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The complement fixation test

in the diagnosis of the

rickettsial diseases of man ,

tick borne relapsing fever,

African human trypanosomiasis ,

and Rift Valley fever.

CONTENTS.

..../ Chapter 6

Part 2.

The complement fixation test in the diagnosis of tick borne relapsing fever.

..../ Part 3.

Part 3.

The complement fixation test in the diagnosis of African human trypanosomiasis.

Part 4.

The complement fixation test in the diagnosis of Rift Valley fever - enzootic hepatitis.

..../ FIGURES

I N T R O D U C T I O N

THE PRINCIPLES OF THE COMPLEMENT FIXATION TEST, ITS APPLICATION AND VALUE IN THE DIAGNOSIS OF DISEASE.

The complement fixation or complement deviation reaction was first described by Bordet(1) in 1898. In 1901 Bordet and Gengou(2) demonstrated that the reaction could be used as a general method for the detection and titration of specific antibodies.

The complement fixation reaction depends upon the principle that an antigen-antibody combination will unite with, or fix complement, so that when sensitized blood cells are added, their haemolysis is inhibited.

However before discussing the reactions involved in the test, it is necessary to define the meaning of certain terms.

Complement. Treffers(3), 1948, says: "Complement is the name given to a group of proteins found in normal serum. The properties of complement include the following:- The haemolysis of sensitized (i.e. antibody coated) red blood cells; the lysis of certain sensitized bacteria; the capacity to kill sensitized bacteria in the absence of bacteriolysis; the opsonization of sensitized bacteria

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in the absence of immune serum; the alteration of thermostable opsonins; the alteration of the rate of aggregation of antigens by their homologous antisera; the alteration of the state of aggregation of antigen-antibody systems; and finally the property of combining with most antigen-antibody systems even in the absence of any visible manifestations.

At present four distinct components of complement are known. These are designated C'1,C'2,C'3 and C'4.All the complements are globulins and contain appreciable amounts of carbohydrates. It is known that all the components are necessary for the haemolysis of sensitized red cells".

Antigen. Quoting Topley and Wilson (4),1936: "An antigen is any substance which,when introduced parenterally into the animal tissues,stimulates the production of an antibody, and which when mixed with that antibody,reacts with it in an observable way".

Antibody. "An antibody is any substance which makes its appearance in the blood stream or body fluids of an animal,in response to the stimulus provided by the parenteral introduction of an antigen into the tissues, and reacts specifically with that antigen in an observable way ".

Haemolysin. Haemolysin or amboceptor are the terms used for the antibody produced in response to the parenteral introduction into an animal,usually the rabbit,of red blood cells,usually those of the sheep. This antibody when mixed with fresh washed sheep's red

..../ blood

blood cells, sensitizes them to the lytic action of complement.

The complement fixation test.

One of the methods of detecting antibodies in sera is by applying the complement fixation test. Both a qualitative and a quantitative analysis can be made of the corresponding antigen. The test consists of an interaction under controllable conditions of (a) the serum suspected of containing a certain antibody, (b) an antigen selected as possibly corresponding to this antibody, (c) a measured amount of complement, the best and most convenient source of which is present in guinea pig serum, and (d) an indicator of sensitized sheep's red blood cells.

The reaction can be summarised as follows:-

1. (a) antigen + antibody + complement = fixation.

   (b) (a) + red cells + haemolysin = no haemolysis.

   = positive reaction.

2. (a) Antigen + antibody absent from serum + complement

   = no fixation.

   (b) (a) + red cells + haemolysin = haemolysis

   = negative reaction.

The complement fixation test has been extensively used in the diagnosis of disease. Perhaps the best known example is the Wassermann reaction, the standard test for the serological diagnosis of syphilis. However this test has been applied for the serological diagnosis of a large number of bacterial, viral ,-

..../protozoal

protozoal and even helminthic diseases. A variety of techniques have been evolved. Recently particular attention has been paid to producing refined antigens. New methods for obtaining abundant growths of the rickettsiae, viruses, spirochaetes and trypanosomes, have facilitated the preparation of suitable antigens for specific differential diagnosis.

This thesis describes the application of this test for the differential diagnosis of the rickettsial diseases, the diagnosis of relapsing fever, trypanosomiasis and Rift Valley fever.

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PART 1.

THE COMPLEMENT FIXATION TEST IN THE DIAGNOSIS OF THE RICKETTSIAL DISEASES OF MAN.

Chapter 1.

Definition of the rickettsiae and their classification among the micro-organisms.

Rickettsiae are small pleomorphic rod shaped or coccoid bacterium-like organisms from 0.3 to 1.0 μ long. They are Gram negative, but stain poorly by this method. They are well stained by Machiavello's method and Giemsa's method. The rickettsiae are obligate intracellular parasites, and parasitize various arthropods including lice, fleas, mites and ticks. Some of them when transmitted to man cause disease including epidemic typhus, murine typhus, Rocky Mountain spotted fever, tick bite fever, rickettsial pox and Q fever.

Rickettsiae resemble bacteria in their morphology and in being readily visible when examined with the oil immersion lens of an ordinary microscope. They resemble viruses in their growth requirements, and so far have not been cultivated in cell-free media, but grow readily in the tissues of living animals and in the embryonic egg, where they occur intracytoplasmically and sometimes intranuclearly. Like the viruses, the rickettsiae have reached a stage of extreme parasitism, depending upon the living cell to

..../ provide

provide enzyme systems necessary for their metabolism and multiplication.

The rickettsiae thus form a group of organisms displaying some of the properties of both the bacteria and the viruses, and so form a link between these two groups of organisms.

Based on clinical features, epidemiological aspects, serological and immunological characteristics, the rickettsial diseases of man can be divided into the following groups:-

I. Typhus group.

(a) Classic epidemic (louse borne) typhus.- Rickettsia prowazeki.

(b) Murine (flea borne) typhus.- Rickettsia mooseri.

(c) Brill's disease. Probably Rickettsia prowazeki.

II. Spotted fever group.

(a) Rickettsia rickettsii.

Rocky Mountain spotted fever - Rickettsia rickettsi
or
Dermacentroxenus rickettsi .

(b) Rickettsia conorii.

(1) South African tick bite fever - Rickettsia rickettsi var pyperi
or
Dermacentroxenus rickettsi var pyperi.

(2) Boutonneuse fever - Rickettsia conori
or
Dermacentroxenus rickettsi var conori.

III. Scrub typhus - Rickettsia tsutsugamushi or orientalis .

IV. Rickettsial pox - Rickettsia akari.

V. Q fever - Rickettsia burneti.

VI. Trench fever - Rickettsia quintana.

..../ Chapter 2

Chapter 2.

A review of the literature dealing with the serological reactions of the typhus group of fevers.

In 1916, Weil and Felix (1) cultivated from the urine of a patient suffering from typhus fever, a proteus-like organism which possessed the property of agglutinating in the presence of typhus sera. Several such organisms were isolated from the urine of typhus patients, and were called Proteus X strains. The most highly agglutinable and the most specific was the Proteus X19 strain Felix (2),1916. Weil and Felix (3),1920, investigated two variants of the organism, (1),a motile,flagellated type,termed "H",and (2) a non-motile, unflagellated type,termed "O". Discussing these variants, Wertman (4),1948, shows that the "H" variant was abandoned for the serological diagnosis of typhus fever,as it was found to agglutinate in the presence of sera from patients suffering from diseases other than those of rickettsial origin, particularly in cases suffering from past or present infections with B,proteus.For the diagnosis of typhus fever, the somatic or "O" type,known as B.proteus "O" X19 was eventually adopted for the purpose. Subsequently two further strains, viz. B.proteus "O" X 2 and B.proteus "O" X K

....(Kingsbury)

(Kingsbury) were found to be of some differential value.Antigens prepared from the three strains - B.proteus "O" X 19, "O" X 2 and "O" X K - today form the components of the standard Weil-Felix test.

Although B.proteus has often been isolated from cases of typhus fever,it is now recognised that this organism has no etiological relationship with the disease. Zarafonetis (5),1948, points out that the Weil-Felix test is non-specific in the sense that it is non-rickettsial. Further that the test is non-specific in that the results cannot be taken to indicate any specific rickettsial infection. Barnes (6),1948, and Carrere and Moustardier (7),1948,have found non-specific reactions occurring in pregnancy,usually in serum dilutions lower than those accepted for the diagnosis of typhus fever.Zarafonetis(5),1948, and Elsdon-Dew (8),1943,found that B.proteus "O" X K was agglutinated to high titre by the sera of cases suffering from louse borne relapsing fever.

The fact that these proteus strains do agglutinate in the presence of typhus sera,has suggested that the rickettsiae and B.proteus are antigenically related. Fulton and Begg (9),1946, have shown that R.prowazeki and R.mooseni share common antigens with B.proteus "O" X 19. Castaneda (10),1934, has demonstrated a carbohydrate common to R.prowazeki and B.proteus "O" X 19.

Agglutination of one or more of these proteus strains has been shown to occur in louse borne epidemic typhus fever, flea borne murine typhus fever, the tick typhus or spotted fever group,and

..../ in scrub

in scrub typhus.

Felix (11),1933,has demonstrated that in louse typhus B.proteus "O" X 19 is agglutinated to higher titre than B proteus "O" X 2 , and that B.proteus "O" X K agglutination is negative.

Scoville (12),1945, obtained similar results on sera from cases of murine typhus fever.

Spencer and Maxcy (13),1930, and Davies and Parker (14),1932, illustrated the variation of high B.proteus "O" X 19 and lower B.proteus "O" X 2 , and low B.proteus "O" X 19 coupled with higher B.proteus "O" X 2 agglutination in sera from cases of Rocky Mountain spotted fever. Results of a similar nature have been shown by Wolstenholme and Gear (15),1945, on the sera of cases of South African tick bite fever. Durand (16),1932, found that sera from cases of boutonneuse fever gave equal titres with B.proteus "O" X 19 and B.proteus "O" X 2.

Fletcher et al (17),1939, while studying typhus fever in the Federated Malay States, noted a significant difference between what were termed urban and rural forms of typhus. Sera from cases of urban typhus agglutinated B.proteus "O" X 19 and were subsequently proved to be flea borne murine typhus fever. Sera from the rural cases failed to agglutinate B.proteus "O" X 19, but agglutinated B.proteus "O" X K to high titre. That these latter cases may have been scrub typhus,was indicated by the work of Blake et al (18), 1945, when studying the reaction on known cases of scrub typhus,

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found that agglutination with B.proteus "O" X K to high titre occurred in the majority.

Derrick (19),1937, found that sera from cases of Q fever did not react with the proteus antigens. Cox (20),1948, has stated that sera from cases of Rickettsial pox fail to agglutinate with the proteus antigens.

The typical responses of sera from cases of these rickettsial diseases are shown diagrammatically in the following table.

TABLE I.

	B.proteus "O" X 19	B.proteus "O" X 2	B. proteus "O" X K.
Epidemic louse borne typhus fever.	+ + + +	+	±
Murine flea borne typhus fever	+ + +	+	±
Tick typhus or Spotted fever group	+ + +	+ + +	+
Rickettsial pox	-	-	-
Scrub typhus fever	-	-	+ + +
Q fever	-	-	-

It will be noted that B.proteus "O" X 19 is agglutinated by sera from cases of louse typhus fever and murine typhus fever.

..../B.proteus "O"X 2

B.proteus "O" X 2 can be agglutinated to equal titre with B.proteus "O" X 19 by sera of the tick typhus group. B.proteus "O" X K is agglutinated by sera from cases of scrub typhus. Sera from cases of rickettsial pox have been found to give negative results. Sera from cases of Q fever are another exception, where titres of agglutination of these proteus strains have been insignificant.

Wertman (4),1948, in discussing the significance of the Weil-Felix test, says, "that in spite of the fact that rickettsial antigens have been introduced as specific diagnostic reagents, the Weil-Felix reaction remains an important diagnostic aid". He asserts that one of the values of the Weil-Felix test is that antibodies are detected somewhat earlier than the specific ones. Further that owing to the simplicity of preparation of the antigens and the technique, the test can be performed in any diagnostic laboratory. He emphasizes the need for the examination of more than one specimen to show a rise in titre, and that no single positive reaction should be regarded as significant. Referring to the limitations of the test,Wertman points out the impossibility of differentiating louse typhus fever,murine typhus fever and Rocky Mountain spotted fever by this method. That cognizance must be taken of the fact that positive reactions do occur in cases other than those of rickettsial origin. Lastly, owing to the transient nature of the proteus agglutinins, the test is not effective for survey purposes to determine past infection.

..../ Felix

Felix (21),1950, maintains that a time lag of not less than five to six days occurs between the appearance of antibodies to the proteus strains and rickettsiae. From the point of view of early diagnosis,this is a serious disadvantage as an aid to clinical diagnosis. Felix holds the view that owing to the fact that the proteus agglutinins disappear within a few weeks or months after an attack of typhus,that the test is of value in epidemiological surveys. Recognising the advantages and disadvantages of the proteus agglutination tests, Felix suggests that fpr accurate diagnosis, the combined use of the Weil-Felix test and the rickettsial complement fixation test should be applied.

Our findings of the value of the Weil-Felix test have been described in detail. The following excerpt from this paper summarizes our conclusions.

" In louse typhus agglutinins develop early and are often demonstrable before the end of the first week of illness.High titres are attained at the time of defervescence. B.proteus "O"X19 is usually agglutinated in titres two to ten times as high as the agglutination of B.proteus "O" X2. Agglutination of B.proteus "O" X19 in dilutions over 1:800 are usual in severe or moderately severe cases, and titres over 1:12,800 are not uncommon.B.proteus "O"X K is also often agglutinated, but rarely in high dilutions. The Weil-Felix test gives positive results in over 90 per cent of severe or moderately severe cases of louse typhus at the time of

.... / defervescence

defervescence. In mild cases the percentage of positive results in diagnostic titre is lower. The "diagnostic" titre of agglutination of B.proteus "O" X 19 is taken as 1:200.

In flea or murine typhus the serological findings are similar, except that the average titre of a number of cases is slightly lower, probably because it is usually a milder disease than louse typhus. However in individual cases no distinction can be made on the results of the Weil-Felix test between louse typhus and murine typhus.

In tick-bite fever agglutinins for the strains of Proteus used in the routine Weil-Felix test do not develop until late in the disease or until convalescence. Positive results are rarely obtained before the tenth day of illness, and the maximum titre is usually two to four weeks after defervescence. On the average over a large series of cases B.proteus "O" X 19 and B.proteus "O" X2 are agglutinated to approximately equal titres. These are appreciably lower than the average titre of agglutination of B.proteus "O" X19 in cases of louse typhus. B.proteus "O" X K is often agglutinated but usually not to high titres.

In individual cases B.proteus "O" X 19 only may be agglutinated or B.proteus "O" X 19 may be agglutinated to higher titre than B.proteus "O" X 2. In these cases on the serological findings alone it is not possible to differentiate tick-bite fever from louse and from flea typhus. In other cases B.proteus "O" X2

..../ is

is agglutinated equally with B.proteus "O" X 19. Such cases are probably but not certainly cases of tick-bite fever, for in some cases of louse typhus similar results are obtained. Cases showing agglutinins for B.proteus "O" X2 only are almost certainly cases of tick-bite fever". Wolstenholme and Gear (15),1945.

In the sera of Q fever cases proved by the complement fixation test, the Proteus strains fail to agglutinate in diagnostic titres. Agglutination reactions rarely rise higher than 1:50 against the three strains B.proteus "O" X 19, "O" X 2 and "O" X K and thus are not of significance. Derrick (19),1937, has remarked upon similar findings.

TABLE 2.

Examples of Weil-Felix titres obtained with sera of the typhus group in the course of this work.

Type of typhus indicated by the complement fixation test.	B.proteus "O" X 19 end point	B.proteus "O" X 2 end point	B.proteus "O" X K end point
Louse typhus	+ 1:800	+ 1:50	- 1:25
"	+ 1:1600	+ 1:200	- 1:25
Murine typhus	± 1:800	± 1:200	+ 1:25
"	+ 1:400	+ 1:50	+ 1:25
South African tick-bite fever	± 1:200	± 1:200	+ 1:25
"	+ 1:1600	- 1:25	- 1:25
"	+ 1:25	+ 1:3200	- 1:25
Q fever	- 1:25	- 1:25	- 1:25
"	± 1:50	+ 1:25	+ 1:25

..../ In this

In this paper the value of a field application of the Weil- Felix test as follows was discussed.

" In South Africa, typhus fever is most prevalent in the Native Territories. None of these as yet has permanent laboratory facilities, and often a week, sometimes longer, elapses between the taking of a sample of blood and the receiving of the laboratory report with the result. In the case of typhus fever this report is often essential before the diagnosis can be confirmed and before anti-typhus measures can be instituted. Such delay may have and often does have serious consequences, as it may result in further spread of the disease. Because of this, a simple "bedside", or preferably "field" test for confirming the clinical diagnosis would be a great boon to the medical practitioners called upon to diagnose and deal with outbreaks of typhus fever in areas far away from laboratory facilities. Several such tests have been described. The methods vary, but the results are available within a few minutes of putting up the test. These tests came into prominence as a result of the war, especially on the Eastern European front.

The field test found to give the best results, was a modification of the method described by Castaneda and Silver (quoted by Gaud (22), 1941. The method consists in mixing on a glass slide a drop of blood taken from the finger with a drop of suspension of B.proteus "O" X 19 stained with methylene blue, sterilized and preserved in 0.3 per cent formalin in 2 per cent citrated normal

...../ saline

saline. In a comparative series it was found that there was very close correlation between the results of the standard Weil-Felix and this slide agglutination test. As this test should be of great value in South Africa, the technique will be described in detail.

Preparation of the antigen.

A smooth agglutinable colony is selected from a plate and inoculated on to five agar slopes. These are incubated for 24 hours at 37°C., and then washed off into 7.5ml. of normal saline containing 0.3 per cent formalin. To this suspension five drops of filtered 0.5 per cent methylene blue are added and allowed to stand at room temperature for 20 minutes. The suspension is then centrifuged at 2,000 r.p.m. for 20 minutes to throw down the organisms. The supernatant fluid is discarded, and 2 per cent sodium citrate solution in normal saline containing 0.3 formalin is added, and the mixture shaken to give an even suspension. This is then tubed in capillary tubes ready for use.

Technique of the test:

The antigen is expressed from the capillary tube by means of a small rubber bulb on to a clean glass slide and a drop of blood from the finger of the patient is added. The blood and the antigen are mixed together with the corner of another glass slide, and by tilting the slide in a rotating movement.

The readings are taken at intervals of one to five minutes. In strongly positive reactions the blue clumps of agglutinated organisms

..../ separate

separate from the blood-antigen mixture within one minute.

In positive reactions the blue clumps are smaller and take from one to four minutes to appear.

In weakly positive reactions the blue clumps are still smaller and separate more slowly, taking five minutes or longer to appear.

If a test gives a weakly positive result it is advisable to repeat it in two or three days time to note whether the agglutination has become stronger, as it most certainly would do if the patient is suffering from typhus fever.

In negative reactions the blood-antigen mixture remains a uniform greenish-brown colour.

Similar results, even clearer to read, are given if serum is used instead of whole blood, but this entails collecting the blood in a tube and waiting for the serum to separate before the test can be done. Clear results are also given with unstained antigen and serum, but if the whole blood is used these results are difficult to read.

The question naturally arises as to whether it would not be worth while to include B.proteus "O" X 2 and B.proteus "O" X K in the test as well. As the agglutination of B.proteus "O" X K is irregular in typhus, no good purpose would be served by including this antigen in the test.

There is better reason for the inclusion of B.proteus "O" X2 in the test outfit. In some cases of tick-bite fever B.proteus "O" X2 only is agglutinated and such cases would give a negative reading

..../ if

if B.proteus "O" X 19 only were tested. However, tick-bite fever is usually easily recognized clinically,and,in any case, the serum usually does not show agglutinins until the patient is convalescent. In louse typhus, B.proteus "O" X19 is nearly always agglutinated to equal or higher titre than B.proteus "O" X 2. So it is considered that the advantages gained are outweighed by the disadvantages resulting from the further complication of the test, and it has been decided not to include B.proteus "O" X2 antigen in the standard test outfit.

The field test has been tried out in many cases in the Transkei and in the Transvaal, and has been found to give very reliable results. The results in one particular outbreak of epidemic typhus fever are given in Table 3, in which they are compared with the standard Weil-Felix test.

..../ Table 3

TABLE 3.

Results of tests on specimens of serum.

Middelburg, Transvaal, 8. 1. 45.

	Weil-Felix test. Agglutination of :			Slide. Agglutination of:
No.	B.proteus "O" X 19	B.proteus "O" X 2	B.proteus "O" X K	B.proteus "O" X 19
1	- 1/50	- 1/50	- 1/50	-
2	± 1/1600	± 1/100	- 1/50	+ + + +
3	± 1/200	- 1/50	- 1/50	+ +
4	± 1/400	- 1/50	- 1/50	+ +
5	± 1/800	± 1/200	- 1/50	+ +
6	± 1/6400	+ 1/50	- 1/50	+ + + +
7	+ 1/800	± 1/50	- 1/50	+ + +
8	- 1/50	- 1/50	- 1/50	-
9	- 1/50	- 1/50	- 1/50	-
10	± 1/1600	± 1/50	- 1/50	+ + +
11	± 1/25	- 1/25	± 1/25	+

Sera giving a positive reaction with the ordinary Weil-Felix test in a dilution of 1:50 or over, usually give a positive reaction with the field slide test. The higher the titre given by the standard Weil-Felix test, the stronger and more rapid is the agglutination

..../ seen

seen in the slide agglutination test.

There appears to be the following rough correlation between the two tests:-

Titre of standard Weil-Felix Test	Reading of slide agglutination test.
1: 800 or over	+ + + + strongly positive
1:200 to 1:400	+ + positive
1: 25 to 1:100	+ weakly positive

Although the slide agglutination test does not indicate an exact titre, for ordinary practical purposes it is easy to recognise a "diagnostic" agglutination.

Indeed, the slide agglutination test appears to be almost as valuable as the standard Weil-Felix test in the diagnosis of typhus fever. It has further the advantage of being applicable in field conditions. It also has the same shortcomings. Probably the greatest of these is that on the results of the Weil-Felix test it is not possible to differentiate louse and flea or murine typhus, nor, in many cases, either of these forms of typhus fever from tick-bite fever. From the point of view of the health official responsible for controlling typhus fever, this differentiation is of paramount importance. The rickettsial complement fixation test is a considerable advance on the Weil-Felix test in this respect."Wolstenholme and Gear (15),1945.

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Rickettsial Complement Fixation and Agglutination Tests.

In 1936, Castaneda (23) as a result of studies on animal sera, suggested that a complement fixation test for the diagnosis of typhus fever showed promise. From this period interest began to move from the study of the Proteus antigens to those prepared from rickettsiae. The early antigens were derived from infected rodent lungs. A more convenient and safer method appeared, with the introduction by Cox,1938, (24), of rickettsial infected yolk sacs of developing chick embryos. The use of di-ethyl ether as an extracting agent,resulted in the preparation of relatively pure suspensions of rickettsiae(Craigie,25).1945. Further differential centrifugation and purification with Kieselguhr, as described by Fulton and Begg (9),1946,resulted in pure concentrated suspensions of rickettsiae.

These techniques provided antigens suitable for rickettsial complement fixation and agglutination tests. This marked a distinct advance, as the antigens had now been prepared from the specific organisms. Many workers were able to demonstrate by means of the complement fixation and agglutination methods, type specific reactions for louse and murine typhus. Fulton and Begg (9),1946,Bengtson (26),1936, Craigie et al (27),1946, Zinsser and Castaneda (28),1932, Giroud and Jadin (29),1948.

Elford and van den Ende (30),1944,and Topping and Shear (31), 1945, showed that a soluble antigen was liberated from the organisms. This soluble antigen, demonstrated in the aqueous phase of

.... / the ether

the ether extraction,has been extensively investigated by Plotz (32), 1948. When used in complement fixation tests, these soluble antigens reveal slight or no differentiation when attempting to determine if either louse or murine typhus antibodies are present. Positive reactions however may appear a little sooner than those obtained using pure,washed,rickettsial suspensions.

Using suspensions of R.prowazeki and R.mooseri washed by centrifuging,Smadel J.E.,Department of Virus and Rickettsial Diseases, Army Graduate School,Washington,D.C.,(personal communication), affirmed that the soluble antigen was almost completely removed. He stated that complement fixation and agglutination tests of a highly specific nature were obtainable. The results of this work confirms this.

Plotz et al (32),1948,have shown that in R.prowazeki and R.mooseri infections, the antibodies appear in the following sequence. First to appear are the Proteus, then group antibodies shown by the soluble antigens, then the rickettsial agglutinins, followed by the neutralizing antibody, and finally the homologous antibody detected by the washed rickettsiae.

(2) Aims of present study.

This study was undertaken to establish the value of the specific complement fixation tests in the diagnosis of the rickettsial diseases occurring in South Africa, and to introduce these tests as a diagnostic procedure to distinguish between the various members of this group of infections.

..../ When

When the value of the test had been established, it was applied to determine the incidence and geographical distribution of the rickettsial diseases in South Africa.

The test was also applied to clarify their epidemiology and ecology, including the part played by various rodents as reservoirs of infection.

..../ Chapter 3

Chapter 3.

A detailed description of the preparation of the reagents required for the test, including the antigens.

(a) The rickettsial strains used for the preparation of the antigen.

With the exception of the Q fever antigen, the antigens were prepared from cultures isolated in South Africa. The Q fever antigen was supplied by the Lederle Laboratories Division, American Cyanamid Company, New York. In November 1950 the Kaplan strain of rickettsial pox was received, and a locally prepared antigen was introduced.

The louse-borne epidemic typhus culture , known as "Ettie", was isolated in January 1939 from a native girl named Ettie suffering from epidemic typhus in the Amersfoort district of the Transvaal. Five cc. of her blood were inoculated intraperitoneally into each of two guinea pigs, both of which developed fever after an incubation period of fourteen days.

Since its isolation, the strain has been maintained continually in guinea pigs by subinoculating brain emulsion approximately every seven to ten days. After an incubation period from five to eight days the temperature of the guinea pigs rises rapidly, usually to over 105° F, is maintained for about a week, when it falls rapidly

..../ by

by lysis to normal. In approximately 10 per cent of the infected guinea pigs there is a slight scrotal swelling and redness, but this reaction is rarely as severe as is usually caused by murine typhus or tick-bite fever infection.

This strain does not cause a febrile reaction in white rats, and rickettsiae are seldom seen in smears made from the peritoneum of animals inoculated intraperitoneally. Guinea pigs recovered from infection due to this strain are usually immune to murine typhus and often, but not always, to tick-bite fever.

Cross immunity and complement fixation tests have shown that this strain resembles the classical Breinl strain.

The flea-borne,endemic murine typhus strain,was isolated from fleas found on a rat sent from Durban,Natal, in 1943. The strain is known as the "Durban Dock" strain. The fleas were homogenized and suspended in sterile distilled water. Two mls. of this suspension were inoculated intraperitoneally into each of two male guinea pigs, both of which developed fever.

Since its isolation in 1943, the strain has been maintained continually in male guinea pigs by subinoculating tunica washings and brain emulsion, or tunica washings alone. After an incubation period from three to seven days the temperature of the guinea pigs rises sharply,usually to over 105°F, is maintained for about five days,when it falls to normal by lysis. Accompanying the temperature rise, and persisting to a lesser degree afterwards, marked scrotal

..../ swelling

swelling occurs. This Neill-Mooser reaction is constantly produced. Occasionally it is possible to demonstrate scanty intracellular rickettsiae in smears made from the tunica and stained by Machiavello's method. In white rats inoculated intraperitoneally this strain causes a febrile reaction, with considerable proliferation of rickettsiae in the cytoplasm of cells from the peritoneum.

Guinea pigs recovered from infection due to this strain usually exhibit immunity to louse typhus and a partial immunity to tick-bite fever.

Complement fixation and agglutination tests on the sera of guinea pigs recovered from infection with the Durban Dock strain, reacted with the standard Wilmington antigen, and with antigens prepared from strains isolated from rats in Natal, and on the Witwatersrand.

The South African tick-bite fever strain, called Malish, was isolated in 1946 from a human case in Johannesburg. Blood was withdrawn from the patient at the time of development of the rash. Intraperitoneal inoculation of the blood into a male guinea pig resulted in a temperature and scrotal reaction. This strain has been continually maintained in male guinea pigs by subinoculating tunica washings at weekly intervals. After an incubation period from three to five days the temperature rises sharply to about 105°F, persists for about five days, when it falls by lysis to normal. Marked scrotal reactions with redness accompany the rise in temperature and

..../ gradually

gradually disappear after the temperature has returned to normal.

Irradiated gerbils when inoculated intraperitoneally with this strain, develop large numbers of rickettsiae in the peritoneal cells. Occasionally closely packed aggregations are seen in the nucleus. This intranuclear situation of the rickettsiae is characteristic of the tick typhus group of fevers.

Complement fixation tests have shown that this tick- bite fever strain is serologically identical with strains subsequently isolated from three species of ticks, namely, Hyalomma aegypticum, Amblyomma hebraeum, and Haemophysalis leachi.

The "Kaplan" strain of rickettsial pox was obtained in the form of a dried culture from Dr. J.E. Smadel of the Division of Virus and Rickettsial Diseases, Army Medical Graduate School, Washington 12, D.C. Male guinea pigs were successfully infected from this culture, and the strain has been maintained by subinoculating tunica washings at weekly intervals. The strains reacts in guinea pigs in the same way as the tick- bite fever cultures, but cross protection tests with several known strains of tick-bite fever have shown that partial protection only is conveyed.

The louse, murine, tick-bite fever and rickettsial pox antigens were prepared from the infected yolk sacs of embryonated hens' eggs.

The Ettie and Durban Dock strains grew equally well and profusely on yolk sac culture, and a single description of the

..../preparation

preparation of an antigen will apply in either case.

The Malish strain however, in common with other tick- bite fever strains, yielded fewer rickettsiae per yolk sac, and a separate description of the preparation of its antigen will be given.

