WASSERMANN AND SIGMA TESTS

COMPARED IN 569 CASES.

By W. T. COLLIER, M.C., B.M. OXF.,
M.R.C.P. LOND.,

ASSISTANT PHYSICIAN TO RADCLIFFE INFIRMARY, OXFORD.

(From the Pathological Laboratory of Radcliffe Infirmary.)

In order to establish the accuracy of Dreyer and Ward's method of estimating the Wassermann substance in the blood (Sigma test)¹ any series of results comparing the new method with the older Wassermann test is of value.

Material.—The present series includes nearly all the samples of blood sent to this laboratory for the routine Wassermann test from July 28th, 1921, to Feb. 2nd, 1922; and is made up as follows:—

		Cases.
V.D.D.	{ 1. Treated syphilis	187
	{ 2. For diagnosis	89
Hospital and private patients undiagnosed		293
		569

The series includes a good many cases examined on more than one occasion.

Technique.¹—1. The Wassermann test was performed by a slight modification of method 4 in the Medical Research Committee's report on the Wassermann test. Each serum was tested both with three and with four times the M.H.D. of complement. The antigen was the same as that used in the Sigma test, except that no cholesterin has been added. The heart extract, prepared according to the method of Bordet and Roulens, has been evaporated to dryness before making up into a saline suspension. It is, perhaps, fair to point out that while we have obtained more reliable results since using this antigen, in that no case has given a positive result at variance with the clinical findings, the readings are possibly less delicate than those obtained with the more concentrated cholesterin-heart extracts generally in use.

2. The Sigma test was carried out in accordance with the technique laid down in the original description by Dreyer and Ward. The same pipette was used throughout the test in any one week, and the only variation of detail was the washing of the pipette in distilled water (twice) and saline (twice), instead of in alcohol and ether and drying in the bunsen flame.

Routine.—The Wassermann was first performed and the sera then placed in the water-bath at 52° to 55° C. for one and a half hours in preparation for the Sigma. For those sera which have given a positive or doubtful Wassermann, the Sigma test was carried to the full nine tubes; for the remainder, the first five tubes only were used.

Interpretation of Results.—(1) In the Wassermann, complete inhibition both with three and four M.H.D. of complement has been recorded as + + + +. + + + signifies 80 per cent., + + 50 per cent., + 20 per cent. and ± a trace of inhibition with three M.H.D. of complement.

+ + + +, + + +, and + + have been regarded as positive.
+ and ± have been regarded as doubtful.
— has been regarded as negative.

(2) In the Sigma, in accordance with Dreyer and Ward's interpretation, four units and over have been regarded as positive in an undiagnosed case, one unit and over as positive in a case of known treated syphilis, and one to four units as doubtful in an undiagnosed case. (See Table.)

Discussion of Results.

In our hands the Sigma test has proved slightly more reliable and more delicate than the Wassermann. Difficulty arises with those sera that give a reading of less than four units. As will be seen, a not inconsiderable proportion of the undiagnosed cases gives this small reading; and some of these were clinically

negative. This naturally throws doubt on the value of a similar small reading in the known syphilitic cases, where it is regarded as positive.

The most that can be said is that a reading of less than four units is suggestive of syphilis. In several cases such a small reading has been obtained on successive occasions, with a negative Wassermann, both in undiagnosed cases that were almost certainly

Results.

Total {	W + S + +	W - S -	W + S -	W - S + +	W - S +	Wd S + +	Wd S -	W + S +
All cases, 569 ..	109	327	2	14	56	27	31	3
Per cent.	19.2	57.3	0.4	2.5	9.8	4.8	5.5	0.5
Treated syphilis, 187 ..	55	63	2	9	29	18	11	0
Per cent.	29.4	33.7	1.1	4.8	15.5	9.6	5.9	0
Undiagnosed, 382 ..	54	264	0	5	27	9	20	3
Per cent.	14.1	69.1	0	1.5	7.0	2.3	5.2	0.8

S + + = more than 4 units and S + = 1-4 units.

W + = + + + +, + + +, or + +. W - = negative.

Wd = doubtful (+, ± or inhibition in control).

syphilitic (especially syphilis of the central nervous system), as well as in old treated cases of syphilis. On the other hand, cases that were clinically negative generally gave a completely negative Sigma at the second examination. With increasing experience of the test, these last have become relatively more infrequent. Finally, it should be noted that in the undiagnosed cases, no blood gave a positive Wassermann as against a negative Sigma. Of the five which gave a positive (S + +) Sigma and a negative Wassermann, four were clinically positive and one was doubtful.

