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A COMPARATIVE STUDY OF CURRENT METHODS FOR DETECTING TREPONEMAL ANTIBODY IN SELECTED POPULATION GROUPS IN SOUTHERN AFRICA.

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A Dissertation Submitted to the Faculcy of Science, University of the Witwatersrand, Johannesburg, for the Degree of Master of Science.

JOHANNESBURG, 1975.

This is to certify that the dissertation 'A Comparative Study of Current Methods for Detecting Treponemal Antibody in Selected population Groups in Southern Africa', presented for the degree of Master of Science at the University of the Witwatersrand, Johannesburg, is my own work and has not been presented at any other University.

SIGNED: N. J. Richardson

Part of this dissertation has been published in the following papers.

1. 'Reproducibility of Results Obtained by the Treponema Pallidum Immobilisation Test (TPI) at the South African Institute for Medical Research'.
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2. 'A Reappraisal of the IgM Fluorescent Treponemal Antibody Absorption Test in the Diagnosis of Congenital Syphilis'.
   Rosen, E.U. and Richardson, N.J. (1974): J.Pediat., In press.

The research on which this dissertation is based was commenced in 1956 and completed in April, 1975.

Abstract

A review of the literature outlines the development of reagin and treponemal tests. Biological False Positive (BFP) results, specificity and sensitivity of reagin tests and reproducibility, specificity and sensitivity of treponemal tests are discussed.

The reproducibility of the Treponema Pallidum Immobilisation (TPI) test at The South African Institute for Medical Research was found to be as good as other laboratories. The Fluorescent Treponemal Antibody-Absorption (FTA-Abs) test correlated well with the TPI test, showing a slightly higher sensitivity. However, lack of adequate clinical details precluded a specificity evaluation. Reproducibility was good, but in the lower activity range the FTA-Abs test is not entirely infallible.

Based on the TPI test, the prevalence of treponematosis in a rural Bantu population of known age in Botswana was shown to be 25,9 per cent. This corroborated the finding of a previous survey, using reagin tests, that treponematosis in the same group of people was high and that BFP reactions were low (maximum 3,5 per cent). Botswana males on the Witwatersrand showed a prevalence of treponemal infection of 46,2 per cent.

Two surveys, with eight years inbetween, carried out on an urban Bantu population near Johannesburg, showed a drop in incidence of those with past or present treponematosis over the age of fifteen years from 33,1 per cent in 1957 to 24,9 per cent in 1965. Congenital syphilis was rare. Statistical analysis of the Standard Tests for Syphilis showed that a combination of the Kolmer Complement Fixation and VDRL flocculation test was significantly more specific than other combinations.

In a predominantly Bantu leprosy population of tuberculoid, borderline and lepromatous types, the overall prevalence of syphilis was 37,2 per cent. BFP reactors were found in 0,8 per cent tuberculoid, 7,4 per cent borderline and 22,8 per cent lepromatous cases. The association between tissue destruction, lepromin reactions and BFP reactors were discussed from an immunological point of view.

A serological assessment of three penicillin treatment schedules was undertaken on a small number of Bantu patients with primary and secondary syphilis. All schedules showed degrees of serological and some clinical relapse and are inadequate and in need of revision. A larger study would test the validity of the recommended schedule.

Using a stated commercial brand of anti-IgM fluorescein conjugated serum, an evaluation study was conducted on the early diagnosis of congenital syphilis in Bantu neonates. Babies with a clinical and radiological diagnosis of congenital syphilis were confirmed serologically using a conjugate dilution of 1:40. This dilution correctly predicted that infants of treated mothers did not have a syphilitic infection on clinical and radiological follow up examinations. Two of three asymptomatic babies showed a 1+ anti-IgM fluorescence and developed clinical signs later; the third showed a false positive reaction.

Absorption tests, using DNA and cell nuclei from calf thymus, were carried out on positive ANF sera ('homogeneous' and 'speckled' pattern of fluorescent staining) which also showed false positive FTA-Abs reactions. Using Phosphate Buffered Saline (PBS) and sorbent as a diluent, the ANF was absorbed in varying degrees. In contrast, with the FTA antibody, absorption with DNA and cell nuclei in PBS as a diluent increased the intensity of fluorescence, whereas with sorbent it was decreased, in many cases to negativity.

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INTRODUCTION

The origin of syphilis is controversial. Some historians believe that syphilis evolved in the New World and was brought to Europe via Spain by Columbus's sailors after his voyage of discovery[1]. Other authorities say that syphilis had been smouldering in the Old World endemically for centuries, and the fact that the disease spread through Europe, rapidly reaching epidemic proportions in the Fifteenth century, was coincidental with the return of Columbus[2].

In South Africa, Lichtenstein (1812)[3] found no evidence of syphilis among the Xhosas during his travels between 1802 and 1806 and fifty years later, David Livingstone[4] reported that the disease was absent among the Botswana tribesmen. It appears that syphilis was introduced into the country by white men and spread with their penetration into the country[5].

With the discovery of the Kimberley diamond fields, there was a rush of White and Bantu men to this area. Within a short time, Kimberley had become the second largest town in South Africa with a population of 8000 White and 10 000 Bantu. It is estimated that between 1871 and 1895, about 100 000 labourers had worked at the mines and later on returned to their homes, dispersed over vast areas of South Africa[6]. The conditions in Kimberley were ideal for the spread of syphilis. As described by Kark (1949)[6], compounds were filthy, drunkenness was common and because of the abnormal social conditions under which the men were

living, promiscuity and prostitution were rife.

The discovery of the Witwatersrand gold fields in 1886 precipitated the second of the Industrial Revolutions in South Africa. As well as the mining industry, all secondary industries which developed required labour, and this unskilled labour was drawn from Bantu men from all areas of the country and the neighbouring territories. And so the spread of syphilis which gained momentum in Kimberley was accelerated on the Witwatersrand[6].

Cogniscance of the seriousness of the morbidity of syphilis was taken by the Government who appointed a Commission in 1906 to assess the incidence of venereal diseases in the Native population. It reported that the incidence of clinical syphilis varied in different parts of the country, that the morbidity was high in the adjacent parts of Botswana, and that an upsurge in cases had occurred in Johannesburg during the past six years[7].

Until Schaudinn and Hoffmann (1905)[8] demonstrated *Treponema pallidum* the only way of diagnosing syphilis was by its clinical manifestations. Further progress was made in 1906, when Neisser, Wassermann and Bruck[9], following the complement studies of Bordet and Gengou, produced a saline extract of a syphilitic liver which gave positive complement fixation reactions with known syphilitic sera. The following year, however, Landsteiner, Müeller and Pötzl[10] found that syphilitic sera fixed complement in the presence of an alcoholic extract of normal mammalian tissue. Not long afterwards it was found that syphilitic sera flocculated this antigen

solution. The antibody so detected was designated rea in. This work heralded the establishment of a serodiagnosis for syphilis.

Using the Wassermann Complement Fixation test, Pijper (1921)[11], found that 36,8 per cent of 500 South Africans, the greater majority being Bantu, gave a 'strongly positive' reaction. The sera were drawn partly from the locations and partly from employees in businesses or households in the city of Pretoria. Cases of primary syphilis were excluded.

All subsequent serological surveys showed a similar high sero-positivity rate. In 1938, Rauch and Saayman[12] reported positive Wassermann tests in 40,5 per cent of 227 Bantu women attending an ante-natal clinic in Germiston and 44,4 per cent of 712 location residents of the same town attending an out-patients dispensary. In a 1937-1939 survey, 35-42 per cent of Bantu women attending an ante-natal clinic in Benoni were sero-positive. In contrast to Livingstone's observations on the absence of clinical syphilis among the Bakwena in Botswana from 1846 to 1951[4], Warren's expedition into that territory in 1885 found many infected natives and in 1909, 3,7 per cent of the Bantu population of Mafeking, Kuruman, Taung and Vryburg were treated for syphilis[13]. The Annual Medical Report of the Bechuanaland (Botswana) Protectorate Government for 1946 reported a sero-positivity rate of 64 per cent in 377 out-patients at Mahalapye[14]

Reviewing the clinical aspects of the syphilis cases reported from Botswana, it is of interest that McArthur and Thornton (1911)[15] reported that 'case-to-case infection without sexual intercourse is the method of infection which gives rise to the major portion of the acquired syphilis

seen in these districts'. After further intensive clinical surveys on syphilis, McArthur (1942)[16] changed his opinion on the nature of the disease by stating that acquired syphilis rarely occurs and that hereditary syphilis was by far the most frequent type of this disease. It was not until 1952 that Murray, Merriweather, Keen and Sachs[17] published their observations on the condition which particularly affects young children. They stated that the disease was, in many ways, similar to other forms of endemic syphilis found in other parts of the world. This type, however. was rarely seen in the large towns and cities.

In 1953, Murray, Merriweather and Friedman[18], sponsored by the World Health Organisation, undertook a comprehensive survey and campaign in the Bakwena reserve of Botswana to control endemic syphilis, known locally as 'dichuchwa', and, at the same time, studied its epidemiological, clinical, social and therapeutic aspects. Using the Kolmer Complement Fixation serological test for syphilis, the sero-positivity rate in the reserve was 37 per cent. Furthermore, the team established that 'dichuchwa' is a childhood and family disease which was usually spread non-venereally. Some clinical aspects differed from yaws and venereal syphilis and it was established that lesions of the cardiovascular and central nervous systems and congenital syphilis were rare.

Later, du Toit (1969)[19] surveyed the incidence of syphilis in a Karoo town and district, and found that endemic venereal syphilis co-existed in that area, and speculated that the survey reflected the pattern of the whole of the Karoo.

Serologically, the antibodies produced by any of the human treponematoses (endemic syphilis, yaws and venereal syphilis) are indistinguishable.

Standard Tests for Syphilis and Biological False Positives

Standard serolog. l tests for syphilis (STS) - Complement Fixation and Flocculation tests - are designed to detect the presence or absence of reagin antibody in a patient. Reagin, however, which is found in the immunoglobulin M(19S, β2M globulin) and G(7S, γ globulin) fraction of the serum[20] or spinal fluid is not specific to syphilis and may be produced to a small degree by numerous other diseases such as malaria, leprosy, respiratory infections, infectious mononucleosis, lupus erythromatosus and some cases of pregnancy. Thus, since the antigen used to detect the reagin is an alcoholic-soluble lipid extracted from beef heart in combination with lecithin and cholesterol and seems not to possess determinants specific for *Treponema pallidum*, false reactions may occur. However, standard serologic tests for syphilis in general use give 80-90 per cent positive reactions with sera from infected individuals, and any test which can forecast infection with an accuracy of more than 80 per cent is regarded as satisfactory as far as sensitivity is concerned[21]. A 'biological false positive' (BFP) may be defined as reagin activity in sera from non-syphilitic patients. In a country such as South Africa where the incidence of syphilis is high, reagin tests are valuable supplementary tools for assessing the prevalence of syphilis. In contrast, countries with a low incidence of syphilis will show a relatively high proportion of

'biological false positives' which tends to undermine the epidemiological value of the test.

Up to 1948, the BFP always constituted a problem for the clinician. If it was not syphilis causing the reagin reaction, then what was it? Fiumara (1964)[22] in his address to the World Forum on Syphilis, so aptly states:- "Time and the recent advances in the nature of the auto-immunologic diseases has jolted us into realizing that reagin antibodies appearing without apparent cause may be the harbinger of an auto-immune disease, such as systemic lupus erythrema osus, rheumatoid arthritis, haemolytic anaemia and others. The joy of yesteryear over a diagnosis of biologic false positive reactions has changed today to a cautious pessimism. One cannot help but philosophize, 'It were better had they had syphilis'"

The Treponema Pallidum Immunobilisation Test (TPI) test

Ever since the discovery of the causative organism of syphilis, *T. pallidum*, attempts have been made to obtain an antigenic component derived from this organism. The first claim to cultivation was made in 1912[23], and there have been others since, but confirmation has never followed. The present day explanation is that the organisms isolated were saprophytic non-virulent spirochaetes which contaminated the lesions allegedly containing *T. pallidum*.

In 1948, Nelson[24] took up the problem of cultivating *T. pallidum* and in conjunction with Mayer[25] established a basal medium in which it was

possible to keep 50 per cent of the spirochaetes alive from six to eight days. No multiplication was noted during this prolonged incubation, but a lengthening of some of the viable organisms was observed. Motile forms retained their virulence, non-motile ones did not.

Although Nelson and Mayer were unsuccessful in culturing *T. pallidum*, during their investigations they found that the addition of syphilitic serum and active complement to a suspension of motile *T. pallidum* immobilized and killed the spirochaetes. This formed the basis of the TPI test as it is now known. It was further established that the antibody which occurs in all syphilitic patients was specific for pathogenic treponema. This antibody was designated immobilin.

Here then was a specific test for pathogenic treponema and a test that would confirm or reject the diagnosis of syphilis in those problem cases falling in the possible 'BFP' category.

Because of the high incidence of reagin-positive sera among the Bantu in South Africa, the possibility exists that some of these reactions may be due to conditions other than syphilis (BFP), such as liver damage due to malnutrition[25]. It was therefore thought possible that some of the serological results may be 'biological false positive' reactions.

Forty reagin-positive sera from the Bechuanaland Survey[18], twenty with a past history and twenty with no history of 'dichuchwa', all with no clinical signs, were sent to the Serological Reference Laboratory at the State Serum Institute, Copenhagen, to be tested by the TPI reaction. These sera, among others, showed marked disturbances of the lipo-proteins and

abnormal liver-function tests. Sera from thirty-nine were reactive to the TPI test. The only non-reactive TPI serum was from a male over sixty years of age. He came from the group with no history of 'dichuchwa', was not treated with penicillin and the VDRL test was non-reactive on re-examination of his blood twelve months later.

Assuming that the TPI test was specific, it was clear from these findings that BFP were uncommon in this group (one in forty).

Because of the controversial prevalence of BFP in Bantu in South Africa, it was decided, in 1955, to introduce the TPI test, to assess the true prevalence of syphilis, extra-venereal treponematosis and allied diseases in the country. The Fluorescent Treponemal Antibody Absorption test (FTA-Abs), developed by Hunter, Deacon and Meyer in 1964[27], was later used to supplement the TPI test.

CHAPTER 1.

TEST METHODS USED IN THE STUDY FOR DETECTING ANTIBODIES PRODUCED IN RESPONSE TO A TREPONEMAL INFECTION.

Two groups of tests are used to detect antibodies to *T. pallidum* and allied pathogenic treponemas.

(A) Complement fixation and flocculation tests, referred to as Standard tests for syphilis (STS), which test for the reagin antibody, so named 'because it reacts'. This antibody is produced not only in response to *T. pallidum* but also in conditions associated with infection by, for example, Plasmodiae, *M. leprae*, rickettsiae and viruses, as well as autoimmune disorders and malignancies[28].

(B) Tests using *Treponema pallidum* as the antigen detect specific antibodies produced by this and allied treponemal organisms.

(A) Standard Blood Tests for Syphilis.

Historical review of the complement fixation and precipitation (flocculation) tests

1. Complement Fixation Tests

Pfeiffer[29], in 1894, observed that *V. cholera* organisms, injected intra-peritoneally into cholera-immunised guinea-pigs, became granular and eventually disappeared spontaneously from the

fluid. In non-immunised guinea-pigs the organisms remained unchanged, showing that something had developed in the immunized animal causing this lysis.

Bordet[30], four years later, reported that *V. cholera* antiserum, heated at 56C, agglutinated *V. cholera* organisms. The addition of fresh normal serum to the mixture resulted in bacteriolysis which also occurred in mixtures of fresh unheated antiserum and *V. cholera*. Thus the immune serum contained two important components: a specific thermostable antibody (agglutinin) and a nonspecific thermolabile substance (alexin or complement). A combination of both components was required to bring about bacteriolysis but if complement was destroyed by heating, only agglutination took place.

Using a system of erythrocytes with corresponding antiserum Bordet and Gengou (1901)[31] found that the red cells haemolysed when mixed with fresh anti-red cell serum but agglutinated when mixed with preheated (56C) anti-red cell serum. Again, the reaction required two components: a specific thermostable antibody (haemolysin or amboceptor) and a nonspecific thermolabile antibody, the complement.

It was thus established that complement was essential for bacteriolysis and haemolysis and that bacteria plus specific antibody or red cells plus anti-red cell serum had the ability to bind or fix complement. Furthermore, Bordet found that complement fixation also occurred with protein antigens and their homologous immune sera.

These observations formed the basis of the complement fixation test.

Following the discovery of *T. pallidum* as the causative organism of syphilis by Schaudinn and Hoffmann (1905)[8], Neisser, Wassermann and Bruck (1906)[9] adapted the complement fixation test to this disease. Using saline extracts of various organs from monkeys inoculated with *T. pallidum* as antigen, and serum from these infected monkeys, they reported that antibodies to syphilis could be detected *in vitro* by means of the complement fixation test. Sera from non-syphilitic patients together with the antigen extract did not bind complement. They assumed that it was essential for the antigen extracts to contain syphilitic substances.

Shortly after the publication of these findings however, two independent groups of workers, Marie and Levaditi (1907)[32], and Landsteiner, Müeller and Pöetzyl (1907)[33] showed that extracts of non-syphilitic tissue fixed complement in the presence of syphilitic serum, but failed to do so with the normal control sera.

It was thus apparent that the reaction differed from that of a conventional antigen-antibody system. The nature of the reacting components in the extract was unknown. However, working on the fact that the 'antigen' was present in an alcoholic extract, it was thought that the active agents were possibly lipoid. Many lipoids and allied compounds were then investigated. Porges and Meier (1908)[34] and Landsteiner, Müeller and Pöetzyl (1908)[35] found that lecithin, to some extent, could replace the alcoholic tissue extract as could some salts of the bile acids investigated by Levaditi

and Yamanouchi (1907)[36], but none of these substances could differentiate as well between syphilitic and non-syphilitic sera as could the crude alcoholic organ extract.

Browning, Cruickshank and McKenzie (1910)[37] noted that an alcoholic solution of lecithin dissolved a considerable amount of cholesterol at room temperature. They showed that the complement fixing properties of a saturated solution of cholesterol in alcoholic lecithin was inferior to that of an alcoholic tissue extract containing a large amount of cholesterol. Nevertheless, they felt that a sero diagnosis for syphilis would be more dependable with a chemically prepared antigen, such as a lecithin-cholesterol combination, which could be standardized.

Sachs (1911)[38] also tried to develop a chemically prepared antigen using lecithin, soap and oleic acid. He found that this combination gave fair results but were less sensitive than extracts of foetal syphilitic livers, as were, but to a lesser degree, those of normal tissue. In an attempt to raise the sensitivity, he combined extracts of normal tissue with his artificial extracts of lecithin, soap, oleic acid and cholesterol in various combinations. His best results came from using a normal heart muscle extract with cholesterol and he reported that this antigen was as sensitive as that of his cholesterolized alcoholic extract of syphilitic liver. Sachs concluded that syphilitic organ extracts were not essential for the Wassermann Complement Fixation test, the name under which it had then become known. Furthermore, he felt that extracts of heart muscle with cholesterol could be easily manufactured on a large scale and be supplied under standard conditions to various laboratories.

The next advancement of note was the isolation by Pangborn[39], in 1941, of a serologically active phospholipid from alcoholic extracts of beef heart. Subsequently, the phospholipid was identified as linoleic acid, oleic acid and a polyester of glycerophosphoric acid and glycerol. It was classified as a complex phosphatidic acid[40]. This substance, called cardiolipin, was found to be comparable with that of the routine antigen when used in conjunction with lecithin and cholesterol in a properly balanced mixture. No one of the three substances was found to be active in the absence of the other two, showing that the antigenic activity depends on at least three components[41].

Pangborn's findings opened a way for standardisation of the antigenic complex, which in turn has led to improved reproducibility of results both within and between laboratories. In addition, the antigenic complex was found to have an adequate sensitivity and specificity without excessive anticomplementary activity[42].

For many years Kolmer[43] had been working on the standardization of the complement fixation test and his modification of refrigerating the antigen-serum-complement mixture for 15-18 hours, followed by the addition of the haemolytic system, was widely accepted. He established a fixed proportion of cardiolipin, lecithin and cholesterol which gave a maximum sensitivity consistent with specificity in testing syphilitic sera and spinal fluids.

2. Precipitation or flocculation tests

The first report on precipitation reactions came from Kraus[44]

in 1897. Various amounts of sterile filtrates of *V. cholera* cultures were mixed with sterile serum from goats immunized with *V. cholera*. After 24 hours at 37C the mixtures exhibited small floccules that gradually settled to the bottom of the tube, resulting in a clear supernatant fluid. Control experiments with heterologous serum did not precipitate. He believed that the substances which were precipitated were part of the bacterial bodies.

Michaelis (1907)[45] was the first worker to carry out precipitation studies using a saline extract of syphilitic liver. Syphilitic sera precipitated this solution whereas normal serum did not. However, precipitation studies using his extract were soon abandoned in favour of those using normal tissue extracted with alcohol. Hecht (1915)[46], using an alcoholic extract of heart muscle - the same antigen as was being used for the Wassermann Complement Fixation test - reported precipitation reactions with known syphilitic serum.

The first time cholesterol was added to an antigen in a precipitation test was in 1918 by Sachs and Georgi[47], using an alcoholic extract of wet heart muscle. Apart from simplifying the test procedure, these workers noted clearer positive results when the antigen was cholesterolized and, together with the syphilitic serum, was incubated 24 hours before reading the test.

However, none of these flocculation (precipitation) tests devised by many workers was as sensitive as the Wassermann Complement Fixation test. This puzzled Kahn in 1920, and it was he and his co-workers who possibly contributed most in developing the flocculation test as a diagnostic

tool for syphilis as we know it today. In extensive studies he established four important factors[48,49] which favoured flocculation in mixtures of syphilitic serum and antigen suspensions; the proper concentrations of the reagents, the physical state of the antigen, the quantitative relation between the antigen suspension and serum and the agitation of the serum-antigen mixture.

Other flocculation tests were developed, in most instances with different methods of antigen production. For example the Eagle[50] test used corn germ sterol in addition to cholesterol; in the Hinton[51] test the tissue extract antigen is diluted with 5 per cent sodium chloride and 50 per cent aqueous glycerol; in the Kline[52] test the cholesterol is first precipitated and the crystals then coated with tissue extract antigen. The Mazzini[53] test employs an antigen prepared from powdered beef heart and powdered egg yolk, and the final extract cholesterolized by a special titration method. Most of the tests, some more practical than others, gave an acceptable degree of sensitivity and specificity and were widely used.

As with the complement fixation test, Pangborn's[39] isolation of cardiolipin paved the way for the standardization of an antigen which was used in what became known as the VDRL flocculation test.

One further flocculation text, the Ide, must be mentioned at this point since a modification of it was used in the early section of the studies which form the basis of this thesis.

Ide and Ide[54], in 1936, added gum benzoin and a mixture of crystal

violet and azure II to their alcoholized extract of ground heart. When a drop of the antigen, diluted in saline, was added to a drop of blood, serum of spinal fluid on a slide and agitated for about 5 minutes, violet blue floccules were observed macroscopically or microscopically if reagin was present.

Following this brief historical review the techniques used in the present studies will be discussed.

Standard Tests for Syphilis (STS)

(a) Crude Antigens

1. Modified Ide Test

In 1947, The South African Institute for Medical Research, Johannesburg, received up to 1500 sera per day for investigation for syphilis. The test used was the Wyler's modification of the Wassermann Complement Fixation test. Although the serologists performing the test agreed that it would be preferable to assess a serum on at least two recognised methods: a complement fixation and a flocculation test, this was impossible because of the work load.

To solve the problem Macnab and Lewin (1948)[55] modified the test described by Ide and Ide[54]. The test was devised as a screen test which could be rapidly performed and was so sensitive that there would be no false negative reactions. Any positives could then be further investigated by using two or more conventional tests and reported accordingly.

The Ide test was modified by preparing the alcoholic extract from finely ground beef heart powder, adding cholesterol and a lecithin sensitizer, extracted from egg yolk, to obtain the required sensitivity. The antigen was dyed with crystal violet and azure II and gum benzoin was added as

an adjuvant which renders the stained particles of antigen and antibody more easily visible.

To assess the sensitivity of the screen test, the author serologists examined 27 225 sera submitted to the laboratory using the Wyler modification of the Wassermann Complement Fixation test and the Modified Ide test.

TABLE 1.

Results of 27 225 sera. tested with the Wassermann Complement Fixation and Modified Ide test.

	Ide negative	Ide doubtful to positive	Total
Wassermann negative	20 177	1336	27 225
Wassermann doubtful to positive	3	3650	
Wassermann doubtful	59	-	

Of the 62 sera showing a doubtful to positive Wassermann and negative Ide reactions 33 gave a negative Eagle flocculation check test and there was insufficient serum on the remaining 29 to check whether the screen or the Wassermann test was unreliable.

There was thus no evidence to suggest that the screen test gave a single certain false negative. The authors postulated that even if all 29 sera were faulty, the error would only amount to 0,1 per cent. In this study the screen test in its comparative simplicity of execution segregated about 74 per cent negative sera (20 177) from the total number of specimens submitted (27 225).

2. The Kahn Test

The technical procedures used in the Kahn flocculation test are identical to those set out in his book 'Serology with Lipid Antigen'[56].

Basically the Kahn antigen is a lipid fraction of heart muscle, soluble in alcohol but insoluble in acetone, with the addition of cholesterol. When this antigen is mixed with saline in certain proportions floccules appear. The antigen is titrated to determine the smallest amount of saline which, when added to 1,0 ml of antigen, produced aggregates which completely disperse on the addition of more saline. Syphilitic serum prevents this dispersion.

The test is based on the mixture of constant amounts of serum with varying concentrations of antigen which are thoroughly mixed by shaking on a Kahn shaking machine at about 280 oscillations per minute for three minutes. After the addition of 1,0 ml of saline to the highest concentration of antigen being more turbid than the next two lower which only get 0,5 ml, the test is read immediately and 10 minutes later. A positive result is indicated by the presence of floccules in the mixture.

3. The VDRL Test

(b) Purified Antigens

Harris (1946)[57] of the Venereal Disease Reference Laboratory, United States Public Health Service, developed a microflocculation test on slides, using cardiolipin antigen (VDRL slide test). Using sera from 1046 syphilitic donors taken before and after treatment he obtained comparative results with the new test and several other current serologic tests for syphilis. Sera from 224 non-syphilitic donors were essentially negative. Later, Harris (1948)[58] developed the VDRL tube flocculation test which, on testing with 5016 sera from syphilitic patients and non-syphilitic donors gave results similar

to those of the VDRL slide flocculation test.

The antigen for this test is an alcoholic solution containing 0,3 per cent cardiolipin, 0,9 per cent cholesterol and between 0,2 to 0,27 per cent purified lecithin to produce standard reactivity, measured against pooled positive and negative sera. Briefly, 0,5 ml of serum heat-inactivated at 56C for 30 minutes is pipetted into a tube followed by 0,5 ml of diluted antigen. The mixture is shaken for 5 minutes on a Kahn shaker, centrifuged for 10 minutes, shaken for 1 minute, and read[59].

Reactive sera show visible aggregates in a clear or slightly turbid medium. Borderline reactions, where there is doubt as to any visible clumping, are reported as non-reactive. Clear mixtures, without visible clumping or aggregation of particles are reported as non-reactive.

Quantitative testing is carried out by serial dilution of the serum from 1:2 to 1:128. The reciprocal of the greatest dilution producing a distinct positive reaction is reported as the titre of the serum. The reagin content of a serum can thus be estimated and fluctuations in titres may be taken as a guide as to the success or failure of treatment.

4. The Kolmer Complement Fixation Test

The result of research by Kolmer and his co-workers, as stated in the historical reviews, possibly contributed more to the standardization of complement fixation tests for syphilis than any investigations. His technique of incubating the antigen, sera and complement for 15 to 18 hours at 8C prior to the addition of the haemolytic system[60] and his research on

the antigen, culminated in the adoption of his improved cardiolipin-lecithin-cholesterol antigen[43]. The technique of the Kolmer test used in this study is as described by Kolmer in his book 'Approved Laboratory Technic'[61]. As with the VDRL test, quantitative tests can be performed by serial dilutions of the patients serum and the results are reported in dilutions or in Kolmer units.

In all reagin tests, standard sera were incorporated in the control systems.

(B) Treponema Pallidum Antigen Tests

Historical Review

Ever since the demonstration by Schaudinn and Hoffmann (1905)[8] of *Treponema pallidum* as the causative organism of syphilis, attempts have been made to obtain an antigenic component derived from this organism. The first claim to cultivation was made in 1912 by Noguchi[23] and there have been others since, but confirmations have been lacking. The present day explanation is that the organisms isolated were saprophytic, non-virulent spirochaetes which contaminated the lesions allegedly containing *T. pallidum*.

As briefly stated in the Introduction another researcher, Nelson (1948)[24], took up the problem of cultivating *T. pallidum*. In an attempt to determine the factors influencing the survival of the organisms *T. pallidum*. he showed that the survival of the anaerobic spirochaetes was considerably improved in a nitrogen atmosphere containing 5 per cent carbon dioxide. This gas mixture was used in all subsequent experiments. To aid anaerobic

conditions reducing agents, as sodium thioglycollate, were incorporated in the survival medium. Substances containing sulph-hydryl groups, glutathione and cysteine, were found to be beneficial in equimolar concentrations; their effect being apparent after 48 hours. The best survival results were obtained at pH 7,0 and 35C. By adding 5 per cent crystalline albumin to the medium, 80 per cent of the spirochaetes survived for 96 hours and 20 per cent for 144 hours.

The *T. pallidum* organisms (Nichol's strain) were inoculated intratesticularly in the rabbit and harvested when a clinical orchitis developed, usually in 7 to 9 days. It was found that the testicular tissue extract was a most important factor in the survival medium. Experiments performed by centrifuging the motile spirochaetes and resuspending them in an identical basal medium without the tissue extract[24] died within 48 hours.

Here then, was a medium in which it was possible to keep 50 per cent of the spirochaetes alive from 6 to 8 days. No multiplication was noted during this prolonged incubation, but a lengthening of some of the viable organisms was observed. Motile forms retained their virulence, non-motile ones were dead.

Having established a basal medium, Nelson and Mayer (1949)[25] in further investigations, found that the addition of syphilitic serum and active complement to a suspension of motile *T. pallidum*, immobilized and killed the spirochaetes. This formed the basis of the Treponema Pallidum Immobilisation test (TPI test).

In experiments with known sera the TPI test was shown to be almost

100 per cent specific; it was less sensitive than the reagin standard tests in primary and to a lesser extent early secondary cases of syphilis[62]. The specific antibody to syphilis - called immobilin - appeared later in the syphilitic infection than did reagin.

That immobilin antibody was different from the reagin was demonstrated by absorbing out the reagin of a syphilitic serum by flocculation with Eagle antigen[25]. The first absorption brought the reagin titre down from 16 to 2 dilutions and it became negative on a second absorption. The immobilin, as measured by the quantitative TPI test, remained unaffected. The two antibodies also differ in their resistance to elevated temperatures. This was shown by heating five separate samples of serum at 60, 64, 68, 70 and 72C. A progressive decrease in the reagin titre was observed, but no corresponding decrease in the immobilizing activity of the serum. Other experiments[63] showed that, in untreated experimental syphilis, the reagin titre, after infection, reached its peak about the first month, then dropped and levelled off at a low titre after six months. The titre of immobilin on the other hand only reached its peak after the tenth month and remained at a high level. Present information suggests that this high titre persists for many years.

The development of the TPI test awakened interest in the use of treponemal antigens in the study of syphilis and related treponemal infections. Mainly because of the technical difficulties involved in performing the TPI test and the inherent danger of laboratory infection while dealing with the live spirochaetes, tests using killed *Treponema pallidum* were developed. These included the Treponema pallidum Agglutination test, the Treponema pallidum Adherance Reaction and the Treponema pallidum Complement

Fixation test.

However, none gained the popularity of the well established TPI test, which, although costly and technically difficult, far from replacing the two current types of reagin tests, complemented them. The patient who had no clinical evidence or history of syphilis and whose serum showed reagin activity or vice versa could have the diagnosis of syphilis confirmed or rejected by the TPI test.

The first fluorescent treponemal antibody test was evolved in 1957 by Deacon, Falcone and Harris[64] and was known as the FTA-5 test, the figure 5 indicating that the serum was diluted 1:5 with buffered saline. Nichol's strain of *Treponema pallidum* was used as an antigen and was obtained from rabbit testicular tissue by extraction. The conjugate was goat antihuman globulin tagged with fluorescein.

Subsequent studies on various categories of syphilitic infections showed the FTA-5 test to be highly sensitive and specific[65]. A new chemical compound, fluorescein isothiocyanate was introduced to produce labelled antiserums of increased potency. Since fluorescein labelled antihuman globulin is the treponemal antibody detector system of the FTA-5 test, an improvement in this reagent was reflected in an apparent increase in test reactivity. It was also found that Tween-80 enhanced antigen-antibody coupling.

With these improvements in the test technique it was found that the FTA-5 test had become considerably more sensitive and there was a frequent occurrence of weakly reactive results obtained in a normal non-syphilitic

category of patients. This lowering of the specificity of the test in terms of syphilis was explained by the presence of antibodies from group antigens shared by both pathogenic and saprophytic treponemas.

Patients sera were then diluted 1:200 which meant that false positive reports were avoided without, as the authors[66] stated 'appreciable loss of ability to detect syphilitic patients'.

The FTA-200 test, which was in use for two to three years meant that any reaction was specific but it lost sensitivity and although false positive reports were eliminated, patients with early syphilis were not diagnosed.

The same group of workers who pioneered the FTA-5 and FTA-200 tests then published details of the FTA-Absorption test in 1964[27]. Work carried out prior to this publication showed that the group antibodies produced by saprophytic treponemes could be absorbed by an extract made from the Reiter treponema which contained this non-specific common antigen. This extract was called "sonnicate" after the process of disrupting the Reiter treponeme, and ultimately 'sorbent' when the extract was prepared from cultures of Reiter treponemes.

Experimental work done on syphilitic sera taken from persons in various stages of syphilis and non-syphilitic persons, showed the FTA-Absorption test to be as specific as the TPI tests and more sensitive in cases of primary syphilis.

The undisputed advantage of the FTA-Abs test was that it was easier to perform than the TPI test and could thus be incorporated into the test

battery in any routine syphilis serology department in order to assist the elucidation of any diagnostic problem cases.

Test Techniques

1. Treponema Pallidum Immobilisation Test[24,25,67].

(TPI test)

The TPI test is an antigen-antibody-complement reaction, the antigen being a suspension of motile, virulent *T. pallidum* (Nichol's strain) in a special medium. In the presence of active complement, usually obtained by cardiac puncture of guinea-pigs under sterile conditions, the antigen is capable of detecting the specific antibody, immobilin, which occurs in the serum of practically all syphilitic patients beyond the early secondary stage. Occasionally the sera contains other spirochaetal components than immobilin, which could give rise to false conclusions. To detect such substances, eg. penicillin, a control tube with heat inactivated complement instead of active complement is set up in parellel with each serum to be tested.

The principle of the TPI test may be described as follows:

A. Motile spirochaetes + immobilizing antibody ——> sensitized spirochaetes.

B. Motile sensitized spirochaetes + inactive complement ——> motile sensitized spirochaetes.

C. Motile sensitized spirochaetes + active complement ——> non-motile spirochaetes.

D. Motile non-sensitized spirochaetes + active complement ——→ motile non-sensitized spirochaetes.

For the test, the pathogenic Nichol's strain of *T. pallidum* is maintained in rabbits by intra-testicular inoculation, the number of spirochaetes in the inoculum being guaged by dark field examination to produce a clinical orchitis in 7 - 9 days in a mature rabbit. Harvesting of the spirochaetes after that period of time is undesirable because of build up of antibody by the rabbit, producing sensitization of the spirochaetes.

The testes are sliced up and placed in a flask with the basal medium. The flask is evacuated and refilled with nitrogen and 5 per cent carbon dioxide containing less than one part of oxygen per million parts of gas mixture. The spirochaetes are liberated by gentle agitation on a mechanical shaker for one and a half hours.

The test is a two tube test, the one containing patient's serum, inactive complement and antigen suspension and in the other, the inactive is replaced by active complement. A control system is included in every batch of tests. The tubes are transferred to a vacuum dessicator from which the air has been evacuated and replaced with the oxygen free gas mixture. Following 18 hours incubation at 35C, 100 spirochaetes are counted under dark ground illumination and the motile ones are recorded on a hand counter.

The control tube containing spirochaetes in basal medium only should show a minimum of 80 per cent motility. The positive serum control,

serially diluted to include a dilution which permits 50 per cent of the spirochaetes to survive after 18 hours incubation, should not vary more than one dilution from batch to batch. A rise in titre may suggest *in vivo* sensitization. The tube with inactive complement, patient's serum and the spirochaetal suspension should show at least an 80 per cent motility while the proportion of motile spirochaetes in the corresponding test tube with active complement will be inversely related to the amount of specific antibody present.

