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THE RELATIONSHIP OF THE "ACID-FAST" BACILLI.

Fred. T. Wills M.B.T.C.
(Edin.)

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Introductory.

Before entering upon our work and its results, we consider it necessary, at once, to offer some explanation under this head, of the very valuable information which has so materially assisted in the work undertaken under the different sub-heads of this thesis. We desire, too, strongly to express here that it is the object of this paper only to record the results presented and information gathered in the course of our work with the "Acid-fast bacilli."

I.

The relationship of the "Acid-fast" bacilli depends not only on their morphological character, but also on their

1. Biological reaction
2. Chemical consistence.

Albuminous bodies — Fatty bodies.

Neutral fat. — (1) Fatty acids.

(2) Lipoids.

Formerly it was believed that only the Albuminous bodies were specifical and able to produce specific

anti-bodies. But recent experiments have shown that even Lipoids are able to produce specific anti-bodies. And still greater progress in our knowledge of the formation of anti-bodies was made when Much[1.] discovered that even purely fatty substances are able as well to produce specific anti-bodies as Albuminous substances.

The first fatty substance that was proved to produce specific anti-bodies was Nastin (Deycke).

When Deycke published his experimental work upon Nastin, the medical world gave greater attention to the therapeutic aspects, and correctly so, than to the merely biological importance of his results.

That a purely neutral fat in crystalline form is able to give a biological reaction in the living organism, not only against leprosy, but even against tuberculosis.

The above fact has been proved by the injection of Nastin into both leprous and tuberculous patients, and in both cases strong reaction was obtained that even could prove dangerous in these cases.

A similar substance to Nastin, prepared from the tubercle bacillus, and called tuberculo-nastin, gives, not only reaction in cases of tuberculosis, but also in leprosy.

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1. Much: Nastin, Munchen, Med. Woch, 1909.

II.

Deycke and Much have established, by further investigations, that they could never obtain such immunity with the Albuminous substance alone of the tubercle bacillus, but only when they mixed the albuminous bodies and the fatty bodies, i.e. Nastin, that they could establish immunity. They say, too, that the immunity obtained by tuberclo-albumen with Nastin is easier to be produced than with Nastin alone.

III.

The fat anti-bodies could be demonstrated in vitro with the aid of that subtle serological method, viz., "the complement fixation.test."

Much found in patients that have been treated with Nastin, complement-fixating bodies against Nastin, while other leper patients that had not been treated with Nastin did not give the same reaction.

After Much had found that leprous cases that had been treated with Nastin (Deycke) gave "complement fixation" against an emulsion of this pure fatty substance, Klein-Schmidt, in Much's laboratory, tested sera of lepers that had been treated with Chaulmoogra oil against an emulsion of this oil. Of five lepers who were undergoing the treatment, two gave a marked positive reaction with an emulsion of Chaulmoogra oil 1:1500. This, we believe, to be the second instance of formation of specific anti-bodies against a

purely neutral fat.

It is very remarkable that the biological activity of these fatty substances (Nastin and Chaulmoogra oil), which have been shewn, by the formation of specific anti-bodies in leprous sera after treatment, should correspond so well with their clinical effects and therapeutic efficiency.

IV.

From the above-mentioned work of Much, some relation appears to exist between leprosy and tuberculosis. The relationship of all acid-fast bacilli has been the subject of further investigations of Much and Hoessli.[1.]

In their "complement-fixation" reactions, they used as antigen for the first time, not the usual bacilli emulsion of Koch, but emulsions of acid-fast bacilli in physiological salt solution and 0.5. per cent Phenel-glycerine, which they themselves prepared in the agate mortar.

They tested human sera that gave "complement-fixation" with tubercle emulsion and tuberculin, against all the following non-pathogenic acid-fact bacilli:-

Urine bacillus, Blind-Worm tubercle-like bacillus (as occur in reptiles), Timothy grass bacillus; - a description of these bacilli appears under Part I.

They found that the sera, that gave positive reaction with tuberculous antigens, gave also a positive reaction with these non-pathogenic bacilli; there was only a

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1. Much and Hoessli; Tuberkulosi studien Beitr 7. Klin. d. Tuberkulosi, 1910.

quantitative difference, i.e. sera that gave a strongly positive reaction with tuberculous antigen, gave only slightly positive reaction with the non-pathogenic, with the exception of Timothy grass bacillus.

Of 19 Sera tested, against:-

Tubercle bacillus	19	positive
Urine bacillus	13	"
Blind Worm bacillus	4	"
Timothy Grass bacillus	0	"

The conclusions they arrived at were:- (1) That the specific complement-fixing substances of tubercle bacillus are the same as those of the other related non-pathogenic, and that the tubercle bacillus only differs quantitatively, i.e., possessing a larger amount of specific substances. (2) The complement-fixation reaction with tuberculin does not always give the same results as the complement-fixation reaction with tubercle bacilli emulsions. Only in 57.6% the reaction is positive with both antigens, nearly the same per centage, i.e., 58.4% has been obtained by Deilman in 118 cases so treated. This difference has to be explained by the difference in quantity of specific substance in the different antigens. Much and Hoessli even found that different tuberculins, e.g., tuberculin, Koch, and tuberculin, Behring, did not always give the same reaction.

V.

"The Specificity Complement-fixation with Tuberculosis Sera." Since the first complement-fixation reaction with tuberculin (Wasserman & Citron), the question has arisen as to whether the complement-fixation is specific or not.

Citron,[2] in a paper entitled as the above stated as his opinion, that the complement-fixation reaction is of practical value in the diagnosis of tuberculosis, when the other methods of clinical diagnosis do not give sufficient certainty.

Cohn,[3] Wolff and Muhsam,[4] Weil and Strauss,[5] found antibodies against tuberculin in the sera of grave cases of tuberculosis as well as in the beginning of the infection which they think to be specific, while others, for instance, Wolff-Eisner and Ascher,[6] Laub and Novotny,[7] hold that complement-fixation with tuberculin is an entirely non-specific reaction. Klein-Schmidt,[8] who in the laboratory of Much, was the first to do complement-fixation, not only with tuberculin, but also with tuberculo-nastin, and Nastin (Deycke), came to the conclusion that "the complement-fixation with tuberculin and tuberculo-nastin is specific as occurring only in cases of tuberculosis and leprosy."

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2. Berlin Klin: Wochen, 1907, No. 36.
3. ibid 1908, No. 28.
4. Deutsche Med. Wochenshrift, 1908, No. 35.
5. Wiener Klin. Wochenshrift, 1908, No. 28.
6. ibid, 1908, No. 37.
7. ibid, 1909, No. 31.
8. Berlin Klin. Wochenschrift, 1910. No. 2.

Much and Hoessli have by their experiments found that the complement-fixation reaction against tuberculin with tubercular patients has to be divided into two classes - Firstly (a) The positive reaction of patients that have not been treated with tuberculin injections is causes by specific anti-bodies in the serum, these specific anti-bodies are produced by substances that are common to all acid-fast bacilli. (b) These sera never give reaction with non-specific antigens, as Peptones, Albumoses, or Bouillon.

Secondly, The positive reaction in cases that have been treated with tuberculin is caused by specific and non-specific anti-bodies (a) the specific anti-bodies are the same as in the cases that have not been treated by tuberculin (b) the non-specific anti-bodies are produced by the non-specific albumoses of tuberculin. These sera therefore give a positive reaction with peptone solution or bouillon, which is much weaker than the reaction against tuberculin.

We have dealt above with cases of specific anti-bodies in the sera of tuberculous patients, but how are we to explain the fact that these same anti-bodies appear in the sera of adults who have no signs of tuberculosis.