Conclusions.

1. The Sigma test is slightly more reliable and more delicate than the Wassermann. It is also less complicated.

2. A reading of one to four Sigma units only must be regarded as doubtful. If obtained on more than one occasion, it is probably evidence of syphilis.

My thanks are due to Dr. A. G. Gibson, under whose general direction the work has been carried out, to Dr. H. K. Ward; and to Mr. A. Chown, who has been responsible for much of the work in connexion with the setting up of the tests.

Note.—Since writing the above, a further 387 Sigma tests have been carried out, in which the readings have been recorded both after 7 and after 24 hours' incubation. As suggested by Dr. Ward, the longer incubation is of great help in assessing the value of a Sigma reading of less than 4 units (after 7 hours' incubation), since a true positive will give a considerably increased degree of flocculation after the longer period. Of the 387 cases, 31 (8 per cent.) gave a reading of less than 4 units. Of these, 27, which were all clinically positive or from known treated cases of syphilis, gave such increased flocculation after the longer incubation. Four, which were all clinically doubtful, gave no such increased flocculation.

THE FORMALIN TEST FOR SYPHILIS:

SOME CONDITIONS CONTROLLING GEL FORMATION.

By A. G. HOLBOROW, F.I.C.,

PUBLIC ANALYST AND BACTERIOLOGIST, GIBRALTAR.

In view of the interest taken by pathologists and physicians in the so-called formalin test for syphilis it was thought desirable to look further into the test, and, if possible, to determine conditions which would make the test more reliable. Recalling the figures of Gate and Papacosta (originators of the formalin test) it is seen that working on 400 sera the Wassermann reaction (W.R.) and the formol-gel reactions accord in 85 per cent. of instances. Dr. Neave Kingsbury,

¹ THE LANCET, 1921, I., 956.

working on 261 sera, found that only 43 per cent. of sera giving a positive W.R. showed a positive formalin test, and that 9.5 per cent. of sera giving a negative W.R. gave a positive formalin. At the Gibraltar laboratories, working with 153 sera, I have found the following:—

No. of specimens.	W.R. ¹	Formalin test.
45	+	 +
19	+	 —
17	—	 +
72	—	 —

Irregularity of Behaviour of Formalin.

Reports from different laboratories are not consistent, testifying to the unreliability of the formalin test. The cause of this irregularity of behaviour of formalin to sera is a matter of much interest. Its importance lies in the fact that a wrong diagnosis may be made by using a test of the chemical and physical nature of which so little is known.

The following experiments were undertaken with the object of determining possible chemical conditions which might influence the test. When neutral formalin was added to white of egg (i.e., the more viscid part) a thick clot was formed by the following morning. The control remained unaffected. This result led to examining other media containing albumin and globulin. It was found that cow's blood serum and sheep's blood serum both set to a stiff gel in 18 hours after addition of formalin. The controls (without formalin) remained fluid indefinitely. The test was carried out in the original way by adding two drops of neutral formalin to 1 c.cm. of the serum. Three sheep's sera were tested, two of which were positive (stiff gel) to formalin in 18 hours. The third sheep's serum remained in a liquid condition for four days without forming a gel. It was suspected that the condition of this serum was not satisfactory for gel formation, and it was thought a gel could possibly be formed artificially. This proved to be so, as will be seen later. Using this same sheep's serum, and also human serum,² a series of tubes were put up as follows:—

1. Serum + formalin.
2. Serum made slightly acid with HCl + formalin.
3. Serum made slightly acid with acetic + formalin.
4. Serum made slightly more alkaline with {NaOH + formalin.
5. Serum made slightly more alkaline with lime-water + formalin.
6. Serum + 2 drops acetone (ketone).
7. Serum + 1 drop hydrogen peroxide + formalin.

Each separate tube was accompanied with a control containing no formalin, and the tubes examined in 18 hours. The results are as below:—

1. Liquid, no change.
2. Stiff gel formation.
3. " " "
4. Liquid, no change.
5. " " "
6. " " "
7. Liquid 18 hours—stiff gel formation 36 hours.

All controls remained fluid.