The specific immobilization (SI) which is defined as the percentage of spirochaetes immobilized entirely as a result of the presence of immobilin in the serum is calculated for each serum tested. This is done as follows:

$$\% \text{ SI} = \frac{\% \text{ motile in the control tube} - \% \text{ motile in the test + tube}}{\% \text{ motile in the control tube}} \times 100$$

In the interpretation of the results, 0-19 per cent SI is reported as non-reactive, 20-49 per cent weakly reactive and 50-100 per cent as reactive. It is essential for the immobilization that complement has been available throughout the incubation period. Therefore, after the examination of each suspension, the test tubes containing active complement are placed in the refrigerator at 4C to minimize any further deterioration of complement. When reading of the suspensions has been completed, the haemolytic system consisting of 2,0 per cent sheep cells and adjusted haemolysin (anti-sheep cell serum), is added to the tubes and incubated at 37C for 30 minutes and the occurrence of haemolysis is taken as evidence of excess complement.

2. The Fluorescent Treponemal Antibody Absorption Test (FTA-Abs test)

The technical details of the FTA-Abs test corresponds with those described in the 'Manual of Tests for Syphilis'[68]. Basically the test is an indirect fluorescent test. The antigen is a suspension of *T. pallidum* (Nichol's strain) extracted from rabbit testicular tissue, fixed and dried on a cleaned slide in a suitably marked area to show a minimum of thirty organisms per high dry field. Prior to adding the serum to the dried antigen on the slide, it is heated for 30 minutes at 56C to inactivate any complement which may possibly interfere with the antigen-antibody complex and thus give false fluorescent readings, and then added to sorbent to give a 1:5 dilution for 15-30 minutes. A minimum time of 15 minutes is required for the sorbent which is a standardized product prepared from Reiter treponemes, to absorb any non-specific treponemal group antibodies from the serum.

After incubation of the sorbent/serum mixture with the antigen and subsequent washing in phosphate buffered saline, suitably diluted fluorescein-labelled antihuman globulin (conjugate) in Tween-80 is added to the slide and again incubated. The slides are washed again, air dried, mounted in glycerine and read under dark ground illumination, using a mercury arc lamp and a high-power dry objective lens.

Controls varying from non-reactive smears (no fluorescence) to strongly reactive (4+) are incorporated in each batch of tests. The most difficult part of this test to the uninitiated is the assessment of the intensity of fluorescence. However, as soon as the reader firmly estab-

lishes in his mind the intensity of a 1+ fluorescence, the problem is solved.

The Treponema Pallidum Haemagglutination (TPHA) test

This test, developed by Rathlev (1965)[69] and Tomizawa and Katsamatsu (1966)[70], is the newest of the specific tests for treponemal disease. Based on the well known immunological principle of the indirect haemagglutination reaction, formalinised tanned sheep red cells are sensitized with antigen components of *T. pallidum* and added to serially diluted serum samples in haemagglutination trays.

The test is simple to perform and read, requires no expensive equipment and the reagents are available commercially in kit form. It is therefore becoming increasingly popular as an acceptable alternative to the confirmatory treponemal tests presently employed for the sero-diagnosis of syphilis.

Evaluation studies vary in the assessment of sensitivity, specificity and reproducibility of the test.

Le Clair (1971)[71] rated the test 96,0 per cent specific on non-syphilis patients, 50,0 per cent sensitive in primary, 100 per cent secondary and 94,0 per cent in latent syphilis. Repeat testing showed a reproducibility of 98,0 per cent. Garner (1973)[72] found a considerable variation in the sensitivity and specificity of three different antigen batches, giving a reproducibility of 90,8 per cent.

Blum et al (1973)[73] reported a false positive rate of 8,8 per cent

and false negative of at least 6,5 per cent for the TPHA test. Ovcinnikov and Timcenko (1974)[74] found non-specific results in cancer patients (7,1 per cent) and patients with certain skin diseases (13,1 per cent).

The test has been performed at this Institute for the last two years, and evaluation shows an acceptable level of specificity and sensitivity[75]. However, in view of the apparently superior specificity of the FTA-Abs test, it was decided not to use the TPHA test in the later studies (Chapter 9 and 10).

CHAPTER 2.

REPRODUCIBILITY, SPECIFICITY AND SENSITIVITY OF SEROLOGICAL TEST FOR SYPHILIS.

Throughout the many years in the development of serological tests for syphilis, much effort has been devoted to the standardization and reproducibility of tests of acceptable specificity and sensitivity. In addition, attention has been paid to technical aspects such as simplicity of performance, speed, and use of mechanical devices when possible.

Standardization

In all tests it is essential that not only the antigen be standardized but reagents and solutions as well. These may be checked by adequate control systems.

Reproducibility

This is measured by the results obtained on the same material (specimen or antigen) in different laboratories or by repeat examination of the same material within a laboratory. It requires exact detailed technical instructions as to the performance of the test including standard measurement of the various reagents. The findings are evaluated with convertional statistical methods.

Specificity and Sensitivity

Ideally, a test for syphilis should detect all cases of infection and be negative in all non-syphilitic conditions. No test has, as yet, been devised to meet both these requirements. In the reagin antibody group of

tests some specificity is lost, since positive serologic reactions are obtained in non-syphilitic individuals with disorders such as leprosy, malaria or systemic lupus erythromatosus. The mechanism of this cross-reactivity is a moot point. In the TPI test, immobilin antibody is not detected until the late primary or early secondary stage of syphilis. This would indicate a loss of sensitivity.

An adequate evaluation of test sensitivity and specificity is based on the reactions obtained in a large series of sera from clinically accurately assessed cases.

Biological False Positive Reactions (BFP) in Standard Serological Tests for Syphilis (STS)

Since specificity and sensitivity of the reagin group of tests is closely related to the 'biological false positive' (BFP) reaction it is pertinent at this point to discuss the problem which has always been a source of concern, not only to the serologist performing the tests but to a far greater degree to the clinician assessing the disorder of his patients.

The term 'biological false positive' (BFP), as described in the introduction denotes the reactivity, with lipoidal and cardiolipin antigens, of sera from patients who do not have or have had syphilis or other trepone-matoses[76].

The lipoidal or cardiolipin antigens, used in the STS, are non-specific in the clinical sense. However the prevalence of the antibody, reagin, detected by these antigens is far higher in treponemal infections

than in other conditions. This makes the tests very useful in the control of syphilis and allied diseases, despite their limitations.

Kahn (1950)[77] established that sera of all human beings contain minute quantities of reagin. They are disregarded by the conventional STS which are adjusted for a lower level of sensitivity. Of great importance, however, are the positive STS reactions in sera of proven non-syphilitic individuals.

Moore and Mohr (1952)[78] state that amounts of reagin detected by STS occur in from 5 to 100 per cent of cases during or after diseases having no relation to syphilis. Summing up the results of a programme of mass blood testing in the United States from 1938 and in the armed forces during World War II, and employing unfractionated cardiolipin, Moore and Mohr estimated that the incidence of BFP reactions in cases of different infections varied from a 'low' (percentage unstated) to 100 per cent in malaria. In non-infectious conditions, BFP ranged from a 'low' in pregnancy to 20 per cent in systemic lupus erythromatosus. It was emphasized that the figures represented averages since the material was collected from several laboratories with similar but not identical techniques.

Moore and Mohr[78] classified non-syphilitic reactions into acute and chronic BFP reactions. The acute category are caused by a variety of infections: bacterial, viral, plasmodial, rickettsial and protozoal. The BFP reactions appear during the disease or the convalescent period and fade spontaneously within a period not exceeding six months.

In the chronic category reagin persists in the blood from six months to years. Leprosy appears to be the only infection associated with chronic

BFP. Reagin is characteristically found in systemic lupus erythromatosus, collagen diseases. rheumatoid arthritis, and a variety of other non-infectious disorders.

The BFP reaction - whether acute or chronic - is often indicative of a serious underlying disorder requiring precise diagnosis and appropriate therapy. These reactions should not be ignored as irrelevant observations but should stimulate further examination of the patient.

With the development of the specific treponemal antibody tests, the task of interpreting STS results in terms of true or false (BFP) reactions was made much easier. This is well illustrated in further studies by Moore and Mohr (1952)[79].

These workers performed the TPI test (Table 2) on 256 selected patients with symptomatic syphilis, the majority presenting obvious clinical evidence of syphilis including benign late gummatous lesions, cardiovascular syphilis or neurosyphilis. A few, diagnosed as latent syphilis, were the mothers of congenital syphilitic children. All had been treated for syphilis by one schedule or another, at some time from one to forty years.

TABLE 2.

The TPI test in treated late syphilis

	TPI test		
Number of patients	Positive	Doubtful	Negative
256	246 (97,3%)*	3	7 (2,7%) **

* Of these, 33 were sero-negative with STS

** Of these, three were sero-positive with STS.

The results of this study indicated a high degree of sensitivity of the TPI test in cases of late syphilis.

Sera from a second group, tested with the TPI reaction, came from 300 patients who showed persistently positive reagin tests in the absence of any clinical evidence of syphilis and were either cases of latent syphilis or BFP reactors (Table 3).

TABLE 3.

The incidence of chronic BFP reactors vs. latent syphilis

	TPI Test	
Number of patients seropositive with STS	Positive (Latent syphilis)	Negative (BFP reactors)
300	164 (54,7%)	136 (45,3%)

Of the 300 patients, 164 may safely be assumed to have or to have had treponemal infections. The remaining 136 patients were persistently STS positive and TPI negative and were therefore in all probability of the BFP type. Re-examination of fifty one of these patients showed that probably thirty-one and possibly as many as forty-five had significant disorders (Table 4).

TABLE 4.

The etiologic background of chronic BFP reactors

Number of patients	Definite diagnosis		Probably diagnosis		(a) Clinically normal but with distinct laboratory pattern	Normal except BFP
	Collagen disease	Other	Collagen disease	Other		
51	5	3*	21	2**	14	6
	(15,7%)		(45,1%)		(27,4%)	(11,7%)

* One each of sarcoid, Hodgkin's and Gaucher's disease

** Sarcoid in both

(a) STS positive (variable titre)
Sedimentation Rate persistently elevated
Cephalin flocculation - ++ to ++++
Thymol turbidity - normal to weakly positive
Serum globulin - sometimes elevated
Urine - sometimes proteinuric and cylindruric

This study provided substantial evidence of the importance of the TPI test in diagnosing chronic BFP reactions.

The possibility of a patient having a BFP superimposed on a specific treponemal infection must always be taken into consideration when assessing 'difficult' cases. This and a number of other variables are presented in Table 5, summarizing present day interpretation of the serological test for syphilis.

TABLE 5.

Interpretation of serological tests fos syphilis

TPI	STS	Interpretation
Non-reactive	Non-reactive	(1) No syphilis (2) Incubating or very early syphilis
Non-reactive	Reactive	(1) Early syphilis (2) Biological false positive
Reactive	Non-reactive	(1) Past syphilis
	Reactive) Non-reactive)	(2) Neurosyphilis[80,81] Tertiary syphilis[80,81]
Reactive	Reactive	(1) Active syphilis, latent or past (2) Cured syphilis with superimposed biological false positive eg. leprosy, lupus erythromatosus (3) Active or latent syphilis with biological false positive

It should be emphasized that syphilis should not be diagnosed nor excluded solely on the basis of serological reactions. The accurate assessment of the disorder requires an interpretation of the total picture: symptoms, clinical signs, darkfield if possible and serology.

Specificity and Sensitivity of Reagin Standard Tests for Syphilis based on Literature Studies

Essentially this section is confined to the tests which were used in the various studies of this thesis: ie. the VDRL macroflocculation tube,

the Kolmer Cardiolipin Complement Fixation and the Kahn flocculation test. Assessment of the Modified Ide Flocculation as a screen test, used at The South African Institute for Medical Research, Johannesburg, is given after the technical details of that test in Chapter 1.

The value of the VDRL flocculation test, was studied by Vogelsang and Haaland (1951)[82]. They examined 15 416 sera with the VDRL tube test, the Wassermann complement fixation using a crude antigen, a complement fixation using cardiolipin antigen and the Meinicke clarification test. Based on accompanying information, the sera could be allocated either to a group drawn from patients with clinical evidence of a history of syphilis but unclassified in any particular stage (1930 sera), or to a group without evidence of syphilis (13 486 sera). The results are shown in Table 6.

TABLE 6.

Evaluation of the four reagin tests

Test	Unclassified Syphilitic group (1930)		Non-syphilitic group (13 486)	
	Positive	Negative	Positive	Negative
VDRL	1197 (62,0%)	733	101 (0,75%)	13 385
W/R Comp. Fix. crude antigen	1207 (62,5%)	623	132 (0,97%)	13 354
W/R Comp. Fix. cardiolipin antigen	1126 (58,3%)	804	83 (0,62%)	13 403
Meinicke	1266 (65.6%)	664	144 (1,05%)	13 342

Comparing the specificity and sensitivity of the four tests, the authors found:

(1) The VDRL and Wassermann reaction with crude antigen showed approximately the same sensitivity but the VDRL was somewhat more specific than the W/R.

(2) The VDRL was more sensitive and as specific as the cardiolipin complement fixation test.

(3) The VDRL was less sensitive but more specific than the Meinicke clarification test.

Although results of the four tests showed a variation in sensitivity, the authors concluded that by virtue of its specificity the cardiolipin antigens both in the complement fixation and the flocculation tests should be regarded as a definite advance in the serology of syphilis. Furthermore, the standardization of the reagents and test techniques improved reproducibility.

In extensive studies, Schmidt (1951)[83] confirmed that cardiolipin was as sensitive as standard crude antigens in testing secondary and old cases of syphilis (patients with infection longer than twelve months). However, cardiolipin antigen was less sensitive in primary and fresh latent cases (patients infected within the twelve months but showing no clinical signs of syphilis). His results are shown in Table 7.

TABLE 7.

Sensitivity results of four tests in sera from 954 syphilitic patients.

Patients	With 'fresh' syphilis					With 'old' syphilis				
Tests	W/R-M	C-W/R	K	MR	VDRL	W/R-M	C-W/R	K	MR	VDRL
Sera examined	186	186	185	182	176	766	765	768	745	698
Positive reactions	159	130	141	149	147	564	569	554	658	621
Percentages of sensitivity	85,5	69,9	76,2	81,3	83,5	73,6	74,4	72,1	92,1	88,9

W/R-M = Wassermann Complement fixation test with crude antigen

C-W/R = Wassermann Complement fixation test with cardiolipin antigen

K = Kahn reaction

MR = Meinicke clarification reaction

VDRL = Slide flocculation test with cardiolipin antigen.

Combining the two syphilitic groups of patients, the VDRL test and Meinicke's clarification test were the most sensitive and Kahn the least of all the STS.

Regarding specificity, it can be seen from Table 8 that Schmidt found the Meinicke reaction to be the most specific, closely followed by the VDRL slide and cardiolipin complement fixation test.

TABLE 8.

Specificity results of four tests in sera from 196 non-syphilitic patients

Positive reactors	Tests				
	W/R-M	C-W/R	K	MR	VDRL
Number	30	5	20	0	3
Per cent	15,3	2,6	10,2	0	2,2

A comparative study of four serological tests for syphilis - VDRL, Meinicke clarification, Kahn and Wassermann (standard antigen) was undertaken by Singh, Singh and Kapoor (1953)[84]. Their material consisted of 304 cases with either a clinical history or a clinical diagnosis of syphilis and 2450 control cases without clinical evidence of syphilis, refugee women from Pakistan, blood donors and cases without clinical notes.

In close agreement with Schmidt's finding, the results in the various groups showed that the Meinicke and VDRL slide tests were more sensitive than the Kahn and Wassermann test in primary and early syphilitic cases but in late and advanced cases all four tests were equally sensitive, and specific in the control group. In treated cases of syphilis, the VDRL test was the last to become negative (ie. most sensitive).

Hill, Buckle and Thomas (1957)[85] in clinically assessed material, evaluated in parallel qualitative and quantitative testing, the VDRL slide, the Eagle flocculation, the Price Precipitation reaction (PPR) and the Kolmer complement fixation test using improved standard (Kolmer) antigen but not cardiolipin. Their material consisted of 919 clinically assessed

cases, comprising 319 clinical syphilitics and 600 non-syphilitics. In addition the specificity of the VDRL and Kolmer tests was assessed in 3655 healthy blood-donors. Table 9 summarizes their results at a qualitative test level (one dilution).

TABLE 9.

Sensitivity and specificity rating of the four reagin tests based on the percentage of positive reactors.

	Sensitivity	Specificity	
Test	319 syphilitic cases	600 non-syphilitic cases	3655 healthy blood donors
VDRL	76,2	5,5	0,68
EFT	74,2	10,3	Not done
PPR	59,8	6,8	Not done
Kolmer CF	54,5	0,5	0,11

All four tests gave a higher sensitivity rating in untreated cases of syphilis but none superior to the others in particular stages of the disease.

The authors concluded that the Kolmer complement fixation test with improved crude antigen and VDRL slide test gave the best combination of sensitivity and specificity. They submitted the following recommendations:

1. Test all sera by the slide VDRL test.

2. Test all VDRL negative sera by the qualitative Kolmer test.

3. Test all VDRL positive sera by the quantitative Kolmer test.

4. Re-test all VDRL positive and VDRL negative (Kolmer positive) sera by the quantitative VDRL test.

These few examples from the literature illustrate the importance of developing an antigen that will achieve a balance between sensitivity and specificity so that it will detect the greatest possible number of treponemal diseases and the lowest number of BFP. Despite all previous research based on syphilitic and non-syphilitic sera, it is generally agreed that there is no certain way in which to be sure that a patient has not had syphilis at some time. This changed with the introduction of the treponemal tests.

Reproducibility, Specificity and Sensitivity of the Treponemal Tests based on Literature Studies.

Treponema Pallidum Immobilization Test (TPI)

Reproducibility

Nielsen (1954)[66], investigating the reproducibility of the TPI test, concluded that the results were highly reproducible except in sera with very low immobil[illegible] content, which, on different days, with the same technique, may give positive, doubtful or negative results. This, he felt, was probably due partly to sensitization of the spirochaetes *in vivo* which introduces a variable of usually insignificant consequences. To prevent sensitization he suggested X-ray radiation of the rabbit followed by

harvesting of the spirochaetes between the seventh and ninth day after inoculation. Using this technique, he illustrated the reproducibility of results of a serum with low immobilin content. The serum was serially diluted and tested in seven consecutive TPI test procedures. The titre of the serum at a Specific Immunobilization of 50 per cent and 18 and 42 hours incubation is shown in Table 10.

TABLE 10.

Serum titres at a Specific Immobilization of 50 per cent on seven different days.

	TPI_{50-18}	TPI_{50-42}
Initial test	130	580
2	160	-
3	120	510
4	160	540
5	135	560
6	125	480
7	150	480

As can be seen, a reproducible titre was obtained at both periods of incubation. Nielsen concluded that an immobilin free serum will never be positive but a serum with a small content of immobilin may be positive.

Sequeira and Wilkinson (1955)[87] assessed the reproducibility of the TPI test by re-testing the sera from 240 patients attending venereal

Disease and other clinics at two London Hospitals. The results of their findings are shown in Table 11.

TABLE 11.

Results of repeat tests on 240 sera.

Second Test		First Test: Positive	Weak Positive	Doubtful	Negative
	*SI	100-76	75-50	49-20	19-0
Positive	100-76	105	3	0	0
Weak Positive	75-50	3	2	3	0
Doubtful	49-20	2	5	8	9
Negative	19-0	0	0	29	71

* SI = Specific Immobilisation.

On the two examinations there was complete correlation between 186 pairs (77,5 per cent) and minor differences (i.e. one doubtful and the other negative, one doubtful and the other weakly positive, or one weakly positive and the other positive) in fifty-two pairs (21,6 per cent) of which thirty-eight occurred between doubtful and negative. A greater difference was observed in only two sera (positive to doubtful), both showing SI results

near the intervening zone. Agreeing with Nielsen that the TPI test has some possible inherent variables such as partial sensitization of the antigen from the rabbit and also that the sensitivity of the test is dependent on the titre of the complement, the authors recommended repeat testing of sera, particularly in cases which gave results varying from doubtful to negative.

Continuing their studies on reproducibility, these workers repeated sera from 283 patients (Table 12).

TABLE 12.

Results of repeat sera from 283 patients.

	First specimen		
Last specimen	Positive	Doubtful	Negative
Positive	165	2	0
Doubtful	4	10	3
Negative	Early syphilis treated 3, Normal infants 2, Tabes treated 1,)6	Early syphilis treated 4, Late syphilis treated 1, ? Congenital syphilis treated 1)8 ?2	85

Among the 283 patients, 260 (92 per cent) showed complete agreement of results of the two specimens. In five (1,8 per cent) there was an increase in the strength of the reaction. In 18 patients (6,0 per cent),

there was an apparent decrease in strength, six changing from positive to negative (shown in Table). The authors considered the variations acceptable and concluded that the reproducibility of the TPI test was satisfactory.

Wilkinson (1954)[88] carried out tests on second specimens of sera from forty-six patients to confirm the original TPI test result. In forty-one cases the result of the second specimen was in agreement with the first. In five (Table 13) there were minor discrepancies but in no case was there a reversal from a frank positive to negative or vice versa.

TABLE 13.

Minor discrepancies on repeat testing of five sera.

Specimen	* SI - First occasion	SI - Second occasion
1	32	51
2	40	76
3	62	33
4	40	20
5	44	12

* SI = Specific Immobilisation.

In specimen five, treatment had been given before the second specimen was taken.

In a second study on reproducibility, Wilkinson, over an 3 month period, tested three ampoules of a WHO positive reference serum, using the 50 per cent immobilisation titre (Table 14).

TABLE 14.

Serial estimations of the 50 per cent Immobilisation Titre on the same serum.

Ampoule	Number of tests	50 per cent Immobilisation titre	Range	S.D.
1	10	334	210-447	64
2	8	266	160-850*	222
3	9	175	135-260	41

* Treponemes sensitized *in vivo*.

If the one value, when the treponemes were sensitized, is omitted, the mean titre for ampoule 2 becomes 183 (with a range of 160-250 and a S.D. of 29) and the results with all three ampoules fall within the ± 2S.D. of the means. He concluded, therefore, that the test can give reproducible results with a quantitative as well as a qualitative test technique.

Specificity and Sensitivity

Zellman (1954)[89] assessed the specificity of the TPI test by analysing the results from eleven laboratories of sera submitted for TPI testing from non-syphilitic individuals. The tests were usually done twice on each serum. Of 1397 non-syphilitic persons, 389 were normal, 615 had diseases other than syphilis - excluded by history and physical examination, and in 393 the distinction between the two groups was not

made. Of these 1397 sera, 99,7 per cent were TPI negative. No further information on the four patients with a positive TPI test was available.

These results indicated a very low incidence of non-specific reactions. Regarding sensitivity, Zellman confirmed the original findings of Nelson and Mayer (1949)[25], that the TPI antibody appears in the blood only after the development of the primary lesion and that the positivity or rise in titre is proportional to the duration of the earlier stages of the infection. Thus in primary and early secondary syphilis the TPI is less sensitive than the STS.

A similar collection of qualitative TPI results was undertaken by Nielsen and Reyn (1956)[90] from a representative selection of papers published on the clinical significance of the TPI test. The results from 38 publications consisted of 10 035 sera. These were divided into 5676 from proven syphilitic cases at various stages of the disease; 682 from cases of doubtful syphilis, 1347 from cases given as 'no syphilis', 474 from normal individuals, 1533 from diseases other than syphilis and 317 giving BFP reactions.

The authors tabulated the results under four categories, three of which are described. The fourth, consisting of sera without a clear differentiation of clinical information, is omitted.

1. Results in which no definite information on treatment was given but where it seemed likely that most of the patients were untreated.

2. Results from untreated cases.

3. Results from treated cases.

This grouping depended mainly on clinical and anamnestic information, but in some cases the reagin tests were also taken into consideration.

The results of categories 1 and 2 were similar for both syphilitic and non-syphilitic sera and are combined in Table 15.

TABLE 15.

Qualitative results of TPI Tests on definitely and probably untreated cases.

Previous diagnosis	Primary syphilis		Secondary syphilis		Early and late latent syphilis		Late Symptomatic syphilis		Congenital syphilis		Normal individuals		Other diagnosis		BFP Reactors	
TPI result	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
Totals	105	72	536	45	610	21	247	1	59	1	0	474	4	991	1	140
	177		581		631		248		60		474		995		141	
Percentage reactive	59,3		92,3		96,7		99,6		98,3		0		0,4		0,7	

In the syphilitic sera series, the sensitivity of the TPI test increased from about 60 per cent in the primary cases to about 90 per cent in the secondary and nearly 100 per cent positive in the older cases.

As expected, a similar but lower trend of reactive TPI sera was seen from treated patients (Table 16).

TABLE 16.

Qualitative results of TPI Tests on treated cases.

Previous diagnosis	Primary syphilis		Secondary syphilis		Early and late latent syphilis		Late symptomatic syphilis		Congenital syphilis		Normal individuals		Other diagnosis		BFP Reactors	
TPI result	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
Totals	176	464	271	268	1232	181	685	32	137	21	None		0	39	0	176
	638		539		1413		717		158		-		39		176	
Percentage reactive	27,2		50,5		87,4		95,4		86,8		-		0		0	

With the exception of the primary and secondary stages where the STS became positive earlier than the TPI test, Nielsen and Reyn stated that the general sensitivity of the test appeared to be higher than the STS, approaching 100 per cent, in cases of untreated syphilis.

Regarding the specificity of the TPI test from this analysis the authors reported that no positive TPI reaction was found among 474 sera from normal individuals and only one positive in the BFP group of 141 sera in the 1st and 2nd categories of sera. In the group 'other diseases' from categories 1, 2 and 3, only four of 1034 (0,4 per cent) were found to react in the TPI test(Table 15). Nielsen concluded that the specificity of the TPI test as measured in sera from normal individuals, sera from patients suffering from diseases other than syphilis and BFP sera was very nearly 100 per cent.

The Fluorescent Treponemal Antibody-Absorption Test (FTA-Abs)

Reproducibility

Two years after their publication of technical details of their FTA-Abs test for syphilis, Deacon, Lucas and Price (1966)[91], instituted a study with five other laboratories to assess the test as a diagnostic tool in syphilis by comparing it with the VDRL and TPI test.

A total of 2252 serum specimens were obtained from patients admitted to venereal disease clinics, patients referred to hospitals as 'problem or BFP' cases, patients without anamnestic evidence of syphilis and suffering from non-syphilitic disorders, and from normal individuals.

The serum from each patient was divided into two portions, half being kept by the laboratory and the other half being shipped to the authors' control laboratory. All five laboratories performed the FTA-Abs test with reagents submitted by the control laboratory and four of the five the VDRL slide test. The control laboratory repeated the VDRL test on its portion of the serum and added the TPI test. When there was any disagreement between the FTA-Abs and the TPI test, the control laboratory repeated both tests.

Initial results of the FTA-Abs test from the five participating laboratories showed an agreement with the VDRL test of 87,6 per cent and with the TPI test of 90,4 per cent.

The results of the repeat testing of 215 (9,6 per cent) sera which on initial testing differed from those of the TPI test are shown in Table 17.

TABLE 17.

Repeat FTA-Abs testing of 215 sera which initially differed from the TPI test.

First Test		Second Test	
FTA-Abs		FTA-Abs	
		+	-
+	178	169	9*
-	37	28	9

* 3/9 were untreated primary syphilis.

Reproducibility between initial and repeat testing was lower (82,8 per cent) than the agreement of 90,4 per cent between the FTA-Abs and TPI tests on initial testing of all specimens. This, the authors state, is to be expected since the 215 specimens came from a highly selective group with critical antibody content.

In the FTA-Abs positive,TPI negative group of 178, nine became negative on repeat testing, three of whom came from cases of untreated primary syphilis (positive dark field examination). Further investigation of this group showed the majority to be from primary syphilitic cases which indicated the greater sensitivity of the FTA-Abs test over the TPI test. Of the FTA-Abs negative, TPI positive group, 28/37 became positive on re-examination, possibly due to a greater confidence at the reference laboratory of interpreting 'borderline' results, or better illumination or different conjugates.

The conclusion drawn was that a good correlation existed between the FTA-Abs and TPI tests (90,4 per cent) showing satisfactory reproducibility between laboratories. Repeat testing of sera also showed good reproducibility since the discrepancies of these critical antibody sera were due mainly to reading difficulties in those laboratories undertaking the initial testing.

Sensitivity and Specificity

Most authors agree that the FTA-Abs test shows a greater sensitivity and a slightly reduced specificity as compared with the TPI test.

Hunter, Norins, Falcone and Stout (1968)[92] reviewing the development, use and status of the FTA-Abs test, discussed four evaluation studies from 1964 to 1968. The sera were drawn from cases of clinical syphilitics, from cases of BFP based on reactive STS and non-reactive TPI tests, from patients with diseases other than syphilis and from normal individuals. The reactivity of the FTA-Abs test was 80-85 per cent in primary syphilis, approximately 100 per cent in secondary syphilis and 92-100 per cent in latent and late syphilis. The FTA-Abs test was much more sensitive in cases of primary syphilis than the TPI test.

Since most evaluations of the FTA-Abs test were based on the TPI test as the standard, the authors stated that the true specificity of the FTA-Abs test would be difficult to assess. They emphasized the need for several evaluation studies in which FTA-Abs tests were performed on all patients admitted to large teaching hospitals. This would give some knowledge of the reactivity of the test in clinical conditions such as cancer, heart disease, kidney disease and surgical conditions.

Mackey, Price, Knox and Scott (1969)[93], in order to evaluate the specificity of the FTA-Abs test obtained sera from 827 individuals. Of these, 335 sera were from treated and untreated clinically diagnosed syphilitic cases and 492 sera from normal controls, from cases with BFP and from diseases other than syphilis. The emphasis on the larger number from the latter group was to determine whether the combination of reactivity to the TPI test was associated with any particular disease.

As had already been firmly established, in primary syphilis the

FTA-Abs test showed greater sensitivity than the TPI, being 89 and 58 per cent respectively.

Of the total of 827 persons tested, five had a confirmed reactive FTA-Abs and a non-reactive TPI test. None of the five had clinical or historical evidence of syphilis and represented 0,6 per cent of the total tested and 1,4 per cent of those with a reactive FTA-Abs test. Two of these had no recorded evidence of disease, one had rheumatoid arthritis, one autoimmune haemolytic anaemia due to lymphosarcoma and the fifth alcoholic cirrhosis of the liver. Assuming there is no false positive TPI, the specificity of the FTA-Abs was 98,6 per cent, but the authors were undecided whether these five cases represented old syphilis or false positive reactions to the FTA-Abs test.

One possible explanation for the specificity of the test being slightly lower than that of the TPI, comes from the observations by Kraus, Haserick and Lantz (1970)[94], who noted that the usual homogeneous pattern of fluorescence of the *T. pallidum* cell antigen was sometimes replaced by a beaded appearance. Four sera from patients with active Lupus Erythematosus (LE) exhibited this unusual phenomenon. This has won general approval and must presently be considered to avoid interpreting such stained treponemas as evidence of syphilis antibodies.

The finding was further corroborated by a controlled study on sera from 150 patients attending a special LE clinic, together with 75 sera from normal controls, matched according to sex, race distribution and age.

The sera were tested at the Venereal Research Laboratory with the

VDRL, FTA-Abs and TPI tests. The sera from the control group were non-reactive in all three tests. In the LE group, 24 had a reactive VDRL, 1 a reactive TPI and 23 some degree of activity in the FTA-Abs of which 11 showed a beaded appearance. None of the 23 had clinical signs or a history of prior syphilitic infection. Considering the high degree of specificity of the TPI test, the patient who gave a reactive TPI was treated, since the possibility of a person having a syphilitic infection and LE running concurrently could not be ruled out.

Although reagin activity (VDRL test) and fluorescence (FTA-Abs test) were present in four LE sera, 20 LE sera with reagin activity had no fluorescence and 19 with fluorescence had no reagin activity. It was thus suggested that since different antibodies are involved in the non-treponemal reagin and treponemal FTA-Abs tests, sera with false positive reagin activity are not necessarily those producing non-syphilitic FTA-Abs fluorescence.

Unable to explain the beading phenomenon, the authors suggested that further studies on related diseases associated with qualitative or quantitative globulin abnormalities may help to provide an answer.

Describing a false positive FTA-Abs test in a case of pregnancy, Buchanan (1970)[95] issued a note of warning that although the high specificity of the FTA-Abs test is not in doubt, false positive reactions do occur and the diagnosis of syphilis should not be made on the basis of a single test. He reiterates that the diagnosis of syphilis still depends on correlating historical, clinical, bacteriological and serological test data.

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VDRL, FTA-Abs and TPI tests. The sera from the control group were non-reactive in all three tests. In the LE group, 24 had a reactive VDRL, 1 a reactive TPI and 23 some degree of activity in the FTA-Abs of which 11 showed a beaded appearance. None of the 23 had clinical signs or a history of prior syphilitic infection. Considering the high degree of specificity of the TPI test, the patient who gave a reactive TPI was treated, since the possibility of a person having a syphilitic infection and LE running concurrently could not be ruled out.

Although reagin activity (VDRL test) and fluorescence (FTA-Abs test) were present in four LE sera, 20 LE sera with reagin activity had no fluorescence and 19 with fluorescence had no reagin activity. It was thus suggested that since different antibodies are involved in the non-treponemal reagin and treponemal FTA-Abs tests, sera with false positive reagin activity are not necessarily those producing non-syphilitic FTA-Abs fluorescence.

Unable to explain the beading phenomenon, the authors suggested that further studies on related diseases associated with qualitative or quantitative globulin abnormalities may help to provide an answer.

Describing a false positive FTA-Abs test in a case of pregnancy, Buchanan (1970)[95] issued a note of warning that although the high specificity of the FTA-Abs test is not in doubt, false positive reactions do occur and the diagnosis of syphilis should not be made on the basis of a single test. He reiterates that the diagnosis of syphilis still depends on correlating historical, clinical, bacteriological and serological test data.

SUMMARY

Summarizing the specificity and sensitivity of the various reagin antibody tests as reviewed in the literature, it appears that the Kolmer cardiolipin complement fixation test and the VDRL slide or tube flocculation test (cardiolipin) show a high degree of sensitivity and are more specific than the Kahn flocculation test. The highly sensitive Modified Ide test is of poor specificity, but serves well as a screen test.

Of the treponemal tests, the TPI test is very specific but relatively insensitive in primary and secondary syphilis. The FTA-Abs test while showing greater sensitivity in the early stages of syphilis when compared with TPI test, falls short of the TPI test in specificity.

CHAPTER 3.

REPRODUCIBILITY OF RESULTS OBTAINED BY THE TREPONEMA PALLIDUM IMMOBILISATION TEST (TPI) AT THE SOUTH AFRICAN INSTITUTE FOR MEDICAL RESEARCH [96].

The technique of the TPI test, introduced at the Institute in 1955, followed the procedure of Nelson and Mayer[25], briefly described under Chapter 1. Since the local gas mixture of nitrogen-carbon dioxide contained traces of oxygen, it was passed over heated copper filings which were subsequently reduced by immersing the red-hot filings in chilled methanol. This, together with doubling the quantity of sodium thioglycollate to the survival medium[97] ensured optimum anaerobic conditions for the fastidious *T. pallidum* spirochaetes. One further modification was the increase of complement in the test to 36,4 per cent[97] which, although increasing the test sensitivity, gave a consistant excess of complement for the duration incubation of 18 hours.

As the sensitivity level of the test varies from batch to batch[98], the TPI_{50-18} (the dilution of a serum which immobilises 50 per cent of the spirochaetes after 18 hours incubation) was measured routinely against a known reference serum. Initially these control sera were supplied by courtesy of the TPI Reference Laboratory in Copenhagen, then, as World Health Organization (WHO) reference sera from the same laboratory.

The degree of reproducibility was examined in three ways:

1. TPI_{50-18} values of the suspension using WHO reference sera

The TPI_{50-18} values were measured against 3 pools of WHO reference

sera (WHO/TPI/7)[99]. From this study it appears that they were of the same order as those found in other laboratories, although the titres classified the sensitivity level as above average. This was to be expected because of the comparatively high concentration of complement in the test[99]. Other TPI laboratories, also using a high percentage of complement, showed the same high sensitivity level. The batch to batch fluctuation in TPI_{50-18} against WHO control serum I ranged from 550 to 760 and against WHO control serum II from 350 to 480, both well within the limits found in other laboratories[99]. Thus, the examination of the TPI_{50-18} values against international reference sera suggest a satisfactory degree of reproducibility on a comparatively high sensitivity level.

