I

INTRODUCTORY REMARKS TO DEMONSTRATION OF FLOCCULATION TESTS

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My appearance in these proceedings is due to the fact that the Council thought that a short account of the development of the flocculation tests would be interesting to members.

The complexity of the Wassermann test stimulated workers at a very early stage to discover a simpler method of demonstrating the interaction between syphilitic reagin and extract. In fact, almost directly after it had been shown by Marie and Levaditi and others, in 1907, that alcoholic extracts of solid organs would act as well in the W.R. as watery extracts of syphilitic liver, and that the reaction was one between syphilitic reagin and lipoids, workers turned to the demonstration of precipitins and of changes in the physical state as offering less complicated tests than the Wassermann.

Quite a large number of tests on these lines were evolved between 1908 and 1914, but they are now only of historic interest, and I will not detain you with them longer than is necessary to show their general nature.

Klausner, in 1908, demonstrated the formation of a precipitate on addition of distilled water to syphilitic serum. Elias, Neubauer, Porges and Salomon, in 1908, published a test in which a precipitate formed on mixture of syphilitic serum with glycocholate of soda. This idea was developed further in the Hermann-Perutz test (1912), in which syphilitic serum was mixed with an alcoholic solution of glycocholate of soda and cholesterol diluted with a watery solution of glycocholate of soda. The Hermann-Perutz test attained some popularity, but it proved less delicate than the Wassermann.

It was not hard to prove that a precipitate formed when syphilitic serum was mixed with any of a variety of extracts of solid organs or with certain chemicals such as glycocholate of soda. The difficulty was to make the precipitate easily visible and to arrange matters so that

* Based on an address delivered before the M.S.S.V.D. on March 27th, 1931.

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positive reactions occurred in a higher proportion of syphilitic cases than with the Wassermann test without overstepping the line of specificity.

Jacobsthal, in 1909, proposed to observe the precipitate in a mixture of syphilitic serum and diluted extract of syphilitic infant's liver under dark-ground illumination, but the method was discarded as interesting, doubtless, but not practicable in routine work.

Amongst other tests which might be mentioned was Weil's, which depended on the resistance of syphilitic blood cells to hæmolysis by cobra venene. Stone (1912) found that a 4 per cent. suspension of normal cells was dissolved by a 1/70,000 or 1/80,000 dilution of cobra venene while syphilitic cells required concentrations of 1/15,000 to 1/40,000. The cobra venene test was found by Kuschakoff not to be specific for syphilis.

Two tests, the epiphanin of Weichardt (1908) and the meiostagmine of Ascoli (1910), depended on changes in surface tension on addition of syphilitic serum to diluted extract.

This was approximately the stage which work on these lines had reached when the war began, and so far it cannot be said that any method had been evolved which approached the W.R. in reliability and uniformity. A study of present-day methods shows, however, that many pre-war workers were on right lines, and all that was required was an increased refinement of technique in the preparation of the extract and in the test conditions.

In 1917 Bruck and Hidaka sought to make the precipitate more visible by the addition of mastic, but their test did not gain any footing, and it was not until Meinicke, followed rapidly by Sachs and colleagues, entered the field that the flocculation tests began to be put on a practical footing.

Meinicke, commencing in 1917, produced in rapid succession three tests, of which only the third obtained any popularity. This, known as the D.M., or Dritte Meinicke, produced in 1919, depended on flocculation of a diluted extract of horse-heart by a syphilitic serum. Meantime, Sachs and Georgi had produced, in 1918, their well-known Sachs-Georgi reaction, which has attained a great popularity. It depends on flocculation (observed through an agglutinoscope) by syphilitic serum of a diluted extract of ox-heart suitably fortified with chole-

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sterol. This addition of cholesterol can confidently be said to have been the first practical solution of the great problem which had baffled previous workers of making the precipitate visible. You will remember that it was mentioned in connection with the Hermann-Perutz reaction, and may easily have been the reason for this method having appeared to have more possibilities than had its contemporaries. The exact way in which cholesterol works in the flocculation and the Wassermann reactions has been greatly debated. Time will not permit of my discussing the different views of its action, but at the end of these remarks I will sketch the explanation offered by Eagle, which appeals to me as the most rational.

The S.-G. reaction was modified and made far more precise by Dreyer and Ward, in 1921, in the Sigma test. Dreyer and Ward showed particularly that the physical state of the diluted extract is a highly important factor and that this physical state is dependent on the method of dilution. Meantime, Vernes, in Paris in 1920, had developed his method, which depends on an increase of turbidity produced by the addition of syphilitic serum to a specially prepared and suitably diluted horse-heart extract. The great feature of the Vernes test is the elimination of the personal factor. The dilution of the extract is done with machinery running at a precisely measured rate, and the reaction is measured by means of a photometer.