The Kaplan strain although yielding better growths than the tick-bite fever strains, did not produce as profuse growths as the louse and murine strains. The antigen was prepared in the same manner as the tick-bite fever antigen.

(b) The preparation of the louse and murine typhus antigens.

Hens' eggs, of reputed high fertility, obtained from flocks vaccinated against bacillary white diarrhoea were incubated at 37°C and 50% humidity for seven days. The eggs were packed in trays, blunt end upwards, and the trays tilted twice daily. A constant temperature and humidity was maintained. The eggs were transilluminated on the seventh day of incubation in a darkened room. The living embryonated eggs were selected, and the dead, moribund and infertile eggs discarded. To prepare a batch of antigen of suitable volume, approximately 150 seventh day living embryonated eggs were required. These eggs were stacked in wire trays in the incubator pending the preparation of the inoculum.

The inoculum was prepared from infected eggs planted on a previous passage of the strain. Usually six living or moribund eggs were selected, after six or seven days incubation. Using the transilluminator, the air sac outline was marked on the eggs with a

..../ pencil

pencil, and by rotating the eggs in turn in a fine oxy-acetylene flame, this area was charred. The eggs were then placed in a sterile manipulating box. These boxes, which were fitted with ultra-violet lamps, were so constructed as to be dust proof, having a glass front for the worker to see through, and admitting the hands only for manipulation. The atmosphere in the box was sterilized before use by exposure to the ultraviolet light for 30 minutes. Using a pair of sterilized forceps, the contents of each egg in turn were poured into a separate sterile dish. A small piece of each yolk sac was torn away and smeared evenly on a microscope slide. After drying by gentle heating the preparations were stained by Machiavello's technique. A yolk sac showing about 100 rickettsiae per microscope field was selected as suitable for the inoculum. The remainder of the selected yolk sac was placed in a sterile 100 ml. Erlenmeyer flask containing approximately 10 grams of glass chips. By rapid shaking the yolk sac was satisfactorily homogenised in about 1/2 a minute. Ten mls. of sterile physiological saline were added, and the flask agitated again to ensure an even suspension of the rickettsiae. This constituted the inoculum for the batch of eggs.

The eggs were then given a preliminary wash in a chloride of lime solution to cleanse and sterilize the shells. Following this, they were stacked in racks of six each, blunt ends pointing upwards. Further cleansing and sterilization of the shells were effected by painting the tops of the eggs with a tincture of iodine solution.

..../ Using

Using a sharp metal probe, a small hole, approximately 2 mm.in diameter,was pierced in the centre of the blunt end of each egg. A sterile 1 ml. tuberculin syringe,fitted with a fine bore 1" needle was charged with the inoculum. The egg racks were transferred in turn to the manipulation box, and by passing the needle through the prepared holes, 0.05 ml. was injected directly into the centre of each egg.

The holes in the eggs were sealed with molten paraffin wax. A little sealing wax was added to the paraffin wax, thereby raising its melting point, and making it tougher and more tenacious when exposed to heat during any future candling process. All the eggs having thus been inoculated, a sample of the inoculum was planted on a blood agar slope and incubated with the batch. This served as a check on any bacterial contamination of the inoculum.

The eggs were stacked in trays,lying on their long axes, and placed in an incubator at 37°C and 50% humidity. On the fourth day after inoculation, the eggs were candled, and any dead ones discarded. Moribund and dead eggs of the fifth and sixth days were set aside in the cool room at 4°C. On the seventh day, dead,moribund and living eggs were added to those previously set aside.

The yolk sacs from all these eggs were then harvested. This process was expedited by placing the eggs in a metal cup mounted on the rotating shaft of an electric gramophone machine. While rotating, the tops of the eggs were charred in a ring with a fine

.... / oxy-acetylene

oxy-acetylene flame at the junction of the air sac. Using relays of sterile forceps, the charred tops were knocked off the eggs and the contents poured into sterile dishes in the manipulating box. The yolk sacs were picked out and placed in a sterile thick-walled pyrex bottle. This bottle was transferred to the deep freezer at -20°C overnight.

The following day the bottle was placed in a 37°C water bath to thaw. The contents were then poured into a sterile stainless steel dish containing about 250 mls. of physiological saline containing 1:10,000 merthiolate. By gently stirring in this solution, the yolk sacs were freed from a considerable quantity of undesirable yolk and haemoglobin. This washing process was repeated three times, the yolk sacs being transferred to a separate vessel containing fresh merthiolate saline on each occasion. The washed yolk sacs were then placed in a sterile 5 litre pyrex bottle stoppered with a non-absorbent cotton wool plug encased in mutton cloth and covered with brown paper. By rotating this bottle vigorously for 10 minutes, the yolk sacs were effectively crushed, and sufficient 1:10,000 merthiolate-saline solution was added to make a 10% yolk sac suspension. A hundred yolk sacs were made up to 1 litre of crude suspension.

This material was decanted into a second sterile 5 litre bottle, the glass chips being left behind. An equal volume of anaesthetic ether was added. It was found that commercial ether was

..../ too acid

too acid, and was unsuitable for the preparation of antigens. The homogenised yolk-sac-ether mixture was well shaken, and the bottle fitted with a sterile rubber bung through which a glass tube was passed to the bottom of the bottle. This introduced atmospheric pressure into the container . A second glass tube through the bung, fitted with a rubber tube and pinch cock, constituted the outlet. When inverted, the apparatus served the purpose ofaseparating funnel. The bottle was allowed to hang in an inverted state overnight in the cool room. During the period three distinct layers formed - (1) a clear upper layer of ether, (2) a dense middle layer of yolk and cell debris, and (3) a lower layer of saline containing some cell debris and rich in rickettsiae. This lower layer was carefully run off into a sterile bottle. A sterile rubber bung pierced with a short glass tube was fitted, and connected to an Edwards vacuum pump. Under a vacuum of about 15 lbs per square inch the greater part of the residual ether was boiled off at room temperature.

This suspension of rickettsiae, although somewhat cloudy in appearance, was almost free from tissue debris and yolk granules. The rickettsial content was about 1000 million organisms per ml. In this state the antigen was stored at 4°C until required for further processing.

Final purification and concentration:

Using sterile 40 ml.pyrex centrifuge bottles, the suspension was centrifuged in an angle centrifuge at 4000 r.p.m. for 1 1/2

.... / hours

hours. The pale amber supernatant fluid was withdrawn and set aside in the cool room in sterile bottles as a future supply of soluble antigen. The deposit of packed rickettsiae, together with some tissue and cell debris was resuspended in 0.5% sterile saline. Six mls. of this solution was added to the deposit in each centrifuge bottle, and the deposit broken up into a dense emulsion with a glass rod.

These resuspended deposits were pooled in a sterile 200 ml. bottle containing 50 gms. of glass chips and stoppered with a rubber bung. The bottle was well shaken to break up any remaining particles. Approximately 150 mls. of this material was obtained from a litre of centrifuged rickettsial suspension. The antigen was then decanted into a second sterile 200 ml. bottle, the glass chips being left behind. To this bottle 1.5 gms. of specially prepared Kieselguhr were added. The bottle was well shaken and allowed to stand overnight at 4°C.

A note on the preparation of the Kieselguhr:

Powdered Kieselguhr as supplied by the B.D.H. (acidwashed) was weighed in 1.5 gm. lots in glass tubes, and washed twice with distilled water. This removed any water soluble impurities. It was then dried in a hot-air oven at 80°C. Care had to be taken not to allow the temperature to rise higher than 80°C as scorching of the material resulted. A stock of such tubes stoppered with rubber bungs were kept on hand for use.

.../ The

The Kieselguhr passing through the suspension had a further purifying effect, removing most of the remaining tissue debris and yolk. After 24 hours in the refrigerator, the bulk of the Kieselguhr had settled in the bottom of the bottle, leaving the rickettsiae in suspension. A further centrifuging of the supernatant fluid in a horizontal centrifuge at 1000 r.p.m. for 20 minutes removed all traces of the Kieselguhr from the antigen. This supernatant rickettsial suspension was centrifuged at 4000 r.p.m. in an angle centrifuge for 2 hours. The dense white deposit of rickettsiae was resuspended in 5 ml. of sterile physiological saline containing 1:10,000 merthiolate and 0.2 ml. of M/2 phosphate buffer at pH 7.0.

This refined concentrated suspension of rickettsiae constituted the stock washed antigen. It was kept at 4°C when not in use, and retained its antigenic value up to six months.

(c) The preparation of the tick-bite fever antigen.

The tick-bite fever antigen differed from the louse and murine typhus antigens mainly in its concentration. This was due to the difficulty experienced in obtaining profuse growths of rickettsiae. The preparation of the inoculum was identical with that for the louse and murine cultures, but the dose per egg was larger, being between 0.1 and 0.2 mls. and containing 5-10 rickettsiae per microscope field. During the candling of the 7th day embryonated eggs, the position of the embryos was noted, and a pencil mark made on the egg near the embryo at the junction of the air sac and its

..../ membrane.

membrane. The needle was pointed towards this mark in the act of inoculating, thereby instilling the inoculum into the region of the yolk sac.

R.rickettsi var. pyperi appeared to be more toxic for the embryos than R.prowazeki and R.mooseri. The average life of the embryos after inoculation was from 4 to 5 days. Better growths too were obtained at an incubator temperature of 35°C. The eggs were candled daily from the third day after inoculation. Dead and moribund embryos on the third day were discarded. Those dying or moribund on subsequent days were kept at room temperature for 24 hours, and then placed in the cool room at 4°C until required for harvesting. Results seemed to indicate that this exposure at room temperature for 24 hours enhanced the growth of the rickettsiae. A possible reason for the increased growth was that the organisms multiplied faster in moribund cells.

Samples of the yolk sacs of each selected egg were prepared for microscopic examination. Yolk sacs which showed from about 20 rickettsiae per microscope field were washed three times in sterile saline to eliminate excess yolk, and homogenised. Sterile saline containing 1:10,000 merthiolate was added to form a 5% suspension of the yolk sacs. The suspension was extracted with an equal volume of anaesthetic ether at 4°C overnight. The lower layer in the extraction apparatus, containing the rickettsiae was then run off. The ether in solution was boiled off at room temperature under vacuum, as

..../ any

any residual ether present would tend to haemolyse the sheep cells when the antigen was used for test purposes.

This suspension constituted the antigen. The tick-bite fever antigen was found to retain its antigenic properties without deteriorating for at least 3 months when kept at 4°C.

The rickettsial pox antigen used first in this work was supplied by the Lederle Laboratories Division , American Cyanamid Coy.,New York. In November 1950, the Kaplan strain of R.akari was obtained, and an antigen was prepared using the same technique as for R.rickettsi var. pyperi. This antigen reacted as well as the imported one and was used for the routine tests.

The Q fever antigen used was supplied by the Lederle Laboratories, prepared from the Nine Mile strain.

(d) The complement.

It was found that the serum of about 10% of apparently normal adult male guinea pigs reacted with the Q fever antigen in low dilutions, e.g.,1:6.25. As a consequence the complement had to be collected separately from the guinea pigs, and tested against the Q fever antigen before pooling. All complement sera reacting against this antigen were discarded as unsatisfactory. The non-reacting complement from at least three guinea pigs was pooled,and dispensed in 3.5 ml. volumes in rubber stoppered test tubes. These tubes were kept in the deep freezer at -20°C until required. Under these conditions the reagent retained its haemolytic potency with

..../ very

very little loss over a period of three weeks. The guinea pigs were bled by heart puncture under an ether-chloroform anaesthetic, and batches of specially pretested animals were kept and bled at intervals of one month.

A note on dry-freezed complement.

Facilities were available for the lyophilizing of complement, and this was tried and found to be successful.

Pooled complement was dispensed in 2 ml. volumes in pyrex ampoules provided with long necks. The serum was "shelled" on the walls of the ampoules in a dry ice-alcohol freezing mixture. The ampoules were then inserted into rubber connection tubes on a specially constructed metal manifold, the lower portion of which was immersed in a dry ice and alcohol freezing mixture contained in a thermos canister. This apparatus was attached by thick rubber tubing to an electric vacuum pump. After about an hour the complement in the ampoules appeared to be dry macroscopically, but drying was continued for five hours. By this time a vacuum of 0.1μ was obtained as registered on a Mcleod vacuum gauge. The ampoules were then filled with nitrogen at atmospheric pressure, and sealed with a fine oxy-acetylene flame. The dried complement was stored in the deep freezer at -20°C. When reconstituted with 2 mls. of distilled water per ampoule, the complement was found to have retained its potency over a period of at least 6 months.

..../ (e)

(e) The haemolysin.

The Kolmer technique was adopted for the preparation of this reagent. A 10% suspension of four times washed sheep cells was prepared, and the rabbits received six or more intravenous injections of this suspension in the marginal vein of the ear at five day intervals, the dose being 5.0 mls. on each occasion. Haemolytic titres of 1:4,000 and higher were found to be satisfactory. Upon a suitable titre being established, usually about 1/4,000, the rabbits were bled by heart puncture or from the marginal vein of the ear. The serum, when separated, was heated at 60°C for 20 minutes, and preserved in an equal volume of best quality glycerine. This haemolysin was stable, and kept for long periods at 4°C.

Apparatus and equipment.

Metal racks: capable of holding four rows of ten 3" X 1/2" glass tubes.

Water baths: capable of holding these racks. One running at 37°C, and a second at 60°C.

Centrifuges: A centrifuge to hold 6" x 1/2" glass tubes and capable of running at 3,500 r.p.m.

An angle centrifuge capable of running at 4,000 r.p.m. for the preparation of the washed rickettsial antigens.

..../ Pipettes

Pipettes : (a) one 10 ml. graduated pipette.

(b) one 1.0 ml. graduated pipette fitted with a rubber teat.

(c) one 0.1 ml. graduated pipette fitted with a rubber teat.

(d) a supply of Pasteur pipettes for the separation of sera and for general use.

Measuring cylinders: one 1000 ml. graduated measuring cylinder.
one 100 ml. graduated measuring cylinder.
one 10 ml. graduated measuring cylinder.

Beakers : 2 x 200 ml. beakers.
4 x 100 ml. beakers.

Glass tubes: (a) a supply of 3" x 1/2" tubes.

(b) a supply of 6" x 1/2" centrifuge tubes.

Saline: a supply of 0.85% saline made up in distilled water.

Anticoagulant: a supply of 2% sodium citrate in physiological saline for storage of sheep cells.

Cleansing solution: a supply of 25% sulphuric acid solution containing saturated potassium bicromate in distilled water, for use in cleansing the glass tubes. This cleansing solution was essential for the proper treatment of the 3" x 1/2" tubes used for the tests. After use the tubes were all washed well in cold tap water and grease pencil marks rubbed off. They

..../ were

were then totally immersed in the cleansing solution for 48 hours. This was followed by a thorough washing in tap water to remove all traces of acid, and a final rinsing in distilled water before drying in a hot-air oven. Properly cleansed tubes, neutral in reaction were necessary for consistent results.

Other glass apparatus was periodically subjected to the same treatment, and at all times was thoroughly washed in cold tap water after use.

..../ Chapter 4.

Chapter 4.

The complement fixation test.

I. The preparation and titration of the reagents used in the complement fixation test.

1. The preparation of the cells.

A fresh supply of sheep cells was obtained for these tests each week. The sheep were bled from the jugular vein, and the blood mixed with an equal volume of 2% sodium citrate solution in physiological saline. With thorough shaking this anti-coagulant proved satisfactory. The cells when not in use were kept in the refrigerator at 4°C, and could be used up to five days. For use, the cells were centrifuged at 3,000 r.p.m. in a horizontal centrifuge for 5 minutes. The supernatant fluid was then pipetted off and replaced with saline. After being well shaken, the cell suspension was centrifuged again. This process was repeated four times. On the fourth occasion the centrifuge was allowed to run at 3,000 r.p.m. for ten minutes. This procedure was adopted on all occasions to ensure as uniform a packing of the cells as possible. After the volume of sensitized cells for any batch of titrations or tests had been estimated, a 1.5% suspension of cells im saline was prepared containing 2 M.H.D. of haemolysin.

The cell suspension was made as follows:- Half the required

..../ volume

volume of saline was placed in a beaker, and washed packed cells added to make a 3.0 % suspension. In a second beaker containing the other half volume of saline 4 M.H.D. of haemolysin was added. After stirring the two beakers separately, the portion containing the haemolysin was added to the cell mixture, resulting in a final preparation containing a 1.5% suspension of cells and 2 M.H.D. of haemolysin. After thorough stirring the beaker was placed in the 37°C water bath for thirty minutes. Sensitization of the cells was found to be complete at the end of this period. The cells kept satisfactorily at room temperature for the rest of the day.

2. The titration of the haemolysin.

Two metal racks were required, each containing nine 3" x 1/2" tubes. Each tube in rack I received 0.4 ml. of saline. The tubes in rack II received the following volumes of saline:-

Rack II.

Tube:	1	2	3	4	5	6	7	8	9
Mls. of saline:	1.9	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9

In a separate tube a 1:100 dilution of the haemolysin was made by adding 0.1 ml. of haemolysin to 9.9 mls. of saline. After thorough mixing 0.1 ml. of this 1:100 dilution was added to tube I in Rack II and mixed. This yielded a 1:1000 dilution of the reagent. 0.2 ml. of this dilution was added to tube 2 in the rack, resulting in a 1:2000 dilution. After adding 0.1 ml. of the 1:1000 dilution to each of the tubes,3 to 9 in rack II, and mixing each in turn,a range of haemolysin dilutions was obtained from 1:1000 to 1:9,000.

..../ Starting

Starting at tube 9 in rack II, 0.2 ml. of this dilution was added to tube 9 in rack I. Working thus from the highest to the lowest dilution, 0.2 ml. volumes of diluted haemolysin were transferred to their corresponding tubes in rack I.

A 3.0% suspension of four times washed sheep cells was prepared and 0.2 ml. added to each tube in rack I. After thoroughly shaking, the tubes were placed in the water bath at 37°C for thirty minutes. During this period sensitization of the cells took place depending upon the amount of sheep cell antibody present. A 1:20 dilution of complement was prepared, and 0.2 ml. added to each tube. After shaking the tubes again, the rack was replaced in the 37°C water bath for a further 30 minutes. At the end of this period the titre of the haemolysin was read.

The titre was defined as that tube containing the highest dilution of haemolysin showing complete haemolysis. This dilution contained one haemolytic dose of the reagent. In all tests, two haemolytic doses of haemolysin were used.

The 0.4 ml. volume of saline added to the tubes, rendered the volume proportions equivalent to those present under test conditions.

3. The titration of the complement.

The complement was titrated on all occasions when sera were tested. The titrations were performed - (a) in the presence of saline alone, and (b) in the presence of each individual antigen.

..../ (a)

(a) Titration in the presence of saline:

After removing a tube of the specially preselected complement from the deep freezer, it was allowed to thaw at room temperature. A range of complement dilutions was made from 1/30 to 1/100 in steps of 10 in cold normal saline. 0.2 ml. volumes of each dilution were added to a corresponding set of tubes in a second rack, starting at the 1:100 dilution and working from right to left down to the 1:30 dilution. 0.4 ml. of cold saline were then added to each tube. This volume substituted for the absence of the serum and antigen present under test conditions. After shaking, the rack was placed in the cool room at 4°C overnight. The following morning, after a lapse of 18 hours, the rack was placed in the 37°C water bath for 10 minutes. A 1.5% suspension of sheep cells sensitized with 2 M.H.D. of haemolysin having been prepared, 0.4 ml. was added to each tube. After shaking, the rack was replaced in the 37°C water bath for thirty minutes, and the result read. One exact haemolytic dose of the complement was taken as the highest dilution to show complete haemolysis. This result was recorded. In addition note was taken of the dilution containing one full haemolytic dose, and was indicated by that dilution showing a trace of unhaemolysed cells. This end point was usually one dilution higher than the exact end point.

(b) Titration in the presence of each individual antigen:

The dilution of the complement for these titrations was performed in the same manner as described in (a) of this section.

..../ Usually

Usually it was found that a series of complement dilutions in steps of 10 from 1/20 to 1/70 covered the active range of the complement in these titrations. Individual rows of tubes were set up in a rack for each antigen required in the tests. Each tube received 0.1 ml. of saline initially as a substitute for the absence of serum normally present under test conditions. Following this, 0.1 ml. volumes of suitably diluted antigen were added to each tube in the row for each particular antigen. Then 0.1 ml. volumes of the diluted complement were added to their corresponding tubes in the rack.

An arrangement was thus obtained of a series of dilutions of the complement from 1:20 to 1:70 in the presence of saline and each individual antigen, the volumes being equivalent to those under test conditions. After shaking, the rack was placed in the 4°C refrigerator overnight. On the following day the rack was placed in the 37°C water bath fpr 10 minutes to restore the temperature of the tubes to the optimal temperature for complement action. Then 0.2 ml. of a 1.5% suspension of sensitized sheep cells was added to each tube, the rack shaken, and incubated in the 37°C water bath for 30 minutes. The results were then read and recorded. Almost invariably it was noted that there was a slight drop in these titres as compared with the titres obtained in the presence of saline alone (ref. section (a) on the complement titration). This slight absorption of complement by the antigens was normal, but note was made that it was not excessive. Complement plus antigen titrations which

..../ reflected

reflected one full M.H.D. end-point, one dilution of lower than the "saline only" complement titration,were found to be satisfactory. On occasions where lower titres were obtained, indicating excessive absorption of complement by the antigen, retitration of the antigen or its replacement had to be resorted to, to avoid non-specific positive and doubtfuk results. A record was kept on each occasion of the one full M.H.D. end-points of these titrations. Two full M.H.D. of the complement was used for the tests proper. Slight variations in the absorption properties of the antigens was the rule. Two full M.H.D. of complement usually ranged from 1.5 to 2.0 M.H.D. of the complement as determined in the presence of saline only.

4. The titration of the antigens.

The antigens were titrated both in the presence of known positive and negative sera. The known positive serum was selected by testing in the presence of a previous batch of the antigen. After heating at 60°C for 20 minutes, the serum was diluted in saline so as to contain 8 antibody units, i.e. it was used in a dilution 8 times stronger than its end point. 0.1 ml. volumes sere pipetted into eleven 3" x 1/2" tubes. A second row of tubes in the rack contained 0.1 ml. volumes of negative serum diluted 1:6.25 in normal saline. This 1:6.25 dilution was equal to the lowest dilution made on sera when tests were performed.

The antigen under test was diluted from 1:10 to 1:100 in steps of ten. Working from right to left 0.1 ml. volumes of the antigen dilutions were added to their corresponding tubes in the two rows.

.... / Tube

Tube eleven in each row received 0.1 ml. of saline, and acted as the anti-complementary control tube for the serum. Following this 0.1 ml. of 2 M.H.D. of complement was added to each tube. After shaking, the rack was placed in the refrigerator overnight. The following day, after warming the rack in the 37°C water bath for 10 minutes, each tube received 0.2 ml. of a 1.5% suspension of sensitized sheep cells. After shaking, the rack was incubated in the 37°C water bath for 30 minutes, and the results read. One reacting dose of the antigen was indicated by the tube containing the highest dilution of antigen to show complete inhibition of haemolysis. Note was made that the anti-complementary control was satisfactory, and further that complete haemolysis had occurred in the corresponding tube containing negative serum. The anti-complementary properties of these antigens were found to be absent or at most, slight. Dilutions of the antigen containing two reacting doses were used in the tests. The commonest dilutions for use of the louse and murine typhus antigens were found to be 1:20. The tick-bite fever antigen owing to its lower rickettsial content was used neat or in a dilution of 1:2. The imported rickettsial pox antigen was diluted 1:8 as recommended by the manufacturers, and latterly the locally prepared ones have been used diluted 1:2. The Q fever antigen was used in a dilution of 1:16.

The following table shows the results of a titration on louse typhus washed rickettsial antigen:-

.../Table 4

Table 4.

Antigen dilution.		1/10	1/20	1/30	1/40	1/50	1/60	1/70	1/80	1/90	1/100	AC Control.
	Tube	1	2	3	4	5	6	7	8	9	10	11
Positive serum diluted to contain 8 antibody units.		+	+	+	+	±	-	-	-	-	-	-
Negative serum diluted 1:6.25.		-	-	-	-	-	-	-	-	-	-	-

Tube 4 indicated the full positive end-point. The antigen dilution in this tube was 1:40. This dilution of the antigen contained one reacting dose, in the presence of 8 antibody units of known positive serum. Two reaction doses were contained in tube 2, where the antigen dilution was 1:20. Note was made that the antigen gave negative results against a known negative serum against all dilutions of the antigen from 1:10. Tubes 11 in each row being the anti-complementary control tubes of the sera were negative and thus the result was satisfactory.

It was determined from this titration that this antigen could be used for future test purposes in a dilution of 1:20 when it would contain 2 reacting doses.

Check was made on the antigens at intervals to determine any change in titre, or the development of anti-complementary and haemolytic properties. The soluble louse and murine typhus

..../ antigens

antigens were usually used in dilutions from 1:5 to 1:10.

II. The performance of the complement fixation test.

On occasions when large numbers of tests had to be done, involving investigations against the complete series of antigens, it was found convenient to dilute the sera the day before the addition of the complement and antigens. Each serum was treated individually, depending upon the information supplied by the clinician. Should the request be for louse or murine typhus, the serum was diluted over a higher range for these two than for tick-bite fever, rickettsial pox or Q fever. In cases where little information was afforded, a screen test was performed where the serum was tested against all of the antigens in the two dilutions, 1:6.25 and 1:50. These two dilutions were selected so as to cover any zone of negative reaction. Sera which gave a positive reaction in these dilutions were later taken to titre.

Sera to be tested were separated from the clot as soon as possible to avoid haemolysis, and kept in the deep freeze refrigerator at -20°C. In addition they were tested as soon as possible, apart from any urgency regarding the results, to avoid the development of anti-complementary properties. The anti-complementary properties of some sera were eliminated by absorption of the specimen of serum with fresh guinea pig complement. It was found advisable as a routine practice to add 0.05 ml. of neat guinea pig complement to 0.2 ml. of all the sera under test. Absorption of the anti-

..../ -complementary

complementary factor took place by incubating the specimens thus treated in the 37°C water bath for 60 minutes. The sera were then heated at 60°C for 20 minutes to destroy their normal and introduced complement.

Following this the sera were diluted in normal saline in a set of tubes in a separate rack. Starting with an initial dilution of 1:6.25 and by the process of doubling dilutions, a final serum dilution of 1:3,200 was obtained. 0.1 ml. volumes of these serum dilutions were pipetted into sets of 3" x 1/2" tubes corresponding to the different antigens against which the serum was to be tested.

Serum controls.

In a separate rack 0.1 ml. of the 1:6.25 dilution of each serum was included to act as an anti-complementary control. 0.1 ml. of saline was added to this tube to substitute for the absence of antigen. A second control tube was included containing 0.1 ml. of the 1:6.25 dilution of each serum.Later when the antigens were added to the series, this tube received 0.1 ml. of normal egg yolk sac antigen. It was deemed advisable to include this control to exclude fixation of complement by any egg protein antibodies in the serum. It was thought possible that the sera of subjects who had had vaccines of egg origin, might contain antibodies to egg protein. If this tube did exhibit fixation of complement, as had happened on rare occasions, then doubt was cast on the validity of any positive reactions obtained in the tests. This antigen consisted of a

..../ 5.0 %

5.0% homogenized suspension of the normal yolk sacs of 12th. day embryonated eggs. After ether extraction and de-etherization, 1:10,000 merthiolate was added as a preservative. The antigen was not anti-complementary and could be used neat.

The following arrangement of tubes was typical of the standard rickettsial complement fixation tests, particularly to distinguish between louse typhus and murine typhus.

Table 5.

	Serum dilutions									
	1/6.25	1/12.5	1/25	1/50.	1/100	1/200	1/400	1/800	1/1600	1/3200
Louse typhus row of tubes	0	0	0	0	0	0	0	0	0	0
Murine typhus row of tubes	0	0	0	0	0	0	0	0	0	0
Tick-bite fever row of tubes	0			0						
Rickettsial pox row of tubes	0			0						
Q fever row of tubes	0			0						
A.C.control tube	0									
Normal egg antigen control tube.	0									

..../ The circles

The circles indicate the position of the tubes used for the serum dilutions against the antigens.

Controls.

Furthermore, on each occasion when tests were done, antigen + saline + complement control tubes were incorporated. The antigens, to be satisfactory, had to give negative reactions. A complement + saline control tube was included containing 2 M.H.D. of complement, which had to give a negative reaction indicating the haemolytic potency of the complement at the time of the tests. Finally, a cell control tube was included containing 0.2 ml. of 1.5% unsensitized cells, saline and complement as used under test conditions. The cells in this tube had to show complete freedom from haemolysis, indicating the absence of any sensitization due to natural causes.

Whenever possible, positive and negative control sera against each antigen were included in every batch of tests.

All of the sera and their controls having thus been diluted, the racks were set aside in the refrigerator overnight at 4°C.

On the afternoon of this day, preparations were begun for the titration of the complement. As outlined in the section on the titration of the complement, provision was made to determine its titre in the presence of each individual antigen , as well as in the presence of saline alone. After allowing the titration racks to remain in the refrigerator at 4°C overnight, sensitized cells were added the following day and the titre of the complement in the

.... / presence

presence of each antigen was determined and recorded.

The following result was typical of a titration of the complement in the presence of (a) saline and (b) louse typhus antigen:-

Table 6.

1. Titration of complement in the presence of saline only.

Complement dilution	1:30	1:40	1:50	1:60	1:70	1:80	1:90	1:100
	-	-	-	-	±	+	+	+

2. Titration of complement in the presence of the antigen.

Complement dilution.	1:20	1:30	1:40	1:50	1:60	1:70
	-	-	-	-	±	+

It will be noted that the full end point in titration (1) was 1:70, in (2) 1:60. The result indicated slight absorption of the complement by the antigen. Note was made that 1 full minimal haemolytic dose of complement in the presence of the antigen was 1:60. For the tests, 2 doses were used and this was contained in a 1:30 dilution of the complement.