2. Duplicate investigations of a number of unknown human sera

The agreement between the first result of an examination and that obtained after several investigations of the same sample is an index of the reproducibility of a test. As shown in Table 18, of 259 sera considered reactive after the first investigation, 234 (90,4 per cent) were finally classified as reactive. Considering the 189 sera giving 90-100 per cent SI on the first occasion, it is noticed that 186 of these (98,4 per cent) remained reactive. Therefore, a result of 90-100 per cent in one test only will carry an error of about 1,6 per cent. This error, could in some cases be attributed to imperfectly cleaned test tubes or slides[100]. Having rectified this, no further false positive results attributable to faulty technique were encountered.

TABLE 18.

Comparison of first and final result of sera tested more than once.

		First result, percentage specific immobilization						
		100	99-90	89-75	74-50	49-20	19- 0	TOTAL
Final result, percentage specific immobilization	100	133	0	0	0	0	0	133
	99-90		18	8	2	0	0	42
	89-75	3	10	15	3	0	0	31
	74-50	3	5	4	16	6	1	35
	49-20	0	0	0	11	25	6	42
	19- 0	2	1	3	8	56	151	221
TOTAL		189					158	504

Of 158 sera initially classified as non-reactive, 1 (0,63 per cent) was finally considered reactive and 6 (3,80 per cent) weakly reactive. Although the antibody content of these sera undoubtedly was low, it is somewhat disturbing to find that one cannot place full reliance on a negative finding in one test only.

Similar observations were made by Sequeira and Wilkinson (1955)[87] and Ledbetter (1956)[101] and although several explanations are possible, it appears most likely and in accordance with the findings of Nielsen (1957)[98] that these results are attributable to variations in sensitivity of the test suspension. The assumption in this study is further supported by the fluctuation in TPI_{50-18} values of the suspensions. The logical answer to

TABLE 18.

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		First result, percentage specific immobilization						
		100	99-90	89-75	74-50	49-20	19- 0	TOTAL
	100	133	0	0	0	0	0	133
	99-90	14	18	8	2	0	0	42
Final result,	89-75	3	10	15	3	0	0	31
percentage	74-50		5	4	16	6	1	35
specific immo-	49-20	0	0	0	11	25	6	42
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this error is to examine a serum in two different treponemal suspensions[98]. In 95,57 per cent of the cases a non-reactive result of the first test was supported by subsequent investigations. As expected, the sera found initially to be weakly reactive were the most troublesome; they were examined repeatedly in order to assay their antibody content as accurately as possible. Nielsen (1954)[86] pointed out that sera containing small amounts of immobilin behave differently in systems of different sensitivity. Therefore, such sera were tested with both 22,2 per cent and 36,4 per cent complement in the same treponemal suspension[87].

Despite the inconsistency of a few observations, it may be concluded that the overall results obtained with repeat tests on the same specimens of serum are, on the whole, reproducible, and are, in fact, very similar to those reported by Sequeira and Wilkinson (1955)[87] and Wilkinson (1954)[88].

3. Results of human sera examined both here and in the TPI Reference Laboratory, Copenhagen.

A direct check of the reliability of the TPI results in this laboratory was made by sending 22 sera to the Reference Laboratory, Copenhagen (Table 19). As can be seen from the table, sera ranged from non-reactive to strongly reactive. With the exception of 2 sera, the results from the Reference Laboratory were in agreement. While 1 of these (No. 335) was considered reactive, the Reference Laboratory classified it as weakly reactive. The opinions were reversed in regard to the other serum (No. 330). Details of the observations of these two sera, together with

another two (Nos. 34B and 36A) in which estimation of immobilin levels proved particularly difficult are given in Figure 1.

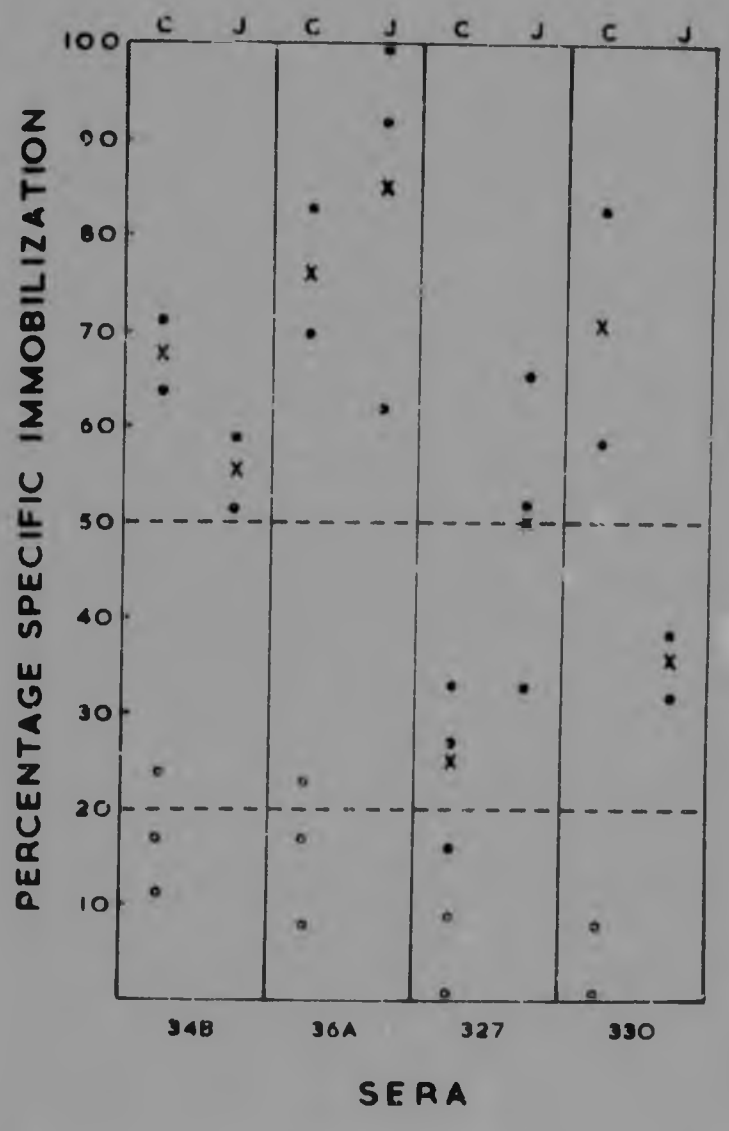
TABLE 19.

Results of 22 sera tested both in Johannesburg and in the Reference Laboratory, Copenhagen.

		Percentage specific immobilization observed in Johannesburg			
		100-90	89-50	49-20	19-0
Percentage specific immobilization observed in Copenhagen	100-90	6			
	89-50		3	1	
	49-20		1	1	
	19- 0				10

Figure 1.

Individual readings of four weakly reactive sera tested in Johannesburg and Copenhagen.



J = Johannesburg

C = Copenhagen

X = Average per cent SI using 36-40 per cent complement

Closed circles = Results, using 36-40 per cent complement

Open circles = Results, using 20 per cent complement

Sera 34B and 36A were positive on all readings in both laboratories when 36-40 per cent complement was used, whereas in the presence of 20 per cent, as employed in some of the investigations in Copenhagen, the specific immobilization percentages were so low that the sera appeared to be non-reactive or only weakly reactive. The fluctuations in titres are more marked with sera 327 and 330. No. 327, in the presence of 40 per cent complement in Copenhagen, gave readings ranging from non-reactive to weakly reactive, while it varied between weakly reactive and reactive in this laboratory. There is, however, no substantial difference between the results, and the same applies to those obtained with serum 330. In both laboratories all four sera showed weak immobilin activity of essentially the same order.

It can thus be concluded that the reproducibility of the qualitative TPI test as performed in this Institute is similar to that reported from most other TPI laboratories.

CHAPTER 4

COMPARATIVE STUDIES OF REAGIN AND IMMOBILIN ANTIBODIES IN BOTSWANA SUBJECTS

Over the past fifty years, many studies have been undertaken to examine the prevalence of syphilis in groups of Bantu, using current complement fixation and flocculation techniques.

Pijper (1921)[11], in an endeavour to establish a reliable incidence of syphilis in Pretoria, reported that in 500 Bantu and Coloured people, 37,0 per cent gave a strongly positive Wassermann test, 11,0 per cent positive and 9,0 per cent weakly positive. Shortly after, he established that over a two and a half year period, 2150 people attended the Pretoria Anti-Venereal Clinic[102]. Of these, 1402 had syphilis clinically and 727 were diagnosed as having syphilis by a positive Wassermann test.

Further evidence for the high prevalence of syphilis is found in the Annual Report of The South African Institute for Medical Research. In 1927[103], 26,6 per cent of sera submitted for the Wassermann test gave positive results and in 1937[104] these had increased to 33,1 per cent.

Purcell (1940)[105], comparing the figures from the Annual Reports of the Cities of Cape Town and Johannesburg, stated that the incidence, in Bantu and Coloureds, of new clinically diagnosed cases of syphilis per 10C 000 in the year 1938-39 and 1937-38 were 851 and 691 respectively.

Acknowledging that many new cases from these cities escaped the clinics and that the figures were an underestimate, the comparison with Great Britain in 1937, was 160 per 100 000 (with an 85 per cent estimate of the total) and with Sweden, 7 per 100 000 (actual number). He concluded that the incidence of syphilis in South Africa, taking all stages in the disease into consideration was 'enormous'.

Based on a Wassermann survey in Pretoria, Cluver (1940)[106] reported that 30,0 per cent of 300 Bantu adults were sero-positive as were 35,0 per cent in a similar group in Germiston.

Kark (1949)[6], reviewing serological studies in the Bantu, based on the Wassermann test from 1921 to 1946, concluded that the incidence of syphilis ranged from an exceptional low 2,0 per cent in a Xhosa group to 48,0 per cent as previously reported[11].

O'Malley and Wilson (1949)[107] however, questioned the assumption that the Bantu population of South Africa was so heavily infected with syphilis. Using the Wassermann reaction, he tested 1000 prospective Bantu male employees arriving in Cape Town from various territories. Twenty Bantu were selected at random from each group of new arrivals. Of the 1000 individuals, 86,8 per cent showed no reaction in the Wassermann test, 7.4 per cent were positive and 5,8 per cent doubtful. In passing, it is interesting that the highest percentage of positives (20,8) were found in the Lesotho tribe, which approaches the figure of 25,5 per cent described by Cluver (1932)[108]. A possible explanation, O'Malley suggested, was that those who wander furthest from their home

surroundings and influences (Lesotho), were more likely to become victims of sex promiscuity. Although confined to one sex and limited in numbers, O'Malley's findings did not support the widely held view that syphilis was rife among the Bantu population. He agreed however, that the figure of 7,4 per cent sero-positivity was an undersirable permeation of syphilis infection in any community, and that a considerable variation in prevalence existed from tribe to tribe.

In contrast to O'Malley's findings, Targowsky (1952)[109] reported a 29,25 per cent sero-positivity among Bantu on the Witwatersrand. In correlating the clinical and serological aspects of syphilis, he found clinical signs in approximately half of the sero-reactors. He concluded that biological false positive reactions were rare and that there was no relationship between reactors and conditions other than syphilis.

Hill, Griffiths and Buckle (1957)[110], on the other hand, thought that a significant proportion of the sero-positive reactors were of the biological false positive type. They based this statement on rare clinical manifestations of syphilis and the low incidence of syphilitic stigmata in post mortem material (5.5 per cent amongst the Bantu and 0,5 per cent amongst the Whites over fifteen years of age) and some different chemical values of Bantu sera compared with sera from Whites. However, Bersohn, Wayburne, Hirsch and Sussman (1954)[111] had previously found that 81 per cent of Bantu mothers showed a reversed albumin/globulin ratio as compared with White mothers, whereas serum protein values and liver function of their babies were similar. Since only

26,1 per cent (of 230 Bantu mothers) showed a positive serological test (Wassermann) it seems more likely that the differences between the sera are possibly due to nutritional and other factors amongst the Bantu, and are unrelated to reagin production.

As indicated briefly in the Introduction, one of the important studies bearing on this problem was undertaken by Murray, Merriweather and Freedman (1956)[18]. Using the Kolmer complement fixation test for syphilis, they found an overall sero-positivity of 37,0 per cent amongst the inhabitants of the Bakwena reserve of Botswana in which endemic syphilis, 'dichuchwa', was rife. The Bakwena reserve, topographically can be divided into two areas, the one, very large and arid which forms the greater part of the reserve and is known as part of the Kalahari desert and the other a smaller section to the East which is more fertile, has more bore-holes, wells and river beds and is nearer the railway line running to Rhodesia. The survey team established that the seropositivity rate in the Kalahari area was much higher (56,3 per cent) than in the more fertile non-Kalahari (30,5 per cent). The average seropositivity of the total number of sera tested from both areas was 37,0 per cent.

Relationship of endemic syphilis (dichuchwa) to other treponematoses

In considering the treponematoses from an epidemiological angle, Grin (1956)[112] classifies them as follows:-

1. Extra-venereal juvenile treponemal infections occurring in endemic form. These are:

(a) Endemic syphilis, comprising similar or identical conditions with different local names such as 'bejel' (Iraq and Syria) 'njovera' (Rhodesia) and 'dichuchwa' (Botswana).

(b) Yaws, the endemic treponematosis of the tropics.

(c) Pinta, the endemic treponematosis of Central America.

2. Predominantly venereal adolescent and adult infections occurring in sporadic form.

As with yaws[112], dichuchwa is a disease of poor hygiene[18], and the most important means of transmission is the close bodily contact of an uninfected person, particularly a child, with a patient with infectious lesions. Murray, Merriweather and Freedman (1956)[18], basing their classification of the various types of treponematosis on environmental and clinical differences, found that dichuchwa was very similar to bejel but different in many clinical aspects from venereal syphilis.

However, the organisms causing the treponemal infections are all morphologically indistinguishable, produce reagin[18,113] and immobilin[114, 115] antibodies and respond to the same treatment schedule[113,116].

It was to get a clearer understanding of the serology of population groups living in areas with 'dichuchwa' that the Treponema Pallidum Immobilisation test was introduced in the serology laboratories of The South African Institute for Medical Research in 1955.

As outlined in various literature reviews in Chapter 2, immobilin, the antibody detected by the TPI test is produced by infections with pathogenic treponemes. A positive reaction, therefore, is considered proof of a past or present treponemal infection but a negative reaction does not exclude early syphilis. This test would thus be well suited to confirm or reject a diagnosis of reasonably advanced treponematosis, be it syphilis, extra-venereal treponematosis or any allied disease. Additional clarification as to the interpretation of reagins in the Bantu would emerge from simultaneous use of STS and the TPI test to appropriate sera.

MATERIAL AND METHODS

The serological evaluation in this study of reagins, as determined by immobilin, is divided into three surveys.

1. Sera from 197 Bantu subjects from Botswana
2. Sera from 142 Bantu subjects from Botswana
3. Sera from 424 Bantu male subjects on the Witwatersrand, recruited as mine labourers from Botswana.

The TPI test was carried out on all specimens of sera according to the technique described by Nelson and Mayer (1949)[25], with some modifications as outlined in Chapter 3. The results were reported as Specific Immobilisation (SI); 0-19 per cent was considered non-reactive, 20-49 per cent weakly reactive and 50-100 per cent reactive.

All sera, with the exception of Survey 1 were subjected to four

reagin tests: quantitative Kolmer Cardiolipin complement fixation; quantitative VDRL tube flocculation; quantitative Kahn - using standard antigen -, and the qualitative Modified Ide test. The Modified Ide test was omitted in Survey 1 but performed in Surveys 2 and 3. The initial dilution in the Kolmer test was 1:2,5 and the VDRL 1:2. The Kahn reaction was done on neat serum and then diluted 1:2. Techniques of all tests were outlined in Chapter 1.

Survey 1. Sera from 197 Bantu subjects from Botswana

Blood specimens were taken from 197 subjects living in the village of Molepolole in the Non-Kalahari area of the Bakwena Reserve of Botswana, about 320 Km North West of Johannesburg.

Most sera were from adults over the age of fifteen. Thirty one sera, without information as to age or sex, are excluded from tables relevant to these factors.

Results

Serological results of the STS were originally classified in three groups according to their behaviour in the Kolmer, Kahn and VDRL tests; the strongly positive, the doubtful, and the negative. However, this was an unfortunate classification. It was necessary to consider even the weakest reagin activity as being positive, otherwise there would have been a loss in sensitivity without any gain in specificity.

TABLE 20

Immobilin and reagin in 197 sera

TPI specific Immobilisation	Total No. of sera	Kolmer		Kahn		VDRL	
		+	-	+	-	+	-
0 - 19%	137	4	133	2	135	4	133
20 - 49%	4	1*	3+	1*	3+	1*	3+
50 - 100%	56	45	11	45	11	46	10
	197						

* = Same serum : Kolmer 20µ, Kahn 20µ, VDRL 20µ.

\+ = These three sera were from individuals over 40 years of age.

Most of the immobilin free sera were non-reactive in the STS. Had the three reagin tests been the only ones used, nearly a quarter of the sera with treponemal antibodies would have been missed. This is shown clearly in Table 21 in which the comparative ratio of the TPI and individual reagin tests is calculated.

TABLE 21

Percentage correlation between TPI and STS results

	TPI		TPI	
	Non-reactive	% Non-reactive STS of negative TPI	Reactive and Weakly Reactive	% Reactive STS of positive TPI
Kolmer	133/137	97,0	46/60	76,7
Kahn	135/137	98,4	46/60	76,7
VDRL	133/137	97,0	47/60	78,3
Agreement with all STS	131/137	95,6	45/60	75,0

According to the non-reactive TPI group, the Kahn reaction in the lowest dilution was slightly more specific than the Kolmer and VDRL. Taking the three STS reactions into consideration, there was only a 95,6 per cent correlation. Of the sixty sera containing immobilin, twelve showed no reagin.

The six immobilin free sera, positive in one to three of the STS are analysed in Table 22 with their respective reagin titres.

TABLE 22

Immobilin free sera with reagin activity

Age	Sex	Kolmer U	Kahn U	VDRL U
40	F	10[(a)]	4	16
15 - 40	M	10	4	-
?	?	5	-	4
40	F	10	-	-
5 - 14	M	-	-	2
?	?	-	-	2

(a) The figures indicate the reagin titre.

It is interesting to see from this table that the reagin titres are comparatively low, and, with the exception of the first serum, the results of the STS are discrepant. Without additional information it is not possible to determine the cause of the presence of reagins. It is quite conceivable that some of them are BFP and others early syphilis, or residual reagin from syphilis treated in the early stages.

In the immobilin reactive group of sixty sera (Table 21), the VDRL reaction was the most sensitive (78,3 per cent) followed by the Kolmer and Kahn, both with a sensitivity of 76,7 per cent. In 75,0 per cent of the sera, both TPI and STS were positive. Of the remaining

fifteen TPI positive sera (25 per cent), twelve showed no reagin activity and three discrepant results.

These three are shown in Table 23 and illustrate the link between immobilin positive sera with and without detectable reagin.

TABLE 23

Immobilin containing sera with discrepant reagin results

TPI/SI %	Kolmer U	Kahn U	VDRL U
100	5(a)	-	2
100	-	2	-
98	-	-	4

(a) The figures indicate the reagin titres.

Of the four sera which gave weakly reactive results in the TPI test (Table 20) three were negative STS. This is understandable, since they probably fall into the same category as the reactive TPI group with no reagin. The fourth serum with all STS positive could be an early case with a rising immobilin titre. A follow-up serum could not be obtained.

It is well known that the greater the number of standard tests performed on a serum sample, the more likely it is that reagin will be detected. The assessment of two simultaneous STS is shown in Table 24.

TABLE 24

Combined sensitivity of two STS in sixty immobilin reactive sera

Tests	Number positive	Percentage
Kolmer and Kahn	47/60	78,3
Kolmer and VDRL	47/60	78,3
Kahn and VDRL	48/60	80,0

The figures in this series show little difference between the various combinations nor when the combinations are compared with the sensitivities of the three tests alone (Table 21). Furthermore, if the Kolmer had been the only test two would have been missed, with the Kahn two and the VDRL one, again showing little difference between the three tests.

Table 25 considers the immobilin and reagin produced in response to treponemal infections by age and sex. Thirty one sera without this information are excluded.

TABLE 25

Immobilin and reagin by age and sex

	TPI reactive			Kolmer +			Kahn +			VDRL +		
	Total	No.	%	No.	% of total	% of TPI+	No.	% of total	% of TPI+	No.	% of total	% of TPI+
5 years	-	-	-	-	-	-	-	-	-	-	-	-
5 - 14 years	10	4	-	2	-	-	1	-	-	3	-	-
15 - 39 years	95	29	30,5	28	29,4	96,5	28	29,4	96,5	28	29,4	96,5
40+	61	24	39,3	13	21,3	54,2	14	23,0	58,3	13	21,3	54,2
Total	166	57	34,3	43	25,9	75,4	43	25,9	75,4	44	26,6	77,2
Males	76	25	32,9	19	25,0	76,0	19	25,0	76,0	19	25,0	76,0
Females	90	32	35,5	24	26,7	75,0	24	26,7	75,0	25	26,8	78,1

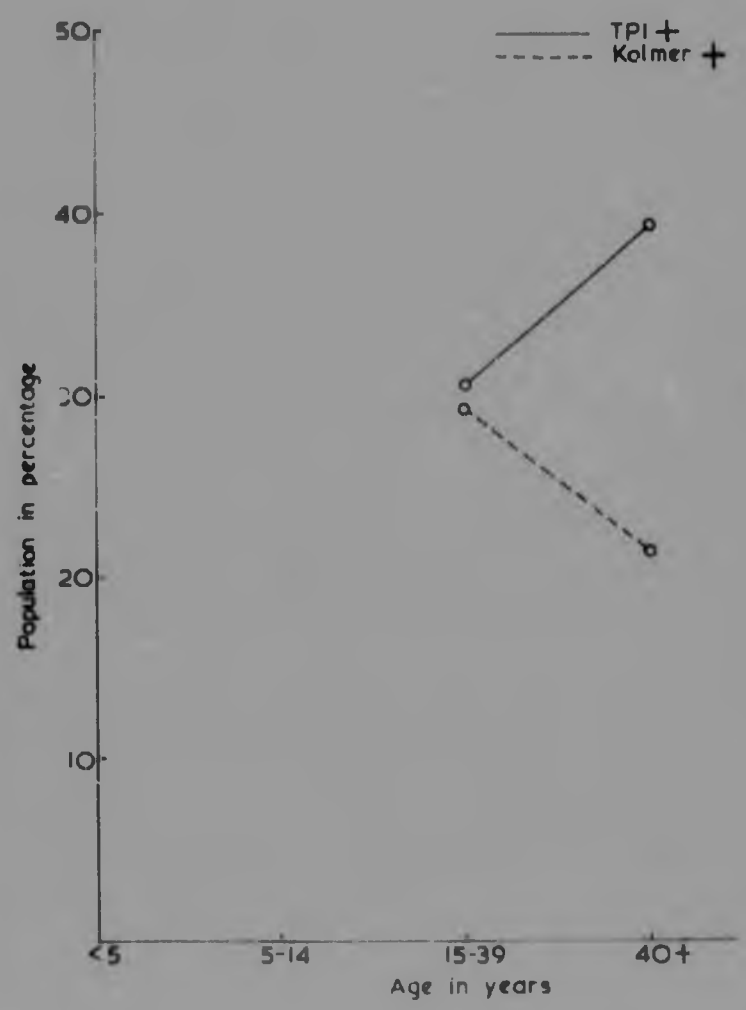
In the 5 - 14 years age group, the numbers are too small to allow any valid conclusions; suffice to point out that four of ten showed evidence of treponematosis. It is striking that the prevalence of immobilin positive sera increases with age while the reagin positive sera reach a peak in the 15 - 39 year old group. In other words, immobilins seem to be more persistent than reagins and the latter may well be associated with the more active types of treponematosis.

This is illustrated in Figure 2, using the Kolmer test as the criteria for demonstrable reagin.

Figure 2

Immobilin and reagin

by age groups.



The sex distribution between the age groups is fairly even (Table 26).

TABLE 26

Age and sex distribution of individuals in Table 25

	Total	Age Groups (166 individuals)		
		5-14	15-39	40+
		Percentage of group		
Males	76	2	27	17
Females	90	4	31	19
	166			

The results of Survey 1 will be discussed in conjunction with Survey 2.

Survey 2. Sera from 142 Bantu subjects from Botswana

It was realized that the age group 5 - 14 years of Survey 1 was too small to allow any opinion on the prevalence of 'dichuchwa' amongst the children of this Botswana population. Furthermore, it was desirable to narrow the post-puberty age groups. It was also decided to take the opportunity to study the performance of the Ide test which was added to the battery of reagin tests.

In the Molepolole area 142 sera were collected; just over two-thirds from children under the age of fifteen years.

In Table 27, the results of the immobilin and reagin antibodies found in these sera are analysed.

TABLE 27

Immobilin and reagin in 142 sera

TPI specific Immobilisation	Total No.of Sera	Kolmer		Kahn		VDRL		Ide	
		+	-	+	-	+	-	+	-
0-19%	120	0	120	(a) 3/117	114/117	0	120	12	108
20-49%	1	0	1	1	0	0	1	1	0
50-100%	21	13	8	17	(b) 4	17	(b) 4	17	(b) 4
	142								

(a) In three cases there was insufficient serum for the Kahn test.

(b) These four sera are not from the same individuals.

Of the immobilin free sera, all were negative in the Kolmer and VDRL tests and three showed activity in the Kahn test. The Ide-positive sera are consistent with increased sensitivity of the antigen. In sera with immobilin activity, the Kolmer was appreciably less sensitive than the other three tests which were negative in about a fifth of the cases (Table 28). Two sera had no detectable reagin.

TABLE 28

Percentage correlation between TPI and STS results

	TPI Non-reactive	% Non-reactive STS of negative TPI	TPI Reactive and weakly reactive	% reactive STS of positive TPI
Kolmer	120/120	100	13/22	59,1
Kahn	114/117	97,4	18/22	81,8
VDRL	120/120	100	17/22	77,0
Ide	108/120	90,0	18/22	81,8
Agreement with all STS	104/117	88,9	12/22	54,6

From this table, it is noteworthy that in the non-reactive TPI group the Kolmer and VDRL were the most specific (100 per cent) followed by the Kahn and then Ide (90,0 per cent). When there was agreement with all four STS the specificity was 88,9 per cent.

Although the Kolmer showed complete specificity it was the least sensitive of the four tests (59,0 per cent). The Kahn and Ide were the most sensitive (81,8 per cent) and the VDRL 77,0 per cent.

Of the 120 immobilin free sera, thirteen showed some reagin activity in one or two of the four STS. These are presented in Table 29.

One serum with a positive Ide and insufficient for the Kahn reaction is omitted.

TABLE 29

Immobilin free sera with reagin activity

Age	Sex	Kolmer µ	Kahn µ	VDRL µ	Ide (Qualitative)
6	F	-	(a)2	-	-
9	F	-	2	-	-
11	F	-	2	-	+
7	M	-	-	-	+
9	F	-	-	-	+
9	F	-	-	-	+
10	M	-	-	-	+
10	F	-	-	-	+
10	F	-	-	-	+
10	F	-	-	-	+
12	F	-	-	-	+
15	F	-	-	-	+
16	M	-	-	-	+

(a) The figures indicate the reagin titre.

These results confirm the lower specificity of the modified Ide test in the younger age groups. Also of note is the low reagin titre of the three positive Kahn tests which may indicate an early treponematosis

or specificity due to some other cause (BFP). Follow-up sera were not available.

TABLE 30

Immobilin containing sera with discrepant reagin activity

TPI SI %	Kolmer μ	Kahn μ	VDRL μ	Ide (Qualitative)
100	(a) 160	-	(a) 128	+
100	-	(a) 4	16	+
100	-	4	8	+
100	-	4	4	+
96	-	4	2	-
83	-	4	-	-
100	-	-	-	+
42	-	4	-	+

(a) The figures indicate the reagin titres.

As in Survey 1 these eight immobilin containing sera illustrate the link between those with and without reagin. The over-sensitive Ide failed in two cases and in four instances the Kolmer was less sensitive than the VDRL. Furthermore, it is difficult to explain why the Kahn test failed entirely in the one serum with an extremely high Kolmer and VDRL reagin titre. Regrettably a repeat test, which would have excluded any technical error, could not be done because of insufficient serum available, nor could an additional serum be obtained.

TABLE 31

Combined sensitivity of two STS in twenty-two immobilin reactive sera

Tests	Number positive	Percentage
Kolmer and Kahn	12/22	54,6
Kolmer and VDRL	13/22	59,1
Kolmer and Ide	13/22	59,1
Kahn and VDRL	16/22	72,7
Kahn and Ide	16/22	72,7
VDRL and Ide	16/22	72,7

In contrast to the equivalent Table in Survey 1 (Table 24), where there was little difference in the sensitivity of the various combinations of the Kolmer, Kahn and VDRL tests, the VDRL, Kahn and Ide combinations were markedly more sensitive than those incorporating the Kolmer. If the Kolmer had been the only test used, nine immobilin containing sera would have been missed, with the VDRL five, the Ide four and the Kahn three.

Three sera which were insufficient for the Kahn test are omitted from Table 32 which shows the sex and age distribution of those individuals with treponematosis, based on immobilin and reagin production.

TABLE 32

Immobilin and reagin by age and sex

		TPI Reactive		Kolmer +			Kahn +			VDRL +			Ide +		
	Total	No.	%	No.	% of total	% of TPI+	No.	% of total	% of TPI+	No.	% of total	% of TPI+	No.	% of total	% of TPI+
0-4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5-9	48	2)	10,5	1)	6,3	60,0	1)	7,4	70,0	1)	7,4	70,0	2)	7,4	80,0
10-14	47	8)		5)			6)			6)			6)		
15-19	33	8)	27,3	4)	15,9	58,3	7)	25,0	91,7	6)	22,7	83,3	6)	22,7	83,3
20-29	11	4)		3)			4)			4)			4)		
30+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	139	22	15,8	13	9,4	54,5	18	13,0	76,8	17	12,2	72,7	18	13,0	76,8
Males	53	8	15,1	6	11,3	75,0	7	13,2	87,5	7	13,2	87,5	7	13,2	87,5
Females	86	14	16,3	7	8,1	50,0	11	12,8	78,6	10	11,6	71,4	11	12,8	78,6

It emerges from Table 32 that nearly three times as many individuals between the age of 15 - 29 years exhibit evidence of treponematosis compared with those below the age of fifteen years. As would be expected from Table 28, the Kolmer reactions are appreciably lower than the other reagin tests. Using the Kolmer test as the index, the decrease in the number of sera showing demonstrable reagin is compared with those with immobilin, by age, in Figure 3.

Figure 3

Immobilin and reagin by age groups

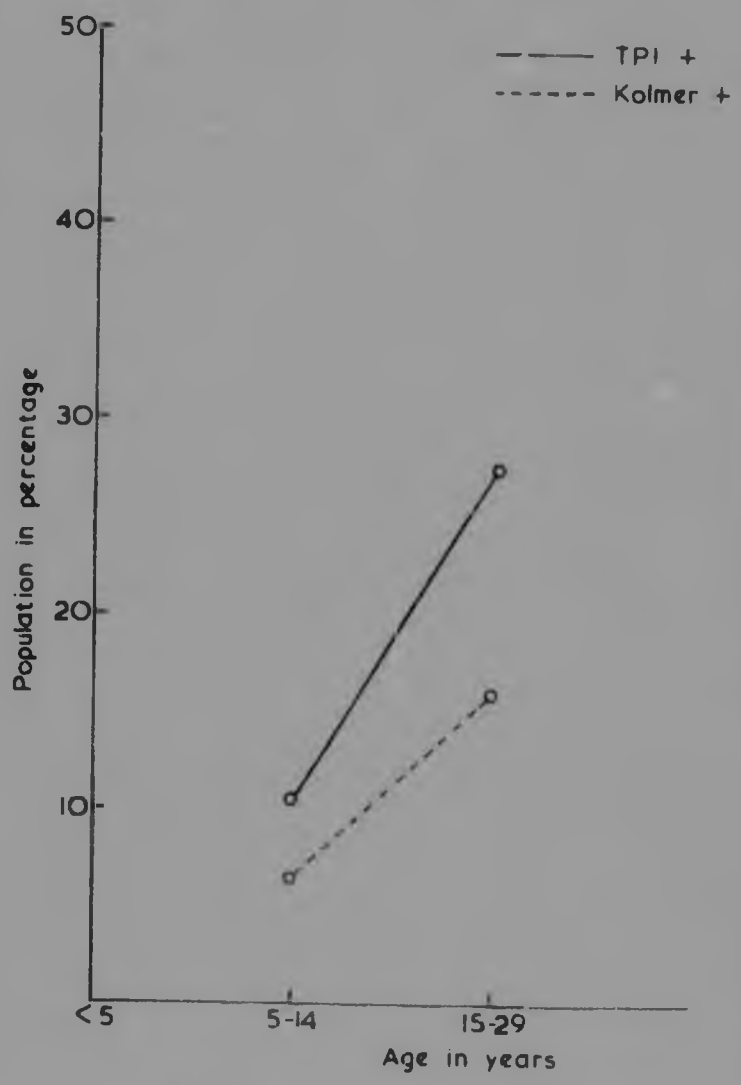


TABLE 33

Age and sex distribution of individuals in Table 32

	Total	Age Groups (139 individuals)				
		5-9	10-14	15-19	20-29	30+
		Percentage of group				
Males	53	11	17	8	2	-
Females	86	24	17	16	5	-
	139					

The sex distribution (Table 33), with a greater majority of females, did not materially influence the overall prevalence of treponematosis (15,8 per cent), a conclusion that might have been drawn from the narrow spread between males (15,1 per cent) and females (16,3 per cent).

Discussion on Surveys 1 and 2.

The purpose of this investigation was to compare the results of the STS and TPI in an indigenous black population. Secondly, to take advantage of the extensive studies by Murray et al[18] the sera were collected in the Molepolole (non-Kalahari) area.

Combining the results of the two surveys, and assuming that the TPI test is both 100 per cent specific and sensitive after the primary stage of the disease, the overall specificity of the Kolmer, VDRL and Kahn tests was 98-99 per cent (Tables 21 and 28). The specificity of the Ide test was 90 per cent. The sensitivity of the tests were: Kolmer 72 per cent; VDRL and Kahn 78 per cent; and Ide 82 per cent. The sensitivity of a

combination of two tests ranged from 72 per cent (Kolmer and Kahn) to 78 per cent (Kahn and VDRL). Thus, the combination of VDRL and Kahn is as specific as any other combination and may be slightly more sensitive. The higher sensitivity of the Ide test gave it certain advantages for screening purposes.

The prevalence of TPI positive sera of the combined study (Tables 20 and 27) was 24,2 per cent. Considering the groups of known age, the overall prevalence of immobilin positive sera was 25,9 per cent (Table 34). The Table demonstrates that the prevalence rose steadily from 13,3 per cent in the 5 - 14 year age group to 39,3 per cent in the over forty.

TABLE 34

Prevalence of treponematosis by age groups in the combined surveys, based on immobilin reactivity

Age group in years	Total	TPI +	
		No.	%
5 - 14	105	14	13,3
15 - 39	139	41	31,3
40+	61	24	39,3
Total	305	79	25,9

This rise parallels the increase in positive sera with age as noted by Murray et al[18].

A comparison of the findings in these surveys and of those made by Murray et al[18] on syphilis serology in sera from Molepolole, is made

in Table 35.

TABLE 35

Comparison of tv studies with an interval of three to four years

Tests	Survey	Previous penicillin treatment	Year of collection	No. Sera	Age range	%+*
STS	Murray et al[18]	Nil	1953-4	13 064	0-40+	30,5
STS	1 and 2	Yes	1956-7	305	5-40+	20,0
TPI	1 and 2	Yes	1956-7	305	5-40+	25,9

* % + for STS = Kolmer and VDRL only.

The present study suggests that at least 25,9 per cent of the individuals suffered or had suffered from treponemal infections and of these 77 per cent (20/25,9) showed reagin activity. In the series by Murray et al[18], the prevalence of reagin positive sera was one third higher (30,5 per cent) than in this study. The difference may be due to a number of factors:-

(1) The World Health Organisation treatment (16 820 individuals) of the population (0,15 million units in asymptomatic infants to 1,8 million units in adults in the symptomatic late stage of 'dichuchwa') in 1954[18], would most likely have lowered the incidence.

(2) Improved hygienic habits due to contact with medical teams impeding the transmission of extra-venereal syphilis.

(3) The decrease in the incidence of treponematosis in the years

between the two studies.

