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CONGENITAL SYPHILIS AND RHEUMATOID FACTOR

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DECLARATION

I, Michael Peter Meyer, hereby declare that this thesis is my own work and has not been presented for any degree at another university.

Signed: Signed Date: 15/3/90

The work reported in this thesis was performed in the Department of Paediatrics and Child Health, University of Cape Town, Cape Town.

TO JILL, SARAH AND TERESA

ABSTRACT

Rheumatoid factor and testing for congenital syphilis

The usefulness of the rheumatoid factor (RF) latex test in the diagnosis of congenital syphilis was investigated in 2 different settings.

Newborn infants at high-risk of congenital syphilis comprised one setting. In this group there were 69 infants born to mothers with untreated or inadequately treated syphilis. Asymptomatic patients born to these mothers were followed up for a minimum of 3 to 4 months to allow serial monitoring of VDRL titres. Altogether, 15 infants developed congenital syphilis according to previously defined criteria. The sensitivities, specificities and positive and negative predictive values of various tests performed at birth were compared.

Tests included the RF latex test, total IgM estimation, radiography of long bones and the FTA-ABS (IgM) test (the latter test was performed after IgG and RF removal). The overall performance of the RF latex test was better than that of the other tests performed on the infants even though the sensitivity was 46.7%. The specificity and positive predictive value of the RF latex test was 100% in the population studied, whilst the negative predictive value was 84.6%. The test was negative in all 85 controls tested. The test is simple, can be rapidly carried out and is relatively inexpensive. As a result of the investigation it was apparent that although a negative RF latex test did not exclude congenital syphilis in an asymptomatic infant, a positive test in the presence of maternal syphilis should lead one to strongly suspect congenital syphilis.

By serendipity it was found that a maternal VDRL titre of 1:32 or more in the last month of pregnancy was a sensitive indicator of which infants would develop congenital syphilis (sensitivity 93.3%). The positive predictive value was 58.3%. The VDRL tube test is inexpensive but is best performed by a central reference laboratory.

The RF latex test and the maternal VDRL test carried out at the time of birth appeared to have a place in patient management. A positive RF latex test obtained before discharge would indicate which high-risk newborns should be further investigated and/or treated. The maternal

VDRL titre result, likely to obtained after patient discharge, could help select which infants should be further managed as outpatients. The use of these measures would allow maximal utilisation of hospital beds, and would allow detection of the vast majority of affected infants.

In a separate study, the RF latex test was evaluated as a simple side-room investigation to screen for congenital syphilis in infants who presented with 1 or more clinical signs suggestive of the disease.

Forty nine infants between the ages 0 and 4 months were investigated. Thirty four of these were finally diagnosed as having congenital syphilis: the RF latex test correctly identified 32 patients (94%). The remainder (15) suffered from a variety of conditions - 3 cases with a congenital infection had positive RF latex tests.

False-positive tests were not found in 85 newborn controls, but 5 of 46 control infants between the ages of 1 and 4 months had positive RF latex tests. These results indicated that the RF latex test is a valuable screen for congenital infection, particularly amongst neonates. The test did not distinguish between different types of chronic intrauterine infection. Its diagnostic value for congenital syphilis would depend on the prevalence of the condition in the study population.

Finally, the effect of eliminating RF interference on the FTA-ABS (IgM) test was investigated. Rheumatoid factor activity was successfully removed from 89% of cases using an immunoprecipitation procedure.

Amongst symptomatic infants with congenital syphilis the FTA-ABS (IgM) test was positive in 34 (92%) of 37 cases prior to abolishing the RF effect and in 29 (78.4%) of 37 cases afterwards. In 12 cases of congenital syphilis who were asymptomatic at birth, 10 had positive FTA-ABS (IgM) tests before RF removal and only 3 had positive tests afterwards. This difference was statistically significant ($p < 0.05$ using the Chi-square test). The false-positive rate of the FTA-ABS (IgM) was less than 5% in newborns and 13% in older infants. The false-positives appeared to be eradicated by the IgG precipitation step.

It was concluded that the improvement in the specificity of the FTA-ABS (IgM) test following IgG and RF removal was at the expense of a loss of

sensitivity, particularly in asymptomatic newborns. As this is the group where sensitive tests are most needed it is important that RF is not removed. In fact, it was shown that if the FTA-ABS (IgM) test was positive the patient was likely to require treatment for congenital syphilis, regardless of whether the test was positive due the presence of RF or specific IgM.

Rheumatoid factor and the immune response in congenital syphilis

To allow more accurate quantitation of the RF concentrations in patients with congenital syphilis an IgM RF ELISA was established. The RF levels obtained in 26 newborns with congenital syphilis (median 29.5 IU/ml) were generally higher than those observed in controls (95th percentile 2.5 IU/ml).

Nevertheless, the IgM RF levels in controls were found to rise outside the immediate newborn period. It was suggested that the RF observed in the controls might represent IgM antibodies against genetic markers (such as the Gm antigens) on maternal IgG.

Further characterisation of the RF response in congenital syphilis indicated that there was little hidden RF and IgG and IgA RF were not detected in the 4 patients studied.

Determining the percentage of the total IgM which was RF led to the conclusion that the mean value was 4%. This was considered to be an interesting finding since RF-producing B cells are more plentiful in cord blood (Hardy et al 1987). One may, therefore, have expected a greater percentage of IgM RF in the infants.

The quantitation of RF in the infants with congenital syphilis allowed the values to be ranked. When non-parametric statistical tests were applied, significant associations ($p < 0.05$) were noted between the RF levels and a number of variables . The most important of these were the severity and extent of the disease and the levels of circulating immune complexes (measured using a C1q binding assay). In addition, liver disease, kidney disease and oedema were also associated with elevated RF levels although it is possible that these findings indicated the presence of more severe disease.

Following treatment, the changes in RF levels and VDRL titres correlated with one another ($p < 0.001$).

Frozen tissue sections from 3 placentae of infants with congenital syphilis were stained with fluorescein-labelled heat-aggregated IgG. Positive fluorescence, thought to be indicative of the presence of RF was noted in many of the small blood vessels and in the stroma of the villi. Minimal positive staining was detected in placentae from control infants.

The results suggested that RF was closely related to the inflammatory process in congenital syphilis. Whilst it was difficult to separate pathological from physiological effects, taken together with evidence from experimental animals it seemed likely that RF may aggravate the inflammatory effect of immune complexes in congenital syphilis. If this is the case, the enhanced tendency to produce RF in early life may partly explain the fact that congenital syphilis is the most severe form of syphilis.

Future research might be directed towards reducing the host inflammatory response in congenital syphilis.

PUBLICATIONS

The following publications related to this thesis:

1. Meyer MP, Malan AF. Rheumatoid factor in the diagnosis of congenital syphilis. S Afr Med J 1987; 72:668-669.

2. Meyer MP, Malan AF. Rheumatoid factor in congenital syphilis. Genitourin Med 1989; 65:304-307.

3. Meyer MP, Woods DL. The treatment of congenital syphilis with penicillin. S Afr Med J 1989; 76:448.

4. Meyer MP. The prevalence of congenital syphilis in Cape Town. S Afr Med J 1990; 77:539.

5. Meyer MP, Malan AF. Risk factors for congenital syphilis. Ann Trop Paediatr (in press).

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SYMBOLS AND ABBREVIATIONS

BSA	Bovine serum albumin
CDC	Centers for Disease Control
CMV	Cytomegalovirus
CNS	Central nervous system
CSF	Cerebrospinal fluid
DNA	Deoxyribonucleic acid
ELISA	Enzyme linked immunosorbent assay
FTA-ABS	Fluorescent Treponemal Antibody Absorption
g	Gram
G	Gravitational force
HaIgG	Heat-aggregated IgG
HIV	Human immunodeficiency virus
IM	Intramuscular
kDa	kilo Dalton
kg	Kilogram
l	Litre
ml	Millilitre
mo	Month
MOU	Midwife obstretric unit
No	Number
PBS	Phosphate buffered saline
PMNS	Peninsula Maternity Neonatal Service
RA	Rheumatoid arthritis
RCWMCH	Red Cross War Memorial Children's Hospital
RF	Rheumatoid factor
Rh	Rhesus
RIA	Radioimmunoassay
RPR	Rapid plasma reagin
SBE	Subacute bacterial endocarditis
STS	Serological test for syphilis
T.pallidum	Treponema pallidum
TPHA	Treponema pallidum haemagglutination
u	Units
UK	United Kingdom
ul	Microlitre
USA	United States of America
VDRL	Venereal Disease Research Laboratory

w	Week
WHO	World Health Organisation
WR	Wasserman reaction
+ve	Positive
-ve	Negative

INTRODUCTION

Congenital syphilis is still a disease of considerable public health importance in underdeveloped countries. Not only is it a common condition (e.g. 1% of live births in a Zambian teaching hospital were reported to have clinical signs of the disease - Hira et al 1982) but it is also associated with a considerable mortality. It has been estimated, for instance, that in many parts of Africa up to 8% of all pregnancies surviving beyond 12 weeks will have an unfavourable outcome due to syphilis (Schultz et al 1987). Furthermore, congenital syphilis has been implicated as a significant cause of perinatal mortality in various areas (e.g. 25-30% of the perinatal mortality rate of 50 per 1000 in Zambia was due to congenital syphilis - Hira et al 1985).

In order to reduce the impact of the disease, medical intervention can be undertaken at several levels.

Primary prevention, which aims to prevent the occurrence of the disease can be achieved by identification and treatment of the pregnant syphilitic woman (Lentz et al 1944). Nevertheless, the high prevalence of congenital syphilis in parts of Africa attests to the fact that primary prevention has not always been successfully carried out. Therefore, secondary prevention (which attempts early diagnosis and treatment of the condition) and tertiary prevention (which seeks to reduce the effects of the disease) are important.

Whilst the early diagnosis of congenital syphilis may be easy in cases presenting with classic symptoms and signs (Rathbun 1983a), in many cases the detection of the disease is hindered by several features. These include the very wide range of possible clinical signs (Malan 1987 pg 624, 629) and the fact that two-thirds or more of infected infants may be asymptomatic at birth (Larsson and Larsson 1970; Pickering 1985),

In such cases the diagnosis depends on serological tests. However, tests such as the Venereal Disease Reference Laboratory (VDRL) which are based on IgG antibodies may be difficult to interpret at birth because of passive transfer of maternal IgG (Kaufman et al 1974). This has meant that prolonged follow-up to monitor antibody levels may be necessary before a definitive diagnosis of congenital syphilis can be

made. To overcome this problem a Fluorescent Treponemal Antibody Absorption test (FTA-ABS) for detection of IgM had been proposed (Scotti and Logan 1968). Unfortunately, this test has been found to have a false negative rate of 35% amongst asymptomatic cases at birth (Kaufman et al 1974). Other tests based on IgM production have been suggested for the diagnosis of congenital syphilis. These include total IgM and rheumatoid factor (Reimer et al 1975; Borobio et al 1980).

Rheumatoid factor was so named because of its frequent association with rheumatoid arthritis (RA) (Carson 1982 pg 114). It became clear, however, that its presence is not limited to RA and it has been found in a wide range of other inflammatory and infectious diseases (Bartfeld 1969). The antibody was discovered to have specificity for the constant portion of the IgG molecule called the Fc fragment (Fudenberg and Kunkel 1961). It was proposed that altered IgG in immune complexes might be a stimulus for rheumatoid factor (RF) production (Williams and Kunkel 1962). Immune complexes have recently been described in congenital syphilis (Dobson et al 1988b). Hence the finding of RF may not be unexpected. Indeed, the successful use of the RF latex test as a screening test for congenital syphilis has been reported in a pilot study (Meyer and Malan 1987). Potential advantages of the test include its simplicity, rapidity and relatively low cost.

However, before the use of the test can be advocated in a clinical setting, it is necessary to further investigate its characteristics. Since congenital syphilis is a treatable condition (Platou 1949), it is important that any proposed screening test should have a high sensitivity. A highly specific test would also be desirable but of lesser importance because the effects of unnecessary treatment are unlikely to be deleterious (Gonin 1985).