On the afternoon of this day, 0.1 volumes of the antigens diluted to contain two reacting doses were added to the appropriate tubes of diluted sera. This was followed by 0.1 ml. of 2 M.H.D. of complement diluted according to the titres obtained against each individual antigen. After thorough shaking, the racks were placed

.... / in the

in the refrigerator for fixation to take place. The next day a 1.5% suspension of sensitized sheep cells was prepared from the washed, packed cells kept over from the previous day in the refrigerator.

The test racks were then removed from the refrigerator, placed in the 37°C water bath for 10 minutes, and 0.2 mls. of sensitized cells added to each tube. After shaking, the racks were replaced in the water bath for 30 minutes, when the results were read and recorded.

The following is a brief summary of the test procedure:-

Each tube contained -

1) 0.1 ml. of heated,diluted serum.
2) 0.1 ml. of appropriate antigen, suitably diluted.
3) 0.1 ml. of complement containing 2 minimal haemolytic doses, titrated under test conditions against the antigen.
4) 0.2 ml. of a 1.5% suspension of sheep cells sensitized with 2 minimal haemolytic doses of haemolysin.

Thorough shaking of the tubes was necessary before the overnight fixation period for 18 hours at 4°C. Before the addition of the sensitized cells, the racks were placed in the 37°C water bath for 10 minutes. After the addition of the sheep cells and thorough shaking, the racks were placed in the 37°C water bath for 30 minutes, when the results were read and recorded.

Note was made that satisfactory anti-complementary controls,

.... / normal

normal egg antigen controls, saline + antigen + complement controls, positive and negative serum controls, and the unsensitized cell control, were obtained.

All sera giving positive reactions were taken to their end point to determine (1) whether thay were reacting at a diagnostic titre and (2) for record purposes to compare with any future specimens submitted.

.... / Chapter 5

Chapter 5.

The results obtained on sera from human cases of the typhus group of fevers in South Africa, and their interpretation.

Four rickettsial diseases affecting man are known to occur in Southern Africa. Three of these belong to the typhus group of fevers, namely

(I) (a) Epidemic louse-borne typhus fever, caused by Rickettsia prowazeki.

(b) Brill's disease.

(II) Murine flea-borne typhus fever, caused by Rickettsia mooseri.

(III) Tick-bite fever, the variety of tick typhus occurring in Southern Africa, caused by Rickettsia rickettsi. var pyperi.

(IV) The other rickettsial disease occurring in this region is Q fever, caused by Rickettsia burneti.

These rickettsial diseases are common in Southern Africa, and indeed in many regions are major health problems. In the case of the typhus group of fevers it is most important to be able to differentiate between the various forms, as the measures of control differ considerably. It is particularly important to differentiate

.... / between

between louse typhus and murine typhus by laboratory tests, as tick-bite fever can usually be distinguished by the characteristic clinical picture.

Laboratory tests to differentiate between the various rickettsial diseases are of great value to the public health administrator, who requires an accurate specific diagnosis to enable him to apply the appropriate control measures.

During this study sera have been received from various localities in South Africa from cases clinically diagnosed as typhus fever. A series of illustrative cases showing the value of the complement fixation test follows;-

I. Epidemic louse borne typhus fever.

Case 1. Patient M.L. A specimen of serum sent from Bloemfontein for typhus fever investigation on the 25.5.50, gave the following result:

Complement fixation test.

	Serum dilution						
:	1/12.5	1/25	1/50	1/100	1/200	1/400	A.C. control
Louse typhus washed antigen	+	+	+	+	+	-	-
Murine typhus washed antigen	±	±	-	-	-	-	
Tick-bite fever antigen	-	-	-	-	-	-	

..../Weil-Felix

Weil-Felix Test

Agglutination antigen	Serum dilution	
B.proteus "O" x 19	+ 1:800	- 1:1600
" "O" x 2	+ 1:50	- 1:100
" "O" X K	- 1:25	

On the 15.6.50, a further specimen from this patient gave the following results:-

Complement Fixation Test

	Serum dilution.								
	1/12.5	1/25	1/50	1/100	1/200	1/400	1/800	1/1600	A.C. control
Louse typhus washed antigen	+	+	+	+	+	+	±	-	-
Murine typhus washed antigen	+	+	+	±	-	-	-	-	
Tick-bite fever antigen	-	-	-	-	-				

Weil-Felix Test

Agglutination antigen	Serum dilution		
B.proteus "O" x 19	+ 1:1600	± 1:3200	
" "O" x 2	+ 1:100	± 1:200	-1:400
" "O" x K	± 1:25	- 1:50	

These two complement fixation results indicated a louse typhus fever infection, with a rise in antibody titre between the examination of the first and second specimens. In addition there was a

..../ rise

rise in titre of the Weil-Felix reaction. The result was further substantiated by a report to the effect that lice had been found on the patient.

Case 2. A specimen of serum was sent by the Government Pathologist, East London, for typhus fever tests. The patient had been ill for eleven days.

Complement Fixation Test.

	Serum dilution.				
	1;12.5	1:25	1:50	1:100	A.C. control
Louse typhus washed antigen	+	+	±	-	-
Murine typhus washed antigen	-	-	-	-	
Tick-bite fever antigen	-	-	-	-	
Q fever antigen	-	-	-	-	

Weil-Felix Test

Agglutination antigen	Serum dilution.
B.proteus "O" x 19	+ 1:1600
" "O" x 2	± 1:200
" "O" x K	- 1:25

This complement fixation result although of low titre was specific, indicating louse typhus infection.

II. Murine flea borne typhus fever.

Case 3. Patient J.M. On the 27.4.50, a specimen of blood was sent from the Superintendent, Baragwanath Hospital, Johannesburg, for

..../ typhus

typhus fever tests. It was subsequently learnt that this patient was employed in a locality where murine typhus had been proved to be present among the rat population.

Complement Fixation Test

	Serum dilution							
	1/6.25	1/12.5	1/25	1/50	1/100	1/200	1/400	A.C. control
Louse typhus washed antigen		+	+	±	-	-	-	-
Murine typhus washed antigen		+	+	+	+	+	-	
Tick-bite fever antigen		±	±	-	-	-		
Q fever	-							

Weil-Felix Test

Agglutination antigen	Serum dilution	
B.proteus "O"x 19	± 1:800	- 1:1600
" "O" x 2	± 1:200	- 1:400
" "O" x K	+ 1:25	- 1:50

The result indicated a murine typhus fever infection. The weak reaction against the tick-bite fever antigen was probably due to a previous infection.

Case 4. On the 1.12.49, a specimen of serum sent by the Medical Officer, Rand Leases, Transvaal, gave the following result -

.... / Complement

Complement Fixation Test

	Serum dilution							
	1/12.5	1/25	1/50	1/100	1/200	1/400	1/800	A.C. control
Louse typhus washed antigen	±	-	-	-	-	-	-	-
Murine typhus washed antigen	+	+	+	+	±	±	-	
Tick-bite fever antigen		-						
Q fever		-						

Weil-Felix Test

Agglutination antigen	Serum dilution.	
B.proteus "O" x 19	± 1:100	- 1:200
" "O" x 2	± 1:100	- 1:200
" "O" x K	- 1:25	

This result indicated murine typhus infection.

In all of these examples, washed suspensions of R.prowazeki and R.mooseri were used. The results show that differentiation between louse typhus and murine typhus was obtained.

When using the soluble antigens however, differentiation although occurring frequently, was less marked. The group end points were often one dilution lower than the final end point, and on some occasions the titres were equal, making differential diagnosis impossible. When sera reacting in this way were further tested with the specific washed rickettsial suspensions, a differential reading was

..../ usually

usually obtained. The soluble antigens were employed in this study for the initial testing of typhus sera. The reasons for this being that they were more easily and cheaply prepared in bulk, and more particularly, according to Plotz (32), 1948, antibodies reacting to the soluble antigens occur a little earlier in the course of these diseases than those reacting to the specific washed rickettsiae.

The following examples illustrate the results obtained using both types of antigen on the sera of louse and murine typhus fever cases.

Case 1. 27.4.51. Patient S, Durban (12543).

Complement Fixation Test

	Serum dilution									
	1/12.5	1/25	1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	A.C. control.
Louse typhus soluble antigen	+	+	+	+	+	+	±	-	-	-
Murine typhus soluble antigen	+	+	+	+	+	+	+	-	-	
Louse typhus washed antigen	+	+	+	+	+	±	-	-	-	
Murine typhus washed antigen	+	+	+	+	+	+	+	±	-	

..../ Case 2

Case 2. 104. 23.1.52. Patient M.,Port Elizabeth.

Complement Fixation Test.

	Serum dilution								
	1/6.25	1/12.5	1/25	1/50	1/100.	1/200	1/400	1/800	A.C. control
Louse typhus soluble antigen	+	+	+	+	+	+	-	-	-
Murine typhus soluble antigen	+	+	+	+	+	+	±	-	
Louse typhus washed antigen	+	+	±	±	-	-	-	-	
Murine typhus washed antigen	+	+	+	+	+	+	±	-	

In these two cases the washed rickettsial suspensions indicated murine typhus more clearly than the soluble type antigens.

A murine typhus immune guinea pig serum gave the following result. Pietermaritzburg rat strain.

Complement Fixation Test

	Serum dilution									
	1/12.5	1/25	1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	A.C. control
Louse typhus soluble antigen	+	+	+	+	+	±	-	-	-	-
Murine typhus soluble antigen	+	+	+	+	+	+	±	-	-	
Louse typhus washed antigen	+	+	+	±	-	-	-	-	-	
Murine typhus washed antigen	+	+	+	+	+	+	+	-	-	

.... / The

The Pietermaritzburg rat strain was isolated from fleas removed from a rat sent to Johannesburg in 1944. The strain has been studied in detail, and found to have the characteristics of the rickettsiae of flea borne murine typhus.

Case 3. 305789/50

Complement Fixation Test

	Serum dilution							
	1/12,5	1/25	1/50	1/100	1/200	1/400	1/800	A.C. control
Louse typhus soluble antigen	+	+	+	+	+	-	-	-
Murine typhus soluble antigen	+	+	+	+	-	-	-	
Louse typhus washed antigen	+	+	+	+	+	-	-	
Murine typhus washed antigen	±	±	-	-	-	-	-	

In this case although the result indicated by the soluble type antigen suggested louse typhus, a definite diagnosis of louse typhus was obtained using the washed rickettsial antigens.

The following results were obtained on the sera of two louse typhus immune guinea pigs. The Jonishwa strain was isolated in 1943 from an adult native ill with typhus fever in the Transkei.

The L 3 strain was also isolated in 1943 from lice removed from a patient with typhus fever in the Transkei.

These strains have been studied in detail, and have been found to have the characteristics of the rickettsiae of louse borne

.... / epidemic

epidemic typhus fever.

a) Complement Fixation Test. Jonishwa strain.

	Serum dilution									
	1/6.25	1/12.5	1/25.	1/50	1/100	1/200	1/400	1/800	1/1600	A.C. control
Louse typhus soluble antigen	+	+	+	+	+	+	+	+	-	-
Murine typhus soluble antigen	+	+	+	+	+	+	+	±	-	
Louse typhus washed antigen	+	+	+	+	+	+	+	-	-	
Murine typhus washed antigen	±	±	-	-	-	-	-	-	-	

b) Complement Fixation Test. L3 strain.

	Serum dilution									
	1/6.25	1/12.5	1/25	1/50	1/100	1/200	1/400	1/800	1/1600	A.C. control
Louse typhus soluble antigen	+	+	+	+	+	+	±	-	-	-
Murine typhus soluble antigen	+	+	+	+	+	±	-	-	-	
Louse typhus washed antigen	+	+	+	+	+	+	+	+	-	
Murine typhus washed antigen	+	+	+	+	±	-	-	-	-	

With occasional exceptions, differentiation between louse typhus and murine typhus by means of the complement fixation reaction was possible. Group reactions were evident in the lower serum dilutions, but specific titres were obtained from one to five dilutions higher

.... / than

than the group end point.

III. Serological Evidence of the occurrence of Brill's disease in South Africa.

In 1898, Brill noted the occurrence in New York of a disease resembling typhus fever. In 1910 Brill (33), noted cases of this relatively mild complaint occurring sporadically along the eastern sea board of the United States, which were not associated with louse infestation. When first described the disease seemed to appear chiefly among Jewish immigrants from South Eastern Europe.

For some time after this the disease was confused with human cases of murine typhus transmitted to man by the rat flea. In 1934, Zinsser (34) in his studies of three strains of rickettsiae isolated from cases of Brill's disease, indicated the close relationship of these rickettsiae to those of classical epidemic typhus rather than to murine typhus. He formed the hypothesis that Brill's disease was a recrudescence of typhus fever in individuals who had suffered an attack of epidemic louse borne typhus years previously in those areas of Europe where classical typhus had occurred for centuries. In 1943, Plotz (35) in support of this hypothesis, found that the sera of 23 patients suffering from Brill's disease gave higher titres with epidemic typhus antigen than with murine typhus antigen, using the complement fixation test.

In the course of this work a few sera from European cases have given positive louse typhus complement fixation reactions, the

..... / histories

histories of which have been difficult to link up with recent typhus infection.

The following examples serve to show the reason why Brill's disease was suspected.

Case 1. Ref.no. 23959/9/2/51. Patient S.

From Drs. R.Polakow and L.Wunsh, Johannesburg.

1st. specimen.

Complement Fixation Test.

	Serum dilution.						
	1/6.25	1/12.5	1/25	1/50	1/100	1/200	A.C. control
Louse typhus washed antigen	+	+	-	-	-	-	-
Murine typhus washed antigen	-	-	-	-	-	-	
Tick-bite fever antigen	-						
Rickettsial pox antigen	-						
Q fever antigen	-						

A further specimen was requested with a view to noting any rise in titre.

2nd. specimen. Ref. no.12359/21/2/51. Duration of illness 14 days. Patient convalescent after treatment with chloromycetin.

..../ Complement

Complement Fixation Test.

	Serum dilution						
	1/6.25	1/12.5	1/25	1/50	1/100	1/200	A.C. control
Louse typhus washed antigen	+	+	+	+	±	-	-
Murine typhus washed antigen	+	+	+	+	-	-	
Tick-bite fever antigen	-						
Rickettsial pox antigen	-						
Q fever antigen	-						

3rd. specimen Ref. no. 12399/14/3/51

Complement fixation test.

	Serum dilution						
	1/6.25	1/12.5	1/25	1/50	1/100	1/200	A.C. control
Louse typhus washed antigen	+	+	+	+	-	-	-
Murine typhus washed antigen	±	+	+	-	-	-	
Tick-bite fever antigen	-			-			
Rickettsial pox antigen	-			-			
Q fever antigen	-			-			

Weil-Felix test

Agglutination antigen	Serum dilution	
B.proteus "O" x 19	+ 1:100	- 1:400
" "O" x 2	± 1:25	- 1:50
" "O" x K	± 1:25	- 1:50

B.proteus "O" x 19 Field test + + +

..../ These

These serological findings indicated that this patient had had epidemic louse typhus fever and not tick-bite fever, the condition suspected.

This patient lived in one of the northern suburbs of Johannesburg where tick-bite fever is prevalent, but where louse infestation of the population is almost unknown. On enquiry it was found that he had served in the Middle East.

Case 2. Ref.no.12440/22/3/51. Edenvale Hospital, Johannesburg.

Patient S., aged 55.

Examination requested : Rickettsial complement fixation tests.

Complement Fixation Test

	Serum dilution					
	1/12.5	1/25	1/50	1/100	1/200	A.C. control
Louse typhus washed antigen	+	+	+	-	-	-
Murine typhus washed antigen	+	+	-	-	-	
Tick-bite fever antigen	±	-				
Q fever antigen	+	-				

B.proteus "O" x 19 Field test negative.

This patient was provisionally diagnosed as suffering from Q fever. The result indicated louse typhus infection, and the weak tick-bite fever and Q fever reactions were not considered of diagnostic significance. His history revealed that he had suffered from louse typhus in Lithuania at the age of 19. As there was no

..../ evidence

evidence of recent exposure to lice, it was suspected that his illness was a recrudescence of his original attack of typhus 36 years before.

Case 3. Ref. no. 12480/7/4/51. Patient O.

Specimen submitted by Dr. M.Coll, Johannesburg.

Examination requested : Rickettsial complement fixation tests.

Complement Fixation Test

	Serum Dilution						
	1/6.25	1/12.5	1/25	1/50	1/100	1/200	A.C. control
Louse typhus washed antigen	±			±			-
Murine typhus washed antigen	+			-			
Louse typhus soluble antigen	+	+	+	+	±	-	
Murine typhus soluble antigen	+	+	±	-	-	-	
Tick-bite fever antigen	-			-			
Rickettsial pox antigen	-			-			
Q fever antigen	-			-			

This patient complained of weakness and perspiration following an attack of fever, during which he developed a rash. He was considerably better after treatment with chloromycetin. His history revealed that he had once lived in Eastern Europe.

The titre of complement fixation was not sufficiently high in these three cases to be dogmatic in forming the opinion that they were cases of Brill's disease. However the suspicion that cases

of Brill's diseases occurred in Johannesburg amongst immigrants from Eastern Europe, was further strengthened by the following case in which the clinical picture was so clear that there was little doubt about the diagnosis.

Case 4. 1st.specimen Ref. no. 267641/31/12/51. Patient B. Aged 73 years. Specimen submitted by Dr. Stakes, Johannesburg. Examination requested : Rickettsial complement fixation tests. Provisional diagnosis: Q fever.

Complement Fixation Test

	Serum dilution										
	1/6.25	1/12.5	1/25	1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	A.C. control
Louse typhus soluble antigen	+	+	+	+	+	+	+	+	+	+	-
Murine typhus soluble antigen	+	+	+	+	+	+	+	±	-	-	
Tick-bite fever antigen	-			-							
Rickettsial pox antigen	-			-							
Q fever antigen	-										

Weil-Felix test

			Serum dilution
Agglutination antigen	B.proteus	"O" x 19	+ 1: 100
	"	"O" x 2	+ 1:100
	"	"O" x K	- 1:25

.... / 2nd.specimen

2nd. specimen 16/1/52

Complement Fixation Test

	Serum dilution										
	1/6.25	1/12.5	1/25	1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	A.C. control.
Louse typhus soluble antigen	+	+	+	+	+	+	+	+	+	±	-
Murine typhus soluble antigen	+	+	+	+	+	+	+	±	-	-	
Louse typhus washed antigen	+	+	+	+	+	+	+	+	±	-	
Murine typhus washed antigen	+	+	+	+	+	+	±	-	-	-	
Tick-bite fever antigen	-			-							
Rickettsial pox antigen	±			-							
Q fever antigen	-			-							
Herpes simplex antigen	-			-							
Lymphocytic choriomeningitis antigen	±			-							
Mumps antigen	+			-							
Lymphogranuloma venereum antigen	+			-							
Psittacosis antigen	-			-							

It will be noted that the agglutination of B.proteus "O" x 19

...../ is not

is not of certain diagnostic value, as the diagnostic titre is taken as 1:200.

In contrast the rickettsial complement fixation test gave positive results in high titre, clearly indicative of epidemic louse typhus.

The positive and trace reactions in the 1:6.25 serum dilution against the rickettsial pox, lymphocytic choriomeningitis, lymphogranuloma venereum and mumps antigens, were not considered of diagnostic significance.

In this case, the patient suffered from a febrile illness in December 1951. Exposure to lice was excluded. It was subsequently learned that he had suffered from louse typhus in Russia at the age of seven, 66 years before. On the serological evidence, it was concluded that he had suffered from a recrudescence of the disease, and that this was in fact a case of Brill's disease.

The results obtained on the first three examples quoted, coupled with the peculiarities of their case histories gave rise at least to the suspicion that these were cases of Brill's disease.

The history and results of case 4 substantiated this suspicion considerably, and apart from the actual isolation of R.prowazeki which would have removed all doubt, the serological evidence indicated that this was a case of Brill's disease which has not previously been reported as occurring in South Africa.

..../ IV. South

IV. South African tick-bite fever.

The South African variety of tick typhus, known as South African tick-bite fever, has been found to occur throughout the Union. The disease does not occur in epidemic form, but sporadic cases are regularly encountered. Most cases occur in the spring and autumn in the temperate parts of South Africa. In the bushveld infection may be contracted all the year round.

The clinical picture presents a primary lesion with a black eschar, regional lymphadenitis, and a relatively coarse muculo - papular rash involving the palms, soles and face of the patient. These features together with a severe headache are so characteristic, that on clinical grounds alone there is little difficulty in diagnosis.

However many cases occur in which the pathognomonic tick bite is not found. In such cases it may be difficult to distinguish this disease from the other rickettsial infections, especially from murine typhus. The results of the Weil-Felix test sometimes are of value in this differentiation.

Thus according to Gear (36),1940, "The diagnosis can be confirmed by finding that the patient's serum, taken after the tenth day of illness agglutinates one of the strains of Proteus used in the Weil-Felix test. Sera of cases of tick-bite fever agglutinate Proteus "O" x 19 and Proteus "O" x 2 and irregularly Proteus "O" x K. These agglutinins are only demonstrable after the tenth day of illness, i.e. convalescence. The titres of agglutination

..../ although

although sometimes high, are low compared with the titres shown by cases of louse typhus. The reaction is therefore a group agglutination in the sense of Felix in his serological classification of the typhus group of fevers. On the average, over a series of cases Proteus "O" x 19 and Proteus "O" x 2 are agglutinated to approximately equal titre. Proteus "O" x K is irregularly agglutinated and rarely to high significant titres. There is, however, considerable variation between individual cases. Some show the presence of agglutinins to Proteus "O" x 19 only, some show high titre agglutinins for Proteus "O" x 19, and low titres for Proteus "O" x 2 , many agglutinate Proteus "O" x 19 and Proteus "O" x 2 to approximately equal titre, and some agglutinate Proteus "O" x 2 only. This variability in serological reactions suggests the existance of different serological strains, but no other evidence favouring this hypothesis has been put forward. At present it seems more probable that one strain is capable of eliciting a different serological response in different individuals. Practically, a series of observations has indicated that cases showing agglutination of Proteus "O" x 2 only are almost certainly cases of tick-bite fever. Cases showing approximately equal agglutination of Proteus "O" x 2 and Proteus "O" x 19 are probably cases of tick-bite fever. On serological findings alone it is not possible to state whether a case showing agglutination of Proteus "O" x 19 to higher titre than Proteus "O" x 2 or of Proteus "O"x K alone is one of tick-bite fever, murine rat flea typhus or

..../ louse

louse epidemic typhus. However, a delay in the appearance of these agglutinins until after the tenth day of illness favours a diagnosis of tick-bite fever. The serological findings in tick-bite fever resemble in many respects those of the other diseases of the tick typhus group. In both Rocky Mountain spotted fever and in fievre boutonneuse the later appearance of agglutinins for Proteus strains has been noted, and the approximately equal average titres for Proteus "0" x 19 and Proteus "0" x 2 described."

In the course of this work, tick-bite fever sera sometimes gave higher B.Proteus "0" x 2 titres than B.Proteus "0" x 19. Out of 23 positive tick-bite fever complement fixation tests, 14 gave higher B.proteus "0" x 2 titres than "0" x 19. Of 14 sera, proved to be louse typhus by the complement fixation test, all gave higher titres with the "0" x 19 antigen than with "0" x 2. Further of 24 sera proved by the complement fixation test to be murine typhus, one gave a higher "0" x 2 titre than "0" x 19.

However, on the results of the Weil-Felix test alone, in many cases it is not possible to state whether the patients have had epidemic typhus, murine typhus, or tick-bite fever. In such cases the rickettsial complement fixation tests have proved to be invaluable.

The following examples illustrate the value of the complement fixation test as specific means for the diagnosis of South African tick-bite fever.

..../ Case 1

Case 1. On the 19.5.50, a specimen of serum was received from the Superintendent, Barberton Hospital, for rickettsial tests.Patient Mrs. L.

Complement fixation test.

	Serum dilution.						
	1/6.25	1/12.5	1/25	1/50	1/100	1/200	A.C. control
Louse typhus washed antigen		±	-	-	-	-	±
Murine typhus washed antigen		±	-	-	-	-	
Tick-bite fever antigen		+	+	+	-	-	
Q fever antigen	+						

Weil-Felix test.

Agglutination antigen	B.proteus	"O" x 19	± 1:200	- 1:400
	"	"O" x 2	± 1:200	- 1:400
	"	"O" x K	+ 1:25	- 1:50

The result indicated that this specimen was anti-complementary, and no conclusion could be drawn. Upon absorbing the serum with fresh guinea pig complement however, the following results were obtained.

..../ Complement

Complement Fixation Test

	Serum dilution.						
	1/6.25	1/12.5	1/25	1/50	1/100	1/200	A.C. control
Louse typhus washed antigen		-	-	-	-	-	-
Murine typhus washed antigen		-	-	-	-	-	
Tick-bite fever antigen		+	+	±	-	-	
Q fever antigen	-						

The result indicated tick-bite fever infection.

Case 2. On the 6.4.50, a specimen of blood was received from Dr. Polakow, Johannesburg, for rickettsial complement fixation tests.

Patient de J.

Complement Fixation Test.

	Serum dilution.						
	1/6.25	1/12.5	1/25	1/50	1/100	1/200	A.C. control
Louse typhus washed antigen		-	-	-	-	-	-
Murine typhus washed antigen		-	-	-	-	-	
Tick-bite fever antigen		+	±	-	-	-	
Q fever antigen	-						

Weil-Felix test — Serum dilution.

Agglutination antigen

B.proteus "O" x 19 — ± 1:200, - 1:400

" "O" x 2 — ± 1:200, - 1:400

" "O" x K — + 1:25; - 1:50

..../ A further

A further specimen submitted a week later gave a rise in titre, confirming the diagnosis of tick-bite fever.

Complement fixation test. Serum dilution.

	1/6.25	1/12.5	1/25	1/50	1/100	1/200	1/400	A.C. control
Louse typhus washed antigen		-	-	-	-	-	-	-
Murine typhus washed antigen		-	-	-	-	-	-	
Tick-bite fever antigen		+	+	+	+	+	-	
Q fever antigen	-							

Weil-Felix test. Serum dilution.

Agglutination antigen

B.proteus "O" x 19	± 1:100
" "O" x 2	+ 1:100
" "O" x K	+ 1:25

Case 3. On the 26.1.50, a specimen of blood was received from the Superintendent, Edenvale Hospital, Johannesburg. Patient A.W.B.

Provisional diagnosis:- Tick-bite fever.

Complement Fixation Test Serum dilution.

	1/12.5	1/25	1/50	1/100	1/200	1/400	A.C. control
Louse typhus antigen	-	-	-	-	-	-	-
Murine typhus antigen	-	-	-	-	-	-	
Tick-bite fever antigen	+	+	+	+	+	-	
Rickettsial pox antigen	+	+	+	+	+	-	
Q fever antigen	-	-	-	-	-	-	

..../Weil-Felix

Weil-Felix Test .		Serum dilution.
Agglutination antigen		
B.proteus	"O" x 19	+ 1:25
"	"O" x 2	± 1:800
"	"O" x K	- 1:25

The result indicated a tick-bite fever infection. It should be noted that the rickettsial pox antigen gave a titre equal to the tick-bite fever antigen, indicating their close antigenic relationship.

..../ Some

Some observations on the effect of antibiotic treatment in rickettsial infections.

During this study, the opportunity occurred for observing the effect of antibiotic treatment on the antibody response of three cases. The following tables show the complement fixation results obtained on one case of murine typhus fever followed by tick-bite fever infection, and on two of tick-bite fever.

Case 1. Mrs. H. A laboratory worker of the Rickettsial Diseases section.

Table 7

Date specimen received	Complement fixation tests.				
	Antigens				
	Louse typhus	Murine typhus	Tick-bite fever.	Rickettsial pox	Q fever
15.6.50 1st. specimen	± 1:25	+ 1:50	- 1:12.5	not done	- 1:6.25
2nd. specimen	± 1:100	± 1:200	- 1:12.5	not done	- 1:6.25
14.4.51 Ill one week	+ 1:25	± 1:100	+ 1:6.25	± 1:12.5	+ 1:6.25
13.3.52 Routine test	+ 1:25	± 1:50	- 1:10	- 1:10	- 1:10

.... / The results

The results of the original illness in June 1950 indicated murine typhus fever infection.

In April 1951 the patient had been bitten by a tick at Hartebeestpoort. After she had been ill one week, complement fixation tests were performed on a specimen of her serum, and the results suggested tick-bite fever infection.

Case 2. Miss C. Employed in the Rickettsial Diseases laboratory, subculturing tick-bite fever strains and a rickettsial pox strain.

Table 8

Date specimen received	Complement fixation tests				
	Antigens				
	Louse typhus	Murine typhus	Tick-bite fever	Rickettsial pox	Q fever
23.5.51 1st. specimen	- 1/6.25	- 1/6.25	- 1/6.25	- 1/6.25	± 1/6.25
6.6.51 2nd. specimen	- 1/6.25	- 1/6.25	± 1/12.5	± 1/25	- 1/6.25
12.3.52 Routine test	- 1/10	- 1/10	- 1/10	- 1/10	- 1/10

The results obtained on the second specimen dated 6.6.51 suggested tick-bite fever infection.

.... / Case 3

Case 3. Miss D. Employed in the Rickettsial Diseases laboratory, exposed to tick-bite fever strains, and a rickettsial pox strain.

Table 9.

Date specimen received	Complement fixation tests. Antigens: Louse typhus	Murine typhus	Tick-bite fever	Rickettsial pox	Q fever
4.3.52 Ill 1 day	- 1/10	- 1/10	- 1/10	- 1/10	- 1/10
21.3.52 Ill 2 weeks	- 1/10	- 1/10	± 1/1600	± 1/100	- 1/10

The results obtained on the second specimen dated 21.3.52 indicated tick-bite fever infection.

These three subjects had received a course of combined louse and murine typhus fever vaccine before commencing work in the Rickettsial Diseases department. They were also given a course of treatment with aureomycin or terramycin (one 250 mgm. capsule 4 hourly for 3 days). It is apparent that although by cutting down the severity and duration of the disease, the titre of response with the respective antigens may be less than in an untreated case, the treatment does not prevent the appearance of these antibodies.

