

FURTHER OBSERVATIONS ON THE WASSERMANN TEST WITH PROLONGED FIXATION AT ICE-CHEST TEMPERATURE (WITH A NOTE ON TESTS WITH BORDET'S ANTIGEN).*

By Temp. Capt. E. J. WYLER, M.C., R.A.M.C., M.D. Lond.,
Pathologist, Military Hospital, Hilsea.

IN a previous contribution to this Journal (1921¹), I summarised my results in 719 Wassermann tests carried out with a technique devised by Griffith and Scott, embodying the use of a highly diluted antigen and prolonged fixation in the ice-box, contrasted with a test of the same sera in strict parallel by the routine method as performed at the Military Hospital, Rochester Row (1918²).

For the ice-box method, I used an antigen prepared as originally suggested by Griffith and Scott, and was able to show that the sensitiveness of the test, at any rate in treated cases, was greatly increased with their technique. But it had the disadvantage that occasional non-specific fixation was liable to occur. This defect would now appear to have been overcome.

In the present series of 405 tests, the antigen in the cold fixation tests was prepared in accordance with the results of their subsequent investigations which have since been published (1920³), and used in 1/640 dilution in saline. The essential principle of its preparation consists in removing the natural cholesterin, together with other substances which are soluble in acetone from the plain extract (Noguchi) and then adding cholesterin in the quantity found by standardisation to be appropriate. The 405 cases now tested comprise 151 treated, 43 untreated (30 primary, 10 secondary, 1 congenital, 2 latent), 151 non-syphilitic, and 60 controls (positive and negative).

The tests were again carried out in strict parallel, and, except for certain minor modifications, the cold fixation technique was as described by Griffith and Scott (*loc. cit.*). They were performed in groups ranging in number of sera from 4 to 34. Donald's drop technique was used throughout. In the case of the cooled tubes the average period of fixation in the ice-chest was twenty hours; the minimum period for any group of tests was eighteen hours; the maximum was twenty-two hours.

The following details of notation and technique are identical with

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those in my previous paper (*loc. cit.*), and are here repeated for the reader's convenience :—

- + = Complete inhibition in tube 1.
- ± = Partial inhibition in tube 1, complete lysis in tube 2.
- ⊥ = Slight inhibition in tube 1, complete lysis in tube 2.
- + ± = Complete inhibition in tube 1, partial inhibition in tube 2.
- + ⊥ = Complete inhibition in tube 1, slight inhibition in tube 2.
- ± + = Partial inhibition in tube 1, complete inhibition in tube 2.
- + + = Complete inhibition in tubes 1 and 2.
- = Complete lysis in tube 1.

and so on.

The first hieroglyphic shows the condition in tube 1 ; the second hieroglyphic shows the condition in tube 2. Where there is no second hieroglyphic this denotes complete lysis in tube 2.

The - sign has been used to indicate *complete* lysis only.

Tubes 1 and 2 contain in the routine test equal amounts of patient's serum, and one volume each of 3 M.H.D. and 5 M.H.D. respectively of complement. In the cold fixation test, tube 1 contains twice as much patient's serum as tube 2, while each tube contains one volume of 1/25 dilution of complement when this has been found by preliminary titration to be of average hæmolytic activity.

The following are abridged data of the intervals of time between the test and the last arsenical injection, and also of the amount of treatment which the cases had received :—

Interval between Date of Test and Last Arsenical Injection.

In 112 cases the interval was approximately 2 to 30 days.

" 9	"	"	"	2 months.
" 6	"	"	"	3 "
" 13	"	"	"	4 "
" 4	"	"	"	5 "
" 7	"	"	"	{ 7, 8, 10, 14, 15, 24, and 26 months.

Amount of Treatment.

89 cases received a minimum of 3·9 grms. "914"* + Hg.†

21 " " 3·75 grms. "914"* + Hg.†

41 " " less than 3·75 grms. "914"* + Hg.†

(Of the 41 cases, 4 had received only one injection each of 0·45 grm. "914.")

Results with 151 Sera of Treated Cases.