Whilst testing for RF might have some worth in congenital syphilis, it is interesting to note that the presence of RF has been regarded as an inconvenience. In the past, attempts have been made to remove RF from sera of infants with suspected congenital syphilis before carrying out tests for specific treponemal IgM (Muller and Sinzig 1982) because RF can result in such tests being falsely positive (Reimer et al 1975). The effect of RF removal on the sensitivity and specificity of tests such as the FTA-ABS (IgM) test has, however, been incompletely studied.

Indeed, the observations of Alford et al (1969a) suggest that nontreponemal IgM (such as RF) is produced before specific treponemal IgM. This would indicate that further investigation is warranted as removal of RF could conceivably reduce the sensitivity of the FTA-ABS (IgM) test.

Therefore, there appear to be several instances relating to secondary prevention where RF may play a role in testing for congenital syphilis.

The first of these is where the newborn is assessed as being at risk of congenital syphilis due to the presence of maternal syphilis which is untreated or inadequately treated.

The second circumstance is that where an infant presents with one or more clinical signs of congenital syphilis.

Thirdly, the effect of eliminating RF interference in the FTA-ABS (IgM) test merits additional examination.

That tertiary intervention is also important in congenital syphilis is emphasized by the high case-fatality rate (e.g. over 50% of neonates with the disease in Zambia died - Hira et al 1985). The host immune response undoubtedly plays a significant role in the pathogenesis of the condition (Benirschke 1974) and may be responsible for some of the untoward effects of the disease. This is implied by the fact that prior to the development of the immune system, spirochaetes present in fetal organs produce little tissue destruction (Silverstein 1962). That congenital syphilis is the most severe form of syphilis (Handsfield and Lukehart 1984) might lead one to believe that differences in the immune response in early life compared to adulthood may account for some of the gravity of the former condition.

One of the peculiarities appears to be a greater propensity for RF production in early life. Evidence for this comes from the fact that RF has been found in over 95% of infants with congenital syphilis but in only 13-25% of adults with syphilis (Bartfeld 1969; Reimer et al 1975; Muller et al 1987; Meyer and Malan 1987). A possible reason for finding RF more often in the infants is that cord blood is richer in RF-producing B lymphocytes (CD5+ cells) than adult blood (Hardy et al

1987).

The effects of RF in congenital syphilis and in a wide range of other conditions such as RA and glomerulonephritis are, however, uncertain.

Nevertheless, using experimental models, it has been shown that RF exacerbates tissue damage in nephrotoxic nephritis and vasculitis (McCormick et al 1969; Floyd and Tesar 1979). Furthermore, in man, the presence of RF has been associated with more severe RA (Carson 1987 pg 1204). In spite of this there are difficulties in separating cause from effect and in ascribing a pathological as opposed to a physiological role to RF (Levinson and Martin 1988).

Whilst it may be desirable to know whether RF exerts a pathological effect in congenital syphilis this knowledge is beyond our grasp at the present time.

It may, however, be possible to acquire circumstantial evidence of an RF effect if the substance is found at sites of inflammation in the disease. Furthermore, it may be demonstrable that factors associated with the induction of RF synthesis in congenital syphilis are similar to those that have been shown to operate in other conditions such as RA and subacute bacterial endocarditis. Such information would allow the comparison of RF production in different diseases and would expand our understanding of the immune response in congenital syphilis. In fact, congenital syphilis may provide a useful model for the further study of the induction of RF synthesis since it occurs at a stage of life when the immune system is developing and RF represents a germ-line response (Levinson et al 1987). Ultimately a clearer understanding of the part of the inflammatory response and the role of RF in particular in the pathogenesis of congenital syphilis may lead to intervention to reduce the mortality of the condition.

For convenience the work undertaken is divided into 3 parts. The first part comprises a review of the literature and deals with the importance of congenital syphilis as well as the diagnostic tests available. In addition, information on RF itself is reviewed.

In the second part studies to determine the part RF may play in testing for congenital syphilis are described.

In the third part RF is investigated as part of the immune response in congenital syphilis.

For the purposes of the study the designation 'RF' or 'rheumatoid factor' is in keeping with the conventional usage of the term to describe an IgM antibody usually detected by an agglutination test (Egeland and Munthe 1983). It should be borne in mind, however, that there are different types of rheumatoid factor; not only may they belong to different immunoglobulin classes (e.g. IgG RF) but they may be directed towards different antigens (or epitopes) on the Fc region of IgG. Where the type of RF is any other than the usual IgM RF this will be specified.

PART I : LITERATURE REVIEW

CHAPTER 1

THE IMPORTANCE AND EPIDEMIOLOGY OF CONGENITAL SYPHILIS

1.1 INTRODUCTION

Syphilis became well known following a pandemic which spread across Europe towards the end of the 15th Century (Dennie 1962 pg 61). Syphilis of the newborn was mentioned by Fracastoro over 450 years ago (Cleugh 1954 pg 73) but it was not until 1854 that Cullerier realized that infection of the newborn was only observed in cases where the mother had the disease (Dennie 1962 pg 89).

The classic clinical description of congenital syphilis (attributed to Diday) is that of a 'little wrinkled old man with a cold in his head' (Dennie 1962 pg 73). Other workers noted the extremely variable signs and symptoms of congenital and acquired syphilis (Diday 1858 pg 119; McDonnell 1881 pg 259). Consideration of the protean manifestations of the disease led Sir William Osler (1917) to suggest that to know syphilis was to know medicine.

Indeed, infants with congenital syphilis may be asymptomatic at birth or born with benign stigmata of the disease. Others are extremely ill with multiple organ systems affected (Hutchinson 1896 pg 73-74).

The disease is usually divided into early and late manifestations, the former appearing in the first 2 years of life, the latter after this time (Krugman and Katz 1981 pg 396).

Frequent signs of early congenital syphilis include low birth weight and disproportionately large placenta, anaemia, skin rashes, oedema, hepatosplenomegaly, metaphyseal dysplasia of long bones, abnormalities of the central nervous system and pneumonia. Among other recorded findings are snuffles, jaundice, petechiae, abdominal distension, leukocytosis,

nephrotic syndrome, joint involvement, pseudoparesis and lymphadenopathy (Sartain 1965; Tan 1973; Budell 1982 pg 924-928; Ingall and Musher 1983 pg 346-351; Wendel 1988).

The hallmarks of late congenital syphilis were first described in 1858 (Hutchinson 1896 pg 431-434). Hutchinson observed a triad of notched incisors, interstitial keratitis and eighth nerve deafness. Other findings are a variety of dental and skeletal deformities, eye signs, central nervous system abnormalities, skin manifestations and cardiovascular defects (Stokes et al 1945 pg 1113; Brown and Moore 1963; Fiumara and Lessel 1970).

Although an extremely common disease prior to the advent of penicillin (e.g. 3% of the childhood population in the United States of America (USA) were thought to be affected - Stokes et al 1945 pg 1075), congenital syphilis is now a rarity in developed countries (Adler 1984).

Nevertheless, there are a number of reasons why congenital syphilis remains a disease of considerable public health importance.

1. The incidence of congenital syphilis is high in many third world countries (Ratnam et al 1982). Although there are fewer cases in developed countries this is partly because existing control measures have had a dramatic effect in reducing the incidence of the disease (Krugman and Katz 1981 pg 390; Pedersen et al 1989).

2. A perinatal mortality rate of between 5 and 16/1000 has been ascribed to congenital syphilis in some areas (Naeye et al 1977; Hira et al 1985). Various studies have found a case-fatality rate (in terms of fetal or perinatal death) of the order of 40-60% (Krugman and Katz 1981 pg 396; Centers for Disease Control 1988c).

3. The long term sequelae in untreated cases are such that it has been estimated that 50% of cases will need institutional care for life while 50% will need special education (Stray-Pedersen 1983).

Thus, the disease is of considerable public health importance and is amenable to intervention at several levels. Adequate antenatal care can largely prevent the disease while early diagnosis and treatment are effective in reducing mortality and morbidity (Budell 1982 pg 921).

Each of these aspects will be discussed in more detail.

1.2 INCIDENCE

The incidence of congenital syphilis partly reflects the incidence of infectious syphilis in women of childbearing age (Kaufman et al 1977). According to the Centers for Disease Control (CDC) 1.5 - 1.8 cases of early congenital syphilis are born per 100 cases of primary and secondary syphilis in the USA (CDC 1974).

It is, therefore, pertinent to describe the frequency with which syphilis occurs in pregnant women.

Syphilis in pregnant women

Syphilis in pregnancy is usually diagnosed on the basis of positive serological tests (Wendel 1988). While the prevalence of syphilis in pregnancy varies widely in different communities, it is generally high in Africa. A survey of mothers delivering in the Peninsula Maternity and Neonatal Service in Cape Town showed that 7.6% of mothers attending the antenatal clinics had serological evidence of active disease (Gonin 1985). Similarly, Naicker et al (1983) found that the prevalence of active syphilis in pregnant women attending the antenatal clinic at King Edward VIII Hospital in Durban was 7.4%. Workers from Baragwanath Hospital in Johannesburg noted that 5.9% of patients attending the antenatal clinic had a positive Rapid Plasma Reagin (RPR) test (Venter et al 1989).

Data from studies elsewhere appear in Table 1.1. It is evident that syphilis in pregnant women is much less common in developed countries.

The incidence of infectious syphilis in the USA, however, has

TABLE 1.1
FREQUENCY OF POSITIVE SEROLOGICAL TESTS FOR SYPHILIS (STS) AMONGST ANTENATAL CLINIC ATTENDERS

COUNTRY	PERCENTAGE POSITIVE STS	REFERENCE	
Fiji	22%	*WHO	(1982)
Brazil	16%	WHO	(1982)
Zambia	12.5%	Ratnam et al	(1982)
Ethiopia	10.9%	Friedmann & Wright	(1977)
Malawi	6.1%	Rampen	(1978)
India	1.4%	WHO	(1982)
Kenya	1.3%	Wafula & Bwibo	(1982)
Australia	0.4%	Garland & Kelly	(1989)
United Kingdom	0.06%	Schofield	(1973)
Norway	0.02%	Skarpaas & Loe	(1980)

* World Health Organisation

recently increased markedly and is now at its highest rate since 1950: more than 14 cases per 100 000 of the population (CDC 1988a). Furthermore, the increase in the USA has been greatest for females in the child-bearing years and in heterosexual males. Concomitant with this increase of syphilis in females of child-bearing years, one may expect a rise in the incidence of congenital syphilis in the near future in the USA.

Incidence of congenital syphilis

Not all the infants born to mothers who have positive serological tests for syphilis (STS) will develop the disease. The risk depends on the stage of syphilis in the mother.

When the mother has untreated primary or secondary syphilis there is little chance of a completely normal pregnancy (Goodwin and Moore 1946). Fiumara et al (1952) stated that 50% of the infants are either preterm, neonatal deaths or stillbirths; the other 50% will have congenital syphilis.

The outcome in mothers with early latent disease is that 20% of the infants will be preterm, 4% neonatal deaths, 16% stillbirths, 40% will have congenital syphilis and 20% will be normal (Fiumara 1975).

On the other hand, late latent syphilis in the mother carries a risk of prematurity in 9%, neonatal death in 1%, stillbirth in 10% and congenital syphilis in 10%; 70% of infants born to such mothers will the normal (Fuimara 1975).

Nevertheless, adequate, early treatment of syphilis in pregnancy can prevent congenital syphilis (Brown and Moore 1963). The incidence of the disease thus partly reflects the adequacy of health care facilities (Malan 1987 pg 623). The true incidence, however, is often uncertain as problems arise with case finding and definition. The recent report from the Centers for Diseases Control (CDC 1988c) will help to define what is meant by 'a case of congenital syphilis'. Lack of clarity in this regard may have led to erroneous reporting in the past (Kaufman et al 1977; WHO 1982). There are, therefore, limitations in the

accuracy of the data which follows.

In 1988, the Centers for Disease Control (CDC 1988c) reported 0.1 cases of congenital syphilis per 1000 livebirths in the USA. Adler (1984) stated that there were 8 cases per year in the United Kingdom. However, underreporting has been suggested in these 2 countries (Rathbun 1983b; Ewing et al 1985).

As might be expected, congenital syphilis is much commoner in developing countries where health services are less well established (WHO 1982). Thus, in a study from Ethiopia, Larsson and Larsson (1970) noted that 3% of newborns developed signs of congenital syphilis. Nearly 1% of live births at a Zambian teaching hospital had clinical signs of the disease (Hira et al 1982).