.... / V. Q fever

V. Q fever.

Q fever is the most recent of the rickettsial diseases to be discovered in South Africa. The evidence for the occurrence of the disease in this country has depended upon the results of serological tests.

The disease was first described by Derrick (19) in 1937, affecting abattoir workers in Australia.

Rickettsia burneti or Coxiella burneti, the rickettsia responsible, was traced to ticks of the species Haemophysalis humerosa collected from bandicoots. Besides infesting rats and cattle, this tick was shown to be capable of transmitting the infection to experimental animals.

In 1938 Davis and Cox (37) isolated a rickettsia, which they named Rickettsia diaporica, from naturally infected ticks near Nine Mile in Montana, in the United States of America. Several laboratory workers while studying this organism contracted an infection characterized by fever and headache associated with pneumonitis. In the same year Dyer (38) noted that the signs and symptoms of Nine Mile fever in man were similar to those of Q fever. Dyer (39), 1939, Bengtson (40), 1941, and Burnet and Freeman (41), 1939, then proved that these rickettsiae, namely R.diaporica and R.burneti, were similar.

Q fever was thus known to occur naturally in both Australia and in the United States of America.

.... / In 1944

In 1944 and 1945, outbreaks of Q fever were found to occur among troops billeted in rural areas in Northern and Southern Italy. R. burneti was also isolated from German troops suffering from an influenza-like illness in Greece. This was followed by reports from North Africa that R.burneti had been isolated from ticks, Thus it was apparent at this period that Q fever had occurred in the countries bordering the Mediterranean Sea.

In 1949 isolation of R.burneti was reported in Great Britain.

The disease was later found to occur not only in Montana, but in several of the States in America, and infection nearly always occurred amongst people associated with cattle and sheep.

As the excreta of infected ticks has been shown to contain enormous numbers of the rickettsiae, it has been presumed that infection is acquired by the inhalation of dust from cattle hides contaminated with the excreta of infected ticks. Further, several cases have been described in which Q fever has been contracted from drinking infected milk.

Clinically, Q fever resembles atypical virus pneumonia, but unlike the typhus group of fevers there is no characteristic rash. The Weil-Felix test gives negative or insignificant results. Diagnosis can however be confirmed by serological tests. The complement fixation test has proved to be of great value, but diagnosis at significant titre cannot be confirmed until after the seventh day of illness.

..../ The

The following examples illustrate the results which have been obtained on cases of Q fever occurring in South Africa, and of one from East Africa.

Case 1. On the 13.10.49, a specimen of serum was received from the District Surgeon, Delareyville, Western Transvaal. The provisional diagnosis was relapsing fever. Patient O.M.

Complement fixation test.

	Serum dilution.					
	1/6.25	1/12.5	1/25	1/50	1/100	A.C. control
Louse typhus washed antigen	-	-	-	-	-	
Murine typhus washed antigen	-	-	-	-	-	
Tick-bite fever antigen	-	-	-	-	-	
Q fever antigen	+	+	+	±	-	

Weil-Felix test

Agglutination antigen	Serum dilution.
B.proteus "O" x 19	- 1:25
" "O" x 2	- 1:25
" "O" x K	- 1:25

This case reported in detail by Gear, Wolstenholme and Cort (42), 1950, was the first to be diagnosed as Q fever on serological evidence in South Africa.

Case 2. On the 9.2.50, a specimen of serum was received from the Superintendent, Edenvale Hospital, Johannesburg. Patient H.D.

.... / Complement

Complement fixation test.

	Serum dilution							
	1/6.25	1/12.5	1/25	1/50	1/100	1/200	1/400	A.C. control
Louse typhus washed antigen		-	-	-	-	-	-	-
Murine typhus washed antigen		-	-	-	-	-	-	
Tick-bite fever antigen		-	-	-	-	-	-	
Rickettsial pox antigen		-	-	-	-	-	-	
Q fever antigen	+	+	+	+	+	±	-	

Weil-Felix test

Agglutination antigen	Serum dilution	
B.proteus "O" x 19	± 1:50	- 1:100
" "O" x 2	+ 1:25	- 1:50
" "O" x K	+ 1:25	- 1:50

This result indicated Q fever infection. The patient, a recent immigrant from Holland, spent two weeks holiday on a farm about 15 miles north of Johannesburg, just before becoming ill.

Case 3. This case was described in detail by Saner and Fehler (43), 1950. The patient,aged 33, came to South Africa from Holland on 14.11.49 and was employed on a farm north of Krugersdorp. He became ill on 19.3.50, and his illness was regarded as a pyrexia of obscure origin. Two specimens of his serum gave the following results: Patient van M.

..../ Specimen

Specimen 1. Ref.no. 305717/6/4/50. 12th. day of illness.

Complement fixation test.

	Serum dilution						
	1/12.5	1/25	1/50	1/100	1/200	1/400	A.C. control
Louse typhus washed antigen	-	-	-	-	-	-	-
Murine typhus washed antigen	-	-	-	-	-	-	
Tick-bite fever antigen	-	-	-	-	-	-	
Q fever antigen	+	+	+	-	-	-	
Relapsing fever (S. duttoni) antigen	-	-	-	-	-	-	

Weil-Felix test

Agglutination antigen	Serum dilution	
B.proteus "O" x 19	- 1:25	
" "O" x 2	± 1:25	- 1:50
" "O" x K	± 1:25	- 1:50

Specimen 2. Ref. no. 305726/13/4/50. 18th. day of illness.

Complement fixation test

	Serum dilution						
	1/12.5	1/25	1/50	1/100	1/200	1/400	A.C. control
Louse typhus washed antigen	-		-				
Murine typhus washed antigen	-		-				
Tick-bite fever antigen	-		-				
Q fever antigen	+	+	+	+	±	-	

..../ Weil-Felix

Weil-Felix test

Agglutination antigen	Serum dilution
B.proteus "O" x 19	- 1:25
" "O" x 2	- 1:25
" "O" x K	- 1:25

The rise in titre against the Q fever antigen from 1:50 to 1:200, indicated that this was a case of Q fever.

Case 4. Ref. no. 247/21/2/52. Dr. Martiny, Johannesburg, submitted a specimen of blood on the 21.2.52, Patient Mrs.B., for rickettsial complement fixation tests. The patient, who had emigrated from Denmark two years before, had been ill for 10 days.

Complement fixation test.

	Serum dilution								
	1/10	1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	A.C. control
Louse typhus soluble antigen	-		-						-
Murine typhus soluble antigen	-		-						
Tick bite fever antigen	-		-						
Rickettsial pox antigen	-		-						
Q fever antigen	+	+	+	+	+	+	+	-	

The result indicated Q fever infection.

Case 5. Ref. no. 810049/21/2/52. Dr. Martiny, Johannesburg, submitted a specimen of blood on the 21.2.52, from a patient, Miss S. This

..../ patient

patient had emigrated from Denmark two years previously, and had been ill for 3 weeks.

Complement fixation test.

	Serum dilution								
	1/10	1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	A.C, control
Louse typhus soluble antigen	-		-						-
Murine typhus soluble antigen	-		-						
Tick-bite fever antigen	-		-						
Rickettsial pox antigen	-		-						
Q fever antigen	+	+	+	+	+	+	+	-	

This result indicated Q fever infection.

Case 6. On the 6.6.51, a specimen of blood was received from the Superintendent, Fever Hospital, Johannesburg. Patient Mrs.A., Wellington, Cape Province. Provisional diagnosis - typhoid fever. Within a week after returning from Wellington, where she had spent a holiday, the patient became ill with severe headache, sweats, feeling of lassitude, anorexia, and abdominal discomfort. She developed a cough and signs of pneumonitis during her fever which persisted in spite of treatment with anti-biotics for over six weeks.

1st. specimen. Duration of illness 10 days.

..../ Complement

Complement fixation test

	Serum dilution.						
	1/6.25	1/12.5	1/25	1/50	1/100	1/200	A.C. control
Louse typhus soluble antigen	-			-			-
Murine typhus soluble antigen	-			-			
Tick-bite fever antigen	-			-			
Rickettsial pox antigen	-			-			
Q fever antigen		±	-	-			

2nd. specimen. Duration of illness 13 days.

Complement fixation test

	Serum dilution							
	1/6.25	1/12.5	1/25	1/50	1/100	1/200	1/400	A.C. control
Louse typhus soluble antigen	±			-				-
Murine typhus soluble antigen	-			-				
Tick-bite fever antigen	±			-				
Rickettsial pox antigen	+	-		-				
Q fever antigen	+	+	+	+	+	+	-	

B.proteus "O" x 19 Field test - negative.

.... / 3rd. specimen

3rd. specimen. Duration of illness 21 days.

Complement fixation test.

	Serum dilution									
	1/6.25	1/12.5	1/25	1/50	1/100	1/200	1/400	1/800	1/1600	A.C. control
Louse typhus soluble antigen	-			-						-
Murine typhus soluble antigen	-			-						
Tick-bite fever antigen	-			-						
Rickettsial pox antigen	+			±						
Q fever antigen	+	+	+	+	+	+	+	+	-	

This case showed an increase in titre from 1:12.5 to 1:800 over the period of illness from the tenth to the twenty first day against the Q fever antigen. This established a diagnosis of Q fever. The plus and trace reactions against the louse typhus, tick-bite fever and rickettsial pox antigens were not regarded as significant.

Case 7. Ref.no. 146541/51. A specimen of blood was received from Dr. H.Epstein, Johannesburg. Patient Mrs. B., a recent immigrant from England, who resided in the country 15 miles north of Johannesburg.

1st. specimen. Duration of illness 2 weeks.

..../Complement

Complement fixation test

	Serum dilution					
	1/6.25	1/12.5	1/25	1/50	1/100	A.C. control
Louse typhus soluble antigen	-			-		-
Murine typhus soluble antigen	-			-		
Tick-bite fever antigen	-			-		
Rickettsial pox antigen	-			-		
Q fever antigen	+	+	+	±	-	

2nd. specimen. 146593/51

Complement fixation test

	Serum dilution								
	1/6.25	1/12.5	1/25	1/50	1/100	1/200	1/400	1/800	A.C. control
Louse typhus soluble antigen	-			-					-
Murine typhus soluble antigen	-			-					
Tick-bite fever antigen	-			-					
Rickettsial pox antigen	-			-					
Q fever antigen	+	+	+	+	+	+	±	-	

The rise in titre from 1:50 to 1:400 indicated Q fever infection.

Case 8. Ref.no. 235098/51. A specimen of blood was received from Dr. A.Jackson, Johannesburg. Patient Mr.B.R. Duration of illness -

..../ 10 days

10 days. Provisional diagnosis - P.U.O.

Complement fixation test.

	Serum dilution				
	1/6.25	1/12.5	1/25	1/50	A.C. control
Louse typhus soluble antigen	-			-	-
Murine typhus soluble antigen	-			-	
Tick-bite fever antigen	-			-	
Rickettsial pox antigen	-			-	
Q fever antigen	-			-	

2nd. specimen. ? Rift Valley fever.

Complement fixation test

	Serum dilution					
	1/6.25	1/12.5	1/25	1/50	1/100	A.C. control
Louse typhus soluble antigen	-			-		-
Murine typhus soluble antigen	-			-		
Tick-bite antigen	-			-		
Rickettsial pox antigen	-			-		
Q fever antigen	+	+	+	+	-	
Rift Valley fever antigen	-			-		

3rd.Specimen. 243853/51

Complement fixation test

	Serum dilution							
	1/6.25	1/12.5	1/25	1/50	1/100	1/200	1/400	A.C. control
Louse typhus soluble antigen	-			-				-
Murine typhus soluble antigen	-			-				
Tick-bite fever antigen	-			-				
Rickettsial pox antigen	-			-				
Q fever antigen	+	+	+	+	+	+	-	

..../ The results

The results indicated Q fever infection.

About 10 days before becoming ill, this patient had spent a day on a farm about 18 miles north of Johannesburg.

Case 9. Ref. no. 243879/51. A specimen of serum was received from Dr. J.du Toit, Pretoria. Patient C.N. Provisional diagnosis - Rift Valley fever.

Complement fixation test.

	Serum dilution.				
	1/6.25	1/12.5	1/25	1/50	A.C. control
Rift Valley fever antigen	-			-	-

As a result of this negative finding, and after a discussion on the case, it was decided to perform the rickettsial complement fixation tests on this specimen.

Complement fixation test

	Serum dilution							
	1/6.25	1/12.5	1/25	1/50	1/100	1/200	1/400	A.C. control
Louse typhus soluble antigen	-			-				-
Murine typhus soluble antigen	-			-				
Tick-bite fever antigen	-			-				
Rickettsial pox antigen	-			-				
Q fever antigen	+	+	+	+	+	±	-	

The result indicated Q fever infection.

..../ Case 10

Case 10. Ref. no. 243594/51. A specimen of serum was received from the Medical Officer, European Hospital, Nairobi, East Africa. Patient Mrs. H. Provisional diagnosis - P.U.O. ? Q fever. The patient had been ill for 10 days.

Complement fixation test	Serum dilution							
	1/6.25	1/12.5	1/25	1/50	1/100	1/200	1/400	A.C. control
Louse typhus soluble antigen	-			-				-
Murine typhus soluble antigen	-			-				
Tick-bite fever antigen	-			-				
Rickettsial pox antigen	-			-				
Q fever antigen	+	+	+	+	+	+	-	

This result indicated Q fever infection.

Case 11. Ref. no. 27462/51. A specimen of blood was received from Dr. S.H. Evans, Kliprivier - 20 miles south of Johannesburg. Patient M. The patient had been ill for 10 days, and was provisionally diagnosed as tick-bite fever, Q fever or murine typhus.

Complement fixation test.	Serum dilution						
	1/6.25	1/12.5	1/25	1/50	1/100	1/200	A.C. control
Louse typhus soluble antigen	-			-			-
Murine typhus soluble antigen	-			-			
Tick-bite fever antigen	-			-			
Rickettsial pox antigen	-			-			
Q fever antigen	+	+	+	+	+	-	

This result indicated Q fever infection.

..../ Case 12.

Case 12. Ref. no. 301499. A specimen of serum was received from the South African Institute for Medical Research, Bloemfontein. Patient B.P. Examination requested - Rickettsial complement fixation tests.

Complement fixation test	Serum dilution.					
	1/6.25	1/12.5	1/25	1/50	1/100	A.C. control
Louse typhus washed antigen	-			-		-
Murine typhus washed antigen	-			-		
Tick-bite fever antigen	-			-		
Rickettsial pox antigen	-			-		
Q fever antigen	+	±	-	-		

Weil-Felix test	Serum dilution
Agglutination antigen	
B.proteus "O" x 19	± 1:25
" "O" x 2	- 1:25
" "O" x K	- 1:25

This result was not positive at a diagnostic titre, and the case was regarded as a suspicious Q fever.

Case 13. Ref. no. 328092/50. A specimen of serum was received from the Government Pathological Laboratory, Durban. Patient P. Provisional diagnosis - Q fever.

..../ Complement

Complement fixation test — Serum dilution

	1/6.25	1/12.5	1/25	1/50	1/100	1/200	1/400	1/800	1/1600	A.C. cont.
Louse typhus washed antigen	-			-						-
Murine typhus washed antigen	-			-						
Tick-bite fever antigen	-			-						
Rickettsial pox antigen	-			-						
Q fever antigen	+	+	+	+	+	+	+	+	-	

Weil-Felix test

Agglutination antigen		Serum dilution
B.proteus	"O" x 19	± 1:25
"	"O" x 2	- 1:25
"	"O" x K	- 1:25

This result confirmed the diagnosis of Q fever.

Case 14. Ref. no. 417/52. A specimen of serum was received from the Senior Pathologist, Union Health Department, Durban. Investigation required - Rickettsial tests. Patient Mrs. P.

Complement fixation test — Serum dilution

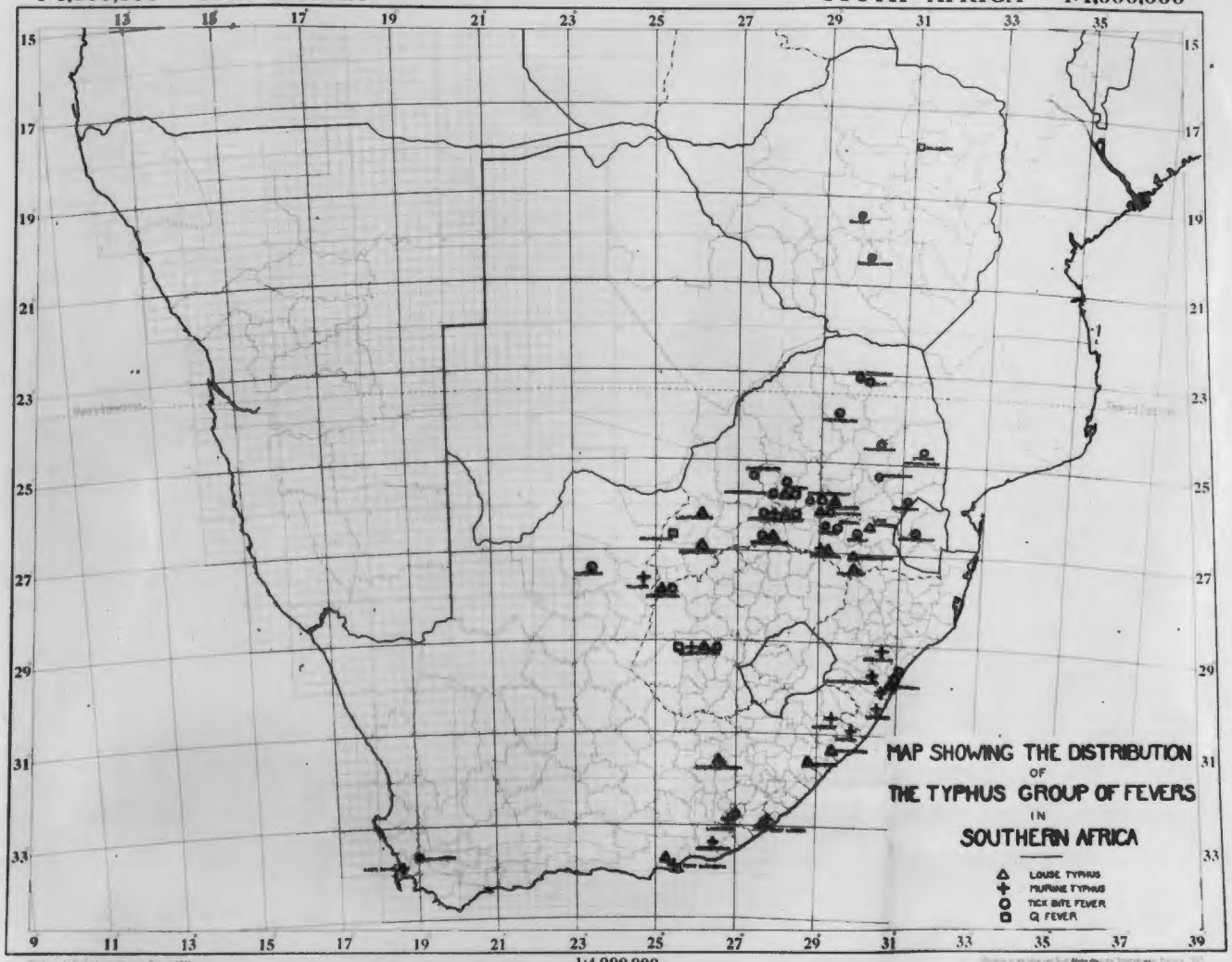
	1/10	1/100	1/200	1/400	1/800	A.C. control
Louse typhus soluble antigen	-	-				-
Murine typhus soluble antigen	-	-				
Tick-bite fever antigen	-	-				
Rickettsial pox antigen	-	-				
Q fever antigen	+	+	+	±	-	

..../ This result

Figure 1.

1:4,000,000 SUID-AFRIKA

SOUTH AFRICA 1:4,000,000



This result indicated that the patient had had Q fever.

It is noteworthy that cases 2,3,4 and 5, proved by the complement fixation test to be Q fever, were recent immigrants from Holland and Denmark, and case 7 was an immigrant from England. These findings suggest that these people have little immunity to Q fever. By contrast, of 429 sera of the indigenous population examined between 19.9.51 and 28.12.51, fifty three or 12.3% gave positive Q fever complement fixation reactions up to a serum dilution of 1:25. During this period 9 sera reacted at diagnostic titres ranging from 1:50 to 1:800. The low titre, i.e. from 1:6.25 to 1:25 so frequently encountered among the indigenous people appears to indicate that Q fever is probably widely spread in South Africa, and that a large proportion of the population, particularly in the rural areas, acquire the infection early in life. They thus have an immunity which is not shared to the same degree by immigrants from North Western Europe.

The findings so far have shown that Q fever occurs in the Transvaal, in areas north and south of Johannesburg, in Pretoria, in Natal in the Ladysmith district, in Wellington in the Cape Province, in the Orange Free State, and in Nairobi in East Africa.

A note on false positive Q fever complement fixation reactions.

In the course of this study, false positive Q fever reactions were occasionally obtained on sera from cases suffering from some of the collagen or hypersensitivity diseases, and in one of chronic

..../ lymphatic

Table 10.

Date specimen received	Complement fixation tests					Remarks
	Antigens					
	Louse typhus	Murine typhus	Tick-bite fever	Rickettsial pox	Q fever	
6/7/50	- 1:12.5	- 1:12.5	- 1:12.5	- 1:12.5	± 1:25	Weil-Felix test - negative. Q fever reaction not diagnostic.
31/8/50	- 1:6.25	- 1:6.25	- 1:6.25	- 1:6.25	± 1:50	This specimen was submitted from the out patients' department. Q fever reaction weakly positive - suggestive but not of diagnostic significance.
20/10/50	- 1:6.25	- 1:6.25	- 1:6.25	- 1:6.25	± 1;6.25	Specimen submitted by Dr. E. Hirshmann, Johannesburg. ? Q fever relapse. Q fever reaction - not diagnostic.
24/11/50	- 1:6.25	- 1:6.25	- 1:6.25	- 1:6.25	± 1:50	Specimen submitted by Hospital. ? Q fever 3rd. relapse. Q fever reaction - weakly positive - suggestive but not of diagnostic significance.

lymphatic leukaemia, and in one of tuberculosis.

The following examples show the reactions obtained:-

Case 1. Patient Miss H.,Johannesburg General Hospital.

This case was provisionally diagnosed as Q fever, but was subsequently proved to be one of chronic lymphatic leukaemia. Over a period of more than four months, the Q fever titre of her serum varied between ± in a serum dilution of 1:6.25 and ± in a dilution of 1:50.

The following table shows the results obtained:-

(See Table 10,opposite page 98)

Case 2. Patient F.M. Coronation Hospital, Johannesburg (native case).

After an illness of four weeks, a specimen of serum was submitted from this case for rickettsial complement fixation tests. Seven specimens were examined over a period of 3 1/4 months, and positive Q fever reactions were obtained varying from ± in a serum dilution of 1:25 to + in a dilution of 1:100.

This case was finally diagnosed as acute rheumatic fever, a diagnosis confirmed by the postmortem examination.

The following table shows the complement fixation results obtained.

(See Table 11 opposite page 99)

..../ Case 3

Table 11.

Date specimen received	Complement fixation tests. Antigens: Louse typhus	Murine typhus	Tick-bite fever	Rickettsial pox	Q fever	Relapsing fever	Remarks
19/10/50	- 1:6.25	-1:6.25	± 1:25	+ 1:6.25	±1:25	- 1:6.25	Patient ill 4 weeks. ? typhus fever, ? tick-bite fever. Reactions not of diagnostic significance.
9/11/50	"	"	- 1:6.25	- 1:6.25	+ 1:100	"	Provisional diagnosis - pericarditis. Q fever reaction - possibly significant.
1/12/50	"	"	"	"	± 1:100	"	Patient ill two months. Q fever reaction - probably not significant in view of duration of illness.
22/12/50	"	"	"	± 1:6.25	± 1:100	"	"
5/1/51	"	"	"	"	+ 1:100		"
24/1/51	"	"	"	- 1:6.25	+ 1:25		? fever reaction - not of diagnostic significance.
3/2/51	"	"	"	+ 1:6.25	+ 1:50		Q fever reaction - of doubtful significance.

Case 3. Patient J.L. The specimen of serum was submitted by Dr. J.Marshall,Johannesburg, 16/5/51.

This patient had been ill for four months. The rickettsial complement fixation tests were all negative, with the exception of Q fever. A ± reaction in a serum dilution of 1:6.25 was obtained with the Q fever antigen.

The patient was clinically diagnosed as suffering from Lupus erythematosus.

Case 4. Patient Mrs. A. A specimen of serum was submitted by Dr. J.Marshall, Johannesburg, on the 21/2/51 for rickettsial complement fixation tests. All the complement fixation tests were negative, except Q fever. A ± reaction in a serum dilution of 1:50 was obtained against the Q fever antigen.

This case was clinically diagnosed as erythema nodosum.

Case 5. Reference no. 3/5503. A specimen of blood was received from the Rand Leases Hospital on the 11/8/50 for complement fixation tests. The case was provisionally diagnosed as relapsing fever. The relapsing fever complement fixation test was negative. With the exception of Q fever, all the rickettsial complement fixation tests were negative. A positive Q fever reaction in a serum dilution of 1:400 was obtained. This patient died of miliary tuberculosis; the diagnosis being confirmed by post mortem examination.

It appears from the results obtained in these cases that the

..../ positive

positive Q fever reactions were not aetiologically related to the patients' current illnesses, and were anamnestic in nature.

In case 1 the titre of reaction did not rise to a diagnostic level, and followed an irregular course not consistant with Q fever.

The slight rise in the Q fever titre to possible significance in case 2 did not progress beyond the diagnostic level, and the rise in antibodies was demonstrated only after an illness lasting between 4 and 7 weeks. This late rise is not characteristic of Q fever.

The results indicate the necessity for care in assessing the value of positive Q fever complement fixation reactions obtained in cases complicated by certain of the collagen or hypersensitivity diseases, chronic lymphatic leukaemia and tuberculosis.

A summary of the results obtained on specimens submitted for serological examination for the typhus group of fevers, between 1/1/49 and 29/9/50

1. Total number of sera examined - 343
2. 161 gave negative complement fixation and Weil-Felix reactions.
3. 22 gave negative complement fixation reactions, the B.proteus "O" x 19 agglutination titres of which varied between - 1:25 and ± 1:1600, and the B.proteus "O" x 2 agglutination titres between - 1:25 and + 1:50.
4. 66 gave negative complement fixation reactions alone, the Weil-Felix tests were not done.
5. 83 gave positive complement fixation reactions indicating either

..../ louse

louse typhus, murine typhus, South African tick-bite fever, or Q fever.

6. Nine gave positive rickettsial agglutination reactions where the complement fixation reactions were either negative, anti-complementary, or where there was no differentiation indicated by the louse and murine complement fixation results.

A. Localities and type indicated by the rickettsial agglutination test.

Durban. Louse typhus - 1 case.

Murine typhus - 4 cases.

B. Localities and type indicated by the rickettsial complement fixation test.

Durban.

Louse typhus - 1 case.

Murine typhus - 6 cases.

S.A. tick-bite fever - 5 cases.

Q fever - 1 case.

Two specimens gave equal titres with the louse and murine typhus antigens, and differential diagnosis was not possible.

Johannesburg and Reef.

Louse typhus - 2 cases.

Murine typhus - 3 cases.

S.A. tick-bite fever - 17 cases.

Q fever - 6 cases.

One specimen gave equal titres with the louse and murine typhus antigens.

..../ Bloemfontein

Bloemfontein.

Louse typhus - 10 cases.

Murine typhus - 6 cases.

S.A. tick-bite fever - 3 cases.

Q fever - 1 doubtful case.

One specimen gave equal titres with the louse and murine typhus antigens.

East London.

Louse typhus - 3 cases.

Murine typhus - 3 cases.

Cape Town.

One specimen gave equal titres with the louse and murine typhus antigens in a dilution of 1:25. Fixation was slightly more complete in the louse typhus test.

Elim. 1 case of tick-bite fever.

Kuruman. 1 case of tick-bite fever.

Bremersdorp. 1 case of tick-bite fever.

Kruger National Park. 1 case of tick-bite fever.

Taungs. 1 case of murine typhus fever.

Christiana. 1 case of tick-bite fever.

Delareyville. 1 case of Q fever.

Bethal. 1 case of tick-bite fever.

Ermelo. 1 case of tick-bite fever.

Sterkstroom. 2 cases of louse typhus fever.

..../ In the

In the complement fixation tests, the average differential dilution difference was 2.7, the range being from one to five. On three occasions completely specific reactions were obtained indicating louse typhus, and on one occasion indicating murine typhus.

Positive louse and murine typhus reactions in serum dilutions from 1:6.25 to 1:25 were considered of doubtful significance, or at the most suggestive of infection. Positive tick-bite fever and Q fever reactions from 1:50 were considered of significance.

The highest serum dilution giving a positive louse typhus complement fixation reaction was in a dilution of 1:1600, and the highest positive murine typhus titre was 1:6400.

The highest serum dilution giving a positive reaction for S.A. tick-bite fever was 1:800. A serum from a case of tick-bite fever was found to react to a titre of 1:200 with the standard tick-bite fever antigen, and to an equal titre with the rickettsial pox antigen.

The highest serum dilution giving a positive reaction against the Q fever antigen, (Nine Mile strain), was 1:200.

The results indicated clearly the specificity of the Q fever reactions. Group reactions, varying in degree, were obtained with the louse and murine typhus antigens. Specific louse and murine typhus reactions were exceptional in the case of human sera, but more commonly found in guinea pig sera.

The rickettsial pox antigen showed a strong group affinity

...../ with

Table 12.

