic sera, heated for half-an-hour at 55°C., three hours before use.

ANTIGEN. Four separate batches are prepared from the same stock cholesterolised heart-extract (A4) by dilution with the same sample of saline. They are all made at the same time and by the same method (Standard Technique; see Appendix 1) and they differ only in volume:

antigen (a); total volume = 6 c.c.
antigen (b); total volume = 6 c.c.
antigen (c); total volume = 6 c.c.
antigen (d); total volume = 90 c.c.

Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
antigen (a).	-	-	+++	+++	-
antigen (b).	-	-	+++	++	-
antigen (c).	-	-	++	++	-
antigen (d).	-	-	+++	+++	-

Antigen.

SERA. Normal and syphilitic sera, heated for half-an-hour at 55°C., ninety-six hours before use.

ANTIGEN. Two separate batches are prepared from the same stock cholesterolised heart-extract (A4) by dilution with the same sample of saline. They are made at the same time and by the same method (Standard Technique; see Appendix 1) and they differ only in volume:

antigen (a); total volume = 6 c.c.

antigen (b); total volume = 18 c.c.

Readings made after 21 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
antigen (a).	-	-	+++	+++	-
antigen (b).	-	-	+++	+++	-

Antigen

SERA. Normal and syphilitic sera, heated for half-an-hour at 55°C., immediately before use.

ANTIGEN. Two separate batches are prepared from the same stock cholesterolised heart-extract (A4) by dilution with the same sample of saline. They are made at the same time and by the same method (Standard Technique; see Appendix 1) and they differ only in volume:

antigen (a); total volume = 6 c.c.

antigen (b); total volume = 60 c.c.

Readings made after 18 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
antigen (a).	-	-	+++	++	-
antigen (b).	-	-	+++	+++	-

Antigen

SERA. Normal serum and syphilitic serum, heated for half-an-hour at 56°C.:-

(1), immediately before use.
(2), 58 hours before use, and stored at room temperature till required for test.
(3), 58 hours before use, and stored in refrigerator till required for test.

ANTIGEN. Two separate batches are prepared from the same stock cholesterolised heart-extract (A4) by dilution with the same sample of saline. They are made at the same time and by the same method (Standard Technique; see Appendix 1) and they differ only in volume:

antigen (a); total volume = 9 c.c.

antigen (b); total volume = 60 c.c.

Readings made after 20 hours at 37°C.

serum c.c.	antigen (a). 0.05	antigen (a). 0.1	antigen (b). 0.05	antigen (b). 0.1
serum.				
NORMAL (1),(2),(3).	-	-	-	-
Syphilitic (1).	++++	++++	++++	++++
Syphilitic (2).	++++	++++	++++	+++
Syphilitic (3).	++++	++++	+++	++
antigen control.	-		-	

Antigen.

SERA. Two NORMAL and two SYPHILITIC sera, heated for half-an-hour at 55°C., seven hours before use.

ANTIGEN. Two separate batches are prepared from the same stock cholesterolised heart-extract (A4) by dilution with the same sample of saline. They are made at the same time and by the same method (Standard Technique; see Appendix 1) and they differ only in volume:

antigen (a); total volume = 9 c.c.

antigen (b); total volume = 18 c.c.

Readings made after 20 hours at 37°C.

	antigen (a)		antigen (b).	
serum c.c.	0.05	0.1	0.05	0.1
serum.				
Normal (1).	-	-	-	-
Normal (2).	-	-	-	-
Syphilitic (1).	++++	+++	++++	++++
Syphilitic (2).	+++	++	+++	+++
antigen control.	-		-	

Antigen.

SALINE. 0.85 per cent. aqueous solution of Sodium Chloride.

SERA. (a) Normal serum (pooled specimen).
(b) Syphilitic serum (pooled specimen).
(c) Syphilitic serum (individual specimen).
The sera are heated for half-an-hour at 55°C. immediately before use.

ANTIGEN. A 4. As soon as it is prepared a portion of the antigen is placed in a water-bath at 100°C. and kept there for three minutes. It is then removed and allowed to cool for twelve minutes before use in the tests.
The remainder of the antigen (unboiled) is also used fifteen minutes after its preparation.

TECHNIQUE. Appendix 9. Readings made after 20 hours at 37°C.

		serum dilution.					
	1 in	5	10	20	40	80	160
Serum.	Antigen.						
(a)	Normal	-	-	-	-	-	-
	Boiled	±	-	-	-	-	-
(b)	Normal	++++	++++	++++	++++	+++	±
	Boiled	++++	++++	++++	++++	+++	++
(c)	Normal	+++	+++	++	+	±	-
	Boiled	++++	+++	+++	+	±	-

Note. No flocculation occurs in the antigen controls.

Antigen.

SALINE:- 0.85 per cent. sodium chloride in distilled water.

SERA:- Normal and syphilitic sera, pooled specimens, heated for half-an-hour at 54°C. immediately before test. Three serum volumes, viz. 0.1, 0.05, and 0.025 c.c. are tested instead of the customary two of the Standard Technique.

ANTIGEN:- A4. The dilution of the cholesterolised heart-extract with saline is carried out in various ways:-

(a). standard technique (appendix 1).
(b). 10 minutes allowed between first and second dilutions.
(c). 15 minutes allowed between first and second dilutions.
(d). 20 minutes allowed between first and second dilutions.
(e). 30 minutes allowed between first and second dilutions.
(f). One volume of extract floated on five volumes of saline and mixed SLOWLY by rotation of tube.
(g). One volume of extract diluted rapidly, all at once, with five volumes of saline.
(h). One volume of extract placed in a wide tube and diluted very slowly by dropping in five volumes of saline from a burette (16 drops per minute), and shaking the mixture continually during the period of dilution.
(a)' to (e)' are similar to (a) to (e) except that, in the primary dilution, 1 volume of extract is mixed rapidly with 0.75 volume of saline (instead of with an equal volume). In the second stage of dilution, sufficient saline is added to make the final dilution of the extract 1 in 6.