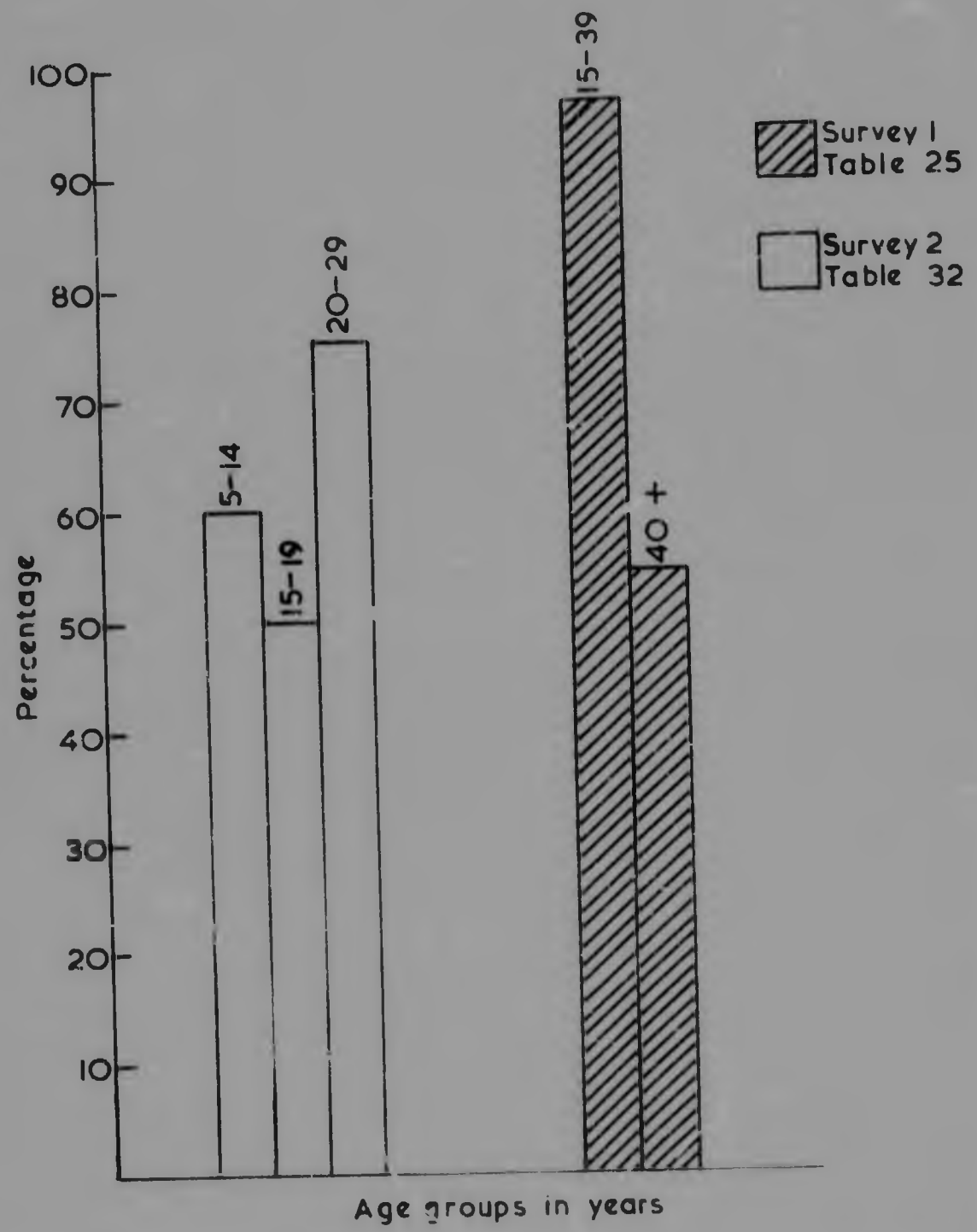
(4) Younger individuals aged 0 - 5 years in the study by Murray et al[18].

It is obvious that treatment for 'dichuchwa'was the main factor responsible for the decline in incidence and the role of the other factors is difficult to assess. It must suffice to state that Murray's finding of a high prevalence of treponematosis in the Molepolole population has been corroborated by the application of the TPI test and that there may be a suggestion that the incidence is on the decline.

Measured by the Kolmer test, Figure 4 illustrates the percentage of individuals with probable active treponemal disease as evidenced by immobilin positive sera which are also reagin positive, in the various age groups of the two surveys,

Figure 4

Percentage of TPI positive individuals with positive reagin (Kolmer), probably indicating an active trenematosis, as a function of age in the two surveys.



It seems possible that the two surveys are dealing with different types of treponematosis. Survey 2 indicates, up to the age of nineteen years, predominantly extra-venereal treponematosis in which the group is losing reagin because of treatment or spontaneous cure (60,0 per cent (6/10) in the 5 - 14 to 50,0 per cent (4/8) in the 15 - 19 age group - Table 32). Thereafter those TPI positive cases with reagin rise to 75,0 per cent (3/4 - Table 32) in the 20 - 29 age group, which may well be due to classical venereal syphilis and, although rare, 'dichuchwa' transmitted venereally[18]. In Survey 1 - Table 25, it appears probable that the high percentage of reagin positive sera shown in the 15 - 39 age group are a result of mainly venereal syphilis with a mean age of the subjects between thirty and forty. In the over forty year old group, the drop in reagin could be attributed to spontaneous cure in some cases and treatment response in others.

Statistical analysis using the Chi square test shows that the Kolmer and VDRL tests are significantly less often positive in the over forty group as compared with the 15 - 39 and 5 - 14 year old groups.

(Kolmer $\chi^2 = 7,95$, $p < 0,012$, VDRL $\chi^2 = 13,08$, $p < 0.001$)

In the combined surveys, ignoring the Ide test in the second group, there were 10 TPI reactive sera (12,2 per cent) showing inconsistent reagin activity. With the exception of one (Table 30) the titres were generally of a low order. It is possible that these sera were from patients cured from treponemal infections following therapy or spontaneously and, as has been intimated before, forms the like between TPI positive individuals with reagin activity and those with none. Three quarters of the sera in the

two surveys were negative to the TPI test and of these excluding the ten which showed a positive Ide only, nine showed some activity in one to three of the Kolmer, Kahn and VDRL test and the titres were usually low (Tables 22 and 29). It is possible that some of the nine sera were biological false positives but some may be from early syphilitic cases where the immobilin is not yet demonstrable in the TPI test. Regardless of the explanation it demonstrates a very acceptable degree of specificity.

Conclusions from Surveys 1 and 2 show that the sensitivity of the STS based on the TPI test is 72,0 - 81,8 per cent, the specificity of the Kolmer, Kahn and VDRL test is at least 98,0 per cent, and that the percentage of TPI and STS reactive sera declines with age. A titre of more than ten units in any of three tests is usually associated with a reactive TPI test.

The number of sera with some reagin (Kolmer, Kahn and VDRL) and no immobilin is low (9/257 = 3,5 per cent) which indicates a possible even lower prevalence of BFP in that population.

Survey 3. Sera from 424 Bantu male subjects on the Witwatersrand, recruited as mine labourers from Botswana

Sera from 424 Botswana men, 16 - 40 years of age, who had offered themselves as blood donors, were examined. Nothing was known about previous illness or treatment, except that they were found healthy on medical examination before admission to the mines.

TABLE 36

Immobilin and reagin in 424 sera

TPI specific Immobilization	Total No.of sera	Kolmer		Kahn		VDRL		Ide	
		+	-	+	-	+	-	+	-
0 - 19%	228	1	227	9	219	3	225	8	220
20 - 49%	6	0	6	0	6	0	6	0	6
50 - 100%	190	129	61	119	71	137	53	134	56
	424								

As in the two previous surveys, most of the immobilin free sera were also reagin free, but in the TPI reactive and weakly reactive group approximately one third failed to show any reagin activity.

TABLE 37

Percentage correlation between TPI and STS results

	TPI		TPI	
	Non-reactive	% Non-reactive STS of negative TPI	Reactive and weakly reactive	% Reactive STS of positive TPI
Kolmer	227/228	99,6	129/196	65,8
Kahn	219/228	96,1	115/196	60,7
VDRL	225/228	98,7	137/196	69,9
Ide	220/228	96,5	134/196	68,4
Agreement with all STS	213/228	93,5	111/196	56,6

From the TPI non-reactive group it is clear that the Kolmer at the lowest dilution was the most specific (99,6 per cent) followed by the VDRL, Ide and Kahn test. Agreement in all negative tests showed a specificity of 93,5 per cent. In the TPI positive group of 196 sera, the VDRL reaction proved to be the most sensitive (69,9 per cent) followed by the Ide, Kolmer and Kahn (60,7 per cent). The fifteen sera showing discrepant STS results are analysed in Table 38.

TABLE 38

Immobilin free sera with reagin activity

No. of sera	Kolmer U	Kahn U	VDRL U	Ide
1	(a) 10	4	-	+
3	-	40-4	-	+
1	-	2	2	-
2	-	20	-	-
2	-	2	-	-
2	-	-	8-2	-
4	-	-	-	+

(a) The figures indicate the reagin titres.

This group of low reagin titred sera follow the pattern of those which may be biological false positive reactors. As was seen in the similar groups in Survey 1 and 2, the sera showed discrepant reagin results; in no case were all STS reactive.

Of the sera showing immobilin activity, 56,6 per cent were positive in all the reagin tests. Of the remaining 43,4 per cent of positive TPI reactive sera, fifty-one had no detectable reagin and thirty-four gave discrepant reagin results. The latter are presented in Table 39.

TABLE 39

Immobilin containing sera with discrepant reagin activity

	TPI SI %	Kolmer U	Kahn U	VDRL U	Modified Ide
3 STS +	100	(a) 5	(a) 4	(a) 4	-
	100	160	-	32	+
	100	160	-	16	+
	100	80	-	32	+
	100	80	-	8	+
	100	40	-	4	+
	100	40	-	4	+
	100	40	-	4	+
	100	20	-	16	+
	100	-	4	8	+
	100	-	4	2	+
	100	-	2	8	+
	96	20	-	8	+
	96	5	-	4	+
	96	-	20	4	+
	95	20	-	8	+
	91	20	-	8	+
	91	5	4	4	-

(a) The figures indicate the reagin titre.

TABLE 39 (continued)

	TPI SI %	Kolmer μ	Kahn μ	VDRL μ	Modified Ide
2 STS +	100	10	-	8	-
	100	5	-	4	-
	100	-	-	2	+
	77	-	4	2	-
1 STS +	100	10	-	-	-
	100	2½	-	-	-
	100	-	4	-	-
	100	-	-	2	-
	100	-	-	2	-
	100	-	-	-	+
	100	-	-	2	+
	98	-	-	2	-
	97	-	-	-	+
	94	-	-	-	+
	82	-	-	-	+
	66	-	-	-	+

In contrast to the immobilin free sera (Table 38) which were invariably of low reagin titre, this group ranged from the highest titres to a positive Ide only. In the series in which three of four reagin tests were positive it is interesting to note that the reputable standard Kahn reaction failed in eleven cases where the Kolmer titre was twenty or higher and the VDRL showed varying degrees of reactivity.

Table 40 indicates the combined sensitivity when using two STS simultaneously.

TABLE 40

Combined sensitivity of two STS in 196 immobilin containing sera

Tests	Number positive	Percentage
Kolmer and Kahn	132/196	67,3
Kolmer and VDRL	133/196	67,9
Kolmer and Ide	140/196	71,4
Kahn and VDRL	138/196	71,9
Kahn and Ide	138/196	71,9
VDRL and Ide	142/196	73,5

Assessing the various combinations, there is little difference in sensitivity between any of them. Compared with the findings in Table 37, the combined sensitivity has increased little above that of the VDRL test alone. However, the sensitivity of the Kahn reaction is comparatively low (60,7 per cent, Table 37) and served little useful purpose in this study, whether alone or in combination. By using two reagin tests, 67,3 - 73,5 per cent of treponemal infections, either active or cured, were diagnosed serologically.

Discussion

Since an immobilin containing serum is considered proof of past or present treponemal infection, it emerges from Table 36 that an incredible 46,2 per cent (196/424) of this group of Botswana mine-workers are or have been infected. It seems probable that there is a combination of endemic syphilis, acquired in Botswana (Murray et al[18]) and Table 32 of this study, and venereal syphilis acquired after puberty. Endemic syphilis is rarely acquired after puberty[18] and is infrequent on the Witwatersrand[17]. Furthermore, since all mine-workers are housed in a hostel environment, the possibility of syphilis being transmitted homosexually cannot be ruled out.

Based on the TPI immobilin results, the sensitivity of the individual STS varied between 60,7 - 69,9 per cent, the VDRL test showing the highest and the Kahn the lowest. A combination of the VDRL and Ide test gave the highest sensitivity (73,5 per cent). The Kolmer and VDRL tests showed the highest specificity (99,6 per cent and 98,7 per cent).

Of the immobilin reactive group, 26,1 per cent showed no reagin activity and 17,3 per cent gave inconsistent results. It is possible that these sera originated from individuals in whom the treponemal infection has been cured, either spontaneously or following therapy.

This survey showed that 53,8 per cent of the sera investigated were negative in the TPI test. Most of these sera were also non-reactive to the STS (Table 37) indicating a high degree of specificity and a low incidence of biological false positive reaction. The fifteen (6,5 per

cent) immobilin free sera (Table 38) which showed various but usually low degrees of reagin activity, may all be 'BFP' reactions, but with the high prevalence of treponematosis shown in this group, it is possible that some of them are from early syphilitic cases. Excluding the Ide test for comparative purposes, the percentage in this group is slightly higher than that found in Surveys 1 and 2 (11/228 = 4,8 per cent).

These surveys support the contention that earlier conclusions, as to the prevalence of treponemal infections among the Bantu deduced from studies of reagins in their sera, were essentially correct.

CHAPTER 5

COMPARATIVE STUDIES OF THE STANDARD SEROLOGICAL TESTS FOR SYPHILIS (STS) WITH THE TREPONEMA PALLIDUM IMMOBILISATION TEST (TPI) IN TWO GROUPS OF BANTU LIVING IN THE SOWETO COMPLEX OF TOWNSHIPS OF JOHANNESBURG.

In the Botswana survey, a group of people living in a family environment in Molepolole and a group of their men living as bachelors on the mines of Johannesburg, were examined. For comparison, a group of non-migratory urban Bantu living with their families in the Soweto complex of townships near Johannesburg, was selected. This complex is a housing scheme built to accommodate a population of about 300 000 persons employed in various walks of life in Johannesburg and is situated about 24 - 32 Km south-west of the centre of the city.

The study, conducted in 1957, is providing additional figures on the reagin and immobilin tests as well as information on the prevalence of treponematosis in an urban population (Survey 1). Furthermore, by examining serum from babies, pre-school and school children up to the age of 14 years, some knowledge may be gained as to the prevalence of congenital or endemic syphilis in this population. For comparative purposes sera from a similar number of whites, aged less than fourteen, were examined.

A follow-up study was performed in Soweto in 1965 when living conditions in the community, including the availability of more clinic facilities, had improved considerably. The purpose was to study the

effect of urbanization on the incidence of syphilis amongst Bantu (Survey 2).

MATERIAL AND METHODS

The subjects came from the Moroka township of Soweto and were divided into age groups ranging from babies to those over thirty years old (Tables 46 and 59). Sera were obtained from 372 people, consisting of adults attending the general clinic of Moroka, school children from a nearby school and pre-school going children accompanying their mothers to the clinic (Survey 1). In addition, blood was taken from eighteen mothers and cord blood from their babies for serological evidence of congenital syphilis. Serum from white babies and children were obtained from 150 consecutive outpatients of the Transvaal Memorial Hospital for Children.

The TPI and STS were performed as described in Chapter 1 and used as in the Botswana studies (Chapter 4). Because of the difficulty of obtaining a suitably pure gum benzoin necessary for the antigen production the Modified Ide test was discontinued in Survey 2.

Survey 1 - Sera from 372 individuals from Moroka Township

TABLE 41

Immobilin and reagin in 372 sera

TPI specific Immobilisation	Total No.of sera	Kolmer		Kahn		VDRL		Ide	
		+	-	+	-	+	-	+	-
0-19%	301	1	300	7/273	266/273	1/262	261/262	13/294	281/294
20-49%	2		2		2		1/1	1	1
50-100%	69	17	52(76%)	18	51(75%)	15/64	49/64(77%)	30	39(56%)
	372								

Note: Where proportions are tabulated there was either insufficient serum for completing the tests or a shortage of VDRL antigen.

Table 41 shows that most of the immobilin free sera were non-reactive in the STS. In contrast, in the TPI reactive group 56 - 76 per cent had no detectable reagin, depending on the test used. The specificity and sensitivity of the individual tests is shown in Table 42.

TABLE 42

Percentage correlation between TPI and STS results

	TPI		TPI	
	Non-reactive	% Non-reactive STS of negative TPI	Reactive and weakly reactive	% Reactive STS of positive TPI
Kolmer	300/301	99,7	17/65	26,2
Kahn	266/273	97,4	18/65	27,7
VDRL	261/262	99,6	15/65	23,1
Ide	281/294	95,5	30/65	46,2
Agreement with all STS	228/245	93,1	13/65	20,0

Six immobilin containing sera, insufficient for completion of the STS are omitted. The Kolmer and VDRL tests showed a high degree of specificity, followed closely by the Kahn reaction and then the Ide. Agreement in all tests gave a 93,1 per cent specificity. As would be expected from a screen test, the Ide was the most sensitive (46,2 per cent) with the other tests showing approximately half (23,1 - 27,7 per cent) that sensitivity.

Those sera with no immobilin and detectable reagin are presented in Table 43.

TABLE 43

Immobilin free sera with reagin activity

Age	Sex	Kolmer µ	Kahn µ	VDRL µ	Ide
8	F	20	4	N.D.	+
?	F	-	N.D.	2	+
16	M	-	20	-	-
16	M	-	20	-	-
16	F	-	4	-	-
17	F	-	4	-	-
18	F	-	4	-	-
19	M	-	4	-	-
4	F	-	-	-	+
6	F	-	-	-	+
12	F	-	-	-	+
18	F	-	-	-	+
18	F	-	-	-	+
19	F	-	-	-	+
26	F	-	-	-	+
30	F	-	-	-	+
32	F	-	-	-	+
36	M	-	-	-	+
39	M	-	-	-	+

The figures under the tests indicate the reagin titres.

With the exception of the first serum the titres are comparatively low and, as has been seen in the previous surveys there is discrepancy in the reagin results. That six positive Kahn reactions were negative in the Ide screen test casts doubt on the value of the test. Unfortunately there was insufficient serum for a repeat technique check. This group constitutes those who may be biologically false positive reactors, early syphilitics or have residual reagin from syphilis treatment in the early stages.

Of the immobilin reactive sera, 34 showed no reagin and 18 gave discrepant reagin results (Table 44).

TABLE 44

Immobilin containing sera with discrepant reagin results

TPI S. %	Kolmer μ	Kahn μ	VDRL μ	Ide
100	80	4	4	-
100	2½	-	4	-
100	-	4	-	-
98	-	4	-	-
12 sera 100-90	-	-	-	+
2 sera 89-80	-	-	-	+

The figures indicate the reagin titre.

In this group the majority of the TPI positives were only reactive

in the more sensitive Ide test which failed however on three occasions, each of which were reactive in the Kahn. This points to the advisability of using at least two simultaneous reagin tests, shown in Table 45.

TABLE 45

Combined sensitivity of 2 STS in 65 TPI reactive sera

Tests	Number positive	Percentage
Kolmer and Kahn	17/65	26,2
Kolmer and VDRL	15/65	23,1
Kolmer and Ide	29/65	44,6
Kahn and VDRL	17/65	26,2
Kahn and Ide	31/65	,7
VDRL and Ide	29/65	44,6

As would be expected from the sensitivity results of the single reagin tests (Table 42), the Kahn and Ide tests together were the most sensitive. However, in order not to sacrifice specificity for sensitivity, the best combination indicated from this series of sera would be either the Kolmer and Ide or VDRL and Ide tests.

In Table 46, the age and sex of the individuals with immobilin and reagin produced by a past or present treponemal infection is considered. Seventeen sera, in which either the STS were incomplete or in cases where the ages of the individuals were unknown, are excluded.

TABLE 46

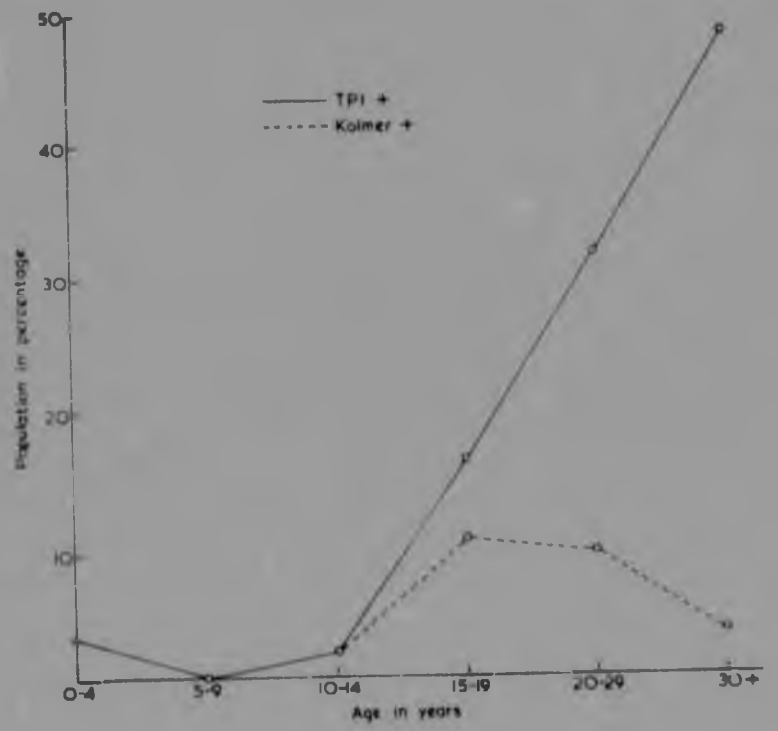
Immobilin and reagin by age and sex

Age	Total	TPI No.	Reactive %	Kolmer + No.	Kolmer + % of Total	Kahn + No.	Kahn + % of Total	VDRL + No.	VDRL + % of Total	Ide + No.	Ide + % of Total
0-4	32	1	3,1	0	0	0	0	0	0	0	0
5-9	86	0	0	0	0	0	0	0	0	0	0
10-14	53	1	1,9	1	1,9	1	1,9	1	1,9	0	0
15-19	49	8	16,3	5	10,4	6	12,3	5	10,4	5	10,4
20-29	75	24	32,0	7	9,3	6	8,0	7	9,3	12	16,0
30+	60	29	48,3	2	3,3	3	5,0	2	3,3	11	18,3
Total	355	63	17,8	15	4,2	16	4,5	15	4,2	28	7,9
Males	88	4	4,5	2	2,3	2	2,3	2	2,3	4	4,5
Females	267	59	22,1	13	4,9	14	4,9	13	4,9	24	9,0

The one serum in the youngest age group which showed a weak immobilin reaction (SI 46,0 per cent) and negative STS was from a four year old girl. It is possible that this case was born of a cured syphilitic mother or, if infected at birth, was treated and is slowly losing her immobilin antibodies. Of interest is the steady increase of those with immobilin from the age of fifteen. Reagin antibodies on the other hand, showed a similar trend up to the age of nineteen years and then start to regress. This is illustrated in Figure 5, using the Kolmer test as an index for reagin.

FIGURE 5

Immobilin and reagin by age groups



As evidenced by Table 47 there were fewer males in the age group 15 - 30+ (3 per cent) than the equivalent group of females (49 per cent). This may account for the greater number of woman showing immobilin (21,5 per cent) than men (4,5 per cent) in the same age group (Table 46).

TABLE 47

Age and sex distribution of individuals in Table 46

	Total	Age groups (355 individuals)					
		0-4	5-9	10 14	15-19	20-29	30+
		Percentage of group					
Males	88	3	14	5	2	0	1
Females	267	6	10	10	11	22	16
	355						

With the possible exception of the one girl, aged four, none of the 118 children aged 0 - 9 years produced any serological evidence of congenital or acquired syphilis. In the 10 - 14 year old group, 1,9 per cent yielded a positive TPI reaction. Of the fifty three children in this group, thirty were collected from children whose mother's sera were also examined. The immobilin results of this group were matched up and are presented in Table 48.

TABLE 48

Immobilin in thirty mothers and their children

		Mothers		
		Reactive	Weakly Reactive	Non-reactive
Children	Reactive			1
10-14 years	Weakly reactive			
Of age	Non-reactive	13	1	15

In this group, fifteen mothers and their children were immobilin free; fourteen positive mothers apparently did not transfer the infection to their offspring. One girl, aged fourteen, was immobilin positive although her mother was negative, suggesting an acquired treponemal infection.

In Table 49, the immobilin results of additional sera from eighteen mothers and cord bloods from their babies are analysed.

TABLE 4[illegible]

Immobilin in the [illegible] of eighteen mothers and cord b[illegible] of their babies

		Mothers		
		Reactive	Weakly reactive	Non-reactive
Babies	Reactive	4		
	Weakly reactive			
	Non-reactive	4		10

The ten TPI negative mothers and their babies were also STS negative. Four mothers with immobilin gave birth to four immobilin free babies; none showed any reagin, suggesting old cured infections, or perhaps latent syphilis, in the mothers. Babies from the other four TPI positive mothers also had immobilin. In two of these pairs, the reagin tests were positive but in the other two, the mothers were reagin negative in contrast to the babies who gave weak reactions. Follow-up specimens for evidence of active infections on the last four babies could not be obtained.

Despite repeated requests, bloods and cord bloods from more mothers and babies were not forthcoming.

From Tables 46, 48 and 49, it would appear that the incidence of congenital syphilis in the township is very low.

The age and sex distribution of the white children whose sera were analysed for comparative purposes with the Moroka children is shown in Table 50.

TABLE 50

Age and sex distribution of 150 white children from the Transvaal Memorial Hospital for Children

	0-4	5-9	10-14	Total
Males	29	33	35	97
Females	21	17	15	53
				150

The results of the TPI test and STS on these 150 children are presented in Table 51.

TABLE 51

Immobilin and reagin in 150 White Children

TPI specific Immobilisation	Total No.of sera	Kolmer		Kahn		VDRL		Ide	
		+	-	+	-	+	-	+	-
0-19%	150	0	150	1/149	148/149	0	149/149	3	147
20-49%	0								
50-100%	0								
	159								

Note:- Where proportions are tabulated there was insufficient serum for completing the tests.

In no instances was immobilin detected in this group of children. The specificity of the various reagin tests is analysed in Table 52.

TABLE 52

Percentage correlation between TPI and STS results

	TPI		TPI	
	Non-reactive	% Non-reactive STS of negative TPI	Reactive	%
Kolmer	150/150	100	-	-
Kahn	148/149	99,3	-	-
VDRL	149/149	100	-	-
Ide	147/150	98,0	-	-
Agreement with all STS	146/149	98,0	-	-

As expected from the results of previous studies, the Kolmer and VDRL tests showed the highest specificity (100 per cent) followed by the Kahn and Ide test. The three sera showing some reagin activity are shown in Table 53.

TABLE 53

Immobilin free sera with reagin activity

Age	Sex	Kolmer U	Kahn U	VDRL U	Ide
13	M	-	4	-	+
12	M	-	-	-	+
4	M	-	-	-	+

Taking into account the high sensitivity of the Ide test, it seems reasonable to assume that the only possible biological false

positive reaction may come from the first child with a low Kahn reaction.

Survey 2 - Sera from 258 individuals from Moroka Township eight years after Survey 1.

Sera for this survey were selected, in so far as age groups were concerned, to match as near as possible those analysed eight years previously. The one exception was the omission of babies up to the age of five. It was felt that sera from this group could be examined at a later date should there be any evidence from the 5 - 14 year old group suggesting any possibility of treponemal infections compared with the first survey. No difference was seen.

With the withdrawal of the Modified Ide test this study is limited to the three conventional reagin tests.

TABLE 54

Immobilin and reagin in 258 sera

TPA Specific Immobilisation	Total No. of sera	Kolmer		Kahn		VDRL	
		+	-	+	-	+	-
0-19%	220	6	214	6	214	6	214
20-49%	3	2	1	1	2	3	0
50-100%	35	25	10	15	20	28	7
	258						

In contrast to Survey 1, nearly three-quarters of those sera with immobilin showed reagin activity. Most of the non-reactive immobilin sera were also reagin free.

TABLE 55

Percentage correlation between TPI and STS results

	TPI			
	Non-reactive	%	Reactive and Weakly reactive	%
Kolmer	214/220	97,3	27/38	71,1
Kahn	214/220	97,3	16/38	42,1
VDRL	214/220	97,3	31/38	81,6
Agreement with all STS	210/220	95,5	15/38	39,6

From this Table it emerges that all three tests attained a high degree of specificity (97,3 per cent). In the group showing immobilin activity however, the Kahn reaction (42,1 per cent) was only half as sensitive as the VDRL (81,6 per cent). The Kolmer test gave a 71,1 per cent sensitivity.

The immobilin free sera with some reagin are tabulated in Table 56.

TABLE 56

Immobilin free sera with reagin activity

Age	Sex	Kolmer U	Kahn U	VDRL U
20	F	2½	4	32
22	F	2½	4	8
17	F	2½	-	2
32	M	2½	-	2
53	F	2½	-	2
19	F	2½	-	-
67	F	-	-	2
15	F	-	4	-
15	M	-	4	-
16	F	-	4	-
53	M	-	4	-

The figures under the tests indicate the reagin titre.

With the exception of the first two sera (VDRL 32μ and 8μ) the titres are low and variable, falling into the pattern of the biological false positive reactors. The possibility of early syphilis, particularly in the first two sera with reagin reactions, cannot be excluded.

In the immobilin reactive group, seven showed no reagin antibody and fifteen discrepant reactions (Table 57).

TABLE 57

Immobilin containing sera with discrepant reagin results

TPI SI %	Kolmer U	Kahn U	VDRL U
100	160	-	64
100	40	-	4
100	20	-	16
100	20	-	2
100	10	-	4
100	5	-	2
100	2½	-	4
100	2½	-	2
72	-	-	8
70	10	-	8
70	-	-	8
69	5	-	2
53	-	-	2
28	2½	-	8
22	-	-	2

The figures indicate the reagin titre.

It is interesting to see from Table 57 how insensitive the Kahn reaction was in this TPI positive group of sera. Had the Kahn been the only test used, all fifteen sera would have been dismissed as non-reactive. Again the importance of using two reagin tests is evidenced in Table 58.

TABLE 58

Combined sensitivity of two STS in thirty-eight TPI reactive sera

Tests	Number positive	Percentage
Kolmer and Kahn	27/38	71,1
Kolmer and VDRL	31/38	81,6
Kahn and VDRI	31/38	81,6

As would be anticipated from Table 55, any combination of tests in which one was the VDRL would give the highest sensitivity and, together with the Kolmer,would be highly specific.

TABLE 59

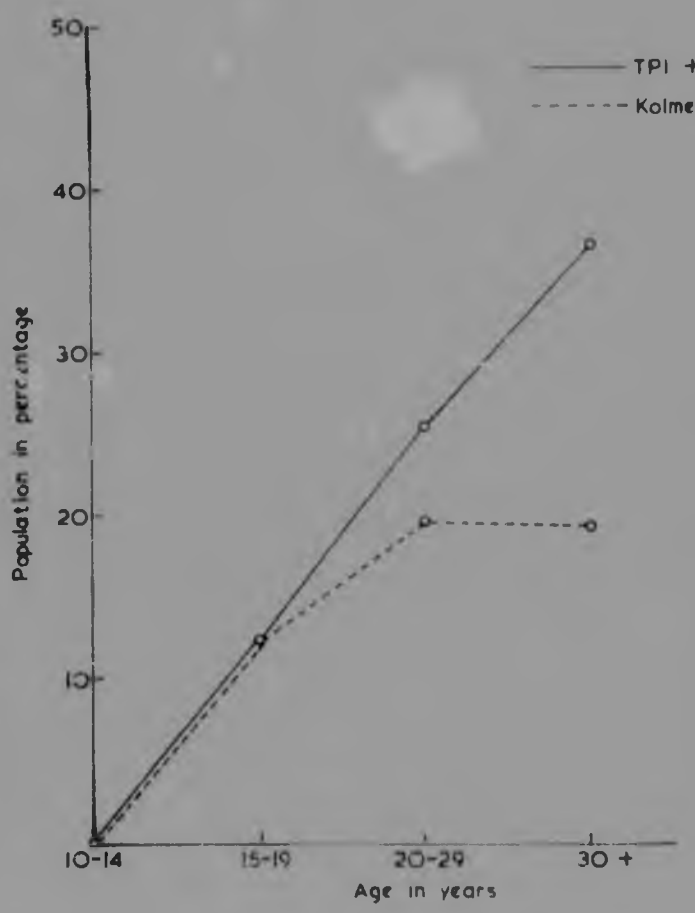
Immobilin and reagin by age and sex

Age	Total	TPI + No.	TPI + %	Kolmer + No.	% of Total	Kahn + No.	% of Total	VDRL + No.	% of Total
5-9	50	0	-	0	-	0	-	0	-
10-14	49	0	-	0	-	0	-	0	-
15-19	56	7	12,5	7	12,5	4	7,4	7	12,5
20-29	51	13	25,5	10	19,6	8	15,7	12	23,5
30+	52	18	36,5	10	19,2	4	7,7	12	23,1
Total	258	38	14,7	27	10,5	16	6,2	31	12,0
Males	116	15	12,9	13	11,2	7	6,0	14	12,1
Females	142	23	15,5	14	9,9	9	6,3	17	12,0

Sera in the age group 5 - 14 were free of immobilin and reagin. The upward trend of positive TPI sera from the age of fifteen follows the same pattern as that in the first survey (Table 46) but there were more with reagin as measured by the Kolmer test (Figure 6). This may possibly be due to an increase in individuals with early syphilis or a laxity in seeking or ensuring successful treatment.

FIGURE 6

Immobilin and reagin by age groups



The sex distribution (Table 60) was more even than in Survey 1, and the prevalence of immobilin was similar in both sexes (Table 59).

TABLE 60

Age and sex distribution of individuals in Table 59

	Total	Age groups (258 individuals)					Total
		6-9	10-14	15-19	20-29	30+	
		Percentage of group					
Males	116	7	7	11	7	13	116
Females	142	12	12	11	13	7	142
	258						

DISCUSSION

Based on the TPI test, the Kolmer, Kahn and VDRL reactions showed a high degree of specificity in both surveys, the Kolmer and VDRL being slightly higher than the Kahn (Tables 42 and 55). The Ide test, in Survey 1, following the pattern of the Botswana survey, is understandably the least specific but more sensitive than the other reagin tests. There was little difference in sensitivity between the Kolmer, Kahn and VDRL tests in Survey 1 (26,2 per cent, 27,7 per cent and 25,1 per cent). Survey 2, however, showed the Kahn test to be much less sensitive than the Kolmer and VDRL (42,1 per cent compared with 71,0 per cent and 81,6 per cent). Although a standard serum was incorporated in the control system and the technical procedure is constant, it is apparent that the Kahn reaction using antigen

in its crude form can produce results of a variable sensitivity.

A statistical analysis was carried out by combining the reagin results of the Kolmer, Kahn and VDRL tests in the three surveys of Botswana subjects and the two from Moroka. Using the Chi square test, the combination of Kolmer and VDRL tests was significantly more specific than either of the other combinations (χ^2 = 6, 84, df = 2, p < 0,05). Regarding sensitivity, the three paired combinations showed no significant differences (χ^2 = 1,21, df = 2, p > 0,5).

Based on the immobilin test, there is no reason to assume that a high proportion of positive STS among the Bantu in Moroka are biologically false positive. In Survey 1, 19/301 (6,3 per cent) of immobilin free sera showed reagin (Table 43). When the Ide test was excluded from this study, however, this dropped to 8/301 (2,7 per cent) which can be compared with 11/220 (5,0 per cent in Survey 2 (Table 56). Disregarding the possibility that some of these sera may be from cases of early syphilis - when immobilin may not be demonstrable by the TPI test - the maximum number of biological false positive reactors (Ide excluded) in the combined surveys is 19/521 (3,6 per cent) which is similar to the maximum possible prevalence of BFP reactors of 11/228 (4,8 per cent), found in the Botswana mine workers' study.

From serological evidence in the two surveys, it would appear that endemic or congenital syphilis in children is rare in Soweto. Combining the 0 - 10 age groups in both studies (217 children, Tables 46 and 59), only one serum showed weakly reactive immobilin with no reagin antibody. In the small study of eighteen mothers and cord blood from their babies, fourteen

babies showed no evidence of immobilin antibody. Although the remaining four cord bloods all showed immobilin and reagin, it is unlikely that two of the children were congenitally infected since their reagins were weak and it was not detected in their mothers. The remaining two babies could well have had an active infection. However, at that time, the technique for differentiating between antibodies of the IgG and IgM types was not available.

No evidence of congenital syphilis was seen in the 150 White children in the comparative age groups of 0 - 14, and, as with the Bantu children, one can conclude that the prevalence of congenital syphilis is very low in the White population.