	Comp ement fixation tests				Weil-Felix test			B.proteus "O" x 19 Field Test
	Louse typhus washed antigen	Murine typhus washed antigen	Tick-bite fever antigen	A.C. control	B.proteus "O" x 19	B.proteus "O" x 2	B.proteus "O" x K	
1st. specimen. III 4 days	- 1:6.25	- 1:6.25	- 1:6.25	-	- 1:25	- 1:25	- 1:25	-
2nd. specimen. III 7 days	- 1:6.25	- 1:6.25	- 1:6.25	-	- 1:25	- 1:25	- 1:25	-
3rd. specimen. III 10 days	- 1:6.25	- 1:6.25	- 1:6.25	-	- 1:25	- 1:25	- 1:25	+
4th. specimen. III 14 days.	+ 1:400 - 1:800	+ 1:12.5 - 1:25	+ 1:200 - 1:400	-	+ 1:200 ± 1:400 - 1:800	- 1:25	- 1:25	+ + +
5th. specimen. III 17 days	+ 1:200 - 1:400	+ 1:25 - 1:50	+ 1:200 - 1:400	-	+ 1:400 ± 1:800 - 1:1600	- 1:25	- 1:25	+ + +
6th. specimen. III 21 days.	+ 1:100 ± 1:200 - 1:400	+ 1:25 - 1:50	+ 1:50 ± 1:100 - 1:200	-	+ 1:400 ± 1:800 - 1:1600	- 1:25	- 1:25	+ + +

with the South African tick-bite fever antigen.

On certain occasions positive reactions on the same serum were obtained with the R.prowazeki, R.mooseri, and R.rickettsi var.pyperi antigens. In interpreting these results, certain factors had to be considered, and this was greatly facilitated by the submission, on the part of the clinician, of a full history of the case. Consideration had to be given to the possibility of

1) previous typhus vaccination, and
2) previous infection by one of the members of this group of fevers.

The following results were obtained on the sera of subjects who had previously been vaccinated with rickettsial vaccine, and who subsequently contracted one of the typhus group of fevers.

Case 1. Mrs. W. She became ill on the 14/12/47, and had previously been vaccinated with epidemic louse borne typhus vaccine in 1943.

The following table shows the results obtained on a series of specimens during the patient's illness and in convalescence.

(see table 12,opposite page 104)

The clinical picture, considered together with a history of exposure to ticks on the patient's dog, suggested that this was a case of tick-bite fever. The positive results obtained with the louse typhus and murine typhus antigens, were regarded as anamnestic reactions resulting from previous vaccination.

..../ Case 2

Case 2. Miss B. This subject, a laboratory worker in the Rickettsial Diseases laboratory, had been vaccinated with a combined louse typhus and murine typhus vaccine. She became ill in June 1950.

The following tables show the results obtained.

Table 13.

	Complement fixation tests					Weil-Felix test.		
	Louse typhus washed antigen	Murine typhus washed antigen	Tick-bite fever antigen	Q fever antigen	A.C. control	B.proteus "O" x 19	B.proteus "O" x 2	B.proteus "O" x K
1st. specimen. 6.6.50	± 1:25 - 1:50	+ 1:25 - 1:50	- 1:12.5	- 1:6.25	-	- 1:25	- 1:25	- 1:25
2nd. specimen. 15.6.50	± 1:100	+ 1:200	- 1:12.5	- 1:6.25	-	± 1:25	- 1:25	- 1:25

The clinical picture, considered together with the significant rise in titre from 1:25 to 1:200 against the murine typhus antigen, gave rise to the conclusion that this was a case of murine typhus infection.

Case 2 (continued). In April 1951 Miss B. again became ill. Her history revealed that she had been bitten by a tick near Hartebeestpoort dam.

..../ Table 14

Table 14.

Specimen April 1951

Complement fixation tests					Weil-Felix test			
Louse typhus washed antigen	Murine typhus washed antigen	Tickbite fever antigen	Q fever antigen	A.C. control	B.proteus "O" x 19	B.proteus "O" x 2	B.proteus "O" x K	B.proteus "O"x19 Field test
+ 1:25 - 1:50	+ 1:50 ± 1:100 - 1:200	+ 1:12.5 - 1:25	+ 1:6.25 - 1:12.5	(±)	- 1:25	- 1:25	± 1:50	±

It was concluded that this was a case of tick-bite fever. The positive reactions against the louse typhus and murine typhus antigens were due to previous vaccination against these two diseases, and to a known previous infection of murine typhus fever.

..../ Chapter 6

Table 15.

Results of tests on sera from cases of louse-borne epidemic typhus fever.

Source of specimen	Rickettsial Complement Fixation tests. Antigens.				Weil-Felix Test. B.proteus antigens			Conclusion
	Louse typhus	Murine typhus	Tick-bite fever	Q fever	"O" x 19	"O" x 2	"O" x K	
1.Bloemfontein	± 1/400	± 1/12.5	- 1/12.5	- 1/6.25	+ 1/1600	+ 1/25	± 1/25	Louse typhus
2. "	± 1/200	- 1/12.5	- 1/12.5	- 1/6.25	± 1/400	± 1/100	+ 1/25	"
3. Durban	± 1/400	+ 1/200	+ 1/50	- 1/6.25	± 1/800	- 1/25	± 1/50	"
4. "	± 1/1600	+ 1/200	± 1/12.5	- 1/6.25	+ 1/800	- 1/25	- 1/25	"
5. "	± 1/800	± 1/25	- 1/12.5	± 1/6.25	± 1/400	± 1/200	- 1/25	"
6. Sterkstroom	+ 1/400	± 1/100	- 1/12.5	- 1/6.25	+ 1/1600	+ 1/50	± 1/50	"
7. Bloemfontein	+ 1/800	± 1/100	+ 1/50	- 1/6.25	± 1/3200	± 1/200	± 1/50	"
8.Johannesburg (Brill's disease)	+ 1/3200	± 1/800	- 1/6.25	- 1/6.25	+ 1/100	+ 1/100	- 1/25	"

Chapter 6.

The correlation between the results of the Weil-Felix test and the results of the rickettsial complement fixation test; with a discussion on some of the advantages and limitations of the Weil-Felix test.

The Weil-Felix test has been the standard diagnostic test for the typhus group of fevers for a number of years. It has proved to be of great value. It is therefore important to compare this test with the rickettsial complement fixation test, and to assess their relative merits. In tables 15,16,17 and 18, results given by the Weil-Felix test are compared with the results given by the rickettsial complement fixation test on sera from patients suffering from, or recently recovered from, one of the typhus group of fevers or from Q fever. The end titres are recorded in each case.

(see Table 15 opposite page 107)

It will be noted that in cases 1 to 7, the Weil-Felix results were positive in diagnostic titres. B.proteus "0" x 19 was agglutinated to higher titre than B.proteus "0" x 2. These findings are in agreement with results previously found in cases of louse typhus fever. It is of exceptional interest that in case no. 8, which was one of Brill's disease, the titres of agglutination of proteus strains were not at diagnostic level. The

..../ positive

Table 16.

Results of tests on sera from cases of fleaborne murine typhus fever.

Sourece of specimen	Rickettsial Complement Fixation tests. Antigens				Weil-Felix Test. B.proteus antigens			Conclusions
	Louse typhus	Murine typhus	Tick-bite fever	Q fever	"O" x 19	"O" x 2	"O" x K	
1. Durban	+1/800	±1/3200	±1/100	+1/12.5	±1/400	±1/200	-1/25	Murine typhus
2. "	±1/1600	±1/6400	+1/50	-1/12.5	+1/3200	-1/25	±1/25	"
3. Bloemfontein	±1/12.5	±1/800	-1/12.5	±1/6.25	+1/1600	±1/100	±1/50	"
4. Dunnotar	+1/400	±1/800	-1/12.5	-1/6.25	+1/3200	+1/200	+1/25	"
5. Kokstad	±1/50	±1/1600	±1/25	-1/6.25	±1/1600	+1/100	±1/25	"
6. Taungs	+1/800	+1/3200	-1/12.5	-1/6.25	+1/200	+1/200	-1/25	"
7.Port Elizabeth	±1/12.5	±1/400	-1/12.5	-1/6.25	±1/200	±1/50	±1/100	"

positive complement fixation reactions against the tick-bite fever antigen in cases 3,4 and 7, and against the Q fever antigen in case 5, were probably due to past infections, and not to a cross reaction resulting from antigenic relationship. The most important point which emerges from this table, is that on the results of the Weil-Felix test alone, it was not possible to give a specific diagnosis of louse borne epidemic typhus. On the other hand the rickettsial complement fixation tests gave specific diagnostic titres. Nevertheless in all cases except one, there was cross fixation with the murine typhus rickettsial antigen at lower titres.

Table 16.

(see table 16 opposite page 108)

In these examples it will again be noted that the Weil-Felix results were positive in diagnostic titres. The proteus agglutinations in case no. 6 could not be taken to titre, as there was insufficient serum available. In all the other cases B.proteus "O" x 19 was agglutinated to higher titre than B.proteus "O"x2. These findings agree with the results previously found in cases of murine typhus fever. They resemble the results found in cases of louse typhus fever. It is apparent then, that on the results of the Weil-Felix test alone it was not possible to give a specific diagnosis of flea borne murine typhus fever. On the other hand the rickettsial complement fixation tests gave specific diagnostic

..../ reactions.

Table 17 .

Results of tests on sera from cases of tick-bite fever.

Source of specimen	Rickettsial Complement Fixation tests. Antigens: Louse typhus	Murine typhus	Tick-bite fever	Q fever	Weil-Felix Test B.proteus antigens: "O" x 19	"O" x 2	"O" x K	Conclusions
1. Benoni	- 1/12.5	- 1/12.5	+ 1/800	- 1/6.25	± 1/100	+ 1/400	± 1/25	Tick-bite fever
2. Durban	- 1/12.5	- 1/12.5	+ 1/100	- 1/6.25	± 1/50	± 1/200	- 1/25	"
3. Bethal	- 1/12.5	- 1/12.5	± 1/200	- 1/6.25	± 1/800	+ 1/200	± 1/25	"
4. Johannesburg	- 1/12.5	- 1/12.5	± 1/200	- 1/6.25	± 1/400	- 1/25	- 1/25	"
5. Elim	± 1/25	± 1/25	+ 1/100	± 1/6.25	± 1/200	+ 1/200	- 1/25	"
6. Ermelo	- 1/12.5	- 1/12.5	± 1/100	- 1/6.25	± 1/50	+ 1/200	± 1/25	"
7. Johannesburg	- 1/12.5	- 1/12.5	+ 1/200	- 1/6.25	± 1/100	± 1/100	+ 1/25	"

reactions. However, in all of these cases there was cross fixation with the louse typhus rickettsial antigen in lower titre . The positive complement fixation reactions with the tick-bite fever antigen in cases 1,2 and 5, and against the Q fever antigen in cases 1 and 3, were probably due to past infections.

Table 17

Results of tests on sera from cases of tick-bite fever.

(see table 17 opposite page 109)

It will be noted that in some cases in the Weil-Felix test, B.proteus "O" x 2 was agglutinated to higher titre than B.proteus "O" x 19. Such results are in favour of a diagnosis of tick-bite fever, rather than louse or murine typhus fever. However, it will also be noted that in other cases B.proteus "O" x 19 was agglutinated to much higher titre than B.proteus "O" x 2. In such cases the Weil-Felix test was of no help in differentiating tick-bite fever from the other two forms of typhus fever. It is in these cases that the rickettsial tests were essential for the serological diagnosis of tick-bite fever. The examples illustrate that the results give a clear cut distinction between tick-bite fever on the one hand, and epidemic and murine typhus on the other. Indeed there is little or no cross reaction between the tick-bite fever antibody, and the epidemic and murine antigens. In only one of the cases quoted was there any cross reaction. In this case, no.5, the positive reactions obtained against the louse typhus,

.... / murine

Table 18.

Results of tests on sera from cases of Q fever.

Source of specimen	Rickettsial Complement Fixation tests				Weil-Felix Test			Conclusions
	Antigens				B.proteus antigens			
	Louse typhus	Murine typhus	Tick-bite fever	Q fever	"O" x19	"O" x 2	"O" x K	
1. Krugersdorp	-1/12.5	-1/12.5	-1/12.5	±1/200	±1/50	±1/25	+1/25	Q fever
2.Johannesburg	-1/12.5	-1/12.5	-1/12.5	±1/200	-1/25	-1/25	-1/25	"
3.Johannesburg	-1/12.5	-1/12.5	-1/12.5	+1/50	±1/25	-1/25	±1/50	"
4.Johannesburg	-1/12.5	-1/12.5	-1/12.5	+1/100	±1/25	-1/25	±1/25	"

murine typhus and Q fever antigens were in low dilutions and not of diagnostic significance.

In addition, it will be noted from the results given in this table, that in cases of tick-bite fever the end titres of complement fixation tended to be lower than those encountered in cases of louse typhus and murine typhus. This may be related to the milder nature of tick-bite fever.

Table 18

(See table 18 opposite page 110).

Results of tests on sera from cases of Q fever.

The results in this table show that there is no correlation between positive Q fever complement fixation reactions, and the agglutination of the Proteus strains used in the Weil-Felix test. Further, the results show that there is no cross reaction between Q fever antibodies, and louse typhus, murine typhus and tick-bite fever antigens.

In most cases there was close correlation between the results of the Weil-Felix test and the rickettsial complement fixation tests. A positive agglutination against B.proteus "O" x 19 or B.proteus "O" x 2, or both, usually indicated recent infection caused by either R.prowazeki, R.mooseri or R.rickettsi var.pyperi.

In some cases however, the Weil-Felix test has given positive results when the rickettsial complement fixation tests were

..../ negative.

negative. The following example of such a case was encountered.

Patient Mr. F.

Complement fixation tests	Serum dilution		
	1/10	1/100	A.C. control
Louse typhus soluble antigen	-	-	-
Murine typhus soluble antigen	-	-	
Tick-bite fever antigen	-	-	
Q fever antigen	-	-	

Weil-Felix test

Agglutination antigen	Serum dilution	
B.proteus "O" x 19	+ 1:50	± 1:200
" "O" x 2	+ 1:50	+ 1:200
" "O" x K	- 1:50	- 1:200

Upon obtaining a positive Weil-Felix reaction such as this, it was expected that the rickettsial complement fixation result would be positive. This did not prove to be the case, and negative reactions were obtained . Further questioning of this patient revealed that he had a proteus infection of the bowel, for which he had been treated. It was concluded that antibodies in response to this infection were the cause of the positive Weil-Felix reaction.

The Weil-Felix test gives a positive reaction a little sooner than the rickettsial tests, but also becomes negative sooner.

..../ Antibodies

Antibodies of the typhus group and Q fever can be detected by the rickettsial complement fixation test years after infection. The rickettsial complement fixation test would then be the test of choice for survey work in determining the presence of past infection in a group of people, and at the same time it would have the added advantage of species identification.

The Weil-Felix test is not a specific test, and does not distinguish between louse borne epidemic typhus, flea borne murine typhus and tick-bite fever. In most cases it has been found that the specific rickettsial tests do distinguish between these diseases.

The Weil-Felix tests however still have a practical value for the small laboratory not equipped for the performance of specific rickettsial tests. Such laboratories, on obtaining a positive Weil-Felix result could submit the serum to central laboratories where identification of the responsible rickettsia could be determined by the specific tests.

..../ Chapter 7

Chapter 7

Comparison of the results of the complement fixation test with the rickettsial agglutination test .

As a supplementary to the complement fixation test, attempts were made to introduce a rickettsial agglutination test in this work.

The technique was particularly applied as a means for differentiating between louse typhus and murine typhus infection. As has been already described, heavy washed suspensions of R.prowazeki and R.mooseri could be prepared. For the agglutination tests, these two antigens were diluted in normal saline to the same opacity as the standard proteus antigens used in the Weil-Felix test.

Serial dilutions of the unheated sera to be tested were made from 1:12.5 to 1:1600. 0.2 ml. volumes of each dilution were pipetted into each of two 3"x 3/8" tubes in a metal rack. The first row of tubes received 0.2 ml. each of the R.prowazeki suspension, and the second row of tubes each received 0.2 ml. of the R.mooseri suspension. The final dilutions of serum thus ranged from 1:25 to 1:3200. 0.2 ml. of each antigen was added to 0.2 ml. of normal saline in separate tubes as a control to exclude auto-agglutinability of the antigens. Further,positive and negative serum controls were included. After shaking, the racks were incubated

..../ for

for 18 hours in a 37°C water bath, when the results were read.

On some occasions the results compared favourably with those obtained using the complement fixation method. The agglutinations were finely granular in nature, and were best read by noting the pattern of agglutinated rickettsiae at the bottom of the tubes, without shaking. Shaking the tubes tended to disperse the agglutinated rickettsiae. Some experience was required to assess the results which were more difficult to read than Proteus agglutinations.

However, at times difficulty was experienced in preparing stable, perfectly pure and even suspensions of the rickettsiae essential for this reaction, and for general diagnostic purposes, the complement fixation test was found to be more reliable.

The preparation of suspensions of R. rickettsi var pyperi for the diagnosis of tick-bite fever by this method, was not practicable owing to the scanty growths obtained.

The following table shows the results obtained on louse and murine typhus immune rabbit sera by the complement fixation and the agglutination tests.

..../ Table 19

Table 19.

	Rickettsial agglutination test. Serum dilution						Saline control	Rickettsial complement fixation test. Serum dilution						A.C. control.
	1/25	1/50	1/100	1/200	1/400	1/800		1/25	1/50	1/100	1/200	1/400	1/800	
Louse typhus rabbit serum														
Louse typhus antigen	+	+	+	+	+	±	-	+	+	+	+	+	±	-
Murine typhus antigen	+	+	+	±	-	-		+	+	+	-	-	-	
Murine typhus rabbit serum														
Louse typhus antigen	+	+	+	±	-	-	-	+	+	+	+	-	-	-
Murine typhus antigen	+	+	+	+	+	±		+	+	+	+	+	±	

The following tables show a comparison of the results obtained by these two methods on two selected human cases, one of louse typhus and one of murine typhus infection.

..../ Table 20

Table 20

Louse typhus case. Serum dilutions.

Complement Fixation	1/12.5	1/25	1/50	1/100	1/200	1/400	Saline control	A.C. control
Louse typhus antigen washed	+	+	+	+	±	-		-
Murine typhus antigen washed	+	±	-	-	-	-		
Tick-bite fever antigen	±	-	-	-	-	-		
Rickettsial agglutination test.								
Louse typhus antigen		+	±	±	-	-	-	
Murine typhus antigen		±	-	-	-	-	-	

Table 21

Murine typhus case.

Serum dilutions.

Complement fixation	1/12.5	1/25	1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400	Sal. con.	A.C. con.
Louse typhus antigen washed	+	+	+	+	+	±	-	-	-			-
Murine typhus antigen washed	+	+	+	+	+	+	+	±	-			
Tick-bite fever antigen	-											
Rickettsial agglutination												
Louse typhus antigen	+	+	+	+	+	+	+	±	-	-	-	
Murine typhus antigen	+	+	+	+	+	+	+	+	±	±	-	

Chapter 8.

The results obtained in a survey of murine typhus on rats in Natal and Johannesburg.

Murine typhus in Natal.

One of the objects of this investigation was to assist in the determination of the incidence and geographical distribution of murine typhus in South Africa. A systematic study of the extent of this disease in rodents was therefore undertaken.

As murine typhus was suspected to occur frequently among both the European and Non European population of the coastal area of Natal, a suspicion which has been confirmed in this study, arrangements were made with the Plague Research Laboratory of the Union Health Department to collect rats in this area. Mr. Rose-Innes who was engaged in a rodent survey in Natal at the time, arranged to send live rodents to this Institute between 28.5.48 and 21.9.48.

On arriving at the Institute, the rats were identified. After being anaesthetised, they were bled by heart puncture and sacrificed. Their hair was then combed for fleas, and when found these were identified. The fleas were homogenised in distilled water and inoculated into male guinea pigs with view to the recovery of any rickettsiae. The brains of each group of rats were pooled, homogenised, and suspended in the pooled saline washings of the

..../ spleens.

spleens. Two mls. of this suspension were inoculated intraperitoneally into each of two male guinea pigs. The temperatures of the guinea pigs were taken daily. It was found advisable to take the temperature of the guinea pigs in the mid-afternoon regularly, and as remote from the previous meal as possible. Guinea pigs normally tend to show a rise in temperature immediately after feeding. Rises in temperature of the guinea pigs 14 to 21 days after inoculation together with slight scrotal reactions, both irregular in nature, resulted in the recovery of Spirillum minus originating from the rat tissue inocula. Concurrent infections of Spirillum minus and R. mooseri did occasionally occur.

Whenever a rise in temperature of the guinea pigs within the first week occurred accompanied by scrotal reactions, attempts were made to cultivate and finally demonstrate the rickettsiae microscopically. The guinea pigs were sacrificed under ether anaesthetic, tunica and brain washings were prepared in sterile distilled water aseptically, and 0.1 ml. of this suspension was inoculated into each of a dozen 7th. day embryonated eggs. The eggs were transilluminated daily, and usually on the 5th or 6th day of incubation became moribund. Smears made from the yolk sacs, stained by Machiavello's method, revealed rickettsiae. Final proof as to the identity of these rickettsiae was obtained in the case of the Blackhurst Location and Amanzimtoti cultures by preparing an inoculum from these infected yolk sacs and inoculating 2mls. intra-

..../ peritoneally

peritoneally into two fresh male guinea pigs. After noting a rise in temperature accompanied by scrotal reactions, these guinea pigs were bled about 4 weeks after inoculation. Their sera gave characteristic murine typhus complement fixation reactions.

Several specimens of "rat lice" (Polyplax spinulosus)were combed from the Amanzimtoti rats on the 27.7.48.

These were found to be engorged with blood. They were homogenised in saline, and the suspension inoculated into a separate pair of male guinea pigs. Characteristic temperature and scrotal reactions occurred in the guinea pigs, and eggs inoculated from their brain and tunica washings yielded a good growth of rickettsiae. This strain proved to be identical serologically with the standard murine typhus strain - the Durban Dock strain. This finding is of special interest, as it indicated the possibility of the transmission of R. mooseri from rat to rat by the rat louse, and confirms that of Mooser,Castaneda and Zinsser (44),1931, who showed that the rat louse acted as a vector of murine typhus amongst rats.

Referring to the table, it will be noted that:-

1. Two species of rat were examined in this study, namely, R.rattus and R.norvegicus. Both of these species gave evidence of murine typhus infection.
2. R.mooseri was isolated from guinea pigs infected with rat tissue on five occasions. Two localities were involved.

....7 (a) Blackhurst

(a) Blackhurst Location, Durban, from one batch of rats.

(b) Amanzimtoti, from four separate batches of rats.

These results showed that at least some of the rats from these sources were suffering from murine typhus infection at the time of examination.

3. The results of the complement fixation tests on sera from the rats indicated the presence of murine typhus in the following localities:-

(a) Stanger

(b) Durban. Three occasions.

(c) Amanzimtoti. Three occasions.

(d) Lamontville.

(e) Isipingo.

Failure to isolate rickettsiae from these rats was probably due to their having had past infections, and living rickettsiae were not present in their tissues.

Positive murine typhus complement fixation reactions were encountered in serum dilutions varying between 1:12.5 and 1:200. Titres higher than 1:200 were not found. A positive reaction in a dilution of 1:12.5 appeared to be significant, for on one occasion from a batch of 6 rats, five of which gave negative reactions and one a positive reaction in a serum dilution of 1:12.5, R.mooseri was isolated.

4. Of 108 rat sera testedinthis study, 25 gave positive murine

..../ typhus

typhus complement fixation reactions. Five of these were weak, reacting in a dilution of 1:12.5 only. Nine sera gave specific reactions, i.e. they did not react with the louse typhus antigen. Such results can be contrasted with those found on human sera where group reactions with murine typhus and louse typhus antigens are common and specific reactions are rare.

5. All of the rats gave negative tick-bite fever complement fixation reactions except two. These two specimens came from Lamontville and both reacted against the tick-bite fever antigen in a serum dilution of 1:25. The Weil-Felix reactions in these two rats were negative.

6. Although not reflected in the table, Weil-Felix tests were performed on the rat sera to note if there was any correlation between this test and the rickettsial complement fixation test.

The following results were obtained:-

(a) Of the 108 sera examined, five reacted with B.proteus "O" x 19 antigen in serum dilutions varying between +1:25 and ± 1:50.

(b) Of these five sera, three gave positive murine typhus complement fixation reactions in dilutions varying between 1:50 and 1:200.

(c) The remaining two sera gave negative murine typhus complement fixation reactions.

(d) All of the sera gave negative reactions against

..../ B.proteus "O" x 2

B.proteus "O" x 2 in a dilution of 1:25.

(e) All the sera reacted with B.proteus "O" x K antigen in dilutions varying between 1:25 and 1:100, the greater number reacting in a dilution of 1:50.

These results showed that the correlation between the murine typhus complement fixation test and the Weil-Felix test was low, and that reliance should be placed upon the complement fixation test when examining rats for evidence of murine typhus rather than on the Weil-Felix test.

7. Spirillum minus was isolated from, and demonstrated in guinea pigs inoculated from the rats of three localities, namely Verulam, Mount Edgecombe Sugar Mills, and Durban.

8. Fleas were found on one occasion only, but as these rats were dipped in a insecticidal solution as a precaution against plague before being sent to the laboratory, their almost total absence is not surprising.

The results of this investigation illustrated the value of the rickettsial complement fixation test as a means for determining present and past infection of murine typhus in rats. They indicated the high incidence of murine typhus infection endemic in the rat population of the coastal belt of Natal.

..../ Table 22

Table 22.

Natal rats.

Locality	No.of rats. Date rcd.	Species.	Result of CF tests on rats' blood.	Result of g.pigs inoc. with rat tissue.	Egg cultivation from g.pigs.	Remarks.
Stanger	8 rats 27.5.48	R.rattus	5 negative 2 positive murine CF. 1 doubtful murine CF. Titres 1:100 specific " 1:50 " 1:12.5 doubtful	Negative	-	Murine typhus serological evidence.
Verulam	5 rats 4.6.48	R.rattus	5 negative	Spirillum minus eventually recovered.	-	-
Durban	4 rats 4.6.48.	R.rattus	3 negative. 1 positive murine CF. End titre 1:100	Negative	-	Murine typhus serological evidence.
Mount Edgecombe	2 rats 10.6.48.	R.rattus	2 negative	Negative	-	-
Black-hurst Location Durban.	3 rats. 11.6.48.	R.rattus	1 negative. 2 positive murine CF. End titre 1:25 (specific). End titre 1:100.	Both g.pigs reaction with temp. over 104 F and scrotal reactions on the 9th day.	Rickettsiae recovered and Murine typhus serological evidence.	Strain still under cultivation. Serological indistinguishable from standard cult.of Durban Dock.

Table 22 (cont.) (2)

Locality	No.of rats. Date recd.	Species.	Result of CF tests on rats' blood.	Result of G.pigs inoc.with rat tissue	Egg cultivation from g.pigs.	Remarks.
Mount Edgecombe Sugar Mills.	4 rats 16.6.48	R.rattus	4 negative	Spirillum minus recovered	-	-
Redhill Durban	1 rat 16.6.48		1 negative	Negative	-	-
Durban	1 rat 17.6.48	R.norvegicus.	1 Positive murine CF. End titre 1:25 (specific).	Spirillum minus recovered.	-	Murine typhus serological evidence,
Durban	4 rats 17.6.48	R.rattus	4 negative.	Negative	-	-
Amansimtoti	6 rats 24.6.48	R.rattus	4 negative. 1 doubtful murine CF. 1 positive murine CF. End titres 1:12:5 1:50	Negative	-	Murine typhus serological evidence,
Lamontville	5 rats 22.6.48	R.norvegicus.	2 negative. 1 murine CF weakly positive, titre ±1:25 (specific). 2 weakly positive tick-bite fever CF.	Negative	-	Murine typhus (doubtful) serological evidence. Tick-bite fever serological evidence.

Table 22 (cont.) (3)

Locality	No. of rats Date rcd.	Species	Result of CF tests on rats' blood	Result of g.pigs inoc.with rat tissue.	Egg cultivation from g.pigs.	Remarks.
Park Ryne	6 rats 1.7.48	R.norvegicus.	6 negative.	Negative	-	-
Isipingo	6 rats 29.6.48	3 R.rattus,3 R. norvegicus.	4 neg. 1 weakly positive murine CF.End titre 1:25. 1 doubtful positive murine CF.End titre 1:12.5. Both R.rattus.	Negative	-	Murine typhus serological evidence,
Amanzimtoti	6 rats 8.7. 48.	R.rattus	4 negative. 1 positive murine CF,1:400 titre. 1 pos.murine CF. 1:200 titre(specific).	Negative	-	Murine typhus serological evidence.
Amanzimtoti	6 rats 13.7.48	R.rattus	3 negative;1 doubtful mur.CF.1:12.5; 1 pos. mur.CF. titre 1:200; 1 pos.mur.CF. 1:100; (specific).	Both g.pigs reacted with a temp. 104 F on the 5th day.Subcult.revealed temp.104-105 F and scrotal reaction on 5th. day.	Rickettsiae recovered.	Murine typhus serological evidence,
Durban Central.	6 rats 15.7.48	2R.rattus,4R. norvegicus.	6 negative.	Negative	-	-

Table 22 (cont.) (4)

Locality	No.of rats Date rcd.	Species	Result of CF tests on rats' blood.	Result of g.pigs inoc.with rat tissue	Egg cultivation from g.pigs.	Remarks.
Amanzimtoti	6 rats 22.7.48	R.rattus	3 negative; 1 pos. murine CF.,1:100 titre (specific).	Both g.pigs reacted with temp. over 105F on 5th. day.Scrotal reactions,positive.	Rickettsiae recovered.	Murine typhus serological evidence.
Amanzimtoti	6 rats 27.7.48	R.rattus	3 neg.;1 pos.mur.CF. titre 1:50;2 pos.mur. CF titre 1:100(specific	Both g.pigs reaction temp.over 104F on 8th.day with scrotal reactions.	Rickettsiae recovered.	Murine typhus serological evidence.
Amanzimtoti	6 rats 29.7.48	R.rattus	4 neg.;1 weakly pos. mur.CF 1:25 titre(specific);1 pos.mur.CF. titre 1:100.	Negative	-	Murine typhus serological evidence.
Amanzimtoti	6 rats 4.8.48	R.rattus	5 neg.;1 doubtful mur. CF. titre 1:12.5.	Both g.pigs react. with temp.105F,& scrotal react.on 9th. day.	Rickettsiae recovered.	Murine typhus doubtful serological evidence.
Amanzimtoti	3 rats 5.8.48	R.rattus	3 negative	Negative	-	-
Umzinto	6 rats 21.9.48	R.rattus	6 negative	Negative	-	-
Durban	2 rats 24.8.48	R.rattus	1 anticompl.;1 pos.mur. CF. end titre 1:200 D.Dock,1:100 Ettie.	Negative	-	Murine typhus serological evidence.