Much with Hoessli in their paper on the subject say, "the investigations of recent years in the field of tuberculosis, have clearly proved that specific reactions do not always express the fact of clinical tuberculosis, we refer to the excellent works of Roemer and to our own researches. If

we get a positive reaction, it is not at all proof that the individual is tuberculous; the person might be only immunized.

The interesting chapter of self immunization against tuberculosis and of latency have clearly proved that a biological reaction of tuberculosis does not always correspond to clinical tuberculosis. We know that even in cases of no clinical tuberculosis the biological reaction of tuberculosis might be positive in a specific sense, i.e., in the sense of self immunization or latency. A positive reaction only means that the individual has come once at least in his life into contact with tuberculosis virus. The data of this coming into contact could only be given by the physician and not the biologist."

The researches of recent years have shewn that with the aid of our most delicate biological reaction of tuberculosis, i.e., by the cutaneous reaction, we obtain a positive result in about 98% of all adults, say from the 15th year; therefore, we cannot be surprised to find in another biological reaction where the same antigen is used against the same anti-bodies, i.e., the complement-fixation, - positive results are obtained in a large number of persons that have no clinical signs of tuberculosis.

Deilman, in our laboratory, has recently tested 238 sera of various persons, and has obtained 118 positive reactions with tubercuIous antigens, i.e., 50%

The fact that the complement-fixation does not give similar high results as the more delicate cutaneous reaction (although it is caused by the same anti-bodies) is to be explained by the existence of haemolysing bodies in the sera; if we take out these Haemolysing bodies by the method that has been worked out by Holmgreen, in the laboratory where we did our work, there is at once a mugh higher percentage of positive reactions of the sera of all adults.

We need not repeat that these complement-fixations with sera of persons shewing no signs of tuberculosis, are entirely specific. We refer to what has been stated above by Much and Hoessli.

Part 1.

The "Acid-fast" Bacilli:- Tubercle, - Leprosy, - Urine, - Blind Worm, - Timothy Grass.

Tubercle bacillus. A description is not necessary in this paper, the bacillus is already widely known, and full details are stated in every text book on bacteriology.

Leper bacillus. The remarks above on the tubercle organism also apply to leprosy; no useful purpose would be served by a repetition of well established facts.

Of the other organisms which we are to deal with, as they are perhaps not so widely known as the above two of the acid-fast group, a short description of them might be useful.

Timothy Grass bacillus[1] of Moëller, isolated from a grass (Phleum arvense), - acid-fast, - grows readily on culture media, - and not so acid fast as the tubercle bacillus.

Urine bacillus: Is very rare.

Culture: On the usual Agar and Bouillon, - grows readily in a few days.

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[1] Hewlett, - Manual of Bacteriology, 3rd ed., p 316.

On tubercle Bouillon, - grows in 3 to 6 days. The culture-growth in bouillon forms a creamy, thick, coherent, dry, crinkled layer on the surface, and it climbs up the wall of the flask. It must float on the surface in order for growth to take place, and from the surface stallactite-like growth are sent down into the bouillon.

Pathogenicity - for rabbits not virulent; for guinea pigs, in usual dose, it is not virulent, but by large doses, viz., 0.4 grams given intra-peritoneally, death quickly takes place from acute plastic peritonitis, - no nodules are found in the organs.

Origin: Found in a case of normal urine.

Blind Worm bacillus. The culture that was used in our experiments had been found in a reptile (blind-worm). This tubercle-like bacillus of reptiles appears in German works as Blind-schleiche - Blind Worm or Slow Worm. For the purposes of this paper, the term Blind Worm bacillus has been adopted throughout for this tubercle-like bacillus of reptiles.

Culture: Grows only on tubercle bacillus bouillon, - culture-growth resembles that of the urine bacillus.

Pathogenicity - Not virulent for warm blooded animals.

All the above bacilli resemble one another,

being like the tubercle bacillus.

1. (a). In form.

   (b). In variety of form, - shew beaded or granular form, - seen in old cultures.

2. Staining reaction:

   (a). Acid-fast.

   (b). Red Colour with Carbol-Fuchsin.

   (c). All are Gram-positive.

   (d). All stain well by Much's modification of Gram, - the Gram-Much being particularly suitable for the granular forms.

Part 2.

The "Acid-fast" Bacilli - their behaviour and relationship in the reaction of complement-fixation.

The important character of the staining reactions and the outward resemblances, microscopically, supply a certain amount of information as regards their relationship.

The writer, working with some of the "acid-fast" bacilli, viz., Tubercle, Leprosy, Urine, Blind Worm and Timothy Grass, desires to record the results obtained with these bacilli; the object in view, as already mentioned, is to state the facts which experiments with the five bacilli just named have shewn, that not only do these organisms - though differing so widely in the very important factor of pathogenicity - exhibit by their biological reaction, and by their chemical consistence with serum, certain definite characteristics which point towards a relationship stronger than mere staining resemblance and outward appearances, but also a kinship from a common stock.

1. Complement-fixation of human sera with acid-fast bacilli as antigen, viz., Tubercle, Leprosy, Urine, Blind Worm and Timothy Grass.

Remarks as to the method of complement-fixation - The bacillus or substance to be used as antigen is made into an emulsion and first tested for self-fixation according to Wasserman, and the "titre" obtained in the usual manner.

The sera and haemolytic system are of course used and prepared after the manner of "Wasserman reaction".

Deilman working at the same time in the laboratory, the writer has had ample opportunities of access to his work at all times, as it would have only meant duplication of labour with similar results, and as Deilman so kindly gave the use of this part of his work, it was considered not necessary to go over the field. His examination of serum was made from time to time, and covered the large number of 238 cases. The method Deilman adopted was (1) the serum was tested by complement-fixation for anti-bodies of tuberculosis, the antigen used being tubercle bacilli emulsion (0.075) and tuberculin (Koch) (0.04). (2) All the sera giving a positive reaction were selected for further examination. (3). The

complement-fixation test was next tried with all the sera showing tuberculosis anti-bodies, against emulsions of Leprosy bacillus, Urine bacillus, Blind Worm bacillus, and Timothy Grass bacillus.

For the purpose of this paper, 18 of the sera so dealt with and the results, are submitted in Table 1.

In Table 1., as would be expected, the tubercular antibodies stand out prominently. This is as it should be, for it would be impossible to conceive, in countries where tubercular disease is very old and widespread, that anti-bodies should not be present in the serum without the presence of active tuberculosis; but it is not our purpose to discuss the specific nature of the anti-bodies of the tubercle organism; much has already been stated in the introductory remarks. Our undertaking is to point out that in so far as the various "acid-fast" bacilli named are concerned, they shew a relationship other than staining reaction and similarity in outward form.

It is to be noted that with the exception of Blind Worm bacillus, which shows only a slight reaction in a single instance of the eighteen sera, all the other bacilli give a strong positive reaction with more than one of the series.

Timothy Grass bacillus, as would be observed, gives

two strong positive reactions, and on each occasion of fixation, the result is similar to that of tuberculin and the tubercle bacillus with the same serum.

Urine bacillus, strongly positive in one case, positive in two other sera, and slightly so in two cases; whether weak or strong the results are in accord with the bahaviour of the tubercle bacillus and tuberculin against the said sera.

Leper bacillus. Here the results are more strongly marked than with the non-pathogenic organisms above. In three instances the complement-fixation test has given very strong positive reaction, and in two others shew a weak positive: all the results are on similar lines with the treatment of the same sera with tuberculin, and the tubercle bacillus: except in one instance the serum with tubercle shewed a negative result.

