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14 who gave no history of syphilis, but in 9 of these 14 cases the Wassermann or Kahn was doubtful. The 4 cases of syphilis consisted of three patients with primary syphilis (*S. pallida* +), and 1 with syphilis of some long standing; it is well known that the Wassermann and Kahn may often be equivocal in such conditions and it seems reasonable to suppose that this might also be so in this case.

Altogether the results suggest that, whereas this flocculation test does not yield false positives, it is rather more sensitive than the Wassermann test as performed in this laboratory.

SUMMARY

The results of the flocculation test of Lorenz for the diagnosis of syphilis on 510 cerebrospinal fluids and, with a modification of the method, on 1,160 samples of blood are described.

The test agrees almost exactly with the Wassermann, and no false positive result was obtained with either blood or fluid.

It is recommended as a parallel test to the Wassermann, or for use in laboratories where facilities do not at present exist for the performance of the Wassermann.

I wish to thank Doctors E. ff. Creed and R. D. Clay for performing a number of the Wassermann tests of the blood.

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V

OBSERVATIONS ON THE LIPOID REINFORCEMENT OF ANTIGENS IN THE GONOCOCCAL COMPLEMENT FIXATION TEST

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ALTHOUGH the serological diagnosis of gonococcal infections by means of a complement fixation test has

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been in use for more than thirty years diversity of opinion continues to be expressed as to the value of the method. It is dismissed, for example, by Kolle and Hetsch (1934) as "of no value for diagnosis, for the methods at present in use do not give sufficiently reliable results." On the other hand Price (1933) has obtained results which would place the test on much the same level as the Wassermann reaction as regards sensitivity and specificity, but Jacoby, Wishengrad, and Koopman (1938) report 13.4% of non-specific reactions with Price's technique and conclude that "the complement fixation test for gonorrhœa . . . has but a limited usefulness and is not reliable for diagnostic purposes."

Attempts to improve the results obtained have been chiefly directed towards enhancing the efficacy of the antigen. In this field there still remain differences of opinion as to the relative merits of simple saline suspensions of gonococci and extracts of the organisms derived in various ways; and as to whether or not it is advantageous or necessary to use multiple strains. Wolffenstein and Pieper (1931) introduced an antigen of a new type. This was intended primarily for use as a therapeutic vaccine. It is described as a "soluble gonotoxin" containing no killed or living gonococcal bodies, but only the "ecto- and endo-toxins," and is apparently the fluid obtained by centrifuging a broth culture (the composition of this medium is not stated) in which gonococci have been grown to exhaustion, *i.e.*, in effect a gonococcal "antivirus." This preparation was marketed in Germany under the name of "Compligon" and used as an antigen in gonococcal complement fixation tests. As such it has been the subject of favourable reports. (Retzlaff, 1932. Poehlmann, 1935.)

A further development derived from the work of Fischer and Gunsberger (1935) on the structure of Wassermann antigen, in the course of which they showed that much stronger reactions were obtained when a lipoid is added to a bacterial antigen, such as is used in the complement fixation tests for gonorrhœa and tuberculosis, and suggested the use of olive oil for this purpose. A similar procedure had previously been employed by Jacobsohn and Schwarz (1932) who added an alcoholic solution of cholesterin to an extract of gonococci and claimed that the method gave increased sensitivity and

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fewer reactions showing partial inhibition of lysis. Brandt (1935) recommended the substitution of castor oil for olive oil and described in detail a method of thus reinforcing "Compligon" for use in the gonococcal complement fixation test, stating that the sensitivity of the test was thereby greatly increased. Subsequent reports on the value of the method appear to be scanty. Poehlmann (1935) saw no advantage in the use of a mixture of alcoholic solution of olive oil and Compligon as recommended by Gunsberger. But Zellweger (1936), who tested Brandt's method on 1008 sera from 951 patients, reported greatly increased sensitivity, while the proportion of non-specific results in 679 sera from non-gonorrhoeal and syphilitic patients was 4.71% as against 3.7% with the unsensitised antigen.