All the antigens are allowed to stand at room temperature for 15 minutes after their preparation before being added to the tests. They are all 1 in 6 dilutions of the same cholesterolised heart-extract with the same saline, and the whole of the tests are placed in the incubator at the same time.

Readings made after 20 hours at 37°C.

(Continued)

Antigen.

	NORMAL serum.			SYPHILITIC serum.			ANTIGEN control
serum c.c.	0.1	0.05	0.025	0.1	0.05	0.025	nil
Antigen							
(a).	-	-	-	+++	+++	+++	-
(b).	-	-	-	+++	+++	+++	-
(c)	-	-	+-	+++	+++	++	-
(d)	-	-	+	+++	+++	++	+-
(e)	+	+	+	++	+++	+++	+
(f)	+	-	-	++	++	+++	-
(g)	-	-	-	+	+-	-	-
(h)	++	++	+	+++	+++	++	+
(a)'.	-	-	-	++	+++	+++	-
(b)'.	-	-	-	+	+++	+++	+-
(c)'.	-	-	-	+	++	+++	+
(d)'.	+-	+-	+-	+	++	++	+
(e)'.	+	+	+	+	++	++	+

Note. (h) is the most turbid antigen. (a)' to (e)' are equally turbid, but less so than (h). (f) is less turbid than the preceding, but more turbid than (a) to (e). (g) is devoid of turbidity but is very faintly opalescent.

(a)' to (e)' are the most turbid antigens which can be prepared from this extract when the dilution is effected in two stages.

Antigen.

SALINE. 0.85 per cent. sodium chloride in distilled water.

SERA:- Normal and syphilitic sera, pooled specimens, heated for half-an-hour at 55°C. six hours before test.

ANTIGEN:- A4. The dilution with saline is carried out in various ways:-

(a). diluted SLOWLY, 9 hours before use, by dropping in saline from a burette and shaking continually during dilution.

(b). routine technique of dilution (appendix 1), nine hours before use.

(c). routine technique, but with extract and saline warmed to 55°C. (15 minutes before use in tests).

(d). routine technique, but with extract and saline chilled to 0°C. (15 minutes before use in tests).

(e). similar to (a), but prepared 15 minutes before use.

(f). routine technique. (15 minutes before use in tests)

All the antigens are 1 in 6 dilutions of the same cholesterolised heart-extract with the same saline, and the whole of the tests are placed in the incubator at the same time.

TECHNIQUE:- Appendix 9. Readings made after 24 hours at 37°C.

(1) NORMAL serum.

Antigen.	1 in 5	10	20	40	80	160	320
(a).	++	++	++	++	-	-	-
(b).	+	-	-	-	-	-	-
(c).	+	-	-	-	-	-	-
(d).	-	-	-	-	-	-	-
(e).	+++	++	+	+	+	-	-
(f).	-	-	-	-	-	-	-

(Continued)

Antigen.

(a) SYPHILITIC serum.

	1 in 5	10	20	40	80	160	320
Antigen.							
(a).	+++	+++	+++	+++	++	+	-
(b).	+++	+++	++++	++++	++	+	-
(c).	++	+++	+++	+++	+	±	-
(d).	++++	++++	+++	+++	-	-	-
(e).	++++	++++	++++	++++	+++	++	+
(f).	++++	++++	++++	+++	+	-	-

	(a)	(b)	(c)	(d)	(e)	(f)
(3) Antigen Controls:	±	-	-	-	+	-

Antigen.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., three hours before use.

SALINE. (Electrolyte solution). A stock 8N/5 solution of Sodium Chloride in distilled water is used, and from this the various strengths required for the tests are prepared by appropriate dilution with distilled water. (The symbol "N" is used with its customary chemical significance).

ANTIGEN. B2. The dilution of the cholesterolised heart-extract is made with distilled water instead of with saline. The antigen is clear and free from any trace of opalescence.

TECHNIQUE. The test is so arranged that, prior to the addition of antigen, each tube contains a total volume of 1.0 c.c. (serum + electrolyte solution). Tubes 3" x 3/8" are used to facilitate readings.

For the purpose of measuring the four smallest amounts of serum, dilutions of the serum are prepared with the weakest electrolyte solution such that 0.03 c.c. of a dilution contains the volume of serum required. The NaCl concentrations shown in the table represent the final concentrations of the electrolyte in the tests, without however adjusting for the serum present. The contents of every tube are mixed thoroughly after the addition both of serum and of antigen.

Readings made after 36 hours at 37°C.

++++ = copious sediment.
+++ = marked sediment
++ = slight sediment
+ = trace of sediment
? = opalescence of mixture but no sediment.

(Continued)

Appendix 172.

Antigen

Normal Serum.

NaCl concentration.	Concentration of serum (per cent).							
	1/8	¼	½	1	2	4	8	16
16N/15	-	-	-	-	-	-	-	-
8N/15	-	-	-	-	-	-	-	-
4N/15	-	-	-	-	-	-	-	-
2N/15	-	-	-	-	-	-	-	-
N/15	-	-	-	-	-	-	-	-
N/30	-	-	-	-	-	-	-	-
N/60	?	++++	++++	++++	?	-	-	-
N/120	?	++++	++++	++++	++++	++	?	-
N/240	?	++	++++	++++	++++	++++	?	-

Antigen

Syphilitic Serum.

NaCl concentration.	Concentration of serum (per cent). 1/8	¼	½	1	2	4	8	16
16N/15	-	-	-	-	-	-	-	-
8N/15	-	-	-	-	-	-	-	++
4N/15	-	-	-	-	-	-	?	+++
2N/15	-	-	-	-	-	-	++++	++++
N/15	-	-	-	-	-	-	++++	++++
N/30	-	-	-	-	-	+	++++	++++
N/60	?	++++	++++	++++	?	?	++	++++
N/120	?	?	++++	++++	++	+	++	++++
N/240	?	?	++++	++++	++++	+	?	++++

Serum controls (with the two highest concentrations of serum) show no precipitation.

