COMPLEMENT FIXATION IN TUBERCULOSIS: A CRITICAL SURVEY.

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EVER since Wassermann and Bruck demonstrated in 1906 the presence in the serum of tuberculous animals of substances capable of fixing complement in the presence of tubercle bacilli a vast amount of work has been done in an endeavour to make use of this fact to obtain a reliable biological test for tuberculosis in human beings.

The present authors have for the last two and a half years been investigating the reliability of a method of performing the complement fixation test for tuberculosis devised by one of us (A. L. P.) as a means of diagnosis and exclusion of pulmonary tuberculosis.

The results upon which we originally formed our opinion that "a positive result is an indication of an active tuberculous lesion in the body, and that a negative result is a reliable indication, with a few rare exceptions to which we shall refer later, of the absence of such lesion" are briefly as follows.

In all, the test was performed in approximately 1,000 pulmonary cases and controls. Of these some 160 were definite cases of pulmonary tuberculosis, the criterion for a definite case being the undoubted presence of tubercle bacilli in the sputum. All save 5 of these gave a positive result. Of the 5 that gave a negative result 3 subsequently gave a positive.

Some 140 presumably healthy people or patients suffering from non-tuberculous diseases were taken as negative controls. Three gave a positive result. Of these 3, in one definite evidence of enlarged presumably tuberculous glands in the neck was subsequently discovered, in another on more careful examination it was impossible definitely to exclude a tuberculous lesion in the lungs, while in the third no evidence of tuberculosis could be discovered. Of the remaining 700 cases some 220 gave a positive result and some 480 gave a negative. These were doubtful cases clinically, so that no definite conclusion could be drawn from them. All that could be said at the time without later investigation was that there seemed to be a reasonable correspondence between the clinical condition on the one hand and the negative or positive result on the other.

The subsequent history of 50 of the cases which were suspicious of pulmonary tuberculosis, but which gave a negative reaction, was then investigated. After the lapse of from ten to twenty months 49 of them showed no further evidence of tuberculous infection. The one exception was a man who was living for a year subsequent to his first examination with a brother suffering from consumption, and it is reasonable to suggest that he became infected while under observation. In the 49 cases the test was negative at the second as well as the first examination.

Finally, we investigated the subsequent histories of 50 cases in which the complement fixation test was positive but in which tubercle bacilli had never been found in the sputum, as far as we were able to determine, prior to the examination of the blood. These were unselected cases and were consecutive as far as the above conditions of a previous absence of tubercle bacilli in the sputum and a positive test were concerned.

Of the 50 cases a reply was received from relatives that 8 had died. In 3 tubercle bacilli had been found in the sputum. Of the remaining 42 cases, 29 attended for re-examination. Ten failed to reply, and 3 replied but were unable to attend. No less than 13 out of these 29 cases had been proved definitely tuberculous between the first and second examinations by the finding of tubercle bacilli in their sputa. An analysis of the 16 as yet unproved cases

showed that 5 were cases of pleurisy when first examined; in 3 no sputum was available; in 8 there were still symptoms and in some further physical signs were present. In 2 there were no symptoms at the second examination.

Since this work was done the test has been carried out in a large number of cases amounting in all to some 2,500. The results of these have fully confirmed our opinion as to the value of the test.

Since 1906 the amount of work done on this subject has been very large and the publications are very numerous. The results have been somewhat variable.

In scientific investigations of this description, as the number of workers increase and the publications multiply and conflicting views are expressed, there is perhaps a tendency for the salient points to be lost sight of and become obscured in a fog of side-issues.

The object of this paper is twofold: (1) By a study of the literature on the subject to determine what, if any, definite advance in knowledge has been made on the subject since 1906—that is to say, what points of agreement have been arrived at by the various workers, and to determine if possible the causes of their points of disagreement. (2) To determine whether the method which we have employed of performing the test might reasonably be expected in the light of ascertained facts to give the reliable results we have obtained.

VARIABILITY OF RESULTS DUE TO EMPLOYMENT OF DIFFERENT ANTIGENS.

There can, we think, be little doubt that the variability of the results obtained can be partially explained by the variety of the antigens used.

Miller¹ has classified the antigens used into four main groups:

1. Suspensions of whole or ground-up tubercle bacilli.
2. Antigens that may be classed as tuberculins.
3. Extracts of derivatives of tubercle bacilli.
4. Extracts of normal or tuberculous tissues.

Has any uniformity of opinion been arrived at as to the relative value of these four groups of antigens? We think we can say without fear of contradiction that antigens belonging to Group 4 are useless as antigens for this test. The relative value of the three other groups of antigens remains to be considered.