The reported prevalence of congenital syphilis varies greatly in different parts of South Africa: from 0.45% at Baragwanath Hospital, Johannesburg (Venter et al 1989) to 0.25% of live births in Durban and 0.05% in Cape Town (Venter et al 1987). This variation is somewhat suprising when the prevalence of syphilis amongst pregnant women is fairly similar in these different centres (Gonin 1985; Manning et al 1985; Venter et al 1989).

It is suggested that congenital syphilis is probably underreported: only cases with clinical signs were reported and it is recognised that 50-60% of cases may be asymptomatic at birth (Alford et al 1975; Pickering 1985). Furthermore, the impact of congenital syphilis on the perinatal mortality rate is reduced by failure to include stillbirths in the figures for Durban and Cape Town.

A recent report from Schultz et al (1987) at the CDC has highlighted the magnitude of the problem of congenital syphilis in Africa. Not only is congenital syphilis a common condition but it is associated with a high mortality rate.

1.3 MORTALITY

The mortality rate due to congenital syphilis varies with the prevalence of the disease and the availability of medical care. Ross and co-workers (1978) compared the perinatal mortality rates from several centres. The extent to which congenital syphilis contributed to these rates was determined by performing postmortems on consecutive perinatal deaths (Naeye et al 1977; Naeye 1977). The diagnosis of congenital syphilis was based on the presence of spirochaetes in the infant's organs.

The perinatal mortality rate due to congenital syphilis was 0.1/1000 in the USA, 3.2/1000 in Durban and 5/1000 in Addis Ababa. Overall, congenital syphilis accounted for 0.2% of perinatal deaths in the USA, 6.25% in Durban and 7.2% in Addis Ababa. Even higher figures, 12.5 - 16.5/1000, were reported from Zambia (Hira et al 1985)

These figures illustrate the impact that a preventable disease can have in developing countries. Indeed, Schulz et al (1987) have estimated that 5-8% of pregnancies surviving past 12 weeks in Africa will have an adverse outcome due to syphilis. Unfortunately, it is not stated what proportion of the perinatal deaths in the African studies quoted above occurred in live born infants. Nor is the neonatal death rate due to congenital syphilis known in most centres. Delport (1988), however, found that congenital syphilis was responsible for 10.5% of all neonatal deaths at Kalafong Hospital near Pretoria.

The reported case fatality rate in treated cases varies widely. For example, Chawla et al (1988) reported a rate of 13%. Sartain (1965) found that 29% of cases died. Tan (1973) and Hira et al (1985) noted case fatality rates of 50% and 54% respectively. The Centers for Disease Control (CDC 1988c) state that there is fetal or perinatal death in 40% of affected cases.

When considering studies reporting case fatality rates, however, one needs to bear in mind that hospital-based studies do not reflect the true situation in the community. This is particularly so with regard to congenital syphilis because ill

infants with clinical signs are likely to be admitted whilst many infected infants are initially asymptomatic (Alford et al 1975, Pickering 1985) and having milder disease may not seek hospital treatment.

Nevertheless, even when considering older infants Naeye et al (1977) found that congenital syphilis was responsible for 5% of postneonatal infant deaths in Addis Ababa. In addition, Mascola et al (1985) noted that the infant mortality rate was 12 times higher for infants with congenital syphilis.

To reduce these high mortality rates efforts should be directed towards prevention of the disease and its effects. This will be discussed in more detail. Not only is there a high mortality rate in congenital syphilis but there is also substantial morbidity.

1.4 MORBIDITY

The true morbidity is unclear, however, as the natural history of untreated congenital syphilis is difficult to ascertain. Hutchinson (1896 pg 75) stated that the early manifestations disappeared over a period of one to two years in survivors. The late manifestations may go unnoticed for many years, although most patients will have signs by adolescence. Budell (1982 pg 928) stated that 40% of untreated cases of congenital syphilis will develop long term sequelae.

The manifestations of late congenital syphilis which are important from a public health standpoint are those which affect the eye, ear, central nervous system and musculoskeletal system (Stray-Pedersen 1983; Rathbun 1983a).

The reported frequency of eye disease varies widely. Most authors describe eye lesions in 30-50% of cases (Stokes et al 1945 pg 1113; Fiumara and Lessell 1970; Budell 1982 pg 929). The most serious features are interstitial keratitis, iritis and optic atrophy. The interstitial keratitis, however, often improves during the natural course of the disease; 70% of patients will recover at least 20/60 vision (Duke-Elder 1965 pg

811).

Eighth nerve deafness occurs in 3-10% of cases (Stokes et al 1945 pg 1113; Ingall and Musher 1983 pg 352).

Involvement of the central nervous system may result in mental retardation, convulsions, hydrocephalus, juvenile general paresis, juvenile tabes dorsalis, cranial nerve lesions, monoplegia and diplegia (Storm-Mathisen 1978 pg 371-372). Reporting on data accumulated from 5 large series, Stokes et al (1945 pg 1113) noted neurosyphilis in 14-27% of cases. Similarly, Platou (1949) found that over the age of 2 years, up to one third of affected children could be regarded as having asymptomatic neurosyphilis on the basis of abnormalities in the cerebrospinal fluid (CSF). Fiumara and Lessell (1970), however, did not find any symptomatic cases of neurosyphilis amongst 271 patients with late congenital syphilis. The latter study may not be comparable with the others as the patients were referred to a skin clinic and CSF examinations, which are an important hallmark of neurosyphilis (Felman and Nikitas 1980), were not performed.

Bone and joint involvement, in the form of sabre tibia and Clutton's joints, occurred in 27-43% of cases described by Stokes et al (1945 pg 1113) and Budell (1982 pg 929). The joint involvement is usually a painless hydrarthrosis (Rathbun 1983a).

The cardiovascular system can be involved but this is rare (Bonugli 1961). The public health importance of these late manifestations will be discussed in the section on tertiary prevention of the disease.

1.5 PREVENTABILITY

The far ranging effects of congenital syphilis mean that prevention is important. In general, prevention of the disease can be undertaken at different levels. Primary prevention is designed to prevent the occurrence of the disease. Secondary prevention attempts, by means of early detection and intervention to reverse, halt or delay the progress of the

condition. Tertiary prevention aims to reduce the effects of the disease and disability among those affected (CDC 1988d).

1.5.1 Primary prevention

Early detection and treatment of syphilis in pregnancy can prevent congenital syphilis (Lentz et al 1944; Ingraham et al 1946; Goodwin and Moore 1946; Nelson and Struve 1956).

Brown and Moore (1963) state, however, that adequate treatment of the mother after the eighteenth week of pregnancy will cure the fetus but cannot prevent the possible bony and dental changes of late congenital syphilis. While Putkonen (1963) found no definitely syphilitic teeth among 30 children whose mothers had been treated for syphilis in the second half of pregnancy, Robinson (1969) found that 5.4% of 148 children had characteristic abnormalities of the teeth. Apart from abnormal scapulae and thickened clavicles, neither author found other late manifestations of congenital syphilis.

It became apparent, however, that treatment late in pregnancy did not always prevent congenital syphilis (Jackson et al 1961; Mascola et al 1985).

Indeed, of 437 infants with congenital syphilis reported to the Centers for Disease Control during the years 1983-1985, 81 (18.5%) of cases were due to antibiotic treatment failure (CDC 1986). Most of the treatment failures (55%) were in the third trimester among patients who had received benzathine penicillin. A further 14% were second trimester failures with the same drug.

Late treatment is often the result of late booking (the booking visit being regarded as the first antenatal clinic attendance). In the Centers for Disease Control report quoted above, the mean gestational age at booking was 22 weeks. In developing countries late booking may be common e.g. at Tygerberg Hospital in Cape Town the mean gestational age at booking was 22.5 weeks (Pattinson and Rossouw 1985), and in Zambia 85% of mothers had their first antenatal clinic attendance at or beyond the 20th week of pregnancy (Ratnam et al 1982).

Furthermore, mothers may not receive treatment despite the detection of positive serology (e.g. 12.5% of 72 mothers delivering infants with congenital syphilis described by Kaufman et al (1977) fell into this group).

Poor compliance with treatment is another potential reason for treatment failure. A study from Durban, South Africa indicated that only about 10% of pregnant patients with syphilis received their prescribed treatment (Manning et al 1985).

Treatment of syphilis in pregnancy with drugs other than penicillin has been reported. Erythromycin is the usual alternative to penicillin but infected infants have been described (South et al 1964). Fourteen percent of treatment failures described by the Centers for Disease Control (CDC 1986) occurred with erythromycin.

Thus, while penicillin given early in pregnancy usually prevents or cures congenital syphilis, treatment in the latter part of pregnancy or with drugs other than penicillin may not do so.

Primary prevention is therefore not always successful. For secondary prevention to be effective early detection and treatment of affected infants is imperative.

1.5.2 Secondary prevention - Early detection

The diagnosis of congenital syphilis may be simple in cases having classical signs and symptoms (Rathbun 1983a). Unfortunately, however, case-finding is often difficult in congenital syphilis (Kaufman et al 1974; Rathbun 1983a; Srinivasan et al 1983, Mascola et al 1985). There are a number of reasons for this which will be discussed in more detail in Chapter 2 but are summarized here.

Features in the history which alert the clinician to the presence of syphilis in the mother may be lacking. This is because most pregnant women with syphilis have latent disease and are asymptomatic (Holder and Knox 1972; Kaufman et al 1977).

Details of the pregnancy e.g. the results of the STS in the

mother may be incomplete because of late booking or failure to book (Kaufman et al 1977; Venter et al 1987). The efficacy of any treatment given to the mother for syphilis in pregnancy may be uncertain because of poor patient compliance (Manning et al 1985). Furthermore, congenital syphilis case finding based on physical examination is unreliable since 50-66% of infected infants may be asymptomatic in early life (Alford et al 1975; Pickering 1985).

For the reasons outlined above, special tests are required to identify infants with congenital syphilis (Kaufman et al 1974; Rathbum 1983a; Pickering 1985). These tests are not without problems, however. The interpretation of tests measuring IgG antibodies is hampered by transplacental passage of maternal IgG, as encountered for example with the VDRL test (Rein and Reyn 1956). A lack of sensitivity in cases of delayed onset disease has been noted with the Fluorescent Treponemal Antibody Absorption test for IgM antibodies (FTA-ABS IgM) and radiographic examination of the long bones (McKelvey and Turner 1934; Kaufman et al 1974).

Other investigations, such as testing for RF or measuring the total IgM level have been incompletely evaluated (Reimer et al 1975; Srinivasan et al 1983). Newer tests such as Western blotting fall into a similar category and are also difficult to perform (Dobson et al 1988a; S'anchez et al 1989).

It follows that the development and appraisal of tests for congenital syphilis is very important for adequate case finding and appropriate early treatment.

Early treatment

The difficulties with accurate case finding in congenital syphilis have resulted in treatment being given to asymptomatic high risk cases (e.g. those whose mothers' have positive STS and inadequately documented therapy). This approach has been suggested when follow-up of the infant is not ensured (Kaufman et al 1977; WHO 1986). The use of a single dose of benzathine penicillin is no longer advised for this purpose by the Centers

for Disease Control (CDC 1988c). This is because treatment failures have been reported and inadequate cerebrospinal fluid levels are obtained (McCracken and Kaplan 1974; Beck-Sague and Alexander 1987). Instead, a 10 day course of intramuscular or intravenous penicillin is recommended (CDC 1988c). In fact, clinical trials of the current standard dosage have never been carried out (Murphy and Patamasucon 1984 pg 370). Cephalosporins such as cephaloridine have been successfully used in treating primary and secondary syphilis in adults. In addition, ceftriazone is as effective as penicillin G in the treatment of experimental syphilis in rabbits (Russo and Thompson 1984 pg 897). None of these preparations have been used in treating congenital syphilis, however.

It can be seen that the widespread implementation of the CDC policy in developing countries would be a major undertaking. Firstly, as already described, the prevalence of syphilis in pregnancy is high. Secondly, adequate treatment of the pregnant syphilitic woman is often not achieved (Ross et al 1978; Manning et al 1985). Thirdly, if the infants were treated for 10 days in hospital overcrowding of nurseries could result. Outpatient treatment would be equally problematic because of the difficulty of ensuring compliance.