Murine typhus in Johannesburg

In June 1948, a dealer at Newton Market, Johannesburg, contracted an illness which was provisionally diagnosed as typhus fever. A specimen of his serum was submitted with a request for rickettsial complement fixation tests.

The following results were obtained:-

Complement fixation test	Serum dilution									
	1/25	1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400	A.C. control
Louse typhus antigen	+	+	+	+	+	+	+	±	-	-
Murine typhus antigen	+	+	+	+	+	+	+	+	+	
Tick-bite fever antigen	±	-								

Weil-Felix test

Agglutination antigen	Serum dilution
B.proteus "O" x 19	± 1:3200
" "O" x 2	+ 1:400
" "O" x K	- 1:25

The result indicated murine typhus fever infection.

The occurrence of this case emphasized the need for a survey of the rat population to determine the incidence and distribution of murine typhus in Johannesburg. An arrangement was therefore made with the Municipal Health department for the dispatch of

..../ live

live rats from a wide range of premises in the city and suburbs of Johannesburg for this investigation.

During the period from the 14th.July 1948 to 25th.November 1949, 390 rats were examined. They were submitted in groups varying between 1 and 4 individuals at the rate of approximately six rats per week. Their sera were tested by the complement fixation test against the louse typhus, murine typhus and tick-bite fever antigens.

The same procedure was adopted as for the Natal investigation regarding the bleeding of the rats for serological tests, the inoculation of guinea pigs with pooled, homogenised rat brains in the spleen washings, and the isolation of rickettsiae on egg culture .

Reference to the table will show that:-

1. Evidence of murine typhus was obtained in twenty-eight batches of rats comprising 60 individuals.

2. Forty-three of these rats gave positive murine typhus complement fixation results in serum titres varying between 1:12.5 and 1:400.

3. R.mooseri was isolated on egg culture from twenty batches of the rats.

4. Positive murine typhus complement fixation tests were obtained on members of eighteen batches of the rats from which R.mooseri was isolated on egg culture.

5. On one occasion R.mooseri was isolated from guinea pigs

..../ inoculated

inoculated with rat tissue, the sera of these rats however gave negative murine typhus complement fixation reactions. It was concluded in this case that one or both of these rats were suffering from an early infection, and that antibodies had not developed at the time of bleeding.

6. The sera of 5 guinea pigs surviving clinically typical murine typhus infection, gave positive murine typhus complement fixation reactions; R.mooseri having been isolated on egg culture from their companions.

7. Positive murine typhus complement fixation tests were obtained on members of seven batches of rats, the isolation of R.mooseri in guinea pigs being negative.

8. Rickettsiae were demonstrated microscopically in smears prepared from the tunica of a guinea pig which had been inoculated with the tissues of a rat. The serum of this rat gave a ± murine typhus complement fixation reaction in a dilution of 1:25.

9. The serum of one rat was anti-complementary, but R.mooseri was isolated on egg culture from a guinea pig inoculated with the rat tissue.

10. Spirillum minus infection occurred on one occasion in guinea pigs inoculated with the tissues of two rats. The sera of these rats however gave positive murine typhus complement fixation reactions.

..../ Table 23

Table 23

Positive results obtained on the rats examined for Murine Typhus between 14.7.48 and 25.11.49 in Johannesburg.

Locality	No.of rats	Complement Fixation test.	Guinea pig result.	Egg culture from g.pig.	Date
Stand 198 Pim St., NEWTOWN.	4	2 + murine CF. 2 - murine CF. End titre ±300 " " ±200	Temperature. No scrotal reaction.	Negative	14.7.48
Stand 104 Pim St., NEWTOWN.	3	1 + murine CF 2 - murine CF End titre ±500	Temperature and scrotal reaction.	Positive. R.mooseri.	20.7.48
Stand 198 Pim St., NEWTOWN.	4	3 + murine CF. 1 - murine CF. End titre ±300 " " ±25 " " ±200	Temperature and scrotal reaction.	Positive. R.mooseri.	20.7.48
Stand 198 Pim St., NEWTOWN.	1	+ murine CF. End titre ±400	Temperature and scrotal reaction.	Positive. R.mooseri.	22.7.48
Stand 156 Crystal St., DENVER	2	2 + murine CF. End titre ± 25 " " ± 25	Temperature and scrotal reaction.	Positive. R.mooseri.	26.8.48.
Stand 105/6, Pim St., NEWTOWN.	3	3 + murine CF. End titre ± 50 " " ± 25 " " ±50	Temperature and scrotal reaction.	Positive. R.mooseri.	19.8.48
Stand 98, Pim St., NEWTOWN.	3	3 + murine CF. End titre ± 50 " " ± 50 " " ±100	Temperature and scrotal reaction.	Positive. R.mooseri.	10.8.48.
Portion B.B. Lower Main Reef Road, George Goch.	1	+ murine CF. End titre ±100	Temperature. No scrotal reaction.	Negative.	28.9.48
Stand 147, Poulton Rd., BOOYSENS.	2	2 + murine CF. End titre ±200 " " ± 50	Temperature and scrotal reaction.	Positive. R.mooseri.	30.9.48
Stand 98, Pim St., NEWTOWN.	1	+ murine CF. Titre ± 100.	Negative	Negative	5.10.48.
Stand 147, Poulton Rd., BOOYSENS.	1	± murine CF. Titre ± 25.	Temp. & scrotal reaction. Rickettsiae demonstrated in tunica.	Negative	22.12.48

..../Table 23 (2).

Table 23 (2)

Locality	No.of rats.	Complement Fixation Test.	Guinea pig result.	Egg culture from g.pigs.	Date.
Stand 111, Malherbe St. NEWTOWN.	2	2+ murine CF. End titre ±200 " " ±100	Temp.& scrotal reaction. Sp.minus infection only.	Negative.	31.3.49
Stand 195/6, Bree St., NEWTOWN.	2	2 + murine CF. End titre ±100 " " ± 50	Negative.	Negative.	5.4.49
Stand 44, Goch St., NEWTOWN.	2	1 ± murine CF. 1 - murine CF. Titre ± 25.	Negative	Negative.	7.4.49
Portion 6, lot B,Farm, TURFONTEIN.	2	1 ± murine CF. 1 - murine CF. End titre ±12.5	Temp. and scrotal reaction.	Positive. R.mooseri.	7.4.49.
Stand 195/6, Bree St., NEWTOWN.	1.	+ murine CF. End titre + 50	Temp. and scrotal reaction.	Positive. R.mooseri.	28.4.49.
Stand 195/6, Bree St., NEWTOWN.	2.	1 + murine CF 1 - murine CF End titre +100	Temp. and scrotal reaction.	Positive. R.mooseri .	10.5.49.
Lower Main Reef Road Farm, DOORNFONTEIN	2	1 + murine CF 1 - murine CF End titre ±400	Temp. and scrotal reaction.	Positive. R.mooseri.	19.5.49
Stand 232, Bree St., NEWTOWN.	2	2 + murine CF. End titre ±100 " " + 50	Temp.& scrotal reaction. CF. test on surviving g. pig + murine typhus .	Positive. R.mooseri.	23.6.49
Lower Main Reef Road Portion 1 of G.G.	2	1 + murine CF 1 - murine CF. End titre + 25	" " "	Positive. R.mooseri.	30.6.49

.:.:/ Table 23 (3)

Table 23 (3)

Locality	No.of rats.	Complement Fixation Test	Guinea pig result	Egg culture from g.pig.	Date
Stand 232, Bree St., NEWTOWN	2	1 + murine CF. 1 - murine CF. End titre + 100	Temperature and scrotal reaction.	Positive. R.mooseri.	30.6.49
Stand 83, Pim St., NEWTOWN	2	2 + murine CF. End titre ± 50 " " ± 100	Temperature and scrotal reaction. CF test on surviving g.pig + Mur.typhus.	Positive. R.mooseri.	5.7.49
Lower Main Reef Rd. Portion 1 of G.G.	2	2 - murine CF.	" " "	Positive. R.mooseri.	5.7.49
Lower Main Reef Rd. Portion 1 of G.G.	2	1 + murine CF. 1 anticomplementary. End titre ± 100	" " "	Positive. R.mooseri.	7.7.49
Stand 232, Bree St., NEWTOWN.	1	+ murine CF. Titre ± 100.	Temperature and scrotal reaction.	Positive. R.mooseri.	14.7.49
Stand 232, Bree Str., NEWTOWN.	4	2 + murine CF. 2 - murine CF. End titre + 25 " " ± 25	" " "	Positive. R.mooseri.	19.7.49
Stand 195, Bree St., NEWTOWN.	1	Anticomplementary.	Temperature and scrotal reaction. CF test on surviving g.pig + mur.typhus.	Positive. R.mooseri.	19.7.49
Stand 232, Bree St., NEWTOWN.	4	3 + murine CF. 1 ± murine CF. Titres ± 25 " ± 50 " ±12.5 " ± 400	Negative	Negative	21.7.49

..../ These

These results showed a close correlation between positive murine typhus complement fixation reactions on rat sera, and the isolation of R.mooseri from their batched brain and spleen washings. In addition it was evident that positive rat serum reactions occurred when rickettsiae were not recoverable from the rat tissues. These positive serum reactions probably indicated past infections of murine typhus.

Attempts to isolate rickettsiae from fleas.

The low incidence of fleas on the rats was probably due to the rats having been dipped in an insecticide before sending to the laboratory. On eleven occasions fleas were combed from the rats. They were homogenised and suspended in distilled water. Two mls. of these suspensions were inoculated intraperitoneally into each of two male guinea pigs. All gave negative results.

Weil-Felix tests on the rat sera.

Weil-Felix tests were performed on the rat sera with a view to noting if there was any correlation between the results of this test and the complement fixation reaction.

The following results were obtained:-

1. Of the 390 rat sera examined, 1.8% gave weakly positive B.proteus "O" x 19 Field test reactions.

2. None of the rat sera reacted against B.proteus "O" x 19 and B.proteus "O" x 2 in a serum dilution of 1:25.

..../ 3. Against

3. Against the B.proteus "O" x K antigen, 3.7% of the sera gave negative reactions in a dilution of 1:25. 15.3% of the sera gave positive reactions in a dilution of 1:25. 76.0% of the sera gave positive reactions in a dilution of 1:50. 5.0% of the sera gave positive reactions in a dilution of 1:100.

It was thus evident that there was no correlation between positive murine typhus complement fixation reactions on the rat sera and agglutination of the B.proteus "O" x 19 and B.proteus "O" x 2 antigens.

Other investigations.

Advantage was taken of pursuing some other investigations on this series of 390 rats, and the following information was obtained:-

a) All of the rat sera gave negative relapsing fever complement fixation reactions using an antigen prepared from Spirochaeta duttoni.

b) Trypanosoma lewisi was noted in the blood smears of 39 rats, i.e. 10%.

c) Spirillum minus was isolated in guinea pigs from four batches of rats in the municipal area.

Between the 29.11.49 and the 13.12.51, a further 730 live rats were submitted from a wide range of suburbs in the Johannesburg Municipal area. Biological tests for murine typhus were discontinued in this series, but the serum of each rat was

..../ tested

tested against the louse typhus, murine typhus and tick-bite fever antigens.

The following table shows the incidence and distribution of murine typhus antibodies in the rat sera of this area.

Table 24.

District.	Number of rats giving:-		
	Positive Murine typhus Complement Fixation tests.	Negative Murine typhus Complement Fixation tests.	Anti complementary sera.
Newtown	35	100	6
City & Suburban	1	8	-
Lower Main Reef Road. Farm Doornfontein	10	17	3
Fordsburg	4	18	-
Troyeville	1	7	-
Observatory .	1	16	-
Rosebank	1	5	-
Linksfield. Huddle Park Golf course.	1	22	-
Other districts	-	466	8

Reference to the table shows that positive murine typhus complement fixation reactions were obtained on 54 of the 730 rat sera examined, i.e. 7.4%.

..../ The incidence

The incidence of murine typhus was highest along the main east-west railway system - through the city from George Goch in the lower Main Reef Road area, City and Suburban, the Newtown market area, and Fordsburg. Single cases were found in the residential areas of Troyeville and Observatory two to three miles east and north-east of the city, and further afield at Rosebank 5 miles to the north, and Linksfield 5 miles to the north-east.

This study also revealed the presence of serum antibodies against tick-bite fever in a considerable proportion.of these rats.

..../ Chapter 9

Chapter 9

The discovery of the presence of South African tick-bite fever infection in house rats and wild rodents in Johannesburg.

Serological evidence of the occurrence of tick-bite fever in the rat population of the Johannesburg Municipal area was obtained first on the 25.8.49. Two rats - Rattus rattus - caught at Linksfield on the north-eastern outskirts of the city both gave positive tick-bite fever complement fixation reactions in a serum dilution of 1:25.

As this suburb borders upon open veld and golf courses, it was then decided to test the veld rodents. Arrangements were accordingly made with the Johannesburg Public Health Department to trap veld rodents in this area. On the 21.9.49 and 28.9.49 twelve vlei rats - Otomys irroratus - and eight striped mice - Rhabdomys pumilio - were received alive at this laboratory. They were anaesthetized and bled by cardiac puncture. The sera separated from these blood specimens were tested by the rickettsial complement fixation test. The results are given on the accompanying table. It will be noted that two vlei rats gave positive tick-bite fever complement fixation tests, the other rodents gave negative results.

..../ Table 25

Table 25.

Linksfield Huddle Park Golf courses.	Number of rodents giving positive tick-bite fever complement fixation reactions.	Number of rodents giving negative tick-bite fever complement fixation reactions.
Otomys irroratus 12.	2	10
Rhabdomys pumilio 8	-	8

During the course of the murine typhus investigation, the rat sera were also tested for evidence of tick-bite fever.

The following table shows the incidence and distribution of tick-bite fever in the 1120 rats - R.rattus - examined between 14.7.48 and 13.12.51.

..../ Table 26

Table 26.

District	Number of rats giving:-		
	Positive tick-bite fever complement fixation tests.	Negative tick-bite fever complement fixation tests.	Anti-complementary sera
Newtown	2	100	6
C.E.D.Compound, City Deep.	1	5	-
Lower Main Reef Road. Farm Doornfontein.	1	17	3
Fairview	1	3	-
Troyeville.	1	7	-
Bellevue	2	2	-
Observatory	1	16	-
Kensington	1	18	-
Parktown	1	27	-
Melrose.	1	6	-
Parktown North.	1	2	-
Greenside	2	4	-
Craighall	2	1	-
Cydna Disposal Works	1	19	-
Parkhurst	5	16	-
Houghton	2	21	-
Northcliff	2	3	-
Industria	3	15	-
Linksfield Huddle Park Golf courses.	5	16	-
Other districts	-	670	8

The table shows that positive tick-bite fever complement fixation reactions were obtained on 35 of the rats examined i.e. 3.43%. Serological evidence of tick-bite fever infection in these domestic rats was found over a wider area than murine typhus. A high incidence was noted in suburbs on the perimeter of the municipal area bordering on open country, namely Greenside, Northcliff, Parkhurst, Craighall and Linksfield ranging from 4 to 6 miles in a north-west to north-east arc from the centre of the city.

As a result of the high incidence of tick-bite fever antibodies found in the sera of the Huddle Park rats, and the fact that two specimens of vlei rats from this area showed evidence of tick-bite fever infection, it was thought possible that the domestic rats may have become infected by contact with the environment of wild veld rodents, i.e. they had been bitten by arthropods infected by the wild rodents.

More extensive evidence of infection in the veld rodents was necessary, and an arrangement was made with Mr. D.H.S.Davis of the Union Health Department to trap veld rodents in this vicinity, and further afield for dispatch to this Institute for examination.

Upon receipt the rodents were bled by cardiac puncture under an anaesthetic. The sera separated from the blood were submitted to the complement fixation tests. The brain and spleen washings from each group were pooled and inoculated into male guinea pigs with a view to isolating rickettsiae.

..../ The following

The following table shows the species, number of rodents, and the results of the tick-bite fever complement fixation tests on the rodent sera. Antibodies to murine typhus were not found.

Table 27.

Results of tick-bite fever complement fixation tests on wild rodents sera between 6.7.51 and 27.11.51.

Rietfontein Hospital area - 6 miles north-east of Johannesburg.

Rodent species.	Number of sera and serum dilutions						
	- 1/6.25	+1/6.25	+ 1/12.5	+1/25	+1/50	+1/100	Anti-complementary
Otomys irroratus 22 sera	8	4	3	3	2	1	1
Mastomys natalensis 3 sera	1	1	-	-	-	-	1
Rhabdomys pumilio 36 sera	16	6	6	4	1	-	3
R.rattus . 17 sera	16	-	-	1	-	-	-

Table 28

Smith's Farm - 8 miles north-east of Johannesburg.

Rodent species.	Number of sera and serum dilutions							
	- 1/6.25	+ 1/6.25	+ 1/12.5	+1/25	+1/50	+ 1/100	+1/200	Anti-complementary
Otomys irroratus 13 sera	2	3	5	-	-	2	1	-
Mastomys natalensis 1 serum	-	1	-	-	-	-	-	-
Rhabdomys pumilio 5 sera	3	1	-	-	-	-	-	-

..../ Reference

Reference to these tables shows that:-

1. Serological evidence of tick-bite fever infection occurs in three species of wild rodent examined, namely Otomys irroratus, Mastomys natalensis and Rhabdomys pumilio.

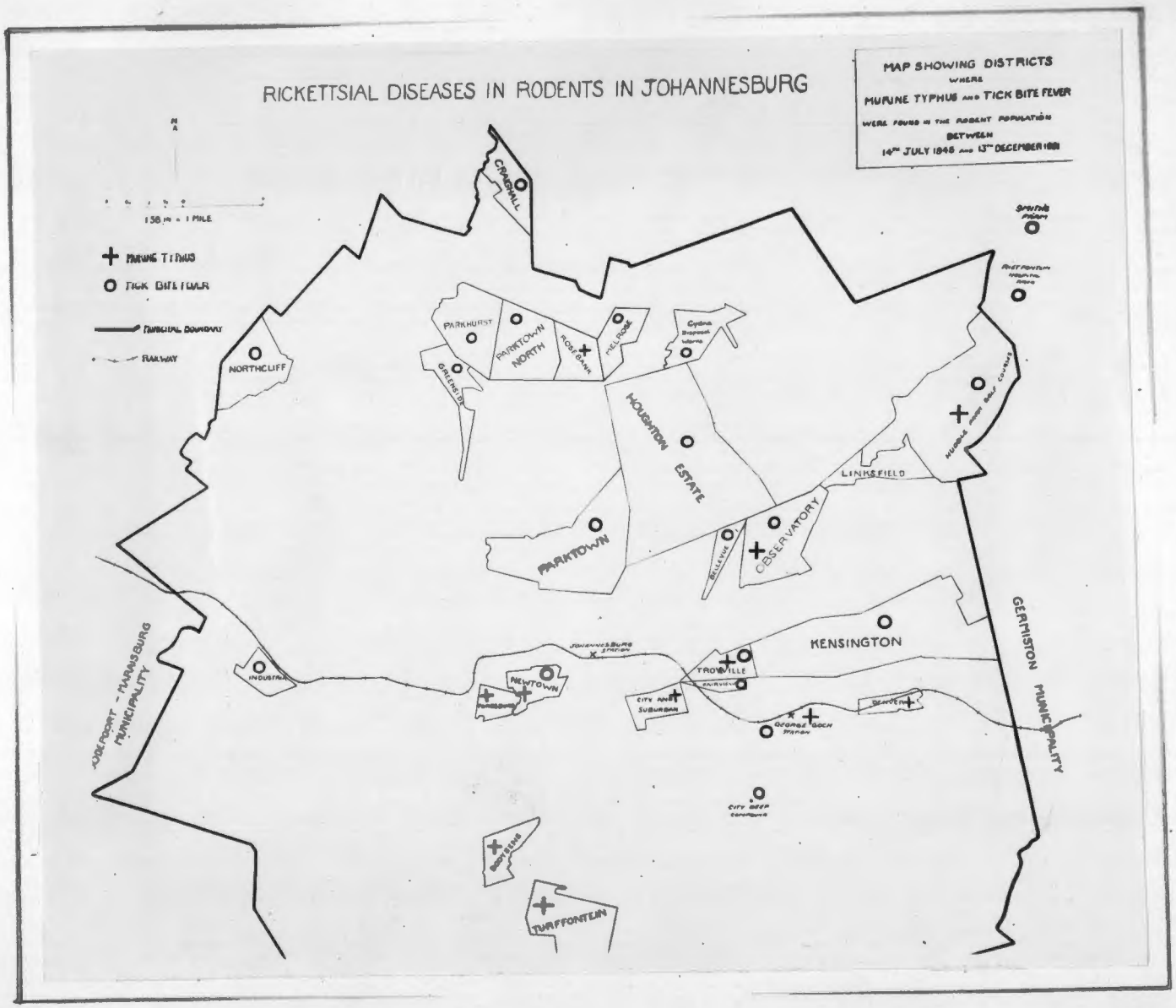
2. That the incidence of infection appears to be particularly high in Otomys and Rhabdomys in these two areas.

The existence of tick-bite fever infection in the vlei rat (Otomys) was conclusively proved on the 27.11.51. A young specimen was received, bled for the complement fixation tests, and its brain and spleen washings were inoculated into male guinea pigs. One of the guinea pigs reacted on the 9th. day after inoculation with a temperature of 104°F. Brain and tunica washings of this guinea pig were subcultured on to two further male guinea pigs which reacted with temperatures and scrotal reactions. One was sacrificed and the brain and tunica washings were subcultured on to 7th day embryonated eggs, in the yolk sac of which rickettsiae were finally demonstrated microscopically, The serum of the surviving guinea pig gave a positive tick-bite fever complement fixation reaction in a dilution of 1:25. This strain of tick-bite fever has since been kept by serial passage both in guinea pigs and on egg culture, and called the Otomys strain.

On the 12.1.51, a strain of tick-bite fever was similarly isolated in guinea pigs and then on egg culture from four rats -

..../ R.rattus -

Figure 2.


RICKETTSIAL DISEASES IN RODENTS IN JOHANNESBURG
MAP SHOWING DISTRICTS WHERE MURINE TYPHUS AND TICK BITE FEVER WERE FOUND IN THE RODENT POPULATION BETWEEN 14TH JULY 1948 AND 13TH DECEMBER 1951
N
1.58 ins = 1 MILE
MURINE TYPHUS
TICK BITE FEVER
MUNICIPAL BOUNDARY
RAILWAY
CRAIGHALL
NORTHCLIFF
PARKHURST
PARKTOWN NORTH
ROSEBANK
MELROSE
GREENSIDE
HOUGHTON ESTATE
PARKTOWN
BELLEVUE
OBSERVATORY
LINKSFIELD
KENSINGTON
TROYVILLE
FAIRVIEW
CITY AND SUBURBAN
PRETORIA MAIN
JOHANNESBURG STATION
INDUSTRIA
TURFFONTEIN
CITY DEEP
JOEL POORT - MARAISBURG MUNICIPALITY
GERMISTON MUNICIPALITY

R.rattus - caught at Huddle Park.

Both the Otomys and Huddle Park immune guinea pig sera reacted identically with the standard tick-bite fever antigen derived from the Malish strain of human origin.

..../Conclusions

Chapter 10

Conclusions.

From this study it is concluded that :-

The Weil-Felix test is a useful screen test for the detection of cases belonging to the typhus group of fevers. The slide agglutination test, using B.proteus "O" x 19 as antigen, is of value under field conditions in South Africa for differentiating the typhus fevers from other clinically similar fevers.

The Weil-Felix test is often negative in cases of Brill's disease, and is negative in cases of Q fever. It may, but not always, differentiate with certainty between cases of tick-bite fever on the one hand, and epidemic louse borne typhus and murine flea borne typhus on the other hand. It does not differentiate between epidemic and murine typhus fever.

The test may help to distinguish between present or recent infection from remote infection.

Positive reactions occur in cases of proteus infection, unrelated to the typhus fevers.

Owing to the simplicity of its technique, the Weil-Felix test will continue to be used for diagnostic purposes in small laboratories not equipped to perform the more recent rickettsial complement fixation and agglutination tests.

Suitable antigens for rickettsial complement fixation and agglutination tests can be prepared from egg cultures of strains of rickettsiae isolated in South Africa.

..../ The most

The most sensitive and satisfactory method for the complement fixation reaction, was a modification of the Kolmer technique employing an overnight fixation period at 4°C.

The South African strain of epidemic louse borne typhus fever - known as "Ettie" - compares identically with the classical Breinl strain, and with other louse borne typhus fever strains isolated in this country. This strain can be used for antigenic purposes in identifying epidemic louse borne typhus fever.

The South African strain of murine flea borne typhus fever - known as "Durban Dock"- compares equally with the standard Wilmington strain , and with other strains isolated from rats and fleas in South Africa. This strain can be used for antigenic purposes in identifying murine flea borne typhus fever.

Both soluble and washed rickettsial antigens prepared from these two strains are capable of giving differential diagnosis when employed in complement fixation tests. Group reactions in low serum dilutions when using these antigens are the rule, and specific reactions are rare. The soluble antigens give less marked differentiation than the washed rickettsial antigens. In most cases differentiation between epidemic louse borne typhus fever and murine flea borne typhus fever is possible.

The louse and murine typhus fever antigens show no cross reaction with South African tick-bite fever, Rickettsial pox, and Q fever sera.

..../ The

The application of complement fixation tests has revealed the occurrence of cases of Brill's disease in South Africa.

Rickettsial complement fixation tests compare favourably with rickettsial agglutination reactions in louse typhus and murine typhus fever.

An antigen prepared from a strain of South African tick-bite fever isolated from a human case - called " Malish" - is effective for the diagnosis of this disease when employed in the complement fixation test. It compares with other strains isolated from several species of ticks. A mixed rickettsial and soluble type antigen is the only one available at present for use in complement fixation tests, owing to the difficulty found in obtaining large numbers of the rickettsiae necessary for agglutination antigens. Tick-bite fever sera show no cross reaction with the louse typhus, murine typhus, and Q fever antigens. They show marked group reaction with Rickettsial pox antigens.

Results obtained with the complement fixation test indicate the presence of Q fever in South Africa. The imported Q fever antigen prepared from the American "Nine Mile" strain appears to be identical with, or at least closely related to, the South African strain. Sera from cases of Q fever show no cross reaction with the louse typhus, murine typhus, South African tick-bite fever, and rickettsial pox antigens. Positive reactions in serum dilutions less than 1:100 are not of diagnostic significance,

..../ unless

unless a rise in titre on a further specimen is obtained. Considering the finding that about 12.0% of normal sera give positive reactions against the Q fever antigen in serum dilutions up to 1:25, it is concluded that Q fever has prevailed in South Africa for some time. Non specific reactions can occur, usually in low serum dilutions, but occasionally in high dilutions, among the collagen or hypersensitivity diseases, in tuberculosis, and in chronic lymphatic leukaemia.

In cases of louse typhus, murine typhus, and tick-bite fever, the correlation between the Weil-Felix test and the rickettsial complement fixation test is usually good. Complement fixation antibodies however persist for years, and a concurrent positive Weil-Felix reaction is more indicative of recent infection.

The examination of more than one specimen during the course of illness, showing a rise in titre, gives more accurate information than obtaining a positive result on a single specimen. This is especially true when the titre of reaction is low.

Complement fixation reactions in serum dilutions from 1:100 upwards against the louse typhus, murine typhus, and Q fever antigens, are diagnostic. Positive reactions in serum dilutions from 1:50 against the tick-bite fever antigen, are significant.

Antibodies can usually be detected by the complement fixation test from the second week of illness in cases of louse typhus, murine typhus, and Q fever infection. Antibodies in cases of

..../South African

South African tick-bite fever, at diagnostic levels, are usually found to occur during convalescence. Treatment of these diseases with antibiotics causes the antibody response to be less than that in untreated cases, but does not prevent the appearance of these antibodies.

The rickettsial complement fixation test is a valuable means for indicating murine typhus and tick-bite fever infection in rat populations. Murine typhus exists in the rat population of the coastal belt of Natal, and in the Johannesburg municipal area. Tick-bite fever is present among the rats of the suburban areas of Johannesburg, and among wild rodents of this city's adjoining country districts.

The Weil-Felix test is of no value in determining the presence of murine typhus and tick-bite fever in rodents.

In practice it is concluded that the Weil-Felix test should not be displaced by the rickettsial complement fixation test, but that the two tests should be performed together. More accurate diagnosis would be obtained from the combined results of the two techniques. Further, that positive Weil-Felix sera from the small laboratory, not equipped to perform the rickettsial tests, should be referred to central or regional laboratories for confirmation and species identification.

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..../ PART 2

PART 2.

THE COMPLEMENT FIXATION TEST IN THE DIAGNOSIS OF RELAPSING FEVER

Chapter 1.

A review of the literature dealing with the serological diagnosis of relapsing fever.

The laboratory diagnosis of relapsing fever has depended largely upon detecting Spirochaeta duttoni in blood smears. The blood smears have to be taken at optimal times, during the febrile periods in the course of the disease, otherwise the organisms may not be seen. Resort has also been made to mouse inoculation with whole blood from suspected cases. Subsequent microscope examination of the mouse blood at daily intervals may show the presence of spirochaetes. Factors which may have an adverse effect upon success with this method, according to Stein (1),1944, are (a) insufficient numbers of spirochaetes present in the inoculum to cause infection of the mice, (b) loss of viability on the part of the organisms, (c) uncontrollable and variable host resistance. From the clinical view point, several infectious diseases, having an intermittant febrile course, may be confused with relapsing fever. Stein quotes

..../ the

the following:- " Malaria, spotted fever, typhus, Weil's disease, and certain enteric diseases particularly in the early stages".

