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DIAGNOSIS OF TUBERCULOSIS

BY THE

COMPLEMENT FIXATION METHOD.

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DIAGNOSIS OF TUBERCULOSIS

BY

FIXATION OF THE COMPLEMENT METHOD.

I. INTRODUCTION.

A. THEORY. To understand thoroughly the principles of sero-diagnosis the elemental theories of Immunity must first be grasped.

Under normal conditions when bacteria invade a host, substances are produced in the serum of the host which effect dissolution of the invading organism. The infecting bacteria are classified as ANTIGENS, and the protective substances formed in the host are known generically as IMMUNE BODIES.

These immune bodies are complex, and fall into many groups:- opsonins, agglutinins, and precipitins which prepare the bacteria for the action of more complex bodies, bacteriolysins, and also for phagocytosis by the white blood cells. Other immune bodies are also produced to neutralise the poisons derived from the bacteria, such have the nature of Antitoxins. The above is the briefest of sketches of the HUMORAL THEORY OF IMMUNITY now generally accepted.

If serum containing immune bodies is taken and heated to 55°C and then mixed with a suitable dose of the antigen, with proper technique the action of the opsonins, agglutinins, and precipitins can be demonstrated, but the bacteriolysins shew no action until fresh unheated serum is added, when they will effect lysis or dissolution of the bacteria.

There is then in the serum another body, thermo-labile in character, which must be present before bacteriolysis is possible. This body which has the nature of a ferment is known as the COMPLEMENT or ALEXIN, and when present permits the bacteriolysins or BACTERIOLYTIC AMBOCEPTORS to effect lysis/

lysis of the antigen, but by doing so it becomes "used up", or fixed.

Similarly, after injection of blood corpuscles from an animal of a different species, immune bodies are formed in the host's blood which will cause lysis of the blood cells. These have the nature of amboceptors, acting only in the presence of free complement, and are known as Haemolysins.

HAEMOLYSIS is the separation of haemoglobin from the cells, the opaque blood solution thereby becoming transparent. In vitro, lysis of blood cells can be effected by the addition of any fluid not isotonic with blood corpuscles, but in bacteriology the term indicates the conversion of blood solution into a transparent colour in an isotonic medium, through the action of a specific amboceptor. As with bacteriolysins, so with haemolysins, if the serum be heated to 55°C and the complement destroyed - a process of INACTIVATION - and then added to the antigenic red blood cells, no haemolysis will occur. The Red cells however are now said to be SENSITISED, as lysis will occur whenever free complement is added.

Bordet and Gengou availed themselves of this property of sensitised blood cells, in the sero-diagnosis of Typhoid Fever. They took serum from a patient suffering from Enteric Fever, which contained both immune bodies and complement, (as the latter had not been driven off) and added to it a suitable dose of antigen, an extract of typhoid bacilli, and incubated it. After a suitable period of incubation sensitised blood cells were added:- No haemolysis/

haemolysis however occurred as the complement had become FIXED to the antigen and amboceptor.

Repeating this experiment with healthy serum they obtained haemolysis, as the complement could not be deviated owing to the absence of a bacteriolytic amboceptor.

As the amount of complement present in an individual's serum is very inconstant, now-a-days it is customary to inactivate all sera to be tested, and then add a definite quantity of complement of pre-ascertained strength.

Pursuing these lines, WASSERMANN employed the principles of sero-diagnosis for syphilitic sera.

He employed as his antigen an alcoholic extract of the liver of a syphilitic foetus - in which organ spirochaetes are very numerous. His results were most startling and his test has been of invaluable service in revealing the pathogenicity of formerly obscure clininal conditions, which of later years have been proved to be of syphilitic origin. It should here be pointed out that a "positive Wassermann reaction," only indicates the presence of an immune body to the spirochaete in the serum tested. Such an immune body is not normally present in healthy serum, but is only formed after infection by the spirochaete, and as its formation, like the others of Nature's defences, requires time, so will a positive reaction only be obtained after a definite period from infection.

Of more recent years a considerable amount of work has been done in endeavouring to employ the fixation of the complement method as a diagnostic means for almost every form/

form of infective disease e.g. Tuberculosis; Influenza; Malaria; Yaws; Hydatid Cysts; and diseases caused by intestinal parasites, nematodes etc. and many others.

"Marmorek, who a short time ago, startled not only the medical world, but the laity as well, with the announcement that he had found a new diagnostic for Tuberculosis, did nothing more than apply the method of Complement Fixation."

B. OPINIONS OF PATHOLOGISTS AND CLINICIANS.

Regarding the value of the results hitherto obtained from Complement Fixation work in Tuberculosis, there is a considerable lack of agreement in the views expressed by pathologists and clinicians of standard repute. This is without doubt, in part due to the want of uniformity in the technique of the test, in part to the difficulty in recognising the protean forms of Tuberculosis, and in part to the fact that it is not sufficiently understood that a positive reaction merely indicates the presence of an immune body to the Tubercle Bacillus in the blood serum, and does not necessarily indicate that an active tuberculous lesion is present, though in this case it certainly does presuppose that the individual has at some time suffered from some form of Tuberculosis - generalised or local - which however might quite conceivably have been overlooked or incorrectly diagnosed. Holding these points in view, it is not difficult to appreciate the want of uniformity in the views of men who have written on the subject; a few of whom it would not be out of place to quote - In discussing the/

the question of the presence of Immune bodies in Tuberculosis - MUIR and RITCHIE , write the "Evidence for the existence of these in Tuberculosis has been sought for by applying the method of Complement Fixation , e.g. the serum of Tuberculous animals being mixed with Tuberculin and the mixture is tested for its capacity of absorbing complement.

Following this line Wassermann and others have found evidence of the presence of an Antituberculin in tuberculous foci and this is taken as the occurrence of a vital reaction against the poisons of an invading organism - generally speaking such an Anti-tuberculin is absent from the blood serum of most tuberculous patients - It may be present in the serum of patients subjected to repeated Tuberculin injections ."

In the same connection Dr. A. BESSON says , "The presence of Immune Bodies is very inconstant in the serum of persons suffering from Tuberculosis. The method of Complement Fixation is therefore not applicable to the diagnosis of Tuberculosis ." This view he has derived from papers on the subject by Widal and Le Sourd; Camus and Pagniez; Wassermann and others .

As opposed to these views , Wolf-Eisner referring to the Diagnostic significance of Complement Fixation in Tuberculosis says "Wassermann explained that the reaction of Complement Fixation in Tuberculosis was of no practical importance since the biologic methods fulfilled all that was required of them . Nevertheless the method of Complement Fixation is of Scientific interest in Tuberculosis/

Tuberculosis researches, and its results afford us valuable hints in regard to the findings obtained by the biologic method of Tuberculin diagnosis. Wassermann and Bruck used Tuberculin as an Antigen, and assumed in consequence, that substances entering into combination with Tuberculin could be present only in persons who had been treated for a considerable period with Tuberculin injections. His experiments confirmed their view."

"Wolff-Eisner was the first to assume that all derivatives of Tuberculin were of the same nature and had a similar effect. Consequently a great many investigators found Tuberculin antibodies in the serum of Tuberculous patients, who were, to be sure, under the influence of substances reabsorbed from their own Tuberculous foci, but who had never been treated with Tuberculin."

"These statements differ in detail, probably owing to a difference in technique and the Antigen applied. As a rule the antibodies are found more frequently in advanced than in initial cases, although there have been instances in which it has been shown beyond a doubt that positive fixations were present in healed or inactive Tuberculosis"

In his same able monograph on Sero-diagnosis, from which the above paragraphs are culled, Wolff-Eisner points out that "it must be remembered that Complement Fixation methods give positive results, only where the reaction substances are present in the serum in comparatively large quantities. "This explains why the reaction often fails in positive syphilitic cases, especially in ititial cases/

cases where the reactive substances in the serum are not yet sufficiently abundant, or in old cases where they are inadequate. Only by assuming that this method fails in the demonstration of small amounts of reactive products, can we explain Wassermann's first contention that reactive bodies were present only in tuberculous patients who had been treated with Tuberculin, a statement which seemed to contradict absolutely the unity of the Tubercle Bacilli toxins now proved by Wolf-Eisner to exist."

"However it soon appeared that Complement Fixation methods yielded results indentical as a whole with vital antigen results. - Calmette's and Von Pirquet's reactions - the only difference being that small amounts of reactive substances were not demonstrable by the former.
"Concurrent investigations of various authors proved the fact, no longer disputed even by Wassermann, that positive Complement Fixation may be found, not only in individuals treated with Tuberculin, but also in those in whom large amounts of Tubercle Bacilli products have been reabsorbed, and in whom a greater production of reactive bodies might be expected in evidence of a reaction of the organism to the stimulus."

HAMMER working along these lines shews himself in sympathy with the view of undoubted reliability and diagnostic value of Complement Fixation work in Tuberculosis. He employed as Antigen, varying quantities of Tuberculin (Koch's O.T.) and an Alcohol-acetone extract of Tuberculous granulations/

granulations from the capsule of a diseased knee-joint. Out of 46 known cases of Tuberculosis, 45 sera gave a positive result, and the one which failed to react he notes was the serum of a possibly cured patient. The above then are views of pathologists on this subject, and it would not now be out of place to mention the opinions of a few clinicians, as it is only by the close co-operation of the diagnostician at the bed-side, and the bacteriologist in the laboratory that a definite advance can be made into the undiscovered realms of sero-diagnosis.

In their "Clinical System of Tuberculosis" BANDELIER and ROEPKE write "Attemps have been made to employ the method of fixation of the complement for the early diagnosis of Tuberculosis. The question whether this is possible under certain conditions must be answered in the negative. According to our experiments the serum of clinically non-tuberculous persons can give the same Complement Fixation results as the serum of tuberculous people. Often too, as with the agglutination test, the results of an old contest between the organism and the bacillus may suffice to produce the reaction.

LANDIS expresses his view that while the test is positive in a large proportion of cases, it is, at present, not of much practical service.

CROCKET and WANG working in collaboration, and employing an antigen of their own preparation, got excellent results from a series of 360 cases. They tested sera of known normal and known tuberculous individuals, and their readings from the test were with few exceptions uniformly good.

They considered that not only was the test of value in the/

the case of known tuberculous subjects but also it threw light on cases shewing no tubercle bacilli in the sputum, and in cases where the clinical signs were inconclusive.

C. PURPORT OF ARTICLE.

In view of the conflicting evidence of the aforementioned authors, our endeavour in this paper will be to allign the results obtained in a series of 365 tested sera with the now generally recognised principles of immunity and sero-diagnosis, and to suggest explanations which may reconcile apparently contradictory results hitherto obtained. The sera investigated have been taken from subjects suffering from Pulmonary tuberculosis at all stages of the disease, from "latent tuberculous" individuals, from healthy people - adults and children, and in a few cases from patients with tuberculosis of glands, bones, joints and skin.

II. THE TEST.

A. REQUIREMENTS FOR THE TEST.

In order to examine for the presence of immune bodies in the human sera, by means of the complement.fixation method, the following are essential.

(1) Inactivated serum to be tested.

(2) Healthy serum containing Complement.

(3) An Antigen of a suitable nature.

(4) Suspension of Sensitised Red Cells. This Haemolytic system is employed as an index for the presence of Free Complement after (1), (2), (3) have been incubated together.

In addition the investigator will require in his laboratory.

(1) A water bath or an incubator thermo-regulated to 37°C.

(2) A water bath as inactivator. This must be set at a mean temperature of 56°C.

(3) Glass Tubes. ½" x 3" and racks for same.

(4) Pipettes graduated 10 c.c.; 1 c.c.; and .1 c.c.

(5) Physiological Saline sterile and freshly made.

If the drop method of Donald be employed, suitable droppers are also necessary. These must be carefully tested to ascertain that the delivery is correct.

(1) SERUM TO BE TESTED. The patient's serum is obtained by puncturing a vein - under surgically sterile conditions - and withdrawing 5 to 10 c.c. of blood. This is received in a sterile glass tube, free from grease and all chemical reagents, and allowed to coagulate, and where necessary

the/

the clot should be separated from the edge of the tube. When the serum has separated it is now pipetted into a sterile serum tube which is then plugged and kept until required for the test. It may here be mentioned that careful labelling is a sine qua non, to avoid subsequent confusion. When several sera are to be separated from their respective clots, it is quite obvious that the pipette must be washed after removing each serum.

Should it be necessary (for any reason) to obtain the serum without delay, the blood should be centrifuged immediately it has coagulated, and the supernatant serum then removed.

When dealing with a very nervous patient, or one too anaemic or too ill to justify vein puncture, sufficient serum may be obtained for one test, by employing a Wright's Capillary Capsule, after having previously sterilised and incised the lobe of the patient's ear. This method of obtaining blood however is unsatisfactory for general work.

On the day of the test, the sera must be <u>inactivated</u> (deprived of Complement), by placing the tubes in a water-bath at 55° to 56° C. for at least 10 minutes. L. W. Harrison recommends 10 minutes inactivation, as he says that prolongedheating deprives the serum of some of its reacting powers. Many authors however heat the sera for 20 to 30 minutes. Crocket and Wang recommended a period of inactivation of 2 hours in the case of tuberculous sera. Personally, although following their advice for a considerable time, we later rejected it, as equally good results/

results were obtained after only 15 minutes heating. Dr. Crocket, after a series of tests conducted with myself, now agrees with this view.

The ANTICOMPLEMENTARY POWER of a serum is its power for absorbing complement even in the absence of any other reagent. (e.g. Antigen). Concerning this feature, although the sera may have been previously inactivated it is necessary to repeat the heating on the day of the test, as, has been pointed out by Browning and Mackenzie, sera, which have been kept for several days developed additional anticomplementary powers which however are for the most part removed by a second heating. Our own investigations on the anticomplementary powers of sera have led us to concur with this. The results of these investigations we shall give later when dealing with the standardisation of reagents.

As regards any undue HAEMOLYTIC POWER of inactivated human sera for an emulsion of sheep's cells - personally we have not observed this phenomena in a single case.

The method of PRESERVING sera for re-examination has been to plug the serum tubes with non-absorbant gauze, and keep them in an ice-chest until required. If asepsis has been maintained such sera will keep for weeks without becoming unduly anticomplementary in action. It is ideal to keep the sera frozen until required, and if so kept their anticomplementary action is no greater than when freshly employed. Should the sera have become contaminated, a scum of bacterial growth appears, unless the sera be frozen/

frozen, and their anticomplementary action is so marked as to interfere with the test.

(2) COMPLEMENT. The serum of guinea-pigs has been employed throughout for complement. To obtain this a guinea-pig is stunned by a blow on the nape of the neck, and while the heart is still beating the throat is cut, and the blood collected by means of a funnel into a large test-tube.

After coagulation, the clot is freed from the sides of the tube and set aside till the serum separates. Harrison employs complement 4 to 8 hours old for his tests. Browning and Mackenzie however do not use it till after 18 hours. We have employed it after 4, 6, 8 and 16 hours, and now never use it until 18 hours old, as the results obtained with such complement have been more permanent than when fresher complement is employed.

Fresh complement appears to be unduly sensitive, and furthermore as it rapidly DETERIORATES at room temperature during the first few hours, if employed too fresh the minimal haemolytic dose (see later) obtained in the titration of the complement will be insufficient by the time the test is set up. Our method then has been to kill the guinea-pig on the night previous to the day of the test, the latter usually taking place in the afternoon.

If the complement be not clear when required for use it should be pipetted off and centrifuged for a few minutes, when the red cells will be driven down. Regarding the HAEMOLYTIC POWER of guinea-pig serum for sheep's cells, we have/

have in no case observed haemolysis of the latter, unless they be previously treated with haemolytic serum.

As guinea-pigs are most prone to both bovine and human types of tuberculous infection, a POST MORTEM EXAMINATION should invariably be held to negative the possibility of using unsuitable serum. Should any lesions be observed, the serum must be discarded. On one occasion although no gross evidence of tuberculous disease was noted, the complement gave positive readings throughout the whole batch of tests, for all sera, even the known normals. This guinea-pig had 3 months previously been inoculated with a sub-lethal dose of an emulsion of tubercle bacilli for experimental purposes, and it is probable that the guinea-pig had developed sufficient immune substance in its blood to combat the infection before any rapid advance of the disease occurred, and that it was these immune bodies which caused the positive readings in the final test in the presence of the tubercle antigen.

The DURABILITY of complement at room temperature as has been pointed out is relatively short. By the end of 48 hours complement is no longer suitable, as too large doses of the guinea-pig serum must be used and the very largeness of the dose of serum is in itself somewhat anticomplementary. There is only one METHOD OF PRESERVING complement and that is by freezing it solid. If merely kept in the ice-chest, the complement is lost. Harrison advises that the serum be pipetted into small capsules, these to be placed in a test-tube, which is capped and immersed/

immersed in a freezing mixture within a good vacuum flask, which is kept within an ice-chest. The capsules should be examined daily and the freezing mixture renewed as necessary.

Only on one occasion have we had sufficient complement to keep for a second batch of tests, and following his instructions we found it had preserved its powers excellently, when used a week later. Frequently for experimental purposes we have kept small quantities of surplus guinea-pig serum in the ice-chest, but never have we found it of use after 48 hours except when frozen solid.

3. ANTIGENS. Inasmuch as the patients, whose sera we have tested, come from almost every walk of life, and as many frequently shew evidence of specific infection, it has been our custom to examine for the Wassermann reaction, as well as for the presence of immune substances to the tubercle bacillus.

(a) SYPHILITIC ANTIGEN. The antigen we have used for the Wassermann test has been that employed by McIntosh and Fildes. The reason for this, is, that this part of the test with us is merely incidental - so to speak - and performed to guide us in the treatment of the patients, and not in the nature of a research. Moreover in the hands of expert investigators, who have tested thousands of sera it has been found more satisfactory than any other antigen, and will in all probability become the standard antigen employed. The antigen consists of a heart-cholesterin extract which is prepared as follows: The fat of the heart having been cut away, the muscular portion/

portion of the left ventricle is minced and ground for one minute with Absolute Alcohol (1 gramme of Heart to 9 c.c. of Alcohol) in a mortar with clean sand.

The mixture is shaken in a mechanical shaker for $1\frac{1}{2}$ hours, and then filtered. The filtrate only is used and should be kept in a dark glass bottle. Our extract was prepared from a guinea-pig's heart as advised by Walker & Swift, in place of the human heart extract first used by McIntosh and Fildes.

A 1% solution of Cholesterin (obtained from Baird & Tatlock) is made in Absolute Alcohol and kept in a separate bottle.

When the extract is required for the test 3 parts of Heart Extract are added to 2 parts of Cholesterin solution, this is diluted with 70 parts of physiological saline: i.e. the antigen employed is a 1 in 15 dilution of Heart-Cholesterin extract.