To avoid these problems, it is clear that the development of sensitive tests for the early diagnosis of congenital syphilis is of considerable importance.

What effect does early treatment have on the outcome of the disease? Following the introduction of penicillin, Budell (1982 pg 936) claims that the mortality rate has dropped to 2%. However, as mentioned previously, the case fatality rate in several large studies has been reported to be much higher, in the range of 13-54% (Sartain 1965; Tan 1973; Hira et al 1985; Chawla et al 1988). The apparent discrepancy may simply reflect the fact that the figures from the large studies are derived from hospitalized patients and do not indicate the overall mortality due to congenital syphilis in the community as a whole.

It is clear, however, that treatment of infected infants with penicillin does not prevent all the fatalities. Other factors, notably the immune response probably play a major role in the unfavourable outcome. This was suggested by the finding that prior to the 5th month of gestation spirochaetes do not produce inflammatory lesions in the fetus (Silverstein 1962). However, by the time that the infected fetus was able to respond immunologically, inflammatory changes and tissue damage were noted around the organism. Other evidence that the host immune response has an effect in the pathogenesis of the lesions of syphilis has been suggested by the presence of immune complexes and RFs in congenital syphilis (Wiggelinkhuizen et al 1973; Reimer et al 1975). More needs to be learnt about these aspects of the disease if the mortality is to be further reduced.

If the infant survives the initial period of therapy it appears that treatment under the age of 2 years usually has an excellent prognosis and permanent sequelae are rare (Budell 1982 pg 936). There are remarkably few studies to support this, however.

Both Rathbun (1983a) and Ingall and Musher (1983 pg 363) have commented on the paucity of available information. Platou (1949) noted a satisfactory response to treatment with penicillin in 100 out of 107 infants with congenital syphilis who were followed up for 6 months to a year. A 'satisfactory' result was taken to mean 'continued well-being or disappearance of all reversible syphilitic lesions, together with progressively declining or negative serologic tests'.

Another study which looked at outcome was that of Putkonen (1963). Thirty six children whose therapy for congenital syphilis was commenced after birth, were followed up between the ages of 10 and 13 years. Twenty one had been treated within the first 3 months of life. None of these children had dental abnormalities or other late manifestations of the disease. Fifteen infants were treated after the age of 3 months. Seven of these had syphilitic dental changes but no other features of late congenital syphilis.

It would seem, therefore, that treatment with penicillin before 3 months may prevent the long term sequelae. Unfortunately, a longer period of follow-up would be necessary to be absolutely sure of this as, for example, neurosyphilis usually only becomes apparent during the teens (Curtis and Philpott 1964). In addition, treatment failures, after apparently adequate penicillin therapy, have been recorded. Such cases have usually occurred in critically ill infants with extensive disease (Boissiere 1967; Hardy et al 1970). Nonetheless, evidence of serological cure following treatment of congenital syphilis with penicillin is the norm, and has been described by several authors (Ingraham 1951; Brown and Moore 1963). Indeed, Platou (1949) observed that 97% of infants treated for congenital syphilis became seronegative by 2 years.

If the outcome following early treatment is satisfactory, what is the outcome in patients who are not treated until over the age of 2 years? Budell (1982 pg 928) states that these patients will usually have some permanent damage. Indeed, it appears that interstitial keratitis, sensorineural deafness and Clutton's joints are unresponsive to antibiotic therapy (Curtis and Philpott 1964; Robinson 1969; Fiumara and Lessell 1970). The other manifestations of late congenital syphilis which are similar to those of acquired syphilis (such as neurosyphilis) respond similarly to penicillin (Idsoe et al 1972). The same authors, reviewing the experience of three decades of the use of penicillin in the treatment of syphilis, note that in the majority of cases of late symptomatic syphilis, penicillin will arrest the pathological process. The prognosis for resolution of existing lesions depends on the degree of damage prior to the commencement of therapy. Once again, these findings stress the desirability of early diagnosis and treatment.

1.5.3 Tertiary prevention

Prevention at this level aims to reduce the effects of the disease. Its importance from a public health perspective lies in the cost of rehabilitation and the loss of manpower.

Stray-Pedersen (1983) estimated these costs whilst evaluating a preventive programme for congenital syphilis in Norway. He found that the prevention of one case of congenital syphilis would save $ 88 000 (as costed in 1979). The savings were high because of the nature of the late manifestations of the disease.

The benefit-cost ratio of the preventive programme was 3.8 to 1 even though the incidence of maternal syphilis was low (0.02%). However, Stray-Pedersen assumed that 'half the infected children surviving the neonatal period would need institutional care for life, while the other half would need special education and training'.

This is likely to be an overestimate as it appears that only 40-50% of affected children will go on to develop late manifestations (Budell 1982 pg 928). In addition, Stray-Pedersen assumed that prenatal care would completely prevent the disease, but this is not invariably the case because of the acquisition of syphilis after serologic screening (Monif et al 1973). Nevertheless, he realized that 'some parameters employed in the calculation could not be estimated precisely' and thus screening costs were set at a maximum and costs of medical and social care were set at a minimum. Furthermore, even if the economic value of lost productivity was disregarded, the programme still gave a benefit-cost ratio of 2,9 to 1. It is likely, therefore, that prenatal prevention of the disease is beneficial from a public health perspective even in those countries, like Norway, where the incidence of the disease is low. This is because of the serious nature of the long-term sequelae and the high cost of tertiary prevention.

1.6 SUMMARY

Congenital syphilis is a major cause of morbidity and mortality, particularly in developing countries. Primary prevention is possible but is not always achieved. Secondary prevention, which entails early diagnosis and treatment is therefore important.

The early diagnosis of congenital syphilis usually requires the

use of various tests and, as discussed in Chapter 2, the existing tests are not without limitations. New or improved methods are needed to facilitate early case finding.

The treatment of congenital syphilis with penicillin has reduced the mortality of the disease but fatalities still occur and may be related to the immune response. A better understanding of the latter may help to further reduce the mortality rate.

CHAPTER 2

THE DIAGNOSIS OF CONGENITAL SYPHILIS

In Chapter 1 the importance of congenital syphilis in developing countries was stressed. From a paediatric perspective, early diagnosis and treatment of the condition are of importance.

The diagnosis of congenital syphilis may be easy in cases where there is a classic history (e.g. positive maternal serology for syphilis) and where characteristic clinical findings are present (Kaufman et al 1977, Rathbun 1983a).

However, difficulties in diagnosis may arise. In symptomatic cases the differential diagnosis may include many conditions (Ingall and Musher 1983 pg 353, 360-362). Furthermore, asymptomatic infants with passively transferred reagin antibodies from the mother present a particularly difficult diagnostic problem (Kaufman et al 1974).

Ideally, a rapid method of screening high risk cases prior to hospital discharge is required (Kaufman et al 1974). Similarly, it would be advantageous to be able to ascribe an infant's symptoms to congenital syphilis rapidly and appropriately.

In fact, the various tests in current use do not reliably detect the infected asymptomatic infant (S'anchez et al 1989), but, rather than treat all potentially infected infants, it is still desirable to make a definitive diagnosis (Platou 1949). This is because full treatment requires a 10 day course of parenteral antibiotics (CDC 1988c). As pointed out in section 1.5.2 these measures could be difficult to carry out in developing countries.

It follows that the diagnosis of congenital syphilis is important, and it is in this context that the possible value of testing for rheumatoid factor (RF) will be discussed. In addition, in this chapter, various aspects of the diagnosis of early congenital syphilis will be reviewed.

2.1 THE HISTORY

In terms of evaluating an infant for possible congenital syphilis details of the mother's pregnancy are important. The history, examination, results of special investigations and particulars of treatment pertaining to the pregnancy, can help to identify the infant at risk.

Features in the pregnancy indicating the presence of maternal syphilis

Ingraham (1935a) found that useful information suggestive of the presence of maternal syphilis could be obtained from the history in 67% of cases.

Pointers to the disease were symptoms suggestive of primary or secondary syphilis: a history of late stillbirths or neonatal deaths in previous pregnancies; a history of previous treatment or positive serology and a history of the husband having syphilis. Ingall and Musher (1983 pg 346) also noted an increased risk among pregnant women who were drug abusers or sexually promiscuous.

Nevertheless, most pregnant women with syphilis have latent syphilis - either early or late latent disease (Holder and Knox 1972; Wendel 1988). They are, therefore, asymptomatic (Kaufman et al 1977), and the risk factors outlined above may be easily overlooked.

Various demographic features indicative of maternal syphilis have been identified in the USA. The mothers are often young, unmarried, of a poor socio-economic background, of African or Spanish ethnicity and have had little or no antenatal care (Fiumara 1951; Fiumara et al 1952; Kaufman et al 1977; Mascola et al 1985; CDC 1986; Wendel 1988).

Not all these factors are important in other centres. In the United Kingdom (UK), for example, Ewing et al (1985) found that ethnicity was not a factor. Ratnam et al (1982), reporting on their experience in Zambia, found that there were no significant differences between seronegative and seropositive pregnant women in terms of their age, marital and economic status, education

and parity. However, seropositive women had a higher incidence of previous abortions and stillbirths.

In South Africa, the major pointer in the history to an at risk pregnancy is a lack of, or inadequate, antenatal care (Venter et al 1987, Venter et al 1989). Indeed, the prevalence of active syphilis in unbooked pregnant women is 2 to 4 times higher than that in booked patients (Hamilton et al 1985; Venter et al 1989; Meyer and Woods 1989). Studies on this population of poor antenatal clinic attenders in South Africa have noted several characteristics. They tend to be young, unmarried and of a lower socioeconomic background (Hamilton et al 1985; Loening and Broughton 1985; Pattinson and Roussouw 1985).

The main features in the history of the pregnancy which suggest a greater risk of maternal syphilis appear, therefore, to be a lack of booking and a history of previous abortions or stillbirths.

Concerning the examination of the pregnant women, Ingraham (1935) found abnormal physical signs in 23% of cases. Most signs, however, related to late disease, which is far less infectious for the fetus and a rarity in these days of penicillin therapy (Ross 1982). Primary or secondary lesions were found in approximately 7% of the cases described by Ingraham.

It follows that in most cases the examination of pregnant women is unhelpful in delineating those at risk of delivering infants with congenital syphilis. The most reliable method of identifying syphilis in pregnancy is by means of performing special investigations.

Spirochaetes may be demonstrated by dark field microscopy of material from primary or secondary lesions or lymph nodes (Jaffe 1975). More usually, however, the diagnosis is based on the presence of positive treponemal and nontreponemal tests (Wendel 1988). Mothers who develop positive STS after initially being negative earlier in pregnancy may be missed by antenatal screening programmes and are therefore at risk. In a study from Durban, Manning et al (1985) found that 4 (11,8%) of 34 patients

who where seronegative at less than 32 weeks of pregnancy had a positive serological test at 36 weeks. Ratnam et al (1982) studied a group of 269 antenatal patients in Zambia of whom 10 (3,7%) developed positive serology after initially having been negative. Five of the 7 mothers giving birth to infants with congenital syphilis in the UK were seronegative when tested at the antenatal clinic (Ewing et al 1985).

It has thus been recommended that in high risk pregnancies, the serology should be repeated after 28 weeks gestation (Wendel 1988).

In spite of these difficulties, STS carried out on the mother are of great importance in identifying high risk situations. As previously described in Chapter 1, the risk depends partly on the adequacy of treatment given to the mother with syphilis.

Although it is difficult to define what constitutes adequate treatment of the pregnant syphilitic, the Centers for Disease Control recommends benzathine penicillin G 2,4 million units intramuscularly (IM) for early syphilis of less than 1 year's duration and benzathine penicillin G 2,4 million units IM weekly for 3 successive weeks for syphilis of more than 1 year's duration (CDC 1985).

A steadily declining titre (using a nontreponemal test) may indicate effective therapy (Wendel 1988). However, such information may not be available, especially in developing countries. This is because of late booking (e.g. 85% of pregnant women book after 20 weeks in Zambia - Ratnam et al 1982) and because of failure to comply with therapy (reported to occur in 90% of cases: Manning et al (1985)).