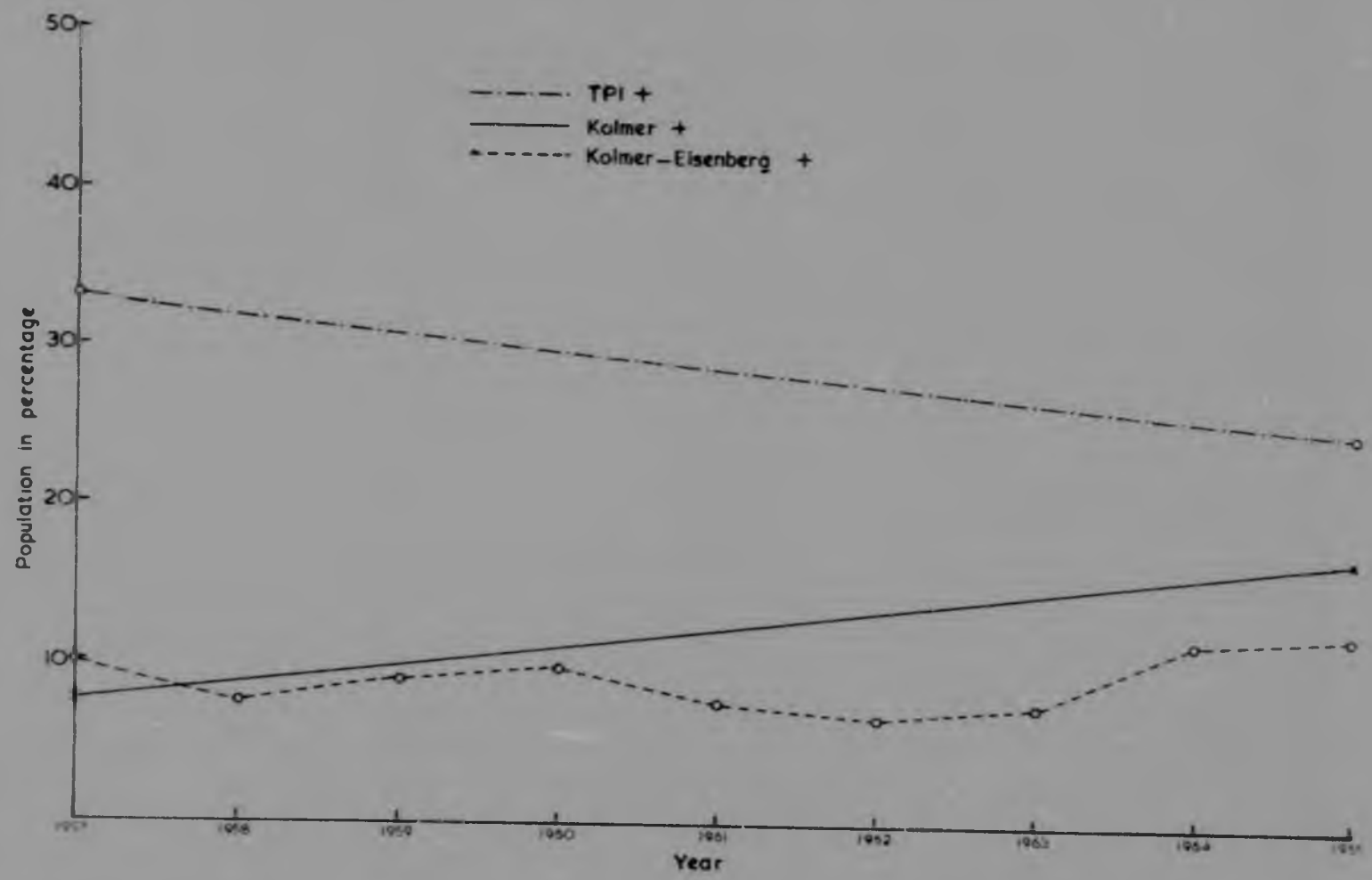
In both surveys the appearance of immobilin antibody coincides with the onset of puberty. This agrees with the study of Dogliotti (1971)[117] at Baragwanath Hospital, of 587 referred cases of syphilis from Soweto township. He reported a predominance of primary manifestations in males aged 16 to 25, and secondary eruptions in females from 16 to 20. Of the total number of cases, 70,4 per cent were in the 16 to 25 age group.

Eisenberg (1973)[118], in order to establish whether the level of syphilis among the child bearing female population of Soweto was rising or falling, analysed the results of Kolmer tests performed on the serum of all females attending eight township ante-natal clinics from 1953 to 1971. He assumed that the results of this analysis would give a general guide as to the incidence of syphilis in the population as a whole.

The results of the Eisenberg study from 1957 - 1965 and of Survey 1 (1957) and Survey 2 (1965), based on individuals over fifteen years of age are presented in Figure 7.

FIGURE 7

Incidence of immobilin and reagin in a Moroka population in 1957 and 1965 and reagin from eight Soweto clinics annually over the same period.



Prior to 1955, syphilis was treated at all clinics with the lengthy arsenic and bismuth schedule. From that date, penicillin was used for all kinds of medical conditions including syphilis. In some measure this could account for the drop in those showing reagin from 20,8 per cent in 1953 to 10 per cent in 1957[118] which is similar to the reagin results of 7,6 per cent found in Survey 1. Similarly, penicillin may also have had some influence on the marked difference seen in Survey 1 between those adults with reagin (7,6 per cent) and immobilin (33,1 per cent) since it is well known that sero-negativity of reagin is attained after treatment whereas immobilin production, apart from early syphilis, may be maintained for many years[119].

In the TPI positive group over fifteen years old a rise in demonstrable reagin, based on the Kolmer test, is shown from 7,6 per cent in 1957 to 17,0 per cent in 1965, a trend also seen by Eisenberg[118] (Figure 7). It is noteworthy that in 1957, in the over 30 age group, 48,3 per cent had a positive TPI (Table 46) whereas in 1965 this had dropped to 36,5 per cent (Table 59). In the same age groups in both surveys, although more marked in Survey 1 than in Survey 2, there is a tendency for reagin to disappear (Tables 46 and 59). Apart from treatment, the possibility of some of these persons having self-limiting benign infections with spontaneous cure should not be discounted.

Comparing the overall incidence of those with evidence of past or present treponematosis over the age of 15 years, as measured by the TPI test, there was a drop from 33,1 per cent in 1957 to 24,9 per cent in 1965 (Figure 7). This may well be partly due to the liberal use of penicillin

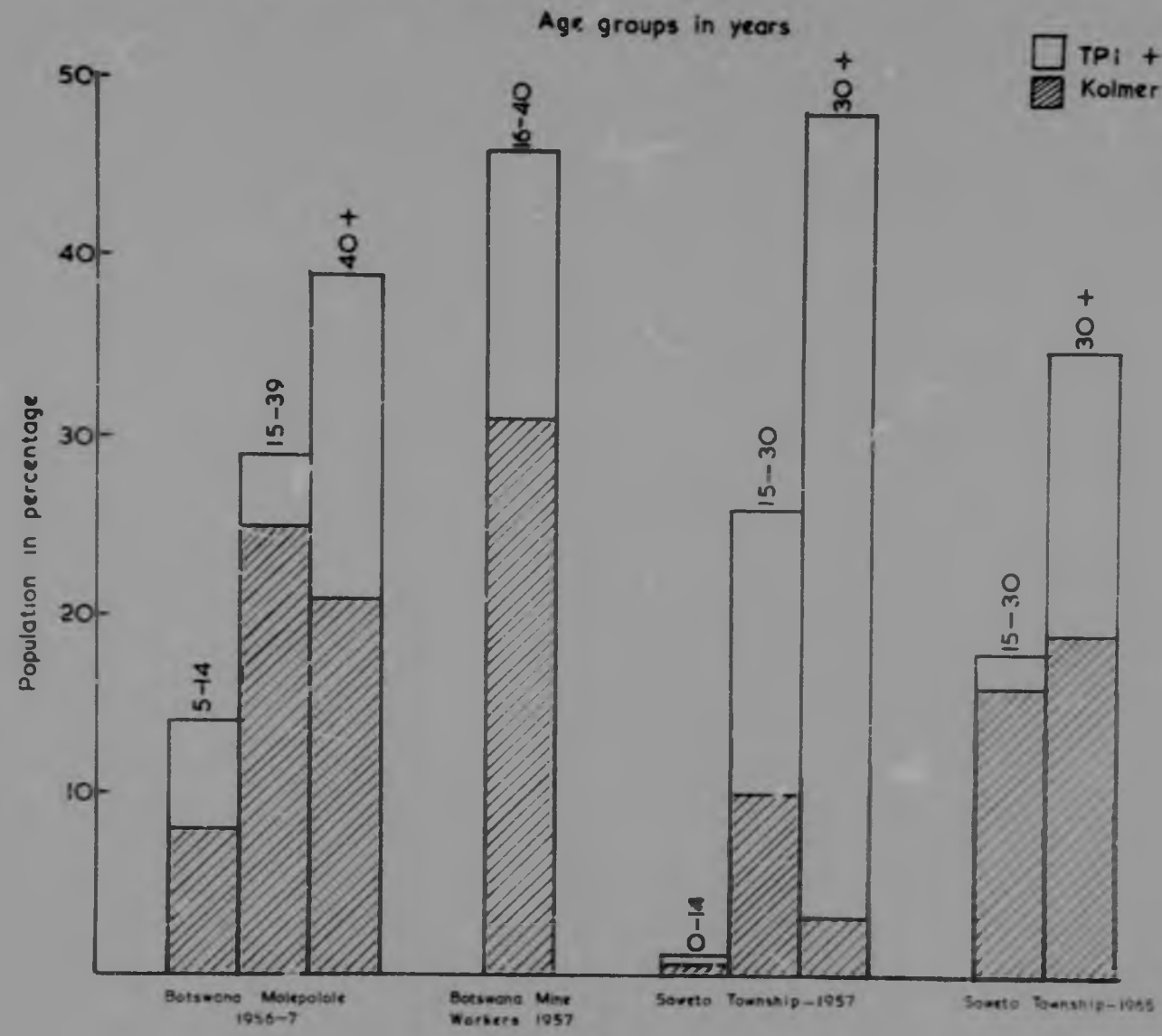
for all ailments, which was observed in other parts of the world during the decade from 1950 to 1960[120]. Furthermore, with increased clinic facilities, syphilis may be diagnosed and treated in the earlier stages with a resultant decrease in latent and tertiary syphilis.

Although an improvement in the incidence of syphilis in this community, during the eight years period, is apparent, the fact remains that syphilis in the Bantu is still wide spread.

As a summary of the incidence of treponematosis in the three Bantu groups studied in Chapters 4 and 5, the immobilin and reagin results in age groups are presented in Figure 8.

FIGURE 8

Immobilin and reagin (Kolmer test) in three Bantu population groups by age



Apart from showing the prevalence of treponematosis in the three groups this summary shows:-

1. Extra-venereal treponematosis (dichuchwa) in Botswana in the younger age group.

2. The lack of congenital syphilis in the Soweto township.

3. The high prevalence of syphilis in the Botswana mine workers, paralleled by an equally high prevalence in Soweto (1957).

4. An improvement in the incidence in Soweto over an eight year period.

5. The prevalence of possible active cases of treponematosis (excluding the small percentage of BFP or very early syphilis) indicated by the Kolmer columns.

CHAPTER 6

THE PREVALENCE OF TREPONEMATOSIS AND BIOLOGICAL FALSE POSITIVE REACTORS IN A LEPROSY POPULATION

Standard serological tests for syphilis using lipid antigens often show positive reactions with sera from non-syphilitic leprosy patients[123, 124].

With the development of the Treponema Pallidum Immobilisation test, it was possible to assess reasonably accurately the prevalence of treponematosis and to estimate the frequency of biological false positive reactors among leprosy patients.

Nelson[125] applied the TPI test to the sera of fifty-seven patients from a leprosy hospital in Louisiana. None of the patients had overt signs of syphilis, but all sera were reactive with the Kolmer complement fixation and VDRL slide tests. Sixteen of the fifty-seven sera (28,1 per cent) immobilised the spirochaetes, which was consistent with the prevalence of the syphilis in the socio-economic groups from which the patients were drawn. The remaining 71,9 per cent of the patients were of the BFP type.

From the same Louisiana hospital Edmundson, Wolcott, Olansky and Ross (1954)[126] examined sera of 204 patients with lepromatous and twenty with tuberculoid leprosy. Positivity of the STS varied from 46,9 - 63,4 per cent while the TPI test was positive in only 11,2 per cent. Although the number in the tuberculoid group were small, it appeared that in the immobilin free group of sera, sero-reactivity was greater in sera from patients with lepromatous leprosy.

In Morocco, Rollier, Pelbois and Chraibi (1956)[127] found that 90

of 197 (45,5 per cent) leprous patients gave positive reagin reactions of which 22,0 per cent were positive TPI. False positive reactors were all in the lepromatous cases with the exception of three which were probably early lepromatous. These two papers are summarized in Table 61.

TABLE 61

Immobilin and reagin in leprosy populations

Authors	No. of patients	TPI +	STS +
Edmundson[126]	224	11,2%	47-63%
Rollier[127]	197	22,0%	45%

The following study describes the serological results of sera from the inmates of a South African leprosy institution to assess the prevalence of treponematosis and biological false positives (BFP) amongst the community.

Material and Methods

The investigation was carried out on sera from 432 Bantu and thirty White leprosy patients from the Westfort Institution, Pretoria.

The patients had been classified into one of three types of leprosy according to clinical and immunological characteristics[128,129].

1. Tuberculoid (Benign)

In tuberculoid leprosy the bacilli fail to establish themselves

in tissues and are rarely seen. Skin lesions are usually annular with an elevated, sometimes erythematous margin and a hypopigmented flat centre which is often insensitive to a pin-prick. The type is generally regarded as a localized disease confined to the skin and nerve trunks and tends to be self-limiting and self-healing. Mitsuda and Fernandez reactions are usually strongly positive.

2. Borderline

This type, as the name implies, is an intermediate form of leprosy and is both near-tuberculoid and near-lepromatous. Bacilli may or may not be seen. Mitsuda and Fernandez reactions may be positive or negative.

3. Lepromatous (malignant)

In lepromatous leprosy, the bacilli multiply in the dermis and the lesions appear as infiltrations in the skin. The areas that are particularly affected are the face, ears and the extremities, though any other site or organ may be affected. Infiltrated patches of skin called plaques may also be present. Acid fast organisms are invariably found in skin incisions or nasal mucosa. Mitsuda and Fernandez reactions are negative.

Mitsuda and Fernandez reactions, which are delayed types of skin tests to lepromin have no diagnostic value, but are considered useful in the classification of patients with leprosy, which in turn, has a bearing on the prognosis. The Fernandez (early) reaction, at its maximum at 48 hours, probably signifies tissue sensitivity to the mixed protein content

of the injected material of tissue cells and fluid, together with leprosy bacilli. The Mitsuda (late) reaction with its maximum at four weeks and running roughly parallel to the Fernandez, indicates potential tissue reactivity to specific antigens derived from the leprosy bacillus[129]. The response to the lepromin test is thus a measure of the ability of the body to put up a successful defence, the degree of positivity being in direct proportion to the intensity of the tissue reaction. A positive test, therefore, is indicative of tuberculoid leprosy while a negative test is consistent with the lepromatous type, showing no resistance of the patient to the infection but may be slightly to moderately positive in phases of 'lepra reaction' which occurs only in lepromatous leprosy.

TABLE 62

Interpretation of Skin Tests in Leprosy

	Negative	Doubtful	Positive		
			1+	2+	3+
Fernandez (read after 48 hours)	0 - 4*	5 - 9	10-14	15-19	>20
Mitsuda (read on the 28th day)	0 - 2	3 - 4	5-7	8-9	>10

* Diameter of skin infiltration in mm.

Although degrees of positivity of the tests are reported as shown in Table 62, the results in this survey are regarded as positive if they show 1+ or more.

Erythema Nodosum Leprosum (ENL) or antibody-mediated tissue damage[130], is a common complication of lepromatous leprosy. It occurs as

an acute, sub-acute or chronic skin eruption with or without fever, and is characterised by showers of red nodules, tender to the touch, 0,5 to 2 cm in diameter. These appear on the surfaces of the limbs, the face and less often on the trunk. The number of lesions appearing in an attack varies from few to several hundreds when they sometimes coalesce to form plaques[131]. ENL was reported when seen.

Results

The distribution of individuals in the three types of leprosy are shown in Table 63.

TABLE 63

Classification of leprosy in the studied material

Race	Classification	Number
Bantu	Tuberculoid	184
	Borderline	37
	Lepromatous	211
White	Tuberculoid	3
	Borderline	1
	Lepromatous	26
Total		462

In the White group, constituting 6,3 per cent of the material 25 of 30 sera showed no reagin or immobilin, two were positive TPI with high reagin titres in all tests and three, with lepromatous leprosy were negative

TPI with discrepant and low reagin titres. With this distribution, it was thought justifiable to analyse the Whites together with the Bantu.

The results of the serological findings are considered separately for each type of leprosy. Any activity in the STS is considered positive and, as previously, the immobilin activity is reported as Specific Immobilisation 0 - 19 per cent non-reactive, 20 - 49 per cent weakly reactive and over 50 per cent reactive.

1. Tuberculoid Leprosy

TABLE 64

Results of TPI test in 187 individuals with tuberculoid leprosy

TPI Specific Immobilisation	Number	%	Average age in years
0-19%	118	63,1	± 47
20-49%	4	2,1	± 30
50-100%	65	34,8	± 43
Total	187		

In this group, sixty-nine (36,9 per cent) have been infected with pathogenic treponema some time in their lives but from the TPI test it is impossible to tell whether the infection is active or cured.

Table 65 shows the break-down of the TPI reactive group with particular reference to the reagin tests.

TABLE 65

Results of STS in 65 immobilin reactive sera by age, sex and lepromin reaction, tuberculoid leprosy

		Total	Male	Female	Age <20	Age 20-40	Age 40-60	Age >60	Mitsuda +	Mitsuda ±	Mitsuda -	Fernandez +	Fernandez ±	Fernandez -	ENL
TPI Reactive		65	33	32	1	22	32	9	13	15	10	10	31	18	0
Kolmer +	No.	46	26	20	1	17	20	7	10	14	6	7	24	13	
	%	70,8	78,7	62,6		77,4	62,6	77,8	77,0	93,3	60,0	77,0	77,4	72,1	
Kahn +	No.	49	26	23	1	19	20	8	9	14	6	6	27	13	
	%	75,3	78,7	72,0		86,4	62,6	89,0	69,2	93,3	60,0	60,0	87,0	72,1	
VDRL +	No.	48	26	22	1	17	21	8	10	15	6	7	25	14	
	%	73,8	78,8	68,6		77,4	65,5	89,0	77,0	100	60,0	70,0	80,0	77,8	
Ide +	No.	49	27	22	1	18	20	8	10	14	6	7	26	14	
	%	75,3	81,7	65,5		81,8	62,6	89,0	77 0	93,3	60,0	70,0	83,3	77,8	

As one would expect from the two previous studies in Botswana and Moroka, the Kahn and Ide test were the most sensitive (75,3 per cent) followed closely by the VDRL (73,8 per cent) and Kolmer (70,8 per cent). When an even sex distribution which also occurred in the age groups, the STS were less sensitive in the females as well as in the 40 - 60 year old group. Although one would expect those over sixty years to show even less reagin, this was not the case. Of nine sera, all but one had reagin, the titres ranging from high (two sera) to medium (two sera) and four with low. There was complete agreement between the immobilin reactive sera and all the reagin tests in 45/65 (69,2 per cent); in 13/65 (20,0 per cent) there was no demonstrable reagin.

On repeat testing of sera from seven individuals over a period of 6 - 12 months, the TPI results remained constantly reactive. Two with positive STS remained positive and two remained negative. The remaining three fluctuated between negative and some tests positive, but in no instance was the titre of any one test higher than four units.

As would be anticipated in this type of leprosy, nearly three quarters of the patients tested in this group showed positive and doubtful reactions (Fernandez 69,5 per cent, Mitsuda 74,0 per cent). Less reagin was detected in the sera from those with positive and negative reactions than those in the doubtful category.

Of the four weakly reactive immobilin sera, all exhibited negative reagin tests. The STS remained negative on repeat tests from one to six months. Three sera remained weakly reactive TPI on repeat testing and one became negative. One person had a doubtful Mitsuda and another a doubtful Fernandez reaction.

TABLE 66

Results of STS in 118 immobilin free sera by age, sex and lepromin reaction, tuberculoid leprosy

		Total	Male	Female	Age				Mitsuda			Frenandez			
					<20	20-40	40-60	>60	+	±	-	+	±	-	ENL
TPI Non-reactive		118	73	45	13	58	31	12	24	29	15	21	45	43	0
Kolmer +	No.	1	1	-	-	1	-	-	-	1	-	-	-	1	
	%	0,8	1,4	0,0	0,0	1,7	0,0	0,0	0,0	3,5	0,0	0,0	0,0	2,3	
Kahn +	No.	21	15	6	1	14	4	1	2	2	-	6	6	6	
	%	17,8	20,5	13,3	7,7	24,2	12,9	8,3	8,3	6,9	0,0	28,6	13,3	14,0	
VDRL +	No.	5	3	2	-	2	2	-	-	2	-	3	-	1	
	%	4,2	4,1	4,5	0,0	3,5	6,5	0,0	0,0	6,9	0,0	14,3	0,0	2,3	
Ide +	No.	5	3	2	1	3	1	-	-	1	-	2	-	2	
	%	4,2	4,1	4,5	7,7	5,8	3,2	0,0	0,0	3,5	0,0	9,5	0,0	4,7	

It emerges from Table 66 that in this group of immobilin free sera the Kolmer was the most specific reagin test (99,2 per cent), followed by the VDRL and Ide (95,8 per cent). The Kahn reaction showed a poor specificity of 82,2 per cent. Based on the TPI test, 22/118 (18,6 per cent) sera showed BFP reactions of which seventeen were positive in the Kahn reaction only, with titres not greater than four units. This showed an appreciable sensitivity over the Ide screen test. Most of the Kahn positive sera came from the 30 - 40 year old group.

Sera with no reagin or immobilin totalled 96/118 (81,4 per cent). One TPI negative serum was positive in all reagin tests with low titres, but four months later these reactions were negative. Four repeat TPI tests remained negative over the same period.

In keeping with the lepromin results of the TPI positive group (Table 65), 53/68 (78,0 per cent) of immobilin negative patients showed a positive and doubtful Mitsuda reaction and 66/109 (60,5 per cent) Fernandez. A higher percentage of positive Kahn reactions were evident in this group compared with the other reagin tests.

2. Borderline Leprosy

TABLE 67

Results of TPI test in 38 individuals with Borderline Leprosy

TPI Specific Immobilisation	Number	Per cent	Average age in years
0-19%	27	71,0	± 31
20-49%	2	5,3	± 40
50-100%	9	23,7	± 36
Total	38		

Of this group, 11/38 (28,9 per cent) have or have had treponematosis. Details of the reactive group are shown in Table 68. (see page 146).

All the STS were positive in five cases and all negative in one. Repeat specimens were examined on five individuals from four to twelve months after the first test. The TPI was repeated in only one instance and remained reactive; the STS on the specimen which showed low titres on first testing became negative one year later. Three positive STS stayed positive on repeat and on one serum remained negative. As would be expected the majority of reactive immobilin sera came from the 20 - 40 year old group. Lepromin showed a lepromatous trend in these patients.

Of two sera with weakly reactive immobilin, one was STS positive and the other STS negative. On examination of further sera one month later the STS negative serum lost its immobilin, while the other serum remained TPI and STS positive.

TABLE 68

Results of STS in 9 immobilin reactive sera by age, sex and lepromin reaction, Borderline Leprosy

	Total	Male	Female	Age				Mitsuda			Fernandez			ENL
				<20	20-40	40-60	>60	+	±	-	+	±	-	
TPI Reactive	9	8	1	1	5	2	1	1	2	4	1	3	5	0
Kolmer +	7	6	1	1	4	1	1	1	2	3	1	2	-	
Kahn +	6	6	-	1	4	-	1	1	2	2	1	2	3	
VDRL +	8	7	1	1	5	1	1	1	2	3	1	3	4	
Ido +	7	7	-	1	4	1	1	1	2	2	1	3	3	

TABLE 69

Results of STS in 27 immobilin free sera by age, sex and lepromin reaction, Borderline Leprosy

		Total	Male	Female	Age <20	Age 20-40	Age 40-60	Age >60	Mitsuda +	Mitsuda ±	Mitsuda -	Fernandez +	Fernandez ±	Fernandez -	ENL
TPI Non-Reactive		27	21	6	7	10	7	2	2	8	7	-	7	19	0
Kolmer	No.	2	1	1	-	2	-	-	-	1	1	-	-	2	
	%	7,4	4,3	16,6		20,0				12,5	14,3			10,5	
Kahn +	No.	1	1	-	-	1	-	-	-	-	1	-	-	1	
	%	3,7	4,3			10,0					14,3			5,3	
VDRL +		-	-	-	-	-	-	-	-	-	-	-	-	-	
Ide +	No.	2	1	1	1	1	-	-	-	-	1	-	-	2	
	%	7,4	4,3	16,6	14,3	10,0					14,3			10,5	

Table 69 (Page 147) shows details of the immobilin free sera. In this small group only two sera showed weak reagin titres and constitute the possible BFP.

Combining the lepromin reactions in Tables 68 and 69, 35,5 per cent of the Fernandez and 50,4 per cent of Mitsuda showed positive or doubtful reactions which is consistent with the borderline classification of leprosy. In this group as a whole, including the two with weakly reactive immobilin, there was a preponderance of males (30/38).

3. Lepromatous Leprosy

TABLE 70

Results of TPI test in 237 individuals with lepromatous leprosy

TPI Specific Immobilisation	Number	Per cent	Average age in years
0 - 19%	145	61,2	34,9
20 - 49%	10	4,2	36,6
50 - 100%	82	34,6	41,3
Total	237		

The number in this group showing evidence of treponematosis, past or present, totalled 92 (38,8 per cent) which approximates the incidence in the tuberculoid series.

In Table 71 the TPI reactive group is analysed.

TABLE 71

Results of STS in 82 immobilin reactive sera by age, sex and lepromin reaction, lepromatous leprosy

		Total	Male	Female	Age <20	Age 20-39	Age 40-60	Age >60	Mitsuda +	Mitsuda ±	Mitsuda -	Fernandez +	Fernandez ±	Fernandez -	ENL -	ENL +	Very strongly +ve sometime or other +++
TPI Reactive		82	40	42	5	33	35	9	-	27	34	3	16	49	41	41	14
Folmer +	No.	67	32	35	4	29	27	7	-	20	28	2	15	46	32	35	12
	%	81,6	80,0	83,4	80,0	88,0	77,2	77,8		74,0	82,3	66,6	93,6	94,0	78,0	85,4	85,6
Kahn +	No.	62	28	34	3	26	26	6	-	19	29	2	15	43	28	34	9
	%	75,5	70,0	81,0	60,0	78,8	74,2	66,7		70,4	85,2	66,6	93,6	87,6	68,4	83,0	64,4
VDRL +	No.	67	29	38	3	30	26	8	-	19	31	2	15	46	31	36	10
	%	81,6	72,5	90,5	60,0	91,0	74,2	89,0		70,4	91,0	66,6	93,6	94,0	75,5	87,8	71,5
Ide +	No.	64	29	35	3	29	25	7	-	20	29	2	15	43	31	33	9
	%	78,0	72,5	83,4	60,0	88,0	71,5	77,8		74,0	85,2	66,6	93,6	87,6	75,5	80,6	64,4

Contrary to the findings in the sera from the tuberculoid group, the Kolmer and VDRL tests showed a higher degree of positivity (both 81,6 per cent) than the Ide (78,0 per cent) and Kahn 75,5 per cent. Males and females were evenly distributed and in contrast with the tuberculoid group, there were more females with reagin than males. Understandably more reagin tests were positive in the 20 - 40 age group than in the others. The nine sera in the over sixty age group, with one exception, all showed reagin, five with high titres, two medium and one weak. These findings are similar to an equivalent number in the tuberculoid group.

Reactive TPI sera with all STS positive numbered 58 (70,7 per cent) and in 12/82 (14,6 per cent) all STS were negative.

Over a period of 2 - 16 months repeat specimens of sera were examined from forty six subjects on two to five occasions. The TPI test invariably remained positive. Sera from twenty-nine patients showed medium to high titres in all reagin tests and four stayed consistently negative. On first testing 13 (28,3 per cent) sera, with low titred or negative but discrepant STS, fluctuated between negative and weakly positive over a period of 8 - 12 months. These sera may possibly constitute the BFP sera due to leprosy within the group of those with evidence of treponematosis, further evidenced by Table 74 which shows that BFP may be associated with high (Kolmer 160) STS titres.

In the lepromatous type of leprosy, all patients were Mitsuda negative and only a few Fernandez positive. In the Mitsuda group, those with doubtful reactions showed slightly less reagin than those with none, but there was little difference in the Fernandez.

Erythema Nodosum Leprosum was noted in 50,0 per cent of the cases but the absence, presence or potency of the condition did not appear to influence the reagin content of the sera.

TABLE 72

Results of STS in 10 weakly reactive immobilin sera by age, sex and lepromin reaction, lepromatous leprosy

	Total	Male	Female	Age				Mitsuda			Fernandez			ENL		
				<20	20-40	40-60	>60	+	±	-	+	±	-	-	+	+++
TPI Weakly reactive	10	3	7	2	3	4	1		2	6		1	8		10	3
Kolmer +	4	3	1	1	-	3	-		1	3		-	4		4	1
Kahn +	4	2	2	1	1	2	-		-	3		-	4		4	1
VDRL +	4	1	3	1	1	1	1		1	3		1	3		4	-
Ide +	5	1	4	1	2	1	1		1	3		1	4		5	1

On first testing (Table 72), all STS were negative in three, positive in two and discrepant in five sera. Two to eight repeat specimens were received over the next 2 - 18 months. Five weakly reactive immobilin sera became non-reactive of which one became reagin negative, two remained negative and two had fluctuating reagin titres. Two sera remained weakly reactive with fluctuating reagin and three ranged between weakly reactive and reactive immobilin, again with fluctuating reagin in two cases; the third remaining consistently positive in all STS. It seems likely that most, if not all these with inconsistent reagin results over the months are due to leprosy and fall into the BFP category.

All the patients showed evidence of Erythema Nodosum Leprosum and the majority were lepromin negative.

TABLE 73

Results of STS in 145 non-reactive immobilin sera by age, sex and lepromin reaction, lepromatous leprosy

		Total	Male	Female	Age				Mitsuda			Fernandez			ENL		Strongly +ve sometime or other
					<20	20-40	40-60	>60	+	±	-	+	±	-	-	+	+++
TPI -		145	62	83	20	76	37	11	1	37	62	2	18	104	64	81	35
Kolmer +	No.	33	15	18	8	19	4	2	-	12	12	-	4	25	12	21	11
	%	22,8	24,2	21,6	40,0	25,0	10,8	18,2		32,4	19,3		22,2	24,0	18,7	25,9	31,4
Kahn +	No.	47	21	26	11	25	6	4	-	13	19	-	6	37	22	25	12
	%	32,4	33,8	31,4	55,0	32,9	16,2	36,2		35,0	30,6		33,3	35,6	34,4	30,8	34,3
VDRL +	No.	29	13	16	8	15	4	2	-	10	10	-	3	21	13	17	7
	%	20,0	21,0	19,3	40,0	19,7	10,8	18,2		27,0	16,1		16,6	20,4	20,3	21,0	20,0
Ide +	No.	39	5	24	9	21	6	2	-	12	16	1	5	29	13	26	11
	%	26,8	24,2	28,9	45,0	27,6	16,2	18,2		32,4	25,8		27,8	27,9	20,3	32,1	31,4

There was a predominance of females in this group (Table 73), detailed analysis showing that it was more pronounced in the age groups up to forty than in the older subjects. Again, with the exception of the Kahn reaction in the over sixty years group, reagin was more apparent in the younger patients.

All STS were negative in 87/145 sera (60,0 per cent) and all positive in 21/145 (14,5 per cent). Based on the TPI test it is thus apparent that 40,0 per cent of the cases show some reagin activity (BFP).

In this group, in which false reactors are expected, the VDRL test showed the highest specificity with 80,0 per cent followed by Kolmer, 77,2 per cent, Ide 73,2 per cent and the Kahn reaction the least with 67,6 per cent. These results, together with the findings of the tuberculoid, immobilin non-reactive, group (Table 66), again show the superiority of the Kolmer and VDRL tests.

Repeat tests were carried out one to eight times on forty-two patients over a period of 1 - 18 months. All non-reactive immobilin sera showed complete reproducibility. Nine sera remained reagin negative, two positive and the balance of thirty-one gave discrepant STS results, some with a striking variation in titres from specimen to specimen. A few sera showed fairly constant and moderately strong reagin activity in most specimens while others had a tendency to become negative. Yet a third group fluctuated between a high reagin titre and negative without any characteristic trend. Details of some of these sera will be presented in Table 74.

In keeping with the immobilin-reactive lepromatous group very few

patients showed positive lepromin reactions. There were also less with doubtful reactions. In the Mitsuda doubtful group there was a slight tendency towards increased reagin compared with those with no reaction. This was not evident, however, in the Fernandez doubtful group.

ENL was observed in 55,8 per cent of patients in the TPI negative group compared with 50,0 per cent in the TPI positive group. There was no consistent difference in reagin as measured by the four STS.

In Table 74, details of reagin results are presented on some of the thirty-one immobilin free sera showing discrepant STS on which repeat tests were carried out over varying periods of time.

TABLE 74

Examples of STS results showing discrepant reagin results on repeat testing of immobilin free sera from lepromatous leprosy cases over a period of 17-20 months

	Patient 12398							Patient 12383					
Date	12/3 56	28/6 56	6/12 56	29/3 57	13/5 57	28/6 57	22/11 57	19/6 56	7/11 56	29/3 57	20/4 57	12/9 57	6/11 57
Kolmer	160	-	-	160	160	80	40	160	40	-	160	-	40
Kahn	80	-	-	20	20	-	4	20	20	-	20	-	20
VDRL	32	-	-	32	4	-	8	16	-	-	-	-	16
Ide	+	-	-	+	+	+	+	-	+	-	+	-	+

	Patient 12807							Patient 8260							
Date	1/5 56	28/6 56	6/12 56	27/3 57	13/5 57	13/7 57	22/11 57	5/7 56	6/9 56	6/12 56	29/3 57	13/5 57	28/6 57	12/9 57	22/11 57
Kolmer	80	-	40	20	160	-	40	80	80	40	160	80	160	-	80
Kahn	120	-	4	-	20	ND	4	40	20	-	40	40	20	-	40
VDRL	64	2	4	2	-	-	8	-	-	-	32	16	16	-	32
Ide	+	-	+	+	+	-	+	+	-	-	+	+	+	+	+

	Patient 11773								Patient 12591							
Date	24/7 56	26/9 56	6/12 56	29/3 57	13/5 57	28/6 57	12/9 57	22/11 57	1/5 56	28/9 56	7/11 56	29/3 57	13/5 57	28/6 57	12/9 57	22/11 57
Kolmer	20	-	-	-	40	-	-	-	-	-	-	-	160	-	-	40
Kahn	4	2	-	-	-	-	-	4	20	-	4	-	4	-	-	4
VDRL	2	-	-	-	4	-	-	4	8	4	2	-	16	-	-	8
Ide	+	+	-	-	+	-	-	+	+	-	+	-	+	-	-	+

These six sera have been selected as representative examples of gross variations in the reagin tests. It is interesting that some sera on some occasions showed medium to high Kolmer titres with negative or low titred flocculation tests (12698, 12883, 12807, 8260), whereas in other cases it was reversed (e.g. 12591). Speculation arises as to whether different types of reagin are produced in leprosy.

TABLE 75

Examples of STS results showing consistent positive and negative reagin reactions on repeat testing of immobilin free sera from lepromatous leprosy cases over a period of 9-13 months

	Patient 665				Patient 13090		
Date	3/7 56	5/9 56	13/12 56	29/3 57	23/1 57	29/3 57	6/11 57
Kolmer	20	10	20	80	80	160	80
Kahn	20	+	4	20	80	20	20
VDRL	4	4	4	2	16	32	8
Ide	+	+	+	+	+	+	+

	Patient 12754			Patient 12891		
Date	12/3 56	12/9 57	24/9 57	19/6 56	26/8 57	12/9 57
Kolmer	-	-	-	-	-	-
Kahn	-	-	-	-	-	-
VDRL	-	-	-	-	-	-
Ide						

These four examples are taken from patients who had, in the first

two cases, positive reagin tests which did not fluctuate unduly and, in the last two, did not appear to produce any reagin at all. Analysis of all these BFP sera did not show any pattern with those with doubtful lepromin reactions.