This article does not pretend to be a complete review of all the literature upon the subject, but merely aims at indicating the conclusions we have arrived at after a perusal of much of it, so that no attempt will be made to give anything like a complete list of all the workers and their results. We shall be content here to give our conclusions with reference to the work of others which appears to us to be illustrative of the bulk of the work in this field of investigation.

Group 1.

It would appear to us that generally the most reliable results have been obtained with antigens belonging to this group. It is antigen of this type—that is to say, a dilute emulsion of living tubercle bacilli—that we have used in our investigation. It is especially in relation to their specificity that antigens of this type appear to be superior.

Dudgeon, Meek, and Weir,² using an antigen belonging to this group, obtained 86 positive results out of 102 "definite cases of pulmonary tuberculosis." But of the 16 cases that gave a negative result, in 4 tubercle bacilli were said to have been present in the sputum two months previously, but were not present at the time the test was performed, and no symptoms or physical signs of pulmonary disease were present. One was a case of old tuberculous leprosy, one was diagnosed by a febrile reaction only. In 2 physical signs and symptoms of pulmonary disease were present, but no tubercle bacilli were present in the sputum. One was in a moribund condition. In 7 tubercle bacilli were present in the sputum. So that excluding the case that was moribund, only 7 could be said definitely to be suffering from pulmonary tuberculosis at the time the test was performed.

McIntosh and Fildes,³ using an emulsion of living tubercle bacilli as antigen, obtained 76 per cent. of positive

results in definite cases of pulmonary tuberculosis. Of 82 controls, all were negative save 3. Of these 3, 2 were cases of leprosy. (We might mention in parenthesis that we have also found cases of leprosy giving a positive result with tuberculous antigen; one was a case of Addison's disease and was therefore in all probability suffering from an active tuberculous lesion.) Miller,⁴ using an antigen made by grinding up tubercle bacilli with salt, obtained a very high percentage of positive results (96.8 per cent.) in cases with tubercle bacilli in the sputum, while of 243 Wassermann positive serums, 7 only gave fixation of complement with tuberculous antigens; of these a diagnosis of tuberculosis was subsequently established in 5 and could not be excluded in the remaining 2.

Moon,⁵ using Miller's antigen, found that 85 per cent. of cases diagnosed clinically as tuberculosis gave a positive result, although 38 out of 100 Wassermann positive serums gave a positive reaction with his tuberculous antigen.

Other workers using antigens of this group have obtained similar results.

Group 2.

The best known of these antigens is Besredka's antigen. He has obtained a high percentage of positive results in definite cases of tuberculosis. But many other workers, including Inman,⁶ Debains and Jupille,⁷ and Bronfenbrenner,⁸ have brought forward evidence to show that this antigen is not specific in that Wassermann positive serums may give a positive reaction when tested with this antigen. The work of the last named is of special interest.

It is well known that the complement-fixing substance in syphilitic serums is not an antibody specific to the spirochaete but a lipotropic substance, and this lipotropic substance may bind complement in the presence of lipoidal substances present in tubercle bacilli. Moreover, the addition of alcohol and other substances to tubercle bacilli may intensify this non-specific lipoidal action of tubercle bacilli.

Bronfenbrenner's experiments are complicated, but they appear to prove that the complement-binding substances present in tuberculous serums are specific antibodies to tubercle bacilli, and not lipotropic in character. In cases where a double infection with the *Spirochaeta pallida* and the tubercle bacillus was present he appears to have been able to demonstrate the presence in the serum of both the lipotropic substances due to the spirochaetal infection and also the specific antibodies to the tubercle bacillus.

In view of these facts it appears to us that a dilute suspension of tubercle bacilli in saline is undoubtedly the best antigen for this test. In such an antigen the lipoidal substances are not present in a sufficient amount to cause any fixing of complement in the presence of lipotropic substances, nor is there any alcohol or other substance added which would intensify this non-specific action. Another and very great advantage that it would appear to us that this antigen possesses lies in the fact that it is not to any appreciable extent anticomplementary. There can be very little doubt that many of the false positives that some workers have obtained have been obtained owing to their antigen giving rise to non-specific summation effects in virtue of slight anticomplementary properties.

Group 3.

Different workers have used various extracts and derivatives of tubercle bacilli as antigens. Calmette and Massol⁹ have used water and peptone soluble preparations. Corper¹⁰ used tubercle bacilli autolysed for ten days at incubation temperature in salt solution; Dudgeon, Meek, and Weir,¹¹ an alcoholic extract of tubercle bacilli; Wang and Crockett¹² tubercle bacilli heated with alcohol, ether, and chloroform; Wilson¹³ an alcohol and ether extract dried and suspended in saline.