In summary, the details of the pregnancy are useful in delineating infants at risk of congenital syphilis. It is clear that where the prevalence of maternal syphilis is high (e.g. in developing countries) many infants may be at risk because of a lack of adequate antenatal care.

To what extent does the physical examination help to identify infants with congenital syphilis?

2.2 CLINICAL EXAMINATION

The abnormal findings described in congenital syphilis have been listed in Chapter 1. Infants that are symptomatic at birth often present with clinical signs which are indistinguishable from those caused by other intrauterine infections. Nahmias (1974) called this the TORCH (toxoplasmosis, rubella, cytomegalovirus, herpes) group of infections, the 'O' standing for other agents including syphilis. Although some clinical features may point to a specific pathogen, most of the abnormal findings are also common to other noninfectious states (Stagno 1981).

It is also apparent that many infants with congenital syphilis are asymptomatic at birth (Stokes et al 1945 pg 1100; Platou 1949; Brown and Moore 1963; Ingall and Musher 1983 pg 345). The exact percentage of asymptomatic newborns is difficult to estimate because of possible under-reporting of these cases (Mascola et al 1985). Pickering (1985) stated that 66% of cases are without clinical signs at birth, while Mascola et al (1985) reported a figure of 38%. A report from the Centers for Disease Control (CDC 1986) indicated that only 12% of notified cases were asymptomatic.

It is only possible, however, to determine the true percentage of asymptomatic cases in a prospective, follow-up study. There are 2 recent studies which meet some of these criteria. Larsson and Larsson (1970), in Ethiopia, followed-up infants born to 141 seropositive mothers for variable periods up to a year. Although repeat serology was not performed on most of the infants, 30 (21,3%) developed signs consistent with congenital syphilis. None of the patients had been symptomatic at birth.

The limitations of the study were that approximately 1 out of 3 cases of the original group did not attend for follow-up and that the diagnosis of congenital syphilis was not confirmed serologically.

Taber and Huber (1975 pg 183-190) reported somewhat similar results. They described 22 infants whose mothers had untreated syphilis in pregnancy. Altogether, 16 infants developed

congenital syphilis with overt clinical signs; 5 were symptomatic at birth and 11 (69%) were later readmitted with obvious disease. Therefore, over two thirds of the infected infants had delayed onset disease. The number may, however, have been higher because another 6 infants with normal physical examinations at birth were treated in the immediate newborn period.

Clearly, the absence of clinical sings at birth does not rule out the diagnosis of congenital syphilis.

Ultimately, however, almost all cases will develop signs by 3 months of age (Nelson and Struve 1956). Although unusual cases where there are no symptoms until 1-2 years of age have been noted (Brown and Moore 1963), the majority of infants with congenital syphilis become symptomatic by 3-8 weeks of age (Stokes et al 1945 pg 1100).

The earliest clinical signs are often nonspecific e.g. irritability, and may be followed by the presence of snuffles, a maculopapular rash and hepatosplenomegaly (Stokes et al 1945 pg 1100; Budell 1982 pg 924).

Owing to the uncertainty of the clinical examination in the early diagnosis of congenital syphilis, special tests are usually carried out.

2.3 SPECIAL INVESTIGATIONS

These are based either upon radiology or the principles of microbiology used in the diagnosis of any infectious disease (Larson 1984 pg 527). The latter include i) demonstration of the agent by microscopy or histochemical methods, ii) serological tests.

In evaluating a diagnostic test one needs to compare the results with a 'gold standard' test (Bourke et al 1985 pg 248-251). There is no such test available for congenital syphilis. Instead, standardization has been attempted by applying a set of criteria to each case and deciding if there is sufficient evidence for a diagnosis of congenital syphilis. The criteria

will be discussed in more detail later.

2.3.1 Diagnosis by radiography

It has been known since 1900 that roentgenograms can demonstrate bony lesions in infants with congenital syphilis (Hochsinger 1904). Typically there are widespread, symmetrical changes of multiple long bones; the metaphyses and diaphyses being primarily involved (McLean 1931). Although earlier workers described the lesions as osteochondritis, osteomyelitis and osteoperiostitis (McLean 1931; Caffey 1939; Platou 1949), Cremin and Fisher (1970) argued that the bony changes represent a widespread disturbance of bone formation rather than the changes of active infection. They proposed, therefore, that the lesions be referred to as metaphyseal dystrophy, an 'osteitis-like' dystrophy and periosteal dystrophy.

Regardless of the pathology, the osseous changes are useful from a diagnostic point of view. Two studies, one of 176 and the other of 202 cases of symptomatic, early congenital syphilis found characteristic lesions in 76% and 90% of cases respectively (Platou 1949; Hira et al 1985). Similar frequencies, between 60% and 100%, have been noted in somewhat smaller studies of symptomatic infants (Sartain 1965; Tan 1973; Rosen and Solomon 1976; Mascola et al 1985; Malan 1987 pg 624-625; Venter and Pettifor 1987). The frequent presence of bony abnormalities in these cases has prompted several workers to use their presence as a major criterion in the diagnosis of congenital syphilis (Kaufman et al 1977; Rathbun 1983b).

Rarely, however, identical lesions have been reported in other conditions. Caffey (1939) reported similar features in cases of bacteraemia, haemolytic anaemia, failure to thrive, birth injury and healing rickets. Rubella may, on occasion, produce similar findings (Cremin and Fisher 1970). More importantly, Malmberg (1944) found periosteal new bone formation in 47% of normal preterm infants. This work was confirmed by Shopfner (1966) who described a periosteal reaction identical to that of congenital syphilis in 34% of preterm infants. In addition, 35% of normal term infants had similar changes. None of the normal infants

had bony abnormalities apart from the periosteal changes.

Not all infants with congenital syphilis will have bony changes at birth, however. There is a latent phase after spirochaetal infection is acquired before bony lesions become apparent. Ingraham (1936) was able to deduce that this period was approximately 5 weeks for the metaphyseal and 'osteitis-like' changes and 4 months for the periosteal dystrophy.

It follows that radiologic evidence of congenital syphilis will probably not be present at birth in those infants who were infected late in pregnancy. A high proportion of infants who do contract syphilis may be infected late in pregnancy (Boissiere 1967) and this may account for the fact that the bone changes are much more likely to be seen after the first few weeks of life than at birth (Krugman and Katz 1981 pg 396; Budell 1982 925). Once again, the percentage of all infants with congenital syphilis (whether symptomatic or asymptomatic) who will have identifiable lesions at birth can only be determined by a prospective follow-up study. In one such investigation, McKelvey and Turner (1934) reported an absence of bony involvement in 43 (69%) of 62 infants with congenital syphilis, but stated that the changes are almost always apparent by the end of the second week of life.

Beck-Sague and Alexander (1987) have stated that 'the long bone roentgenogram is the most sensitive screening test for evidence of clinically inapparent tissue damage'. The authors have cited the paper by Hira et al (1985) as providing evidence for their statement. This appears to be unjustified because the number (if any) of asymptomatic cases is not stated and no other screening tests were carried out. In summary, the use of radiography in the diagnosis of congenital syphilis suffers not only from false positive results but more importantly may give erroneously negative results in asymptomatic cases.

2.3.2 Demonstration of *Treponema pallidum*

i. Darkfield microscopy

Treponema pallidum belongs to the order *Spirochaetales*. It can be differentiated from other members of the order by its characteristic motility (WHO 1982). Moist skin lesions and nasal secretions are potential sources of the organism, but in most series this is an uncommon method of diagnosis (Boissiere 1967; Alpert and Plotkin 1986). Of the 50 cases reported by Mascola *et al* (1985), none were diagnosed in this way. A report from the Centers for Disease Control (CDC 1986) noted that 50 (11%) of 460 cases notified were proven on dark field microscopy.

Scrapings of the wall of the umbilical vein have also been used as a source of the organism. McKelvey and Turner (1934) regarded this method as being 'too cumbersome' for routine use. Ingraham (1935b), however, found organisms in 19 (34%) of 35 cases examined in this way. There have been no recent reports which have specifically utilized this technique.

Budell (1982 pg 932-933) and Lukehart (1986) have pointed out that the usefulness of dark field microscopy depends on the skill of the microscopist. This is partly because other spirochaetes found in the oral, rectal and genital mucosae (and regarded as commensals) may be difficult to distinguish from *T.pallidum*.

ii. Immunofluorescent tests

Treponema pallidum in body fluids and secretions can also be identified by direct immunofluorescence using either polyclonal or monoclonal antibodies labelled with fluorescein (Edwards 1962, Lukehart *et al* 1985). Although shown to be sensitive and specific tests in the diagnosis of adult syphilis (Daniels and Ferneyhough 1977, Hook *et al* 1985) the tests have not been evaluated in congenital syphilis.

iii. Other means of identification

Organisms can be demonstrated histologically in the placenta or other organs (Dippel 1944, Harter and Benirischke 1976). Silver stains have been useful for this purpose (Oppenheimer and Hardy 1971) but have been superceded by immunofluorescent tests (WHO 1982).

The tests outlined above which involve demonstration of the organism are useful confirmatory tests, especially in cases where there are suitable lesions. Negative tests, however, do not preclude the diagnosis of congenital syphilis.

2.3.3 Examination of the placenta

Macroscopically, the placenta has been described as being enlarged and pale in cases of congenital syphilis (Russell and Altshuler 1974; Braunstein 1978). Wendel (1988) also describes hydropic changes, but these do not appear to be universal (Benirschke 1974). The ratio of the placental to fetal weight may be above the normal 1:6 (Budell 1982 pg 923). Twenty eight (38%) of 74 symptomatic cases of congenital syphilis described by Malan et al (1990) had placental weights above the 90th percentile.

The microscopic features are a focal villitis with a lymphocytic and plasma cell infiltrate, relative immaturity of the villi and vascular obliteration due to endovascular and perivascular proliferation (Russell and Altshuler 1974).

The placental changes are nonspecific (Benirschke and Driscoll 1967) although spirochaetes may, on occasion, be demonstrated (Dippel 1944). McKelvey and Turner (1934) evaluated the macroscopic and microscopic features of the placenta as an aid to the diagnosis of congenital syphilis. Of 578 pregnancies occurring amongst women with syphilis, the placenta appeared normal in 518 cases. Of these, 20% of the infants later developed features of congenital syphilis. In 41 pregnancies the placenta was classified as 'syphilitic', approximately 88% of these infants were infected. Where the placental findings

were questionable (19 cases), all the infants were later shown to have congenital syphilis.

Thus, examination of the placenta can provide useful information, but it appears, from the study of McKelvey and Turner, to lack sensitivity and the presence of a normal placenta does not rule out the diagnosis of congenital syphilis. Furthermore, as histological investigation can be both time consuming and labour-intensive it is not an ideal method to apply to large numbers of cases at risk.

2.3.4 Pathological changes in other organs

These have been well described by other authors (Morison 1970 pg 602-609; Oppenheimer and Hardy 1971). The changes are of value in making the diagnosis in cases coming to postmortem.

2.3.5 Serological tests

These may be broadly divided into 2 groups depending on whether they measure IgG or IgM antibodies. Tests in the latter category have the advantage that they measure only the infant's serological response (Scotti and Logan 1968). Investigations relying on the presence of IgG antibodies may be difficult to interpret because of the transplacental passage of these antibodies to the fetus (Gitlin 1964). The maternal IgG has a half-life of approximately 30 days, so that it usually disappears after the first 6 months (Altemeier and Smith 1965; Gitlin 1967). It is also necessary to remember that some tests are nonspecific and measure the total antibody response (e.g. total IgM levels - Alford 1971) while others are more specific and measure only antibodies directed towards treponemal antigens.

The sensitivity and specificity of the various tests needs to be taken into account. Sensitivity refers to the ability of a test to be positive in the presence of disease. Specificity is the ability of the test to be negative in persons without the disease (Jaffe 1975).

As congenital syphilis is treatable and has potentially serious long-term sequelae, one ideally needs tests which are highly sensitive and have a high predictive value for the absence of infection (Hennekens and Buring 1987 pg 334).