Antigen controls show commencing precipitation (turbidity) with NaCl concentration 4N/15 and upwards.

These controls are set up as in the standard Sachs-Georgi technique.

Antigen.

SERUM. A Wassermann-negative serum from a case of jaundice, heated for half-an-hour at 56°C., immediately before use. (The serum is eleven days old and it has been stored frozen hard in the refrigerator).

SALINE. (Electrolyte solution). A series of dilutions of a stock 8N/5 solution of Sodium Chloride (N/5, N/10, N/20, N/40, N/80) are prepared with distilled water, and with each of these a test is set up containing a range of increasing serum concentrations, such that the total volume in every tube is 1.0 c.c. (serum + electrolyte solution).

Each tube then receives 0.5 c.c. antigen.

ANTIGEN. B 2. The cholesterolised heart-extract is diluted with distilled water instead of with saline and the diluted antigen is free from any trace of opalescence. The same antigen is used throughout.
The contents of each tube are mixed thoroughly after the addition of both serum and antigen and the completed tests are incubated at 37°C for 36 hours.
A duplicate set of tests in which the antigen is replaced by 0.5 c.c. of a 1 in 6 dilution of absolute alcohol in distilled water is carried out with serum concentrations of 8 per cent. and 16 per cent. (serum control).

Antigen controls (electrolyte solutions 1.0 c.c. + antigen 0.5 c.c.) are also included.

Precipitation is recorded as in Appendix 171.

(Continued)

Antigen

Main Test (with antigen).

NaCl concentration.	Concentration of serum (per cent). ¼	½	1	2	4	8	16
2N/15	-	-	-	-	++	+++	++++
N/15	-	-	-	-	-	+	+++
N/30	-	-	-	-	-	-	+
N/60	?	+	+++	+++	+	-	-
N/120	?	+++	++++	++++	++	?	-

Serum control (without antigen).

2N/15	+++	++++
N/15	+	+++
N/30	-	+
N/60	-	-
N/120	-	-

Antigen controls show no precipitation, or opalescence.

Antigen

SERA. Five Wassermann-positive sera (individual specimens) heated for half-an-hour at 56°C., immediately before use. (The sera are eleven days old and they have been stored frozen hard in the refrigerator).

SALINE. (Electrolyte solution). As detailed in appendix 171.

ANTIGEN and TECHNIQUE. As detailed in appendix 171.

Serum 1.

NaCl concentration.	Concentration of serum (per cent). ¼	½	1	2	4	8	16
2N/15	-	-	-	-	+	++	+++
N/15	-	-	-	-	+	++	+++
N/30	-	-	-	-	+	+++	+++
N/60	?	++	+++	+++	+++	+++	+++
N/120	?	+++	+++	+++	+++	+++	+++

Serum 2.

2N/15	-	-	-	+	+	+++	+++
N/15	-	-	+	+	+	+++	+++
N/30	-	-	+	+	+	+++	+++
N/60	?	+	+	+	+	+++	+++
N/120	?	++	++	?	+	++	+++

(Continued)

Antigen

Serum 3.

NaCl concentration.	Concentration of serum (per cent). ¼	½	1	2	4	8	16
2N/15	-	-	-	-	+	++	+++
N/15	-	-	-	+	+	++	+++
N/30	++	+++	+++	?	+	++	+++
N/60	+++	++++	++++	+++	+	++	+++
N/120	++++	++++	++++	++++	+++	++	+++

Serum 4.

2N/15	-	-	+	++	+++	+++	+++
N/15	-	-	?	++	+++	+++	+++
N/30	?	+	+	+++	+++	+++	+++
N/60	+++	++++	++++	+++	+++	+++	+++
N/120	+++	+++	++++	++++	++++	+++	+++

(Continued)

Antigen

Serum 5.

NaCl concentration.	Concentration of serum (per cent). ¼	½	1	2	4	8	16
2N/15	-	-	-	+	+	++	+++
N/15	-	-	-	+	+	+	++
N/30	+++	++++	?	?	?	+	+++
N/60	++++	++++	++++	+++	++	+++	+++
N/120	++++	++++	++++	++++	+++	+++	+++

Serum controls and antigen controls show no precipitation.

Antigen

SERA. One Wassermann-negative serum (6).
Five Wassermann-positive sera (7) - (11).
The sera are heated for half-an-hour at 56°C. immediately before use. All are individual specimens. They are 7-14 days old and have been stored frozen hard in the refrigerator.

SALINE. (Electrolyte solution). As detailed in appendix 171.

ANTIGEN and TECHNIQUE. As detailed in appendix 171.

Serum 6.

NaCl concentration.	Concentration of serum (per cent) ¼	½	1	2	4	8	16
2N/15	-	-	-	-	-	-	-
N/15	-	-	-	-	-	-	-
N/30	-	-	-	-	-	-	-
N/60	++	+++	+++	++	?	-	-
N/120	+++	++++	++++	++++	++++	?	-

Serum 7.

2N/15	-	-	-	-	+	++	+++
N/15	-	-	-	-	+	++	+++
N/30	?	+	+	+	+	+	+++
N/60	+	++++	++++	?	?	?	++
N/120	?	++++	++++	++++	+++	?	+

Antigen

Serum 8.

NaCl Concentration.	Concentration of serum (per cent). ¼	½	1	2	4	8	16
2N/15	-	-	-	-	?	+	+
N/15	-	-	-	-	?	+	++
N/30	-	-	-	-	?	+	++
N/60	?	++++	++++	+++	+++	+	++
N/120	++	++++	++++	++++	+++	++	++

Serum 9.

2N/15	-	-	-	-	++	+++	++++
N/15	-	-	-	-	++	+++	++++
N/30	-	-	-	-	++	+++	++++
N/60	++	++++	++++	+++	+++	+++	++++
N/120	?	+++	++++	++++	+++	+++	++++

(Continued)

Antigen

Serum 10.