This was approximately the position in 1921, which is noteworthy as being the year in which the Health Section of the League of Nations began an investigation that has stimulated enormously improvements in syphilitic serum tests. In the latter end of 1920, or beginning of 1921, a member of the Office International d'Hygiene Publique, discussing the Brussels Agreement, expressed a generally felt wish that the Wassermann test could be standardised so that sera (for example, of merchant seamen) tested in different laboratories would give comparable results. When asked about it I expressed the view (knowing the nature of pathologists) that standardisation would be impracticable, but possibly something might be done by comparing on the same sera the different methods used in each country and then comparing similarly the best methods of different countries with one another. In this way I thought we might eventually be able to make a scale so that a clinician reading the report of a test of a

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patient's serum done in a strange laboratory could estimate with fair accuracy the sort of reaction his own pathologist would have obtained with it. I may say that this is the stage we are approaching in this country ; at any rate, I know from study of a report on a test carried out in one of the laboratories approved under the English V.D. scheme what result would have been reported by, say, Dr. Wyler in the Ministry's laboratory, Dr. Osmond at St. Thomas's, or Dr. Thomson at St. Paul's. But this is a digression.

I put forward my suggestion for comparison of tests on the lines I have sketched at the North European Red Cross Congress held in Copenhagen in May, 1921, and it was taken up keenly by Dr. Madsen, President of the Health Section of the League of Nations, quite possibly because he had been thinking on somewhat similar lines. At any rate, at an international conference organised by the Health Section of the League of Nations in London in November, 1921, a great comparison of serum tests was organised. The broad principle was that in each of a number of European laboratories one worker should test the same sera by (1) his own method of the Wassermann ; (2) the S.-G. method ; (3) the Sigma ; and (4) the Meinicke III. or D.M.

The subsequent history is interesting as showing how difficult it is to calculate when one is dealing with different personalities. At the November, 1921, conference I had made a great point of each worker, in carrying out any test in this comparison, following precisely the methods of its author, and had got a resolution passed to this effect. In accordance with this resolution, I arranged that Dr. Wyler, who was entrusted with the testing work in this country, should spend as many days as necessary practising the Sigma at Oxford, the S.-G. at Sachs's laboratory in Heidelberg, and the Meinicke in Meinicke's laboratory in Hagen. He did not leave any of these places until his technique was a precise copy of Dreyer and Ward's, Sachs's and Meinicke's respectively. I believe that Dr. Wyler was the only one of the different testers who complied exactly with the terms of the resolution to follow precisely the author's directions. At the end of a year the first results were presented to a conference in Paris, and they were most various and interesting. All but Dr. Wyler had modified the various tests entrusted

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to them, and the order of merit of the various tests varied considerably. Thus Wyler had found the Sigma the most delicate and yet specific of the four under comparison, but most of the others placed it much lower. It was obvious that comparison in different laboratories was not reliable, and in 1923 the workers engaged in the comparison, with some representatives of other European laboratories, were convened at Copenhagen to test the same approximately 530 sera by the methods mentioned. The results must have been a revelation to some workers, who had imagined their methods to be the last word in delicacy and specificity, but the broad effect was a considerable stimulus to improvement. It is interesting that at this conference the best of the Wassermann tests proved more delicate than the best of the flocculation methods, but the conditions under which the Sigma test was carried out were not precisely as prescribed by the author of the test. Otherwise I feel sure that, as had been found by Wyler in England, it would have proved more delicate than the Wassermann.

At the end of the conference Meinicke announced frankly that Meinicke III. was dead, and its place was taken by the Meinicke Trübungs Reaktion (M.T.R.), published in 1923, and also tested at this conference, where it proved approximately equal to the Sachs-Georgi. It depends on an increased turbidity produced by syphilitic serum in a diluted extract of horse-heart, fortified by balsam of tolu and benzoic acid.

The conference showed clearly to workers the variety of results which could be afforded by different workers with apparently the same test, such as the Sigma, when the workers varied the technique of the author, however slight the modifications might appear on occasional study. It showed why the four methods under comparison had afforded such different results in different laboratories, and justified my insistence at the first conference on the workers adhering strictly to the letter of the author's technique, as learnt in the author's laboratory.

The next conference on similar lines was in 1928, and showed great advances in the delicacy and reliability of the flocculation methods. Müller had published his Ballungs Reaktion in 1925, and Kahn's test, produced in 1922, had become very popular in U.S.A. These two tests proved at the 1928 conference to be the most

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delicate and yet specific, far in advance in these respects of the S.-G., the M.T.R. and the Vernes, as shown in this table :—

	448 Syphilitic Sera. Positive.	365 Controls. Positive.
Best Wassermann	190	0
Kahn	277	0
Müller (M.B.R.)	287	1
Meinicke (M.T.R.)	225	5
Vernes	171	2

To Kahn is due the credit of showing first the importance of keeping the reagents as concentrated as possible and of shaking. In the matter of concentration he has been copied by Sachs and Witebsky in a modification which they introduced in 1928, called the Citochol reaction, to distinguish it from the original S.-G. test, called the Lentochol. In the Citochol test the extract used for the ordinary S.-G.—the Lentochol—test is evaporated to dryness and then taken up with only three parts of alcohol, to which is added sufficient cholesterol. The concentrated cholesterol extract is diluted with only two parts of saline instead of five, as in the original. The concentration speeds up the reaction so that it can be read in half an hour instead of eighteen to twenty-four hours, as in the Lentochol method. The Lentochol and the Citochol compared with others as shown in these results obtained at the conference :—