If Table 1. be referred to, and the following sera, viz., 1. 2. 3. 4. 5. 6. 16. 17. 18. be given some attention, the results point to a connecting link, though slight in some instances, yet of sufficient note for interest, as further observations in other directions have helped materially to strengthen what appears here to be of slender evidence; but to return, we must note that in at least two of the series of sera, namely, 1. and 6., anti-bodies appear constant with four of the

organisms used as antigen, in other words, the results of the experiments point to some property or anti-bodies present in the serum and common to all the organisms. We may record the same facts in another form, as follows:-

Complement-fixation of sera shewing tuberculosis anti-bodies against the "acid-fast" bacilli as antigen- Of 18 cases:-

Blind worm bacillus	-	5%	positive.
Timothy Grass	"	11%	"
Urine	"	27%	"
Leprosy	"	27%	"
Tubercle	"	83%	"

11. Complement-fixation of Goat's immuned sera[1] against the acid-fast bacilli as antigen.

Much and Beschke have kindly consented to the inclusion, in this thesis, of their really excellent work in preparing two goats for immunization against tuberculosis.

The writer has on nearly all the occasions been present, and assisted when the injections were made. The sera of the goats have been from time to time

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[1] Much: Ueber neure Verfahren zur Tuberkelbazil-enanlshliessung.

Leschke: Usber Aufschllessung von Tuberkelbazillen- und, etc.

Munch: Med: Wochenshrift No. 11, 1911.

tested, and the results were always good and of high specific quality.

The goats were prepared in the following manner:-

Goat 1.

Injections of solutions of tubercle bacilli in lactic acid - namely, 5 grams tubercle bacilli in 100 grams lactic-acid 1%.

The dose given on each occasion was 5 c.c.

This Goat received on the whole 5 injections, at intervals of a fortnight, the dose being the same on each occasion, the injections being given subcutaneously.

Goat 11.

Was treated by injection of solution of tubercle bacillus in Tartaric Acid, namely, 5 grams tubercle bacilli in 100 grams Tartaric Acid 1%. The dose here was increased to 10 c.c., and within the course of a month this goat received two injections of 10 c.c. each. The injections, as in the case of Goat 1., were administered subcutaneously.

For the purposes of examination, blood was taken from the ear of each goat, collected in tubes, immediately centrifugalised, and the serum tested. As already said, the testing of the sera of these goats was regularly made (partly by the writer) and the state of immunity reached in these animals must prove of far-reaching value in the question of immunization of

animals against the Bac: tuberculosis.

The complement-fixation test has been made with the serum of each of the goats against the acid-fast bacilli with which this paper is treating. The results are given in Tables 11 and 111.

Unfortunately, the first testings recorded in Table 11 had to be made without the leper bacillus, as the supply of the laboratory had run short. Just a glance at this table, and information of value is supplied. Perhaps it may not be surprising to some that anti-bodies of tuberculosis should be presemt in such weak dilutions of sera against the tubercle bacillus but that the urine bacillus, with these specific sera so highly charged with anti-bodies of tubercle, should give, in such dilutions, so strong a complement-fixation undoubtedly points to some property or properties present in both of these sera that must be also common to both the tubercle and urine bacillus. As must be noted (but we do not think with surprise) the blind worm bacillus and timothy grass bacillus have given negative results with such weak solutions as 0.1 c.c. of specific sera, but it might be mentioned that with 0.2. c.c. of the sera both of the organisms have given positive results.

Table 111 represents the testing of the immunized

serum of Goat 1 against all the acid-fast bacilli: this was done at a later time than Table 11. The tubercle and urine bacilli remain unaltered, the complement-fixation being strong, but with them must now be included the blind worm bacillus, which has also given strong positive results. The bacillus of leprosy and timothy grass have also fallen into line with the other bacilli, their reactions not being so marked, the leprosy bacillus even giving a weaker result than that of the Bac: timothy grass. This is to be explained by the fact that the leper bacilli could not be used in pure culture but from anti-formin solutions of lepromata. As they possess a large amount of self-fixating bodies, much weaker dilutions have to be used as compared with the other acid-fast bacilli. The main point, though, is not the quantative, but the fact that even the weak dilutions of leper bacilli have given a positive reaction with tuberculous anti-bodies.

The positive reaction of the tubercle-bacillus might be looked upon as a matter of course, but that urine, blind worm, and timothy grass bacilli with serum so richly charged with anti-bodies of the tubercle organism should give a positive reaction, seems to give these organisms a relationship to the tubercle bacillus, not only, as is well known, morphologically, and in

their staining powers by Ziehl Neelsen and Gram-Much methods, but also a connection far deeper and stronger, that is to say, inherent qualities which are common to these organisms in chemical consistence and biological reaction. If the results of these Tables (viz., 11 and 111) do not assert this conclusion, what then could be the cause for the complement - fixation of immuned bodies of the tubercle bacillus with the bacillus of leprosy, urine, blind worm, and timothy grass? The more, as the goats had been treated with the pure substances of the tubercle bacilli without any other non-specific substances (as are contained in tuberculin). Is it not that there is a definite property common to all these micro-organisms? The results of further examination may perhaps supply facts of a yet more definite character.

III. Let us view now the results of the reaction of other specific sera, namely, Leper Sera, with the acid-fast bacilli with which we are at present concerned. For this purpose thirteen sera have been obtained as follows:- 11 leprous and 2 tuberculous, these are all positively known cases, and are at present under treatment. It may be well to mention here that one of the cases - No.11 - has been treated with Nastin (Deycke).

Table IV. gives, we believe, ample proof in support of previous remarks regarding the family connection of the acid-fast bacilli; in this instance two different series of human specific sera have been tested with these organisms as their antigen, that these sera should contain immune bodies against their specific micro-organism is beyond doubt.

It may be well to mention, too, what is of common knowledge to workers with both of these specific sera, that the complement fixation test is not positive in every instance where it is expected to be, i.e., where the disease is active, as in tuberculous disease, the serum of many cases when tested with the tubercle bacillus as the antigen, complement-fixation does not always take place. It is also admitted that it cannot be adopted for diagnostic purposes, but for confirmation of diagnosis it is certainly valuable, as is strongly demonstrated by the non-specific Wasserman reaction in cases of Syphilis. On the other hand, as just said, the value in cases of tubercular disease may not be

as great as we desire. Hewlett[1], concludes that tuberculous anti-bodies appear to be absent in cases of localized or latent tuberculosis, and the test cannot therefore be applied. But this opinion can no longer be held according to what we have learnt from the work of Much, Hoessli, and Deilman. For further information we refer to "The Specificity of Complement-fixation with Tuberculous Sera," V. of introductory remarks.

We contend that the complement-fixation test with leprosy is specific; this fact has been disputed by many authorities, because it has been found that leper sera gave complement fixation, not only with antigen, prepared with leper bacilli, but also with many other antigens.

Wasserman's reaction with syphilitic antigen has been found by certain investigators to give positive results.

Positive complement-fixation reaction has been also obtained against tuberculin. Mayer, Platineann and Danielopolu, Frugoni, and Pisani[2] and Kleinschmidt[3] found this reaction to be positive in more than half of the cases.

Complement fixation with extracts from lepromata has been tried for diagnostic purposes with satisfactory results by Gaucher and Abramz[4] and Suger.[5]

Again, positive complement-fixation has been obtained against extracts of tumors (carcinoma and sarcoma) by

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1. Hewlitt Man. Bact. 3rd Ed. P. 299.
2. Berlin klin: Wochenschrift 1909 pt.33.
3. ibid 1910 pt. 2
4. Lepra 1909 Vol. 8
5. Archiv. f. Dematol u. Syph. 1909 Vol. 95.

Frugoni and Pisani.