The remarks that we have made with regard to Group 2 apply with equal force to this group.

CAUSES OF DIFFERENCES OBTAINED BY DIFFERENT WORKERS USING THE SAME ANTIGEN.

The varying results obtained by different workers using different antigens is no doubt due in part to the difference

of the antigen. The differences obtained by various workers using the same antigen do not at first sight seem easy to explain. But a little consideration will, we think, show certain possible contributory causes.

1. In the first place, and especially in regard to antigens of Groups 2 and 3, it appears to us that the description of the methods of their preparation are often wanting in details, and are frequently impossible of constant preparation, so that it is often impossible to be certain that one is using exactly the same antigen as that employed by the author whose technique one is endeavouring to follow.

2. In their publications many authors do not give a detailed description of the technique they have employed. They mention the antigen they have used, but they do not give in detail any quantitative data of the other reagents used in the test. Now Dean¹⁴ has pointed out that there is a definite favourable quantitative relationship between antigen, antibody, and complement. It is not surprising, therefore, that variable results have been obtained by various workers if the technique is not adhered to in every detail.

3. A third manner in which differences of opinion as to the exact reliability of the test may arise is through incomplete control of the cases on the clinical side and through the employment of incomplete and inaccurate classifications of the cases. In all our cases we have investigated, as far as lay in our power, most carefully the clinical aspect of the patients, and we feel that part of the success in the results we have obtained has been due to this very careful clinical control. As an instance of what we mean, Stoll and Newman,¹⁵ using Wilson's antigen, state that 58 per cent. only of cases of definite pulmonary tuberculosis gave a positive result. But among their cases of definite pulmonary tuberculosis they include a considerable number of cases without tubercle bacilli in their sputum. Now, for the purpose of controlling a test for pulmonary tuberculosis the only definite criterion for a case of phthisis should be the presence of tubercle bacilli in the sputum, for there is no combination of symptoms and physical signs which is pathognomonic of tuberculosis and which cannot be simulated by other diseases.

4. Finally, there is one other point in connexion with the method of performing the test we have adopted which is of importance in explaining the high degree of accuracy attained by this method. This relates to the amount of fixation of complement necessary for the reaction to be regarded as positive. Having, as we believe we have, in a dilute emulsion of tubercle bacilli an antigen possessing no non-specific complement fixing properties, we might reasonably expect to be able to detect relatively small amounts of specific antibodies. There can be no doubt that in some cases of active pulmonary tuberculosis the antibodies are present in the serum in small amounts only. We have found that any appreciable inhibition of haemolysis in the presence of one and a half H.D. of complement as estimated by titration in the presence of antigen should be regarded as a positive reaction.

There is one point upon which nearly all workers appear to agree. No test is likely to give 100 per cent. degree of accuracy in all cases. In our series some 2 to 3 per cent. of definite cases of pulmonary tuberculosis have given a negative result. It is the very acute and advanced case which may give a negative result. Many other workers have found also that moribund cases may react negatively.

CONCLUSION.

We have tried to suggest some of the possible causes of the uncertainty which up to the present time has existed as to the value of the complement fixation test in tuberculosis. In spite of much contradictory evidence we think there has been a steady improvement in the method employed and the results obtained. The work that we have done appears to be in line with the work that has gone before, to which it gives merely the finishing touch.

It would appear to us that the work above described gives clear proof of the great value of the complement fixation test in the diagnosis and exclusion of active or recently active pulmonary tuberculosis.

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COMPLEMENT FIXATION TESTS AND TUBERCULOSIS.

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THE present contribution continues some work published previously,¹ but some modifications of the technique originally used have been made.

For the present purpose two antigens were used.

Antigen Wang was an extract prepared after the method of Wang and Crockett,² and was practically the same as the one used for the former work. It was made from seven strains of tubercle bacilli grown on glycerine veal broth—three human (sputum, adults), three human (bone), one bovine (milk). It is essentially the residue of a mass of bacilli left after repeated alternate extraction with alcohol, ether, and chloroform.

Antigen Besredka was prepared from a culture of one human strain grown on Besredka's medium³ consisting of egg yolk and water for three weeks. The contents of the flask were sterilized in the autoclave and shaken as recommended by Besredka and a little phenol added.

The dilution in which each antigen could be used was found by a technique similar to that used in testing extracts for Wassermann tests. The dosage for routine work was determined by finding the lowest dilution which was devoid of anticomplementary action. Thus Wang could be used 1 in 40; Besredka, 1 in 20.