This paper records some observations on Brandt's method. Unfortunately it has not been possible, for various reasons, to complete the investigation as originally planned nor to follow up certain points of interest which arose in the course of the work. Nevertheless the results are thought worthy of record with a view to directing attention to the method and its possible application to complement fixation tests in other bacterial infections, *e.g.*, cerebro-spinal meningitis, in which the prior use of the sulphonamides has created difficulties in laboratory diagnosis by the usual microscopical and cultural methods. (See Cruickshank, 1941.)

Compligon,* used as a simple antigen, is diluted with saline in the proportions stated on each bottle. The dilution is said to vary slightly with different batches, but in the samples used in this work has remained constantly at 1 in 5. The method of reinforcing Compligon by Brandt's technique is as follows: A 10% solution of castor oil in absolute alcohol is made up as stock. For use a 0.2% solution is prepared from this in 95% alcohol. This is termed "R." At room temperature 1.0 c.c. of Compligon is added to 1.0 c.c. of "R." This is allowed to "ripen" for 30 seconds, during which the mixture takes on a dense colloidal appearance with a faint yellow colour derived from the Compligon. Three c.c. of saline are now added and the reagent is ready for use after 2 minutes and remains active for one to two hours. Beyond this time a slight oily skin is formed and

* Supplied by Messrs. Griffin and Tatlock, Manchester.

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the activity of the preparation may become altered. The reagent thus prepared is intended for use with the complement fixation technique employed by Muller, the amount used in this test being three drops. If, however, the technique employed is one which calls for equal volumes of the various reagents then the antigen should be further diluted by adding 3 parts of saline to 5 parts of R-Compligon. Whatever the original antigen used, it must always be mixed with "R" in equal amounts, *i.e.*, any adjustment required to obtain a greater final dilution must be made by first preparing an appropriate dilution of the original antigen.

The complement fixation technique employed corresponded in essentials to that of the M.R.C. No. 1 Wassermann method (Report, 1929), using the larger amount of patient's serum recommended by Wyler (1932). Complement consisted of pooled guinea-pig serum preserved by the addition of boric acid and sodium acetate (Osborn, 1937; Green, 1938), 3 m.h.d. being used for the serum controls and tubes with 3 and 4 m.h.d. for the test proper. Fixation was allowed to proceed for 30 mins. at room temperature, followed by 30 mins. at 37° C. After adding sensitised cells the tubes were returned to the water bath and the results read as soon as lysis was complete in the negative control. Parallel tests were set up with (a) Compligon diluted 1 in 5 with saline, and (b) Compligon reinforced as described above. The results, differentiated simply as positive, doubtful, and negative are set out in Table I.

TABLE I—COMPLIGON

	Diluted with Saline				
		+	±	—	Total
Reinforced .	+	241	97	274	612
	±	2	14	140	156
	—	2	8	626	636
Total .		245	119	1,040	1,404

In order to ascertain the effect of reinforcement on antigens of different types two other small series of tests have been made. The antigens used were both commer-

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cial preparations, "A" being a lysate of gonococci obtained by treatment with NaOH, and "B" a simple saline suspension of multiple strains of the organism. For the test with the saline-diluted antigen "A" Thomson's method (M.R.C. Report, 1923) involving fixation in the ice-box overnight was followed, but for the other test the technique was that previously described. The results obtained are set out in Tables II and III.

TABLE II—ANTIGEN "A"

	Diluted with Saline				
		+	±	—	Total
Reinforced .	+	37	9	34	80
	±	3	8	22	33
	—	4	5	235	244
Total .		44	22	291	357

TABLE III—ANTIGEN "B"

	Diluted with Saline				
		+	±	—	Total
Reinforced .	+	43	9	37	89
	±	2	3	19	24
	—	1	8	201	210
Total .		46	20	257	323

It is clear from these results that, with each of the antigens used, the effect of the lipoid addition under the conditions described is to produce a marked increase of sensitivity, the extent of which is indicated in Table IV.