Opinion has therefore been held that all these complement fixating bodies in the sera of lepers are non-specific, but this view, though at first sight it appears convincing, is in our opinion incorrect. It must be admitted that leprous sera, more than perhaps any other sera have a great tendency to complement-fixation with any reactive antigen; also more than with any other sera with which we have dealt, they seem to have a tendency to self-fixation. These tendencies, we believe, explain the non-specific reactions with extracts of different organs and tumours. But they do not explain the complement-fixations with specific antigens, and the quantitative differences in the strength of these reactions corresponding perhaps with the quantitative differences contained in the specific substances of the different acid-fast bacilli. If the lepra sera had given a strongly positive reaction throughout with all the acid-fast bacilli, the specific nature of the reaction could then be doubted, but the differences which we have found in the complement-fixations of the lepra sera under review against the acid-fast bacilli, exactly correspond with the same differences in the specific anti-bodies of tubercular sera with the acid-fast organisms, clearly proving that the complement-fixation of leprous sera with specific antigens, i.e., antigens prepared from acid-fast bacilli, is wholly specific.

The above are not the only facts in favour of the

the specific nature of the complement-fixation of leprous sera. The next in support we take from the results obtained, for leper serum does not give complement-fixation with other micro-organisms or other albuminious bodies. We have tested our sera in the same manner as was done with the acid-fast bacilli, against streptococci and staphylococci emulsions, and the usual sterile bouillon, and it would be seen from Tables V and VI that no complement-fixation has been obtained from these non-specific organisms.

It has been sufficiently shown that the complement-fixation with leprous sera against acid-fast bacilli is specific - firstly, from the quantitative differences shown with the individual acid-fast organisms. Secondly, by the negative results obtained with the non-specific antigens.

Besides, there exists a non-specific reaction against the same antigens as with Wassermann's reaction.

To return to Table IV. As to be expected, the leper organism has not given a positive reaction with all its corresponding sera, nevertheless, the fact of a strongly positive reaction in 6 cases from 11 tests should mark the reaction as important, and the more remarkable, as has been before pointed out, that much weaker solutions have been used as compared with the other acid-fast organisms. This fact explains the negative result in some cases, when the reaction with Tubercle bacilli is positive. In the two tubercular cases, though the tests do not shew very strong posi-

tive reactions with leper bacilli, yet the result with serum No.12 is sufficiently marked.

From the results revealed in this table, could it be upheld that these acid-fast bacilli are distinct and apart from one another, and that by the coincidence of their form and colour-staining reaction they are to be classed together? Certainly not. The whole drift of the results tends in one direction - to the proof of their strong ties by properties common to each group of the acid-fast class.

Again, it is to be questioned why independent organisms used as antigens to specific sera should react in a similar manner to the specific organisms when used as antigen for their corresponding sera. Is it mere resemblance in form and resistance to acids that account for:-

(a) Leper Sera - the urine bacillus, giving a strong positive reaction in 5 of 9 cases, blind worm bacillus 4 positive reactions in 9 cases, to be noted that in one case the reaction was strong; timothy grass bacillus, 5 positive fixations in 9 cases, three of the reactions being strongly positive.

Though it is intended to deal separately with the strong links that appear to connect leprosy and tubercle bacilli more closely even then the other non-pathogenic organisms, the strong positive reactions of both human and bovine tubercle bacillus, as shewn with the series of specific leprous sera, must not be overlooked even at this stage. We

cannot help noting that of 9 of the leprous sera treated with human tubercle bacillus as antigen, strong positive reaction was obtained in 6 instances, and of 11 leper sera tested with bovine tubercle bacillus as antigen, complement-fixation was strong in 7 cases.

This slight difference of course is of no importance. Surely it is not correct to regard the types human and bovine tubercle as different species.

It will be observed that where the readings of the different antigens against the sera did not remain the same during 24 hours, the results have been omitted above; yet sufficiently interesting facts remain and may be expressed thus:-

Testing of Leper sera against Acid-fast bacilli emulsions as antigen:

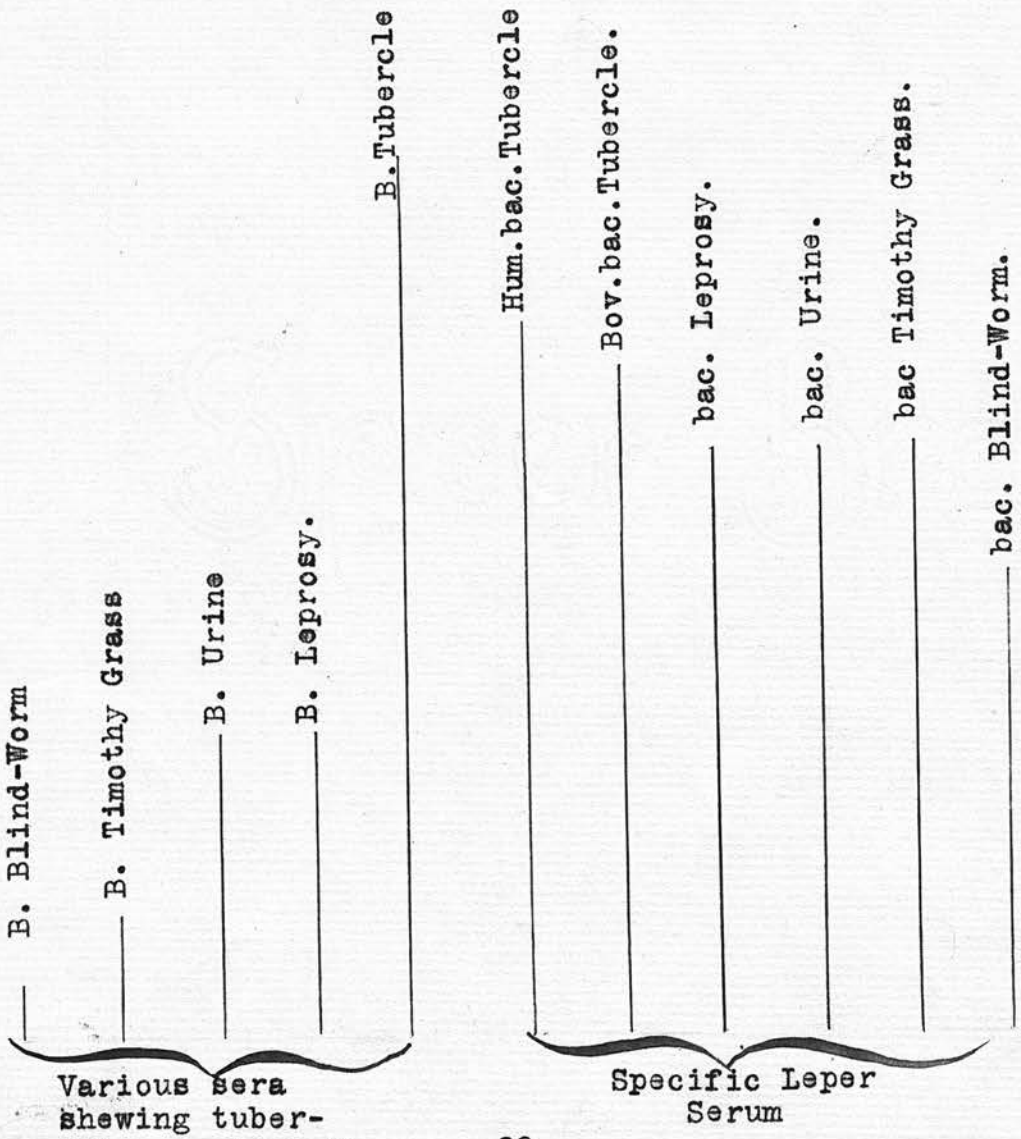
Leper bacilli positive after 24 hours					55%
Human tubercle	"	"	"	"	66%
Bovine "	"	"	"	"	63%
Urine	"	"	"	"	55%
Blind Worm	"	"	"	"	44%
Timothy Grass	"	"	"	"	55%

(b) The tuberculous sera, too, offer sufficient for attention; all the micro-organisms, viz., human tubercle, bovine tubercle, leper, blind worm, urine, timothy grass, giving a positive result with one or other of these specific sera.