NaCl concentration.	Concentration of serum (per cent). ¼	½	1	2	4	8	16
2N/15	-	-	+	++	+++	+++	++++
N/15	-	-	+	+++	+++	+++	++++
N/30	+	+++	++++	++++	++++	++++	++++
N/60	++++	++++	++++	++++	++++	++++	++++
N/120	++++	++++	++++	++++	++++	++++	++++

Serum 11.

2N/15	-	-	-	-	?	++	+++
N/15	-	-	-	-	?	++	+++
N/30	-	-	-	-	?	++	+++
N/60	+++	++++	++++	+++	+	+++	+++
N/120	+++	++++	++++	++++	+++	+++	+++

Serum controls and antigen controls show no precipitation.

Antigen

SERA. One Wassermann-positive serum (12).
Five Wassermann-negative sera (13) - (17).
The sera are heated for half-an-hour at 56°C., immediately before use. All are individual specimens. They are not more than two days old and have been kept in a cool place since the withdrawal of the blood samples.

SALINE. (Electrolyte solution). As detailed in appendix 171.

ANTIGEN and TECHNIQUE. As detailed in appendix 171.

Serum 12.

NaCl concentration.	concentration of serum (per cent). ¼	½	1	2	4	8	16
2N/15	-	-	-	-	+	++	++++
N/15	-	-	-	-	?	++	++++
N/30	?	++	+	?	+	+++	++++
N/60	++++	++++	++++	+++	++	+++	++++
N/120	++++	++++	++++	++++	++++	++++	++++

Serum 13.

2N/15	-	-	-	-	-	-	-
N/15	-	-	-	-	-	-	-
N/30	?	+	+	-	-	-	-
N/60	++++	+++	+++	+	?	-	-
N/120	++++	+++	+++	+++	++	+	-

(Continued)

Antigen

Serum 14.

NaCl concentration.	concentration of serum (per cent). ¼	½	1	2	4	8	16
2N/15	-	-	-	-	-	-	-
N/15	-	-	-	-	-	-	-
N/30	-	-	-	-	-	-	-
N/60	++	+++	+	?	?	-	-
N/120	++++	++++	+++	++	+	?	-

Serum 15.

2N/15	-	-	-	-	-	-	-
N/15	-	-	-	-	-	-	-
N/30	-	?	+	-	-	-	-
N/60	++	+++	++++	+++	?	-	-
N/120	+++	+++	++++	++++	+	?	-

(Continued)

Antigen

Serum 16.

NaCl concentration.	concentration of serum (per cent) ¼	½	1	2	4	8	16
2N/15	-	-	-	-	-	-	-
N/15	-	-	-	-	-	-	-
N/30	-	-	-	-	-	-	-
N/60	+++	+++	+++	+	?	-	-
N/120	+++	++++	++++	++++	++++	?	-

Serum 17.

2N/15	-	-	-	-	-	-	-
N/15	-	-	-	-	-	-	-
N/30	-	-	-	-	-	-	-
N/60	+++	++	+++	+	?	?	-
N/120	++++	++++	++++	++++	+++	?	-

Serum controls and antigen controls show no precipitation.

Antigen

SERA. (1) Pooled normal serum.
(2) Pooled syphilitic serum.
(3) Individual syphilitic serum (Wassermann reaction: - strong positive.)

ANTIGEN. A 4.

TECHNIQUE. Appendix 9. A series of increasing dilutions of each serum is prepared with saline such that the total volume of each dilution is 1.0 c.c. Antigen (0.5 c.c.) is then added to every tube and the tests are incubated at 37°C for 18 hours.
The tests are therefore standard Sachs-Georgi tests in which an extended range of serum volumes is used.

	serum dilution						
1 in	5	10	20	40	80	160	320
Serum (1)	-	-	-	-	-	-	-
Serum (2)	++	+++	++++	++++	+++	++	+
Serum (3)	-	-	-	-	++	++++	++++

The tubes with serum dilutions 1 in 20 and 1 in 10 constitute the standard Sachs-Georgi test.

Antigen

SERA. Normal and syphilitic sera, heated for half-an-hour at 55°C., four hours before use.

SALINE. (a). 0·8 per cent. NaCl.
(b). 0·2 per cent. NaCl.

ANTIGEN. B2. Three batches of antigen dilution are prepared:-

(1), diluted with saline (a) as in Standard Technique.
(2), diluted with saline (b) as in Standard Technique.
(3), diluted rapidly, to 1 in 6, with distilled water.

To portions of antigen (3) sufficient 8·0 per cent. NaCl solution is added to bring their NaCl concentrations to 0·2 per cent. and 0·8 per cent. respectively. These are shown as antigens (4) and (5).

The tests are so arranged that the final concentration of NaCl is as shown in the table. Readings made after 24 hours at 37°C.

		SYPHILITIC serum (per cent).							
		1	2	3	4	5	6	8	10
Antigen.	NaCl %								
(1)	0·8	++	+++	++++	++++	++++	++++	++++	+++
(5)	0·8	-	-	+	++	+++	++++	++++	++++
(2)	0·2	++++*	++	+++	++++	++++	++++	++++	++++
(3)	0·2	-	-	-	+	++++	++++	++++	++++
(4)	0·2	-*	-	-	++	++++	++++	++++	++++

* In the duplicate test with NORMAL serum, flocculation (++) occurs in these two tubes. There is no flocculation in any of the others.

Antigen controls and serum controls show no flocculation.

Antigen

SERA. Normal and syphilitic sera (individual specimens) heated for half-an-hour at 55°C., immediately before use.

SALINE. A stock 10 per cent. solution of Sodium Chloride in distilled water is used and the tests are arranged so that the concentration of the electrolyte in the tubes, prior to the addition of antigen, is as shown. (Total volume in each tube of completed test = 1.5 c.c.)

ANTIGEN. B 2. (a) diluted with 0.85 per cent. saline as in Sachs-Georgi technique.
(b) diluted rapidly with distilled water.