Discussion

The only form of treponematosis observed clinically in this leper institution was syphilis. However, it is possible that some of the TPI positive patients admitted from rural areas, may previously have had endemic syphilis. As no early cases of syphilis had been recorded among the patients during the last ten years, it would be reasonable to assess the true prevalence of syphilis on the results of the TPI test. Thus for all practical purposes a reactive TPI test would be proof of syphilitic infection, past or present. This interpretation would consequently give the minimum prevalence of syphilis.

Table 76 shows the TPI test results of all sera in the study, extracted from Tables 64, 67 and 70. Weakly reactive immobilin results are taken as evidence of treponematosis and are included with the reactive results.

TABLE 76

Prevalence of syphilis - active or cured - in 462 leprosy patients based on serological investigation with the TPI test

Type of leprosy	Total	Reactive and Weakly reactive	%	Non-reactive	%
Tuberculoid	187	69	36,9	118	63,1
Borderline	38	11	29,0	27	71,0
Lepromatous	237	92	38,8	145	61,2
Total	462	172	37,2	290	62,8

The overall prevalence of syphilis was 37,2 per cent with little variation in the different types of leprosy. This is similar to the prevalence of treponematosis found in the Botswana and Soweto studies. Reproducibility of the TPI test was found to be of a high order. On many repeat tests carried out over 1 - 18 months, reactive sera remained reactive and non-reactive stayed non-reactive. On occasions weakly reactive sera fluctuated from test to test when the same serum was repeated but on average a weakly reactive result was observed in each case. This was confirmed by parallel examination of sera in the International reference laboratory of Copenhagen. Some weakly reactive sera understandably lost their immobilin over the months between testing. The reproducibility of the TPI test in this survey is contrary to the findings of Ruge (1968)[132]. In a similar study on sera from leprosy patients he did not consider the TPI test totally reliable, particularly in non-reactive sera, and recommended that specimens from all cases be repeated twice on different occasions

before a verdict of 'treponematosis or not' is passed.

Sera from all three types of leprosy proved the Kolmer and VDRL tests to be more specific than the Kahn and Ide, corroborating the findings in the Botswana and Moroka survey. In the tuberculoid group the Kahn and Ide tests were more sensitive than the Kolmer and VDRL but this was not the case in the lepromatous, although there were several occasions when the Kahn was the only test showing any evidence of reagin.

In keeping with previous studies[126-127], biological false positive reactors occurred almost exclusively in lepromatous leprosy patients. In the tuberculoid group, because of the high sensitivity of the Kahn test (Table 66) the number of TPI non-reactive sera showing reagin was 22/118 (18,6 per cent). Had the Kolmer and VDRL been the only tests used, these would have been reduced to 5/118 (4,2 per cent) which is similar to the BFP findings in the Botswana and Moroka surveys. In the immobilin free lepromatous group, (Table 73) however, with the Kolmer and VDRL tests as the only reagin tests, 39/145 (26,9 per cent) were positive. When the result of the Kahn reaction was included, the number of BFP reactors rose from 39 to 55/145 (43,8 per cent). There were only three sera which showed flocculation in the Ide test only.

TABLE 77

Calculation of Biological False Positives in a leprosy population

TPI Reaction	Clinical Classification	Number	Kolmer +		Theoretical BFP
			Number	Per cent	
+	Lepromatous	92*	71	77,2	22,8
	Borderline	11**	8	72,7	7,4
	Tuberculoid	69***	46	66,7	0,8
-	Lepromatous	145	33	22,8	
	Borderline	27	2	7,4	
	Tuberculoid	118	1	0,8	

* Tables 71 and 72

** Table 68 and two weakly reactive TPI

*** Table 65 and four weakly reactive TPI.

Table 77 clearly demonstrates that the large majority of BFP occurs in the lepromatous cases and is probably related to the tissue destruction in this disease. As tissue destruction occurs in both the lepromatous and tuberculoid forms of leprosy, it is probably that not only the degree but the type of tissue destruction, induced by the numerous leprosy bacilli in lepromatous leprosy, is important. Supporting the concept that BFP in lepromatous leprosy may be due to antigens released with tissue destruction is the finding of other autoantibodies e.g. cryoglobulins,antinuclear factors, rheumatoid factors, antithyroglobulin antibodies and cold agglutinins which have been described in lepromatous leprosy[133].

It is reasonable to assume that some of the 77,2 per cent reagin positive patients in the syphilitic (TPI+) group (Table 77) were due to

non-syphilitic reagin production as a result of lepromatous leprosy per se. If one assumes that the incidence of non-syphilitic reagin positive reactors in the TPI positive and TPI negative (22,8 per cent positive reagin reactors) lepromatous groups is similar, then the reagin reactors due to syphilis only would be about 54 per cent. In contrast, 66,7 per cent of tuberculoid patients were TPI positive.

This finding of an apparently lower prevalence of reagin antibodies, based on the Kolmer test in lepromatous leprosy patients with syphilis, is surprising. One could reasonably expect to have a similar prevalence of active syphilis in the tuberculoid and lepromatous groups. The apparently lower (54 per cent) number of those with possible active syphilis in the lepromatous group may be due to a lower percentage of antibody forming B lymphocytes recently reported in lepromatous leprosy patients. The B lymphocytes may be functionally affected by the high antigen load originating from the numerous leprosy bacilli found in macrophages of patients with lepromatous leprosy[134,135]. Antigenic competition is known to affect cell mediated and humoral immune responses and the constant antigenic stimulation of *M. leprae* organisms in lepromatous leprosy could therefore affect the antigen response to other organisms[136], in this case *T. pallidum*.

The abnormal immune system of lepromatous leprosy is also a likely factor responsible for the increased number of BFP in this group. Increased tissue destruction has already been proposed as an explanation. Non-specific stimulation of antibody production by macrophages, loaded with *M. leprae* bacilli, may be an alternate explanation[137].

In examining sera from 821 cases of leprosy (types not stated) in India, Kvittingen, Cutler, Amador Guevara, McCullough, Rose and Ford (1952)[138] observed 11,8 per cent positive Meinicke slide test reactions against 25,0 per cent VDRL and 65,5 per cent Kahn. However, with no TPI test, the true prevalence of syphilis and BFP could not be determined. Kvittingen (1952)[139] attributed the infrequency of BFP results with the Meinicke test to the absence of cholesterol in the antigen. Similar results were reported by Ruge (1955)[140] from a leprosy survey in Egypt. Based on past history and any clinical evidence of syphilis, he found less BFP with the Wassermann test (cardiolipin antigen) than with the VDRL. The Meinicke test was practically free of BFP in all types of leprosy. These and other authors listed factors that influence the reagin tests:

(1) the concentration of cholesterol[139] and lecithin[141] in the antigen;

(2) the crudeness of the antigen used in the Kahn test, since it was assumed that it might have a wider range of reactivity than purified cardiolipin[140];

(3) the high concentration of cholesterol[140] and lipoprotein[142] in the serum of patients.

The suggestion that cross reacting cardiolipin antigen which may be present in leprosy bacilli may account for the BFP results[133] is not in accordance with the findings of Schmidt (1961)[141] using cardchol (lecithin-free cardiolipin) antigen. He concluded that there were different reagins produced in syphilis and leprosy and thought that the kind of anti-lipoidal antibodies detected by cardchol may be more typical for leprosy

than for syphilis. Furthermore, he felt that the reagin antibodies from leprosy may fluctuate more than those produced in syphilis.

Supporting this theory, Holst (1964)[143] and Holst and Weiss Bentzom (1965)[144] using the same antigens as Schmidt[141] examined the behaviour of positive sera from non-syphilitic lepromatous patients and syphilitic sera towards prolonged heating (30 - 120 minutes) at 56C. They showed that cardchol antigen in lepromatous sera reacted with a relatively heat stable antibody while cardiolipin did not. Sera from syphilitic patients, on the other hand, showed a reverse trend.

The results of this study showed many fluctuating titres as described by the quoted authors. Examples of these in TPI non-reactive sera over a period of 9 - 20 months are shown in Table 74. They are all from lepromatous cases of leprosy and show not only marked variation in all the tests from one test day to another (12698, c.f. results 12/3/56 and 28/6/56) but also differences in the tests themselves (12698 c.f. results of 13/5/57 and 28/6/57). In a follow-up survey of patients at a leprosy institution in India, Kvittingen[138] obtained an increase in positive reactions with the VDRL, Kahn and to a lesser extent with the Meinicke test. Quoting observations of physicians in that and another institution that patients usually give positive Kahn reactions during a 'lepra reaction', he suggested that all serological reactions which give rise to false positive results in leprosy may be affected by such aggravations of the disease. Although no notes were made of the clinical state of each patient when repeat tests were taken in this study this supposition may well account for the fluctuations in the repeat tests. That 33/145

(22,8 per cent) negative TPI lepromatous sera showed a positive Kolmer reaction compared with 1/118 (0,8 per cent) in the tuberculoid group (Table 77) adds further support to Kvittingen's theory.

Both Fernandez and Mitsuda lepromin reactions carried out in this survey are in agreement with the clinical diagnosis of the two types of leprosy. Consideration of the frank positives (>10 mm for Fernandez and >5 mm for Mitsuda) reveals that in the tuberculoid group 18,3 per cent were Fernandez positive and 34,9 per cent Mitsuda, whereas the lepromatous patients gave 2,6 per cent Fernandez and 0.6 per cent positive Mitsuda reaction. A comparison of the reagin results in the TPI reactive tuberculoid group shows a slight increase in those with positive Mitsuda reactions over the negative but appreciably higher in the doubtful (Table 65). If one assumes that the doubtful Mitsuda results in fact denote a slightly increased cell mediated immune activity as opposed to the little or none in Mitsuda negative patients, then it is possible that increased T cell co-operation with B cell lymphocytes in the Mitsuda doubtful cases would stimulate increased reagin production. The lower prevalence of reagin positive results in the Mitsuda positive patients compared with the doubtful group is not unexpected. It is plausible that in the Mitsuda positive group, the production of reagin which does not originate from syphilis is negligible, whilst the Mitsuda doubtful group will behave, from an immunological point of view, more like lepromatous leprosy. This may give rise to some non-specific reagin production being related to leprosy rather than syphilis. This explanation is supported by the findings in Table 66 giving the results of TPI negative tuberculoid patients, where reagin antibodies (except for the Kahn test), although uncommon, were all found in the doubtful Mitsuda group.

In this same group of lepromin positive individuals (Table 66) the Kahn test was positive more often than other STS. Schmidt[141] found that 13/30 (60 per cent) lepromatous, non-syphilitic, lepromin negative individuals reacted positively with his sensitive cardchol test as against 2/11 (18 per cent) lepromatous, non-syphilitic, lepromin positive patients. The cardiolipin test was less sensitive. It is clear that more clearly defined investigations are needed to decide whether there is any significant correlation between the occurrence of circulating reagin antibodies and cell mediated immunity as demonstrated by intracutaneous test reactions.

Apart from treatment of leprosy which, during the course of this survey was DDS (diaminodiphenylsulphone), penicillin (PAM) was generally given as antisyphilitic treatment when STS reactions were positive. Prior to the introduction of the TPI test, STS negative syphilitic patients did not receive penicillin treatment. Whether to treat or not should be decided by assessing each individual case. Some of these cases might well be successfully treated individuals or burnt out benign cases, effecting a spontaneous cure[145], but exhibiting persistent TPI reactions. It is interesting that in the tuberculoid group showing immobilin, 13/65 (20,0 per cent) were without any reagin whereas in the equivalent lepromatous group there were only 12/82 (14,6 per cent). The possibility that in lepromatous leprosy patients, reagin antibody production in response to active syphilis may be depressed, was previously discussed. It would therefore seem advisable to give antisyphilitic treatment to all lepromatous patients with TPI positive results and not, as is generally accepted, on reagin positive results.

In agreement with the findings of Ruge[140], there were more female than male leprosy cases with evidence of syphilis (39,0 per cent and 36,8 per cent).

In Table 78, evidence of syphilis and type of leprosy in patients is analysed in the four age groups.

TABLE 78

Type of leprosy and evidence of syphilis by age

Type of leprosy	TPI Result	Age <20	20-40	40-60	>60	Total
Tuberculoid	Reactive	2 (3,1)	22 (33,3)	33 (50,0)	9 (13,6)	66
	Non-reactive	13	58	31	12	114
	Total	15 (8,3)	80 (44,4)	64 (35,6)	21 (11,7)	180
Borderline	Reactive	1 (11,1)	5 (55,6)	2 (22,2)	1 (11,7)	9
	Non-reactive	7	10	7	2	26
	Total	8 (22,8)	15 (42,9)	9 (25,7)	3 (8,6)	35
Lepromatous	Reactive	7 (7,6)	36 (39,2)	39 (42,4)	10 (10,8)	92
	Non-reactive	20	76	37	11	144
	Total	27 (11,4)	112 (47,5)	76 (32,2)	21 (8,9)	236

* Figures in parenthesis represent percentages of the respective totals.

Analysis of this Table reveals that with the exception of the small borderline group, those with the highest prevalence of syphilis came from the 40 - 60 year old group. This is in agreement with Survey 1 of

the Botswana study and the trend observed in both Moroka suverys. The lower prevalence of TPI reactive individuals shown in the over 60 year old group may well be due to a higher mortality of syphilitics in a leprosy group or to a regression of immobilin antibodies over a long period. Regarding leprosy, there were more patients in the younger age group of 20 - 40, with little difference in type distribution.

Summary

According to the results of the TPI test, this survey on leprosy patients from Westfort Institution is summarised as follows:-

(1) Regardless of the type of leprosy, 35 - 40 per cent of the patients were or had been infected with syphilis. The largest percentage occurred in the 40 - 60 year old group.

(2) On all repeat specimens of sera, the TPI test was reproducible.

(3) The Kolmer and VDRL tests, in agreement with the two previous studies, proved to be the most specific and, in combination, to have a satisfactory sensitivity.

(4) Biological false positive reactors using reagin tests for syphilis were confined almost exclusively to the lepromatous type of leprosy.

(5) The BFP reactors ranged from 26,9 per cent when the Kolmer and VDRL tests were used to 43,8 per cent when the Kahn reaction was added.

(6) Sera tested at various intervals from the same BFP patients often varied in titre, e.g. ranging in several instances from positive

with 160 Kolmer units to negative.

(7) Some association of reagin production with lepromin reactions was found. There was no apparent relationship between BFP and the appearance of Erythema Nodosum Leprosum

(8) Some immobilin reactive sera were negative reagin, indicating either successful treatment or burnt out cases of syphilis.

(9) The theoretical percentage of BFP in TPI positive cases of lepromatous leprosy was calculated and discussed.

(10) As evidences by BFP in lepromatous cases, there appears to be circumstantial evidence of an association between tissue destruction and/or a defective immune system on the one hand, and reagin production on the other.

CHAPTER 7

EVALUATION OF THE FLUORESCENT TREPONEMAL ANTIBODY-ABSORPTION (FTA-Abs) TEST FOR SYPHILIS, BASED ON THE RESULTS OF THE TREPONEMA PALLIDUM IMMOBOLISATION TEST (TPI)

The technique of the FTA-Abs test followed that described by the Staff, Venereal Disease Research Laboratory[146]. Reagents were obtained commercially but later, *T.pallidum* antigen was prepared in the laboratory, standardized to 100 - 150 treponemas per high dry field and stored at 4C. Smears were made and fixed on each test day from the stock solution. A Zeiss dark-field fluorescence microscope assembly equipped with an Osram HBO-200 mercury lamp, a Schott BG-12 exciter filter and matching 51 barrier filter was used for reading the processed slides.

As recommended[146], intensity of fluorescence was based on the Minimally Reactive (1+) control slide as the reading standard. However, difficulty was experienced in judging the subtle difference between a weak but definite (±) and 'vaguely visible' fluorescence of the spirochaete. In order to assess antibody content in a serum as objectively as possible, fluorescence seen as weak but definite was reported as 1+ reactive, and 'vaguely visible' as non-reactive. All sera showing any fluorescence in these categories were repeated. Repeat specimens of sera were requested when necessary. Reporting was carried out according to the recommendations of the V.D. Reference Laboratory[146].

Over an 18 month period, 668 sera submitted to the TPI laboratory were examined both by the FTA-Abs and the TPI tests. These sera came from: (a) patients with positive reagin tests in whom there was no clinical evidence or history of syphilis: (b) patients with clinical signs or symptoms

[illegible]

[illegible]

[illegible]

related to late syphilis b.t with negative reagin tests; (c) patients with diseases other than syphilis showing reagin reactivity, and (d) pregnant women with positive reagin tests. Clinical notes rarely accompanied the sera, nor were they forthcoming on request. The assessment of the value of the FTA-Abs test in this study, therefore, is not based on the clinical findings, but rests entirely on the results of the TPI test.

In Table 79, the Specific Immobilisation (SI) of the TPI test as described in Chapter 1 is compared with the degree of fluorescence of the FTA-Abs test.

TABLE 79

Comparative reactivity of the TPI and FTA-Abs Tests on sera from 668 diagnostic problem patients

		TPI/SI								
		80-100		50-89		20-49		0-19		
		No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	Total
FTA-Abs	4+ and 3+	234	98,3	63	75,0	25	49,0	5	1,7	327
	2+	4	1,7	19	22,6	15	29,4	10	3,4	48
	1+	-	-	1	1,2	6	11,8	11	3,7	18
	Non-reactive	-	-	1	1,2	5	9,8	269	91,2	275
Total		238	-	84	-	51	-	295	-	668

In the group of sera showing immobilin activity as measured by the TPI test (SI 20-100) 367/373 (98,4 per cent) were 1+ ——> 4+ fluorescent reading. Conversely 269/295 (91,2 per cent) sera were non-reactive in

both tests. Thus, there was complete correlation in 636/668 (93,8 per cent) sera.

Not surprisingly, most of the discrepancies, 26/295 (8,8 per cent), occurred in the TPI non-reactive group which raises the question of the specificity of the FTA-Abs test. Absence of demonstrable immobilin in the early stages of syphilis renders the TPI test invalid in determining the specificity of the fluorescence test. The paucity of clinical information did not help either. However, it may be stated that the finding of positive FTA-Abs tests in the TPI non-reactive group, is in accordance with published observations that the FTA-Abs test is more sensitive than the TPI test in early cases of syphilis[91, 147], in latent syphilis [91,148] and, more rarely, may be associated with non-specific reactions in cases of lupus erythematosus and pregnancy[94,95].

As regards the sensitivity of the FTA-Abs test, 6/373 (1,6 per cent) of the sera with immobilin were FTA-Abs negative. No reason could be found for the failure of the FTA-Abs test to detect antibodies in these cases. Beam, Dedeaux and Humes (1967)[149] report a similar failure, with no explanation, in 9 of 237 (3,8 per cent) sera.

Reproducibility of the FTA-Abs test was assessed in a series of 116 consecutive sera submitted for the TPI tests (Table 80). Another group of ninety-eight sera were repeated when, on first testing, the FTA-Abs test results did not correlate with the expected results of the TPI tests, e.g. with a TPI/SI of 100 per cent and FTA-Abs of 2+ instead of 3+ ⟶ 4+, or a non-reactive TPI serum with a 1+ FTA-Abs result. These sera are presented in Table 81.

TABLE 80

Repeat FTA-Abs testing of 116 consecutive routine sera

		2nd Test							
		4+ or 3+		2+		1+		Non-reactive	
1st Test	Total	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
4+ or 3+	35	34	97,1*	1	2,9	-	-	-	-
2+	16	2	12,5	13	81,3*	1	6,2	-	-
1+	11	-	-	2	18,2	8	72,7*	1	9,1
Non-reactive	54	-	-	-	-	3	5,6	51	94,4*
Total	116								

* Percentages in blocks represent identical results of repeat tests.

As experienced in other laboratories[150], Table 80 shows that the greatest reproducibility of results occurred in the moderate, the strongly positive and the non-reactive sera, with an over-all agreement of repeat tests in 106/116 (91,4 per cent) sera. In the non-reactive group of sera three, negative in the TPI test, gave a 1+ fluorescence on second testing, but were non-reactive when repeated a third time, suggesting a technical error on the second occasion.

TABLE 80

Repeat FTA-Abs testing of 116 consecutive routine sera

		2nd Test							
		4+ or 3+		2+		1+		Non-reactive	
1st Test	Total	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
4+ or 3+	35	34	97,1*	1	2,9	-	-	-	-
2+	16	2	12,5	13	81,3*	1	6,2	-	-
1+	11	-	-	2	18,2	8	72,7*	1	9,1
Non-reactive	54	-	-	-	-	3	5,6	51	94,4*
Total	116								

* Percentages in blocks represent identical results of repeat tests.

As experienced in other laboratories[150], Table 80 shows that the greatest reproducibility of results occurred in the moderate, the strongly positive and the non-reactive sera, with an over-all agreement of repeat tests in 106/116 (91,4 per cent) sera. In the non-reactive group of sera three, negative in the TPI test, gave a 1+ fluorescence on second testing, but were non-reactive when repeated a third time, suggesting a technical error on the second occasion.

TABLE 81

Repeat FTA-Abs testing of 98 selected sera

		2nd Test							
		4+ or 3+		2+		1 +		Non-reactive	
1st Test	Total	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
4+ or 3+	25	19	76,0*	5	20,0	-	-	1	4,0
2+	37	10	27,0	25	67,6	2	5,4	-	-
1+	29	-	-	4	13,8	19	65,5	6	20,7
Non-reactive	7	-	-	-	-	3	42,8	4	57,2

* Percentages in blocks represent identical results of repeat tests.

In this selected group of sera, repeat tests were identical in 67/98 (69,4 per cent) occasions. However if one were to disregard the slight difference of intensity of fluorescence observed between a 2+ and 3+, and a 2+ and 1+, the number of sera showing acceptable correlation would rise to 88/98 (89,7 per cent) which is comparable with the first group (Table 80). The results perhaps provide some motivation for replacing the mercury lamp, which is known to gradually lose it's intensity with time, in favour of the constant output halogen lamp.

There was an obvious technical error in which one serum gave a 3+ reading on the first occasion and was non-reactive on two further testings with both treponemal tests. In the non-reactive group of sera, three gave weakly reactive immobilin results (SI 23-39 per cent) in the TPI test and were negative reagin. A 1+ degree of fluorescence was seen on second

testing. Had these sera not been subjected to the TPI reaction, false FTA-Abs results would have been issued.

Conclusions

The results of this study indicate a high level of correlation between the two treponemal tests. The FTA-Abs test has a greater sensitivity than the TPI test but because of the absence of adequate clinical details, evaluation of the specificity of the test could not be done. However, the figures in Table 79 show that the FTA-Abs test approaches the high specificity of the TPI test.

Reproducibility on both series of specimens was good. It is clear however, that in the low activity range the test is not entirely infallible. In order to avoid the occasional discrepancy, possibly due to a fault in technique, and to reduce the chances of reporting erroneous results, all sera with a 1+ fluorescent reading should be repeated. If discrepant results are found in the repeat test, a further specimen of serum fromthe patient should be requested. Furthermore any serum should be repeated which shows conflicting results with other serological tests or results not in accord with the patients clinical signs or history.

The study also underlines the necessity of working with a constant source of illumination and as this is rarely achieved with a mercury lamp, it may be beneficial to change to the more reliable halogen light source.

CHAPTER 8

SEROLOGICAL ASSESSMENT OF THREE PENICILLIN TREATMENT SCHEDULES ON BANTU PATIENTS WITH PRIMARY AND SECONDARY SYPHILIS

The introduction of penicillin for the treatment of human syphilis by Mahoney, Arnold and Harris (1943)[151], represented a significant milestone in the control of both venereal syphilis and the extra-venereal treponematoses[152-155]. Over the following years, the development of derivations of penicillin on the one hand and therapeutic application to the various stages of syphilis on the other, first supplemented and then replaced the traditional therapy of arsenicals and bismuth.

Although *Treponema pallidum* is one of the most penicillin-sensitive organisms, extensive treatment over prolonged periods of time may be required[156]. The therapeutic effect in treponematoses depends on the stage of syphilis, the duration of treatment and the dose of the drug. A minimal concentration of 0,03 International Units of penicillin per ml of serum should be maintained for at least seven to ten days in the early stages of the disease[157,158]. This would prevent any surviving treponemes from multiplying.

The ideal treatment schedule for syphilis would be one where a single or at the most a few injections of penicillin would give an effective penicillinaemia of sufficient duration to eliminate the treponemes rather than the impractical schedule in which effective blood concentrations require the administration of several injections daily. This concept led to the development by Buckwalter and Dickison (1948)[159] of a long-acting drug, procaine penicillin G in oil with aluminium monostearate (PAM).

Eagle (1949)[160] showed experimentally that the treponemicidal effect

of a given amount of penicillin was directly related to the number of treponemes present. Thus, the earlier the course of syphilis treatment is started, the less important is the dose/time relationship. It follows therefore that as the duration of the infection increases, so are higher doses needed. To ensure a safety margin the WHO Expert Committee on Venereal Infections and Treponematoses[161] recommended that the minimum dose of penicillin administered for any stage of early syphilis should equal the dose required for secondary syphilis. Minimum production standards for PAM preparations were also established by the WHO Committee.

Recommended Treatment Schedules[161]

(a) One injection of 600 000 units of PAM every second day until the total dose of 6 000 000 units has been reached. The concentration after ten injections (twenty days) will reach 0,5 units per ml of serum with a subsequent gradual fall to the minimum therapeutic level over the next eight days; this will give a total of 28 days effective treatment.

(b) Five injections at three to four day's interval of 1,2 million units per injection. Adequate penicillin levels are maintained for at least 20 days.

(c) A single injection of 4,8 or 6,0 million units. Adequate penicillin concentrations are maintained for 8 - 10 days.

In practice, treatment schedule (a) of 600 000 units every second day is commonly used in syphilis[162,163]. However, under certain circumstances, for example when patients cannot be relied upon to return for

further treatment or when the prevention of spread of infection is a consideration, a larger single dose is given. This will rapidly render the patient non-infectious and will cure early syphilis in approximately 85 per cent of cases[164].

A significant simplification of therapy was achieved in 1951, by the introduction of benzathine penicillin G (Szabo)[165]. Following a single injection of 2,4 million units, the treponemicidal penicillinaemia of about 0,03 units per ml persists for up to three to four weeks, proving the drug to be as long-acting as PAM[166]. Evaluation studies on primary and secondary cases of syphilis have shown that treatment with benzathine penicillin G is at least equal to the results achieved with PAM[167,168].

One disadvantage of benzathine penicillin G is that it causes local pain in approximately half the patients. In contrast, only 5,0 per cent of patients treated with procaine penicillin complain of pain at the site of injection[169,170]. On the other hand, the risks of allergic reactions to penicillin are reduced when the single injection technique is used[171].

In the era of metal chemotherapy a case of early syphilis was considered permanently cured after negative serology had been observed for four years. With penicillin, a patient may be considered cured after only two years observation. Most failures in the treatment of the early infection occur within six to nine months of treatment and are seldom observed after two years[172]. Furthermore, long-term observations have shown that late involvement of cardiovascular and nervous systems are apparently very rare after penicillin therapy[172].

The criteria for cure in primary and secondary syphilis is generally accepted as being the disappearance of clinical manifestations together with regression of reagin and sometimes immobilin antibody with ultimate serological reagin non-reactivity[156].

The purpose of this study was to observe over a twelve month period the clinical and serological results of Bantu patients with primary and secondary syphilis after treatment schedules with PAM and benzathine penicillin G.

Material and Methods

Clinically diagnosed cases of primary and secondary syphilis came from the Out-patients clinic at Baragwanath Hospital - the hospital serving the Soweto complex of townships near Johannesburg. Specimens of blood were taken for serological tests and treatment started according to the following schedules:-

(a) Series 1.

One injection of 2,4 million units benzathine penicillin G (Bicillin).

(b) Series 2.

One injection of 2,4 million units benzathine penicillin G (Bicillin) followed by a further injection of 2,4 million units, four-five weeks later.

(c) Series 3.

Daily injections of 600 000 units procaine penicillin in oil with aluminium monostearate (PAM) for ten days.

After each course of treatment was completed, the patients were instructed to present themselves every three months for at least a period of one-year for clinical and serological evaluation. This proved problematical; many came back for the first examination, never to be seen again. As a result, only those who presented themselves on at least two occasions after termination of treatment are included in this investigation.

Quantitative Kolmer Complement fixation and the VDRL tube flocculation test both with cardiolipin antigen were used to test for reagin antibody and the TPI and FTA-Abs test for specific treponemal antibody.

The serological results of those patients with primary and secondary syphilis, treated with one injection of 2,4 million units of Bicillin are shown in Tables 82 and 83.

TABLE 82

Primary syphilis : serological results before and after treatment with one injection of 2,4 million units of Bicillin (Series 1)

Patients Number	Age in years	Sex	Date of Treatment and first serological test	Serological follow-up in months	Colmer Units	VDRL Units	TPI Specific Immobilization	FTA-Abs
5903	7	M	4/6/69		160	64	100	4+
				2	4	8	100	4+
				10	160	128	100	4+
6029	20	M	4/8/69		160	16	100	4+
				2	10	4	100	4+
				6	10	2	100	4+
6128	31	M	18/9/69		20	16	100	4+
				4	10	4	100	4+
				6	2½	2	100	4+
5877	20	M	22/5/69		40	32	100	4+
				4	2½	2	100	4+
				8	-	-	65	3+
6070	18	M	26/8/69		160	64	100	4+
				2	2½	8	83	4+
				5	-	2	42	3+
6090	23	M	4/9/69		160	32	100	4+
				2	80	16	100	4+
				8	10	-	31	2+
6071	20	M	26/8/69		40	8	65	4+
				2	-	-	29	2+
				5	-	-	13	1+
				9	-	-	2	1+
6107	20	F	15/9/69		20	-	45	3+
				3	-	2	40	2+
				8	5	-	29	1+
5855	29	M	9/5/69		160	32	100	4+
				3	-	-	83	3+
				10	-	-	7	-
6011	21	F	23/7/69		-	2	3	1+
				5	-	-	0	1+
				10	-	-	11	1+

Patient 5503 was the only one in this group that showed evidence of serological relapse; there were no clinical indications of reinfection. Three sera became reagin free after 4 to 10 months and 6 showed marked regression of immobilin antibody both with the TPI and FTA-Abs test. Two sera (6071, 5855) became TPI negative after 9 and 10 months and one (5855), non-reactive in the FTA-Abs test. The last serum in Table 82 (6011) was a very early case of primary syphilis and illustrates the increased sensitivity of the FTA-Abs test.

TABLE 83

Secondary syphilis : serological results before and after
Treatment with one injection of 2,4 million units of Bicillin (Series 1)

Patients Number	Age in	Sex	Date of Treatment and first serological test	Serological follow-up in months	Kolmer Units	VDRL Units	TPI Specific Immobilization	FTA-Abs
6032	16	M	5/8/69		160	16	100	4+
				4	-	2	86	4+
				9	40	4	95	3+
5956	21	M	26/6/69		160	32	100	4+
				2	5	4	100	4+
				7	10	32	100	4+
6075	26	M	27/8/69		160	64	100	4+
				2	20	8	100	4+
				8	160	64	100	4+
6227	19	M	30/10/69		160	8	100	4+
				3	10	4	100	4+
				12	160	64	100	4+
5939	17	F	20/6/69		80	32	100	4+
				3	5	4	100	4+
				7	40	16	100	4+
				15	40	4	100	4+
6110	27	F	15/9/69		160	16	100	4+
				2	80	16	100	4+
				8	10	1	100	3+
6067	28	F	26/8/69		160	32	100	4+
				3	20	2	100	4+
				5	5	4	100	4+
				3	10	2	100	4+
6114	22	M	16/9/69		40	16	100	4+
				2	-	-	69	3+
				4	-	-	38	3+
6045	22	M	12/8/69		160	32	100	4+
				3	-	2	78	3+
				5	2½	2	63	3+
				9	10	-	23	2+
6047	26	F	12/8/69		160	32	100	4+
				3	20	-	88	4+
				9	-	-	11	1+
6056	18	M	18/8/69		160	64	100	4+
				1	20	32	100	4+
				8	-	-	36	2+
6174	19	F	18/9/69		80	32	100	4+
				2	5	2	93	4+
				4	-	-	41	3+
				5	10	2	30	3+
				8	-	-	26	2+
5909	21	F	6/6/69		160	32	100	4+
				5	10	2	64	4+
				7	-	-	24	3+
				13	-	-	41	3+
6104	19	M	9/9/69		-	-	46	2+
				4	-	-	28	2+
				8	-	-	9	-

As with the primary syphilis patients in Series 1, all secondary cases appeared to be clinically cured at the last examination. Serologically on the other hand, based on the Kolmer and VDRL tests, the first five patients in Table 83 (6032, 5956, 6075, 6227 and 5939) showed evidence after two to four months of serological relapse. Of the remaining nine cases, five became reagin negative and three showed a marked regression in the reagin titres. A drop in immobilin antibody titre was seen in seven sera. Patient 6104 had a weakly reactive immobilin reaction with a 2+ FTA-Abs test and no reagin. It appeared that he had received Bicillin for gonorrhoea one month before presenting himself at the clinic with symptoms of secondary syphilis. This prior treatment would explain the absence of reagin when the initial serum was tested.

TABLE 9

2864	22	M	3/7/69	- 1 2	160 40 20	4 1 4	1/4 1/4 1/4	1 1 1
6142	24	F	24/7/69	- 1 4	160 - 20	4 2 1	1/4 1/4 1/4	1 1 1
6874	28	F	27/8/69	- 3 8	160 20 4	4 2 1	1/4 1/4 1/4	1 1 1
6230	27	M	22/10/69	- 2 5	160 40 20	4 2 1	1/4 1/4 1/4	1 1 1
6252	22	M	10/11/69	- 3 8	160 20 -	4 2 -	1/4 1/4 1/4	1 1 1
6001	16	F	15/7/69	- 2 7	80 40 -	4 1 -	1/4 1/4 1/4	1 1 1
5904	22	M	4/6/69	- 3 6 8 12	160 160 40 10 20	4 4 1 1 1	1/4 1/4 1/4 1/4 1/4	1 1 1 1 1

*AC = Anticomplementary

TABLE 84

Secondary syphilis - serological results before and after treatment with two injections of 2,4 million units of Bicillin with 4-5 weeks in between each (Series 2)

Patients Number	Age in years	Sex	Date of treatment and first serological test	Serological follow-up in months	Kolmer Units	VDRL Units	TPI Specific Immobilization	FTA-Abs
5864	20	M	16/5/69	-	160	32	100	4+
				3	40	8	100	4+
				10	160	64	95	4+
6142	16	F	24/9/69		160	16	96	4+
				2	-	16	100	4+
				5	20	8	100	4+
6074	28	F	27/8/69		160	64	100	4+
				5	20	16	100	4+
				8	40	4	98	4+
6230	27	M	31/10/69		160	64	100	4+
				2	40	16	100	4+
				5	20	16	93	4+
6252	21	M	10/11/69		160	8	100	4+
				3	20	16	98	4+
				8	-	-	30	4+
6001	16	F	19/7/69		80	64	100	4+
				2	AC*	4	95	4+
				7	-	-	71	3+
5904	22	M	4/6/69		160	2	63	4+
				3	160	8	100	4+
				6	40	8	96	4+
				8	10	8	87	4+
				12	20	2	93	3+

*AC = Anticomplementary

Patient 5864 on examination after ten months showed a clinical reinfection which may account for the increase in reagin titre over the third month result. Patient 5904 initially had a large primary penile chancre and relapsed into secondary syphilis in the third month. According to the serological results the treatment schedule appeared to be successful but after twelve months, clinical examination showed evidence of reinfection. Additional treatment was given but the case was lost for further studies. An upward trend in the Kolmer titre of patient 6142 indicated a possible serological relapse five months after treatment. No follow-up specimens were obtainable. The remaining cases in this group, none with clinical symptoms, showed a drop in reagin titres, two to negativity, suggesting successful treatment.

TABLE 35

Five primary and five secondary cases of syphilis : serological results before and after treatment with 10 daily injections of 600 000 units of PAM (Series 3)

PRIMARY SYPHILIS

Patients Number	Age in years	Sex	Date of treatment and first serological test	Serological follow-up in months	Kolmer Units	VDRL Units	TPI Specific Immobilization	FTA-Abs
6186	21	M	15/10/69		5	-	23	2+
				7	10	-	23	2+
				12	160	64	100	4+
6211	24	M	24/10/69		5	2	93	4+
				3	2½	2	38	3+
				12	160	64	100	4+
6154	18	M	20/9/69	-	40	16	70	4+
				8	10	-	28	2+
				13	160	64	100	4+
6226	19	F	30/12/69		160	16	100	4+
				2	20	16	100	4+
				12	160	64	100	4+
6148	26	M	25/9/69		160	32	95	4+
				2	40	4	100	4+
				5	5	2	100	4+

SECONDARY SYPHILIS

6189	16	F	16/10/69		160	64	100	4+
				2	10	8	100	4+
				4	40	16	83	4+
6199	37	F	21/10/69		160	32	92	4+
				3	5	2	37	3+
				8	-	-	16	1+
6178	17	F	7/10/69		160	64	100	4+
				2	-	2	61	3+
				4	5	2	32	2+
				7	-	-	22	2+
6185	20	M	13/10/69		160	64	94	4+
				2	5	2	53	3+
				5	-	-	11	-ve
6276	31	F	24/11/69		20	2	83	4+
				2	10	2	14	3+
				6	5	-	15	1+

Of the five cases of primary syphilis, four, after 12 months, showed a marked increase in reagin titres compared with the second examination, indicating serological relapse attributable either to reinfection or treatment failure. No case presented any clinical symptoms of syphilis on the last examination. As measured by the TPI and FTA-Abs tests, increases in immobilin antibody occurred in the first three cases between the second and third period of testing.