The results obtained fromthe use of this antigen have been trustworthy, repetition with fresh sera in positive syphilities has given identical results.

(b) TUBERCLE ANTIGEN. Before discussing the various antigens which we have tried, a few considerations of the necessary qualifications of a good antigen might be mentioned. The ideal antigen should in itself not be anticomplementary to any degree to interfere with the test; it should give positive readings with Tuberculous sera/i.e. those in which immune substances to the tubercle bacilli are present; and negative readings when these are absent. It is obvious/

obvious that a weak antigen may fail to give positive readings where the amount of immune substances is relatively small, and that too strong an antigen may conceivably give positive readings with normal sera. The CRITERIA we have observed have been that the antigen is not anticomplementary, that it gives positive readings with sera of undoubted cases of active tuberculosis, that it gives negatives with known normal sera EVEN WHEN DOUBLE THE USUAL DOSE OF ANTIGEN is employed, by this means a weak positive reaction leaves little doubt as to the class to which the tested serum belongs.

Along these lines many Antigens have been tested.
(i) CROCKET AND WANG employed an emulsion of tubercle bacilli after their fatty substances had been removed. They took a fresh growth of tubercle bacilli on Veal broth, transferred it to a sterile bottle and dehydrated with Absolute Alcohol, after repeated changings, the Alcohol was replaced by Ether in the proportion of 100 c.c. to 10 grammes of the wet bacilli. The bottle was then shaken in a mechanical shaker for $2\frac{1}{2}$ hours, and then its contents centrifuged. The residue was treated with Chloroform in the same amount as Ether and shaken as before. Three volumes of Ether were then added, and the bottle shaken for another two hours, after which Centrifugalisation was again carried out. Alternately this process was repeated with Ether and Chloroform until 5 extractions had been completed.. The dry residue was then stored in the dark.

A fluid antigen was made up by grinding 100 mgm. of residue in 3 c.c. saline in a mechanical mill over night.

The/

The fluid was then drawn off, and the mask and balls washed with repeated changes of saline. The washings were pooled and saline added to 100 c.c. Phenol .5% was added as a preservative, and the antigen then stored in the dark.

This antigen they employed for a series of 360 cases, and claimed good results. We tried the same antigen in varying doses but failed to get lasting readings - not infrequently all the tests would shew lysis after half-an-hour. Owing to the instability of the results this antigen was no longer employed by us.

(ii.) HAMMER'S ANTIGEN. Hammer employed a mixture of Koch's old Tuberculin, along with an Alcohol extract, and an Ether extract of tuberculous granulations from the knee. He made his mixture in a proportion of 1 part of Tuberculin to 10 parts of extract, and employed various doses. He reports on 46 cases only, but his results were good. Owing in part to our inability to obtain suitable granulations, and in part to our having obtained good results from various tuberculins alone we did not pursue this line of research.

(iii.) Our investigations have been concerning the relative antigenic values of exotoxic and endotoxic tuberculins of standard strength prepared in laboratories of reputed fame; to wit the Tuberculins of Burroughs Wellcome & Co., of Duncan Flockhart, of Lucius & Büning, and of Denys in Belgium.

The following is a list of the various undiluted tuberculins whose Antigenic properties we have titrated with known normal and known tuberculous sera:-

(a)/

Tuberculin.	Antigenic Value.

(a) Burroughs Wellcome's.

Old Tuberculin (T.) Human..... Fairly good.
" " (P.T.) Bovine.. Weak.
Tuberculin Filtrate (TOA) Human.. Fairly good.
" " (P.T.O) Bovine....Fairly good.
New Tuberculin (W.) Human..... Fairly good.
" " (B.E.) Human.....good.
" " (P.B.E.) Bovine..... good.

(b) Lucius & Brüning Ltd.

Koch's Old Tuberculin (O.T.) Human..... good.
" " " (P.T.) Bovine.... good.
New Albumose free Tuberculin (T.A.F.) Human... good.

(c) Duncan Flockhart's.

New Tuberculin. (B.E.) Human..... Fairly good.
" " (P.B.E.) Bovine.. Fairly good.

(d) Deny's.

Tuberculin Filtrate..... Not suitable.

Excellent results were obtained from all tuberculins manufactured by Lucius & Brüning, and from the new Tuberculins of Burroughs Wellcome. We now regularly employ as our tubercle antigen a MIXTURE OF BURROUGHS WELLCOME, P.B.E. and B.E. TUBERCULINS for three reasons, firstly, their relative cheapness; secondly, their guaranteed standardisation; and thirdly, that the results obtained have been in accordance with the necessary qualifications of a good antigen. The reason for mixing the human and bovine types is the theoretical one, that generally speaking, tuberculous lesions in children are due to the bovine type of bacillus, while with adults the affections are usually caused by the human type. In practice we have not infrequently found that a stronger positive reading was obtained with sera from tuberculous children when the P. B. E. element was introduced into the antigen.

We are in full agreement with the theory originally extended by Wolf-Eisner that "All derivatives of

Tuberculin/

Tuberculin were of the same nature and had a similar effect," but there is little doubt that the nearer the antigen resembles the causative type of organism, the sharper will be the results derived from the use of that antigen. This would explain why notwithstanding that positive results are obtained from the sera of tuberculous children with the B.E. Antigen yet stronger results are gained by employing the P.B.E. tuberculin.

The EXOTOXIC Tuberculins - i.e. the Filtrates - are suitable as Antigens if employed in sufficiently large doses, generally about ten times the dose of the endotoxic tuberculins. Deny's Filtrate is an exception to this, the results when employing it as antigen, were uniformly poor and unstable. Under the heading of exotoxic tuberculins must also be classified the Old Tuberculins (O.T.) and (P.T.). The Antigenic properties of these is mid-way between those of the Filtrates and the new Tuberculins. Those manufactured by Lucius & Brüning however are more potent than the same tuberculins of Burroughs Wellcome.

Regarding the ENDOTOXIC tuberculins, one or two features are noteworthy. The New Tuberculin (W.) is lipoid free and water-soluble, in other words it is practically equivalent to the Antigen of Crocket and Wang. As it contains less bacillary substance, than the Bacillary emulsions (P.B.E. and B.E.) it was not surprising to find that it was not quite such a strong antigen. Although good it is too expensive/

expensive for use on a large scale.

When employing tuberculins as antigen the question naturally arises as to whether the sera of patients who are undergoing a course of tuberculin therapy would not necessarily give strong positive results irrespective of the activity of their conditions.

This must be answered in the negative. Equally varying reactions are obtained from sera of patients both who have, and who never have undergone such treatment. In this connection it may be pointed out that all actively tuberculous individuals are constantly inoculating themselves with the tuberculin from their own foci. This point will be fully dealt with later on.

(4) THE HAEMOLYTIC SYSTEM. This is a suspension of red blood cells sensitised by the addition of the inactivated haemolytic serum from an animal which has been immunised against the same type of red cells.

In all our tests we have employed a suspension of Sheep's cells, while the haemolytic serum used has been from rabbits immunised to sheep's cells. For the most part the haemolytic amboceptor used has been serum prepared by Burroughs Wellcome which we have found quite satisfactory. Our technique for the preparation of the red-cell suspension has been along the usual lines. SHEEP'S BLOOD is obtained from the slaughterhouse in a sterile bottle containing pieces of broken glass.

The blood is immediately shaken vigorously to ensure defibrination. Tubes are then filled with the whipped blood and centrifuged at a high speed. When the cells have been well driven down the supernatant serum is removed and replaced with/

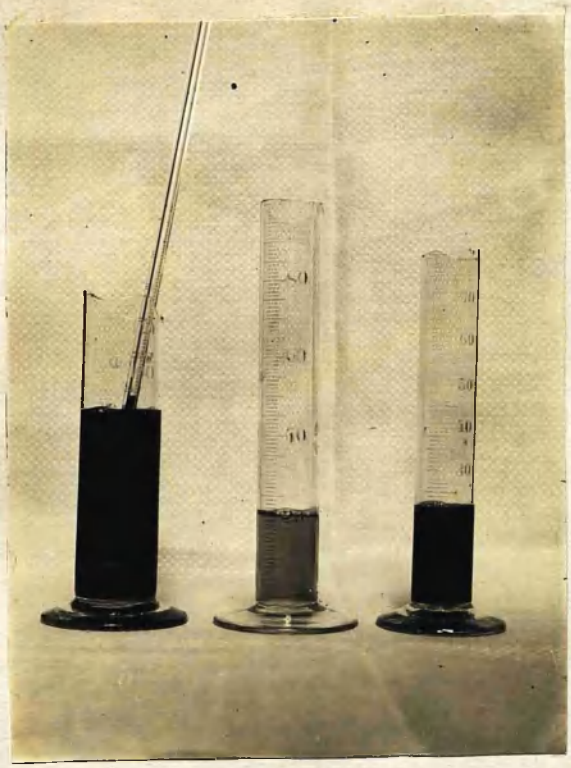
with 0.8 per cent saline. The tubes are shaken and again centrifuged. This operation is repeated four or five times. Finally the supernatant saline is removed and a 5 per cent suspension of the washed cells is made in physiological saline - By the addition of an equal quantity of a suitable dilution of haemolytic serum in saline, this suspension is converted into a $2\frac{1}{2}$ per cent. suspension (which strength we have been in the habit of using.)

The HAEMOLYTIC SERUM can be readily obtained by injecting rabbits with washed sterile sheep's cells either intravenously, intraperitoneally, or subcutaneously. Small quantities of blood are from time to time removed from the rabbit's ear, and the serum titred, until a serum of adequate potency is obtained, when the animal is bled to death. The serum is collected under full aseptic precautions, inactivated, and stored in an ice-chest until required for use. It should be retitred every three months.

As we have not been in the habit of preparing our own haemolytic serum, I have purposely avoided enlarging on this subject in this paper.

Defibrinated sheep's blood tends to undergo AUTOLYSIS after 48 to 72 hours even if kept in the cold. If for any reason it is necessary to keep the red cells for work on a subsequent day, a better plan, with us, has been to make a 5 per cent suspension in physiological saline without however adding any haemolytic serum. On the day of the test this suspension is well shaken up, and made into the $2\frac{1}{2}\%$ suspension of sensitised blood cells as previously described.

THE HAEMOLYTIC SYSTEM.



On the extreme right is a 5 o/o suspension of washed Red Blood Cells.

In the middle is an equal bulk of Haemolytic Serum, so diluted that each dose of the resultant mixture of red cell suspension and immune serum will contain 8 M.H.D. of immune body (to Red blood cells)

The left tube contains the "Sensitised Red Cells" — 2½ o/o red cell suspension in physiological saline containing haemolytic serum 8 M.H.D. to each dose (·5 c.c.)

B. STANDARDISATION OF REAGENTS.

In complement fixation work it is necessary to bear in mind that serum alone, antigen alone, and antigen together with "healthy" serum will all deviate a certain amount of complement.

The crux of the test is that the "diseased" serum in combination with its specific antigen will deviate complement in considerable excess to any of the above. The reagents employed then must so be standardised that a "diseased" serum does not give a negative result, nor a "healthy" serum a positive result owing to the presence of the reagents in such relative excess as to be anticomplementary of themselves.

Before the test proper is set up, each reagent is standardised or titrated to avoid inconsistency in the work.

(1) TITRATION OF HAEMOLYTIC AMBOCEPTOR. (Immune body to sheep's red cells). A dilution of 1 in 1000 of haemolytic serum is made in sterile physiological saline. A suspension

of red cells is then made of the same strength as that to be employed in the test, with us this is 2½%. A row of small test tubes is then set up, and into each is placed .5 c.c. red cell suspension, and in rotation .05 c.c., .1 c.c., .15 c.c. .2 c.c., .25 c.c., and so on to .9 c.c. of the diluted Immune body. Saline is now added to make the bulk up to 1.9 c.c. in all tubes. The tubes are incubated for 15 minutes at 37°C. and then into each is dropped .1 c.c. of a 1-in-10 dilution of complement. After carefully shaking the tubes and incubating them again for 15-30 minutes, the tube containing the smallest amount of immune body which shows complete haemolysis is noted. The dilution of haemolytic serum in this tube represents the minimal haemolytic dose (M.H.D.) of the titrated serum for the specific quantity of red cells employed; here .5 c.c. of a 2½% suspension.

It is necessary to ensure that the bulk of the reagents in all titrations is the same as that used in the test, as the titre is affected by concentration. In our work the final bulk has always been 2 c.c.

Tubes.	1.	2.	3.	4.	5.	6.	7.	8.
R.B.C. 2½%	.5.	.5.	.5.	.5.	.5.	.5	.5	.5.
I.B. 1 in 1000.	.05	.1.	.15	.2.	.25.	.3.	.35.	.4.
Saline (to 2c.c)	1.35	1.3.	1.25.	1.2	1.15	1.1	1.05.	1.
Complement 1 in 10.	.1.	.1	.1	.1	.1	.1	.1	.1.
Readings.	N.H.	N.H.	N.H.	N.H.	P.H.	C.H.	C.H.	C.H.

In the above diagram .3 of 1/1000 dilution would represent I.M.H.D. of Immune body.

It/

It is our custom to set up in addition three CONTROL tubes as in diagram.

TUBES.	1.	2.	3.
R.B.C. 2½%	.5	.5	.5
I.B. 1 in 1000.	.9	-	-
Saline - to 2 c.c.	.5	1.4	1.5.
C. 1 in 10.	.1	.1	-
Readings.	N.H.	N.H.	N.H.

Should tube 3 show lysis, the red cells suspension must be discarded as useless, this however is extremely unlikely to occur.

Should Tube 2 then lysis it indicates the presence of a haemolytic character in the complement which must be discarded.

Haemolysis in tube 1 would suggest two possibilities, either the presence of complement in the red cells, unlikely if they have been properly washed, or the presence of complement in the haemolytic serum. In this case the latter should be inactivated and the test repeated.

Inasmuch as haemolytic Amboceptor is very stable, this titration need only be carried out once every 3 months or whenever a fresh supply of the serum is being used.

Having obtained the M.H.D. of haemolytic serum, the next step is to prepare the Sensitised red cells. Now as 1 M.H.D. of Immune body does not produce complete haemolysis in the presence of only 1 M.H.D. of Complement, for in the above/

above titrations, an excess of complement has been used, and furthermore as minute quantities of substances causing haemolysis with sheep's cells are present in human sera, it is advisable to sensitise the sheep's cells with an excess of immune body in the first place, so that later the addition of human serum will not cause any obvious interference with the test. For this reason various authors employ from $2\frac{1}{2}$ to 10 M.H.D. of immune body to each dose of sensitised red cells. We have habitually employed 8 M.H.D.

Assuming that 25 sera are to be tested, that 4 tests are set up for each serum, and that a margin of 40 doses will be allowed for subsequent titrations, then 140 doses of sensitised cells will be required, each dose to contain 8 M.H.D. of Immune body.

The bulk of each volume of sensitised cells we fix at .5 c.c. therefore 70 c.c. will be required.

Supposing 1 M.H.D. of Immune Body to be .3 c.c. of 1 in 1000 dilution, then the necessary quantity will be .3 x 8 x 140 ÷ 1000 c.c. i.e. .236 c.c. of Immune Body. Physiological saline up to 35 c.c. is added to this amount.
35 c.c. of a 5% suspension of red cells is made, and the two are now well mixed, and incubated for 15 minutes at 37°C.

2. TITRATION OF COMPLEMENT. Complement being unstable must be titrated on the day of the test, and furthermore with each guinea-pig employed, as the amount of complement present in any serum varies to a considerable degree. A series of tubes are set up as before, each containing 1 volume .5 c.c. of sensitised red cells,

Complement in varying amounts is added, and lastly saline/

saline to make the bulk the same as that of the test. The tubes are incubated for 15-30 minutes and then the tube containing the smallest amount of Complement which will allow of complete haemolysis is noted. This amount represents 1 M.H.D. of Complement.

Tube.	1.	2.	3.	4.	5.	6.
R.B.C. + I.B.	.5.	.5	.5	.5	.5	.5
∅. 1/30.	.05.	.1	.15	.2	.25.	.3
Saline to 2 c.c.	1.45	1.4.	1.35.	1.3.	1.25.	1.2.
Readings.	N.H.	N.H.	C.H.	C.H.	C.H.	C.H.

Our method has been to dilute with saline the complement 1 in 30, and then add varying quantities of this dilution to the tubes. The most common reading for complete lysis to be obtained was in the tube containing .15 of 1 in 30 dilution.

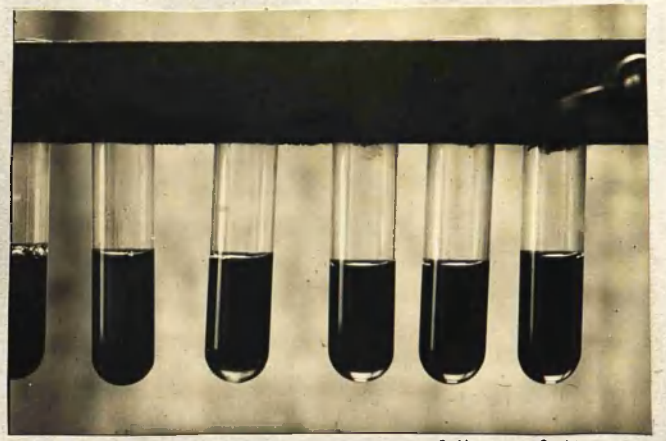
Now as it has been our technique so to dilute the complement for the test proper that .1 c.c. always represents 1 M.H.D. of complement, in the above case it would be necessary to dilute the neat complement containing serum in the proportion of 1 in 20.

as .15 ÷ 30. = .1 ÷ 20.

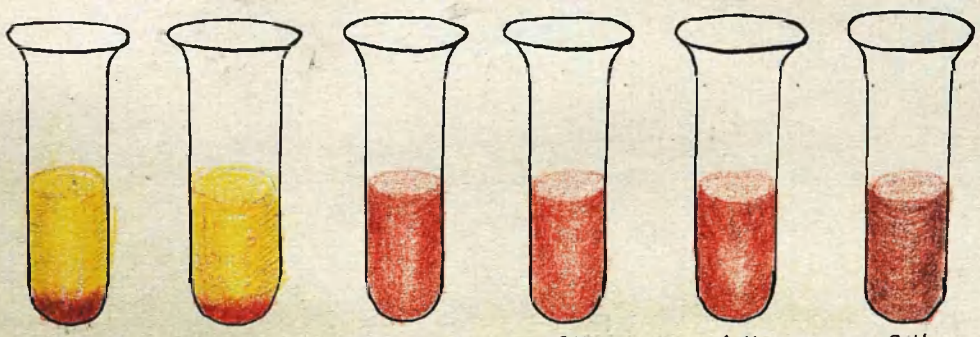
If the complement for the test be made up in factors of 30 c.c. the amount of neat serum necessary for each 30 c.c. of diluted complement to enable .1 c.c. to represent 1 M.H.D., will be the quantity noted in the titration with the decimal point moved back one place:- here 1.5 cc = 1 in 20 as required.