Readings made after 20 hours at 37°C.

		NORMAL serum.		SYPHILITIC serum.	
serum c.c.		0.05	0.1	0.05	0.1
NaCl concentration.	Antigen.				
N/8	(a)	-	-	++++	++++
	(b)	-	-	++++	++++
N/16	(a)	-	-	+++	++
	(b)	-	-	++++	++++
N/32	(a)	-	-	++++	+++
	(b)	-	-	+++	++++
N/64	(a)	-	-	++++	++++
	(b)	-	-	++++	++++
N/128	(a)	-	-	++++	++++
	(b)	-	-	++++	++++

No flocculation occurs in either of the antigen controls.

Antigen.

SERA. Normal and syphilitic sera (individual specimens) heated for half-an-hour at 56°C., immediately before use.

SALINE. A stock 10 per cent. solution of Sodium Chloride in distilled water is used and the tests are arranged so that the final concentration of the electrolyte in the tubes is as shown. (Total volume in each tube of completed test = 1.5 c.c.)

ANTIGEN. B 2. (a) diluted with 0.85 per cent., or with 0.425 per cent., saline as in Sachs-Georgi technique.
(b) diluted rapidly with distilled water.

Readings made after 20 hours at 37°C.

NaCl concentration		concentration of serum (per cent). 0.5	1.0	1.25	2.5	5.0	7.5	10.0
		Normal serum.						
N/8	(a)	-	-	-	-	-	-	-
	(b)	-	-	-	-	-	-	-
7N/200	(a)	+++	++	++	-	-	-	-
	(b)	-	-	-	-	-	-	-
		Syphilitic serum.						
N/8	(a)	+++	++++	++++	++++	++++	++++	++++
	(b)	+	++	+++	++++	++++	++++	++++
7N/200	(a)	++++	+++	+	+	-	-	-
	(b)	-	+	+	++	+++	++++	++++

No flocculation occurs in either of the antigen controls.

Antigen

OPTIMUM ANTIGEN.

Titration of optimum antigen of a new batch of heart-extract, as detailed in Appendix 6-8.

SERA. Normal and syphilitic sera, heated for half-an-hour at 55°C., four hours before use.

Readings made after 19 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
antigen.					
A 1.	+	+	+	++	-
A 2.	- silky	-	silky		silky
A 3.	silky		silky		silky
A 4.	-	-	+++	+++	-
B 1.	-	-	++	++	-
B 2.	-	-	+++	+++	-
B 3.	silky		silky		silky
B 4.	silky		silky		silky
C 1.	-	-	+	+	-
C 2.	silky		silky	-	silky
C 3.	silky		silky		silky
C 4.	±	-	+	++	-

Note. The term "silky" denotes a watered-silk appearance suggestive of partial separation of the cholesterol from the mixtures. It renders the estimation of flocculation almost impossible.

Antigen.

OPTIMUM ANTIGEN.

SERA. Individual sera (whose Wassermann reactions are known) heated for half-an-hour at 56°C., four hours before use

ANTIGEN. A4 and B2. These were selected as the two best antigens in the titration shown in Appendix 189.

Readings made after 21 hours at 37°C.

		Antigen A4.		Antigen B2.	
	serum c.c.	0.05	0.1	0.05	0.1
Serum.	Wassermann reaction.				
1.	weak positive	+	+++	+	++
2.	negative	-	-	-	-
3.	weak positive	+++	+++	++	+++
4.	positive	+	+	±	+
5.	negative	-	-	-	-
6.	weak positive	+++	+++	+++	+++
7.	positive	++++	++++	++++	++++
8.	negative	-	-	-	-
9.	negative	-	±	-	±
10.	negative	-	-	-	±

No flocculation occurs in either of the antigen controls.

Antigen

OPTIMUM ANTIGEN.

SERA. Individual sera (whose Wassermann reactions are known) heated for half-an-hour at 55°C., four hours before use.

ANTIGEN. A4 and B2. These were selected as the two best antigens in the titration shown in Appendix 189.

Readings made after 20 hours at 37°C.

		Antigen A4.		Antigen B2.	
	serum c.c.	0.05	0.1	0.05	0.1
Serum.	Wassermann reaction.				
11.	suspicious	-	-	-	-
12.	positive	-	-	-	-
13.	negative	-	-	-	-
14.	weak positive	±	-	±	+
15.	negative	±	-	+	+
16.	positive	-	-	-	+
17.	negative	-	-	-	-
18.	negative	-	±	-	-
19.	positive	-	-	-	-

No flocculation occurs in either of the antigen controls.

OPTIMUM ANTIGEN.

SERA. Individual sera (whose Wassermann reactions are known) heated for half-an-hour at 55°C., four hours before use.

ANTIGEN. A4 and B2. These were selected as the two best antigens in the titration shown in Appendix 189.

Readings made after 20 hours at 37°C.

		Antigen A4.		Antigen B2.	
	serum c.c.	0.05	0.1	0.05	0.1
Serum.	Wassermann reaction.				
20.	suspicious	±	±	-	-
21.	positive	-	-	-	±
22.	positive	±	±	±	+
23.	positive	-	-	±	+
24.	negative	±	±	+	++
25.	suspicious	±	-	-	±
26.	negative	-	-	-	-
27.	negative	±	+	±	+
28.	positive	±	+	+	++

No flocculation occurs in either of the antigen controls.

Antigen

SERA. Individual sera (whose Wassermann reactions are known) heated for half-an-hour at 55°C., four hours before use.

ANTIGEN. A4 and B2. These were selected as the two best antigens in the titration shown in Appendix 189.

Readings made after 20 hours at 37°C.