As a consequence of the limitations of these methods, a number of investigators have attempted to develop serological techniques. Some have confined their work to the serological study of various species of spirochaetes and their antibody production in laboratory animals. Others have reported favourably on the results obtained on both experimental animal and human sera. Cunningham and Fraser (2), 1935, and Cunningham (3),1925, have conducted agglutination tests. Using a microscopic method, they have been able to demonstrate antibodies both in animal and human sera. Yano (4),1936, has determined the presence of complement fixation immune bodies in guinea pigs.

The principal factor for a successful serological method, was that of obtaining an antigen of pure spirochaetes. This was overcome by Stein, who utilized an antigen obtained from heavily infected rat and mouse blood. The whole blood obtained by heart puncture, was mixed with 2% sodium citrate solution in physiological saline. It was then subjected to the haemolysing action of saponin. Separation and thorough washing of the spirochaetes was finally obtained by differential centrifugation. The resulting antigen consisted of spirochaetes suspended in 1:15000 merthiolate saline. This antigen was successfully used both by agglutination and complement fixation methods. The reagent was stable, and kept

..../ for

for four months in the refrigerator. His investigation was concerned with the study of both animal and human sera, using antigens derived from two species of relapsing fever spirochaetes. A positive reaction in a human serum, diluted 1:500, was obtained by both methods. This case had been infected with mouse blood. Suitable controls were included to determine the possible presence of mouse protein antibodies, and these were found to be absent. Stein records that convalescent sera from cases of typhus fever, malaria, Rocky Mountain spotted fever, Weil's disease, syphilis, and typhoid fever, failed to give positive results.A note was made that the suspensions appeared to possess broad antigenic qualities for the three species under consideration, namely, S.novyi, S.hermsi, and S.obermeieri. He suggested that the complement fixation test might be of value for the diagnosis of relapsing fever. The agglutination test, although easier to perform, was considered to be more difficult to read, and in addition the complement fixation method was found to be more sensitive.

The question of the influence of S.pallidum infection has been investigated by several workers. Roaf (5),1922/23, has asserted that a transient positive Wassermann reaction occurred during the acute stage of the disease. Ts'Un and Chung (6),1938), have found 7.95% false positive Kolmer Wassermann reactions, Kahn and Kline tests occurred in known cases of relapsing fever. Wolstenholme and Gear (7),1948, found that of thirty nine + +

..../ positive

positive Wassermann sera, four gave ± reactions in a serum dilution of 1:12.5 with S.duttoni antigen. Reactions in a dilution of 1:12.5, although not considered diagnostic of relapsing fever, might be considered as suspicious.

Elsdon-Dew (8),1943, observed that relapsing fever sera agglutinated B.proteus "O" x K antigen to high titre. Of 650 known cases, he obtained 211 positive B.proteus "O" x K reactions in a dilution of 1:100 or more. In one case an agglutination in a serum dilution of 1:6400 was obtained, falling to 1:50 after the last relapse. Although not of a specific nature, this unusual finding in a group of people - apart from scrub typhus infection - would appear to indicate that the presence of relapsing fever should be considered. This outbreak,thought to be of louse origin, occurred in Ethiopia.

The conclusions reached are that -

1. The complement fixation technique appears to be a reliable and sensitive method for the diagnosis of relapsing fever.
2. This test, although more complicated in its performance, has the advantage over the agglutination method of being easier to read.
3. For bulk work this serological method is less time consuming than the slide and animal tests.
4. The test can detect the presence of antibodies irrespective of the presence of spirochaetes in the blood.

..../ Chapter 2

Chapter 2.

The development of a technique for growing Spirochaeta duttoni.

On the 2.7.46, a white mouse was received from Dr. D.Ordman of the South African Institute for Medical Research, Johannesburg, which had been infected with S.duttoni. The mouse appeared to be ill, possibly as a result of exposure on the previous night. It was sacrificed under ether, the heart exposed, and blood withdrawn into a sterile 2 ml. all glass syringe. Leishmann stained films, and dark ground illumination of the blood revealed scanty spirochaetes. 0.1 ml. of the blood was inoculated intraperitoneally into each of two further mice. This was done mainly with a view to perpetuating the strain. The idea of attempting to grow spirochaetes in eggs occurred at this time. Consequently 0.05 ml. of whole mouse blood was inoculated into the allantoic cavity of each of 8 seventh day embryonated eggs. The precaution of replacing the syringe needle with a sterile one before inoculating the eggs was taken in an attempt to avoid contamination.

On the 3.7.46, two of the eggs were found to be contaminated, and spirochaetes were not found. Samples of blood taken from the tail veins of the two mice showed no spirochaetes.

..../ 4.7.46

4.7.46 : Further samples of blood from these mice revealed no spirochaetes, and the eggs had all died, having been contaminated.

5.7.46 : Blood samples from both of the mice revealed a few motile spirochaetes by dark ground examination. One of the mice was sacrificed under ether, the heart exposed, and blood withdrawn into a sterile salinified syringe. After replacing the syringe needle with a sterile one, the blood was immediately inoculated into eight 8th day embryonated eggs - 0.05 ml. of blood per egg. At the same time two fresh mice received 0.1 ml. of the blood intraperitoneally. A dark ground check of this inoculum revealed a few motile spirochaetes.

8.7.46 : The eggs inoculated on the 5.7.46 were found to be alive. One was opened, and the blood examined under dark ground illumination, but spirochaetes were not found. Blood samples of the mice inoculated on the 5.7.46 revealed a few motile spirochaetes.

9.7.46 : All of the remaining eggs inoculated on the 5.7.46 were found to ba alive. One was opened but spirochaetes were not found.

10.7.46 : All of the remaining eggs were found to be alive. Two were opened, and blood samples from each were examined. One egg was negative, the second contained a few motile spirochaetes. A fresh batch of twenty four 7th day embryonated eggs was inoculated. Each egg received 0.1 ml. of a suspension comprised of blood cells, allantoic fluid and scanty spirochaetes gleaned from the positive

..../ egg.

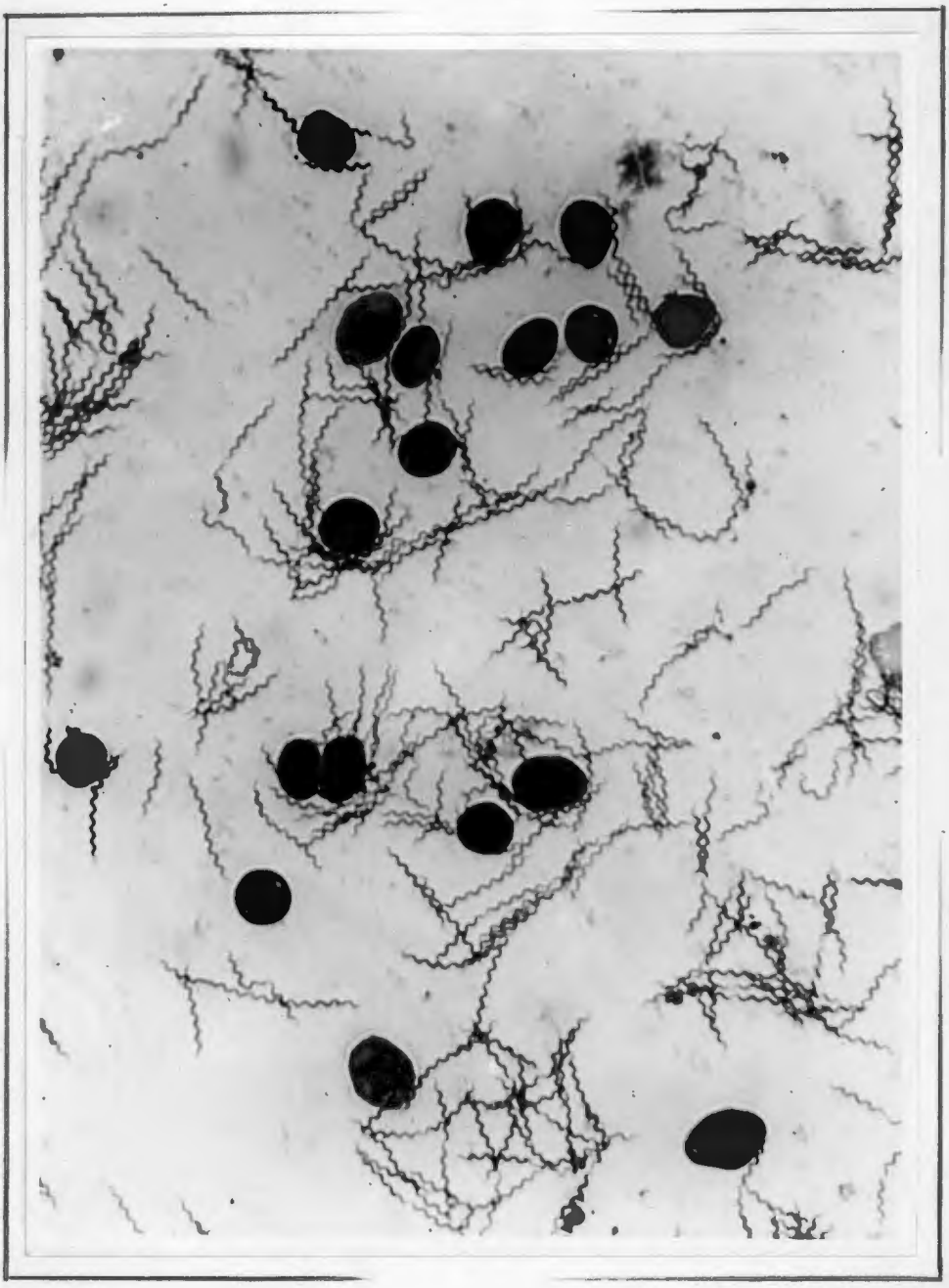
Figure 3.

Spirochaeta duttoni.

Photomicrograph x 840 Wright's stain.

Thin film prepared from the

blood of an infected chick embryo.



egg.

17.7.46 : On transillumination, 14 of the eggs inoculated on the 10.7.46 were found to be alive. Each one was examined in turn for the presence of spirochaetes, and the degree of infection varied from nil in some, to abundant spirochaetes in two.

This culture of S.duttoni had been established on eggs, and has been subinoculated at weekly intervals ever since.

Although this technique for the growing of relapsing fever spirochaetes was subsequently found to have been described, considerable satisfaction was experienced by this achievement. Priority is given to Oag (9),1939, who grew relapsing fever spirochaetes in eggs.

The dark ground illumination procedure for this work consisted of the following:-

Having charred the air sac end of a living infected egg in an acetylene flame, the charred shell was removed exposing the dry air sac membrane. Using sterile forceps this membrane was carefully peeled away exposing the allantoic membrane with its blood vessels intact. By carefully tearing this tissue aside, the blood vessels were allowed to rupture and flow into the allantoic fluid. A drop of this blood and allantoic fluid was placed on a microscope slide using a sterilized platinum loop and mounted under a cover slip.

The Bausch and Lomb microscope used, was provided with a dark ground illumination stop which was fitted into the condenser, Using

...../ the high

the high power objective, the spirochaetes moving among the blood cells could easily be seen. The source of light was a 100 watt frosted electric globe housed in a standard microscope lamp box.

The egg inoculation technique was the same as that described for growing the rickettsiae, i.e., directly into the egg through a small hole pierced in the blunt end over the air sac. The inoculum on each occasion was selected from an infected living egg of the previous passage. An egg displaying a good growth of spirochaetes was selected, i.e., from 2 to 4 times as many spirochaetes per dark ground field as there were blood cells. One minum of such a suspension was found to be the most suitable to yield a good growth in living eggs after a further 7 days incubation at 37°C.

It should be noted that the inocula were only obtained from living eggs as the spirochaetes tended to disappear once the egg had died.

White mice and white rats inoculated at intervals from the egg culture, showed infection from the third day. An attempt at infecting rabbits with strong doses of 2 ml. intravenously, failed.

Subsequently four further strains of S.duttoni were isolated on eggs via white mice from the blood clots of known cases of relapsing fever. The blood clots on these occasions were homogenised, and 0.2 ml. of this material was inoculated intraperitoneally into each white mouse. The mice were then checked daily for infection, and when this was established, their blood was inoculated into eggs

..../ as previously

as previously described. It is interesting to note that two of these blood clots had been exposed to 4°C overnight before being inoculated into the mice. The spirochaetes had withstood this temperature.

Morphology and behaviour of the spirochaetes.

When examined under dark ground illumination, the spirochaetes from the mouse blood showed distinct ability to bend through a wide arc, and their movement across the field was rapid. The spirals were large and sometimes irregular, Their general appearance was like that revealed in stained human blood smears. In eggs, however, the spirochaetes appeared to be more rigid, and the spirals were closer together and more regular, resembling S.pallidum. When freshly mounted from a warm egg they showed considerable motility, which rapidly decreased with the drop in temperature.

Chapter 3.

A description of the preparation of antigens of S.duttoni, and the technique of the relapsing fever complement fixation test.

Following on the remarkably abundant growths of S.duttoni obtainable on egg culture, it was decided to attempt the preparation of an antigen for serological diagnosis. The problem presenting itself was to separate the spirochaetes in the pure state from the embryonic blood cells and allantoic fluid. This was successfully effected by carefully controlled centrifuging of the harvested material.

Twenty four 7th day embryonated eggs were inoculated. Each received 1 minum of a suspension of spirochaetes, blood cells, and allantoic fluid gleaned from a living infected egg of a previous subculture. These eggs after a further 7 days incubation at 37°C were candled, and the living set aside for individual examination. The blood,allantoic fluid and spirochaete mixture of the positive eggs was pooled by pipetting the suspensions into a sterile bottle, The average number of eggs worthy of harvesting from an initial planting of 24, was twelve. The usual yield of fluid from these eggs was 60 mls. The bottle of harvested material was allowed to stand overnight at room temperature. By the following day the blood

..../ cells

cells had settled to the bottom, leaving about 50% of the spirochaetes in the supernatant fluid, the remainder having settled with the cells. Approximately two thirds of the supernatant fluid was pipetted into sterile centrifuge tubes. The sedimented cells and the remaining spirochaetes were then thoroughly stirred into the remaining allantoic fluid. This was pipetted into two separate centrifuge tubes, and centrifuged at 500 r.p.m. for three minutes. A small portion of the cell-free supernatant fluid containing spirochaetes was removed into fresh centrifuge tubes. After stirring the deposited cells again, this process was repeated until the cells had been washed free from spirochaetes. The centrifuge tubes now contained allantoic fluid and spirochaetes in suspension, free from blood cells. A further centrifuging at 2000 r.p.m. in a horizontal centrifuge for 20 minutes, resulted in a dense white deposit of spirochaetes. After discarding the supernatant allantoic fluid, the spirochaete deposit was resuspended in 12 mls. of physiological saline containing 1:10,000 merthiolate. This suspension constituted the antigen. When examined under dark ground illumination, abundant spirochaetes were observed together with an occasional blood cell. The anti-complementary properties of these antigens were low, and they were usually used in the neat state or diluted so as not to absorb more than 0.5 M.H.D. of complement under test conditions. The antigens were found to keep for at least 3 months at 4°C before beginning to become anti-complementary.

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The technique for performing the relapsing fever complement fixation tests was the same as that described for the rickettsial tests. The complement was titrated in the presence of the antigen and saline - as a substitute for serum in the tests - and 1 full M.H.D. of complement was determined as the highest dilution to show a trace of cells. Two full M.H.D. of complement was used in the tests. This titration was performed on the day prior to the performance of the tests, so as to allow a fixation period of 18 hours in accordance with that used for the tests.

The sera under test, after absorption with complement to remove anti-complementary properties, were heated at 60°C for 20 minutes. They were then diluted serially from 1:6.25 to 1:800 - the usual range of reaction. Provision was made in addition for an anti-complementary, and a normal egg antigen control of the serum. The fixation period was 18 hours at 4°C, and the haemolytic system consisted of 1.5% washed sheep cells sensitized with 2 M.H.D. of haemolysin. 0.1 ml. volumes of all reagents were used in the tests, with the exception of the sensitized cells, where 0.2 ml. was added to each tube. The results were read after 30 minutes in the 37°C water bath.

..../ Chapter 4

Chapter 4

A discussion of the results obtained, and a comparison with other diagnostic tests.

The results obtained have proved to be of diagnostic value, and today the test is regularly applied. The test appears to be specific, and parallel tests with sera giving positive Wassermann reactions were negative or of low titre, i.e., in 1:12.5.

When the test was first evolved, smears were made from the blood clots submitted for serological diagnosis with a view to finding the spirochaetes when stained by Giemsa's method. On all occasions when spirochaetes were found in smears from these blood clots, the complement fixation tests were positive. In addition, the homogenised blood clots were inoculated into white mice, and spirochaetes were later demonstrated in the blood from some of the specimens giving positive complement fixation reactions.

The following results were obtained from 26.7.46 - the date of inception of the test - until 23.7.48.

Positive complement fixation reactions ranged from a serum dilution of 1:25 to 1:1600. Reactions in dilutions of 1:6.25 and 1:12.5 were regarded as suspicious or of doubtful significance.

1. Positive complement fixation

   Positive thick smears from blood clots 4 cases

   Spirochaetes demonstrated in mouse blood.

..../ 2.

2. Positive complement fixation.
   Positive thick smears from blood clots. — 5 cases.

3. Positive complement fixation.
   Negative thick smears from blood clots. — 18 cases.

4. Positive complement fixation.
   Negative thick smears from blood clots.
   Spirochaetes demonstrated in mice. — 6 cases.

5. Positive complement fixation.
   Negative thick smears from blood clots.
   Spirochaetes not demonstrated in mice. — 38 cases.

6. Positive complement fixation.
   Smear and mouse tests not done. — 13 cases.

7. Doubtful complement fixation.
   Negative thick smears from clots.
   Spirochaetes not demonstrated in mice. — 12 cases.

8. Doubtful complement fixation.
   Smear and mouse tests not done. — 5 cases.

9. Negative complement fixation.
   Positive thick smears from blood clots.
   Spirochaetes demonstrated in mice. — NIL

..../ 10.

10. Negative complement fixation.

Negative thick smears from blood clots. 20 cases.

Spirochaetes not demonstrated in mice.

11. Negative complement fixation.

21 cases.

Smear and mouse tests not done.

The results indicate that the relapsing fever complement fixation test is of value in the diagnosis of the disease. The test is helpful in those cases where spirochaetes are difficult or impossible to find on direct blood smear examination. It appears to be more sensitive than the mouse inoculation test, and is less time and space consuming.

The test promises to be of value in assessing the antibody content of sera before, during, and after, treatment. Furthermore its value for large-scale survey purposes would be considerable, and in such circumstances would be less laborious and time consuming than the direct smear or mouse inoculation techniques.

The results quoted were submitted from known or suspected relapsing fever cases. On two occasions however, relapsing fever was suspected and tick-bite fever infection was proved by complement fixation. On one occasion typhus fever was suspected, and relapsing fever infection was proved by the complement fixation test.

The following results were obtained from a case of relapsing fever taken over a period. A gradual increase in titre was indicated.

..../ Patient

Patient (Robert).

Specimen no.	Serum dilution									Duration of illness
	1/12.5	1/25	1/50	1/100	1/200	1/400	1/800	1/1600	A.C. control	
1.	±	-	-	-	-	-	-	-	-	8 days.
2.	+	+	+	±	-	-	-	-	-	11 days
3.	+	+	+	+	±	±	-	-	-	14 days
4.	+	+	+	+	±	±	-	-	-	15 1/2 days
5.	+	+	+	+	±	-	-	-	-	22 days

Sera giving positive or trace reactions up to a dilution of 1:25 were regarded as suspicious, and a positive diagnosis could only be reported on a rise in titre determined on examination of a further specimen.

Following on the work of Middlebrook and Dubos (10),1948, and Fisher and Keogh (11),1950, a red cell sensitization test was attempted. Although little success was obtained by trying to sensitize sheep's cells with the spirochaetes, good results were obtained by utilizing the embryonic blood corpuscles which became sensitized in vivo. These chick cells, when freed from excess spirochaetes by differential centrifuging, were found to be susceptible to the haemolytic action of complement in the presence of the antibody. Control sera containing no spirochaete antibody exhibited no haemolysis in the presence of complement.

..../ Serial

Serial dilutions were made in saline of the decomplementized serum, and 0.2 ml. volumes added to a series of 3" x 3/8" tubes. To each of these tubes 0.2 ml. of a 1% suspension of twice washed spirochaete sensitized chick embryo blood cells were added. After incubation in a 37°C water bath for 1 hour, 0.2 ml. of 2 M.H.D. of complement were added to each tube; haemolysis occurred in the lower dilutions of serum after a further 30 minutes incubation in the 37°C water bath. The haemolysis gradually decreased in intensity as the antibody content of the serum was diluted out. The 2 M.H.D. of complement was determined using an equivalent strength of sheep's corpuscles. The results obtained were opposite in effect to a complement fixation reaction, namely - a positive reaction showed complete haemolysis, and a negative reaction absence of haemolysis. In addition, it was noted that the sedimented chick red cell nuclei at the bottom of the tubes showing complete haemolysis, i.e. in positive reacting tubes, had agglutinated as a tenuous deposit. Microscopic examination showed massed clumping of the chick red cell nuclei, free from the cell membranes.

This test compared favourably with the standard complement fixation reaction, and was easier to perform and took less time. However, it should be noted that this technique can only be performed in laboratories where S.duttoni is kept on routine egg culture, as a source of supply of sensitized chick cells. Whereas the complement fixation antigen can be distributed for use in any laboratory.

..../ The following

The following results were obtained from a case of suspected relapsing fever of 18 days duration.

Complement fixation test.

Antigen	Serum dilutions					A.C. control.	Normal egg antigen control.
	1/6.25	1/12.5	1/25	1/50	1/100		
S.duttoni	+	+	+	±	-	-	-
Normal serum control	-	-	-	-	-	-	-

Sensitized chick cell - haemolysis test.

Antigen indicator.	Serum dilutions					Saline control.
	1/6.25	1/12.5	1/25	1/50	1/100	
Chick cells sensitized in vivo with S.duttoni	+	+	±	±	-	-
Normal serum control	-	-	-	-	-	

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.... / PART 3

PART 3.

THE COMPLEMENT FIXATION TEST IN THE DIAGNOSIS OF AFRICAN TRYPANOSOMIASIS.

Chapter 1.

A review of the literature dealing with the serological diagnosis of trypanosomiasis.

Serological methods for the diagnosis of trypanosomiasis have been sought by many workers, and reference to published results reveals some considerable degree of success. As an aid to, and perhaps as a substitute for blood smear examination, these methods could be of great value. For the examination of a large number of suspected cases, or for survey work, a reliable serological method would be considered almost essential. However, it is apparent from the literature on this subject, that (1) some technical experience would be required both for the performance of the tests, and their interpretation, and (2) a supply of the reagents and cultures, properly controlled and standardised, would have to be at hand. It appears that the performance of some of the tests at least would have to be conducted at some central institution or specially equipped station.

The methods described can be grouped into (A) non-specific, and (B) specific tests. The non-specific tests comprise the formol-gel

..../ reaction

reactions, and the mercuric chloride precipitation test. The specific tests consist of the red-cell adhesion test, agglutination, precipitation, and complement fixation tests.

Non-specific tests. Dye (1),1926, while emphasising the non-specificity of the formol-gel reaction, and pointing out its doubtful significance in areas where Kala-azar is present, recommended this test when slide examination fails. The author indicated some of the pitfalls likely to be encountered, and illustrated the optimal conditions required for obtaining satisfactory results. Cookson (2),1947, considered the test of real value for the diagnosis of Trypanosoma rhodesiense infection,especially as a method for rapid survey of a suspected area. Hope-Gill (3), 1938, maintained that a positive formol-gel reaction within 60 minutes was strongly in favour of sleeping sickness infection. Mention was made again, however, that the reaction could only be regarded as of significance in the absence of Kala-azar. He claimed that interference by syphilis was negligible.

Wilson (4), 1930, described the results he obtained with the mercuric chloride precipitation test. The reaction was considered to be of value when there are clinical signs in cases from infected areas. The results were concluded to resemble those obtained with the formol-gel reaction.

Specific tests. Wormall (5),1933, and Davis and Brown (6),1927, have obtained results with the red cell adhesion test. The technique,

..../ when

when properly performed, was complicated and exacting. Some experience was required for interpreting the results and excluding non-specific reactions. However, Brown (7),1933, has reported on a case of T.gambiense infection. Clinically a case of sleeping sickness, the blood slide examinations were negative on 22 days. Red cell adhesion tests gave positive results using three strains of T.gambiense . These results indicated recourse to monkey inoculation, which finally revealed T.gambiense infection. The test was judged to be of value as it pointed to more exhaustive investigation,leading to the final isolation of the causative trypanosome.

Packchanian (8),1940, using an antigen of T.cruzi derived from Novy and MacNeal's medium, successfully obtained agglutination reactions on immune rabbit and fowl sera. He was able to show that T.cruzi sera gave positive reactions ranging in titre from 1:256 to 1:1024, whereas nagana and murina sera gave a maximum titre of 1:32.

Muniz (9),1947, has described the results of a precipitation test in the diagnosis of Chagas' disease. The test was considered to be of value in early cases only, but not when the condition became chronic.

Research on the complement fixation test has been mainly conducted by American workers on T.cruzi infection. Davis and Sullivan (10),1946, were able to prove as a result of a survey of over 2000 sera in Texas, that American trypanosomiasis infection,if present

..../ at all

at all, was extremely rare.

Kelser (11),1936, has recommended an antigen of T.cruzi grown on Bonacci's medium. Over 400 specimens of man and animal sera were tested. Known cases gave constantly positive results, and the negative control sera were satisfactory. No cross reactions appeared with positive Wassermann sera.

Johnson and Kelser (12),1937, tested 1251 sera in Panama, and determined an infection rate of 3.83%. Positive reactions were found in cases of 1 to 5 years duration, and in many cases where trypanosomes could not be demonstrated on blood smear examination.

Pellegrino and Borrotchin (13),1948, in their investigation said, "The complement fixation test (Schizo trypanum cruzi) culture antigen, owing to its specificity and sensibility, proved to be very useful for the laboratory diagnosis of chronic Chagas' disease".

In conclusion, it appears that the laboratory worker has an extensive range of techniques for the serological diagnosis of sleeping sickness. That in the event of failing to find trypanosomes by blood slide examination, one of the various serological techniques might aid in the diagnosis. The formo-gel and mercuric chloride tests, after due consideration regarding their non-specificity, would act as a general guide as to the prevalence of the disease in an area. Judging by the results obtained, the complement fixation test appears to be the method of choice, giving the most accurate and easily readable results.

..../ Chapter 2

Chapter 2.

The culture of Trypanosoma rhodesiense on the developing chick embryo.

In March 1945, a guinea pig was received which had been inoculated intraperitoneally with the blood of a patient suffering from sleeping sickness in Ngamiland. After trypanosomes had been found in the peripheral blood of the guinea pig, the strain was transferred to further guinea pigs by syringe passage. An attempt was made at this time to establish the strain on embryonated hens' eggs. A guinea pig possessing large numbers of trypanosomes in its blood stream was selected, and anaesthetized with ether. Using a sterile 2 ml. all glass syringe, 2 mls. of blood were withdrawn from the heart, and replacing the used needle with a fresh sterile one, 0.1 ml. of the whole blood was inoculated immediately into each of a batch of twenty four 7th.day embryonated eggs. Using a 1 1/2 " 26 G. needle the inoculum of whole blood was injected through the centre of the blunt end of each egg, passing through the air sac directly into the allantoic cavity. The batch of eggs was incubated at 37°C and a humidity of 50% for five days. At the end of this period, the eggs were candled, and samples from dead and living eggs were examined microscopically for trypanosomes. One of the living eggs so examined showed an occasional trypanosome in the

..../ allantoic

allantoic fluid into which the blood vessels had been allowed to flow. The eggs were left in the incubator for a further two days. Candling at this stage revealed a 50% death rate of the embryos. All the remaining eggs were opened. By removing the air sac shell area, and tearing away the air sac membranes with sterile forceps, the blood vessels of the allantoic membrane were broken and allowed to flow into the allantoic fluid. Samples from each of the twelve eggs were examined by the dark ground illumination procedure described in the section on S.duttoni. Trypanosomes were found in varying degree, from extremely scanty to numerous.

An egg possessing numerous trypanosomes, i.e. approximately ten per microscope field, was selected for further passage. The allantoic fluid, blood cells and trypanosome mixture was withdrawn, and this suspension inoculated into a batch of eggs; each egg receiving 0.1 ml. In this way the strain was passaged at weekly intervals for 233 weeks. At times difficulty was experienced in maintaining the strain, but with experience this was overcome by varying the volume of the inoculum from 0.1 ml. to 0.75 ml. per egg, depending upon the number of trypanosomes present. It was found essential, however, to passage the strain from living eggs on each occasion, as the trypanosomes tended to die rapidly after the death of the embryo.

Examination of the embryo, the yolk sac, and the allantoic and amniotic fluids of infected eggs did not reveal trypanosomes in large

..../ numbers.