The final simplicity of this form of titration, commends itself/

TITRATION
OF
COMPLEMENT.



N.H. N.H. C.H. C.H. C.H. C. H.



N.H. N.H. C. H. C.H. C. H. C. H.

	1	2	3	4	5	6
Saline (to 2 c.c.)	1·45	1·4	1·35	1·3	1·25	1·2
R.B.C. + I.B.	·5	·5	·5	·5	·5	·5
Compl. $\frac{1}{30}$	·05	·1	·15	·2	·25	·3
	N.H.	N.H.	C. H.	C. H.	C.H.	C. H.

·15 c.c. of $\frac{1}{30}$ dilution of Complement = 1. M.H.D.

∴ if ·1 cc is to represent 1 M.H.D of C.
the Complement must be dilated 1 in 20.

∴ for each 30 c.c. of diluted Complement necessary for the test, in order that ·1 c.c. will contain 1 M.H.D of C.
1·5 c.c. of Neat Serum must be added to 28·5 c.c. Saline.
This equals a $\frac{1}{20}$ dilution of Neat C.

itself to us, in preference to the method advocated by Harrison and others.

When carrying out this titration, should complete haemolysis not occur in tube 6, the complement must be discarded, as sufficient dilution of the guinea-pig serum would not be possible. This would allow of substances in the serum which are inimicable to the test being present in so concentrated a form as to be disturbing.

In this case fresh serum must be obtained, and titred. (L. W. Harrison and others).

(3) TITRATION OF SERA. The powers of sera alone for fixing complement should be known, otherwise, sera which are unduly anticomplementary may be classified among the positive results when by rights they may belong to the negatives.

The serum may be titrated for each individual, but this lengthy process is unnecessary when testing numerous sera. By an adequate system of control tubes such Anti-complementary action of any one serum can instantly be be diagnosed.

For this titration our plan is to collect in two tubes the poolings of several sera, in the one tuberculous, the the other normal sera. The dose is fixed meanwhile arbitrarily - see later - at .1 c.c.

Two series of 4 tubes are set up, in each tube of the one set is placed a dose of tuberculous serum, in the other of the pooled normal serum.

Complement 1, 2, 3, and 4 M.H.D. is added to each tube of the two series, also saline so that the final bulk is/

is 2 c.c.

The tubes are incubated for 45 minutes at 37°C, and 15 minutes at room temperature, and the haemolytic system is then added. After having been shaken, the tubes are incubated for a further ½ hour, and the results then noted.

	A.					B.			
Tubes.	1.	2.	3.	4.		1.	2.	3.	4.
Serum T.B.	.1	.1	.1	.1	Serum Normal.	.1	.1	.1	.1
Compl. M.H.D.	.1	.2	.3	.4		.1	.2	.3	.4.
Saline to 2c.c.	1.3	1.2	1.1	1.0.		1.3	1.2	1,1	1.0.
R.B.C. ½ 1 B.	.5	.5	.5	.5		.5	.5	.5.	.5.
	N.H.	I.C.H.	C.H.	C.H.		P.H.	C.H.	C.H.	C.H.

It is worthy of note that TUBERCULOUS SERA are almost without exception always MORE ANTICOMPLEMENTARY than normal sera. The former usually deviate rather more than 1 M.H.D. of complement, the latter rather less than 1 M.H.D.

Occasionally one has met with sera which are more anticomplementary than usual. Sera from two psoriatic tuberculous individuals tested fixed 2 M.H.D. of complement.

The anticomplementary action of sera may become so marked as to interfere with the results of the test when either the serum used is not clear owing to partial haemolysis prior to inactivation, or when contamination of serum by bacterial growth has been allowed. In such cases fresh serum should be obtained and centrifuged till clear, or when not obtainable an extra amount of complement sufficient to allow for their individual anticomplementariness must be added to the usual amount -

4 M.H.D. employed in the tests.

In the latter case however the results are less reliable than when fresh clear serum is employed.

(4). TITRATION OF ANTIGENS (a) SYPHILITIC. It is not our purpose to discuss the titration of this antigen at length, as details are available in most standard text-books of bacteriology. We have observed from frequent titrations that .2 c.c. of a 1 in 15 dilution of heart-cholesterol extract - diluted quickly with saline in the manner advocated by McIntosh and Fildes. - is the most convenient dose for one test. The readings obtained have invariably been reliable.

(b) TUBERCLE. As the P.B.E + B.E. mixture of tuberculins is the antigen we most frequently have employed. I shall deal only with the titrations of this, the method of titrating other antigens is of course similar.

(i). To ensure that the antigen is not unduly anticomplementary it is necessary to ascertain the COMPLEMENT FIXING POWERS OF THE ANTIGEN employed, the dose of the latter being arbitrarily fixed.

As previously, a series of tubes is set up, and into each one dose of antigen is placed, varying quantities of complement are added, viz., 1, 2, 3, 4, M.H.D. Saline is added so that the final bulk when the sensitised cells are present will be 2 c.c.

The tubes are shaken, incubated at 37°C for 45 minutes, and then at room temperature for 15 minutes after which the haemolytic system .5 cc. is added, the tubes again shaken and incubated.

Tubes/

Tubes.	1.	2.	3.	4.
Complement (.1 c.c.= 1 M.H.D.)	.1	.2	.3	.4
Antigen. (P.B.E. + B.E.)	.03	.03	.03	.03.
Saline.	1.4	1.3	1.2	1.1
R.B.C. + 1 B.	.5	.5	.5	.5
Readings.	P.H.	C.H.	C.H.	C.H.

It is usually observed that while haemolysis is complete in the second tube, it is only partial in the first tube, from which it is to be inferred that the antigen alone will partially deviate 1 M.H.D. of complement.

(ii.) TITRATION OF ANTIGEN IN PRESENCE OF NORMAL AND TUBERCULOUS SERA.

The complement fixing powers of both antigen alone and serum alone being known, two series of tubes are set up, one set containing known normal serum, the other containing known tuberculous serum. Varying amounts of antigen are added, likewise an excess of complement - with us 4 M.H.D. - and saline as before. The tubes are incubated as previously and the haemolytic index added. Readings are taken after 20 minutes incubation at 37°C.

	Set A.							Set B.	
Tubes.	1.	2.	3.	4.	5.	6.		1.	6.
Saline (to 2 c.c)	1	1	1	1	1	1		1	1
Complement (4 M.H.D.)	.4	.4	.4	.4	.4	.4		.4	.4
Antigen (PBE.& BE)	.01	.015	.02	.025	.03	.035.		.01	.035.
Tuberculous Serum.	.1	.1	.1	.1	.1	.1	Normal Serum	.1	.1.
R.B.C. & 1 B.	.5	.5	.5	.5	.5	.5		.5	.5.
Readings.	P.H.	P.H.	N.H.	N.H.	N.H.	N.H.		C.H.	C.H.

We have usually employed .025 c.c. AS THE DOSE OF ANTIGEN as it invariably prevented any haemolysis, with/

with tuberculous sera while even larger doses allowed complete haemolysis in the presence of normal sera.

Burroughs Wellcome's Bacillary Emulsions are standardised so that 1 c.c. of emulsion contains 5 mgms. bacillary substance. .125 MGMS. OF BACILLARY SUBSTANCE then is present in each dose of antigen. Comparisons with the strengths of the other tuberculins we investigated gave almost corresponding results.

(iii.) TITRATION OF ANTIGEN WITH VARYING QUANTITIES OF SERA.

This titration, while not essential, is useful in demonstrating the ideal quantities of these two reagents to use in the test proper.

6 rows of Tubes are set up.

A row contains .05 c.c. Tuberculous serum.
A' " " .05 c.c. Non. Tuberculous serum.
B " " .1 c.c. Tuberculous serum.
B' " " .1 c.c. Non " "
C " " .15 c.c Tuberculous "
C' " " .15 c.c. Non " "

Into each row graduated doses of antigen are measured, also each tube receives 4 M.H.D. of complement, saline to 2 c.c., and finally sensitised cells. The technique is carried out as usual.

From frequent titrations we have learnt that, ceteris paribus, the smaller doses of tuberculous serum required larger doses of antigen in order to inhibit haemolysis, and that the readings so obtained are less trustworthy. When large doses of serum are employed, normal sera may partially inhibit haemolysis especially when relatively large doses of antigen/

antigen are employed. This phenomenon which is most undesirable is no doubt due to the combined anticomplementary substances of Antigen and sera being in relative excess. In our test then, par excellence, we fix the dose of serum at .1 c.c., and of antigen at .025 e.c. P.B.E. + B.E. Tuberculin.

(iv.) COMPLEMENT FIXING POWERS OF TUBERCLE ANTIGEN IN ASSOCIATION WITH SERA - NORMAL AND TUBERCULOUS.

This titration well merits mention. Tubes are set up each containing 1 dose of antigen, 1 dose of serum, and saline to the requisite amount.

In series A containing tuberculous serum, complement is added in increasing amounts from 1 to 12 M. H. D.

In series B containing normal serum, only 4 tubes are necessary, into which complement is placed 1, 2, 3, and 4 M.H.D. respectively. The remainder of the technique is as previously. The results obtained are very interesting: in series A it is not uncommon to find complete deviation of complement up to 10 M. H. D. especially with sera "strongly positive" to tuberculosis. On the other hand in series B haemolysis is usually incomplete in the second tube, and complete in third and fourth, in other words just over 1 M.H.D. of complement is fixed, which after all is exactly the sum of the anticomplementary values of serum and antigen alone as previously demonstrated.

C. METHOD OF SETTING UP THE TEST.

1. DOSAGE OF REAGENTS. From the preliminary titrations already discussed the doses of reagents are fixed:-

(i)/

(i) Haemolytic system. .5 c.c. of 2½% suspension of red blood cells sensitised with 8 M.H.D. of Haemolytic Amboceptor.

(ii) Complement - 4 M.H.D. are employed. This is more than sufficient to allow for the anticomplementary actions of serum and antigens, which, combined is approximately under 2 M.H.D.

(iii) Antigens. .2 c.c. of 1 in 15 dilution in saline of Heart - cholesterin extract - for syphilis.

.025 c.c. of P.B.E. + B.E. mixture Tuberculin, for Tuberculosis.

(iv.) Serum. .1 c.c. of inactivated human serum to be tested.

(v.) Saline - Sufficient physiological saline is added to each tube as diluent to make the fimal bulk 2 c.c.

2. NUMBER OF TUBES. We employ a series of 4 tubes for each serum to be tested.

A. is a control tube to demonstrate any disturbing anti-complementariness of the serum tested. No antigen is present. As complete lysis of the red cells should occur it acts as a contrast for negative readings.

B. is a test for Wassermann reaction; it contains a dose of syphilitic antigen.

C. and D. are tests for Tuberculosis - each contains a dose of tubercle antigen. C. contains 4 M.H.D. of complement and is a QUALITATIVE test; D. on the other hand as it contains 6 M.H.D. of complement is rather a QUANTITATIVE test, as obviously where 6 M.H.D. are completely fixed a stronger positive reading is to be interpretated, than where only

4/

4 M.H.D. are deviated. Originally we employed D as a second control tube, omitting antigen and complement, to check any undue haemolytic element of the serum, and also as a contrast for positive readings, as no haemolysis should occur. If the serum has been properly inactivated no complement can be present, and therefore haemolysis can never occur. We have therefore discarded this control tube in favour of the quantitative test for tuberculosis we now employ.

Series of tests for each serum.

Reagents.	A.	B.	C.	D.
Saline (to 2 c.c.)	1.0	.8	1.0	.8
Serum.	.1	.1	.1	.1
Syph. Antigen.	-	.2	-	-
T.B. Antigen.	-	-	.025.	.025.
Complement.	.4	.4	.4	.6
R.B.C. + 1 B.	.5	.5	.5	.5.
Readings.	C.H.	C.H.or N.H.	C.H. or N.H.	C.H. or N.H.

3. TECHNIQUE. The saline, serum, antigens and complement are first carefully measured into each tube. The tubes are shaken to ensure thorough mixing and placed in the incubator at 37°C for 45 minutes, and then allowedto stand at room temperature for a further 15 minutes. By this time any binding of complement to serum by its appropriate antigen will have occurred if immune bodies are present in the serum. A dose of sensitised red cells is then added as the/

the haemolytic index, the tubes are again shaken and placed in the incubator at 37°C.

They are carefully watched and when haemolysis is complete in tube A of each series, an extra minute or two is allowed and then the tubes are removed from the incubator for reading.

D. RULES TO BE OBSERVED IN READING THE TEST.

(i) Known tuberculous, known syphilitic, and known normal sera should be included among the sera tested as guides. If incorrect readings are obtained from these sera the whole batch of tests must be discarded, and a fresh set put up using fresh reagents.

(ii) If Tube A of any series fails to shew complete haemolysis the series must be discarded. Possibly the absence of haemolysis may be due to an anticomplementary action of the serum, in which case the serum alone should be titrated, before setting up a new series.

(iii.) If the control syphilitic serum shews inhibition of haemolysis then inhibition in any tubes of row B may be taken to indicate a "positive Wassermann;" complete haemolysis on the other hand would indicate a "negative Wassermann."

(iv.) Similarly with rows C and D. If the control tuberculous serum checks haemolysis, while the control normal serum permits of full haemolysis, then any inhibition of lysis of the red cells in tubes C and D indicates a "positive" reading for Tuberculosis, while complete lysis points/

points to a "negative" result.

(v.) For convenience a special NOTATION is employed by us to facilitate expression of results.

Complete Inhibition of Haemolysis.	=	N.H.
Partial " " "	=	P.H.
Incomplete haemolysis.	=	I.C.H.
Complete haemolysis.	=	C.H.

Finer graduations are obtained by using the signs + or - after the notation, e.g.

faintest trace of Commencing haemolysis	=	N.H. -
slightly greater trace " "	=	P.H. +

(vi) In reporting on the positive results obtained we employ a definite scale to indicate the strength of reaction - Thus with the Syphilis row B:-

N.H.	=	+ + +	(very strong positive).
P.H.	=	+ +	(strong positive)
I.C.H.$^{+}$	=	+	(positive).
I.C.H.	=	$\pm$	(weak positive).
I.C.H.$^{-}$	=	$\mp$	(doubtful).

With tuberculous sera/the sum of the reactions in rows C and D is taken into account, thus:-

C.	D.			
N.H.	N.H.	=	+ + +	(v. strong positive).
N.H.	P.H.	=	+ + +	(v. strong positive).
N.H.	I.C.H.	=	++	(strong positive).
N.H.	C.H.	=	+ +	(strong positive).
P.H.	P.H.	=	+ +	(strong positive).
P.H.	I.C.H.	=	+ +	(positive.)
P.H.	C.H.	=	+	(positive).
I.C.H.	C.H.	=	$\frac{+}{-}$	(very weak positive).
I.C.H.$^{-}$	C.H.	=	$\mp$	(doubtful).
C.H.	C.H.	=	—	(negative).

(vii)/

(vii) When doubtful readings are obtained, if possible the sera should be retested, and if an identical result is obtained it must duly be reported on as doubtful.

From practice we have ascertained that doubtful readings are usually very weak positives, in-as-much as 2 M.H.D. of complement is all that is necessary to allow of complete haemolysis with normal sera, and in the test 4 M.H.D. is employed. Notwithstanding, one is hardly justified in attaching much value to a doubtful reading.

E. GENERAL ROUTINE.

1. Obtain sheep's blood - defibrinated - and wash cells.
2. Kill healthy guinea-pig, and collect serum.
3. Titrate Haemolytic amboceptor - If this has been performed recently this step may be omitted.
4. Make up suspension of Sensitised Red cells.
5. Titrate complement.
6. Titrate antigen in presence of known normal and tuberculous sera. This does not need to be done with each batch of tests, but it is advisable, to ensure accuracy of results.
7. Set up tests.
8. Read after $\frac{1}{4}$ to $\frac{1}{2}$ hour from final incubation, and check readings two hours later.

III. ANALYSIS OF 365 TESTS.

Regarding the results of the 365 tests which are recorded below one or two points require elucidation.

(i) Of the 229 sanatoria patients whose sera was tested, a few were doubtful cases of Pulmonary Tuberculosis; notes have been appended on such cases.

(ii) The sera of 103 epileptics were investigated, usually by way of negative controls. Numerous cases of healed glandular, or inactive pulmonary tuberculous disease were unfortunately present.. Where positive readings were obtained a careful examination has been made of the patients.

(iii) 11 children who had applied to emigrate to Canada (from Quarrier's Homes) but who were rejected on health grounds were the subject of investigation. Notes on their physical conditions have been appended.

(iv). All other cases marked O.H.S. and Private were cases of obscure clinical signs, and of whose sera, it was thought, an examination by the complement fixation method might throw light upon the diagnosis.

The actual results have been divided into 8 series, partly for convenience in analysis, and partly because different antigens were employed in the first four series.

To the left the laboratory findings are given, and on the right of the middle line are notes on the clinical conditions of the patients whose sera were tested.

The CLASSIFICATION is that of Sir. Robt. Philip:- L_1, L_2, L_3 represents the stage of pulmonary disease; S. S_1, S_2, S_3 represents the amount of systemic toxaemia clinically recognisable.

A/

A SPUTUM ANALYSIS is included, it is interesting to note that in many unquestionable cases of Phthisis no tubercle bacilli are evident in the sputa.

Under the heading "CLINICAL" any outstanding feature of the case is noted.

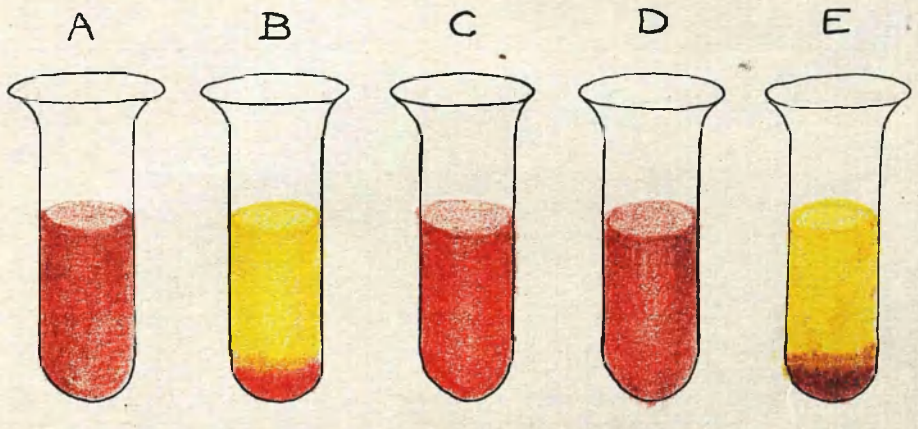
The column T represents TUBERCULIN TREATMENT:- Thus, "T -" means patient has never received Tuberculin of any form.