		Antigen A4.		Antigen B2.	
	serum c.c.	0.05	0.1	0.05	0.1
Serum.	Wassermann reaction.				
29.	weak positive	++	++	++	+++
30.	weak positive	++	+++	+++	+++
31.	positive	+++	++++	+++	++++
32.	negative	-	-	±	±
33.	negative	±	±	±	±
34.	negative	+	++	+	+
35.	negative	-	-	±	±
36.	positive	+++	+++	++	+++
37.	negative	-	-	±	±
38.	weak positive	-	++	±	±
39.	positive	-	-	±	±

No flocculation occurs in the antigen control A4, but doubtful flocculation (±) is present in the antigen control B2.

Antigen

OPTIMUM ANTIGEN.

SERA. Individual sera (whose Wassermann reactions are known) heated for half-an-hour at 55°C., four hours before use.

ANTIGEN. A4 and B2. These were selected as the two best antigens in the titration shown in Appendix 189.

Readings made after 20 hours at 37°C.

		Antigen A4.		Antigen B2.	
	serum c.c.	0.05	0.1	0.05	0.1
Serum.	Wassermann reaction.				
40.	negative	-	-	±	±
41.	negative	-	-	±	±
42.	negative	-	-	±	-
43.	weak positive	++++	++++	+	++
44.	positive	-	-	±	±
45.	negative	+	++	±	±
46.	negative	-	-	±	±
47.	positive	+++	+++	±	+
48.	negative	-	±	±	±
49.	negative	-	-	±	±

No flocculation occurs in the antigen control A4, but doubtful flocculation (±) is present in the antigen control B2.

Antigen

OPTIMUM ANTIGEN.

Summary of Appendix 189 to Appendix 194.

As a result of the preliminary titration (appendix 189) extract dilutions A 4 and B 2 were chosen as the two best. Subsequently, parallel tests were carried out with 49 individual sera, using these two cholesterolised extracts as antigens. The results of these tests and of the Wassermann reactions are shown in Appendix 190 to Appendix 194.

Antigen.			A 4.	B 2.
Wass.R.	+	S-G.R. + or ±	14	15
Wass.R.	+	S-G.R. -	8	7
Wass.R.	±	S-G.R. ±	1	nil
Wass.R.	±	S-G.R. -	2	3
Wass.R.	-	S-G.R. ± or +	5	4
Wass.R.	-	S-G.R. -	19	20

Note day of comparative failure of both antigens (appendix 191-192)

Antigen

OPTIMUM ANTIGEN.

Titration of optimum antigen of a new batch of heart-extract, as detailed in Appendix 6-8.

SERA. Normal and syphilitic sera, heated for half-an-hour at 56°C., immediately before use.

Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
antigen.					
A 1.	±	±	++	++	-
A 2.	-	-	++	++	-
A 3.	silky		++	++	silky
A 4.	silky		++	+++	silky
B 1.	silky		+	++	silky
B 2.	-	-	+++	+++	-
B 3.	silky		+++	+++	silky
B 4.	±	±	+++	++++	-
C 1.	silky		+++	+++	silky
C 2.	silky		+++	+++	silky
C 3.	silky		+++	+++	silky
C 4.	silky		+++	+++	silky

Note. The term "silky" denotes a watered-silk appearance suggestive of partial separation of the cholesterol from the mixtures.

Antigen.

OPTIMUM ANTIGEN

Titration of optimum antigen of the same batch of heart-extract and the same cholesterol solution, on different occasions, using three different syphilitic sera (pooled specimens).

SERA. Syphilitic sera, heated for half-an-hour at 55°C., not more than three hours before use.
A normal serum is included as a control with each set of tests.

TECHNIQUE. As detailed in Appendix 6 - 8.

Readings made after 20 to 24 hours at 37°C.

serum c.c.	SERUM (1). 0.05	SERUM (1). 0.1	SERUM (2). 0.05	SERUM (2). 0.1	SERUM (3). 0.05	SERUM (3). 0.1
antigen .						
A 1.	-	+	±	+	+	++
A 2.	±	+	±	+	++	++
A 3.	±	++	+	+	+	+
A 4.	+++	+++	++	+++	++	+++
B 1.	±	+	+	+	+	+
B 2.	++	++	++	++	+	+
B 3.	+++	++	+++	+++	++	+
B 4.	++	+	+++	+++	++	++
C 1.	++	+	+	+	+	±
C 2.	++	±	+++	+++	+	+
C 3.	++	+	+++	+++	++	+
C 4.	+++	+++	+++	++++	+++	++

Note. No flocculation occurs in any of the antigen controls, but the C4 antigen shows flocculation (+) in the presence of NORMAL serum, at all tests.

Antigen.

OPTIMUM ANTIGEN.

Titration of optimum antigen of the same batch of heart-extract and the same cholesterol solution on three occasions with portions of the same syphilitic serum (pooled specimen).

SERUM. Syphilitic serum, separate portion of which are heated for half-an-hour at 55°C. immediately before use.

TECHNIQUE. As detailed in Appendix 6 - 8.

Readings made after 20 to 22 hours at 37°C.

	1st. test.		2nd. test.		3rd. test.	
serum c.c.	0.05	0.1	0.05	0.1	0.05	0.1
antigen.						
A 1.	±	+	±	+	±	+
A 2.	±	+	±	+	±	+
A 3.	+	+	+	+	+	++
A 4.	++	+++	+++	+++	+++	+++
B 1.	+	+	+	++	+	+
B 2.	++	++	+++	++	++	++
B 3.	+++	+++	++++	++++	+++	+++
B 4.	+++	+++	++++	++++	++++	+++
C 1.	+	+	++	++	+	++
C 2.	+++	+++	+++	+++	+++	+++
C 3.	+++	+++	+++	+++	+++	+++
C 4.	+++	++++	+++	++++	+++	+++

Note. No flocculation occurs in any of the antigen controls, but the C4 antigen shows flocculation (+) in the presence of NORMAL serum, at all tests.

Antigen

OPTIMUM ANTIGEN

Titration of optimum antigen of the same batch of heart-extract and the same cholesterol solution on three occasions with portions of the same syphilitic serum (pooled specimen).

SERUM. Syphilitic serum, separate portions of which are heated for half-an-hour at 56°C. immediately before use.