TABLE IV

Antigen	No. of Tests	% (and S.D.) of Positive Results :	
		Saline-diluted	Reinforced
Compligon .	1,404	17·4 ± 1·0	43·5 ± 1·3
"A" .	357	12·3 ± 1·7	22·4 ± 2·2
"B" .	323	14·2 ± 1·9	27·5 ± 2·5
Total .	2,084	16·0 ± 0·8	37·4 ± 1·1

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Apart from this increase of sensitivity it will be observed that sera were occasionally met with which gave a positive reaction with the saline-diluted antigen but a doubtful or negative result with the same antigen when reinforced. This phenomenon was also noted by Zellweger, who therefore recommended that simultaneous tests should always be made with both preparations. Table IV suggests that Compligon is both more sensitive, and more susceptible to reinforcement, than either of the other antigens used. As regards the former, however, it should be noted that three different groups of sera are concerned and the clinical material in each may not be comparable. The latter may be a real effect, possibly related to the physical state of the different antigen preparations. It may be added that while with most gonococcal antigens a rather troublesome difficulty is that they tend to exhibit from time to time considerable variation in their antigenic and anti-complementary properties, we have found Compligon to be remarkably uniform in both respects. It is also claimed (Retzlaff, 1932) to be very stable, showing little or no loss of antigenic activity even when kept for a period of twelve months or more.

It has not been possible to carry out, on all those patients whose sera gave divergent results with the two methods used, the careful clinical re-examination which would be necessary for an accurate assessment of the specificity of the modified test, but such observations as have been made suggest that the proportion of false positives is notably increased, and further, that such results are especially liable to occur with Wassermann positive sera. The latter is a point which will obviously require careful investigation, the more so as the question of the occurrence of such cross-reactions even when saline diluted antigens are used cannot be said to have been finally disposed of.

Oliver (1929) records 73 (12%) positive G.C.F.T. results among 608 Wassermann positive sera, but found no evidence of a falsely positive reaction among them. Thomson (1923) had reached a similar conclusion as regards 18.5% positive reactions among 89 syphilitics who denied gonorrhœa, and the opinion that the gonococcal complement fixation test is uninfluenced by Wassermann positivity is supported by a number of

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other authorities (Tulloch, 1929, Freudenthal *et al.* 1929, Freudenthal & Heymann, 1930, Osmond, 1938).

On the other hand, Engelhart and Summent (1930), who used a variety of antigens, consider that Wassermann positivity can influence the result of a gonococcal complement fixation test. Poehlmann (1935) believes that Wassermann positive sera not seldom give cross-reactions. He included such serum among his G.C.F.T. on each of 115 test days, and on 15 (13.6%) the Wassermann positive serum gave an apparently non-specific positive reaction. Retzlaff (1932) also records her experience that syphilitic, non-gonococcal sera have repeatedly given positive G.C.F.T. Both these latter workers were using Compligon, but they apparently do not regard such cross-reactions as being peculiar to this antigen. Rullens (1932) concluded that Wassermann positive syphilis influenced the G.C.F.T. in 18.6% of cases and that the greatest modification was in sera from late syphilis. Brandt (1936) is of opinion that strongly positive Wassermann sera have a tendency to give complement fixation with a diversity of antigens, and that accordingly a positive complement fixation test in gonorrhœa (or tuberculosis) is of unequivocal diagnostic value only if the Wassermann reaction is shown at the same time to be negative.

Using Thomson's method and antigen "A" gonococcal complement fixation tests have been carried out on strongly positive Wassermann sera from 141 patients. Twenty (14.1%) gave positive results, a figure which, it will be noted, does not differ materially from those obtained by Thomson and by Oliver. In no fewer than 13 of these however clinical re-examination failed to bring to light any history or furnish any evidence of gonococcal infection. Two of the patients were congenital syphilitics, aged 13 and 15 respectively. Similar tests were then carried out on a further 265 Wassermann positive sera, antigen "B" being used in 103 and Compligon in 162. The results closely paralleled those previously obtained. There were 23 positive reactions, but in 17 of these no present evidence of gonococcal infection could be obtained.