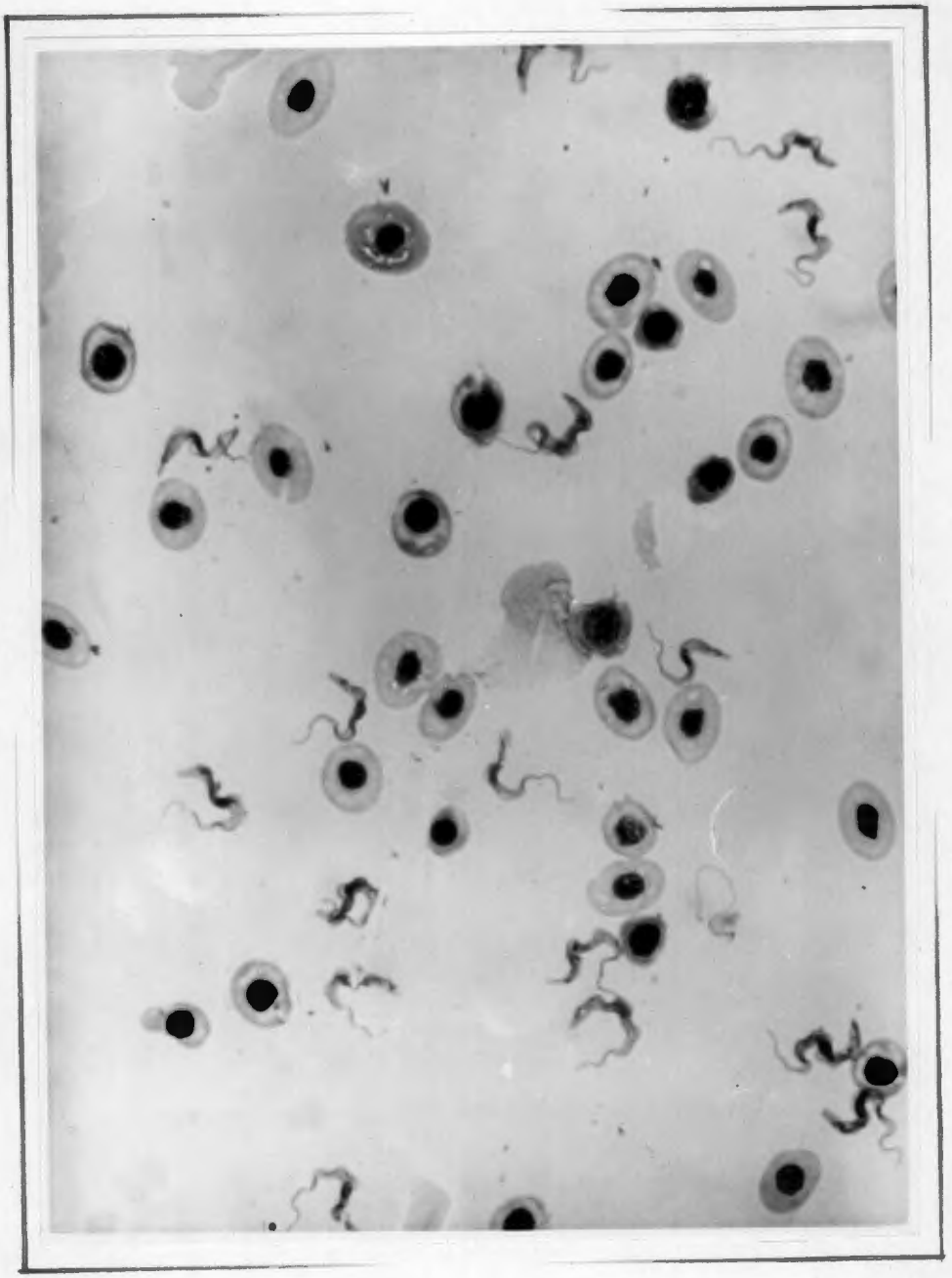
Figure 4.

Trypanosoma rhodesiense.

Photomicrograph x 840 Wright's stain.

Thin film prepared from the

blood of an infected chick embryo.



numbers. They appeared to be confined to the embryonic blood stream, where they multiplied by binary fission. Rounded and crithidial forms were not found. Preparations stained by Wright's stain revealed organisms displaying normal characteristics with an occasional posterior nucleated form peculiar to the species.

As a medium for growing trypanosomes, the embryonated egg had undoubtedly proved successful. Large numbers of the organism could be separated from the allantoic fluid and blood mixture. It was found that the trypanosomes remained motile in this suspension for about 2 hours, after which time loss in motility was noted with the gradual drop in temperature. It was subsequently found that priority was due to Longley et al (14),1939, for using the egg as a medium for growing trypanosomes. So successful, rapid and simple was the method, that further study was indicated. The preparation of a complement fixation antigen was attempted. An argument in favour of a trypanosome antigen prepared from egg culture, was the concept that the parasites would not be influenced by immune bodies.

..../ Chapter 3

Chapter 3

The preparation of antigen for the complement fixation test.

The procedure for obtaining suspensions rich in trypanosomes, was the same as that previously described for the collection of spirochaetes. Seventh day living eggs were obtained from routine passage of the strain, examined individually, and the allantoic fluid, blood and trypanosome mixture pipetted into centrifuge tubes. By the process of differential centrifuging, a deposit of practically pure trypanosomes was obtained. Physiological saline containing 20% ethyl alcohol was added to the deposit up to 10% of the original volume of harvested material. After thorough shaking, the organisms were immediately immobilized and partially disintegrated. This suspension of alcohol extracted trypanosomes constituted the antigen. The product proved to be more sensitive than a simple saline suspension as a complement fixation antigen. The titre of these antigens was usually 1:3. This titre was obtained by testing the antigen in varying dilutions fromaneat state to 1:10 in the presence of known negative human serum against the complement. The strongest dilution of antigen was chosen which did not absorb more than 0.5 M.H.D. of complement. These antigens kept well

..../ at 4°C

at 4°C for about 2 months, after which time they tended to become anti-complementary. The general procedure for performing the trypanosome complement fixation test was the same as previously described for the rickettsiae and S.duttoni.

..../Chapter 4.

Chapter 4.

The results obtained in tests on sera of human cases of trypanosomiasis and their interpretation.

In March and April 1947, by arrangement with Dr. S.Buck of Lusaka, Northern Rhodesia, several specimens of serum were obtained from suspected and known cases of trypanosomiasis.

The first two sera were preserved with chloroform. One gave a positive trypanosome complement fixation reaction in a dilution of 1:1600, and the second in 1:800.

On a further occasion five sera were submitted with histories, and gave the following results:-

Specimen no.	Trypanosome complement fixation result.	Details submitted.
1.	Positive in a serum dilution of 1:100	(Adult female). No treatment. Blood slides positive. Clinically moderately advanced. No glands. Moderate anaemia. C.S.F. protein 47 mgms.%. Globulin not increased.
2.	Positive in a serum dilution of 1:400.	(Male aged 18 years). No treatment. Blood slides positive. Clinically early case. Glands enlarged. Slight anaemia. Formol-gel reaction positive. Serum albumen 3.5, and globulin 3.1 mgms.%.
3.	Positive in a serum dilution of 1:50.	(Adult: male). Blood slides negative. No clinical details available. Suspected case.

.../ 4.

Specimen no.	Trypanosome complement fixation result.	Details submitted.
4.	Positive in a serum dilution of 1:100	(Adult: Male). Blood slides positive. Clinically secondary stage. No glands. Moderate anaemia. Two months history of illness.
5.	Positive in a serum dilution of 1:200.	(Adult: Male). Blood slides positive. Clinically early secondary stage. Febrile. Glands enlarged. E.S.R. 28 mm. in 1 hour. One month history of illness.

A positive control serum was included, givingareaction in a dilution of 1:800. A normal serum in this series gave a negative reaction in a dilution of 1:6.25.

In June 1949, two specimens of serum were received from Maun, Bechuanaland. They gave positive reactions in dilutions of 1:100 and 1:25 respectively. In January 1950, the serum of a suspected case from Maun gave a positive reaction in a dilution of 1:25. Normal human sera were included on these occasions, and gave negative reactions in dilutions of 1:6.25.

Hence between March 1947 and January 1950, a few specimens were examined as outlined. The results of these complement fixation tests showed considerable promise as an aid to the diagnosis of sleeping sickness. Further, over a period of nearly three years the antigens prepared from the egg passaged strain of T.rhodesiense

.... / appeared

appeared to be consistant and specific. A check on the specificity of the reaction was performed in March 1947. Three positive relapsing fever sera giving complement fixation titres of 1:800, 1:100 and 1:400 respectively were employed. They gave negative trypanosome complement fixation results in a dilution of 1:6.25. In each case the Wassermann, Ide and Kahn reactions were negative.

It appeared from the little evidence available, that positive trypanosome complement fixation reactions in a serum dilution of 1:50 upwards, could be regarded as diagnostic of sleeping sickness. Reactions in serum dilutions lower than 1:50 were to be regarded as suspicious, and requiring the testing of further specimens to ascertain the possibility of a rise in titre.

Discussion of the value of the complement fixation test in the diagnosis of sleeping sickness or African trypanosomiasis.

The test, although not well known, appears to be of considerable value for diagnosis. Once again, as in the case of relapsing fever, the difficulty often encountered in finding the causative organism microscopically, can be overcome in part at least by recourse to the complement fixation test. This technique is less time consuming than animal inoculation procedures, and would greatly facilitate survey work on occasions when large numbers of suspected cases required examination.

One difficulty was encountered in the form of the numerous

..../ anti-complementary

anti-complementary sera received. Owing to the great distances some specimens had to travel, exposure, age and contamination had affected the specimens. The addition of a drop of chloroform as a preservative before dispatching, together with absorption of the sera with complement before testing, tended to overcome this difficulty.

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PART 4 .

THE COMPLEMENT FIXATION TEST IN THE DIAGNOSIS OF RIFT VALLEY FEVER - ENZOOTIC HEPATITIS.

Chapter 1.

A review of the literature dealing with the serological diagnosis of Rift Valley fever.

Two methods have been employed for the serological diagnosis of Rift Valley fever, (a) the mouse protection test, and (b), the complement fixation test.

(a) Findlay (1),1932, noted that of all laboratory animals, white mice appeared to be peculiarly susceptible, dying 33-96 hours after inoculation. It was shown that as a result of active acquired immunity, Rift Valley fever immune bodies could be demonstrated in the sera of human beings 4 to 5 years after infection.

Sabin and Blumberg (2),1947, have detected protective antibodies as early as four days after onset of the disease, and as long as 12 years after recovery.

Smithburn et al (3),1949, have reported on eight cases of Rift Valley fever infection caught in the laboratory. The development of neutralizing antibody during convalescence was shown in all of these cases. Further, the virus was isolated from the blood

..../ of

of each case.

The influence of yellow fever antibodies was investigated by Findlay (1),1932. It was shown that three monkeys who had suffered from yellow fever 18 months previously, and one which had had yellow fever vaccine, all became infected with Rift Valley fever when challenged. This information illustrates the specific nature of the Rift Valley fever virus in respect to that of yellow fever.

(b). Broom and Findlay (4),1932, have obtained specific complement fixation reactions with the sera of men,monkeys, sheep, rats and mice, recovered from Rift Valley fever infection. Negative results were obtained on a number of non-susceptible animals and birds. Immune yellow fever and dengue fever sera gave negative results, as did two secondary syphilitic sera. They found the reaction well developed 14 days after infection, and it lasted for at least 6 months. Positive results were also found on the sera of people repeatedly exposed to infection, but who suffered no definite symptoms. Their results indicated that the titres were roughly proportional to the severity of the illness. It was found that rats and mice produced only small quantities of both protective and complement fixing antibody.

Their antigens were prepared from infected mouse and rat livers. Control antigens of normal mouse and rat livers gave negative results.

..../ Chapter 2

Chapter 2.

The cultivation of the virus from infected human cases

In May 1951, an outbreak of Rift Valley fever occurred in the Orange Free State. In addition to extensive infection among the sheep, several human cases were established clinically. The virus was isolated from the blood clots of two human cases by intraperitoneal inoculation in white mice. One of these strains was utilized successfully in mouse protection tests on the sera of several of the suspected human cases. Passage of these strains has been continued on white mice, and infected livers periodically set aside in the deep freezer at -20°C, for future antigen preparation.

The preparation of antigen for the complement fixation test.

The method used for the preparation of this reagent was a modification based on the work of Broom and Findlay (4),1932.

Owing to the highly infectious nature of the virus, it was deemed advisable to kill the culture before attempting purification. This was effected by heating the livers at 56°C for 45 minutes. This procedure did not appear to affect the antigenic properties of the material.

..../ The weight

The weight of two mouse livers used was found to be 1.91 gms. After removal from the deep freezer, the bottle containing the livers was placed in the 37°C water bath to hasten thawing. This was followed by a period of 45 minutes in the 56°C water bath. The livers were then decanted into a sterile 200 ml. Erlenmeyer flask containing about 20 gms. of glass chips. Homogenisation was completed in five minutes by vigorous agitation of the flask. 100 mls. of sterile physiological saline were then added, and after stirring, the liquid contents were decanted into a second sterile 100 ml. bottle. This was heated again for 45 minutes at 56°C to ensure the death of the virus particles. The bottle was then placed in the refrigerator at 4°C overnight. On the following day, a heavy chocolate coloured flocculant precipitate had formed, leaving a slightly turbid supernatant fluid. Sufficient merthiolate was added making a concentration of 1:10000. After stirring, the antigen was centrifuged in an angle centrifuge for 15 minutes at 4000 r.p.m. The supernatant fluid, now almost clear, comprised the antigen. The death of the virus was inferred by the fact that white mice failed to become infected when inoculated with this antigen.

This reagent was found to be free from anti-complementary properties when used in the neat state both in the presence of saline and normal human serum diluted 1:6.25. So far, it has been found that the antigen has remained stable for at least 4 months

..../ when

when kept at 4°C when not in use.

A preliminary test on the serum of a recovered case of Rift Valley fever infection, yielded a positive complement fixation reaction in a dilution of 1:50. On this occasion the antigen was used neat. Further titration of the antigen indicated that it could be used in a dilution of 1:4. In this dilution, the antigen possessed two reacting doses. One reacting dose was defined as the highest dilution of the antigen to show full fixation in a serum dilution of 1:50. This antigen dilution was 1:8.

The tests were conducted on the same principle as that adopted for all the tests described in this work, namely:-

0.1 ml. of serially diluted serum from 1:6.25 to 1:200.

0.1 ml. of antigen diluted 1:4.

0.1 ml. of complement containing 2 M.H.D., titrated by the overnight fixation method in the presence of saline and the antigen diluted 1:4.

After shaking, fixation was allowed to take place in the refrigerator at 4°C for 18 hours. The following day 0.2 mls. of 1.5% washed sheep cells sensitized with 2 M.H.D. of haemolysin, were added. The results were read after 30 minutes at 37°C. The reactions were clear cut and stable. Anti-complementary and normal mouse liver controls of the sera were included. A serum reacting in the presence of normal mouse liver antigen, prepared under the same conditions as the test antigen, would be regarded as unsuitable for the test.

Chapter 3.

The results obtained in tests on human and animal sera, and their interpretation.

Forty five sera were tested in the course of this investigation. Some were from convalescent cases, others from contacts, and from veterinary personnel exposed during the field investigation.

A detailed list of the results follows:-

Case	Complement fixation serum dilution.						A.C. control	Protection test	Remarks.
	1/6.25	1/12.5	1/25	1/50	1/100	1/200			
1. T.	+	+	+	-	-	-	-	Positive	
2. F.	+	+	+	-	-	-	-	Positive	
3. S.1	-	-	-	-	-	-	-	Negative	
4. S.11	+	+	+	±	-	-	-	Positive	
5. V.	+	+	±	-	-	-	-	Positive	
6. C.	+	+	±	-	-	-	-	Positive	
7. M.	-	-	-	-	-	-	-	Negative	2 suspected cases from Bloemfontein at time of outbreak.
8. R.	-	-	-	-	-	-	-	Negative	
9. Spring hare	-	-	-	-	-	-	-	Negative	
10. D.	+	+	+	+	-	-	-	Positive	
11. K.	-	-	-	-	-	-	-	Negative.	Piccanin. Not ill. Not associated with sheep.

..../ 12.

Case	Complement fixation serum dilutions.						A.C. control	Protection test	Remarks.
	1/6.25	1/12.5	1/25	1/50	1/100	1/200			
12.W.M.	+	+	±	-	-	-	-	Positive	
13.H.M.							+	Positive	Serum anti-complementary.
14.P.M.	+	+	+	-	-	-	-	Positive	Was ill. Worked with sheep.
15.K.	+	+	+	±	-	-	-	Positive	
16.M.M.	+	+	±	-	-	-	-	Positive	Not ill.
17.S.M.	-	-	-	-	-	-	-	Negative	Not ill.
18.T.M.	+	+	+	+	-	-	-	Positive	Was ill.
19.A.M.	-	-	-	-	-	-	-	Negative	
20. Duck	-	-	-	-	-	-	-	Negative	
21.Mrs. V.	-	-	-	-	-	-	-	Negative	
22.Cape hare	-	-	-	-	-	-	-	Negative	
23.E.	+	+	+	-	-	-	-	Positive	Not ill.
24.Native Silo	-	-	-	-	-	-	-	Negative	
25.Mrs. M.	+	+	+	+	-	-	-	Positive	
26. K.	-	-	-	-	-	-	-	Negative	Worked with sheep.Not ill.
27.Mr.C	-	-	-	-	-	-	-	Negative	
28.S.	-	-	-	-	-	-	-	Negative	Not ill.
29.Mr.P.	+	+	+	±	-	-	-	Positive	

..../ 30.

Case	Complement fixation serum dilutions						A.C. control	Protection test.	Remarks.
	1/6.25	1/12.5	1/25	1/50	1/100	1/200			
30.Flowers 1.	-	-	-	-	-	-	-	Negative	
31.Flowers 11	-	-	-	-	-	-	-	Negative	
32.S.	-	-	-	-	-	-	-	Negative	
33.M.1	-	-	-	-	-	-	-	Negative	
34.M 11	-	-	-	-	-	-	-	Negative	
35.P.L.L.	-	-	-	-	-	-	-	Negative	
36.Mrs.S.	+	+	+	+	±	-	-	Positive	
37.Mr.T.	-	-	-	-	-	-	-	Negative	Suspected case on Reef.
38. L.	+	+	+	±	-	-		Positive	
39.S.	+	+	+	+	-	-		Positive	
40.Dr.L 1	-	-	-	-	-	-	-	Negative	Exposed to infection
41.Dr.L. 11.	+	+	±	-	-	-	-	Positive	"
42.Dr.A. 1	-	-	-	-	-	-	-	Negative	Exposed to infection.
43.Dr.A. 11.	+	+	+	-	-	-	-	Partial	"
44.Dr.M. 1	-	-	-	-	-	-	-	Negative	Exposed to infection
45.Dr.M. 11	±	-	-	-	-	-	-	Positive	"

..../ In addition

In addition, twelve positive Wassermann sera were tested against the antigen to reveal any possible Wassermann antigen present. Eleven of the sera were negative, and the twelfth proved to be anti-complementary.

The results obtained with protection tests have been included in the chart. These tests were performed by Dr.J.H.S.Gear and Miss R.Harwin of the South African Institute for Medical Research, and the results were published separately. Extremely close agreement will be noted between the two tests. The results obtained on the sera of the veterinary officers confirm the findings of Broom and Findlay (6),1932. Antibodies had developed by simple exposure , without illness. It appeared that a positive complement fixation reaction in a serum dilution of 1:6.25 was significant. The results indicate that the complement fixation test as described, should prove of considerable value for the diagnosis of Rift Valley fever in South Africa.

..../ References

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..../ SUMMARY

SUMMARY

Introduction.

The principles of the complement fixation test, and the reagents required, are explained and defined. Mention is made of the widespread application of the test for the diagnosis of diseases ranging from those of virus, bacterial, and protozoal to those of helminthic origin. This has been possible through the recent development of new techniques for growing the organisms responsible for these diseases, and methods for obtaining concentrated pure antigens from them.

This thesis describes the application of the test for the diagnosis of the rickettsial diseases, tick-borne relapsing fever, African human trypanosomiasis, and Rift Valley fever.

Part 1.

The complement fixation test in the diagnosis of the rickettsial diseases.

The rickettsiae are defined and classified. Their characteristics are compared with the viruses and bacteria, and it is noted that they form a link between these two orders.

The literature dealing with the serological reactions of the typhus group of fevers is reviewed, from the time of the introduction of the Weil-Felix test in 1916, to the modern rickettsial

..../ tests

tests elaborated during the past ten years. The advantages and disadvantages of these two tests are discussed. The B.proteus x antigens used in the Weil-Felix test are non-specific in that they are non-rickettsial. False positive reactions are shown to occur in diseases not of rickettsial origin. However, the test has proved to be of value in indicating the presence of louse typhus, murine typhus, tick-bite fever - a member of the spotted fever group - and scrub typhus; but is of little or no differential value for the specific diagnosis of these diseases.

The value of the B.proteus "O" x 19 slide agglutination test is discussed, and the test is described in detail. The Weil-Felix test was found to be of no assistance in the diagnosis of Brill's disease and Q fever. In practice the Weil-Felix test continues to be of value for indicating the presence of the typhus group of fevers, it is simple to perform, and can be done in most bacteriological laboratories.

With the introduction of methods for growing rickettsiae in the yolk sacs of embryonated hen's eggs, and in rodent lungs and peritoneal cavities, pure specific antigens became available for use in complement fixation and agglutination tests. Results of a specific nature were obtained using these techniques, and it became possible to define the rickettsiae responsible for these diseases by serological examination.

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This study was undertaken to establish the value of the specific complement fixation tests, and to introduce these tests as a differential diagnostic procedure. When this was established, the test was applied to determine the incidence and distribution of the rickettsial diseases in South Africa, including the part played by rodents as reservoirs of infection.

A detailed description of the preparation of the reagents required for the test, including the antigens, is followed by a description of the performance of the complement fixation test. The overnight fixation method at $4^{o}C$ was found to be the most suitable and sensitive technique. The necessity for the pre-selection of complement to exclude positive reacting guinea pig serum to the Q fever antigen is mentioned. The necessity for titrating each antigen individually in the presence of complement, is emphasized.

The results of complement fixation tests obtained on sera from human cases of the typhus group of fevers in South Africa, and their interpretation, are tabulated. These confirmed the presence of three members of the typhus group of fevers known to occur in South Africa, namely:-

1) Epidemic louse borne typhus fever, caused by Rickettsia prowazeki.

2) Murine flea borne typhus fever, caused by Rickettsia mooseri.

3) Tick-bite fever, the variety of tick typhus occurring in Southern Africa, caused by Rickettsia rickettsi var pyperi.

4) In addition they have revealed the occurrence in this region

..../ of Q fever

of Q fever, caused by Rickettsia burneti.

The importance of being able to distinguish between typhus fevers of louse and flea origin is indicated, so as to enable public health authorities to take appropriate measures to eradicate the different vectors of these diseases. The results obtained by the rickettsial complement fixation tests were of value in this respect.

During the course of this study, serological evidence of the occurrence of Brill's disease in South Africa was noted.

In the section on tick-bite fever mention is made of sera from some cases giving equal titres with B.proteus "O" x 19 and B proteus "O" x 2 antigens, and in others where B.proteus "O" x 2 antigen reacted alone, or to a higher titre than B.proteus "O"x19. Although such reactions may suggest tick-bite fever infection, it was concluded that the rickettsial complement fixation test was the only sure means for specific diagnosis.

Examples are given of the results obtained on subjects who had previously been vaccinated with rickettsial vaccines, and also illustrating the influence of antibiotic treatment on the antibody response as shown by complement fixation tests.

During the course of this study, serological evidence of the occurrence of Q fever in South Africa was noted. Subsequent to this, many other cases were revealed from country districts bordering on the towns, and extending from Wellington in the

..../ Cape Province

Cape Province as far north as Nairobi in Kenya. It was noteworthy that a high proportion of these cases occurred in recent immigrants to this country. It was found that about 12% of the indigenous population gave positive Q fever complement fixation reactions in low serum dilutions. This suggests that this disease is widespread in South Africa, and that most South Africans have acquired immunity from previous infection. Recent immigrants do not possess this immunity to the same degree.

The occurrence of false positive Q fever reactions was noted in the sera from some cases suffering from the collagen or hypersensitivity diseases, and in a case of chronic lymphatic leukaemia, and in one of tuberculosis. The results indicated the necessity for care in assessing the value of positive Q fever complement fixation tests on sera from patients possibly suffering from one or other of these conditions.

Tables illustrating the correlation of the results of the rickettsial complement fixation tests and the Weil-Felix test, show close agreement in cases of louse typhus fever, murine typhus fever, and tick-bite fever. In Q fever the Weil-Felix test gives a negative result, and so is of no diagnostic value in this disease.

Tables illustrating the correlation between the results of of the rickettsial complement fixation test and the rickettsial agglutination test, show close agreement in louse typhus and

..../ murine

murine typhus fever. Difficulty was experienced at times in preparing suitable antigens for the agglutination tests, and the results were found to be difficult to read.

A map is included, illustrating centres where louse typhus fever, murine typhus fever, South African tick-bite fever, and Q fever were found to occur by the rickettsial complement fixation tests.

A survey was conducted to determine the incidence of murine typhus infection in the rat populations of the coastal belt of Natal, and in the Johannesburg municipal area.

Of 108 rat sera examined from Natal between 28.5.48 and 21.9.48, twenty five gave positive murine typhus complement fixation reactions, involving the following localities:-
Blackhurst location, Durban, Amanzimtoti, Stanger, Lamontville and Isipingo. R.mooseri was isolated on egg culture on several occasions from these specimens, thus confirming the positive serological evidence obtained. It was found that the Weil-Felix test was of no value in indicating typhus infection in these rodents.

In Johannesburg, a survey involving the examination of 1120 rats between 14.7.48 and 13.12.51, showed that 8.7% of their sera gave positive murine typhus complement fixation reactions. Confirmation was also obtained by the isolation of R.mooseri on egg culture from several batches of these rats. The incidence was highest in the Newtown market area of the west-central city, and extended along the main east-west railway system, with occasional cases in the suburbs.

..../ On the

On the 25.8.49, serological evidence of the occurrence of tick-bite fever infection in domestic rats was obtained from two specimens caught at Linksfield on the north-eastern outskirts of the city. During the period 14.7.48 to 13.12.51, further evidence of tick-bite fever infection in domestic rats was obtained in eighteen suburbs, mainly on the northern perimeter of the municipal area. The incidence rate of positive tick-bite fever complement fixation reactions in the rat sera, was 3.43%.

The examination of wild veld rodents caught in the country districts to the north of the city, revealed that antibodies to tick-bite fever were present in the three species examined, namely:- Otomys irroratus, Mastomys natalensis, and Rhabdomys pumilio. Further confirmation of these findings, was obtained in the isolation of R.rickettsi var pyperi from a specimen of Otomys irroratus on egg culture. As a result of this discovery, it is surmised that tick-bite fever is essentially a disease of the veld rodents, and that it is spread amongst them by ticks which can maintain the infection indefinitely from one generation to the next. Occasionally and accidentally, these arthropod vectors parasitize Man and the domestic rat, and so transmit the infection to them.

A map is included of the Johannesburg municipal area, illustrating the suburbs in which murine typhus and tick-bite fever infection in domestic fats was proved to occur by complement fixation tests.

..../ Part 2.

Part 2.

The complement fixation test in the diagnosis of tick borne relapsing fever.

The literature dealing with the serological diagnosis of relapsing fever, is reviewed. Serological methods have been sought to augment the diagnosis of this disease by the usual methods of blood slide examination and mouse inoculation. These two methods suffer from certain limitations. In blood smear examination, the spirochaetes are often difficult to find, and biological tests are subject to several uncontrollable factors. Successful agglutination and complement fixation techniques have been described for the diagnosis of relapsing fever. The main limiting factor for success with serological methods, was in obtaining suitable antigens prepared from the spirochaetes.

The development of a satisfactory antigen for the complement fixation test, prepared from embryonated hens' eggs infected with Spirochaeta duttoni is described. The spirochaetes were separated by a process of differential centrifugation. The basic technique for the test was the same as that used for the rickettsial complement fixation tests.

The results of the complement fixation test, are compared with blood slide examination and mouse inoculation tests on a number of specimens from known or suspected cases. It is evident that there is close correlation between these methods, and that in

..../ many

many cases the complement fixation test gives positive reactions where the other two methods fail to reveal infection.

Promising results were obtained using a red cell sensitization test, based on the work of Middlebrook and Dubos in the haemagglutination test for the diagnosis of tuberculosis.

A microphotograph of a thin blood smear prepared from an infected chick embryo, illustrates the growth of S.duttoni obtained in this medium.

Part 3.

The complement fixation test in the diagnosis of African trypanosomiasis.

The literature dealing with the serological diagnosis of trypanosomiasis is reviewed. The methods were divided into (a), non-specific tests, and (b), specific tests. The non-specific, comprising the formol-gel and mercuric chloride precipitation tests, are concluded to be only rough guides for detecting the presence of sleeping sickness. The specific tests, including the red cell adhesion test, agglutination and precipitation reactions, are of a highly specialised nature, and somewhat limited in application. Antigens of Trypanosoma cruzi , prepared from cultures on artificial media, have shown promise for the diagnosis of American trypanosomiasus using complement fixation methods.

..../ The development

The development of a suitable antigen derived from embryonated hens' eggs infected with Trypanosoma rhodesiense , is described.

An alcohol extract of the parasites, separated from the egg culture by differential centrifugation, was found to be the most sensitive antigen for the purpose. The results obtained on a few sera from cases known to be suffering from trypanosomiasis were promising, and the test would appear to be of value in cases where the trypanosomes could not be demonstrated microscopically,

A microphotograph of a thin blood smear prepared from an infected chick embryo, illustrates the growth of T.rhodesiense in this medium.

Part 4.

The complement fixation test in the diagnosis of Rift Valley fever - enzootic hepatitis.

The literature dealing with the serological diagnosis of Rift Valley fever, is reviewed. The two methods employed are (A) the mouse protection test, and (B) the complement fixation test. The mouse protection test has been shown to be an accurate and reliable method for the detection of antibodies against the Rift Valley fever virus. Complement fixation tests, using antigens derived from infected mouse livers, were found to be equally as sensitive as the mouse protection test, and the titres of reaction in general were found to be proportional to the severity of the

..../ illness.

illness.

In May 1951, an outbreak of Rift Valley fever occurred in the Orange Free State, affecting both Man and sheep. The virus was isolated from the blood clots of two human cases, and has since been maintained in white mice in the Virus and Rickettsial Diseases Department at the South African Institute for Medical Research.

The preparation of a suitable antigen, derived from infected mouse livers, and its use in complement fixation tests, is described.

Forty five sera were tested in the course of this investigation from convalescent cases, contacts, veterinary personnel - exposed during the outbreak -, and from some animals.

The complement fixation results were tabulated, and compared with those of the mouse protection test. It was evident that there was complete correlation between the two methods, and that the complement fixation test should prove of considerable value for the diagnosis of Rift Valley fever in South Africa.

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