Findings of the VON PIRQUET test on the sanatoria patients are also given for purposes of comparison.

* an ASTERISK indicates that a special note is appended.

DIAGRAMS OF TESTS.

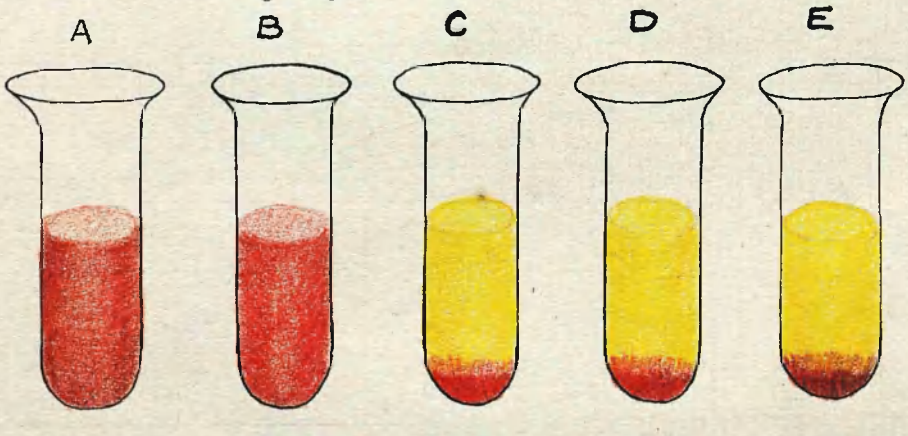
1. Test with Syphilitic - but Non-Tuberculous - Serum.



— Control Tube.		Syphilis Tube.		Tubercle Tube.		Tubercle Tube.		+ Control Tube.
Saline	1·0		·8		1·0		·8	1·4
Serum	·1		·1		·1		·1	·1
Antigen.	—	(Syph.)	·2	(P.B.E+B.E.)	·025	(P.B.E.+B.E.)	·025	—
Complement.	·4		·4		·4		·6	—
R.B.C.+I.B.	·5		·5		·5		·5	·5

RESULT :— SYPHILIS +++.

2. Test with Non-Syphilitic - Tuberculous Serum.

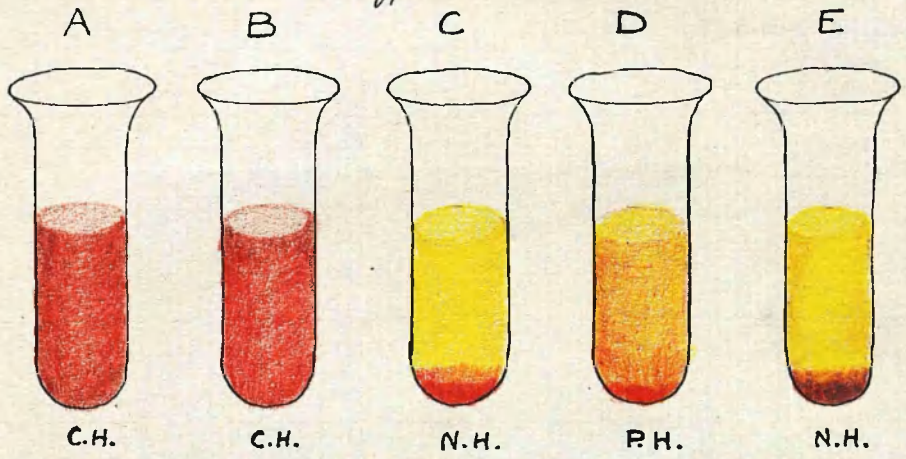


— Control Tube.		Syphilis Tube.		Tubercle Tube.		Tubercle Tube.		+ Control Tube.
Saline	1·0		·8		1·0		·8	1·4
Serum.	·1		·1		·1		·1	·1
Antigen.	—	(Syph.)	·2	(PBE+BE)	·025	(PBE+BE)	·025	—
Complement.	·4		·4		·4		·6	—
R.B.C.+I.B.	·5		·5		·5		·5	·5

RESULT :— TUBERCLE +++.

DIAGRAMS CONT?

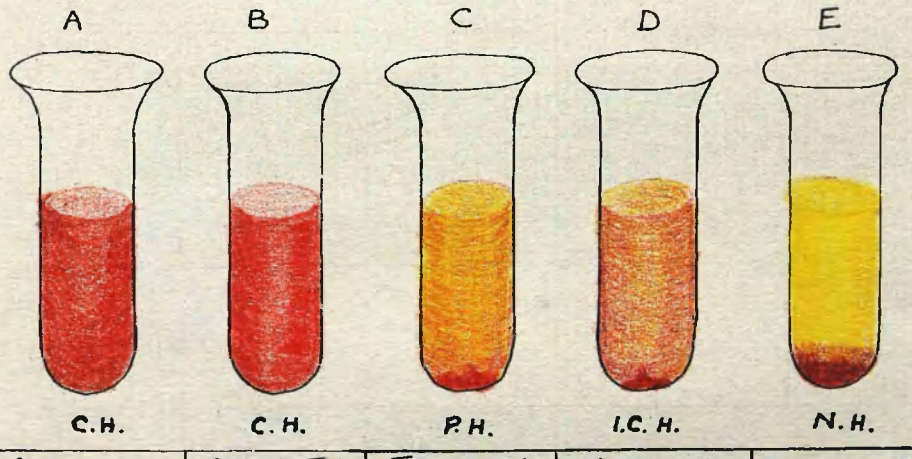
3. Test with -Non-Syphilitic- Tuberculous Serum.



— Control Tube.		Syphilis Tube.		Tubercle Tube.		Tubercle Tube.		+ Control Tube.
Saline.	1·0		·8		1·0		·8	1·4
Serum.	·1		·1		·1		·1	·1
Antigen.	—	(Syph.)	·2	(PBE + BE)	·025	(PBE + BE)	·025	—
Complement	·4		·4		·4		·6	—
R.B.C.+I.B.	·5		·5		·5		·5	·5

RESULT :— TUBERCLE + + + .

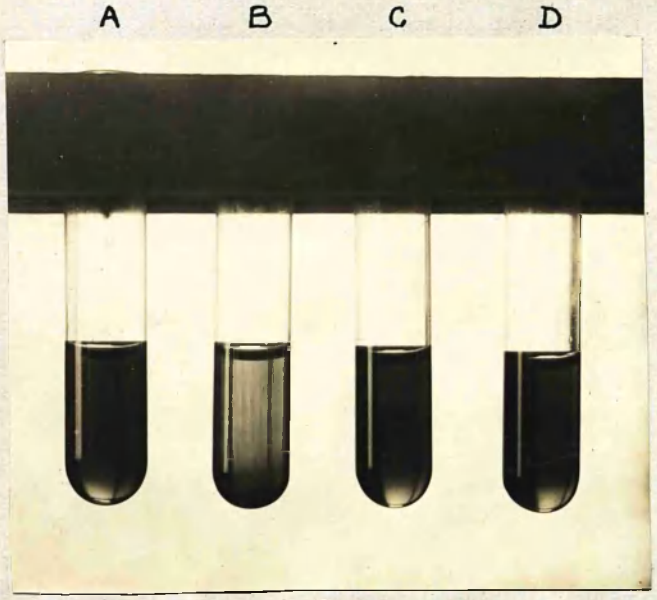
4. Test with - Non-Syphilitic - Tuberculous Serum.



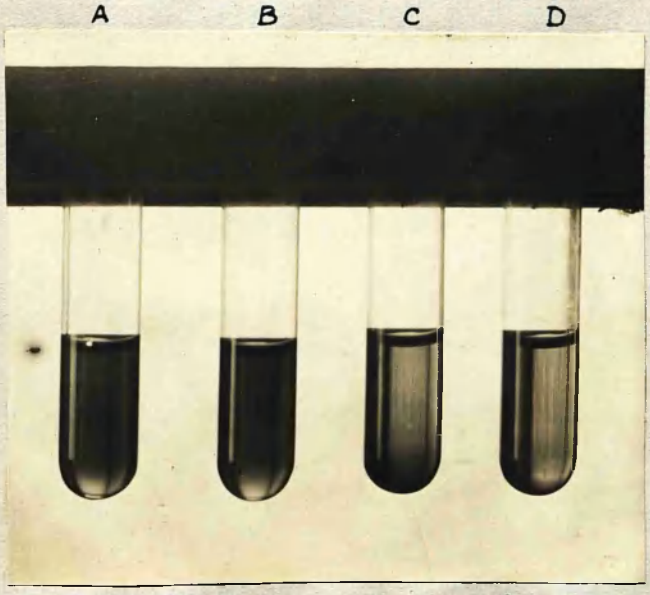
— Control Tube.		Syphilis Tube.		Tubercle Tube.		Tubercle Tube.		+ Control Tube.
Saline.	1·0		·8		1·0		·8	1·4
Serum.	·1		·1		·1		·1	·1
Antigen.	—	(Syph.)	·2	(PBE+BE)	·025	(PBE+BE)	·025	—
Complement.	·4		·4		·4		·6	—
R.B.C. + I.B.	·5		·5		·5		·5	·5

RESULT :— TUBERCLE + + .

TESTS FOR SYPHILIS AND TUBERCLE.



B is the Syphilis Tube - No Haemolysis has occurred; the red cells have fallen to the bottom, leaving the supernatant fluid clear.



C & D are Tubercle Tubes - The reds cells have fallen to the bottom - A & B however shew complete haemolysis.

SERIES I. (1-77).

No.	A.	B.	C.	D.	Sy.	Tb.	Class.	Sputum.	Clinical.	T.	V.P. H.	V.P. B.	Remarks.
1.	C.H	CH	NH.	NH.	–	+++	L_3 S_1	TB. Num.	Very pale.	+	+	+	Sanatorium.
2.	CH.	CH.	NH.	NH.	–	+++	L_2 S_1	TB. Nil.	Anaemia.	+	–	–	"
3.	CH.	CH.	NH.	NH.	–	+++	L_2 S_2	TB. Nil.	Lupus.	+	++	++	"
4.	CH.	CH.	NH.	NH.	–	+++	L_2 S_1	TB. Nil.	Cyanosis.	+	+	+	"
5.	CH.	CH.	NH.	NH.	–	+++	L_2 S_1	TB. Nil	Dyspnoea.	+	++	+	"
6.	CH.	CH.	ICH.	NH.	–	+	L_1 S_1	TB. Nil.	Dyspnoea.	+	++	++	"
7.	CH.	CH.	PH.	N.H.	–	++	L_2 S_1	TB. Nil.	Cyanosis.	+	+	+	"
* 8.	CH.	CH.	CH.	NH.	–	–	L_2 S_1	TB. Nil.	Lividity.	+	+	+	"
9.	CH.	CH.	PH.	NH.	–	++	L_3 S_2	TB. Scatt.	Larynx.	–	+	+	"
10.	CH.	PH.	PH.	NH.	+	++	L_2 S_2	TB. Num.	Cyanosis.	+	+	+	"
11.	CH.	CH.	CH.	NH.	–	–	–	–	–	–	–	–	Epileptic.
12.	CH.	NH.	PH.	NH.	+++	++	L_3 S_1	TB. Num.	Aneurysm. Dyspnoea.	–	+	+	Sanatorium.
13.	CH.	CH.	NH.	NH.	–	+++	L_3 S_1	TB. Few.	Seborrhoea.	+	+	+	"
14.	CH.	CH.	NH.	NH.	–	+++	L_2 S_1	TB. Few.	Cyanosis.	+	+	+	"
15.	CH.	CH.	PH.	NH.	–	++	L_2 S_1	TB. Nil.	Larynx.	+	+	–	"
16.	CH.	PH.	NH.	NH.	+	+++	L_3 S_1	TB. Few.	Cyanosis.	+	+	+	"
17.	CH.	CH.	PH.	NH.	–	++	L_2 S_2	TB. Nil.	Lividity.	+	++	++	"
18.	CH.	CH.	NH.	NH.	–	+++	L_3 S_2	TB. Num.	Dyspnoea.	–	+	–	"
19.	CH.	PH.	NH.	NH.	+	+++	L_3 S_1	TB. Few.	Had Adenitis.	+	+	+	"
20.	CH.	CH.	NH.	NH.	–	+++	L_3 S_2	TB. Num.	Haemoptysis.	+	+	+	"
21.	CH.	CH.	CH.	NH.	–	–	–	–	–	–	–	–	Epileptic.
22.	CH.	CH.	CH.	NH.	–	–	–	–	–	–	–	–	Epileptic.
23.	CH.	CH.	NH.	NH.	–	+++	L_3 S_2	TB. Num.	Cyanosis.	+	++	+	Sanatoria:
24.	CH.	CH.	CH.	NH.	–	–	–	–	–	–	–	–	Epileptic.
25.	CH.	CH.	CH.	NH.	–	–	–	–	–	–	–	–	"
26.	CH.	CH.	CH.	NH.	–	–	–	–	–	–	–	–	"
27.	CH.	CH.	ICH.	NH.	–	+	L_2 S_1	TB. Few.	Dyspnoea.	+	+	+	Sanatorium.
28.	CH.	PH.	CH.	NH.	++	–	–	–	Congenital Syphilis.	–	–	–	Private Case.
29.	CH.	CH.	PH.	NH.	–	+	L_2 S_2	TB. Nil.	Larynx.	+	+	+	Sanatorium.
* 30.	CH.	CH.	ICH.	NH.	–	±	L_3 S_2	TB. Few.	C.S:Dyspnoea	–	–	–	"

Sy. = Syphilis. TB. Num = TB. Numerous. C.S. = Coloured sputum.
Tb. = Tubercle. TB. Scatt = TB. Scattered.

V.P. = Von Pirquet.
H. = Human Tuberculin.
B. = Bovine Tuberculin.

No.	A.	B.	C.	D.	Sy.	Tb.	Class.	Sputum.	Clinical.	V.P. T.	V.P. H.	V.P. B.	Remarks.
31.	CH.	CH.	NH.	NH.	–	+++	$L_2 S_2$	T.B. Few.	Dyspnoea.	+	+	+	Sanetorium.
32.	CH.	CH.	NH.	NH.	–	+++	$L_3 S_2$	TB. Num.	C.S..Anaemia	+	+	+	"
33.	CH.	CH.	PH.	NH.	–	++	$L_2 S_2$	TB. Few.	Cyanosis.	+	+	+	"
34.	CH.	CH.	NH.	NH.	–	+++	$L_3 S_2$	TB. Num.	Dyspnoea.	+	++	++	"
35.	CH.	CH.	NH.	NH.	–	+++	$L_3 S_2$	TB. A Few.	Anaemia.	+	+	+	"
* 36.	CH.	CH.	CH.	NH.	–	–	$L_3 S_1$	TB. Nil.	Bronchitis.	+	+	+	"
37.	CH.	CH.	PH.	NH.	–	++	$L_3 S_2$	TB. Num.	Deaf.	+	+	+	"
38.	CH.	CH.	NH.	NH.	–	+++	$L_2 S_2$	TB. Few.	Lupus.	+	sl.	sl.	"
39.	CH.	CH.	PH.	NH.	–	++	$L_2 S_2$	TB. Few.	Cyanotic.	+	+	+	"
40.	CH.	CH.	NH.	NH.	–	+++	$L_3 S_2$	TB. Num.	Toxic.	+	+	+	"
41.	CH.	CH.	ICH.	NH.	–	+	$L_3 S_2$	T.B.Num.	Anaemia.	+	++	++	"
42.	CH.	CH.	PH.	NH.	–	++	$L_2 S_2$	TB. Few.	Larynx	+	+	+	"
* 43	CH.	CH.	ICH.	NH.	–	±	$L_2 S_1$	None.	Had traumatic Empyema.	–	+	+	"
44.	CH.	CH.	PH.	NH.	–	++	$L_2 S_2$	TB. Nil.	Dyspnoea.	+	+	+	"
45.	CH.	CH.	ICH.	NH.	–	±	–	–	(No symptoms)	- - -	- - -	- - -	Epileptic.
46.	CH.	CH.	CH.	NH.	–	–	- - -	- - -	- - -	- - -	- - -	- - -	"
47.	CH.	CH.	CH.	NH.	–	–	- - -	- - -	- - -	- - -	- - -	- - -	"
48.	CH.	CH.	CH.	NH.	–	–	- - -	- - -	- - -	- - -	- - -	- - -	"
49.	CH.	CH.	CH.	NH.	–	–	- - -	- - -	- - -	- - -	- - -	- - -	"
50.	CH.	CH.	NH.	NH.	–	+++	$L_2 S_1$	TB. Few.	Larynx.	+	+	+	Sanatorium.
* 51	CH.	CH.	CH.	NH.	–	–	$L_2 S_2$	TB. Num.	Acne.	+	++	++	"
52.	CH.	CH.	NH.	NH.	–	+++	$L_3 S_1$	TB. Scatt.	Cachectic.	+	+	–	"
* 53.	CH.	CH.	ICH.	NH.	–	±	$L_2 S_3$	TB. Nil.	Ischio-Rectal Abscesses.	–	–	–	"
* 54.	NH.	NH.	NH.	NH.	?	?							Private Case.
55.	CH.	CH.	CH.	NH.	–	–	- - -	- - -	- - -	- - -	- - -	- - -	Epileptic.
56.	CH.	CH.	ICH.	NH.	–	+	$L_2 S_2$	TB. Nil.	Pleurisy.	+	–	+	Sanatorium.
57.	CH.	NH.	ICH.	NH.	+++	+	$L_3 S_1$	TB. Nil.	Syphilis.	+	sl.	sl.	"
58.	CH.	CH.	NH.	NH.	–	+++	$L_3 S_2$	TB. Num.	Cavity.	+	+	+	"
59.	CH.	CH.	NH.	NH.	–	+++	$L_3 S_1$	TB. Few.	Cavity.	+	+	+	"
60.	CH.	CH.	NH.	NH.	–	+++	$L_2 S_2$	TB. Few.	Adenitis.	+	+	+	"

No.	A.	B.	C.	D.	Sy.	Tb.	Class.	Sputum.	Clinical.	T.	V.P. H.	V.P. B.	Remarks.
61.	CH.	NH.	NH.	NH.	+++	+++	L_2 S_1	TB, Few.	Dyspepsia.	+	+	+	Sanatorium.
62.	CH.	CH.	NH.	NH.	–	+++	L_2 S_1	TB. Nil.	Dyspnoea.	–	–	–	"
63.	CH.	CH.	NH.	NH.	–	+++	L_3 S_2.	TB. Few.	Cyanosis.	+	–	+	"
64.	CH.	CH.	PH.	NH.	–	++	L_2 S_2.	TB. Nil.	Larynx.	+	±	±	"
65.	CH.	CH.	NH.	NH.	–	+++	L_2 S_2	TB. Nil.	Pale Pleurisy.	–	+	+	"
66.	CH.	CH.	NH.	NH.	–	+++	L_3 S_2	TB. Num.	C.S.	–	–	–	"
67.	CH.	CH.	NH.	NH.	–	+++	L_2 S_2	TB. Few.	Cyanosis.	+	+	++	"
68.	CH.	CH.	CH.	CH.	–	–	–	–	–	–	–	–	Epileptic.
69.	CH.	CH.	CH.	NH.	–	–	–	–	–	–	–	–	"
70.	CH.	CH.	CH.	NH.	–	–	–	–	–	–	–	–	"
* 71.	CH.	CH.	PH.	NH.	–	++	–	–	R. Apex. (healed.)	–	–	–	"
* 72.	CH.	CH.	CH.	NH.	–	–	L_3 S_2	TB. Nil.	Bronchiectasis	+	–	–	Sanatorium.
* 73.	CH.	PH.	ICH.	NH.	+	+	L_3 S_2	TB. Nil	"	–	±	–	"
* 74.	CH.	CH.	ICH.	NH.	–	+	–	–	Healed Adenitis.	–	–	–	Epileptic.
75.	CH.	CH.	CH.	NH.	–	–	–	–	–	–	–	–	"
* 76.	CH.	CH.	NH.	NH.	–	+++	L_3 S_2	TB.Scatt.	Perineal) Fistula)	+	+	+	Sanatorium.
* 77.	CH.	NH.	ICH.	NH.	++	+	L_2 S_2	TB. Nil.	Sinuses.	+	+	+	"

ANALYSIS OF SERIES I.