Tests based predominantly on IgG antibodies

Nontreponemal Tests:

These tests measure reagin antibodies in the serum (Rudolph 1976). The Wasserman reaction (WR) which depends on complement fixation was the initial test to be evaluated in the diagnosis of congenital syphilis. However, flocculation tests such as the VDRL or RPR tests or alternatives to the complement fixing tests are more widely used today (Robertson et al 1980 pg 74). Non-treponemal tests are commonly used for screening purposes at birth or for serial follow-up in cases of suspected infection.

Serological tests at birth:

Cord blood or infant serum has been used for testing. While Stokes et al (1945 pg 1098) and Robinson (1969) agree that there are no major differences in reagin titres obtained from these 2 sources, Breasette (1979) states that cord blood is unsuitable. This is because of the possibility of contamination either with maternal blood or mucoid material from the cord. The latter can produce false negative reactions by neutralizing syphilitic antibodies directed towards lipoidal antigens.

Numerous studies have evaluated the use of reagin tests at birth to diagnose congenital syphilis. McKelvey and Turner (1934) found that of 283 infants having a negative WR, 86% turned out to be normal and 14% were infected. Of 54 infants with a positive WR, 81.4% had congenital syphilis. They felt that a positive test indicated the need for further close study of the infant.

Stokes et al (1945 pg 1096-1097), state that tests for reaginic antibodies are of limited diagnostic value in the first few weeks of life. They noted that of 63 newborns with detectable reaginic antibodies 25 (40%) were not infected. Conversely,

only 36 (35%) of 104 infants with congenital syphilis were seropositive at birth.

Brown and Moore (1963) and Curtis and Philpott (1964) are in agreement with these latter authors. Mascola et al (1985) found that 5 out of 50 infants with congenital syphilis had negative nontreponemal tests at birth. A report on cases of congenital syphilis from 1983-1985 in the USA (CDC 1986) found that 10 of 191 cases were seronegative at birth.

These negative results may reflect maternal infection late in pregnancy, since the nontreponemal tests take 4-8 weeks to become positive (Boissiere 1967; Sparling 1971; Felman and Nikitas 1980).

Reagin tests may have diagnostic value at birth where the infant's titre is at least four times greater than that of the mother (Rein and Reyn 1956). This, however, is a relatively unusual event (Stokes et al 1945 pg 1098; Curtis and Philpott 1964). Mascola et al (1985) found a diagnostically raised titre in 3 out of 50 cases, while Srinivasan et al (1983) noted the feature in 1 out of 8 cases. Thus, the diagnostic value of reagin tests at birth may not be great.

One of the reasons for the lack of specificity of nontreponemal tests in the diagnosis of congenital syphilis is the passive transfer of maternal IgG across the placenta (Gitlin 1964) e.g. the VDRL may be positive because of the presence of maternal anti-treponemal IgG regardless of whether the infant has syphilis or not (Rein and Reyn 1956). Even if the mother has a biologically false positive VDRL, the reagin may be transferred to the infant (Miller et al 1960; Harris and Cave 1965).

Indeed, it was because of the inability of the reagin tests to distinguish between active infection of the newborn and passive transfer of maternal antibodies, that the search for IgM antibodies was initiated (Scotti and Logan 1968). Although tests for these antibodies will be discussed in more detail later, it should be noted that RF is an example of one of the IgM class antibodies produced (Reimer et al 1975).

In spite of these difficulties with reagin tests, few authorities would disagree that these tests are useful screening tests which should initiate a search for active disease in the infant (Breasette 1979; Budell 1982 pg 931; Rathbun 1983a; Ingall and Musher 1984 pg 366).

Serial follow-up:

The nontreponemal tests are extremely useful in the diagnosis of congenital syphilis when performed on a serial basis. In fact, monitoring serial VDRL titres is regarded as the standard laboratory test for congenital syphilis (Jaffe 1975; Breasette 1979). When the testing is combined with careful clinical examination a definitive diagnosis can usually be made (Boissiere 1967; Kaufman et al 1974; Breasette 1979).

In cases where there is passive transfer of reagin from mother to infant, the nontreponemal tests usually become negative between 2 and 4 months (Rein and Reyn 1956; Robinson 1969; Felman and Nikitas 1980). On occasion, the tests take longer to become negative (Robinson 1969; Johnston 1972; Wendel 1988) but 6 months is regarded as the outside limit (Brown and Moore 1963; Taber and Huber 1975 pg 188; Srinivasan et al 1983; CDC 1988c).

In order to exclude a diagnosis of congenital syphilis, some workers have felt that follow-up for a period of 1 year may be necessary (Ingraham et al 1946; Rathbun 1983a). Roberts (1933), however, stated that if there was no clinical or serological evidence of disease by 4 months, the infant had probably escaped infection. Similarly, Goodwin and Moore (1946) felt that it was very unlikely that the diagnosis would need to be changed in an asymptomatic infant who had negative reagin tests after a 3 month period of observation. Most other authors are in agreement with this. Thus, Fiumara et al (1952) state that a 3 month follow-up period is needed before an infant can be regarded as not infected. Nelson and Struve (1956), Johnston (1972) and Budell (1982 pg 932) give a similar time period. Woody et al (1964) noted that infants with congenital syphilis will develop rising titres or clinical signs by 4 months of age.

It is, therefore, possible to make a definitive diagnosis in time although the need for serial follow-up is a serious disadvantage.

Ideally, a decision as to whether a newborn infant is infected or not should be made in the first few days of life prior to hospital discharge (Kaufman et al 1974). To achieve this other tests are needed, and the RF latex test, which can provide a rapid result may have theoretical advantages.

Treponemal tests

Treponemal tests performed on the infant also suffer from the problem of reflecting the mother's antibody status because of the passive transfer of IgG across the placenta (Rudolph 1976). This applies to tests such as the TPHA and FTA-ABS (IgG). Although usually negative by 6 months (Curtis and Philpott 1964; Taber and Huber 1975 pg 188), the tests may take as long as 12-15 months to become negative in uninfected infants (Taber and Feigin 1979; Rathbun 1983a; Ingall and Musher 1983 pg 359).

Owing to the difficulties described above, the treponemal tests are less useful for serial follow-up, and Huber (1984) believes that they have no place in the diagnosis of early congenital syphilis.

Tests based on IgM production

These tests are useful in that they measure antibodies produced by the fetus or infant as IgM does not cross the placental barrier (Alford 1971).

All the IgM class antibody can be measured (total IgM level) or IgM directed against Treponema pallidum can be detected (e.g. using the FTA-ABS IgM test). Rheumatoid factor may also be part of the IgM response in congenital syphilis (Reimer et al 1975) and can be identified using the RF latex test. There are, in addition, several newer serological tests and these will be described in section 2.3.6.

Total IgM levels

Serum levels of IgM can be measured by means of a radial immunodiffusion plate; a procedure which requires relatively small amounts of serum and from which one may obtain a result within 24 hours (Fahey and McKelvey 1965). Umbilical cord serum can be used although it is necessary to ensure that there is no admixture with maternal blood (Alford et al 1969b).

Normal newborn serum contains only small amounts of IgM (Stiehm et al 1966). IgM production may be markedly increased in response to intrauterine infections (McCracken and Shinefield 1965; Alford 1965). Although an elevated IgM level is not specific for any particular pathogen, congenital syphilis and rubella appear to give the highest levels (Alford et al 1969b). Dudgeon (1975) states that while 50-60% of patients with congenital rubella had raised IgM level, congenital infection with toxoplasmosis and syphilis are more likely to give consistently elevated levels.

Nevertheless, there may be overlap between levels of IgM found in normal infants and those with infection. It is, therefore, difficult to define an 'elevated level'. It is desirable to determine a level where there are a minimum of false negatives without an overwhelming number of false positives (Alford 1971). For this reason most workers in the United Stated have chosen values of 18 - 20 mg % which is between the first and second standard deviations above the mean (Alford 1971).

The usefulness of this level has been demonstrated in studies by Alford et al (1969b) who performed IgM estimations on the cord sera of 2916 live births. Elevated IgM levels were identified in 123 (34%) of the infants. Infection was found in 42 (34%) of this group - 18 (43%) being intrauterine infections. Compared with the control group, there was a 30-fold increase in the rate of infection in newborns with raised IgM levels.

Although there is no apparent association between IgM level and socioeconomic status in the USA (Alford 1971), workers in South America have found that 40-60% of infants from urban slums and

rural areas had raised IgM levels (Lechtig and Mata 1971). It is, therefore, necessary to define normal values for the population one is dealing with.

Alford (1971) has suggested that measuring IgM levels in newborns is useful in two main circumstances:

i. A raised IgM is useful confirmatory evidence that an infants symptoms are due to intrauterine infection. Specific tests are then needed to identify the precise agent.

ii. As a screening test to detect subclinical infection.

In the first category (that of symptomatic infants) raised IgM levels have frequently been demonstrated in congenital syphilis. Thus, Tan (1973) found that 5 out of 6 such infants had raised IgM levels. Similar results, also with relatively small numbers of patients have been noted by other authors (e.g. Mamunes et al 1970; Taber and Huber 1975 pg 187; Borobio et al 1980; Srinivasan et al 1983; Ewing et al 1985). A larger series was reported by Malan (1987 pg 628) who found elevated IgM levels in 17 out of 18 cases. Similarly, Meyer and Malan (1987) published a series in which raised levels were demonstrated in 10 out of 12 infants at birth.

Sever et al (1969) described a different method of detecting raised IgM levels. They used a latex reagent which they found to be quick and gave good agreement with the gel method. Tympner and Neuhaus (1972) used the test to detect levels of IgM greater than 30 mg %. All cases of congenital syphilis studied by them had a positive IgM latex test. Al-Nakib et al (1985) have also used this method to detect elevated IgM levels in cord blood.

Using raised IgM levels to screen for infants with subclinical congenital syphilis has been the subject of fewer publications. However, soon after the introduction of radial immunodiffusion tests it was realized that normal amounts of IgM could be found in asymptomatic infants. Thus, Alford et al (1967) described 2

infants who in spite of normal IgM levels at birth went on to develop features of congenital syphilis.

Mamunes et al (1970) were able to adequately observe 37 infants who had positive cord blood VDRL tests. Four of the infants developed definite congenital syphilis. Three had signs at birth and had raised IgM levels; one infant was asymptomatic at birth and had a normal IgM level at this time. A further two cases were asymptomatic in the neonatal period and had normal IgM levels. They were treated and the outcome in these 2 cases could not be definitely resolved.

Borobio et al (1980) described a series of 9 infants with congenital syphilis. Two of the cases were definitely asymptomatic at birth and a further case was presumably asymptomatic. All 3 cases were found to have raised IgM levels and positive FTA-ABS (IgM) tests. According to the criteria of Kaufman et al (1977) or Mascola et al (1985) the 3 asymptomatic infants would have been regarded as possible cases of congenital syphilis. Another study which determined IgM levels in asymptomatic patients was that of Srinivasan et al (1983). These workers found normal IgM levels in all 61 high-risk infants who did not develop congenital syphilis. There were 4 infants who were asymptomatic but had raised levels of IgM. The results of all the other investigations were normal. The authors were uncertain as to whether these infants would develop congenital syphilis or not. They opted to treat them but suggested that raised IgM levels could be used to screen out infants needing further investigation.

The work reviewed here would suggest that most symptomatic infants with congenital syphilis will have raised levels of IgM. Furthermore, raised levels may be useful in identifying a group of asymptomatic infants who would benefit from further surveillance and testing. Conversely, from the small number of patients reported, it appears that a normal level cannot be used as a screening test to rule out congenital syphilis in an asymptomatic infant. A large prospective study determining outcome in relation to IgM levels in this group of babies remains to be done.

Fluorescent Treponemal Antibody Absorption Test for IgM (FTA-ABS (IgM))

Scotti and Logan (1968) modified the FTA-ABS test for use in congenital syphilis. The test is performed by adding the patient's serum to a slide which has been coated with Nichol's strain of Treponema pallidum. (Nonspecific group antibodies are first removed with 'sorbent' prepared from nonpathogenic treponemes). Fluorescein-labelled anti-human IgM is then added after incubation and washing steps. Fluorescence indicates the presence of IgM antibody attached to the fixed treponemes (Jaffe 1975, Hart 1986).

Kaufman et al published a review of the use of the test in the diagnosis of neonatal congenital syphilis in 1974. The authors point out difficulties in comparing the 7 studies reviewed. Problems included the use of different criteria in the diagnosis of congenital syphilis, the selection of cases was not the same and there was no uniformity in the test conditions. Furthermore, there was a lack of information about the ages of patients and the source of the test specimens.