In contrast, in the group of secondary syphilis, only one patient (No. 6189) showed a rise in reagin antibody two months after the second examination. This was attributed to a clinical reinfection. The other four patients showed a dramatic regression of reagin and immobilin antibodies five to eight months after treatment.

Discussion

The choice of treatment is determined by a variety of factors, the most important being the clinical, closely followed by the serological response. From this study, the results of these two factors are presented in Table 86.

TABLE 86

Comparison of serological and clinical response in each treatment schedule

Treatment	STAGES OF SYPHILIS									
	PRIMARY					SECONDARY				
	Total	Sero-logical relapse	Clinical relapse	Regression		Total	Sero-logical relapse	Clinical relapse	Regression	
				Reagin(1)	Immobilin(2)				Reagin(1)	Immobilin(2)
Series 1 2,4 million units Bicillin	10	1 (10 months)	0	9 (8-10 months)	5 (5-10 months)	14	5 (7-14 months)	0	9 (4-13 months)	7 (4-13 months)
Series 2 2 x 2,4 million units Bicillin with interval of 4-5 weeks	0	-	-	-	-	7	2 (5-10 months) (1 reinfection)	1 (3 months)	5 (5-12 months)	2 (7-8 months)
Series 3 10 x 600 000 units PAM daily	5	4 (12-13 months)	0	1 (5 months)	0	5	1 (4 months) (reinfection)	0	4 (5-8 months)	4 (5-8 months)

(1) = Four-fold drop in titre or positive to negative

(2) = 30 per cent drop in Specific Immobilisation

Table 86 shows that schedules 1 and 3 did not present any patients with clinical relapse, whereas there was one, treated initially for primary syphilis, in Series 2. Series 1 and 3 each had one case of reinfection. In contrast, serological relapse occurred in one of ten patients in Series 1 with primary, and five of 14 with secondary syphilis. Series 3 gave more patients with increased antibody activity in the primary syphilis group (4/5) than the secondary (1/5). Despite the one case of clinical relapse, the treatment schedule of Series 2 only produced two of seven serological failures.

Smith, Kamp, Olansky and Price (1956)[171] evaluated Bicillin and PAM in a series of seropositive primary and secondary cases of syphilis (Table 87).

TABLE 87

Comparison of Benzathine Penicillin G and PAM in the treatment of Primary and Secondary syphilis

Treatment schedule	Stage of syphilis	No.of cases	Percentage retreated			Percentage seronegative after 2 years
			Total	Clinical or Serological failure	Reinfection	
Benzathine Penicillin G (2,5 million units 1 injection	Primary sero-positive	67	4,0	2,0	2,0	96,0
	Secondary	155	5,5	0,9	4,6	94,5
PAM (4,8 million units (1 injection)	Secondary	166	7,7	3,8	3,9	91,0
(2-4 injection)	Secondary	415	11,7	7,8	3,9	88,3

As shown in Table 87 their results, following treatment of secondary syphilis with one injection of Benzathine Penicillin G, are slightly better than those obtained with 4,8 million units of PAM, administered either in a single or two to four sessions.

With a similar treatment schedules to those of Smith[171], Schroeter, Lucas, Price and Falcone (1972)[173] found the cumulative percentage retreated after two years, due to both reinfection and treatment failures,to be 11,4 per cent for Benzathine Penicillin G and 10,9 per cent for PAM (three injections). Fiumara (1964)[174] states that with either schedule, one can after one course, expect a treatment failure rate of two per cent in primary and 5 - 10 per cent in secondary syphilis.

The prevalence of syphilis in the community under study may have a bearing on the number of cases showing serological relapse; the majority in population groups with a high prevalence of syphilis presumably being due to reinfection rather than treatment failure. This may explain the differences in serological relapse rates in this study and those quoted above.

Notwithstanding the small number of patients in this survey, it is clear that each treatment series has its quota of patients with serological relapse, based on increased reagin titre in both the Kolmer and VDRL tests and, in some instances, immobilin antibody. In only two cases (Series 2 and 3) was there any evidence of a clinical reinfection. It is therefore very difficult in the other 11/41 asymptomatic cases to assess whether or not the relapse was due to reinfection or treatment failure. Bauer (1951)[175] found that relapses, after penicillin treatment, occur early in the disease,

usually between four and nine months, and Merrell (1951)[176] that the majority of treatment failures were due to reinfections. However, what ever the reason for the relapse, a second course of treatment is indicated.

Regression of reagin, based on a four-fold drop in titre or from positive to negative, showed that in the relatively short period of time in which follow-up examinations were undertaken, 28/41 had either become non-reactive or were approaching seronegativity. It appears from the high reagin and in the majority of cases immobilin titres, that most of the primary cases presented themselves for treatment in the advanced primary stage.

The rapidity with which sero-reversal occurs after treatment of early syphilis depends mainly on the duration of the disease before treatment[177]. However, serological tests behave differently because of different degrees of sensitivity and the nature of the antibodies detected by them.

As measured by the Specific Immobilization of the TPI reaction, all series showed sera with some degree of immobilin antibody. In the group as a whole, 18/41 (Table 86) showed a regression of 30 per cent or more SI over a period of four to thirteen months. Five became non-reactive. In keeping with its higher sensitivity the FTA-Abs test did not show such a marked response over the observation period; only three became non-reactive.

In all series there were some sera that maintained a high degree of Specific Immobilisation despite the marked drop in reagin antibody titre. This emphasizes the importance of reagin over specific treponemal tests in

assessing the success of treatment particularly in latent and late syphilis cases where the TPI test in almost all cases remains active many years after cure[178,179]. This finding has led to the suggestion that the continued positivity of the TPI and FTA-Abs tests may be due to the persistence of treponemes in the body. Experimental evidence to support this possibility was provided by Collart, Borel and Durel (1962)[180], who demonstrated the presence of treponemes in the lymph-nodes of rabbits adequately treated with penicillin two years after infection with *T.pallidum*. Yobs Rockwell and Clark (1964)[181] found treponemes in five of forty five inguinal nodes from men who had in the past received adequate treatment for late latent syphilis. In two instances the organisms were successfully transferred to rabbits, with the development of dark-field positive testicular lesions. In a further publication, Yobs and her co-workers[182] retreated the five patients and again found treponemes in three of them and in six of the original group.

Conclusions

Although small in numbers, the results of this survey show that all treatment schedules are inadequate and in need of revising.

Since there is no other reliable alternative, the criteria for cure after treatment in early syphilis must be the disappearance of clinical manifestations and the permanent reversal of STS. In order to achieve this, it is obvious that a clinical and serological examination of the patient should be undertaken regularly for two years. Fiumara[174] suggests a further course of treatment in all primary seropositive syphilis patients if seronegativity is not obtained after nine months and with secondary after one year.

However, in the population group described in this study the return to the hospital clinic for a regular clinical and serological examination is, at best, haphazard. Therefore the schedule described by Durst, Sibulkin, Trunnell and Allyn (1973)[183] would possibly be the most positive recommendation. In a small study on forty patients with secondary syphilis, these workers found a reversal to seronegativity in all patients within two years with a total dose of 6 000 000 units of benzathine penicillin G. The schedule consisted of 2 400 000 units followed by a similar dose three to five days later and 1 200 000 units again three to five days later,

It is obvious that a further larger study with regular clinical and serological examinations over a two year period is necessary to establish the efficiency of this recommended treatment regimen in this group.

CHAPTER 9

EVALUATION OF THE MONOSPECIFIC ANTI IgM FLUORESCENT TREPONEMAL ANTIBODY-ABSORPTION TEST (IgM FTA-Abs) FOR THE EARLY DIAGNOSIS OF CONGENITAL SYPHILIS IN BANTU NEONATES

The diagnosis of congenital syphilis in new born babies presents no difficulty when there are positive dark-field lesions or obvious clinical stigmata. The problem of diagnosis arises however, when the baby is asymptomatic and presents positive serology both in non-treponemal (Kolmer and VDRL) and treponemal (TPI and FTA-Abs) tests. A rise in reagin or an increase of treponemal antibodies or the appearance of lesions, will establish that the first results were due to an active infection and not to passively transferred antibodies from the mother. However, these considerations are mainly academic since one cannot afford to postpone treatment until such findings have corroborated the diagnosis. Also, there is a real risk that the infant will not be brought back for regular observation; treatment, therefore, is often given initially, irrespective of a final diagnosis, and often to normal babies.

Since IgG antibodies may be placentally transferred from the mother and specific IgM antibodies do not pass the placenta[191], the presence of IgM in the neonate would indicate an in-utero infection of syphilis. It is well documented that antibodies in the three major gamma globulin classes react in the FTA-Abs test[185-190]. Both IgG and IgM can be shown in the early stages of syphilis[185,186,187,190], whereas IgG predominates in the later stages [186,187,189,190]. IgA is present in small amounts in early syphilis[185,186,187,190] and not in late[189]. Modifying

the FTA-Abs test by substituting the conjugated antihuman 'total globulin' serum with a fluorescien-labeled globulin, monospecific for IgM, Scotti and Logan (1968)[192] reported positive results with three babies with obvious syphilitic dark-field lesions. The conventional FTA-Abs and VDRL tests were also reactive. Six normal infants chosen as controls, were non-reactive in all tests, and ten cases of clinically negative infants with reactive VDRL and FTA-Abs tests were negative with the IgM FTA-Abs test. In a further publication, Scotti, Logan and Caldwell (1969)[193] reaffirmed the effectiveness of the IgM FTA-Abs test in distinguishing between antibodies associated with actual infection and antibodies passively transferred in the neonate, but stated that the sensitivity of the test had not been established. This, they added, could only be done by withholding treatment in asymptomatic infants with positive serology and initially without IgM treponemal antibodies.

Following these reports, further studies were carried out to evaluate the accuracy and usefulness of the test[194-197]. Although the assessment of the results was based on small numbers of cases of congenital syphilis, the authors agreed that the test was valuable in the diagnosis of the disease in neonates. However, doubts as to the specificity of the test were expressed[196] and the need for standardization of technical procedures was emphasized[197].

Most of the babies born at the Baragwanath Hospital are from mothers living in the Soweto complex of townships where the prevalence of syphilis, past or present, is high (Chapter 5). This study was undertaken in an attempt to standardize and assess the reliability of the monospecific IgM

FTA-Abs test in the diagnosis of both symptomatic and asymptomatic early congenital syphilis in infants.

Materials and Methods

The infants were divided into the following three groups:-

Group 1. Twenty normal neonates, whose mothers were seronegative for syphilis. This control series would establish false positive reactions to the IgM FTA-Abs test.

Group 2. In order to evaluate the sensitivity of the test, 114 infants aged one day to five months, all with definite clinical and/or radiological stigmata of congenital syphilis, were chosen.

Group 3. All fifty neonates, sero-tested within seven days of birth, were clinically normal and without radiograph signs of syphilis in the long bones. As measured by the VDRL test, the mothers showed reagin, ranging from 2 to 128 units, either during pregnancy or immediately after delivery. The majority (35/50) had received luetic treatment during their gestation period. No information on clinical symptoms or history was available. The babies were left untreated, regardless of serological findings and were checked at three weekly intervals until they were nine weeks old. Regular attendance for follow-up examination was ensured by a social worker. Should any infant show clinical or radiological evidence of congenital syphilis during this period, penicillin treatment was instituted immediately. The results from this group would tend to clarify the discriminatory value of the IgM FTA-Abs test.

The Kolmer Complement Fixation, VDRL, conventional FTA-Abs, IgG FTA-Abs and IgM FTA-Abs were performed on all infant's sera. Conjugate of fluorescien labeled monospecific anti IgM serum, obtained from Wellcome Reagents Ltd., was diluted 1:10, 1:20 and 1:40 and the sera tested with each dilution. Subsequently, as many Group 2 sera as possible were tested using a conjugate dilution of 1:80. Positive readings were assessed on the degree of fluorescence and were graded 1+ to 4+.

Results

Group 1

All twenty infants, whose mothers showed negative serology for syphilis were completely non-reactive in the two reagin, the FTA-Abs, IgG FTA-Abs and IgM FTA-Abs tests.

Group 2

The serological results of those babies who had definite clinical signs of congenital syphilis are shown in Table 88. Most of them were born of mothers who had received no treatment during pregnancy (102/114). Of the remaining twelve, three admitted that they had failed to complete the treatment.

TABLE 88

Serological results of 114 Symptomatic Neonates

Age	No.	FTA		IgG		IgM						Kolmer Units			VDRL Units**		
						1/20			1/40								
		4+,3+	2+,1+	4+,3+	2+,1+	4+,3+	2+	1+	4+,3+	2+	1+	20+	2j+-10	Non-reactive	10+	2-8	Non-reactive
1-7 days	29	29	-	27	2	14	12	3	10	12	7	26	1	AC*, Ins*	27	2	-
8-14 days	8	7	1	6	2	4	3	1	4	3	1	6	1	1	6	1	1
15-28 days	21	21	-	21	-	14	6	1	12	7	2	19	1	AC*	20	1	-
4-8 weeks	36	36	-	31	5	19	13	4	15	11	10	27	4	AC*[1] (4)	30	5	Ins*
9-12 weeks	10	10	-	7	3	5	2	3	5	7	3	10	-	-	10	-	-
3-5 months	10	10	-	10	-	6	3	1	5	4	1	8	-	ND*[1]	9	-	1
Total	114	113	1	102	12	62	39	13	51	39	24	96	7	3	102	9	2

* AC = Anticomplementary
Ins = Insufficient
ND = Not done

** Some of these results were obtained by using the commercial RPR technique.

Of 114 infants, the sera of 113 gave a strongly positive (3+–4+) FTA-Abs test; the remaining one showed a 2+ fluorescence. The monospecific IgG FTA-Abs test was positive in all infants sera, 102 with a 3+–4+ fluorescence. The greater majority gave reagin reactions with high titres (96/106 Kolmer and 102/113 VDRL). Only two babies had no detectable reagin; their mothers both showed VDRL titres of 32 and were treated during their last month of pregnancy. The monospecific IgM FTA-Abs test was positive in all 114 babies at conjugate dilutions of 1:10, 1:20 and 1:40. However, when a dilution of 1:80 was used, 28/52 (52 per cent) of the sera tested were non-reactive.

All babies were given penicillin therapy and follow-up sera from 14, over a period of 1 to 9 months showed a marked regression of reagin antibodies. The intensity of fluorescence in the IgM FTA-Abs test dropped in the 1:20 and 1:40 dilution of conjugate; many becoming negative in the latter dilution.

Of the twelve babies who died, five had developed gastro-enteritis (two proven salmonellosis and one shigellosis). Of the remaining seven, six died of syphilis within 11 weeks of birth and permission for a post-mortem was refused on the seventh.

Group 3

From the results of the FTA-Abs, the monospecific IgG and IgM FTA-Abs tests, carried out during their first week of life, this group of asymptomatic infants with possible treponemal infection (mothers serologically positive) fall into three groups (Table 89).

TABLE 89

Fifty untreated infants, born from seropositive mothers and classified according to seroreactivity during their first week of life

				IgM FTA-Abs		
				Conjugate dilution		
Sub-group	No. of infants	FTA-Abs	IgG FTA-Abs	1:10 and 1:20	1:40	Kolmer and VDRL
a	4	NR	NR	NR	NR	NR
b	43	R	R	R	NR	NR and R
c	3	R	R	R	R	R

NR = Non-reactive

R = Reactive

Sub-group a

Throughout the study, these four cases remained clinically and radiologically normal and all fluorescent and both reagin tests were consistently non-reactive. Three of four mothers had been treated. Two mothers showed 32 and two, 2 VDRL units.

Sub-group b

This group (Table 90) is of infants with reactive FTA-Abs and IgG FTA-Abs tests but non-reactive IgM FTA-Abs tests in conjugate dilutions of 1:40. The two reagin tests were variable. Apart from positive VDRL tests (titres ranging from 2 to 128) in all mothers, no information on

clinical symptoms or past history was available. Of the forty-three mothers, thirty-one had received some form of penicillin therapy during their pregnancy.

TABLE 90

Forty-three Neonates with variable seroreactivity but negative IgM FTA-Abs 1:40

		IgM							
		IgG		1:10		1:20		1:40	
	No.	3-4+	1-2+	+	-	+	-	+	-
FTA 3-4+	32	27	5	21	11	19	13	0	32
1-2+	11	0	11	2	9	0	11	0	11

About half of the sera showed reagin activity, ranging from 2½ - 20 Kolmer units and half the sera were reagin negative (not tabulated). Table 90 shows a definite correlation between the results obtained with all three FTA-Abs tests. Only IgM 1;40 was capable of correctly predicting that these children did not suffer from a syphilitic infection as evidenced by the follow-up. Clinical and radiological findings remained consistently negative.

The gradual loss of IgM reactivity over a nine weeks observation period, despite lack of treatment, is shown in Table 91.

TABLE 91

Number of infants with reactive IgM FTA-Abs tests over a nine week period

Age in weeks	Monospecific anti IgM conjugate dilutions		
	1:10	1:20	1:40
1	23	19	0
3	11	7	0
6	10	6	0
9	1	1	0

All forty-three infants remained clinically and radiologically normal until the conclusion of the study.

Sub-group c

Case 1: This child's mother, whose serum showed 32 VDRL units after delivery, was untreated during her pregnancy. Within the first week of birth, the infant serologically showed 10 Kolmer and 8 VDRL units. Her FTA-Abs and IgG FTA-Abs test both showed 3-4+ readings; the IgM 1:10 was 2+ and IgM 1:40, 1+. At three weeks her serological tests were unchanged but she developed clinical and radiological evidence of clinical syphilis (snuffles, hepatosplenomegaly and periostitis of long bones). Penicillin treatment was instituted immediately. At six weeks there was no change in the results of all the fluorescent tests, but both reagin tests had become negative.

Case 2: The infant's mother had received no treatment during pregnancy and her serum showed 128 VDRL units after delivery. The serum from the baby showed 80 Kolmer and 32 VDRL units just after birth, with a 4+ FTA-Abs and IgG FTA-Abs test reading. His IgM 1:10 was 2+ and IgM 1:40, 1+. At three weeks of age the baby was treated with gentamycin and cloxacillin for three days for an upper respiratory tract infection. Radiological changes (lytic lesions and periostitis of multiple long bones), consistent with the diagnosis of congenital syphilis were noted. No serology was done on this occasion.

On presentation at six weeks of age, the Kolmer and VDRL reactions on the child's serum had dropped to 20 and 8 respectively. All fluorescent tests remained positive including the IgM FTA-Abs at a conjugate dilution of 1:40. Bone lesions showed a marked improvement and no further treatment was given. The mother was instructed to present her baby for a final examination at a later date.

Case 3: No serological results were available from the mother. At birth, serological reactions of this infant showed 5 Kolmer and 2 VDRL units with strongly positive FTA-Abs and IgG FTA-Abs tests. IgM 1:40 gave a 3+ reaction. Three and six weeks later the IgM had dropped to 1+ in 1:40 and became quite negative ten weeks later. The baby remained well throughout the sixteen weeks of observation.

This case represents a false positive reaction in the IgM FTA-Abs test.

Discussion

Assessing the IgM FTA-Abs test in the early diagnosis of neonatal syphilis, Mamunes, Cave, Budell, Andersen and Steward (1970)[195] reported 5/33 (15,0 per cent) false positive reactions and Sepetjian, Tissot Guerraz, Nivelon and Thivolet (1972)[196] 10/40 (25,0 per cent). Johnston (1972)[197] on the other hand, found that 6/25 (24,0 per cent) asymptomatic infants who subsequently developed clinical signs of syphilis, gave negative readings on first testing. However, in each of these studies variable anti IgM conjugate and serum dilutions were used, as well as different methods for removing non-specific treponemal antibodies from the sera.

In an attempt to standardize the test procedure it was decided to adhere to the test technique described by The Staff, Venereal Disease Research Laboratory[146], and to only vary the dilution of the anti IgM conjugate, obtained from one commercial firm only. Thus, by using sera from clinically or radiologically obvious congenital syphilitic infants, it should be possible to establish a conjugate titre which would give consistently positive IgM FTA-Abs results.

By serial dilution of the conjugate from 1:10 to 1:40 it was found that the highest dilution which gave positive results in all 114 infants with congenital syphilis (Group 2) was 1:40. With IgM 1:80, 52,0 per cent were non-reactive. As the ages of the children ranged from one day to five months and their clinical manifestations from mild to severe, it was theorized that a positive reading with IgM 1:40 would be the optimal test for detection of congenital syphilis although a few false negative and false positive reactions had to be anticipated.

When the serial conjugate dilutions were applied to asymptomatic neonates (Group 3), the results of the IgM FTA-Abs test were often positive in conjugate dilutions of 1:10 and 1:20, whereas with a dilution of 1:40 there were no false negatives and only one false positive (1/50). Such a false positive reaction is to be expected in small numbers as stated above. However it is of interest that transplacental leakage of small amounts of maternal blood into the cord has been described in approximately 2 per cent of deliveries[198]. That this was the mechanism could not be substantiated since studies for iso-agglutinins were not carried out[199].

For the total globulin conjugate for the FTA-Abs test, it is recommended that the dilution selected for use is one doubling dilution lower than the highest giving maximum fluorescence. This was not found applicable to the anti IgM conjugate in this study, since the suitable dilution would then have been 1:20 which has been shown to give a large number of false positives (19/43) (Table 90).

It is difficult to explain why it should be necessary to dilute the conjugate to eliminate false positive results. Mamunes et al[195] suggest that the necessity may originate from minute amounts of residual Treponema-specific IgM antibodies produced by a foetus previously infected but subsequently treated in utero. Remington and Desmonts (1973)[200], discussing false positive results obtained in the IgM fluorescent antibody response to congenital toxoplasmosis, suggest that there is some factor which reacts with the anti IgM conjugate having been passively transferred through the placenta from the previously infected mother to her uninfected foetus. This might also apply in cases of congenital syphilis.

Under the test conditions described, it was possible to demonstrate IgM antibodies for syphilis in both asymptomatic (sub-group c) and symptomatic (Table 88) neonates in the first week of life. The test thus fulfills a useful function in assessing, with a minimal degree of error, whether or not the infant has an infection of T. pallidum. Although these results, as with most other serological information, require clinical interpretation and follow-up, the necessity for prolonged observation of the patients, born of sero-positive mothers, before a definite diagnosis could be made or the alternative of implementing treatment in every suspected case of congenital syphilis would be obviated. This is particularly important in a community where follow-up visits to the hospital are not guaranteed.

During the course of the survey it was occasionally found that a vial of anti IgM conjugate gave an inferior fluorescence with a known 4+ reactive serum and was discarded. Results also varied to some extent with conjugate prepared by a different commercial firm[201].

Anti IgM conjugate, to give maximum sensitivity and specificity, should be standardized by testing multiple dilutions of conjugate on a number of reliable reference sera.

Summary

Anti IgM conjugate, diluted 1:40 was by far the most reliable test for predicting whether or not an infant suffered from congenital syphilis. However, it must be emphasized that a few false positive IgM 1:40 can be expected. These observations are valid for the particular

commercial brand used in these experiments.

Reagin tests are still satisfactory for the screening of possible syphilitic patients. Although many reagin reactors were observed in non-syphilitic infants, only 2/114 infants with congenital syphilis gave a negative reagin test. If all reagin positive infants are examined with IgM conjugate diluted 1:40 (or an IgM test of corresponding sensitivity using other proprietory brands of conjugate), very few patients with congenital syphilis will be missed. However, to avoid missing the occasional case of congenital syphilis due to negative reagin reactions, ideally, the serum of babies of all reagin positive mothers should be tested with the routine FTA-Abs test as well as reagin and if positive, be further investigated with the IgM test.

CHAPTER 10.

POSITIVE FLUORESCENT TREPONEMAL ANTIBODY-ABSORPTION (FTA-Abs) AND REAGIN REACTIONS IN NON-SYPHILITIC PATIENTS WITH ANTINUCLEAR ANTIBODIES

Systemic lupus erythematosus (SLE), rheumatoid arthritis, scleroderma and dermatomyositis are typical examples of a group of diseases of connective tissue, autoimmune in nature, involving immune complex mechanisms. These are known as 'the collagen diseases'[202]. As described in Chapter 2, Moore and Mohr[79] found that in these disorders, BFP reactions for syphilis may persist for many years and also that a prolonged follow-up of apparently healthy BFP reactors showed that some subsequently developed signs of collagen disease, on occasions, many years later. According to Harvey and Shulman,[203] 10 to 20 per cent of SLE cases give positive reagin tests, the majority as measured by the TPI test, being BFP reactors.

It is classical teaching that lipids, released from cells damaged in the course of syphilis, become autoantigens evoking the production of antilipid antibodies[204]. As previously discussed, these are detected in the reagin tests for syphilis; cardiolipin antigen used in these tests being a tissue phospholipid present in the organs of many species. It is thought that reagin antibodies giving rise to positive results in collagen diseases (BFP) may be produced by a similar process and are autoimmune in character[205].

Following the development of the FTA-Abs test by Hunter, Deacon and Meyer (1964)[27], evaluation studies[91,92] showed the test to be more sensitive than the TPI in early syphilis. However, it was shown that the test was slightly less specific than the TPI, since some patients with

diseases often associated with globulin abnormalities such as rheumatoid arthritis, autoimmune haemolytic anaemia due to lymphosarcoma and alcoholic cirrhosis, were found to have a reactive FTA-Abs but were without evidence of prior syphilitic infection[93]. Kraus, Haserick and Lantz (1970)[206] reported an atypical pattern of treponemal fluorescence in the FTA-Abs test with sera from four patients with active lupus erythematosus. The usual homogeneous pattern of fluorescence was replaced by a beaded appearance of treponemes. In a further study on 150 consecutive patients attending a special LE clinic, these workers reported twenty-four with a reactive VDRL, one a reactive TPI and twenty-three, none of whom had clinical or historical evidence of syphilis, with some degree of reactivity in the FTA-Abs test. The atypical beaded pattern of the FTA-Abs fluorescence was seen in eleven of the twenty-three sera[94].

Antinuclear Factor (ANF)

The discovery of the LE cell phenomenon by Hargraves, Richmond and Morton (1948)[207] and its dependence on serum factors present in lupus patients, heralded a series of observations on the immunology of SLE. In 1950, the serum factor necessary for the production of LE cells was identified as a gamma globulin[208]. Using the immunofluorescent technique of Coons[209], several workers subsequently detected antinuclear factors in SLE and sera from other collagen diseases capable of reacting with nuclei[210,211,212].

The term 'antinuclear factor' is thus used to describe any gamma globulins reacting with nuclear material using immunofluorescence and other serological techniques. Briefly, in the immunofluorescence test, a section of any fixed tissue, smear of blood or other nucleated cell suspension is

exposed to a drop of serum with suspected autoimmune antibodies, washed and stained with fluorescein labelled rabbit antibody to human gamma globulin. Using ultraviolet microscopy, such a preparation shows marked fluorescence of the cell nuclei due to their uptake of ANF which is specifically combined with the fluorescent antiglobulin reagent[212].

In SLE, there is a close agreement between the results of the cell tests and ANF tests, whereas in patients with rheumatoid arthritis ANF occurs without demonstrable LE cell activity[204]. Holborow and Johnson (1965)[213] observed that in rheumatoid sera the ANF is often destroyed by heating at 65C, while in lupus sera, ANF withstands this treatment. Antinuclear activity as detected by immunofluorescence does not always correlate with the LE cell phenomenon and suggests the presence of more than one antibody. These two diseases are clinically often difficult to separate and intermediate clinical presentations occur. It should also be realised that the pathogenesis of these diseases depends on the deposition of immune complexes consisting of Antinuclear antibody (ANF) and nuclear antigens.

Immunological techniques including precipitin[214] complement fixation[215] fluorescent antibody staining[211,216] and absorption experiments[216] have been used to demonstrate that cell nuclei, deoxyribonucleic acid (DNA) and nucleoprotein may be involved as antigens of the ANF reaction. These techniques, in addition, have shown that a variety of autoantibodies such as reagin, antismooth muscle, antimitochondrial and antireticulin, are a prominent feature of SLE and other connective tissue diseases[217,218].

The participation of DNA has been implicated since precipitin and complement fixation reactions take place between lupus serum and pure DNA preparations[219]. Furthermore, reactivity of the antigen is destroyed by

DNA-se digestion. Borba, Seligman and Joly (1960)[220] showed that thermally denatured calf thymus DNA reacted as well as the native species. Stollar and Levine (1961)[219] however, considered denatured DNA to be a more reactive antigen than native DNA.

Using cell nuclei and DNA (type unstated) in the complement fixation test, Robbins, Deicher and Kunkel (1957)[215] found that sera from patients with active SLE fixed complement with nuclei from calf thymus and DNA from various sources, including calf thymus, in roughly parallel titres. However, cross-absorption experiments with nuclei and DNA suggested the presence of two distinct serum factors.

Variations in the patterns of staining produced by human sera containing antinuclear antibodies were reported by Beck (1961)[221]. He observed the following three distinct patterns of nuclear fluorescence in sections of rat liver stained with the serum form thirty patients with SLE, Sjögren's syndrome and other collagen diseases: (1)'Homogeneous', in which the nucleus was stained throughout. (2) 'Speckled', in which the nucleus showed small uniform points or streaks of fluorescence scattered throughout its substance. (3) 'Nucleolar' in which intense homogeneous staining of the nucleolus was associated with dull homogeneous fluorescence of the rest of the nucleus. A fourth pattern was recognized by Casals, Friou and Teague (1963)[222] which they called 'shaggy' (peripheral or ring or membranous). Here, there was a diffuse or irregular margin of nuclear staining, showing a 'shaggy' appearance. These sera came from patients with SLE. It is thus clear from the literature that a number of antibodies to different nuclear constituents are identifiable in SLE and other collagen disease sera.

Investigating sera from non-syphilitic patients with connective tissue disorders, notably SLE, Neblett, Burnham Merriam and Fine (1966)[223] found that two antibodies appeared to be responsible for the simultaneous occurrence of ANF and FTA-200 reactions. They absorbed these sera with a sonicate of Reiter treponemes, whole tumour homogenate, cytoplasmic residue and cell nuclei sonicate and repeated the FTA-200 and ANF tests. The results are shown in Table 92.

TABLE 92

Absorption data on reactive FTA-200 and ANF sera from non-syphilitic patients

(From Neblett *et al.* - 1966)[223]

FTA-200 result	Reiter sonicate	Tumour homogenate	Cytoplasmic residue	Cell nuclei sonicate
No change	1	8	2	8
Slight change	4	12	6	4
Marked change	13	8	4	0
TOTAL	18	28	12	12
ANF result				
No change	6	1	1	2
Slight change	4	5	1	2
Marked change	0	6	6	6
TOTAL	10	12	8	10

Using Reiter's sonicate, 13/18 became FTA negative but none ANF negative. Whole tumour homogenate resulted in the reaction becoming negative

or reduced in 20/28 with ANF and 11/12 FTA. Cytoplasmic residue absorption showed a trend towards a reduction of the ANF while leaving the FTA variable. Cell nuclear sonicate either did not alter the fluorescence brilliance of the original FTA or, in some cases, increased it, suggesting the removal of a blocking antibody; the ANF was removed by the sonicate in 6/10 cases and reduced in two. Sera from six known syphilitic cases with negative ANF showed essentially no or only slight change in the FTA-200 test after absorbing with the same agents.

Jokinen, Lassus and Linder[224] found nine FTA-200 reactions out of 135 sera with ANF. With the FTA-Abs test three of the nine were negative and two were positive TPI. Of the remaining four cases (ANF detectable in titres ranging from 1:100 to 1:12 500), three changed from a 1+ fluorescence to non-reactive after absorption with 50 mg per ml serum of cell nuclei isolated from calf thymus. In the fourth case, the FTA-Abs reaction (originally 2+) persisted after cell nuclei absorption. It was established in this instance that although TPI negative, the patient had a past history of syphilis. All sera were negative ANF after cell nuclei absorption.

In order to establish a possible relationship between antibodies produced by SLE and other collagen diseases, this study was undertaken to investigate the behavior of reactive ANF, FTA-Abs and/or reagin sera after absorption experiments. Only small quantities of sera were available in most instances which would limit the variety of absorption tests necessary for such an investigation. It was thought, therefore, that the most representative results may be obtained by using cell nuclei from calf thymus and DNA isolated from the same source. In addition, it would be possible

to assess the prevalence of non-specific FTA-Abs and reagin antibodies in patients with positive ANF.

Material and Methods

The ANF-fluorescent antibody test was performed by the indirect immunofluorescence method with cryostat sections of rat liver as antigen[225]. All slides were read at a magnification of 320x.

Over a period of eighteen months, sera from 337 patients which showed an ANF reaction at a serum dilution of 1:10 or higher were stored at -20C. These sera had been submitted routinely for ANF testing in the investigation of collagen disease disorders. Regrettably, adequate clinical information in many instances was poor or lacking. From eight confirmed SLE patients, two or more sera were investigated. FTA-Abs and reagin (Kolmer complement fixation and the RPR modification of the VDRL flocculation) tests were then carried out on all sera; only those showing positive reactions were kept for further study. To exclude patients with specific antibodies to syphilis, the TPI test was performed on all sera showing a positive FTA-Abs and/or reagin test.

For absorption tests, cell nuclei form calf thymus were prepared according to the method of Mirsky and Pollister[226] and lyophilized. Native DNA was obtained commercially from Merck. Initially, absorption with DNA was carried out by adding 0,1 ml of serum to 0,5 ml phosphate buffered saline (PBS) containing 0,5 mg of DNA (serum dilution 1:6), but later, PBS was replaced by commercially available sorbent (Difco Laboratories). The reasons for this will be shown in the results. Similarly, for absorptions with cell nuclei, 0,1 ml of serum was added to 0,4 ml of PBS, later replaced

by sorbent, (serum dilution 1:5) containing 10 mg cell nuclei. Tubes containing DNA absorptions were shaken in a water bath at 37C for one hour and stored overnight at 4C. Tubes with cell nuclei were shaken for two hours in a 37C water bath, centrifuged and the supernatant again added to 10 mg of fresh cell nuclei for a further two hours incubation. The supernatant was also stored overnight at 4C. It was established later that no further absorption of the antibodies took place on prolonged storage.

ANF and FTA-Abs tests were then carried out on the two absorbed and their corresponding unabsorbed serum to obtain comparative results under the same test conditions. When unabsorbed positive ANF sera, originally screened at a dilution of 1:10, were titred, the first dilution was 1:16. With absorbed sera, the initial dilution in the diluents (PBS and sorbent) was taken into consideration when preparing the first dilution of 1:16.

Results

TABLE 93

FTA-Abs, reagin and TPI results on sera from 337 patients with positive ANF reactions : titres ranging from 1:10 to 1:4096

	Reactive FTA-Abs 1+ → 4+	Reactive FTA-Abs and reagin	Reactive reagin only	Reactive TPI and FTA-Abs	Total AC* to Kolmer test	Total AC* in reactive FTA-Abs and/or RPR sera
Number	51/337	6/337	14/337	5/337	48/337	9/65
Per cent	15,1	1,8	4,2	1,5	14,2	13.8

* AC = Anticomplementary.

Of the 51 sera showing reactivity in the FTA-Abs test, five were TPI positive, one of which was also positive in both reagin tests. One further serum, repeatedly anticomplementary in the TPI test, was excluded entirely from the study. Thus, 45/331 (13,6 per cent) sera showed false-positive FTA-Abs reactions. Of the 45 patients, 29 (65,0 per cent) were female and 14 (31,0 per cent) male; information on the sex of two patients was not available.

There were 14 (4,2 per cent) reagin positive sera, and all were TPI negative. Sera anticomplementary in the Kolmer complement fixation test totalled 48/337 (14,2 per cent). FTA-Abs and/or reagin activity did not appear to have any bearing on anticomplementary sera; in this group 9/65 (13,8 per cent) were anticomplementary.