TECHNIQUE. As detailed in Appendix 6-8.

Readings made after 20 to 24 hours at 37°C.

	1st. test.		2nd. test.		3rd. test.	
serum c.c.	0.05	0.1	0.05	0.1	0.05	0.1
antigen.						
A 1.	++	++++	++	+++	++++	++++
A 2.	++++	++++	++++	++++	++++	++++
A 3.	++++	++++	++++	++++	+++	++++
A 4.	++++	++++	++++	++++	+++	+++
B 1.	++++	++++	+++	+++	++++	++++
B 2.	++++	++++	++	++	++	+++
B 3.	++++	++++	+	++	++++	+++
B 4.	++++	++++	++	+++	++	+++
C 1.	+++	+++	++	+	+	++
C 2.	++++	++++	++	++	++	++
C 3.	++++	++++	++	++	+++	+++
C 4.	++++	++++	+++	++++	++++	++++

Note. No flocculation occurs in any of the antigen controls, but the C4 antigen shows flocculation (++) in the presence of NORMAL serum, at all tests.

Antigen.

OPTIMUM ANTIGEN.

Titration of optimum antigen of the same batch of heart-extract and the same cholesterol solution with three different syphilitic sera (pooled specimens), the tests being carried out in parallel at the same time.

SERA. Syphilitic sera, heated for half-an-hour at 56°C., immediately before use.
(A normal serum is also tested, as a control).

TECHNIQUE. As detailed in Appendix 6-8. Each antigen is tested with three syphilitic sera.

Readings made after 20 hours at 37°C.

	SERUM (1).		SERUM (2).		SERUM (3).	
serum c.c.	0.05	0.1	0.05	0.1	0.05	0.1
antigen.						
A 1.	++++	++++	+	++	++++	++++
A 2.	++++	++++	+++	+++	++++	++++
A 3.	+++	+++	+	+++	+++	++++
A 4.	+++	++	+++	++++	+++	+++
B 1.	++++	+++	+++	++++	++++	++++
B 2.	++	++	+++	+++	++	+++
B 3.	+++	++	++++	++++	++++	+++
B 4.	+++	+++	+++	+++	++	+++
C 1.	+	++	+++	+++	+	++
C 2.	+	++	+++	+++	++	++
C 3.	+++	+++	+++	+++	+++	+++
C 4.	++++	++++	++	+++	++++	++++

Note. No flocculation occurs in any of the antigen controls, but the C4 antigen shows flocculation (++) with NORMAL serum

Antigen

PREPARATION of HEART-EXTRACT (after Meinicke).

(Ether-insoluble lipoids).

Human hearts which have been carefully freed from surface fat are minced finely.

The minced heart is dried in a hot-air oven at 50°C., or on a water-bath at the same temperature, and when completely dry it is ground to a fine powder. (A coffee-mill is useful for this purpose).

5.0 grammes of the powdered heart are extracted for one hour, in a shaking machine, with 45.0 c.c. of anaesthetic ether. The mixture is then filtered through paper and the residue on the filter is dried in the incubator at 37°C.

When thoroughly dry it is treated with 45.0 c.c. of absolute alcohol and extraction is allowed to proceed at room temperature for twenty-four hours. The mixture is shaken repeatedly during this period. It is finally filtered through paper and the filtrate constitutes the extract of ether-insoluble lipoids used in the test instead of the usual Sachs-Georgi extract.

Antigen.

OPTIMUM ANTIGEN.

Titration of optimum antigen of a batch of heart-extract prepared according to Meinicke's method (see Appendix 201).

SERA. Normal and syphilitic sera, heated for half-an-hour at 55°C., twenty-four hours before use.

TECHNIQUE. As detailed in Appendix 6-8, but using the Meinicke extract as the basis for the antigens instead of a Sachs-Georgi extract.

Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
antigen.					
A 1.	-	-	++++	++++	-
A 2.	-	-	++++	++++	-
A 3.	-	-	++++	++++	-
A 4.	-	-	++++	++++	-
B 1.	-	-	++++	++++	-
B 2.	-	-	++++	++++	-
B 3.	-	-	++++	++++	-
B 4.	-	-	+++	++++	-
C 1.	-	-	++++	++++	-
C 2.	-	-	+++	++++	-
C 3.	-	-	++	+++	-
C 4.	-	-	+	++	-

Note. Where flocculation is recorded above as ++++, the flocculi are even coarser than those usually seen when a Sachs-Georgi extract is used.

Antigen.

OPTIMUM ANTIGEN.

Titration of optimum antigen of a batch of heart-extract prepared from the acetone-insoluble lipoids of human heart.

EXTRACT. 5.0 grammes of powdered heart (prepared as detailed in Appendix 201) are extracted for forty-eight hours at room temperature with 45.0 c.c. of acetone, and shaken frequently. The mixture is then filtered through paper and the residue is dried on a water-bath at 60°C., When thoroughly dry it is treated with 45.0 c.c. of absolute alcohol and extraction is allowed to proceed at room temperature for forty-eight hours. The mixture is shaken repeatedly during this period. It is finally filtered and the filtrate constitutes the extract used in the test instead of the usual Sachs-Georgi extract.

SERA. Normal and syphilitic sera, heated for half-an-hour at 55°C., twenty-four hours before use.

TECHNIQUE. As detailed in Appendix 6 - 8.

Readings made after 20 hours at 37°C.

	NORMAL SERUM.		SYPHILITIC serum.		ANTIGEN control.
serum c.c.	0.05	0.1	0.05	0.1	nil
antigen.					
A 1.	-	-	-	+	-
A 2.	-	-	-	+	-
A 3.	-	-	+	++	-
A 4.	-	-	+	++	-
B 1.	-	-	+	++	-
B 2.	-	-	++	+++	-
B 3.	-	-	++	+++	-
B 4.	-	-	++	++++	-
C 1.	-	-	++	++++	-
C 2.	-	-	++	++++	-
C 3.	-	-	+++	+++	-
C 4.	-	-	+++	++++	-

Antigen

H - ion concentration.