	491 Syphilitic Sera. Positive.	425 Controls. Positive.
Best Wassermann	206	0
Kahn	299	0
Müller	311	1
M.T.R.	239	9
Sachs-Georgi original (Lentochol)	206	0

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	490 Syphilitic Sera.	421 Controls.
Best Wassermann	205	0
Kahn	298	0
Müller	310	1
M.T.R.	238	8
Sach-Witebsky's . . . (Citochol)	250	0

showing that the Lentochol was no more delicate than the Wassermann, but the Citochol, which, like the Kahn, affords results very quickly, was much more so, though not equal to the Kahn.

Amongst others, an interesting method shown at the 1928 conference was the Murata, which depends on the formation of a ring at the junction of serum and extract, the extract having been floated on the serum, as is urine on nitric acid in a test for albumen. It is probably the easiest of all the tests to carry out, but unfortunately does not seem to be sufficiently reliable.

You will see in the tables the great advances in delicacy which have been made in certain flocculation tests. The Wassermann shown in these tables was that which had proved the most delicate at the 1923 conference, when it was more delicate than any of the flocculation tests then compared.

The tables show also the value of testing the serum of a treated case by at least two methods, a Wassermann and a flocculation, since the Wassermann alone is apt to leave one in a fool's paradise.

The conference had two great effects: (1) Meinicke improved on his M.T.R. by producing the clearing reaction (M.K.R.), and (2) Müller, seeing that his test would attain no great popularity unless it was simplified and made to afford results more quickly, modified his M.B.R. to M.B.R. II., which gives results in two to three hours, while a second modification, M.B.R. II. K., in which the mixtures are centrifugalised after being in the water bath a few minutes, gives results in half to three hours.

In U.S.A., Kline first adapted the Kahn test to a slide

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precipitation test and then modified considerably the method of making the antigen.

Meinicke claims now that the M.K.R. is as delicate and specific as the Müller or the Kahn, and Kline that his test is as good as the Kahn, but judgment on these claims must be reserved until they have met at such a conference as those I have mentioned.

These conferences are terrible disillusionisers, as was found again at a third serum conference held in Monte Video last autumn, when the Kahn maintained its reputation, but some others, formerly greatly extolled, were found to be sadly wanting in reliability.

I have said enough to show the great service the Health Section of the League of Nations has rendered the control of syphilis in the matter of serum testing, and also to show how careful one must be in accepting reports on the value of a given test in comparison with others. One worker will place a number of methods in one order and the next will reverse it. It is only when the two are side by side testing the same sera that one discovers the explanation. It always lies in modifications of the author's technique. Again, the serum conferences have demonstrated most clearly the fallacy of a pathologist developing a test out of touch with the clinician. It is easy enough for the pathologist to sit up aloft and when a positive result is not in accord with the clinical data to say that probably the clinician is wrong in his diagnosis. Such a method of working may keep a pathologist in a fool's paradise until he takes his method to a conference where, alongside with other workers, he has to test several hundreds of unknown sera. Then his positive reactions with clinically non-syphilitic sera, standing out like blots on the landscape amongst the negatives obtained by most of the other workers, must be for him a painful sight.

I should like now to summarise very shortly an explanation of the serum reactions which has lately been put forward by Eagle.

According to Eagle, who supports his views with convincing evidence, the complement fixation method and the flocculation are only two ways of demonstrating the same phenomenon, which, shortly, is as follows. An alcoholic extract of heart when added to a watery solution becomes a colloidal suspension of lipoid particles, which are ultra-microscopic, or visible only under dark-

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ground illumination. If a non-syphilitic serum is added to this suspension, the particles become coated with protein, and the suspension has now the physical properties of protein particles, but the protein can be washed off, leaving the lipoid as before. On the other hand, if a syphilitic serum is added to the lipoid suspension, a combination occurs between the specific reagin contained in its globulin and the lipoids, and the suspension is now very different. The globulin cannot be washed off the lipoid, the particles are easily clumped by electrolytes (as in saline), and they have a great avidity for complement.

The *rôle* of cholesterol, which is used with most extracts, is this. When an alcoholic solution of lipoid and of cholesterol is added to a watery solution the lipoid coats the cholesterol particles, and the suspension behaves towards non-syphilitic and syphilitic serum respectively, just as does an ordinary lipoid suspension, but the lipoid particles, swelled by their cores of cholesterol, are bigger, *i.e.*, in a given dilution they are closer to one another, meet more easily, and therefore clump more easily; similarly they have a greater avidity for complement. Cholesterol is thus an agent which makes the phenomena of agglutinability and of avidity for complement which occur on addition of syphilitic serum to a lipoid suspension more easily detected.