TABLE 94

Degree of fluorescence and reagin results of sera from 45 patients with false positive FTA-Abs results

Degree of fluorescence	Number	Kolmer of test			RPR test	
		+	-	AC*	+	-
4+	2	1°	-	1	1°	1
3+	9	-	8	1	1	8
2+	20	1*	17	2	2*	18
1+	14	-	11	3	-	14
TOTAL	45	2	36	7	4	41

* AC = Anticomplementary

°* = Same patient

From Table 94 it can be seen that the majority of sera gave negative reagin reactions indicating that, as in the case of syphilis, two different antibodies are involved in these BFP reactions. On only four occasions was the beading phenomenon, described by Kraus, Haserick and Lantz[94], observed in the FTA-Abs test.

Absorption tests were carried out on nine sera using DNA dissolved in PBS and cell nuclei suspended in PBS. As a comparison, and in order to keep the FTA-Abs test standard by using sorbent to absorb any possible non-specific treponemal antibodies, absorption of the same sera were repeated using sorbent in place of PBS. In four sera there was insufficient serum for the DNA/sorbent absorption. Table 95 shows the results of the ANF and FTA-Abs tests performed on neat sera together with the parallel absorptions of DNA and cell nuclei in both diluents.

TABLE 95

Comperative results of serum absorptions with DNA and cell nuclei diluted in Phosphate Buffered Saline and sorbent

Patients Number	ANF titre before absorption	Pattern of staining	ANF titre after absorption with PBS: DNA	PBS: Decrease in titre	PBS: Cell nuclei	PBS: Decrease in titre	Sorbent: DNA	Sorbent: Decrease in titre	Sorbent: Cell nuclei	Sorbent: Decrease in titre	FTA-Abs before Absorption	FTA result after absorption with PBS: DNA	PBS: Cell nuclei	Sorbent: DNA	Sorbent: Cell nuclei
9553	1/16	Speckled	-	2 x	-	2 x	-	2 x	-	2 x	2 +	2 +	2 +	1 +	1 +
0545	1/64	Homogeneous	-	8 x	-	8 x	-	8 x	-	8 x	2 +	2-3+	2 +	1 +	1 +
7692	1/128	Homogeneous	1/64	2 x	-	8 x	-	16 x	-	16 x	1 +	2 +	2 +	±	±
3670	1/128	Speckled	1/128	Nil	1/128	Nil	1/128	Nil	1/128	Nil	1-2+	2 +	2 +	-	-
5000*	1/32	Homogeneous	1/16	2 x	-	4 x	-	4 x	-	4 x	1-2+	3 +	2 +	±	-
4475*	1/64	Homogeneous	-	8 x	-	8 x	INS[x]	/	-	8 x	1-2+	2-3+	3 +	INS[x]	-
1462	1/64	Speckled	1/16	4 x	-	8 x	INS	/	-	8 x	1 +	1-2+	1-2+	INS	-
1120	1/32	Homogeneous	1/16	2 x	1/16	2 x	INS	/	-	4 x	1 +	1-2+	1-2+	INS	-
4606	1/64	Homogeneous	1/16	4 x	1/16	4 x	INS	/	-	8 x	1-2+	2 +	1-2+	INS	-

x INS = Insufficient serum for absorption with DNA in sorbent

* = Same patient

ANF titres of most of the sera were reduced to some extent (2x-8x) by DNA and cell nuclei, regardless of whether PBS or sorbent was used to dilute the sera. In two sera, (1120, 4806) cell nuclei absorbed slightly more ANF in sorbent than PBS. Unfortunately there was insufficient serum for a DNA/sorbent comparison. The ANF in serum 7692 was slightly reduced by DNA (2x) in PBS but was removed by DNA in sorbent as well as with cell nuclei. One serum with a 'speckled' pattern of staining (3670) was not absorbed by either DNA or cell nuclei in either medium. This non-absorption however, was not peculiar to the 'speckled' fluorescence, as two other sera (9553, 1462) both showed a reduction in ANF titre.

Three sera with 'homogeneous' and three 'speckled' patterns of ANF staining, with titres ranging between 1/64 to 1/4096, showed no drop in titre when tested with sorbent alone.

In contrast, in the FTA-Abs test, sera absorbed with cell nuclei and DNA behaved quite differently when PBS and sorbent were used as a diluent. Compared with the standard FTA-Abs test results, the intensity of fluorescence with DNA in PBS, increased with 8/9 sera and remained the same in one. Similarly, cell nuclei in PBS showed a brighter fluorescence with 6/9 sera and equal in three. When sorbent was used on the other hand, the intensity of fluorescence was reduced by DNA and cell nuclei in all instances, some becoming non-reactive.

It was thus apparent that the combination of DNA and cell nuclei with sorbent influenced the absorbtion of non-specific FTA antibody in the sera. With these observations, it was decided to process the remaining sera, if sufficient, with DNA and cell nuclei in sorbent.

Sera were classified according to the pattern of ANF staining. Only two types were observed; the 'homogeneous' (Table 96a) and 'speckled' (Table 96b). Those sera in which the ANF was not completely absorbed showed the same fluorescent pattern as the original test. As observed by Bonomo, Tursi and Dammacco (1965)[227] in no instance did the 'homogeneous' pattern appear to mask the 'speckled'. In these cases, only one serum was received from each patient.

To assess any possible difference in the absorbing capacity of DNA and cell nuclei to sera exhibiting the two patterns of ANF staining, the degree of decrease of the ANF and FTA absorptions are shown in Table 97.

TABLE 96(a)

ANF and FTA-Abs results after absorption with DNA and cell nuclei in sorbent on sera showing a 'homogeneous' pattern of ANF staining

Patients Number	Sex	ANF titre before absorption	ANF titre after absorption with				FTA-Abs before absorption	FTA result after absorption with		Provisional Diagnosis
			DNA	Decrease in titre	Cell nuclei	Decrease in titre		DNA	Cell nuclei	
9229	M	1/64	1/16	4 x	-	8 x	2 +	-	-	Auto-immune disease
6377	F	1/256	1/64	4 x	1/64	4 x	2 +	-	-	Not stated
2515	F	1/16	-	2 x	-	2 x	2 +	1 +	1 +	Myeloma
0545	F	1/64	-	8 x	-	8 x	2 +	1 +	1 +	Rheumatoid arthritis
7692	M	1/128	-	16 x	-	16 x	1 +	±	±	Not stated
3207	M	1/64	-	8 x	-	8 x	1 +	-	-	Not stated
7684	F	1/64	-	8 x	-	8 x	1 +	-	-	Not stated
9240	F	1/64	-	8 x	-	8 x	2 +	-	-	Not stated
1120	?	1/32	INS*	/	-	4 x	1 +	INS	-	Anti-DNA antibodies
4806	F	1/64	INS	/	-	8 x	1-2+	INS	-	Jaundice
5134	F	1/64	INS	/	-	8 x	1 +	INS	-	Arthritis. SLE
6101	F	1/10	INS	/	-	/	2 +	INS	±	Jaundice
2117	F	1/256	INS	/	1/64	4 x	3 +	INS	-	SLE
9517	F	1/256	INS	/	1/64	4 x	3 +	INS	3 +	Rheumatoid arthritis

* INS = Insufficient serum for absorption with DNA.

TABLE 96(a)

ANF and FTA-Abs results after absorption with DNA and cell nuclei in sorbent on sera showing a 'homogeneous' pattern of ANF staining

Patients Number	Sex	ANF titre before absorption	ANF titre after absorption with				FTA-Abs before absorption	FTA result after absorption with		Provisional Diagnosis
			DNA	Decrease in titre	Cell nuclei	Decrease in titre		DNA	Cell nuclei	
9229	M	1/64	1/16	4 x	-	8 x	2 +	-	-	Auto-immune disease
6377	F	1/256	1,64	4 x	1/64	4 x	? +	-	-	Not stated
2515	F	1/16	-	2 x	-	2 x	2 +	1 +	1 +	Myeloma
0545	F	1/64		8 x	-	8 x	2 +	1 +	1 +	Rheumatoid arthritis
7692	M	1/128	-	16 x	-	16 x	1 +	±	±	Not stated
3207	M	1/64	-	8 x	-	8 x	1 +	-	-	Not stated
7834	F	1/64	-	8 x	-	8 x	1 +	-	-	Not stated
9240	F	1/64	-	8 x	-	8 x	2 +	-	-	Not stated
1120	?	1/32	INS*	/	-	4 x	1 +	INS	-	Anti-DNA antibodies
4806	F	1/64	INS	/	-	8 x	1-2+	INS	-	Jaundice
5134	F	1/64	INS	/	-	8 x	1 +	INS	-	Arthritis. SLE
6101	F	1/10	INS	/	-	/	2 +	INS	±	Jaundice
8117	F	1/256	INS	/	1/64	4 x	3 +	INS	-	SLE
9597	F	1/256	INS	/	1/64	4 x	3 +	INS	3 +	Rheumatoid arthritis

* INS = Insufficient serum for absorption with DNA.

TABLE 96(t)

ANF and FTA-Abs results after absorption with DNA and cell nuclei in sorbent on sera showing a 'speckled' pattern of ANF staining

Patients	Sex	ANF titre before absorption	ANF titre after absorption with				FTA-Abs before absorption	FTA result after absorption with		Provisional Diagnosis
			DNA	Decrease in titres	Cell nuclei	Decrease in titre		DNA	Cell nuclei	
7490	F	1/2048	1/64	32 x	1/64	32 x	2 +	-	-	SLE
5094	F	1/256	1/64	4 x	1/64	4 x	2 +	-	-	Pulmonary TB
7113	F	1/256	1/64	4 x	1/32	8 x	2 +	-	-	Not stated
3670	F	1/128	1/128	Nil	1/128	Nil	1-2+	-	-	Not stated
5066	M	1/32	1/16	2 x	-	4 x	1 +	-	-	Not stated
7650	F	1/256	1/64	4 x	1/64	4 x	2 +	1 +	1 +	Rheumatoid arthritis. SLE
9366	F	1/256	1/64	4 x	1/32	8 x	3 +	1 +	1-2+	Not stated
3545	F	1/128	1/64	2 x	1/64	2 x	3 +	1 +	1 +	Not stated
9553	F	1/16	-	2 x	-	2 x	2 +	1 +	1 +	SLE
9166	F	1/512	INS*	/	1/16	16 x	2 +	INS*	±	SLE
8119	F	1/2048	INS	/	1/256	8 x	3 +	INS	2 +	Not stated
1426	F	1/64	INS	/	-	8 x	1 +	INS	-	Raynaud's Disease

* INS = Insufficient serum for absorption with DNA.

TABLE 97

Comparison of the absorbing capacity of DNA and cell nuclei on ANF and FTA-Abs antibodies based on the ANF 'homogeneous' and 'speckled' pattern of staining

Pattern of staining	Sera absorbed with	Decrease in ANF titre								Decrease in FTA fluorescence intensity					
		8x or greater		4x		2x		No change		To negativity		Reduced		No change	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Homogeneous	DNA	5/8	63	2/8	25	1/8	12	-	-	5/8	63	3/8	37	-	-
	Cell nuolei	8/13	62	4/13	30	1/13	8	-	-	9/14	64	4/14	28	1/14	8
Speckled	DNA	1/9	12	4/9	44	4/9	44	-	-	5/9	55	4/9	45	-	-
	Cell nuclei	6/12	50	3/12	25	2/12	17	1	8	6/12	50	6/12	50	-	-

Although the number of tests performed is too small to draw statistical conclusions, DNA appeared to remove more ANF in the 'homogeneous' group than the 'speckled' (in the '8x or greater' column, 5/8 = 63 per cent 'homogeneous' compared with 1/9 = 12 per cent 'speckled'). With cell nuclei, there was little difference in the degree in which the ANF titre was reduced in the two groups. With the FTA test, all sera, with one exception, showed reduction in fluorescence or became negative. That 9597 (Table 96a) showed no decrease in titre after absorption is difficult to explain.

More than one serum was submitted from eight patients who were finally diagnosed as having SLE. A 'homogeneous' pattern of staining was seen in all sera of seven cases and 'speckled' in eighth. The absorption results of these sera are presented in Table 98.

TABLE 98

ANF and FTA-Abs results after absorption with DNA and cell nuclei on sera from eight patients, clinically diagnosed as SLE, from whom more than one serum was submitted

Patient	Serum	Sex	Type of fluorescence	ANF titre before absorption	ANF titre after absorption with				FTA-Abs before absorption	FTA-Abs result after absorption with	
					DNA	Decrease in titre	Cell nuclei	Decrease in titre		DNA	Cell nuclei
1	3646	F	Homogeneous	1/256	1/32	8 x	-	32 x	3 +	-	-
	16700			1/256	1/32	8 x	-	32 x	2 +	-	-
	0304			1/128	1/16	8 x	-	32 x	3 +	-	-
2	7169	F	Homogeneous	1/512	INS*	/	1/64	8 x	2 +°	INS*	-
	0132			1/64	INS	/	-	8 x	3 +°	INS	-
	6764			1/128	1/16	8 x	-	16 x	3 +	-	-
3	0244	F	Homogeneous	1/64	-	8 x	-	8 x	3 +	-	-
	8034			1/256	1/16	16 x	-	32 x	3 +	-	-
	9698			1/128	-	8 x	-	8 x	4 +°	-	-
4	0100	F	Homogeneous	1/256	1/32	8 x	1/16	16 x	2 +	-	-
	8234			1/64	1/16	4 x	1/16	4 x	2 +	-	-
5	1727	F	Homogeneous	1/32	-	4 x	-	4 x	2 +	-	-
	7441			1/64	1/16	4 x	-	4 x	1 +	-	-
6	5847	M	Homogeneous	1/64	1/16	4 x	1/16	4 x	2 +	-	-
	26700			1/512	1/16	32 x	-	64 x	3 +°	-	-
7	4475	M	Homogeneous	1/32	INS	/	-	4 x	1-2+	INS	-
	5600			1/64	-	8 x	-	8 x	1-2+	±	-
8	4258	F	Speckled	1/4096	1/512	8 x	1/512	8 x	3 +	1 +	2 +
	3633			1/4096	1/512	8 x	1/512	8 x	2 +	1 +	±
	6383			1/128	1/128	Nil	1/128	Nil	2 +	1 +	2 +

* INS = Insufficient for absorption with DNA

° = Beaded appearance of fluorescence

With this group of sera from patients with confirmed SLE, a much clearer pattern of results emerge. With one exception (patient 7, sera 5600), DNA and cell nuclei removed the fraction which produces the FTA fluorescence in all sera which showed a 'homogeneous' type of ANF fluorescence. In all instances there was at least a four fold drop in ANF antibody titre. In contrast, the three sera from patient 8 which produced a 'speckled' pattern of ANF fluorscence only showed a slight reduction in intensity of fluorescence after absorption; none became negative. The ANF titre was reduced in two of these sera by DNA and cell nuclei but the third (6383) was unaffected, as was serum 3670 (Table 96b). It appears that the ANF in sera with a 'speckled' staining tend to be less affected by absorption, particularly with DNA (Table 97), than the 'homogeneous'.

The beading phenomenon was present in four sera from three patients, all in the 'homogeneous' group. One could speculate that beading is peculiar to this group, but confirmation would require many more observations.

The results of a control group of sera consisting of four of the five TPI reactive sera (Table 93) with positive ANF, all showing 'homogeneous' staining, and three positive TPI with negative ANF are presented in Table 99.

TABLE 99

ANF and FTA-Abs results on TPI positive sera
before and after absorption with DNA and cell nuclei in sorbent

Patient's number	TPI specific Immobilisation	ANF ticre before absorption	ANF after absorption with		FTA-Abs before absorption	FTA-Abs after absorption with	
			DNA	Cell nuclei		DNA	Cell nuclei
6811	50	1/32	-	-	2 +	2 +	2 +
2180	72	1/16	-	-	3 +	3 +	3 +
0110	68	1/256	1/64	1/32	3 +	3 +	3 +
3929	83	1/16	INS*	-	3 +	INS	3 +
6067	100	-	ND°	ND	4 +	4 +	4 +
1709	100	-	ND	ND	3 +	3 +	3 +
1901	100	-	ND	ND	4 +	4 +	4 +

INS* = Insufficient for absorption with DNA

ND° = Not done

The four ANF positive sera again showed a 2-8 times decrease in titre after absorption with DNA and cell nuclei. However, with the FTA-Abs test, no decrease in fluorescent intensity was noted, indicating a syphilis-specific antibody. Two non-reactive FTA-Abs, positive ANF sera, a 'homogeneous' and a 'speckled' type, showed a characteristic drop in titre after absorption.

On initial testing, reagin positive, FTA-Abs non-reactive sera (14/337, Table 93) showed, with one exception, low antibody content. Unfortunately the low titred sera became non-reactive after storage for several months at -20C. The one serum, originally showing a Kolmer titre of 32 units and RPR positive (not titred) had dropped to four Kolmer units and eight RPR units. The serum was absorbed twice at 37C with 25 mg cell nuclei per 0,5 ml serum for two hours and re-tested. Both the Kolmer and RPR tests were negative. However, a further study on fresh sera showing positive ANF and reagin tests would be necessary before any conclusions on the absorbing property of cell nuclei for reagin antibody could be drawn.

DISCUSSION

In this group of patients with positive ANF sera, 45/331 (13,6 per cent, Table 93) showed a false positive FTA-Abs test. Although in only fourteen cases was there a definite diagnosis of SLE, the prevalence of 13,6 per cent with false positive FTA-Abs tests in this group of patients showing autoimmune ANF antibodies, is similar to that of 15,3 per cent (23/150) patients attending a L.E. clinic reported by Kraus, Haserick and Lantz (1970)[94]. As in their study, the majority of patients were females.

Measured by the complement fixation and flocculation test, the number of reagin reactors was low compared with studies by other workers; only 20/331 (6.0 per cent) being evident. Lee (1956)[228] estimated 25-40 per cent of SLE cases gave reagin BFP reactions. Rein, Chargin and Kelcec (1957)[229] found sera from 35 of 79 (44 per cent) SLE patients with positive STS in one or more of five tests in the reagin battery. However, as reported by Haserick and Long (1952)[230], reagins rise and fall with the clinical course of the disease. It may well be that many of the sera in this group are from patients already undergoing some form of treatment. Again, the diagnosis of SLE was only confirmed in a small number of sera.

It is well known that an increased number of sera from SLE patients give anticomplementary results. Lange, Wasserman and Slobody (1960)[231] found 25 per cent of sera from cases of active SLE were anti-complementary and the remaining all showed markedly lowered serum complement levels. Rein, Chargin and Kelcec (1957)[229] described a similar prevalence of 20 per cent anticomplementary sera. Morse, Müller-Eberhard and Kunkel (1962)[232] suggest that the low serum complement levels are due to complement consumption by immune complexes *in vivo* in cases of SLE, and anticomplementary results are due to the consumption of complement by 'autoantibodies' *in vitro*. In this series of positive ANF sera, 14,2 per cent (Table 93) were anticomplementary, which, as with the reagin reactors, is slightly lower than in other studies. That some of these sera were not necessarily from SLE patients, may again account for this difference.

The results seen in Table 94 suggest that the FTA-Abs antibodies are not associated with reagin in non-syphilitic patients with positive ANF tests. Of these sera only 2 and 4 of 45 sera gave positive complement

fixation and flocculation reactions respectively. Furthermore, 14 BFP positive reagin sera showing ANF antibodies were non-reactive in the FTA-Abs test.

Antinuclear antibody absorptions with DNA and Cell nuclei

The literature in this field indicates that a number of different antibodies to different nuclear constituents are identifiable in SLE and other connective tissue disease sera.

Antibody to Deoxyribonucleoprotein (DNAP) gives rise to the LE-cell phenomenon and 'homogeneous' nuclear staining[217,233]. This antibody is absorbed by DNAP but not by either DNA or histone alone and is regarded as specific to DNAP[221,233]. The antibody giving rise to the 'speckled' pattern of nuclear staining has been shown to be from a saline soluble protein antigen, present in nuclei, which is not part of the DNAP complex[233]. This antigen corresponds to the buffer-extractable nuclear antigens described by Holman, Deicher and Kunkel (1959)[234] in their complement fixation studies. The third nuclear antibody which reacts with the nucleoli of liver cell nuclei may be due to a protein which reacts with the structural ribonucleic acid of the nucleolus[233]; these have only rarely been found in SLE. Finally, the fourth nuclear antibody shown to react with the peripheral part of the nucleus ('shaggy' pattern of immunofluorescent staining) has been identified as antibody to DNA[222].

In a study of 539 patients with autoimmune and clinically related conditions, Beck (1961)[221] showed that 'homogeneous' and 'speckled' patterns of staining were far more common than 'nucleolar' or 'shaggy'. In the SLE group of 57 cases in their series, 33/48 showed a 'homogeneous'

and 15/48 a 'speckled' type of fluorescence at a serum dilution of 1:16. Bickel, Barnett and Pearson (1968)[235] however, found that patterns of any type may occur with sera containing anti-DNA antibody and the selection of nuclear substrates could also influence the patterns. In this present study, not limited to SLE cases alone in which there was sufficient serum to carry out absorption tests, 14/26 showed the 'homogeneous' staining pattern and 12/26 'speckled'. Of the confirmed SLE cases, the 'speckled' staining was seen in the serum of only one of eight patients, the others showing 'homogeneous'.

Irrespective of the ANF pattern of fluorescent staining, some measure of absorption by DNA and cell nuclei was observed in practically all the sera shown in Tables 96(a) and 96(b). With DNA, more ANF was absorbed in the 'homogeneous' group than the 'speckled' (Table 97 - column : 8x or greater). This is understandable, since one would expect the saline-soluble protein antigen from cell nuclei rather than DNA to absorb the antibody responsible for the 'speckled' appearance[233].

That DNA was capable of absorbing some part of the ANF in the 'homogeneous' group is contrary to the findings of other workers who have established that absorption with DNA alone would not lower the amount of ANF in a serum[221,227]. If absorption is only effective with a DNAP one must consider that the commercial DNA used in this study contained a protein impurity to obtain these results. Information from the manufacturers of the DNA as to the purity of the product was not forthcoming. However, a report of an analysis of one commercial DNA preparation indicated 10 per cent amino acid by weight of DNA[210], and another, a protein content of up to 1 per cent[214]. As a screening test, the addition of trichloacetic acid to the commercial DNA preparation used in the present study showed

traces of protein. A more accurate analysis, using an automated modification[236] of the Lowry method[237] of protein analysis, showed protein contamination of the DNA of almost 1,0 per cent. This may have had some bearing on the absorption results.

Comparing the efficacy of cell nuclei to absorb ANF in the two groups (Tables 96a and 96b), little difference was observed. Cell nuclei with their multiple antigen content, including DNA, nucleoprotein and saline-soluble protein, were capable of removing some of the ANF in both groups. Why the two sera both showing a 'speckled' type of fluorescence (3670 - Table 96a and 6383 - Table 96b) were not absorbed at all by DNA or cell nuclei is difficult to understand. It would appear that these two sera contained yet another antibody, dissociated from the antigens found in cell nuclei or the DNA complex, but which still gave a positive ANF test.

DNA and cell nuclei varied in their ability to absorb the ANF in both 'homogeneous' and 'speckled' groups. In all sera where the ANF titre was 1/256 or higher, DNA did not entirely absorb out the ANF. The same applied to the majority of sera when absorbed with cell nuclei. Considering the sera with low titres, many were absorbed entirely while in others the titre was only reduced two or four-fold. Since the quantity of DNA and cell nuclei used were regarded as sufficient to block any ANF[224, 238] one could speculate that many of the sera contained additional antibodies unaffected by these substances. In no instance of incomplete absorptions was the pattern of fluorescence seen to alter (e.g.'homogeneous' to 'speckled') as has been reported by some workers[227].

FTA absorptions with DNA and cell nuclei

The role of sorbent

The absorbing agent of ultrasonically disintegrated Reiter treponemes, originally used in the FTA-Abs test is difficult to prepare commercially on a large scale. Because of this, sorbent, which consists of a heated, concentrated filtrate of a Reiter treponeme broth culture, was developed. As described in Chapter 2, evaluation studies using sorbent showed the FTA-Abs test to be more sensitive than and almost as specific as the TPI test. It was thought that its activity was due to the antigenic liberation of material from the Reiter treponeme which reacted with a non-specific group antibody common to *T. pallidum* and other commensal treponemes. Thereafter, several workers investigated the properties of sorbent. Cannefax, H nson and Skaggs (1968)[239], testing the uninoculated medium and media components, demonstrated that pancreatic digests of casein and yeast extract were capable of sorbing or blocking the reactivity of non-syphilitic sera when used in place of the usual sorbent of the FTA-Abs test.

Rathlev (1968)[240] suggested that factors, such as low pH, concentration of the ingredients and physical adsorptions of the serum components by the sorbent are partially responsible for the decrease in the degree of fluorescence. Wilkinson and Ferguson (1968)[241] confirmed the sorbing activity of the medium as being equal to the same mediu n which the Reiter treponemes had been cultured. This they found was due to the casein digest in the medium. In a further study, Wilkinson and Wiseman (1971)[242] reported

(a) the presence of Reiter antigens in sorbent which could be one of the factors responsible for removing group-reactive anti-tremonemal antibody

(b) that a 10 per cent sodium chloride solution with a high osmolarity similar to sorbent could abolish the reactivity of some non-specific sera and also reduce the fluorescence shown by syphilitic sera

(c) that ultrasonicate of Reiter treponemes was a more effective and reliable reagent than sorbent but of limited practical value as previously discussed. These workers felt that the blocking ability of sorbent was, to some extent, due to an antigen-antibody reaction but mainly to the non-specific effect from the high concentration of salts and protein breakdown products in the medium.

It appears from this investigation that sorbent played an important role in the absorption of non-specific antibody demonstrable by the FTA-Abs test in ANF reactive sera. From the preliminary absorptions seen in Table 95, the intensity of fluorescence of the sera tested by the standard FTA-Abs test was increased in eight of nine cases when the serum was diluted in PBS containing DNA and cell nuclei instead of sorbent. This could possibly be due to two factors: the sera may have contained some measure of commensal treponemal group reactive antibody which would have been removed by the sorbent, and secondly, DNA and cell nuclei may have partially removed a blocking antibody as hypothesized by Neblett *et al.*[223]. Since the addition of DNA and cell nuclei to sorbent reduced the intensity

of fluorescence, on many occasions to nil, it appears that these two substances or parts thereof combine with sorbent to form a complex which absorbed the antibody causing fluorescence of the *T. pallidum* antigen.

Comparing the decrease in FTA fluorescence in the two different ANF staining patterns following absorption procedures (Table 97), there appeared to be little difference. However, it is interesting that in those patients in which a firm clinical diagnosis of SLE was made (Table 98), patient 8 whose sera produced a 'speckled' ANF type of fluorescence, DNA and cell nuclei only slightly reduced the FTA fluorescence in all three sera. This was contrary to the findings in the sera of the other seven patients with 'homogeneous' staining. It could be that, in some cases, a different 'FTA' antibody is produced in those SLE patients with a 'speckled' ANF. Although clinical information was in most cases inadequate, similar results of incomplete fluorescent absorption were apparent in sera of three of four cases showing a 'speckled'ANF pattern, provisionally diagnosed as SLE (Table 96b).

When sera containing specific antibody to syphilis (Table 99) were subjected to similar absorptions, no change in intensity of fluorescence could be noted. While this appears to verify the specificity of treponemal antibody, this could only be confirmed by obtaining similar results on unabsorbed and absorbed titrated sera. Absorptions on the four ANF positive sera paralleled the results of non-syphilitic sera. It is also possible that the fluorescent staining of the spirochaete antigen by the positive ANF sera could be attributed to both specific and non-specific antibody, in which case some absorption of antibody would be apparent on titration whereas there may be none in sera containing only specific syphilis antibody.

In this study, the beading phenomenon was observed in only four sera from three of eight patients with confirmed SLE. This prevalence is lower than reported by Kraus *et al.* (11/23)[94]. The difference could be attributed to the fact that the sera had been stored for some months at -20C. McKenna, Schroeter, Kierland, Stilwell and Pien (1973)[243] noted the beading effect to be labile in 25 per cent of their specimens kept in the refrigerator or stored at -70C. It is interesting that these workers experienced the beading effect in connective tissue disorders in addition to patients with SLE.

In electron microscopy studies on the beading phenomenon with LE sera, Strobel and Kraus (1972)[244] used ferritin-conjugated antihuman IgG. While conceding that beading may possibly be due to rabbit nuclear material clinging to the *T. pallidum* antigen, these workers favoured the suggestion that it appeared more likely that LE antibody, as detected by the ferritin marker system, reacts at breaks or twists in the organisms outer envelope. The cell's DNA and nucleoprotein would then be exposed and react with the ANF antibody. In a previous study, Kraus, Haserick, Logan and Bullard (1971)[245] found that the occurrence of beaded FTA-Abs fluorescence was associated with the presence of anti-nucleoprotein, anti-DNA and LE cell factors. Incubating sera with DNA or nucleoprotein, or by treating the *T. pallidum* antigen with DNA-se, either inhibited the reaction resulting in the beaded FTA-Abs fluorescence or changed it to a homogeneous appearance. Furthermore seven of their LE sera with homogeneous FTA-Abs fluorescence were unaffected by treatment with DNA or nucleoprotein. It is not clear however, which mechanism determines the homogeneous fluorescence of the *T. pallidum* cell.

Absorption findings of the sera showing beaded fluorescence in this study agree with the above results where cell nuclei effectively removed the factor causing fluorescence in all four sera and two in which DNA was used (Table 98). In contrast, however, all sera (9597 - Table 96a excepted) with homogeneous FTA staining either showed a reduction in intensity of fluorescence or became non-reactive after absorption with DNA and cell nuclei. This may be explained by differences in absorbing techniques applicable to the two studies. Kraus *et al.*[243] absorbed their sera by incubating the DNA and cell nuclei in saline whereas sorbent was used in this study. This difference parallels that experienced when sorbent was used in place of PBS (Table 95).

This investigation has demonstrated that sorbent in conjunction with DNA and cell nuclei is necessary for partial or complete absorption of the antibody in some reactive ANF sera which produce fluorescence of the *T. pallidum* antigen. A further detailed study would be necessary to determine which fraction or fractions of sorbent in combination with DNA or cell nuclei would give the same absorption properties.

From the absorption results of the present study and those of other workers, it is obvious that several antibodies reacting with nuclei and cross reacting with *T. pallidum* are present in patients with collagen diseases. This may be due to tissue breakdown caused by soluble immune complexes, resulting in the production of multiple autoantibodies. However, evidence of deficient T- lymphocyte function has been reported in animals with a predisposition to collagen disease[246]. T- lymphocytes are known to exert a helper-function in the production of antibodies by B- lymphocytes and plasma cells. If the modulating effect of T- lympho-

cytes is disturbed, it is possible that uncontrolled B- lymphocytes may produce a variety of antibodies, including autoantibodies, much more easily[247].

Conclusions

Absorption experiments in this investigation have shown that, in most instances, cell nuclei results have paralleled those of DNA. This is understandable since cell nuclei contain DNA as well as other components. Variations in the patterns of ANF fluorescence were seen in the sera examined but the antibodies in almost all were absorbed by DNA and cell nuclei. Why it was not possible to absorb sera with high titres completely may relate to other factors. That more than one antibody is produced by collagen diseases was shown by the fact that more antibody, attributed to a saline soluble nuclear antigen, was absorbed by cell nuclei in the 'speckled' group than by DNA. However, in two cases no reduction was seen, possibly indicating response to a different source of antigenic stimulus or perhaps some blocking factor in the serum, inhibiting the absorption process.

Evidence of a further antibody is apparent in the FTA-Abs test. Here, absorption is only expedited when sera are incubated in sorbent containing DNA and cell nuclei. It appears that DNA and components of sorbent, compounded in some form are necessary to evoke this absorption. The presence of an additional antibody is suggested by three confirmed SLE sera showing speckled ANF staining in which the FTA was only slightly reduced whereas in the 'homogeneous' ANF group all became FTA negative.

However, by virtue of the absorption results of DNA and cell nuclei, there are indications that both ANF and FTA antibodies share common factors. As previously stated, too little evidence is presented to draw any conclusions regarding an association between reagin and the ANF and FTA antibodies.

The results of this investigation have, above all, outlined the complexity of this field of study. It is apparent that a further project, with clearly defined clinical information, additional absorbing material (e.g. nucleoprotein, single and double stranded DNA) and further tests, such as precipitation, complement fixation and specific enzyme inhibition studies, desirable. Such an investigation in greater detail may clarify the association between the various autoimmune antibodies produced in connective tissue disorders.

SUMMARY

1. Of 337 patients with positive ANF reactions, 51 (15,1 per cent) gave a reactive FTA-Abs test; five (1,5 per cent) were positive TPI. Only 14/337 (4,2 per cent) sera showed reagin and as expected disorders in sera from patients with connective tissue disorders 14,2 per cent were anticomplementary in the Kolmer Complement Fixation test. The FTA-Abs reactive sera did not appear to correlate with those showing reagin. The beading phenomenon was noted on only four occasions in the FTA-Abs test.

2. Nine reactive ANF and FTA-Abs sera were absorbed with DNA and cell nuclei in PBS and sorbent. In PBS and sorbent, only one serum failed to show a reduction in ANF titre. With FTA on the other hand, intensity of fluorescence either increased or was equal to the standard FTA-Abs reading in PBS, but in sorbent, decreased, in many cases to negativity. This suggests that a combination of DNA and cell nuclei with sorbent com[illegible]s is necessary to absorb the FTA antibody. Sorbent was used as a diluent for the rest of the study.

3. Positive ANF and FTA-Abs sera were divided into their ANF fluorescent staining patterns. Only 'homogeneous' and 'speckled' patterns were seen. DNA and cell nuclei absorbed some ANF [illegible]i-body in a large majority of sera in both groups. In the 'homogeneous' group however, DNA removed more ANF then in the 'speckled'. This is in keeping with previous reports that the 'speckled' pattern is due to antibodies produced by saline-soluble nuclear antigens. With cell nuclei there was little difference in the degree in which the ANF titre was reduced in the two groups. With the FTA test, with one exception, all sera, including those in which SLE was confirmed, showed reduction in fluorescence or became negative. In the SLE group, sera with a 'speckled' ANF pattern were not absorbed to the same extent as the 'homogeneous'.

4. That DNA absorbed ANF sera with 'homogeneous' staining is contrary to the findings of other workers who established that deoxyribonucleoprotein was responsible for the production of this

antibody. This is possibly due to contamination of the DNA preparation with protein.

5. Absorption of positive TPI sera with DNA and cell nuclei did not show any reduction of the FTA-Abs antibody. ANF titres were not affected by sorbent alone.

6. That one FTA and two ANF positive sera were unaffected by absorptions suggest antibodies to different antigens. Furthermore, presumed adequate quantities of DNA and cell nuclei were unable to completely absorb ANF reactive sera with high titres.

7. While antibodies demonstrated in the ANF and FTA-abs fluorescent reaction have been shown to some extent to share common antigens, those few sera which behaved differently would appear to contain a[illegible]ties from other sources. Furthermore, the fact that the FTA antibody requires sorbent in addition to DNA and cell nuclei for absorption and the ANF sera do not, suggests that different factors are involved.

8. A more extensive study is required for further investigation of autoimmune antibodies in collagen diseases.

CONCLUDING REMARKS

It was through the introduction of the specific treponemal test, the Treponema Pallidum Immobilisation (TPI), that it was possible to assess the true prevalence of treponematosis in some population groups in Southern Africa. This confirmed the previous contention, based on reagin tests, that treponematosis in the Bantu was high. Furthermore, it was established that there were relatively few 'biological false positive' (BFP) reactors in the groups studied. In such population groups the comparatively inexpensive but less specific non-treponemal tests such as the Kolmer Complement Fixation and VDRL flocculation are invaluable. However, as illustrated in the group of leprosy patients, the use of specific treponemal tests is essential in assessing 'difficult' cases where there is a possibility of patients with positive reagin tests being BFP reactors. These two types of tests, properly utilized and interpreted, leave relatively few diagnostic problems.

Following the development of a further specific treponemal test, the Fluorescent Treponemal Antibody-Absorption (FTA-Abs) test, a fluorescent-antibody conjugate, specific for IgM antibody, made it possible to distinguish between an infant who is merely carrying antibodies passively transferred from the mother and an infant who is actively infected with syphilis.

The problem of venereal disease remains one of great magnitude, not only in developing countries such as Southern Africa but in the developed countries of the Western world. This is inspite of the continuing effectiveness of penicillin, the efforts to educate the public of

the seriousness of the disease and improved medical education.

Modern treponemal tests for syphilis have played a vital role over the last two decades in the understanding of the disease. Research activities continue to be directed towards the improvement of serological tests for the diagnosis of syphilis and a vaccine against the invasion of the bacteria. However, more fundamental knowledge about the nature of the organisms is essential; how they divide and multiply; their structure and chemistry; the disease process and immunological aspects in the body.

When a method of cultivating *Treponema pallidum* in the laboratory has been accomplished, great strides will have been made towards solving these problems and the development of an effective immunization agent on a large scale.

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