A. Control. C.H. Tubes. NO Antigen.
B. Syphilis.
C. Tubercle. Antigen (W.) Tuberculin (.035 c.c. dose)
D. Control. N.H. Tube: NO complement.

Sanatorium patients.

	-	±	+	++	+++	Total.
L_1	0.	0.	1.	0.	0.	1.
L_2	2.	2.	4.	9.	13.	30.
L_3	2.	1.	3.	3.	17.	26.
	4.	3.	8.	12.	30.	57.

*

Negatives. - (8) Repeated in test (96.) N.H. results apparently some error, possibly antigen omitted.

(36) .TB. always nil in sputum.

(51) Repeated twice with similar results (cf. 155). Case not reacting to treatment.

(72.) Signs of small cavities in chest. TB. always Nil.: Possible case of Bronchiectasis.

DOUBTFULS. ± (30) Much coloured sputum. Very cachectic. Not reacting to treatment.

(43) Wound-Empyema. Never TB in sputum. Doubtful if tuberculous.

(53) Very Ill. Many Ischio-rectal abscesses. Prognosis bad.

EPILEPTIC PATIENTS.

	-	±	+	++	+++	Total.
	15.	1.	1.	1.	0.	18.

DOUBTFULS. ± (45). No definite signs of disease.

POSITIVES ++ (71). R. Apex. Healed Phthisis.
+ (74). Had Cervical Adenitis. Cleared up under treatment.

PRIVATE CASES.

?.	-	
1.	1.	2

(28) Congenital Syphilitic.
(54). Serum anticomplementary.

No.	A.	B.	C.	D.	Sy.	Tb.	Class.	Sputum.	Clinical.	T.	V.P. H.	V.P. B.	Remarks.
78.	CH.	PH.	NH.	NH.	+	+++	L_2 S_1	TB. Few.	Pale; much Pleurisy.	+	+	-	Sanatorium.
79.	CH.	CH.	PH.	PH.	-	++	L_2 S_2	TB. Few.	Cachectic.	+	-	-	"
*80.	CH.	CH.	CH.	CH.	-	-	L_2 S_1	TB. Nil.	Doubtful case.	+	sl.	sl.	"
81.	CH.	CH.	NH.	NH.	-	+++	L_3 S_3	TB. Num.	Cavity. C.S.	-	-	-	"
82.	CH.	CH.	PH.	PH.	-	++	L_2 S_1	TB. Nil.	Sl. Dyspnoea	-	sl.	++	"
*83.	CH.	CH.	CH.	CH.	-	-	L_2 S_2	TB. Nil.	(Anaemia (Doubtful case	-	-	-	"
84.	CH.	CH.	ICH.	PH.	-	++	L_2 S_2	TB. Nil.	Bronchitis.	-	sl.	sl.	"
85.	CH.	CH.	PH.	PH.	-	++	L_2 S_2	TB. Num.	Pleurisy.	-	++	++	"
86.	CH.	CH.	ICH.	NH.	-	+++	L_3 S_2	TB. Scatt.	V. Cachectic.	-	+	+	"
87.	CH.	CH.	CH.	CH.	-	-							Epileptic.
88.	CH.	NH.	ICH.	NH.	++	+++	L_3 S_3	TB. Num.	Hectic.	+	-	-	Sanatorium.
89.	CH.	NH.	ICH.	NH.	++	+++	L_3 S_2	TB. Num.	Pleurisy	-	sl.	sl.	"
90.	CH.	PH.	PH.	PH.	+	++	L_2 S_2	TB. Nil.	Cyanosis.	-	sl.	sl.	"
91.	CH.	CH.	PH.	NH.	-	+++	L_2 S_2	TB. Nil.	Sinuses.	-	-	*	"
92.	CH.	CH.	NH.	NH.	-	+++	L_2 S_2	TB. Num.	Hectic.	-	±	±	"
93.	CH.	CH.	ICH.	PH.	-	++	L_3 S_2	TB. Num.	Larynx Cavity	-	+	+	"
*94.	CH.	CH.	ICH.	PH.	-	+	L_3 S_2	TB. Num.	v. Cachectic	*	-	+	"
95.	CH.	CH.	PH.	PH.	-	++	L_2 S_2	TB. Nil.	Pleurisy,	-	+	+	"
*96.	CH.	CH.	NH.	NH.	-	+++	L_2 S_2	TB. Nil.	Livid.	+	+	+	"
*97.	CH.	CH.	CH.	CH.	-	-	L_2 S_1	TB. Nil.	Wound-Empyema	+	sl.	sl.	"
*98.	CH.	CH.	CH.	CH.	-	-	L_2 S_1	None.	Fibroid. Pneumonia.	-	±	-	"
*99.	CH.	CH.	ICH.	PH.	-	+			V. Anaemic.				Private.
100.	CH.	ICH.	CH.	CH.	+	-							Epileptic.
101.	CH.	CH.	CH.	CH.	-	-							"
102.	CH.	CH.	CH.	CH.	-	-							"
103.	CH.	CH.	CH.	CH.	-	-							"

ANALYSIS OF SERIES II.

A. Control Tube for C.H.
B. Syphilis.
C.) Tubercle. (B.E. Tuberculin .02 c.c.
D.) (P.B.E. + B.E. " .02 c.c.

SANATORIUM PATIENTS.

	-	±	+	++	+++	
L_1	0.	0.	0.	0.	0.	0.
L_2	4.	0.	0.	6.	4.	14.
L_3	0.	0.	1.	1.	4.	6.
	4.	0.	1.	7.	8.	20.

NEGATIVES. - (80). Wound chest. Doubtful if actually tuberculous.
(83) Gassed: TB. always nil in sputum: Doubtful case.
(97). Wound Empyema: TB. always Nil: Doubtful case.
(98). Interstitial Pneumonia (acute pneumonia unresolved) TB. always Nil. Doubtful case.

POSITIVES. + (94) L_3 case. Very ill: Chest active: Prognosis bad.
(96) See Note (8).

EPILEPTIC PATIENTS.

-	±	+	++	+++	
5.	0.	0.	0.	0.	5.

PRIVATE PATIENTS.

+	
1.	1.

POSITIVE: (99) Girl aged. 18 yrs. Very weakly and anaemic.

SERIES III. (104-129.)

No.	A.	B.	C.	D.	Sy.	Tb.	Class.	Sputum.	Clinical.	T.	V.P. H.	V.P. B.	Remarks.
104.	CH.	CH.	PH.	PH.	–	++	L_3 S_2	TB. Nil.	Bronchiectasis	–	–	–	Sanatorium.
105.	CH.	CH.	NH.	NH.	–	+++	L_2 S_2	TB. Num.	(Pleurisy (Diazo(+ve)	+	+	+	"
106.	CH.	CH.	NH.	NH.	–	+++	L_3 S_2	TB. Few.	Larynx.	–	–	–	"
107.	CH.	CH.	PH.	PH.	–	++	L_2 S_1	TB. Nil.	Quiescent	+	–	–	"
108.	CH.	CH.	NH.	ICH.	–	++	L_3 S_2	TB. Num.	V. Thin.	–	+	+	"
109.	CH.	CH.	NH.	NH.	–	+++	L_3 S_1	TB. Nil.	V. Anaemic	–	–	++	"
110.	CH.	CH.	NH.	PH.	–	++	L_2 S_2	None.	Amenorrhoea	–	–	–	"
111	CH.	CH.	NH.	ICH.	–	+	L_2 S_2	TB. Num.	Glands.	–	+	+	"
112.	CH.	CH.	PH.	ICH.	–	+	L_2 S_1	TB. Nil.	Larynx.	–	–	–	"
113.	CH.	CH.	PH.	ICH.	–	+	L_3 S_1	None.	(Pleurisy (Larynx.	–	–	–	"
114.	CH.	CH.	NH.	NH.	–	+++	L_3 S_2	TB. Num.	Glands.	+	+	+	"
115.	CH.	ICH.	NH.	NH.	±	+++	L_3 S_2	TB. Few.	Anaemia.	–	+	+	"
116.	CH.	CH.	NH.	NH.	–	+++	L_3 S_2	TB. Num.	Dyspnoea.	–	+	+	"
* 117.	CH.	CH.	NH.	NH.	–	+++	L_3 S_2	None.	(Pott's (Pleural (Effusion.	–	+	+	"
118.	CH.	CH.	NH.	NH.	–	+++	L_3 S_3	TB. Num.	Glands.	+	–	–	"
119.	Ch.	CH.	NH.	NH.	–	++	L_2 S_1	None.	Chilblains.	+	+	+	"
120.	CH.	CH.	NH.	PH.	–	++	L_3 S_2	TB. Few.	(Nasal (Catarrh.	–	+	+	"
121.	CH.	CH.	NH.	NH.	–	++	L_3 S_2	None.	Pleurisy.	–	++	++	"
122.	CH.	CH.	NH.	NH.	–	++	L_2 S_1	TB. Nil.	Dyspnoea.	–	+	+	"
123.	CH.	CH.	NH.	NH.	–	++	L_2 S_2	TB. Nil.	T.B. Knee	–	++	++	"
124.	CH.	CH.	CH.	CH.	–	–							Epileptic
* 125.	CH.	CH.	ICh.	PH.	–	+							"
* 126.	CH.	CH.	CH.	ICH.	–	±							"
127.	CH.	CH.	CH.	CH.	–	–							"
128.	CH.	CH.	CH.	CH.	–	–							"
129.	CH.	CH.	PH.	PH.	–	+	L_2 S_2	TB. Nil.	Cough.	–	±	±	Private case.

ANALYSIS OF SERIES III.

A. Control Tube for C.H.
B. Syphilis.
C.) Tubercle. (P.B.E. Tuberculin. .03 c.c.
D.) Tubercle. (PBE + BE. " .03 c.c.

SANATORIUM PATIENTS.

	-	±	+	++	+++	
L_1.	0.	0.	0.	0.	0.	0.
L_2.	0.	0.	3.	5.	1.	9.
L_3	0.	0.	1.	4.	7.	12.
	0.	0.	4.	9.	8.	21.

EPILEPTIC PATIENTS.

-	±	+	++	+++	
3.	1.	1.	0.	0.	5.

Dountful case ± (126) Repeated in Test (235)
No active signs.

Positive. + (125) Very Pale. Occasionally Basal Pleurisy - Dry.

SERIES IV. (130 - 157).

No.	A.	B.	C.	D.	Sy.	Tb.	Class.	Sputum.	Clinical.	T.	V.P. H.	V.P. B.	Remarks.
130.	CH.	PH.	PH.	CH.	+	+			Both Apices.				Rejected Emigrant.
131.	CH.	PH.	PH.	CH.	+	+			Both Apices.				" "
132.	CH.	CH.	CH.	CH.	-	-			-				" "
133.	CH.	CH.	NH.	PH.	-	+++			R.Apex. active; adenitis.				"
134.	CH.	CH.	CH.	CH.	-	-			-				"
135.	CH.	CH.	CH.	CH.	-	-			-				"
136.	CH.	PH.	CH.	CH.	+	-			-				"
137.	CH.	CH.	PH.	CH.	-	+			L.Apex. active.				"
138.	CH.	CH.	CH.	CH.	-	-			-				"
139.	CH.	CH.	CH.	CH.	-	-			-				"
140.	CH.	CH.	ICH.	ICH.	-	+			R. Apex. sl. active.				"
141.	CH.	CH.	CH.	CH.	-	-			-				Epileptic.
142.	CH.	CH.	NH.	NH.	-	+++			Adenitis.				"
143.	CH.	CH.	CH.	CH.	-	-			Healed Hip Joint Disease.				O.H.S.
144.	CH.	PH.	ICH.	ICH.	+	+			Both apices.				O.H.S.
145.	CH.	CH.	PH.	ICH.	-	+			Retro-Mammary Abscess.				Private Case.
146.	CH.	CH.	PH.	ICH.	-	++			Psoas Abscess.				O.H.S.
147.	CH.	CH.	PH.	ICH.	-	+	L_2 S_2	TB. Nil.	Lividity.		++	++	Sanatorium.
148.	CH.	PH.	NH.	NH.	+	+++			Glands.				O.H.S.
149.	CH.	PH.	PH.	PH.	+	++			Synovitis.				O.H.S.
*150.	CH.	CH.	CH.	CH.	-	-			C.S. Fluid.				O.H.S.
151.	CH.	PH.	ICH.	ICH.	++	+	L_2 S_2	TB. Nil.	Congenital Syphilis.	+	+	+	Sanatorium.
152.	CH.	NH.	PH.	ICH.	+++	++	L_3 S_1	TB. Nil.	Dyspnoea.	+	sl.	sl.	"
153.	CH.	CH.	PH.	ICH.	+	++	L_3 S2	TB. Num.	Anaemia.	+	++	++	"
154.	CH.	CH.	ICH.	ICH.	-	+	L_3 S_1	TB. Nil.	Bronchitis.	+	+	+	"
*155.	CH.	CH.	CH.	CH.	-	-	L_3 S_2	TB. Num.	Acne.	+	++	++	"
*156.	CH.	CH.	CH.	CH.	-	-	L_2 S_2	TB. Nil.	V. Anaemia.	+	-	-	"
157.	CH.	CH.	CH.	CH.	-	-	L_2 S_1	TB. Nil.	Pleurisy.	+	-	+	"

ANALYSIS OF SERIES IV.

A. Control Tube.
B. Syphilis.
C. PBE + BE Tuberculin .025 c.c. + 4 MHD of complement.
D. do. + 6 MHD " do.

SANATORIUM PATIENTS.

	-	±	+	++	+++	
L_1.	0.	0.	0.	0.	0.	0.
L_2.	2.	0.	2.	0.	0.	4.
L_3.	1.	0.	1.	2.	0.	4.
	3.	0.	3.	2.	0.	8.

NEGATIVES. (155) Not reacting to treatment. cf. (51)
(156) Gassed: Doubtful case: cf. (83).
(157) Pleural adhesions and slight dyspnoea - No TB. in sputum. Chest quiscent.

EPILEPTIC PATIENTS.

-	±	+	++	+++	
1.	0.	0.	0.	1.	2.

Positive +++ (142) Cervical Adenitis.

REJECTED EMIGRANTS.

-	±	+	++	+++	
6.	0.	4.	0.	1.	11.

Positive. + (130) (131) (140) (147) all shew apical activity. All are thin and anaemic.
+++ (133) R. Apex. active. Has Cervical adenitis.

ORPHAN HOMES CHILDREN.

-	±	+	++	+++	
2.	0.	1.	2.	1.	6.

NEGATIVES. - (143) HEALED. Hip Joint Disease.
(150) CEREBRO-SPINAL FLUID. Child died. P.M. exam. shewed gliomatous tumour.

Positive. + (144) Slight cough. Thin: Apices are doubtful.
++ (146) Psoas Abscess.
(147) Hydrops of Knee. Syphilitis also.
+++ (148) Cervical Adenitis.

Private Case. Positive + (145) Tuberculous Retro-mammary abscesses.

SERIES V. (158 - 204).