SALINE. (a) 0.75 per cent. solution of NaCl in distilled water pH = 5.8
(b) the same + weak NaOH solution to give pH = 6.4
(c) the same + weak NaOH solution to give pH = 7.5
(d) the same + weak NaOH solution to give pH = 9.0

SERA. One normal serum and two syphilitic sera (pooled specimens) heated for half-an-hour at 56°C., two hours before use.

TECHNIQUE. Parallel Sachs-Georgi tests in each of which one of the above salines is used throughout (i.e., for diluting the serum and for the preparation of the antigen - A 4).

Readings made after 20 hours at 37°C.

	NORMAL serum.		SYPHILITIC serum (1)		SYPHILITIC serum (2)	
serum c.c.	0.05	0.1	0.05	0.1	0.05	0.1
Saline.						
(a)	-	-	++++	++++	+++	++++
(b)	-	-	++++	++++	+++	++++
(c)	-	-	++++	++++	+++	++++
(d)	-	-	++++	++++	+++	++++

No flocculation occurs in any of the antigen controls.

Antigen

H - ion concentration.

SALINE. (a) 0.85 per cent. solution on NaCl in distilled water pH = 5.6
(b) the same +,weak NaOH solution to give pH = 6.8-7.0
(c) the same + weak NaOH solution to give pH = 7.6
(d) the same + weak NaOH solution to give pH = 9.5
(e) the same + weak NaOH solution to give pH = 10.5+

SERUM. Syphilitic serum, heated for half-an-hour at 55°C., immediately before use.

TECHNIQUE. Parallel Sachs-Georgi tests in each of which one of the above salines is used throughout (i.e.,for diluting the serum and for the preparation of the antigen - A 2).

Readings made after 20 hours at 37°C.

	SYPHILITIC SERUM.				ANTIGEN control.
serum c.c.	0.1	0.05	0.025	0.0125	nil
Saline.					
(a)	+++	+++	++	+	-
(b)	+++	+++	++	+	-
(c)	+++	+++	++	+	-
(d)	++++	+++	++	+	-
(e)	++++	+++	++	+	-

Note. A normal serum, tested im parallel, as control, showed no flocculation in any of the tubes.

Antigen

H - ion concentration.

Titration of optimum antigen using (a), heart-extract and absolute alcohol without adjustment of their reactions, (b), the same heart-extract and absolute alcohol with reaction of each adjusted to pH = 5.6 (approx).

The same cholesterol solution is used throughout.

SALINE. 0.85 per cent. NaCl solution. pH = 5.6 (approx).

SERUM. Syphilitic serum heated for half-an-hour at 56°C., immediately before use.

Readings made after 24 hours at 37°C.

Reaction :-	Unadjusted.		Adjusted.	
serum c.c.	0.05	0.1	0.05	0.1
Antigen.				
A 1.	±	+	-	+
A 2.	±	+	++	+++
A 3.	+	+	+++	++++
A 4.	++	+++	++++	++++
B 1.	+	+	+++	+++
B 2.	++	++	+++	+++
B 3.	+++	+++	+++	+++
B 4.	+++	+++	+++	++++
C 1.	+	+	+++	+++
C 2.	+++	+++	+++	+++
C 3.	+++	+++	+++	+++
C 4.	+++	++++	+++	++++

No flocculation occurs in any of the antigen controls, but NORMAL serum flocculates,(+) in the presence of the C4 antigen.

Antigen

H - ion concentration.

Titration of optimum antigen using (a), heart-extract and absolute alcohol without adjustment of their reactions (b), the same heart-extract and absolute alcohol with reaction of each adjusted to pH = 5.6 (approx).

The same cholesterol solution is used throughout.

SALINE. 0.85 per cent. NaCl solution. pH = 5.6 (approx).

SERUM. Syphilitic serum, heated for half-an-hour at 56°C., immediately before use.

Readings made after 24 hours at 37°C.

REACTION:-	UNADJUSTED.		ADJUSTED.	
serum c.c.	0.05	0.1	0.05	0.1
Antigen.				
A 1.	+	++	++	+++
A 2.	++	++	++++	++++
A 3.	+	+	++++	++++
A 4.	++	+++	++++	++++
B 1.	+	+	+++	+++
B 2.	+	+	++	++
B 3.	++	+	+	++
B 4.	++	++	++	+++
C 1.	+	±	++	+
C 2.	+	+	++	++
C 3.	++	+	++	++
C 4.	+++	++	+++	++++

No flocculation occurs in any of the antigen controls, but Normal serum flocculates (+) in the presence of the C 4 antigen.

Antigen.

H - ion CONCENTRATION.

SERA. Sera, whose Wassermann reactions are known, heated for half-an-hour at 55°C. immediately before use.

ANTIGEN. (a), stock cholesterolised heart-extract (A4).
(b), the same with its reaction adjusted to pH = 5.6 by the addition of 0.14 c.c. N/1 NaOH per 100 c.c.
These are diluted with saline (pH = 5.6 in the usual manner (see Appendix 1) for use in the tests.

TECHNIQUE. Appendix 9. Readings made after 20 hours at 37°C.

serum dilution:-	1 in	Antigen.	10	20	40	80	160
Wass.R.							
(1) positive		(a)	++	++	++	+	±
		(b)	+++	+++	++	+	±
(2) suspicious		(a)	±	-	-	-	-
		(b)	±	±	-	-	-
(3) weak positive		(a)	+++	+++	++	+	±
		(b)	+++	+++	++	+	±
(4) positive		(a)	±	±	±	-	-
		(b)	+	±	-	-	-
(5) negative		(a)	-	-	-	-	-
		(b)	-	-	-	-	-
(6) positive		(a)	++	++	+	±	-
		(b)	+++	++	+	±	-
(7) negative		(a)	-	-	-	-	-
		(b)	-	-	-	-	-

No flocculation occurs in either of the antigen controls.