If the reactions of the following sera be looked at, namely 2, 3, 8, 12, it would be immediately recognised that whether the serum be leper specific or tuberculous specific, the several micro-

organisms have behaved in the same manner, showing only a difference in intensity. The leper sera even show a greater percentage of positive re-actions than the above tested tuberculous sera and sera of normal persons with no active tuberculosis. (Part 2(1).

In order to obtain better information as to the further proof of the relationship of these micro-organisms, the complement-fixation test of the substances of the tubercle bacillus as antigens have been made against specific sera of leprosy and tuberculosis, but before passing on, the chart below might demonstrate more clearly the facts just stated.



IV. The Complement-fixation test of Leper and Tuberculosis Sera against the substances of tubercle bacillus.

The tubercle bacillus if split up into its component parts is as follows:-

(Taken from a paper of Much: Neuere Ergeb. uber d. Biol. des Tuberkelbazillus. Erge. d. w. Med. Marz, 1911.)

Tubercle bacillus.

1. Albumen (Albuminous bodies)

2. Fatty substances

(a) Neutral fat (c) Fatty acids

(b) Fatty Alcohol (d) Lipoids

2 (a) (b) Tuberculo Nastin

" c Acid-fast Substances (red staining with Carbol-Fuchsin)

" b Little acid-fast (rose coloured)

1. The Fatty acids - They are obtained by the method described by Deycke in the following manner:-

Tubercle bacilli are boiled with K.O.H. (caustic potash) until all the acid-fast substance is saponised and disintegrated; it is then neutralised with ~~H.Cl~~ H.Cl.; the sediment thereby formed is washed out and extracted with absolute alcohol, the alcoholic extract contains the fatty acids, i.e. the acid-fast substance and the lipoids.

In order to obtain a good emulsion for serological purposes, we use the method of Much, which consists in

dissolving the fatty acids in absolute alcohol 1:20; from this standard solution further solutions are prepared with physiological salt solution, and then tested for self-fixation. The dilution used was 1:3000.

2. Tuberculo-Nastin - This is obtained from the residuum after the extraction of the fatty acids, by further extraction with ether. This ether extract contains the neutral fat and a high molecular fatty alcohol, and has been named tuberculo-Nastin. As Nastin is not soluble in water it is difficult to get a homologous emulsion. Much in his paper on Nastin has recently described a method of obtaining an emulsion which is fit for complement-fixation reactions. He heats 1 grain Nastin and 20 C.C. absolute alcohol until a clear solution appears; to this he adds 80 C.C. boiling distilled water, a milky emulsion is then formed, this is then further diluted with physiological salt solution.

After some time a deposit is formed. Then the emulsion has to be boiled again, until it becomes quite homogenous.

We used as antigen 1 C.C. of a solution 1:5000.

3. Tubercle bacillus albumen has been prepared by Deycke & Much in the following manner:-

The fatty substances of the tubercle bacillus were removed by Benzyl-chloride, the residuum contains nothing but albuminous bodies. For our purposes, an emulsion with distilled water was prepared by rubbing it in the agate

mortar: and the solution used was 1:2500.

Table VII. (a) Leper Sera. The first of the antigen tests stated is tuberculin (Koch). It contains all the complement-fixing properties of the tubercle bacillus, and like the tubercle bacillus it will be seen that strong complement-fixation of nearly all the specific sera of leprosy is the main feature of the results of the reaction, a strong positive fixation being obtained in 7 cases of 9 tested.

Tuberculo-fatty acids - With this substance as antigen, the complement-fixation is also strong and marked, the result shewn is that of 10 sera tested, 7 giving strong positive reaction.

Tuberculo Nastin. - The results here shew poor fixations, there being only 3 positive reactions of the 10 sera tested.

Tuberculo Albumen - The results here shewn are of importance in our thinking, and it must be noted that only two instances give positive results in 9 tests.

(b) Tuberculous sera. The reaction of tuberculin with these sera is in order, both sera shew strong complement-fixating bodies.

Tuberculo-fatty acids - Give here exactly similar results with both sera as tuberculin.

Tuberculo-Nastin and Tuberculo-Albumen - Both of these substances shew a negative reaction.

Our results of the reaction set out in Table VII may be stated thus:-

Tuberculin (Koch) positive reaction (after 24 hours)					77.7%
Tuberculo-fatty acid	"	"	"	"	70.%
" -Nastin	"	"	"	"	3 %
" -Albumen	"	"	"	"	2.2%

It is obvious that the fixating bodies in this series of specific leper sera belong to the Tuberculo-Nastin and Tuberculo-fatty acid group, and that the Tuberculo-Albumen is of little importance.

Deilman, working with sera of tubercular cases and persons shewing anti-bodies of tuberculosis - immunized adults - in an examination of a number of cases, used as his antigen the tubercle bacilli emulsion, tuberculin (Koch), and the substances of the tubercle bacillus. He obtained positive reactions as follows:-

Tubercle bacillus emulsion		69.2%
Tuberculin (Koch)	"	86.6%
Tuberculo-Nastin	"	56.2%
Tuberculo-fatty acid	"	15.0%
Tuberculo-Albumen	"	7.1%

These results are in accord with our own. We can now state that the very important property in common with all these acid-fast organisms is their fatty substances, and the capacity for development by their presence in the serum of similar anti-bodies, which give so complete a complement-

fixation not only with the specific micro-organism, but with the others of the acid-fast class. Our comparison scales under subhead III fully demonstrate our remarks.

We might remark that lepra-sera give more complement fixation with the fatty acids (70%) than with the neutral fat (3%), whereas sera with tubercle-immune-bodies contain a higher percentage of anti-bodies against Nastin (56.2%) than against the fatty acids (15%). Perhaps this fact, after Much's opinion, explains the clinical efficiency of Nastin with cases of leprosy, because it starts the production of the wanted fat-anti-bodies.

Our conclusions are similar to those of Much - that the specifical complement-fixing substances of the tubercle bacillus are the same as those of the other related non-pathogenic organisms, vide Introductory remarks IV.

Our results entitle us to add that (1) Both pathogenic and non-pathogenic acid-fast bacilli possess common properties. (2) That their thorough similarity,

(a) Morphologically,

(b) With regard to biological reactions,

(c) With regard to chemical reactions and consistence, shew that these acid-fast organisms are of a common stock, and that their differences are due to the development of certain special qualities peculiar only to the individual organism.

PART 3.

Quantitative Complement-fixation.

In order to get further information we have tested some of the sera quantitatively. The results are shewn in the following tables:-

Table VIII. Quantitative Complement-fixation of Leper Sera 3 and 5 (vide Table IV) against antigens of the acid-fast bacilli.

1. Serum 3. Postive reactions, it will be seen, have taken place with all the antigens, though tested against such weak dilutions of sera; but as we intend only to treat here with tuberculosis and leprosy bacillus, we call attention to the fixation of both organisms with a dilution of 0.01^{cc} serum.

2. Serum 5. The results are completely negative with leprosy, but positive with tubercle bacillus. The negative reaction against leper bacilli is rather surprising, as the serum contains strong anti-bodies against tuberculous antigen. But we have to remember that the leper bacilli could not be tested in the same strength of solution as the other acid-fast bacilli (as already mentioned).

Table IX. Quantitative Complement-fixation of the substances of the tubercle bacillus as antigen against Leper Sera.