In the previous work most of the tests were made with a dose of complement determined for each set by a preliminary titration of the guinea-pig serum used, in the presence of antigen. In the present series this preliminary titration was omitted and the tests were made with a constant dose of 0.5 of 1 in 10 serum. This plan incurs the risk of missing some cases not capable of giving a strong reaction, but probably eliminates some fallacious reactions. The complement was obtained from the pooled serums in three or more healthy male guinea-pigs. Its minimum haemolytic dose was always at least 1 in 40 (0.5 of 1 in 40 in 2.5 fluid) and frequently higher. None of the antigens were used in such concentration as would fix 1 minimum haemolytic dose of whatever complement was in use for the day's work, in the absence of patients' serum. The patients' serums were inactivated by heating for thirty minutes at 56° C. Whenever possible they were examined soon after collection or were kept in a refrigerator and heated a second time for ten minutes on the day of examination. The serum dosage was 0.5 of 1 in 5. Most of the specimens of blood were taken from either the finger or the ear of the patient.

Cerebro-spinal Fluid.—These specimens were not heated but were kept in a refrigerator if they could not be tested when quite fresh. Some specimens had to be rejected because they were found to be anticomplementary. There

is no reason why cerebro-spinal fluid should not be heated, and probably heating would have saved some specimens which were found unsatisfactory.

The following scheme shows the routine method of performing the tests:

	Tube 1.	Tube 2.	Tube 3.
Serum dilution 1 in 5 or cerebro-spinal fluid undiluted	0.5	0.5	0
Complement 1 in 10...	0.5	0.5	0.5
Antigen dilution ...	0	0.5	0.5
Saline ...	0.5	0	0.5

Incubation one hour at 37° C. Sensitized corpuscles, 1 c.cm. added. Reincubation thirty minutes. The sensitized corpuscles were prepared by mixing a 5 per cent. suspension of washed sheep's corpuscles with an equal volume of diluted haemolytic rabbit-sheep serum of such concentration that at least 10 minimum haemolytic doses were present in each cubic centimetre of the mixture.

The results were recorded as positive, negative, or doubtful. A positive result was recorded when there was either no haemolysis in Tube 2 or only a trace, a negative when haemolysis was complete, and a doubtful when haemolysis was incomplete. In the various tables some of the doubtful reactions are included as positive, but none were regarded as positive in which there was not clear evidence that some fixation of complement had occurred. The method of reading tends towards the inclusion of some negative results as positive, but as the amount of complement used was large it appeared better to adopt this interpretation of the results.

Table I shows the results obtained with specimens of blood. The patients were under treatment in hospital and most of them were under observation for many months. They were all children aged 15½ to 6 years—twenty-six boys and thirty-one girls, suffering from some form of chronic tuberculosis of bones or joints or lymphatic glands (one case only of cervical adenitis) including spine, hip, ankle, knee, finger (one case only), tuberculous pyelitis (one case), without evidence of visceral lesions and none of them having tuberculin treatment. Each specimen was tested with two antigens.

TABLE I.—Blood; Tuberculosis of Bones and Joints: Children.

	Positive.	Negative.	Percentage of Positives.
Wang ...	27	30	40.7
Besredka ...	37	20	64.9

Some disagreements were noted on comparing the results given by the two antigens, but in every instance where Wang gave a positive Besredka was also positive. No Wassermann tests were made with these specimens.

Control Specimens.—Eight serums from cases of various conditions, including hydrocephalus, anaemia, rheumatic fever, synovitis, cerebral tumour, mastoid disease, and malnutrition, gave negative results with both antigens. Two serums with a strongly positive Wassermann also gave negative results. In four instances, all giving a negative reaction, the clinical diagnosis of tuberculosis was open to some doubt. One patient, F., aged 8 years, had pyelitis, but no tubercle bacilli were found in the urine; when 6 years old she had an abdominal operation and a tuberculous lymphatic gland was removed; there was no other evidence of tuberculosis. One patient (aged 1 year and 4 months) had a tuberculous dactylitis (M., aged 1 year and 4 months); tubercle bacilli were not found in scrapings. Another case (aged 7 years) was diagnosed clinically as abdominal tuberculosis, but not confirmed. The fourth case was diagnosed as tuberculous meningitis, but a definite tuberculous infection could not be established; the cerebro-spinal fluid gave a positive result. In all the other cases the diagnosis of tuberculosis of some form was not open to doubt.

Cerebro-spinal Fluid.—These specimens were from cases of meningitis or suspected meningitis, and in ten instances