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.

BY

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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

the clinical diagnosis was confirmed either by *post-mortem* examination or by finding tubercle bacilli by microscopic examination. All of them were more or less pathological fluids, but none showed more than a trace of turbidity. With two exceptions the patients were all children.

TABLE II.—*Cerebro-spinal Fluid: Tuberculous Meningitis.*

	Positive.	Negative.	No. of Specimens.	Percentage of Positives.
Wang	13	5	18	72.2
Besredka	5	12	17	29.4

This table does not enable a comparison to be made between the properties of the two antigens, as the specimens were not all tested with both. The results given with seventeen specimens tested with both are shown in Table III.

TABLE III.—*Cerebro-spinal Fluid: Two Antigens.*

	Positive.	Negative.	Percentage of Positives.
Wang	12	5	70
Besredka	6	11	35

In six instances Besredka gave negative and Wang positive results. In all cases where Besredka gave a positive Wang was also positive. Thus it appears as if Wang were the better of the two for testing cerebro-spinal fluid.

Cerebro-spinal Fluid Controls.—The cases for which these specimens were obtained included acute and chronic conditions with no evidence of tuberculosis, cerebro-spinal fever (1), meningo-encephalitis of doubtful nature (1), encephalitis lethargica (1), septic throat with meningeal symptoms (1), cerebral tumour (1), uraemia (1), meningocoele (1), hydrocephalus (3), and some other conditions of doubtful nature.

TABLE IV.—*Cerebro-spinal Fluid: Controls.*

	Positive.	Negative.	Total.
Wang	7	13	20
Besredka	2	14	16

Sixteen specimens were tested with both antigens, and of these there were three in which Wang was positive and Besredka negative. In every instance where Besredka was positive, Wang was also positive. Apparently Wang is liable to give more fallacious reactions than Besredka.

REMARKS.

Complement fixation tests in tuberculous constitutions have been the subject of much study and many workers have found the test to be of high diagnostic value. In concluding my previous communication I said I was not satisfied that complement fixation tests afford a reliable means of clinical diagnosis in tuberculous cases, and I remain of the same opinion. The reaction is a specific one in the sense that under certain conditions the mixture serum *plus* extract will fix more complement than either of two alone, but it seems clear that the property of fixing complement in the presence of an antigen derived from tubercle bacilli is shared by serums from cases other than cases of tuberculosis. A positive result does not necessarily mean a definite tuberculous infection. Probably the number of such false diagnostic reactions is not large, but the fact that they occur must not be ignored. One of the chief aims of the present work was to see whether Besredka was better than Wang, and the results are encouraging. The results with control specimens of cerebro-spinal fluid were disappointing.

Prognostic Value of the Test.

Through the kindness of Mr. Telford, some observations were made on the prognostic value of the test. The patients

were children aged 6 to 15 years, inmates of the Swinton School for Crippled Children under treatment for various forms of chronic tuberculosis of spine, hip, and knee. They were under close observation for long periods, and it was possible in twenty-nine instances to make two tests with two antigens on each occasion. The specimens of blood were collected by the matron of the hospital, Miss M. Draper. The first series of tests were made between November, 1921, and February, 1922, and the second during the latter part of 1922. In July, 1922, and March, 1923, Mr. Telford made a special clinical examination of the children and stated in each instance whether the progress of the case had been good or bad. In giving his opinion he was guided by such clinical evidence as seemed of importance in the light of his long experience of such cases. There was usually something definite, such as abscess formation or loss of weight, to help him, but in some instances it was difficult for him to make a decision. The terms "good" and "bad," therefore, must be interpreted liberally—that in his opinion as surgeon the progress had been more or less satisfactory. The results given by the two antigens were not always in agreement. Every patient gave at least one positive reaction with one or other of the antigens on either the first or second examination. The following table shows the results obtained with each antigen, the number of positive and negative reactions, and the number of cases in which the progress in the period intervening between the collection of the sample of blood and the time of clinical review had been good or bad.

	First Series.				Second Series.			
	Positive Reaction.		Negative Reaction.		Positive Reaction.		Negative Reaction.	
	No.	Progress.	No.	Progress.	No.	Progress.	No.	Progress.
Wang ...	9	Bad 1 Good 8 (88%)	20	Bad 6 Good 14 (70%)	21	Bad 5 Good 19 (20%)	5	Bad 0 Good 5
Besredka	18	Bad 2 Good 16 (88%)	11	Bad 5 Good 6 (54%)	19	Bad 5 Good 14 (26%)	10	Bad 0 Good 10

Apparently a positive reaction in a case of active tuberculosis is more favourable as regards prognosis than a negative, but where there are no signs of active tuberculosis a negative result points to a healed tuberculosis. A negative reaction followed by a positive indicates that improvement is setting in. These points are shown in the following diagram, which shows the number of instances where the reaction was unchanged or changed. The figures above the short horizontal line are "progress good" and below it "progress bad." The figures to the left relate to antigen Wang and to the right to antigen Besredka.

Positive Unchanged.	Positive to Negative.	Negative Unchanged.	Negative to Positive.
6 9 — — 0 0	3 9 — — 0 0	2 1 — — 0 0	13 6 — — 4 4

The number in the various categories are small and it is unsafe to draw conclusions from them. The three cases included in "negative unchanged" were very slow chronic cases, and on first clinical examination in July, 1922, were progressing badly. They subsequently did better and were probably healed at the time of the second test.

On the whole it cannot be maintained that the indications furnished in this work as regards prognosis could have been of any great assistance to the clinician.

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