From these results it is seen that if the serum be made slightly acid to litmus a stiff gel will be formed with formalin, and no gel occurs if the alkalinity be increased. Increased alkalinity of a serum would appear to inhibit gel formation. Carrying this yet further, more of the serum (human and sheep's) was taken, and its alkalinity decreased with acetic acid; it was allowed to remain alkaline to litmus. The effect of formalin was to produce a soft gel in 20 hours and a stiff gel in 48 hours. The controls remained fluid. The formation of a gel in tube 7 would be due to the oxidation of the formaldehyde to formic acid by the hydrogen peroxide added. If these results are to be interpreted and an explanation given of the interchange of alkalis and acids in the body during life, a very complex metabolism is met, impossible of emulation in the laboratory.

Dual Nature of Acid-combining Power of Blood.

As the acid-combining power of the blood is of dual nature, due to (1) free or diffusible alkali salts, and (2) the combining power of the protein with acid, it is a matter of conjecture which is affected when acid is added in small quantities, insufficient to make the serum acid to litmus. It is known that in normal blood the degree of alkalinity fluctuates before and after a meal. In excessive exercise or deficient nitrogen in diet the alkalinity is decreased. In pathological conditions the deviation from normal in the same direction becomes marked, as in uræmia, anæmia, acidosis, &c. In conditions causing decreased alkalinity of the blood it is feasible that a sufficient decrease may be reached whereby a positive formalin test is obtained, for, as mentioned already, two sheep's sera showed positive formalin test, and the other did not.

This might explain why some human sera give a positive formalin test but negative W.R. Whatever the true explanation demands, it can be shown that weak acids like dihydrogen-sodium-phosphate (NaH_2PO_4) are effective in reducing the alkalinity of blood sufficiently to produce gel formation with formalin. This substance might well be an intermediate product in the formation of the disodium-hydrogen-phosphate found in blood, and more especially if the bases present should be insufficient to convert it.

Tube A. Human serum + NaH_2PO_4 (alkalinity decreased) + formalin.

Tube B. Human serum + NaH_2PO_4 (made neutral) + formalin.

Tube C. Human serum + NaH_2PO_4 (made acid slightly) + formalin.

Result.—Tube A, gel in 40 hours. B, gel in 30 hours. C, gel in 24 hours. Controls unaffected. Acid sodium phosphate took longer to act than was the case with stronger acids.

Deductions and Conclusion.

When serum forms a gel with formalin it is due to the direct action of the reagent upon serum albumin or serum globulin, or both, and not due to any action upon the toxins of *Sp. pallidum* direct. It is probable that acid-protein produces a gel with formalin and alkali-protein does not. If formalin forms a gel of human serum it is an indication of decreased alkalinity of that serum, but does not decide the cause of the decreased alkalinity. The evidence obtained from the foregoing experiments is, in my opinion, sufficiently adverse to regard the formalin test for syphilis with much reserve.

A NOTE ON LUMINAL.

By W. JOHNSON, M.D. LOND.,

ASSISTANT PHYSICIAN, ROYAL SOUTHERN HOSPITAL, LIVERPOOL,
AND ROYAL LIVERPOOL CHILDREN'S HOSPITAL.

SOME years have elapsed since luminal was first recommended as a drug useful in epilepsy.¹ The object of this note is to draw attention to its value and to recommend its use to a much greater extent than appears to be the case at the present time. My experience with luminal in the treatment of epileptics extends over the last two years. I began using it for those cases—so well-known in the out-patient department—where drugs had been used for several years and the patient has wandered from hospital to hospital without any improvement in the condition being obtained. The result proved so surprising that I commenced prescribing it and noting results in all varieties of "fits." The beneficial effect of the drug has seemed to be extraordinarily wide.

Pharmacologically, luminal is closely allied to veronal, one of the ethyl groups of this latter drug being replaced by a phenyl group; the full chemical name is phenyl-ethyl-barbituric acid. The dose given is 1½ to 5 gr. It is insoluble in water, but is easily suspended in a mucilaginous mixture, and in this form is readily taken without unpleasant effects.

¹ Method described by Colonel L. W. Harrison, D.S.O., R.A.M.C., as used at Rochester Row Military Hospital.

² Wassermann and formalin negative.

¹ Hauptmann: Münchener medizinische Wochenschrift, 1912.