Kaufman and co-workers divided the results into those obtained in symptomatic and asymptomatic neonates. Pooling the results obtained by different investigators, they found that 88% of symptomatic neonates had a positive test. Of the infants who were initially asymptomatic, 65% had a positive test.

False positive test results were obtained in 8.4% of controls, mostly in the group who had passive transfer of maternal antibodies. In well infants with negative STSs the test was 100% specific.

Kaufman et al pointed out the limitations of their study. Nevertheless, they were of the opinion that a 'technically consistent, prospective study of carefully delineated clinical groups' was needed.

A series, published by Rosen and Richardson (1975), met most of these pre-requisites.

Of 115 infants (33 presenting in the first week of life) with 'definite clinical and/or radiologic stigmata of congenital syphilis' 114 had positive FTA-ABS (IgM) tests. Of 51 asymptomatic patients at risk for congenital syphilis, all but 4 had negative FTA-ABS (IgM) tests. Three of these 4 later developed features of congenital syphilis, the remaining patients with negative tests proved to be normal on follow-up (albeit after a rather short period of 9 weeks). Thus, although there were only 3 asymptomatic patients the test was positive in all and there was only 1 false positive result. The test was negative in 20 controls whose mothers had negative serology.

However, Kaufman et al (1974) suggested that a group of patients with nonspecific features of infection who later proved not to have congenital syphilis be studied. Including patients with CMV or rubella for example, may have indicated that the FTA-ABS (IgM) test as performed by Rosen and Richardson and others was not specific for congenital syphilis. This was suggested by Reimer et al (1975).

These workers mixed purified infant's IgM with the serum of an adult with syphilis whose IgM had been removed. The immunofluorescence observed was almost obliterated by absorbing the infant's serum with insolubilized IgG (which would extract RF). The likely sequence of events was that the anti-treponemal IgG in the adult serum bound to the treponemes in the immunofluorescence test. The IgM RF from the infant then bound to the IgG already attached to the treponemes and was detected by the anti-human IgM-fluorescein conjugate. As shown by Reimer et al (1975) infants with various congenital infections produced RF. Thus, it was postulated that a nonspecific result could be obtained unless steps were taken to eliminate RF interference.

Demonstration of specific IgM antibodies by immunofluorescence techniques has been used as a diagnostic test for many chronic intrauterine infections (Alford et al 1975). To improve the specificity of the tests RF has been removed by a variety of methods (Stagno et al 1980, Tuomanen and Powell 1980, Chonmaitree et al 1982). Müller and Sinzig (1982) prevented RF interference by removing the IgG using gel filtration.

In addition to improving the specificity of the FTA-ABS (IgM) test, IgG removal may enhance the latter's sensitivity by preventing competition between IgG and IgM antibodies for binding sites (Cohen et al 1967).

The modification of the FTA-ABS (IgM) test whereby testing is performed only on the IgM fraction has become known as the FTA-ABS 19S IgM test.

Müller and Sinzig (1982) carried out both specific IgM tests on a group of 26 children with congenital syphilis. The full clinical details of the patients whose ages ranged from 2 days to 14 months were not given but in 14 cases definite clinical signs of the disease were present. In the remaining cases the diagnosis was suggested by the history of untreated syphilis in the mother, positive 19S IgM tests, and by the presence of a reactive reagin antibody test beyond the age of 3 months in 2 asymptomatic patients. The FTA-ABS 19S IgM test was positive in all 26 cases, whilst the FTA-ABS (IgM) test, which was done in 25 instances, was reactive in only 16 patients.

The study has several limitations. The tests were not all performed at birth but were done at various ages. Thus, the predictive value of the tests at birth cannot be assessed. In addition, a 'gold standard' was not applied in making the diagnosis of congenital syphilis, but rather the results of the FTA-ABS 19S test were used. It was evident, however, that the diagnosis of congenital syphilis could have been substantiated in 16 of the 26 cases by the clinical signs and results of reagin testing.

The FTA-ABS 19S IgM test was positive in all 16 of these patients whilst the FTA-ABS (IgM) test was positive in 12 out of 15 cases. The differences between the 2 tests may, therefore, not be so great as suggested at first glance.

In spite of incomplete evaluation, and the fact that the FTA-ABS 19S IgM test is technically complicated and expensive (Müller et al 1987), the investigation has become the standard procedure

for detecting anti-treponemal IgM in Holland (Ijsselmuiden et al 1989).

In 1985, Cerny and co-workers reported the results of using a simpler immunoprecipitation method to remove IgG from sera prior to testing for anti-treponemal IgM. These investigators were able to show that the technique not only removed RF but improved the sensitivity of the specific IgM test in adults.

The use of this easy step to remove RF and IgG has not been evaluated in the diagnosis of congenital syphilis. One could anticipate an increase in the specificity of the FTA-ABS (IgM) from the work of Reimer et al (1975). In addition, by preventing competition between IgM and IgG antibodies, the sensitivity of the test may be enhanced (Cohen et al 1967). On the other hand, given that nontreponemal IgM (which would presumably include RF) appears to increase before specific IgM (Alford et al 1969a), removal of the RF may make the test less sensitive. These aspects need further study.

It is apparent, however, that the main drawback of the FTA-ABS (IgM) test is the fact that it's sensitivity in asymptomatic patients is only 65%. From the information currently available there is no reason to suggest that the FTA-ABS 19S IgM test is superior in this regard. As previously described, when dealing with a treatable condition such as congenital syphilis with serious long-term sequelae, a test with high sensitivity is required. Another disadvantage of the fluorescent test for IgM antibodies is that specialized laboratory procedures are necessary. Apart from being expensive, this may lead to the results being unobtainable prior to the newborn's discharge from the nursery.

A far simpler test for another group of IgM antibodies produced in congenital syphilis is the RF latex test.

Rheumatoid factor

Reimer et al (1975) reported the presence of RF using the RF latex test in congenital syphilis in 1975. There were 2 earlier reports describing anticomplimentary reactions in the disease

(Lighter 1953; Lassus et al 1969). The anticomplementary reaction, in which serum was able to inhibit haemolysis in an antigen-free mixture of serum and complement, rendered sera unsuitable for complement-fixation tests (e.g. the Wasserman reaction). It was found that RF, hypergammaglobulinaemia and cryoglobulins could all produce the anticomplementary effect. Lighter (1953) noted that the effect occurred commonly in congenital syphilis. Lassus et al (1969) also reported the phenomenon in congenital syphilis and performed RF latex tests on a group of syphilitics. it is not clear if patients with congenital syphilis were included in the group so tested.

The study of Reimer's group was, therefore, the first major report on the presence of RF in congenital syphilis. The authors suggested that the infants produced large amounts of RF and relatively small amounts of IgM specific for Treponema pallidum. They found that 26 of 27 infants with congenital syphilis, 13 of 16 with cytomegalovirus infection, 4 out of 6 with congenital rubella and a single newborn with congenital toxoplasmosis all had RF present. The RF was detected by means of the latex agglutination test. The infants with congenital syphilis had a wide range of RF titres, but 22 of the 26 had endpoint titres of 1:16 or above. In contrast to the large number of infected babies with positive RF tests, only 5 of the 42 sera from normal infants were positive.

The authors pointed out that the test was simple, rapid and economical and appeared to differentiate infected from healthy infants. The study was, however, retrospective and was really of a preliminary nature and as such it had several limitations.

The latex tests were performed on banked sera supplied by investigators who 'thought' the infants were infected with the relevant diseases mentioned. Neither the criteria for diagnosis nor the clinical features were stated. Thus, whether any of the infants were asymptomatic at the time of serum collection or whether any infants were newborn is unknown.

Furthermore, the control infants were not clearly described. The age of the controls is not stated; as discussed below this

has an important bearing on the number of false positives likely to be encountered. None of the controls were reported as having passively acquired reagin antibodies from their mothers. Therefore, it was unknown if the RF latex test could distinguish active infection from a state of passively acquired immunity.

It is also obscure from the work of Reimer et al (1975) if testing for RF has any application in determining whether an infant who displays clinical signs of congenital syphilis is in fact suffering from this condition. Such a question cannot be answered from Reimer's study, which, while raising several interesting possibilities, cannot be construed as indicating that the RF latex test is necessarily a very useful test for congenital syphilis.

Borobio et al (1980) performed RF latex tests on 9 patients with congenital syphilis. These tests were negative in all patients at a dilution of 1:25. It is difficult to explain why the test was negative in these cases. The sensitivity of the RF latex test used is not mentioned, however, and it is not stated if the tests were repeated at other dilutions. It is possible, therefore, that a less sensitive detection system was used compared to that of Reimer et al (1975).

Meyer and Malan (1987) described a preliminary study designed to clarify some of these points. Of 12 neonates with congenital syphilis, 11 were symptomatic. All 12 had positive RF latex tests. Total IgM levels were increased in 10 of these 12 cases. A further 3 cases presented after the first few weeks of life. All 3 patients had positive RF latex tests. The RF latex test was negative in 80 normal newborns who acted as controls and in 14 infants whose mothers had positive serological tests but whose infants had no features of congenital syphilis at birth. Infection in this last group could not be excluded as the infants were not followed-up. Nevertheless, the results confirmed the findings of Reimer et al (1975) and suggested that testing for RF may enable a distinction to be made between

newborns with passively transferred reagin antibodies and those with active infection.

The studies quoted above used the RF latex test to detect IgM RF. Other techniques are available, however, including an RF ELISA (Faith et al 1982). This was utilized by Dobson et al (1988a) who demonstrated IgM RF in 3 out of 6 cases of congenital syphilis. Tests for IgG RF were negative.

Pedersen et al (1989) have also recently reported RF ELISA results in infants with congenital syphilis. Their study is described more fully in Section 2.3.6, but in summary 7 of 9 symptomatic infants with congenital syphilis had RF present. In contrast, the RF ELISA was negative in 15 infants born to mothers who had been treated antenatally for syphilis. As these infants were not followed-up it was not possible to determine their outcome. The results of these workers appear to confirm those of Meyer and Malan (1989).

The work on RF in congenital syphilis to date has not considered the problem of asymptomatic infants. There is, however, a report describing the use of the RF latex test in asymptomatic infants with congenital CMV.

Stagno et al (1980) compared diagnostic procedures for CMV. Of 20 infected newborns, all of whom were asymptomatic, 7 (35%) had RF in their cord sera. Total IgM levels were elevated in 33% of infected infants. The authors also noted that none of the sera of 676 controls gave positive RF results at birth, while 21 of the controls had raised IgM levels at this time. However, by the end of the first month 1 of 7 controls (14%) had positive RF tests. Four of 32 controls (12%) had positive tests by 3 months and another 2 out of 17 (12%) tested positive at 6 months. It was evident that, in asymptomatic newborns, a positive RF test was the most convenient serological screening test for congenital CMV.

There are a number of pertinent questions concerning RF and tests for congenital syphilis.

i. How useful would the RF latex test be to screen for

infection in a group of infants at high-risk of congenital syphilis (eg.those born to untreated or incompletely treated mothers with positive STS)?

ii. Would the RF latex test be of value in distinguishing infants with congenital syphilis from those with other conditions who may present with similar clinical signs?

iii. What effect would RF removal have on the usefulness of the FTA-ABS (IgM) test for congenital syphilis?

2.3.6 Recent advances in antigen detection and serodiagnosis

Lukehart (1986) has recently reviewed the advances in this field. The newer techniques may involve antigen or antibody detection.

Antigen detection

The genes encoding T.pallidum antigens have been cloned and attempts are being made to obtain purified antigens. Monoclonal antibodies can then be raised to the relevant antigens and the former used to detect antigens in body fluids or secretions (Norgard et al 1984). In addition, DNA encoding treponemal antigens could be used as a DNA probe to identify treponemes (Norgard et al 1986).

Antibody detection

Whole or purified treponemal antigens can also be used to detect antibodies. For example, IgM antibodies can be measured using an ELISA method (the so called Treponema pallidum specific IgM ELISA or Tp-IgM-ELISA). Alternatively, the Western blotting technique can be employed for the same purpose.