No.	A.	B.	C.	D.	Sy.	Tb.	Class.	Sputum.	Clinical.	T.	V.P. H.	V.P. B.	Remarks.
158.	CH.	CH.	NH.	CH.	-	+++	L_2 S_2	TB. Nil.	Anaemic.	+	+	+	Sanatorium.
159.	CH.	CH.	NH.	NH.	-	+++	L_3 S_2	TB. Nil.	Dyspnoea.	+	+	+	"
160.	CH.	CH.	NH.	NH.	-	+++	L_3 S_2	TB. Num.	Dyspnoea.	+	-	-	"
161.	CH.	CH.	CH.	CH.	-	-							Epileptic.
162.	CH.	CH.	CH.	CH.	-	-							"
163.	CH.	CH.	CH.	CH.	-	-							"
164.	CH.	CH.	CH.	CH.	-	-							"
165.	CH.	CH.	CH.	CH.	-	-							"
* 166.	CH.	CH.	CH.	CH.	-	-	L_3 S_2	TB. Nil	Lupus.	-	-	-	Sanatorium.
167.	CH.	CH.	CH.	ICH.	-	+	L_3 S_2	TB. Num.	Amenorrhoea.	-	sl.	sl.	"
168.	CH.	CH.	ICH.	CH.	-	+	L_2 S_2	TB. Nil.	Larynx.	-	+	+	"
169.	CH.	CH.	ICH.	CH.	-	+	L_2 S_2	TB. Nil.	TB. Abdomen	-	+	+	"
170.	CH.	CH.	NH.	PH.	-	+++	L_1 S_1	TB. Nil.	Cough.	-	+	+	"
171.	CH.	CH.	NH.	PH.	-	+++	L_2 S_2	TB. Few.	Cough.	-	+	+	"
172.	CH.	CH.	ICH.	CH.	-	+	L_2 S_2	TB. Nil.	Sl. Cough.	-	+	+	"
173.	CH.	CH.	PH.	CH.	-	++	L_2 S_2	TB. Nil.	-	-	+	-	"
174.	CH.	CH.	NH.	NH.	-	+++	L_2 S_2	TB. Nil.	Cough.	-	+	+	"
175.	CH.	CH.	PH.	ICH.	-	++	L_1 S_2	TB. Nil.	Glands.	-	++	++	"
176.	CH.	CH.	PH.	ICH.	-	++	L_2 L_2	TB. Nil.	Glands.	-	++	+	"
177.	CH.	CH.	ICH.	CH.	-	+	L_1 S_1	TB. Nil.	-	-	-	-	"
178.	CH.	CH.	ICH.	CH.	-	+	L_2 S_2	TB. Num.	Otorrhoea.	-	+	+	"
179.	CH.	CH.	ICH.	CH.	-	+	L_2 S_2	TB. Nil.	-	-	+	+	"
180.	CH.	CH.	ICH.	CH.	-	±	L_1	None.	-	-	-	-	"
181.	CH.	CH.	ICH.	CH.	-	+	L_2 S_2	None.	Phlycten.	-	+	+	"
182	CH.	CH.	NH.	NH.	-	+++	L_1 S_3	None.	T.B. Spine) Glands)	-	+	+	"
* 183.	CH.	CH.	CH.	CH.	-	-	L_2 S_2	None.	TB. Abdomen	-	-	-	"
* 184.	CH.	CH.	PH.	ICH.	-	+	L_3 S_2	TB. Nil.	Pleural Fluid.	-	-	-	"
185.	CH.	CH.	NH.	ICH.	-	++	L_3 S_2	TB. Nil.	Dyspnoea.	-	+	+	"
186.	CH.	CH.	ICH.	ICH.	-	+	L_1 S_2	TB. Nil.	V. Pale.	-	sl.	sl.	"

No.	A.	B.	C.	D.	Sy.	Tb.	Class.	Sputum.	Clinical.	T.	V.P. H.	V.P. B.	Remarks.
187.	CH.	PH.	NH.	NH.	++	+++	L_3 S_2	TB. Num.	Pleurisy.	-	+	+	Sanatorium.
188.	CH.	CH.	PH.	ICH.	-	+	L_2 S_1	TB. Scatt.	Cyanosis.	-	+	+	"
189.	CH.	CH.	NH.	NH.	-	+++	L_3 S_3	TB. Num.	C.S..Toxic.	-	-	-	"
190.	CH.	CH.	NH.	PH.	-	+++	L_2 S_2	None.	Adenitis.	-	++	++	"
191.	CH.	CH.	NH.	NH.	-	+++	L_3 S_2	TB. Nil.	Dextrocardia.	-	+	+	"
192.	CH.	CH.	NH.	NH.	-	+++	L_3 S_2	TB. Scatt.	Otitis Media.	-	+	+	"
193.	CH.	CH.	NH.	NH.	-	+++	L_3 S_2	TB. Few.	(Larynx, and (Pleural Effusion	-	sl.	sl.	"
194.	CH.	CH.	NH.	NH.	-	+++	L_3 S_2	TB. Num.	-	-	sl.	sl.	"
195.	CH.	CH.	NH.	ICH.	-	++	L_3 S_3	TB. Few.	-	-	+	+	"
196.	CH.	CH.	NH.	NH.	-	+++	L_2 S_2	TB. Scatt.	Indigestion.	-	+	+	"
197.	CH.	CH.	NH.	NH.	-	+++	L_3 S_2	TB. Num.	Mitral.	-	+	+	"
198.	CH.	CH.	NH.	NH.	-	+++	L_3 S_2	TB. Num.	Pleural Effusion	-	+	+	"
* 199.	CH.	CH.	CH.	CH.	-	-	L_3 S_3	TB. Nil.	Adenitis Larynx.	-	+	+	"
200.	CH.	CH.	PH.	PH.	-	++	L_3 S_2	TB. Scatt.	-	-	-	+	"
201.	CH.	CH.	NH.	NH.	-	+++	L_2 S_2	TB. Num.	-	-	+	+	"
202.	CH.	CH.	NH.	NH.	-	+++	L_2 S_2	TB. Scatt.	Larynx. Pleural Effusion)	-	+	+	"
203.	CH.	CH.	PH.	ICH.	-	+	S_3.	None.	TB. Ulcers	-	+	+	"
204.	CH.	CH.	PH.	CH.	-	+	S_2	None.	Lupus of Neck.	-	+	+	"

ANALYSIS OF SERIES V.

A. Control for C.H.
B. Syphilis.
C. P.B.E. + B.E. Tuberculin + 4 M.H.D. of Complement.
D. do. + 6 M.H.D. "

SANATORIUM PATIENTS.

	−	±	+	++	+++	
L_1	0.	1.	2.	1.	2.	6.
L_2	1.	0.	7.	2.	7.	17.
L_3	2.	0.	2.	3.	10.	17.
Non- Pulmonary.	0.	0.	2.	0.	0.	2.
	3.	1.	13.	6.	19.	42.

Negatives − (163) Very ill case. Not reacting to treatment.
(166). Lupus of Face.
(199) Not reacting to treatment. Very toxic.

Doubtful ± (180) Early case. Symptons slight.

Positives. + (184) Pleural Fluid employed for Test not Blood serum.

EPILEPTIC PATIENTS. 5 − all negatives − Patients are apparently healthy.

SERIES VI. (205 - 254).

No.	A.	B.	C.	D.	Sy.	Tb.	Class.	Sputum.	Clinical.	T.	V.P. H.	V.P. B.	Remarks.
205.	CH.	CH.	NH.	NH.	-	+++	L_2 S_2.	None.	T.B. foot) Larynx.)		+	+	Sanatorium.
206.	CH.	CH.	NH.	NH.	-	+++	L_3 S_3	TB. Nil.	Dextracardia.		++	+	"
207.	CH.	CH.	NH.	NH.	-	+++	L_2 S_2	TB. Scatt.	Cyanosis.		+	+	"
208.	CH.	CH.	NH.	NH.	-	+++	L_2 S_2	TB. Num.	Cyanosis.		+	+	"
* 209.	CH.	CH.	NH.	CH.	-	++			(Adenitis Scars (? L. Apex.				Epileptic.
210.	CH.	CH.	CH.	CH.	-	-							"
211.	CH.	CH.	CH.	CH.	-	-							"
212.	CH.	CH.	CH.	CH.	-	-							"
213.	CH.	CH.	CH.	CH.	-	-							"
* 214.	CH.	CH.	NH.	PH.	-	++			Early Phthisis				Private case
* 215.	CH.	CH.	ICH.	CH.	-	±			Contact case.				"
216.	CH.	CH.	CH.	CH.	-	-			No symptoms.				"
217.	CH.	CH.	NH.	NH.	-	+++	L_2 S_2	TB. Scatt.	Amemorrhoea.				Sanatorium.
218.	CH.	CH.	NH.	NH.	-	+++	L_2 S_2	TB. Few.	Anaemia.		+	+	"
219.	CH.	CH.	NH.	NH.	-	+++	L_2 S_2	TB. Few.	Indigestion.		+	+	"
220.	CH.	CH.	PH.	ICH.	-	++	L_2 S_2	TB. Few.	Sl. Dyspnoea.		+	+	"
221.	CH.	CH.	PH.	PH.	-	++	L_3 S_2	TB. Nil.	Mitral.		+	+	"
222.	CH.	ICH.	NH.	PH.	+	+++	L_3 S_2	TB. Few.	Adenitis.		+	+	"
223.	CH.	CH.	NH.	ICH.	-	++	L_2 S_2	None.	TB. Hip (healed)		-	-	"
224.	CH.	CH.	NH.	NH.	-	+++	L_3 S_2	TB. Nil.	-		+	+	"
225.	CH.	CH.	NH.	NH.	-	+++	L_3 S_2	TB. Num.	-		+	+	"
226.	CH.	CH.	NH.	NH.	-	+++	L_3 S_2	TB. Few.	Pyorrhoea.		+	+	"
227.	CH.	CH.	NH.	PH.	-	++	L_1 S_1	None.	-		+	+	"
228.	CH.	CH.	NH.	NH.	-	+++	L_3 S_2	TB. Scatt.	(Adenitis. Lupus (Larynx.		+	+	"
229.	CH.	PH.	NH.	PH.	-	+++	L_3 S_2	TB. Scatt.	Larynx.		+	+	"
230.	CH.	CH.	NH.	PH.	-	++	L_2 S_2	TB. Few.	-		+	+	"
231.	CH.	CH.	NH.	PH.	-	++	L_3 S_2	TB. Num.	Larynx.		sl.	sl.	"
232.	ICH.	ICH.	NH.	NH.	-	+++	L_3 S_2	TB. Few.	Adenitis.		+	++	"
* 233.	CH.	CH.	PH.	ICH.	-	+			V. pale, no other symptoms.		-	-	Epileptic.

No.	A.	B.	C.	D.	Sy.	Tb.	Class.	Sputum.	Clinical.	T.	V.P. H.	V.P. B.	Remarks.
234.	CH.	CH.	CH.	CH.	–	–			–				Epileptic.
*235.	CH.	CH.	ICH.	CH.	–	±			No symptoms.				" (c.f.186)
236.	CH.	CH.	CH.	CH.	–	–			–				"
* 237.	CH.	CH.	NH.	ICH	–	+++			Hydrops Knee				" (c.f.101)
* 238.	CH.	CH.	ICH.	CH.	–	+	L_3 S_3	TB. Num.	(Moribund (Consumption.	+	++	++	Sanatorium.
* 239.	CH.	CH.	CH.	CH.	–	–	L_3 S_3	TB. Num.	Meningismus.	+	++	++	" (C.S.Fluid)
240.	CH.	CH.	CH.	CH.	–	–			(No evidence (of Tuberculosis				Private Case.
241.	CH.	CH.	PH.	CH.	–	++	L_1 S_2	TB. Nil.	Larynx.	–			Sanatorium.
242.	CH.	CH.	CH.	CH.	–	–			(No Symptoms (TB. Family (History.				Private Case
* 243.	CH.	CH.	NH.	NH.	–	+++			Adenitis scars) Quiescent) Phthisis.)				Epileptic.
244.	CH.	CH.	CH.	CH.	–	–			–				"
* 245.	CH.	ICH.	PH.	ICH.	±	+			(Quiescent Phthisis.				"
* 246.	NH.	NH.	NH.	NH.	?	?			Anticomplementary.				"
* ×247.	CH.	ICH.	NH.	NH.	±	+++			(Died later of (Cerebral Tumour. (Pleurisy, glands.				"
248.	CH.	CH.	ICH.	CH.	–	±			"Butterfly Rash") on face.)				"
249.	CH.	CH.	CH.	CH.	–	–			–				"
250.	CH.	CH.	CH.	CH.	–	–			Eczema.				"
251.	CH.	CH.	CH.	CH.	–	–			–				"
* 252.	CH.	NH.	NH.	CH.	+++	++			Puny child.				"
253.	CH.	CH.	CH.	CH.	–	–			–				"
* 254.	CH.	CH.	ICH.	CH.	–	±			Adenitis Scars.				"

ANALYSIS OF SERIES VI.

Reagents in Test as for Series V.

SANATORIUM PATIENTS.

	−	±	+	++	+++	
L_1	0.	0.	0.	2.	0.	2.
L_2	0.	0.	0.	3.	6.	9.
L_3	1.	0.	1.	2.	8.	12.
	1.	0.	1.	7.	14.	23.

Negative − (239) C. S. FLUID from Patient very ill, with terminal cerebral symptoms. P.M. No meningitis: No tumour.

Positive + (238) BLOOD SERUM from same patient when moribund. c.f. (41) always very toxic.

EPILEPTIC PATIENTS.

?	−	±	+	++	+++	
1.	11.	3.	2.	3.	2.	22.

Doubtful. ? (346) Anticomplementary.

± (235) NO symptoms. (c.f. 126).

(248) No symptoms beyond "Butterfly Rash" on face.

(254). Cervical adenitis.

Positive + (233) Very pale. No active signs of Phthisis.

(245). No active signs, but not robust.

++ (209) Healed R. apex. Cervical adenitis scars. c.f. (71).

(237) Hydrops Knee. c.f. (101).

(252) Very puny delicate child.

+++ (243) Signs of Healed Phthisis: Adenitis scars.

(247). Pleurisy: Adenitis scars: Died of Cenebral Tumour.

PRIVATE PATIENTS.

−	±	+	++	+++		
3.	1.	0.	1.	0.		5.

Negatives. All Non-tuberculous.

Doubtful ± (215) "Contact Case" 8 Brothers, 3 Sisters died of Phthisis. X-Ray exam. suggests early lesion of lungs.

Positive ++ (214) Phthisical active: Corroborated by X-Ray Exam.

SERIES VII. (255 - 304).

	No.	A.	B.	C.	D.	Sy.	Tb.	Class.	Sputum.	Clinical.	T.	V.P. H.	V.P. B.	Remarks.
*	255.	NH.	NH.	NH.	NH.	?	?			Anticomplementary				Epileptic.
*	256.	CH.	CH.	NH.	NH.	–	+++			Lungs) suspicions)				"
	257.	CH.	CH.	CH.	CH.	–	–			–				"
*	258.	CH.	CH.	NH.	NH.	–	+++			(Glands				"
										(Pleurisy.				
	259.	CH.	CH.	CH.	CH.	–	–							"
*	260.	CH.	CH.	ICH.	CH.	–	±			(Health Phthisis				
										(Both apices.				"
	261.	CH.	CH.	CH.	CH.	–	–							"
	262.	CH.	CH.	ICH.	CH.	–	±			Chilblains.				"
*	263.	CH.	CH.	CH.	CH.	–	–	L_3 S_3	TB. Nil.	Pleural) Effusions.)	–	–	–	Sanatorium.
	264.	CH.	CH.	CH.	CH.	–	–	–		No signs.				Private case.
	265.	CH.	CH.	NH.	ICH.	–	++	L_1 S_1	TB. Nil.	Cyanosis.	–	++	++	Sanatorium.
*	266.	CH.	CH.	CH.	CH.	–	–		None.	Synovitis				
										(? Sarcoma)	–	–	–	"
*	267.	CH.	CH.	CH.	CH.	–	–	–	None.	? Sarcoma.	–	–	–	"
*	268.	CH.	CH.	CH.	CH.	–	–	L_2 S_2	TB. Nil.	V. Toxic.	–	–	–	"
*	269.	CH.	CH.	ICH.	CH.	–	+			Amenorrhoea.				Epileptic.
*	270.	CH.	CH.	ICH.	CH.	–	+			Adenitis Scars.				"
	271.	CH.	CH.	CH.	CH.	–	–			--				"
*	272.	CH.	CH.	PH.	PH.	–	++			Chronic Phthisis				"
	273.	CH.	CH.	CH.	CH.	–	–			--				"
	274.	CH.	CH.	CH.	CH.	–	–			--				"
	275.	CH.	CH.	CH.	CH.	–	–			--				"
*	276.	CH.	CH.	PH.	ICH.	–	++			Fibroid Phthisis				
										R. Apex.				"
*	277.	CH.	CH.	ICH.	CH.	–	±			Healed Apices.				"
	278.	CH.	CH.	CH.	CH.	–	–			–				"
	279.	CH.	CH.	CH.	CH.	–	–			–				"

No.	A.	B.	C.	D.	Sy.	Tb.	Class.	Sputum.	Clinical.	T.	V.P. H.	V.P. B.	Remarks.
280.	CH.	CH.	CH.	CH.	–	–			–				Epileptic.
281.	CH.	CH.	CH.	CH.	–	–			–				"
282.	CH.	CH.	CH.	CH.	–	–			–				"
283.	CH.	CH.	ICH.	CH.	–	+	L_3 S_2	TB. Num.	Dyspepsia.	–	±	–	Sanatorium.
284.	CH.	CH.	ICH.	CH.	–	+	L_1 S_1	TB. Nil.	Larynx.	–	–	+	"
* 285.	CH.	CH.	ICH.	CH.	–	±	L_2 S_2	None.	Pleurisy (Trauma.)	–	–	+	"
286.	CH.	CH.	ICH.	CH.	–	+	L_2 S_1	TB. Nil.	Pleurisy.	–	–	–	"
287.	CH.	CH.	ICH.	ICH.	–	+	L_3 S_1	TB. Num.	Fibroid Phthisis.)	–	++	++	"
288.	CH.	CH.	NH.	NH.	–	+++	L_3 S_2	TB. Num.	Stomach.	–	–	–	"
289.	CH.	CH.	NH.	NH.	–	+++	L_3 S_2	TB. Few.	V. Pale.	–	++	+	"
* 290.	CH.	CH.	ICH.	CH.	–	±	L_3 S_2	TB. Num.	Larynx.	–	++	++	"
291.	CH.	CH.	NH.	NH.	–	+++	L_2 S_2	TB. Nil.	(Pains in Chest (Psoriasis.	–	–	–	"
292.	CH.	CH.	NH.	NH.	–	+++	L_2 S_2	TB. Few.	Undoubted Phthisis.	----------			Private case.
293.	CH.	CH.	ICH.	ICH.	–	+	L_2 S_2	TB. Nil.	Dyspepsia.	–	–	–	Sanatorium.
294.	CH.	CH.	PH.	ICH.	–	++	L_2 S_1	TB. Nil.	TB. Spine.	–	±	±	"
295.	CH.	NH.	PH.	ICH.	+++	++	L_2 S_2	TB. Nil	C.S., Fibroid.	+	+	+	"
296.	CH.	CH.	NH.	PH.	–	+++	L_2 S_1	TB. Nil.	Pale.	+	–	–	"
297.	CH.	NH.	PH.	ICH.	+++	++	L_3 S_1	TB. Nil.	Chronic disease.)	+	sl.	sl.	"
298.	CH.	CH.	PH.	ICH.	–	++	L_3 S_2	TB. Num.	V. Pale Toxic.	+	–	+	"
299.	CH.	PH.	NH.	ICH.	++	++	L_2 S_1	TB. Few.	Pleurisy.	+	sl.	–	"
300.	CH.	CH.	PH.	PH.	–	++	L_2 S_2	TB. Nil.	Adenitis:) Lupus.)	+	++	++	"
301.	CH.	NH.	NH.	NH.	+++	+++	L_2 S_2	TB. Nil.	Pains in Chest	–	–	–	"
302.	CH.	CH.	ICH.	ICH.	–	+	L_1 S_1	TB. Nil	Pale:Pleurisy	–	–	–	"
303.	CH.	CH.	CH.	CH.	–	±	L_2 S_1	TB. Scatt	Cyanosis.	+	+	+	"
304.	CH.	CH.	NH.	NH.	–	+++	L_3 S_2	TB. Num.	Dyspepsia.	–	++	++	"

ANALYSIS OF SERIES VII.

Reagents as for Series V.

SANATORIUM PATIENTS.