The assessment of specificity is a problem beset with difficulties. In the first place it is primarily dependent on clinical judgment, which in turn can only be arrived at by a consideration of the history, symptoms, and signs,

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any of which may be, and frequently are, of an equivocal nature, especially when they have to serve as the basis for a retrospective diagnosis. It is not without interest to note in this connexion that of the 73 positive reactions in Oliver's series the validity of the result in 30 cases is supported only by "a suspicious history or some physical sign suggestive, but not certainly diagnostic of, gonorrhoea." A further difficulty is that it is as yet undetermined whether, and for how long, antibodies may persist in the serum of patients in whom clinical cure has been effected. And finally, as Carpenter and Westphal (1940) have shown, it is possible for gonococcal infection to be present without there being any clinically demonstrable lesion: the absence of clinical evidence is not therefore in itself a proof of the non-specific nature of a positive serological test.

Such difficulties emphasise the empirical nature of this, as of many other, laboratory tests, and nothing seems more likely to contribute to their elucidation than a collective investigation on the same lines as the laboratory conferences previously organised for the comparative examination of methods in the serodiagnosis of syphilis. For the present we can only record that our experience, in which the clinical and laboratory investigations were carried out independently, leads us to be sceptical of the value of a positive G.C.F.T. in Wassermann positive sera, and especially so if reinforced antigens are used, since when the 265 sera referred to above were tested in this way 79 out of a total of 89 positive results were classified as non-specific so far as clinical assessment could determine.

This last finding is in contrast to that of Brandt, who states that with reinforced Compligon as used by him non-specific reactions with Wassermann positive sera are "relatively infrequent." Nevertheless he agrees that such reactions do occur and recommends that in carrying out the G.C.F.T. on Wassermann positive sera the Wassermann antibody should first be removed. For this purpose he makes use of a technique devised by d'Alessandro and Sofia (1935) whereby the Wassermann reagin is adsorbed on to a complex obtained by treating kaolin with heart extract which has previously been evaporated and the residue taken up with saline. We have found this procedure completely effective for the

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removal of Wassermann antibody, but only a few experiments have so far been made in the application of the method to Wassermann positive sera followed by a G.C.F.T. on the adsorbed serum. The results obtained, however, (assuming that treatment with kaolin-heart-extract does not, of itself, affect the G.C.F.T.), tend to support the clinical opinion that a large proportion of positive gonococcal complement fixation reactions obtained with reinforced antigens on Wassermann positive sera are of a non-specific character.

SUMMARY

1. Attention is directed to a method of increasing the sensitivity of the gonococcal complement fixation test by means of the reinforcement of antigens with a lipoid in the form of an alcoholic solution of castor oil, as recommended by Brandt.

2. Some results are recorded of the application of the method to three different types of antigen. With each of these preparations the modified test showed greatly increased sensitivity, but this appears to have been attained at least partly at the expense of specificity, especially so in the case of Wassermann positive sera.

3. The question of cross reactions between the Wassermann and gonococcal complement fixation tests is discussed and the results of some preliminary experiments presented. We are of opinion that this point requires much closer investigation, and suggest that with saline-diluted antigens a positive G.C.F.T. obtained with a Wassermann positive serum should be regarded with caution, and that no significance should be attached to a similar result obtained with a reinforced antigen unless the serum has first been rendered Wassermann negative.

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VI

PYRETO-THERAPY BY INTRAVENOUS T.A.B. VACCINE IN THE TREATMENT OF SULPHONAMIDE RESISTANT GONORRHŒA AND NON-GONOCOCCAL URETHRITIS*

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INTRODUCTION

THE purpose of this paper is to attempt an assessment of the value of T.A.B. vaccine injected intravenously in the treatment of cases of gonorrhœa and non-gonococcal urethritis which have failed to respond to various drugs of the sulphonamide group. At the same time schemes of dosage and methods of administration are reviewed in relation to the height and duration of temperature produced.

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