Serum 3. (a) Tuberculo Albumen has shewn only one positive reaction, and that with the usual amount of serum.

Tuberculo-Nastin. This antigen was too weak as compared with the other substances. After the usual hour, positive results were shewn with 0.15 and 0.1, but both of these disappeared on standing.

Tuberculo-fatty acid and Tuberculin both shew interesting results:

Sera 5. The results throughout are negative.

Table X. Quantitative Complement fixation of Leper Nastin and the substances of the tubercle bacillus as antigen against immuned Serum (Goat).

These results are from experiments of Much and Leschke.

With such weak dilutions of the serum, they obtained positive reactions with all the antigens. This furnishes ample proof of the value of the specific nature of the serum.

The value and importance of the discovery of Much that purely fatty substances are able to produce specific antibodies can never be over estimated. By this knowledge, the fixating properties of the specific serum of the acid-fast bacilli are now definitely known. It should also be of immense value in morbid conditions due to the presence of pathogenic acid-fast bacilli.

The work of Deycke in the discovery of Nastin is also of immense value, this purely fatty substance having given ample proof of its high fixating qualities, as shewn in the table now under consideration.

These results at the same time shew that Nastin could be prepared in the same way from any other acid-fast bacillus, as the fatty substances of all these bacilli are so nearly related to one another.

Our conclusions are based on the following:-

(1) The results arrived at by Deycke when he found that inoculation of Nastin into both leprous and tuberculous patients produced similar results, vide Introductory I. (2) The results of work in another field with the acid-fast bacilli, when there was found more corresponding relationship between leper and tubercle bacillus than between the other acid-fast organisms. (3) The results of our own work as set out in the tables, all of which substantiate and are in thorough agreement with 1 and 2.

We therefore say that the leper and tubercle bacillus are not alone morphologically alike, but that they are of a common stock.

PART 4.

OPSONIC INDEX.

Lepra and tuberculous sera against B. Tubercle, B. Leper, B. Blind-worm, B. Urine, B. Timothy grass.

Besides the complement-fixation method, we used the opsonic index for testing the immune bodies of specific sera of leper and and tuberculosis.

The opsonic index has been determined by the methods used at the laboratory where our work took place, viz:-

Leucocytes: Fresh human blood was taken with Sod. Cit. 10% and the plasma centrifugalised, to allow the leucocytes to fall to the bottom; the serum was next poured off, the leucocytes washed with physiological salt solution and again centrifugalised, this washing and centrifugalising being repeated about twice.

Bacilli: These were rubbed up in the agate mortar and emulsions made with physiological salt, and controlled by microscopic examination.

Sera: To each C.C. of serum used, one drop of fresh complement was added so as to reactivate the serum.

The opsonic index was determined with the following sera, viz:- 8 leper and 2 tubercular.

I.

A. The non-pathogenic organisms.

Timothy grass bacillus. The opsonic index is high and fairly steady, and may be said to be within what is termed normal range, with the exception of sera Nos. IV., VII and XIII.

Blind-Worm Bacilli - Here phagacytosis is much the same as with Timothy Grass bacilli; there is nothing of special note, but attention might be again drawn to Sera IV, VII and XIII.

Urine Bacilli - So far as known, this bacillus is also non-pathogenic to warm blooded animals, and only in largely increased doses intra-peritoneally are fatal to guinea pigs. In this instance, a little interest appears to lie in the fact that (1) The opsonic index is on the whole lower than that of the Blind-Worm bacil. and Timothy Grass Bacil. respectively. (2) That the differences between the several sera are more marked. Sera IV, VII and XIII, also give results worthy of consideration.

B. The Pathogenic Organisms.

Tubercle Bacillus. There is a material lowering or drop in the opsonic index when the sera are not specific for the tubercle micro-organisms, and when specific as in the cases XIII and XII, there is at once a range within the laid-down normal area and also a considerable heightening of the index which at once demand consideration.

Leper Bacillus - As in the case of the tubercular sera, there

is not only the coming to normal, but the strong heightening that characterises the whole of these specific sera, viz., I to IX (of the opsonic index table) with the leper bacillus, and we have here also what might be termed a turning of the table with the leper bacilli against tubercular sera, as seen with sera XII and XIII.

II.

The Sera. It is not intended to treat each serum separately in this small paper, neither can certain interesting facts be passed over without some comment. Nor do we lay claim to the establishment of new matter, but our sole desire is to express what is strikingly appreciable in the facts obtained from the specimens which have come under our notice.

It is difficult to select, but for the purposes of this thesis let us see the information that can be obtained from sera IV, VII and XIII.

Serum IV. Leper - Its opsonic index is the highest of the series when treated with its specific bacilli, and it maintains a very heightened index also with all the other non-pathogenic organisms, but when dealt with by another pathogenic specific micro-organism of the said group, viz., the tubercle bacillus, it immediately gives results in the direct opposite, that is, the opsonic index falls to about the lowest in the series of the leper sera. From this and the behaviour of the other leper sera to the tubercle bacilli, the impression seems incontestable that the governing force of phagacytosis depends upon whether the serum is specific to the organism and also as

to whether the organism is specifically pathogenic or not.

Serum VII. Leper. The opsonic index here is with the lowest of the leper group, thus differing in a great measure to the above serum (IV), but it is certainly worthy of note that in this instance where the index is so low as regards its own specific micro-organism, the opsonic index is heightened to a marked extent when treated with the tubercle bacillus. As would be seen, it is in line within the normal area, and slightly lower than that for its own specific organism; it is also very noteworthy and can hardly be passed by without mention that Serum V. (also leper) has behaved in an exactly similar manner. With respect to the non-pathogenic organisms, there is nothing of interest to note.

Serum XIII. Tuberculosis. This is a specific serum of the tubercle microbe, and like the leper sera, its index is a long way heightened by its own specific micro-organism; and its drop towards the leper organism is distinct. Serum XII. also tubercular shews the same characteristics as Serum XIII in its behaviour with the leper bacillus. With the non-pathogenic organisms its tendency is somewhat within line with that of Serum V., viz., to maintain a fair range.

Before turning away from this part of the work, we would like to add the very important fact that but for the Gram-Much manner of staining, much of the information arrived at could not have been obtained, as many of the organisms (whether from age or not) lose much of their fatty properties, and as the Ziehl Neelsen method only brings out the micro-organism through its fatty properties, it is readily recognised of what excellent value

is the Gram-Much.

Mention ought to be made that when the opsonic index could not be obtained with some of the specimens at our disposal with the Ziehl-Neelsen, the specimens were restained by the Gram-Much method and phagacytosis was readily shewn.

The opsonic index, in so far as we have been able to deal with it, is of value and points, as a help, to diagnosis and treatment in these diseases.

The writer has determined in other instances the opsonic index with immuned sera (tubercle) against the tubercle baccilus and the opsonic index with the sera of two immunized goats against the tubercle bacillus. In both of the above instances it was greatly increased, corresponding with the present results of human specific tuberculous serum against the tubercle bacillus and leper serum against the leper bacillus.

The remarkable difference between leper and tuberculosis sera is very clearly demonstrated by the opsonic chart. The sera are represented thus:-

Red: Leper Sera

Blue: Tuberculous Sera.

Carbol-Fuchsin:-------

Gram-Much: -------

We see that there is an entire agreement in the results obtained from leper and tuberculous sera; leprous sera having a very high opsonic index against leper bacilli, a very low one against tubercle bacilli, and vice-versa, tuberculous sera a very low opsonic index against leper bacilli, and a high one

against its specific bacilli.

We only state these remarkable facts without drawing theoretical conclusions from them. However, we must remember that the circumstances of opsonic reaction are more difficult to explain than any other reactions of immune bodies, because not only a higher, but also a lower opsonic index proves the existence of specific immune bodies.