Same result by both methods :—

82 (54·3 per cent.) { 20 were + + by both methods.
62 were - " "

Less fixation by routine method, 66 (43·7 per cent.): 16 of these were completely negative by the routine method and + + by cold fixation; of the remaining 50, 29 were completely negative by the routine method and showed varying degrees of inhibition (less

* Or the equivalent amount of "606."

† Mercury in all cases was given by intramuscular injection.

than ++ by cold fixation, while 21 showed inhibition by the routine method, but of a less degree than by cold fixation.

Three cases showed less fixation by cold fixation than by routine. The results in these cases were:—

	Routine.	Cold Fixation.
(a)	\pm	—
(b)	$+\pm$	$\pm\pm$
(c)	$+\pm$	\pm

Case (a) had completed a total of 1.35 grms. "914" twelve days before the test. *Sp. pallida* had been found on admission to hospital.

Case (b) had been intermittently treated with, as nearly as could be ascertained, fourteen arsenical injections, the last about four months before the test. When tested one month later, after further treatment the result was \pm by routine, and $\pm\pm$ by cold fixation. Case (c) had received one injection of 0.45 grm. "914" four days before the test. *Sp. pallida* had been found on admission when the result of the parallel test had been \pm by routine and negative by cold fixation.

The following details of 8 cases are of particular interest. These had shown, after treatment, less fixation by routine than by cold fixation, and I was fortunate in being enabled to retest them by the routine method after a further interval:—

Case 1.—Routine test —, cold fixation test \pm . Had completed a course of 3.9 grms. "914" and 8 grs. Hg. one week previously. Sixteen weeks later routine test was ++.

Case 2.—Routine test —, cold fixation test —. Had completed a course of 3.9 grms. "914" and 3 grs. Hg. three days previously. Two weeks later routine test was ++.

Case 3.—Routine test —, cold fixation test ++. Three days previously had completed 2.55 grms. "914" and 6 grs. Hg. Fourteen weeks later routine test was ++, the patient having meanwhile completed a course of 3.75 grms. "914."

Case 4.—Routine test —, cold fixation test ++. Three days previously had completed 2.55 grms. "914" and 5 grs. Hg. Seven weeks later routine test was ++, the patient having meanwhile completed a course of 4.65 grms. "914."

Case 5.—Routine test —, cold fixation test $\pm\pm$. One week previously had completed 3.75 grms. "914" and 5 grs. Hg. Twelve weeks later routine test was ++.

Case 6.—Routine test \pm , cold fixation test ++. A case of latent syphilis. Had received six injections "914" (exact amount not ascertained) and 2 grs. Hg. four months earlier, and one week before the parallel test had completed 2.55 grms. "914" and 3 grs. Hg. One week later the routine test was ++.

Case 7.—Routine test —, cold fixation test $\pm\pm$. A case of latent syphilis. Had received four injections "606" (exact amount not ascertained) and 3 grs. Hg. fourteen months previously. Four weeks after the parallel test, the routine test was \pm .

Case 8.—Routine test —, cold fixation test ++. Ten days previously had completed 1.35 grms. "914" and 2 grs. Hg. Fourteen weeks later, without further treatment (the patient having been demobilised), the routine test was —.

The following case is also of particular interest. The clinical diagnosis was bulbar paralysis. The patient had received a course

of arsenical treatment (details not ascertained) six months before the parallel test, which was $\pm \pm$ by routine and $++$ by cold fixation. The routine test of the cerebro-spinal fluid was positive in all dilutions (10, 5, 2, and 1 times serum strength), the globulin test was positive, and there were 56 lymphocytes to the c.mm.

Results with 43 Sera of Untreated Cases of Syphilis.

(a) Primary. Thirty sera were tested. In 27 *Sp. pallida* was found. The supposed age of the sore ranged from two to thirty-five days. Eleven of the cases were negative by both methods. Ten showed complete or almost complete inhibition of lysis by both methods. One was negative by routine and gave almost complete inhibition by cold fixation. Two were negative by cold fixation but showed more or less inhibition by routine. Four showed partial inhibition by both methods. In two there was weak inhibition by routine and complete inhibition by cold fixation. It may be mentioned that both of these, after the first injection of "914," gave complete inhibition by routine ("provocative" effect). This also occurred with two out of the four cases that showed partial inhibition by both methods, and with one of the two cases that showed partial inhibition by routine and was negative by cold fixation.