	−	±	+	++	+++	
L_1	0.	0.	2.	1.	0.	3.
L_2	1.	2.	2.	4.	3.	12.
L_3	1.	1.	2.	2.	3.	9
Non. Pulmonary.	2.	0.	0.	0.	0.	2.
	4.	3.	6.	7.	6.	26.

Negatives. − (266) (267). Sarcoma of Knee-Joint, sent in as "TB. Knee" SANGUINARY EFFUSION from Joint, and blood serum both tested.
(268) Very Toxic case, not doing well.
(263) PLEURAL EFFUSION used in place of Blood serum, of patient very ill with acute Phthisis.

Doubtfuls ± (285) Gassed at War: No sputum at present, Dountful case.
(290) Fairly toxic case: Undoubtedly phthisical.
(303) Phthisical. doing well at present.

EPILEPTIC PATIENTS.

?	−	±	+	++	+++	
1.	12.	3.	2.	2.	2.	22.

Anticomplementary ? (258).

Doubtfuls. ± (260) Healed Apex.
(262) No symptoms in chest, but sluggish circulation. Has chilblains.
(277) Quiescent Phthisis of apices.

Positives. + (269) No Chest symptoms: Anaemia, Amenorrhoea.
(270) Scars of C. Adenitis.
++ (272) Shews signs of past disease in lungs.
(276) do. do.
+++ (258) Pleuritic pains: Enlarged glands of neck.
(256) No dulness; but R.M. very poor over R. Lung.

PRIVATE CASE.

−	+++	
1.	1.	2.

Negative − (264) No signs. Healthy individual.

Positive +++ (292) T.B. in Sputum.

SERIES VIII. (305 - 365).

	No.	A.	B.	C.	D.	Sy.	Tb.	Class.	Sputum.	Clinical.	V.P. T.	V.P. H.	V.P. B.	Remarks.
	305.	CH.	CH.	PH.	ICH.	–	+	L_2 S_2	TB. Nil	Hectic.	–	++	++	Sanatorium.
	306.	CH.	CH.	NH.	NH.	–	+++	L_3 S_2	TB.Scatt.	Pleurisy: V. Pale	–	+	+	"
	307.	CH.	NH.	PH.	ICH.	+++	++	L_2 S_1	TB. Few.	Pale:Dyspepsia	*	+	+	"
	308.	CH.	CH.	ICH.	ICH.	–	+	L_2 S_2	TB. Nil.	Pleurisy.	–	±	–	"
	309.	CH.	CH.	ICH.	CH.	–	±			No Signs.				Epileptic.
*	310.	CH.	CH.	ICH.	ICH.	–	+			Phthisical.				"
*	311.	CH.	CH.	NH.	ICH.	–	++			No obvious disease.				"
	312.	CH.	CH.	CH.	CH.	–	–							"
*	313.	CH.	CH.	ICH.	ICH.	–	+			Mother died of Phthisis				"
	314.	CH.	CH.	CH.	CH.	–	–							"
*	315.	CH.	PH.	ICH.	CH.	+	+			ARgyrism.				"
	316.	CH.	CH.	CH.	CH.	–	–							"
*	317.	CH.	CH.	NH.	ICH.	–	++			No obvious disease.				"
	318.	CH.	ICH.	CH.	CH.	±	–			Child in poor health).				Private case,
	319.	CH.	CH.	PH.	CH.	–	+	L_2 S_2	TB. Nil.	Thin: Pale.	–	++	±	Sanatorium.
	320.	CH.	CH.	ICH.	CH.	–	+	L_2 S_2	TB. Nil.	Pale.	–	++	++	"
*	321.	CH.	CH.	PH.	CH.	–	+	L_3 S_3	TB. Few.	V. Ill.	–	–	–	"
	322.	CH.	NH.	NH.	PH.	+++	+++	L_3 S_3	TB. Few.	Dyspnoea.	–	+	+	"
	323.	CH.	PH.	NH.	ICH.	++	+++	L_2 S_2	TB. Few.	V.Chronic case.	–	++	++	"
	324.	CH.	CH.	NH.	PH.	–	++	L_2 S_1	TB. Num	Albuminuria.	–	+	+	"
	325.	CH.	CH.	NH.	NH.	–	+++	L_3 S_3	TB. Scatt.	Hectic.	–	++	++	"
*	326.	CH.	CH.	ICH.	CH.	–	±	L_3 S_2	TB. Num.	? Cerebral.	–	++	++	"
	327.	CH.	CH.	PH.	CH.	–	+	L_3 S_2	TB. Nil.	Pleurisy.	+	±	++	"
	328.	CH.	NH.	NH.	PH.	+++	+++	L_3 S_2	TB. Num.	Pale.	+	++	++	"
	329.	CH.	CH.	NH.	CH.	–	+++	L_3 S_2	TB. Num.	Cachectic.	–	+	+	"
	330.	CH.	CH.	NH.	PH.	–	+++	L_2 S_1	TB.Scatt.	Fibrosis.	+	++	++	"

	No.	A.	B.	C.	D.	Sy.	Tb.	Class.	Sputum	Clinical.	T.	V.P. H.	V.P. B.	Remarks.
	330.	CH.	CH.	NH.	PH.	–	+++	L_2 S_1	TB. Scatt.	Fibrosis.	+	++	++	Sanatorium.
*	331.	CH.	CH.	NH.	PH.	–	+++	L_3 S_2	TB. Num.	Larynx, C.S.	+	+	+	"
*	332.	CH.	CH.	CH.	CH.	–	–	L_2 S_2	None.	T.B. Knee.	+	+	+	"
*	333.	CH.	CH.	CH.	CH.	–	–	L_2 S_1	TB. Nil.	Cough.	+	+	+	"
	334.	CH.	NH.	ICH.	CH.	+++	+	L_2 S_2	None.	Cough. Ulcers of legs.	+	+	+	"
	335.	CH.	CH.	ICH.	CH.	–	+	L_3 S_1	None.	Cough.	–	+	+	"
	336.	CH.	CH.	NH.	PH.	–	+++	L_3 S_2	TB. Num.	Dyspeptic.	–	+	+	"
	337.	CH.	CH.	NH.	ICH.	–	+++	L_1 S_2	None.	Adenitis: Cough	–	+	+	"
	338.	CH.	CH.	NH.	ICH.	–	+++	L_2 S_2	None.	Neurasthenic.	–	++	++	"
	339.	CH.	CH.	NH.	PH.	–	+++	L_3 S_2	TB. Num.	Larynx.	–	++	++	"
	340.	CH.	CH.	PH.	CH.	–	+			No active disease				Private case
	341.	CH.	CH.	CH.	CH.	–	–			—				Epileptic.
	342.	CH.	CH.	CH.	CH.	–	–			—				"
*	343.	CH.	CH.	NH.	CH.	–	++			V. Pale. No symptoms.				"
	344.	CH.	CH.	CH.	CH.	–	–			—				"
*	345.	CH.	CH.	PH.	CH.	–	++			Congenital Cataract.				"
	346.	CH.	CH.	PH.	PH.	–	++			Active Phthisis.				Private case
	347.	CH.	CH.	NH.	PH.	–	+++			Active Phthisis.				"
	348.	CH.	CH.	NH.	PH.	–	+++	L_2 S_1	TB. Few.	C.S.	–	+	+	Sanatorium.
	349.	CH.	CH.	NH.	NH.	–	+++	L_2 S_2	TB. Nil.	V. Pale.	–	++	++	"
	350.	CH.	CH.	NH.	PH.	–	+++	L_3 S_3	TB. Few.	Cachectic.	–	–	–	"
	351.	CH.	CH.	PH.	PH.	–	++	L_3 S_2	TB. Few.	V. Pale.	+	+	+	"
	352.	CH.	CH.	NH.	PH.	–	+++	L_3 S_2	TB. Nil.	Hectic.	–	++	++	"
	353.	CH.	CH.	CH.	CH.	–	–			—				Epileptic
*	354.	CH.	CH.	PH.	CH.	–	+			R. Apex. Scoliosis.	+			"
*	355.	CH.	ICH.	NH.	PH.	+	++			Infantile Paralysis				Epileptic.
*	356.	CH.	PH.	PH.	CH.	++	+			Adenitis.				"

No.	A.	B.	C.	D.	Sy.	TB.	Class.	Sputum.	Clinical.	V.P. T.	V.P. H.	V.P. B.	Remarks.
* 357.	CH.	PH.	PH.	ICH.	+	++			(TB. Family history (Puny Child.				Epileptic.
358.	CH.	CH.	CH.	CH.	–	–			—				"
* 359.	CH.	PH.	ICH.	CH.	+	±			Healed L. Apex.				"
* 360.	CH.	PH.	PH.	PH.	+	++			Delicate.				"
361.	CH.	CH.	CH.	CH.	–	–			—				"
* 362.	CH.	CH.	PH.	CH.	–	+			(Adenitis. (Chest sl. active				Epileptic.
363.	CH.	CH.	CH.	CH.	–	–			—				Private case
* 364.	CH.	CH.	CH.	CH.	–	–	L_3 S_3	TB. Nil.	Pleural Effusion.	–	–	–	Sanatorium.
* 365.	CH.	CH.	CH.	CH.	–	–	L_3 S_3	TB. Num.	? Cerebral tumour.	–	++	++	"

ANALYSIS OF SERIES VIII.

Reagents as for series V.

SANATORIA PATIENTS.

	-	±	+	++	+++	
L_1	0.	0.	0.	0.	1.	1.
L_2	2.	0.	5.	2.	5.	14.
L_3	2.	1.	3.	1.	10.	17.
	4.	1.	8.	3.	16.	32.

Negatives - (332) TB. Knee.
(333) Cough, but never TB. in sputum.
(364). EFFUSION: Very ill, acute Phthisis c.f. (263).
(365) CEREBRO-SPINAL FLUID: Cerebral symptoms.

Doubtful. ± (326). Blood serum from same case as (365); very toxic.

Positive. + (321). Patient very toxic: c.f. (79) ++ when patient was not so ill.

EPILEPTIC PATIENTS.

-	±	+	++	+++	
9.	2.	6.	7.	0.	24.

Doubtful. ± (309) No signs of disease.
(359) L. Apex. healed.

Positives. + (310) Quiescent disease.
(313). No symptoms. TB. Family history.
(315) Argyrism.
(354) R. apex doubtful. Scoliosis.
(356) Cervical adenitis.
(362) Chest slightly active: Adenitis.

++ (311) Small and puny child.
(317) Apparently healthy. No active disease.
(343) Very pale, but no active symptoms.
(345) Phthinoid chest: Congenital cataract.
(355) Infantile Paralysis: R.M. weak over chest.
(357) Puny delicate child.
(360) Thin, flat chested: delicate; has chilblains.

PRIVATE CASES.

-	±	+	++	+++	
2.	0.	1.	1.	1.	5.

(340) No active disease at present. Very high coloured: had glands during childhood.

SUMMARY OF ANALYSES.

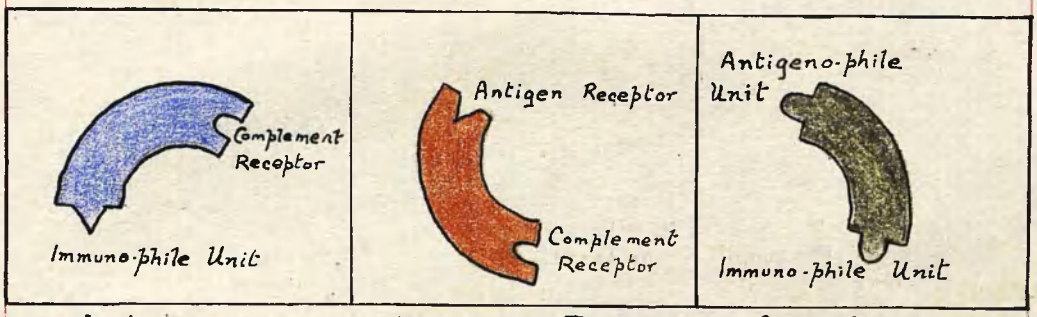
		?	-	±	+	++	+++	Totals.
SANATORIA PATIENTS.	Non-Pulmonary	0	2	0	2	0	0	4.
	L_1	0.	0.	1.	5.	4.	3.	13.
	L_2	0.	12.	4.	23.	31.	39.	109.
	L_3	0.	9.	3.	14.	18.	59.	103.
		0.	23.	8.	44.	53.	101.	229.
EPILEPTIC PATIENTS.		2.	61.	10.	12.	13.	5.	103.
ORPHAN HOMES CHILDREN.		0.	2.	0.	1.	2.	1.	6.
REJECTED EMIGRANTS.		0	6.	0.	4.	0.	1.	11.
PRIVATE CASES.		1.	7.	1.	3.	2.	2.	16.
	Grand Totals.	3.	99.	19.	64.	70.	110.	365.

Of 229 SANATORIA cases the (1) Positives Number 198 or 86.5%
(2) Doubtfuls " 8 3.5%
(3) Negatives. " 23. 10.0%

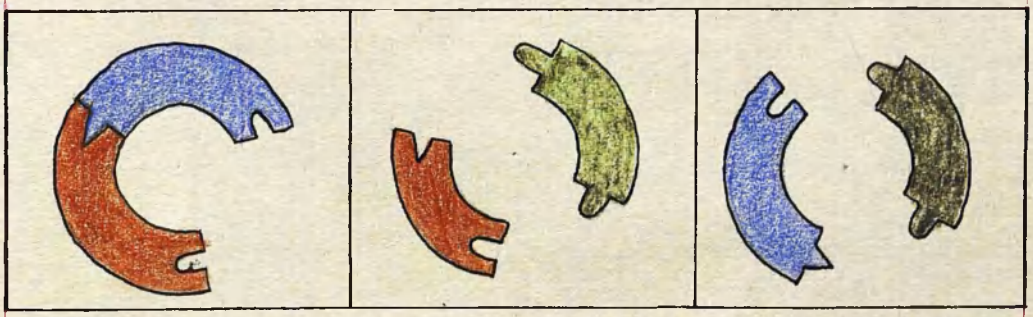
Of 103 EPILEPTIC. the (1) Positives number 30 or 29.1%
(2) Doubtfuls " 10. 9.7%
(3) Negatives " 61. 59.2%
(4) Anticomplementary 2. 2%

Of 33 MISCELLANEOUS cases the (1) Positives number 16 or 48.5%
(2) Doubtfuls " 1. 3 %
(3) Negatives " 15. 45.5%
(4) Anticomplementary 1 3 %

A SCHEMATIC REPRESENTATION OF FIXATION OF COMPLEMENT.



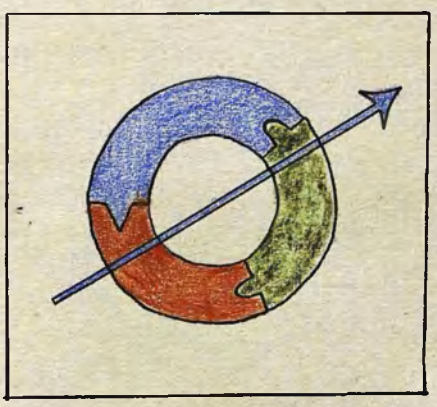
Antigen- Immune Body. Complement-



Immune Body linked onto Antigen, by virtue of its special Receptor Antigen is now "Sensitised".

Complement can only unite when both its "phile" units can be received at the same time into their respective Receptors; i.e. can only unite with a "Sensitized" Antigen.

ANTIGENOLYSIS.



Complement has been taken up, i.e Fixed, by Antigen-Immune Body System. Lysis of Antigen can now occur.

e.g. Bacteriolysis,
Haemolysis,
Cytolysis. &c.

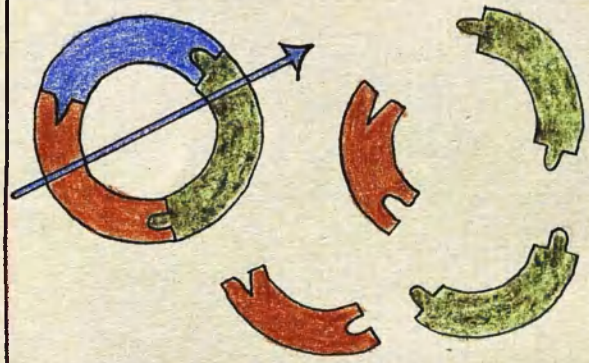
A SCHEMATIC REPRESENTATION OF NORMAL AND TUBERCULOUS SERA.

1.

NORMAL SERUM.

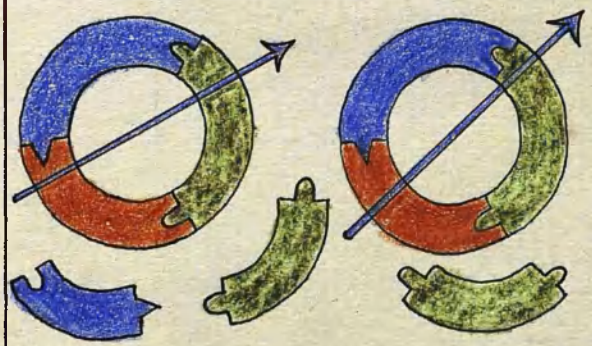
No Antigen- (Bacteria)
No Immune Body-
Complement, however, is normally present.

2.



TUBERCULOUS SERUM.
(of patient "doing well".)
Complement.
Excess of Immune Body.
Bacteria unable to proliferate to any great extent-
Bacteriolysis therefore Slight, consequently Toxaemia mild.

3.



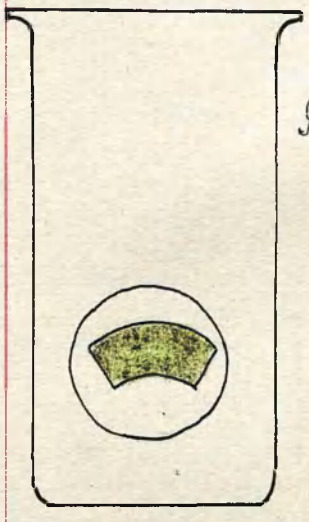
TUBERCULOUS SERUM.
(of patient who is "very ill.")
Complement.
Bacteria have upper hand.
Marked degree of Bacteriolysis, therefore Severe toxaemia.

In the ensuing diagrams sera of types 1 and 2 only are portrayed. Type 3, owing to the relative absence of Immune Bodies, will give results in the Complement Fixation Tests similar to type 1.

A SCHEMATIC REPRESENTATION OF THE COMPLEMENT FIXATION TEST.

Normal Serum - Immune Serum.

1.



Inactivation of Serum -

Complement is rendered inert.

2.

Addition of Antigen -

Antigen links onto Immune body if latter present.

3.

Addition of Fresh Complement -

Complement united with "Sensitised" Antigen.