The apparent inconsistency that even a lower opsonic index is caused by specific anti-bodies has been demonstrated by many experiments of recent years, but a sufficient explanation is still wanted.

We therefore may say that in our cases the tubercle bacilli, as well as the leper bacilli, have given a specific reaction with leper sera as well as with tuberculous sera. However, we state that there is a remarkable difference as well as a remarkable responsive action with regard to the increase and decrease respectively in the behaviour of these sera against these two micro-organisms.

General Conclusions.

1. There are definite substances common to all acid-fast bacilli.

2. These substances are able to produce specific anti-bodies.

3. They belong to the fatty group (fatty acids, lipoids and neutral fats).

4. An organism that has been in contact with the virus

of tuberculosis, not only gives a reaction against substances of the tubercle bacillus, but even against those of other pathogenic and non-pathogenic acid-fast bacilli with but quantitative differences.

5. In the same manner an organism under the influence of leper virus, not only reacts against the substances of leper bacilli, but also against substances of other acid-fast bacilli, chiefly those of tuberculosis.

6. The relationship of all acid-fast bacilli is established by the fact that their characteristic chemical bodies can be extracted from the bacilli, and these bodies have to be regarded as the property which these organisms possess in common.

7. With regard to leprosy, anti-bodies are produced more against the fatty acids and lipoids than against the neutral fat. This fact explains the therapeutic efficiency of Nastin as causing the formation of fatty anti-bodies. With regard to tuberculosis the formation of anti-bodies seems to be somewhat different from what we have seen with leprosy (Much and Leschke).

8. The existence of the anti-bodies against neutral fatty bodies that have been discovered by Much is very important. They even exist in a much larger amount than Much himself at first thought.

9. All the non-pathogenic acid-fast bacilli shew nearly normal opsonic index.

10. All these re-actions against acid-fast bacilli are to be regarded as entirely specific.

TABLE I.

Human Sera - Shewing Tuberculosis Anti-Bodies.

Complement Fixation with Acid-fast Bacilli.

No.	Serum (quantity)	Tuberculin 0.04	Tubercle Bacilli Emulsion 0.075	Leper Bacilli Emulsion 0.1	Urine Bacilli Emulsion 0.1	Timothy Grass Bacilli Emulsion 0.06	Blind-worm Bacilli Emulsion 0.2	Clinical signs of Tuberculosis.
1	0.2cc	x x x	x x x	x	x	x x x	0	None
2	"	x x x	x x x	x x x				None
3	"	x x x	0	x x x	0	0	0	Tuberculosis of Lungs
4	"	x x	x x	x x x	0	0	0	None
5	"	x x	x x	x	0	0	0	None
6	"	x x x	x x	0	x	x x x	x	None
7	"	x x x	x x	0	0	0	0	None, but positive cutaneous reaction
8	"	x x	x x	0	0	0	0	None
9	"	x x x	x	0	0	0	0	Tuberculous family, himself no Tuberculosis
10	"	x x x	x	0	0	0	0	None
11	"	x	x	0	0	0	0	None
12	"	x	x	0	0	0	0	None

TABLE I (Continued)

Human Sera - Shewing Tuberculosis Anti-Bodies.

Complement Fixation with Acid-fast Bacilli.

No.	Serum quantity	Tuberculin. 0.04	Tubercle Bacilli Emulsion 0.075	Leper Bacilli Emulsion 0.1	Urine Bacilli Emulsion 0.1	Timothy Grass Bacilli Emulsion 0.06	Blindworm Bacilli Emulsion 0.2	Clinical signs of Tuberculosis.
13	0.2cc	x	x	0	0	0	0	None
14	"	x	0	0	0	0	0	None
15	"	x x	0	0	0	0	0	Carries Pelvis
16	"	x	x		x x	0		
17	"	x	x x		x x	0		
18	"	x x x	x		x x x	0		

TABLE II.

Immunised Sera of Goats 1 & 2

against

The Acid-fast Bacilli.

Serum (quantity)	Tubercle Bacilli Emulsion		Urine Bacilli Emulsion		Blind-worm Bacilli Emulsion		Timothy Grass Bacilli Emulsion	
	Goat 1	Goat 2	Goat 1	Goat 2	Goat 1	Goat 2	Goat 1	Goat 2
0.2cc	x x x	x x x	x x x	x x x	x	x	(x)	(x)
0.1cc	x x x	x x x	x x x	x x x	0	0	0	0
0.075cc	x x x	x x x	x x x	x x x	0	0	0	0
0.05cc	x x x	x x x	x x x	x x x	0	0	0	0
0.025cc	x x x	x x x	x x x	x x x	0	0	0	0
0.01cc	x x x	x x x	x x x	x x x	0	0	0	0
0.0075cc	x x x	x x x	x x x	x	0	0	0	0
0.045cc	x x	x	x x	0	0	0	0	0
0.0025cc	x x	x	x	0	0	0	0	0

TABLE III.

Goat 1.

Serum (quantity)	Leper Bacilli Emulsion	Tubercle Bacilli Emulsion	Urine Bacilli Emulsion	Blind-worm Bacilli Emulsion	Timothy Grass Bacilli Emulsion
0.2cc					
0.1cc	x	x x x	x x x	x x x	x x

TABLE IV.

Complement Fixation Leper Sera

against Acid fast Bacilli.

No. of Serum	Serum & quantity	Leper Bacilli Emulsion 0.1	Human Tubercle Bacilli Emulsion 0.06	Bovine Tubercle Bacilli Emulsion 0.075	Urine Bacilli Emulsion 0.1	Blindworm Bacilli Emulsion 0.2	Timothy Grass Bacilli Emulsion 0.06
1	Lepra 0.2 cc	(1) x x x		(1) x x x			
2	" "	(1) x x x (2) x	(1) x x x	(1) x x x	(1) x x x	(1) x x x (2) xx	(1) x x x
3	" "	(1) x x x (2) x	(1) x x x	(1) x x x	(1) x x x	(1) x x x	(1) x x x
4	" "	(1) 0	(1) 0	(1) 0	(1) 0	(1) 0	(1) 0
5	" "	(1) 0	(1) x x x	(1) x x x	(1) x x x	(1) 0	(1) x x x
6	" "	(1) 0	(1) x x x	(1) (2) 0 x	(1) (2) 0 x	(1) 0	(1) x x x (2) x
7	" "	(1) 0	(1) (2) 0 x	(1) 0	(1) (x) (2) 0	(1) 0	(1) (2) 0 x
8	" 0.1cc	(1) x x x	(1) x x x	(1) x x x	(1) x x	(1) x x	(1) x
9	" 0.2cc	(1) 0	(1) (x) (2) 0	(1) 0	(1) 0	(1) 0	(1) 0
10	" 0.1cc	(1) x x x	(1) x x x	(1) x x x			
11	Leper 0.2cc	(1) x x x		(1) x x x	(1) x x x	(1) x (2) x (x)	(1) (2) 0 x
12	Tuberculosis 0.2cc	(1) x x	(1) (2) x x x x x	(1) (2) x x x x x	(1) (2) x x x x	(1) (2) x x x x	(1) x x x
13	" "	(1) x	(1) x x (2) x	(1) x x (2) x	(1) x x x (2) 0	(1) x x x (2) 0	(1) x x x

(1) Result at examination 1 hour after
(2) " of " 24 " "

TABLE V.

Leper-Sera against Bouillon.