Of the three cases in which *Sp. pallida* was not found, two gave complete, and one gave partial inhibition of lysis by both methods.

(b) Secondary.—Ten cases were tested. All gave complete inhibition of lysis by both methods.

(c) Tertiary.—None.

(d) Congenital.—One serum, $++$ by both methods.

(e) Latent.—Two sera, $++$ by both methods.

Results with 151 Sera of Non-syphilitic Cases.

The clinical diagnoses of these cases were as follows:—34 soft chancre, 1 eczema, 1 impetigo, 31 balanoposthitis, 8 papillomata, 3 scabies, 2 herpes, 2 psoriasis, 65 gonorrhœa, 1 tonsillitis, 1 urticaria, 2 epididymitis. Of these 151 cases, none yielded any trace of inhibition of lysis by either method.

CONCLUSIONS.

Treated Cases.—Consideration of the data given under this head indicates that the cold fixation method yields a much higher proportion of positive results than the routine method, but that very occasionally there may be less fixation by the cold method.

Untreated Cases.—From the data given it would appear that cold fixation is not appreciably more sensitive than the routine method of fixation, and that, in some cases, it is less so.

The results obtained in both types of case are closely similar to

those of Boyd (1921⁴) working on the same lines as in the present investigation with 150 sera, and to those obtained in my previous series of 719 tests (*loc. cit.*); but in contrast to this earlier series of cases non-specific fixation did not occur by the method as now applied. The difference in the results by the warm and cold methods in treated and in untreated cases (to which Boyd also draws attention) is striking, and offers a wide field for inquiry. Whilst in untreated cases the readings are very much alike in the two methods, in treated cases they are, in many instances, widely divergent. The 8 treated cases, details of which are set out above, which were retested by the routine method after an interval, are of particular significance and, as far as any conclusion can be drawn from so small a number, would suggest that in the cold fixation method we have a better guide to the control of treatment than in the warm method.

In the 30 sets of tests performed in the course of the present investigation, no undue fixation of complement by antigen was found on the second day of the test such as occurred with the earlier technique.

The disadvantages of the cold fixation method appear to be (i) that it cannot be completed in one day. But, as pointed out by Colonel Harrison in his Introduction (1920³), "It is compensated by the advantage that the important process of reading results is conducted in the morning, when the light is good and the observer fresh"; (ii) that considerable labour is involved in the preparation and standardisation of the antigen.

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NOTE ON BORDET AND RUELEN'S ANTIGEN.

This was tried, using in other respects the routine technique, in 88 of the above cases and in parallel with the ordinary routine and the cold fixation methods. The antigen, prepared according to Bordet's directions which render it as nearly as possible free from cholesterin, was used without added cholesterin and suspended in saline strictly according to his description (1919⁵). The number and variety of cases tested is, of course, too small to enable any definite conclusions to be drawn, but it seemed worth while briefly to set out the results.

The cases tested comprise 23 treated, 14 untreated primary, 2 untreated secondary, 29 non-syphilitic, and 20 controls (positive and negative).

Of the 23 treated cases the results agreed with the routine method

in 15, 9 being negative and 6 ++. The 6 positive cases were also ++ by cold fixation. Of the 9 negative cases, 5 were also negative by cold fixation, the remaining 4 showing varying degrees of inhibition (but less than ++).

In 7 treated cases there was less fixation with Bordet's antigen than by the routine method, 4 of these being clear negatives with the former. By cold fixation 6 out of these 7 cases were ++, the remaining case being \pm .

In 1 treated case the routine test was — while the result with Bordet's antigen was \pm . The cold fixation result was ++.

Of the 14 untreated primary cases in all of which *Sp. pallida* was found, the results in the 3 tests were identical in 10, 8 being negative and 2 ++. The remaining 4 were as follows:—

	Routine.	Routine using Bordet's Antigen.	Cold Fixation.
1.	—	—	++
2.	\pm	\pm	\pm
3.	++	—	++
4.	\pm	\pm	++

The 2 untreated secondary cases were ++ by the three tests.

„ 29 non-syphilitic „ — „ „
 „ 20 controls gave identical results „ „

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