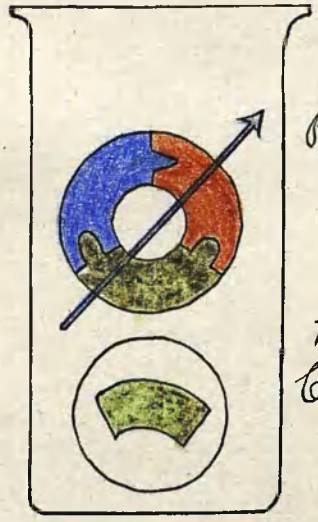
CONTINUATION OF SCHEMA.

Normal Serum. Immune Serum.

4.

Incubation of Reagents.

Complement is still free.



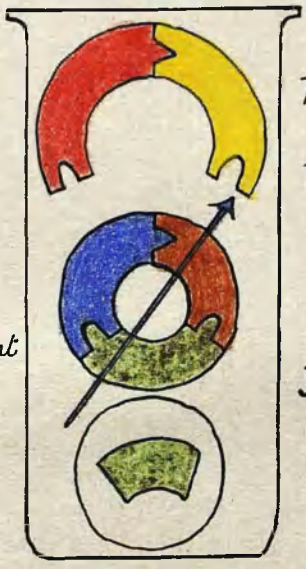
Bacteriolysis.

No free Complement now.

5.

Addition of Sensitised Red Cells.

Free Complement is able to unite.



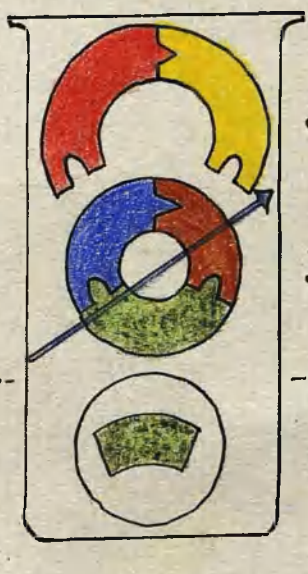
No free Complement remains to unite with the Haemolytic System.

6.

Incubation.

Haemolysis is effected.

-Negative Reaction-



Therefore No Haemolysis.

- Positive Reaction-

IV. CONCLUSIONS FROM THE ANALYSES.

A. ARGUMENTATIVE.

1. SOME PRINCIPLES IN IMMUNITY. Before drawing conclusions from the analysis of the 365 tested sera, it will be advantageous to discuss more fully some of the principles of Acquired Immunity.

Two forms of immunity are to be differentiated:-

(i) ANTIOXIC IMMUNITY, where substances are formed in the host to neutralise the poisons secreted and excreted by the infecting bacteria.

(ii) ANTIBACTERIAL IMMUNITY in which the reactive antibodies are directed against the bacteria alone. To this latter group belongs the bacteriolysins which effect dissolution of the bacteria. By their action, although the 'casus belli' is removed, endotoxins having the nature of heterogeneous albuminous material are liberated into the circulation from the bodies of the bacteria. Where a great proliferation of bacteria has occurred prior to lysis, the amount of toxins freed by their death may cause so excessive a toxaemia as to render the continuance of life impossible.

The virulence of the bacteria becomes evident only after their death; during their propagation no subjective symptoms are experienced. As WOLFF-EISNER says "only by dissolution of the bacteria, can death (of the host) occur at all."

"PFEIFFER/

"PFEIFFER has likened this to a serpent biting and killing after its head has been struck off."

Under favourable conditions the bacteriolytic antibodies are produced in sufficient quantities to kill off the bacteria before they are able to proliferate to any great degree. The amount of endotoxins freed then is relatively small, and only mild symptoms of toxaemia are caused, e.g. pyrexia, sweating, malaise, anorexia &c.

However, should the lysins not be produced in sufficient quantities to check proliferation of the bacteria, the outlook is bad, in-as-much as once the protective forces are formed, large quantities of bacteria undergo dissolution, and consequently the amount of heterogeneous albumen liberated into the circulation is very great. Moreover, since the lysins are used up by the "self-sacrificing" bacteria, the others are enabled to proliferate, and so the vicious cycle continues.

In tuberculosis, as in syphilis, the antibodies are formed somewhat slowly, and a considerable period must elapse after the infection, before lysins can be demonstrated to be present in large quantities.

When dealing with healthy, non-tuberculous individuals, even after a long course of tuberculin treatment, the amount of lysins demonstrably present in the serum is very small. Our inability to produce an artificial immunity to tuberculosis in healthy people is unfortunate, as were it otherwise, by legal enforcement of tubercle-vaccination of all infants, the "white scourge" might be eliminated

in/

in one generation.

However, an immunity to tuberculosis can only be obtained by a previous infection. The presence of the tubercle bacilli in the system gives rise to the formation of antibodies which persist long after the former have become "walled off" by a local reaction on the part of the tissues to the source of irritation. The bacteriolytic substances which remain afford a high degree of immunity; "a protection, however which has been well likened to the arsenal of a fort, in which the protective power may at any moment be transformed into a destructive force, by an explosion.

Under the influence of psychical and somatic injuries, the latent processes may at any time become active."

2. METHODS OF DEMONSTRATING THE PRESENCE OF BACTERIOLYSINS.

The action of these antibodies on bacteria may be DIRECTLY demonstrated by PFEIFFER'S bacteriolytic experiment. Into the peritoneal sacs of several guinea-pigs is injected a mixture of bacteria and immune-serum. After varying intervals the animals are killed and the injected material examined microscopically. The bacteria are then seen to become first swollen, then globular, then their outlines become indistinct, until finally they can no longer be observed.

The complement fixation test is an INDIRECT method of ascertaining the presence or absence of lytic substances, according as the complement is "fixed," or not.

3. BIOLOGIC METHODS OF SERO-DIAGNOSIS. These are important as they can be conducted in vivo. These methods/

methods are based on the introduction of small quantities of the antigen - tuberculin - into the system, when if lytic antibodies are present a definite reaction will be obtained for the dead bacteria will be resolved from non-toxic substances into poisonous material of a heterogeneous albumen nature.

A short digression may elucidate this. To the normal individual pollen dust is non-toxic, but to the susceptible - i.e. those prone to Hay Fever - whenever such dust is inhaled lytic bodies come into play and form toxic material which gives rise to very definite symptoms. Such individuals are said to shew a reaction of HYPERSENSITIVENESS. "Hypersensitiveness is the exaggerated response by the organism to an excitant, after the same excitant has acted one or more times."

Similarly in the tuberculous then, owing to the presence of tuberculo-bacteriolysins in the serum, the introduction of an antigen will cause a hypersensitive reaction, be it local, focal, or general.

There are various methods of introducing the antigen, but only one need be mentioned, as the results from this biologic test alone have been tabulated in the analysis. It is the CUTANEOUS TEST OF VON PIRQUET. A small scarification is made on the surface of the skin, and a drop of Koch's old tuberculin rubbed in, the object being to obtain a local erythematous reaction whereby the presence of lytic substances may be indicated.

Unfortunately the hypersensitive reaction obtained is seldom a criterion as to whether the infection is latent or active, as the lytic substances remain in the circulation long/

long after the original lesion has been healed.

Positive results then are frequently obtained in the apparently healthy, and as LANDIS writes, "it must be clearly kept in mind that there is a great difference between Tuberculosis that is clinically recognisable, and a hypersensitiveness to tuberculin. Hypersensitiveness is extremely common and in encountered in a large proportion of healthy people, the frequency with which it is met increasing rapidly from the second decade."

A reaction, however, in a healthy individual certainly does argue a previous infection of a tuberculous nature.

On the other hand a negative reaction with the Von Pirquet test does not necessarily indicate an absence of active tuberculosis, for such negatives, as will be noticed in the analysis, are frequently obtained among the tuberculous. It is usually of bad omen. It cannot be said that it is due to an entire absence if lytic bodies, for not infrequently weakly positive readings are obtained by the complement fixation method. It is the failure to react any longer to the antigen.

4. A COMPARISON OF THE METHODS OF SERO-DIAGNOSIS.

Positive readings obtained by either the complement fixation, or the biologic method, indicate the presence of the same lytic reactive substances in the serum.

The complement fixation method is a crude one, for where only relatively small amounts of lytic antibodies are present, doubtful positive or definitely negative readings will be obtained.

Such small amounts, however, would normally be sufficient/

sufficient to cause reactions to the biologic methods. On the other hand the organism may shew an inability to react biologically to the introduction of the antigen, and yet lytic bodies still be present, and demonstrably so, by the complement fixation method.

Again, positive readings may be obtained by both methods, in individuals no longer suffering from active disease, even after the lapse of many years.

5. A.COMPARISON WITH THE WASSERMANN TEST.

Unlike the positive results obtained with sera of healed-tuberculous individuals, in healed syphilis, negative readings are almost always recorded. Possibly antibodies are present after complete recovery from syphilis, but they must be in small quantities, or else tend rapidly to disappear, in which case the immunity is short lived.

Again, malignant cases of Syphilis give very doubtful positive, or negative readings. This, however, is to be expected, as the lytic bodies never obtain the upper hand, and consequently are never present in relative excess.

This same phenomenon is obtained in Tuberculosis. Generally speaking however, a positive Wassermann reaction is indicative of active syphilis, though a negative reading by no means argues an absence of the spirochaete, for negative readings may be transformed into positives by the influence of mercurial treatment.

Nor is a negative reaction in active disease of such serious portent as it is in tuberculosis.

In syphilitics, it is possible that mercurial therapy acts either by destroying the antigenic spirochaetes without the aid of the bacteriolytic antibodies, or by stimulating

an/

an added formation of the latter.

B. THE VALUE OF THE COMPLEMENT FIXATION TEST IN TUBERCULOSIS.

1. IN PULMONARY TUBERCULOSIS. From the statistics appended to the analysis of 365 tested sera, the value of this test as a diagnostic means cannot be gainsaid.

Of the 229 sanatoria patients, many have all along failed to shew tubercle bacilli in their sputa, yet without doubt are for the most part frankly tuberculous. This is borne out by the test:- 86.5% positive readings were obtained.

(a) Early cases of Phthisis, who are doing well, and bear no history of previous infection, give readings + and ++. The relative paucity of lytic antibodies present argues that for their formation a considerable interval of time must elapse. Usually the longer the period which has elapsed from the onset of their illness, the stronger will be the positive reading.

(b) Cases of Phthisis, graded L_2 and L_3, but who are reacting well to treatment, almost without exception give +++ readings. It is clinically noticeable that the toxaemia in these individuals is sufficiently mild to permit of their performing 4 hours manual labour a day in addition to their household duties, without producing any appreciable untoward results.

(c) On the other hand Pulmonary cases L_2 and L_3 who are not improving but rather on the retrograde, shew a dimination in the amount of lytic antibodies.

This/

This is borne out in the case of a patient whose serum was tested both before and after an acute exacerbation. Prior to the spread of the disease the reading was ++, following it only +; see (79) and (321).

(d) From the same series of patients 10% negatives, and 3.5% doubtful readings were obtained. Among these, however, were sera of non-tuberculous individuals suffering from (1) Bronchiectasis, (2) Sarcoma of knee-joint admitted as a supposed case of tuberculosis of knee-joint, and (3) interstitial pneumonia following (a) traumatic empyema, and (b) failure of resolution in a lobar pneumonia.

Some of the sera of this last mentioned class gave doubtful readings and where a previous tuberculous infection can be negatived, it is possible that an active tuberculosis is now being superimposed. Such patients are therefore being treated as ordinary consumptives. So far, it must be admitted however, their sputa have failed to shew the presence of the tubercle bacillus, which after all is the supreme criterion.

(2) There is however, excluding these, still a margin of frankly tuberculous patients not infrequently shewing numerous bacilli in their sputa, who not-with-standing gave weakly positive or negative results, both to the von Pirquet and to the complement fixation methods. These almost without exception, shewed severe degrees of toxaemia. The doubtful, or frankly negative results obtained argue a scarcity of lytic substances in the serum. This is to be expected on the grounds already explained in detail, because asrapidly as they are being formed the lytic/

amboceptors are being used to effect dissolution of the active bacteria, and this destruction by introducing large quantities of toxins into the circulation, gives rise to symptoms of profound toxaemia.

2. IN NON-PULMONARY TUBERCULOSIS. It is regrettable that only a few cases of purely non-pulmonary tuberculosis were available. Frequently, however, the pulmonary symptoms were more or less in abeyance and the extra-pulmonary lesions were the chief source ofcomplaint. A careful analysis of such cases shews that lytic bodies are not produced in great quantities when the lesion is localised, but when in addition there are signs of active pulmonary disease then the antibodies are usually found to be present in larger quantities.

(a) Tuberculosis of skin - LUPUS - see (166), (204) (228), and(300) the readingsaverage a ++ result.

(b) Tuberculosis of glands - CERVICAL ADENITIS, see (60), (148), (175), (176), (190), (232), (337), for the most part ++ and +++ readings were obtained. In all of these there was a concomitant pulmonary condition. Serum (74) from a case of healed adenitis with no lung affection gave a reading + .

(c) Tuberculosis of Bones. POTT'S DISEASE WITH POSOAS ABSCESS. see (156), (182), (294). These all gave strongly positive results.

(d) Tuberculosis of Joints - KNEE - see (121), (123) strong positive readings were obtained.

HIP. Both cases recorded were healed; (143) gave - result, while (223) which still had active pulmonary symptoms gave a ++ reading.

(e) Tuberculosis of Mamma - RETRO MAMMARY ABSCESS. -

Case/

Case (145) amputation of the breast had been performed previously, and the patient gives a tuberculous family history, a positive reading was obtained.

(f) Tuberculosis of Ischio-rectal Fossa - PERINEAL ABSCESS. - see (53) and (76). Both these cases had active pulmonary disease. The former was extremely toxic and in consequence gave only a weakly positive reading, the latter was responding well to treatment, and the positive result recorded was correspondingly strong.

3. IN EPILEPTICS USED AS CONTROLS. As the epileptic patients were for the most part apparently free from active tuberculosis, at first considerable disappointment was experienced when positive readings were obtained, all the more so as with definitely known normal sera double the dose of antigen employed in the test proper, gave negative results, and a titration of the complement absorbing bodies of the epileptic sera shewed no undue anticomplementary action except where noted.

Notwithstanding these facts, nearly 30% of the sera gave positive readings, all errors of technique negatived by a repetition of the tests.

A careful clinical examination of the patients was then made, and further light was obtained. Most shewed definite signs of former disease, either glandular - evidence the cervical scars, or pulmonary - evidence the apical contraction of the lungs. Some indeed shewed signs of slightly active disease. Mention may be made here of the results observed from two sera; the one (270) from a woman who has recovered from cervical adenitis within the last 3 years, her serum gave/

gave a ++ reading; the other (209) from a man who had a cervical adenitis and an apical pulmonary lesion during adolescence, but who claims to have been free from disease for over 15 years gave a similar reading. From such results it is inferrible that an immunity to Tuberculosis is of long duration.

It is of interest to point out that both the patients whose sera proved anticomplementary, have received a prolonged course of Silver Nitrate medication, and now shew signs of Argyrism.

4. CONCERNING SERA OBTAINED FROM PLEURAL EFFUSIONS.

In place of testing blood serum, on three occasions serum from pleural effusions was employed. Case 184 - gave a positive reading (one +), and a special annotation made on the day of the test points out that the reading was sharp and stable, and that the serum employed, itself absorbed no more than 1 M.H.D. of complement.

On the other two occasions the effusions were aspirated from the same patient (263) and (364), who was rapidly going downhill and is now deceased. Negative results were both times recorded. This individual was too ill to allow of vein-puncture, but it is most probable that a negative reading would also have been obtained from the blood serum, certainly the von Pirquet test was negative.

It has been suggested that when an effusion is poured out, at first the concentration of lytic bodies present in it is practically the same as in the blood serum, but later they become readsorbed into the circulation or are used up in causing dissolution of the bacteria present in the pleural cavity/

cavity, and the concentration is therefore weaker.

It was unfortunate that we were unable to pursue this line of research, but the condition of the patient did not permit.

5. EXAMINATION OF THE CEREBRO-SPINAL FLUID.

Only three specimens from cases showing cerebral symptons were examined and the readings were all negative. Little however can be argued from these results, as a post-mortem examination on (150) shewed the presence of a sarcomatous tumour over the Corpus Callosum; case (239) also died and in the post-mortem examination held, no evidence of either a meningitis, encephalitis, or tumour was present. Possibly the symptoms were those of a toxic "meningismus". At the same time the serum was tested, (238), and a result only weakly positive was obtained. This patient had advanced pulmonary disease, and at the time of withdrawing the fluids, the toxaemia was profound. The third case (365) is living and the cerebral symptoms are now in abeyance. The reading from this test was also negative.

It is probable that except with an early case of a tuberculous meningitis or tumour, a negative reading would be obtained, owing to the relative paucity of the lytic antibodies present in the more severe stages.

c. SUMMARY OF CONCLUSIONS.

1. No authoritative statement either prognostic or diagnostic, regarding the presence of a tuberculous lesion, should be made from the readings obtained from a complement fixation test until the clinical facts have been considered.

Notes/

Notes of the history, and the findings of an examination of the patient, should accompany each serum to be tested.

2. Frankly negative readings do not necessarily indicate an absence of tuberculosis, but merely that the immune bodies are not demonstrably present. The omen is bad when clinical symptoms and signs of an active tuberculosis are present, and the prognosis should be made accordingly.

3. Other things being equal, when the dose of antigen employed in the test is half that amount which will still give a negative reading with a normal non-tuberculous serum, and when the serum to be tested is not in itself unduly anti-complementary, then a doubtful ± reading is usually indicative of the presence of small quantities of lysins. In other words, it is a very weak positive.

Unless the symptomatology presented by the patient be in accordance, too much value should not be attached to such a reading, and in any case it is wise to repeat the test.

4. Positive readings +, ++, and +++ are all indicative of the presence of bacteriolysins in the serum, and where there are symptoms of active disease, such readings are of great diagnostic value.

5. In cases in whom clinical signs and symptoms are in abeyance, a positive reading is by no means of bad portent, for its presence argues an immunity.

The patient, however, should be warned that overstress of any form may cause the latent processes to become active.

6. Early cases of Pulmonary Phthisis L_1 usually give positive reactions to this test, even long before the presence of tubercle bacilli can be demonstrated in the sputum. Also in the so-called cases of "closed" tuberculosis /

tuberculosis this test is of immense value.

7. A pleural effusion, especially if it be of recent origin, may be tested in place of the blood serum, when the results obtained will be practically identical with those from the latter.

8. Even a prolonged course of tuberculin treatment has LITTLE effect on the strength of the reaction obtained. Negative and weakly positive readings have been got from tuberculous patients who have received many subcutaneous injections of tuberculin. It has already been mentioned that it is practically impossible to obtain an immunity by means of this form of treatment. It is the tuberculin which patients absorb from their own fomi that gives rise to the formation of antibodies.

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