No.of Serum.	Serum quantity	Tuberculin Koch 0.04.	Urine Bacilli Bouillon 0.04	Blind-worm Bacilli Bouillon 0.05	Leper Bouillon Culture. 0.45	Bouillon 0.5
2	0.2cc	x x x	x x x	x	x x x	
3	"	x x x	x x	x (x)	x x x	0
4	"	x x x	0	0	x x	
5	"	x x x	x x	0	x x x	
7	"	x 0	x (x)	trace	x x	0
9	"	x 0	x	0	0	0
11	"	x x	0	0		0

TABLE VI.

Complement Fixation Leper Sera

against different Non-Specific Antigens

No of Serum	Serum & quantity	Control	Chaulmoogra Oil 1:1500	Wassermann Reaction	Cocci. Staphylo	Cocci. Strepto
1	Leper 0.2cc	0	(2) x 0			
2	" "	0	(2) x 0	x x x	0	0
3	" "	0	(2) x x x X	0	0	0
4	" "	0	0	x x x	0	0
5	" "	0	0	x x x	0	0
6	" "	0	0	0	0	0
7	" "	0	0	0	0	0
8	" 0.1	0	x	0	0	0
9	" 0.2	0	0	0	0	0
10	" 0.1	0		0	0	0
11	" 0.2	0		x x x	0	0
12	Tuberculosis	0	0	0	0	0
13	"	0	0	0	0	0

(2) Result of examination 24 hours after.

TABLE VII.

Complement Fixation Leper Sera

against Substances of the Tubercle Bacillus

No of Serum	Serum & quantity	Tuberculin Koch 0.04	Tuberculo Fatty Acids 1:3000	Tuberculo Nastin 1:5000	Tuberculo Albumen. 1:2500	
1	Leper 0.2cc					
2	" "	(1) x x x	(1) x x x	(1) (x)(2)0	(1) 0	
3	" "	(1) x x x	(1) x x x	(1) x x	(1) x x	
4	" "	(1) x x x	(1) 0	(1) 0	(1) 0	
5	" "	(1) x x x	(1) x x x	(1) 0	(1) 0	
6	" "	(1) x x x	(1) x x x(2)XX	(1) 0	(1) 0	
7	" "	(1) x x x(2)0	(1) x x(2)0	(1) 0	(1) 0	
8	" 0.1cc	(1) x x x	(1) x x x	(1) x	(1) x	
9	" 0.2cc	(1) x x(2)0	(1) 0	(1) 0	(1) 0	
10	" 0.1cc		(1) x	(1) x		
11	0.2cc	(1) x x x(2)X X	(1) x x x	(1) 0	(1) x x(2)0	
12	Tubercular"	(1) x x x	(1) x x x	(1) 0	(1) 0	
13	" "	(1) x x x	(1) x x x	(1) 0	(1) 0	

1 Result of examination 1 hour after

2 " " 24 " "

TABLE VIII.

Quantitative Complement Fixation

Leper Serum 3.

erum (Quantity)	Leper Bacilli Emulsion 0.1	Human Tubercle Bacilli Emulsion 0.06	Bovine Tubercle Bacilli Emulsion 0.075	Urine Bacilli Emulsion 0.1	Blind-Worm Bacilli Emulsion 0.2	Timothy Grass Bacilli Emulsion 0.06
eper 0.2cc	x x x	x x x	x x x	x x x	x x x	x x x
0.15cc	x x x	x x x	x x x	x x x	x x x	x x x
0.1cc	x x x	x x x	x x x	x x x	x x x	x x x
0.05cc	x x x	x x x	x x x	x x x	x x x	x x x
0.025cc	x x x	x x x	x x x	x x x	x x (0)x	x
0.01cc	x x x	x x	x x	x (2)0	0	0

TABLE VIII. Cont'd

Quantitative Complement Fixation.

Leper Serum 5.

Serum (Quantity)	Leper Bacilli Emulsion 0.1	Human Tubercle Bacilli Emulsion 0.06	Bovine Tubercle Bacilli Emulsion 0.075	Urine Bacilli Emulsion 0.1	Blind-Worm Bacilli Emulsion 0.2	Timothy Grass Bacilli Emulsion 0.06
Leper 0.2cc	0	x x x	x x x	x x x	0	x x x
" 0.15cc	0	x x x	x x x	x x x	0	x x x
" 0.1cc	0	x x x	x x x	x x x	0	x x x
" 0.05cc	0	x x x	x x x	x x x	0	x x
" 0.025cc	0	x x x	x x x	x x	0	x (2) 0
" 0.01	0	x	0	(x) (2) x	0	0

(2) Result of examination 24 hours after.

TABLE IX.

Quantitative Complement Fixation.

Leper Serum 3.

Serum (quantity)	Tuberculin Koch 0.04	Tuberculo Fatty Acid 1;3000	Tuberculo Nastin 1:5000	Tuberculo Albumen 0.04
Leper 0.2 c.c	x x x	x x x	x x x	x x
" 0.15 "	x x x	(2) x x (x) x x	(2) (x) x x	0
" 0.1 "	x x x	(2) x x (x) x x	(2) 0 x x	0
" 0.05 "	x x x	(2) 0 x	0	0
" 0.025 "	x x	0	0	0
" 0.01 "	0	0	0	0

TABLE IX. Cont'd

Quantitative Complement Fixation

Leper Serum 5.

Serum (quantity)	Tuberculin Koch 0.04	Tuberculo Fatty Acid 1:3000	Tuberculo Nastin 1:5000	Tuberculo Albumen 0.04
" 0.2 c.c.	x x x	0	0	0
" 0.15 "	x x x	0	0	0
" 0.1 "	x x x	0	0	0
" 0.05 "	x x x (2) x (x)	0	0	0
" 0.025 "	x (2) 0	0	0	0
" 0.01 "	0	0	0	0

(2) Result of examination 24 hours after.

TABLE X.

Quantitative Complement Fixation

Immunised Serum (Goati)

against Leper Nastin & Tubercle Bacilli Substances.

Serum (quantity)	Nastin 1;750	Tuberculo-Nastin 1:5000	Tuberculo Fatty Acids 1:3000	Tuberculo Albumen 1:2500	~~Tuberculo~~
0.2cc	x x x	x x x	x x	x x (x)	
0.1cc	x x x	x x x	x x	x (x)	
0.075cc	x x x	x (x)	x	x (x)	
0.05cc	x x	trace		trace	
0.025cc	x				
0.01	x				

OPSONIC INDEX TABLE.

Leprous and Tuberculous Sera

Against the following bacilli,

Leper, Tubercle, Urine, Blind-Worm,

and Timothy Grass.

No. of Serum	Tubercle Bacilli C-F.	G-M.	Lepra Bacilli C-F.	G-M.	Urine Bacilli C-F.	G-M.	Blind Worm Bacilli C-F.	G-M.	Timothy Grass Bacilli C-F.	G-M.
I	0.23	0.35	2.7	2.3	0	0.58	0.61	0.66	0	0.73
II	0.4	0.52	2.2	2.0	0	0.67	0.69	0.66	0	0.86
III	0.2	0.29	3.0	3.5	0	0.58	0,8	0.64	0	0.8
IV	0.29	0.29	3.7	4.0	0	0.79	0.8	0.78	0	0.8
V	0.43	0.61	1.3	1.5	0	0.64	0.79	0.85	0	0.6
VII	0.85	1.1	1.9	1.7	0	0.41	0.79	0.9	0	0.53
VIII	0.29	0.31	2.1	2.1	0	0.44	0.58	0.64	0	0.8
IX	0.38	0.4	3.0	3.0	0	0.64	0.71	0.75	0	0.66
XII	0.9	0.9	0.5	0.8	0	0.47	0.76	0.65	0	0.66
X(I)	2.02	2.0	0.3	0.5	0	0.64	0.56	0.55	0	0.86

The Relationship of the "Acid-Fast" Bacilli

Frd. T. Wills M B V. (Ca