Initially, a Tp-IgG-ELISA was described (Veldkamp and Visser 1975). Later, Lindenschmidt et al (1983) and others modified this to detect IgM antibodies. In addition, the treponemal antigen was also purified.

Both Cerny et al (1985) and Muller et al (1987) used sonicated T. pallidum as the antigen for the ELISA. The latter workers

carried out the test on a group of adult patients with syphilis and found a sensitivity and specificity of over 97%.

Until recently, however, one of the limitations of the test has been a lack of commercially available antigens (Ijsselmiuden et al 1989). These authors have now evaluated an IgM capture ELISA. As a result of finding IgM antibody in 5 infants with symptomatic congenital syphilis and an absence of such antibody in 21 uninfected neonates with passively acquired maternal antibodies, the workers have suggested that the test is useful for screening for congenital syphilis. This statement appears to be premature as a group of asymptomatic infants with delayed onset disease were not included in the study. It is, nevertheless, conceivable that this test, or modifications of it, may be shown to be a suitable screening test for congenital syphilis in time to come.

In fact, Pedersen et al (1989) have very recently, published the results of using an ELISA for IgM antibodies against purified treponemal flagella. The study compared the IgM flagellum ELISA, the FTA-ABS (IgM) test and an ELISA for IgM reactive with the VDRL antigen. Three rather heterogeneous groups of patients were studied. Group 1 comprised 84 newborn infants from Botswana. Most of the mothers of these infants had negative screening tests for syphilis, but 2 had positive tests. One of these 2 infants was healthy at birth and tested negative for IgM with all 3 tests listed above. The other infant had congenital syphilis and all 3 tests were positive. The remaining infants in group I did not have congenital syphilis and had negative IgM tests.

Group 2 consisted of 10 infants born to mothers from Zambia with untreated syphilis. All 10 patients had clinical signs of congenital syphilis; 9 had positive IgM tests (using all 3 techniques).

In group 3 (also from Zambia) there were 15 newborns whose mothers had been treated for syphilis at varying stages of

pregnancy. Four of the infants had hepatomegaly; 1 of these 4 patients had positive IgM tests. The other 11 infants were asymptomatic and the IgM tests were negative except for 1 case whose mother had only been treated at 30 weeks.

The study has seveal limitations. The authors do not state what their 'gold standard' for the diagnosis of congenital syphilis is. They do not appear to have used a set of criteria for this. Hence, for example, it is not clear whether they regarded the 4 infants with hepatomegaly as having the disease or not. A similar lack of clarity exists with regard to the asymptomatic patients. There was 1 such patient in group 1 whose mother had untreated spyphilis. As this case was not followed-up the significance of the negative IgM tests could not be determined. Any of the asymptomatic patients in group 3 may or may not have been infected.

What points can be made from the study? All 3 IgM tests were able to detect symptomatic congenital syphilis. The tests had equal sensitivity in this regard. The VDRL IgM ELISA has potential advantages over the FTA-ABS (IgM) and flagellum ELISA in its simplicity and the fact that the quantitative results of an ELISA are likely to be easier to assess than those of the rather subjective fluorescent antibody tests (Griffiths et al 1982). On the other hand, it could be argued that the FTA-ABS (IgM) test has been in use for many years and its limitations are known (Kaufman et al 1974).

The study is of little value in determining the usefulness of the tests in asymptomatic but infected infants - the very group where sensitive tests are needed (Kaufman et al 1974). In addition, if the 4 infants in group 3 who had hepatomegaly are regarded as having congenital syphilis, the IgM tests would have been falsely negative in 3 out of the 4 cases. Perhaps the authors would have produced a simpler paper by considering only 2 groups - those who definitely did not have congenital syphilis (by virtue of being asymptomatic and having mothers with negative STS) and those who definitely had the disease (by criteria other than the IgM tests under review).

Western blotting has been used by Dobson et al (1988a) to demonstrate the IgM response to treponemal antigens. The workers were able to show that the sera of all 6 infants with congenital syphilis demonstrated reactivity against 47 and 37 kilodalton (kDa) antigens, whilst these findings were absent in 13 control sera.

S'anchez et al (1989) extended the observations to 12 symptomatic infants with congenital syphilis, 9 asymptomatic infants with uncertain infection and 18 well infants of mothers previously treated for syphilis. Each of the symptomatic infants had IgM antibodies directed against the 47kDa antigen. In addition, 2 of the asymptomatic infants showed a similar pattern of reactivity. A lack of response to the same antigens was noted in 13 control sera. The remainder of the infants did not show a response to the 47kDa antigen.

The findings of Dobson's and S'anchez's groups are important but these new approaches have not yet been shown to be of value in asymptomatic infants with delayed onset congenital syphilis. It is not known if the 2 asymptomatic infants with IgM reactivity to the 47kDa antigen described by S'anchez et al (1989) were actually infected as they were treated with penicillin. Furthermore, the final outcome of the other 7 infants in the group with uncertain disease is not stated.

It follows that much work still needs to be done in order to evaluate the Western blotting technique fully.

2.3.7 Cerebrospinal fluid (CSF) examination

Platou (1949) described CSF 'abnormalities' in 63,6% of 172 cases of early congenital syphilis. Most authorities agree that elevations in white cell count, raised protein concentrations, the presence of spirochaetes and a positive VDRL may be found in congenital neurosyphilis (Rathbun 1983a). However, the values regarded by Platou as abnormal (5 or more cells per mm^3 and protein above 45 mg %) would now be considered normal in newborns (Naidoo 1968, Sarff et al 1976). Another potential problem is passive transfer of antibody of maternal origin from

blood to CSF in infants. Thorley et al (1975) demonstrated that this occurs with diphtheria and tetanus antibodies. The extent to which this affects VDRL results in the CSF in congenital syphilis is unknown.

Srinivasan et al (1983) evaluated the role of CSF examinations in the diagnosis of congenital syphilis. All 61 infants who were asymptomatic at birth (but at high-risk of developing congenital syphilis) had normal CSF tests. They were treated with benzathine penicillin (which is unlikely to produce adequate CSF levels - McCracken and Kaplan 1974) and the 25 who returned for follow-up were clinically normal. Of eight infants with clinical disease or raised IgM levels, two had abnormal CSF tests. All nine infants presenting with congenital syphilis in the postneonatal period had normal CSF findings. The authors noted that neurosyphilis appeared to be uncommon and that asymptomatic infants were unlikely to have CNS involvement as detected by lumbar puncture.

Recently, Mascola et al (1985) noted that 18 (53%) of 34 infants with congenital syphilis had a positive VDRL test of the CSF. Similarly, Hira et al (1985) found that 44% of newborns with the disease had an abnormal CSF. A publication from the Centers for Disease Control (CDC 1986) suggests a different picture. Of 460 patients with suspected congenital syphilis under 1 year of age, 241 (53%) were regarded as definite or probable cases. Altogether, 34 infants had clinically significant CNS involvement, but only 5 had CSF serological evidence of neurosyphilis. Unfortunately, the total number of infants who had CSF examinations is not stated.

A recent publication from the Centers for Disease Control (CDC 1988c) on congenital syphilis, states that the role of CSF examination in the diagnosis is debatable. As the most recent treatment protocols from the Centers for Disease Control (CDC 1988c) advise treatment of all infants with a regimen effective for neurosyphilis, no change in antibiotic policy could be expected from performing a lumbar puncture. It is uncertain whether there would be a change in the prognosis.

It is also noteworthy that the performance of a spinal tap may have a high failure rate (e.g. the procedure was unsuccessful in 64/142 of 45% of the cases described by Hira et al 1985).

2.3.8 Other investigations

Other laboratory investigations may suggest or lend support to a proposed diagnosis of congenital syphilis. These findings have been reviewed by Ingall and Musher (1983 pg 347-348).

The full blood count may demonstrate anaemia with or without features of haemolysis, leucocytosis, leucopaenia or thrombocytopenia.

The blood chemistry may indicate hyperbilirubinaemia (either conjugated or unconjugated), hypoalbuminaemia and/or raised liver enzymes (e.g. alanine aminotransferase).

Urinary abnormalities including proteinuria may be present.

The wide range of abnormal laboratory findings described serves to stress the protean manifestations of the disease. In general, however, the tests are of an ancillary nature and none are so consistently associated with congenital syphilis as to be of value as screening tests.

2.4 DIAGNOSTIC CRITERIA FOR CONGENITAL SYPHILIS

The problem of a lack of a 'gold standard' test in the diagnosis of congenital syphilis has already been outlined.

It will be recalled from Section 2.3.5 that for practical purposes a diagnosis of congenital syphilis can be excluded if the VDRL titre has declined to zero and there are no clinical signs by 3-4 months.

Several groups have described sets of criteria by which the diagnosis can be 'ruled in'.

2.4.1 Criteria of Kaufman et al 1977

Kaufman and co-workers (1977) developed a set of criteria for the diagnosis of neonatal and early congenital syphilis. The

criteria are shown in Table 2.1 and include clinical, serological and epidemiological data. While useful for assigning disease status, some points require clarification and there are difficulties with the 'probable' cases.

i. From the list of criteria it appears that the diagnosis of probable congenital syphilis can be made on the basis of one major and one minor clinical feature. This interpretation of the criteria is correct if the STS are positive. The diagnosis is stated as being unlikely in the absence of positive STS, and in their 'Discussion', the authors note that 'upon initial examination the ideal test should be at least a quantitative VDRL test'. If the proviso of initial serological testing is ignored the criteria would be non-specific as findings such as radiographic changes in the long bones and hepatosplenomegaly have been noted in symptomatic infants with congenital CMV (Mahboubi 1981; Stagno et al 1984 pg 109).

ii. The VDRL usually reverts to nonreactive in uninfected infants by 4 months (serologic criterion D). Several authors have noted that 6 months may be the outside limit (Brown and More 1963; Taber and Huber 1975; Srinivasan et al 1983; CDC 1988c).

iii. The use of a reactive FTA-ABS (IgM) test along with 2 minor criteria needs clarification. Reimer et al (1975) suggested that a positive test may be obtained in the presence of RF. Infants with intrauterine infections other than congenital syphilis (e.g. toxoplasmosis, cytomegalovirus) may produce RF and have 2 of the minor clinical criteria (e.g. hepatosplenomegaly and central nervous system signs). Thus, one needs to specify that interference from RF is removed prior to the FTA-ABS(IgM) test.

iv. The presence of a reactive FTA-ABS (IgG) test and one major clinical criterion may also be relatively nonspecific in certain populations. For example, in many parts of South

Africa approximately 6-8% of antenatal clinic attenders have positive serology for syphilis (Naicker et al 1983; Gonin 1985) The FTA-ABS (IgG) may be positive in the infant because of passive transfer of IgG antibodies (Curtis and Philpot 1964). In a population where syphilis is prevalent it is conceivable that periostitis, itself a nonspecific feature (Shopfner 1966), may be present in infants of adequately treated mothers.

2.4.2 Criteria of Rathbun (Rathbun 1983b)

Rathbun published a modified set of criteria in 1983 (Table 2.2). To the clinical criteria of Kaufman et al (1977) were added a positive reagin test of the cerebrospinal fluid and bullous lesions or a palmar/plantar rash. Pneumonitis, oedema/ascites, placental abnormalities and intrauterine growth retardation were added to the minor clinical criteria. These findings are nonspecific (Benirischke and Discoll 1967 pg 269-270; Malan 1987 pg 627) and the value of intrauterine growth retardation is particularly doubtful for several reasons. Firstly, in some communities it is extremely common and without obvious cause (e.g. nearly 6% amongst Cape Coloureds in Cape Town - Malan et al 1967). Secondly, Naeye (1971) has shown that congenital syphilis has little effect on fetal growth.

The serological criteria have been modified to include the development of a positive treponemal antibody test after birth. This would presumably include the FTA-ABS (IgM) test and would be subject to the same limitations as previously discussed. The presence of a positive treponemal antibody test after 1 year of age has also been included. The limitations of this are that the treponemal tests may remain positive for up to 15 months in uninfected cases (Taber and Feigin 1979; Rathbun 1983a).

The epidemiologic criteria have also been expanded to include untreated early syphilis in the mother within 4 weeks of delivery. Unfortunately, the duration of maternal infection is often unknown (Ingraham 1936). This is a serious disadvantage as it means that one of the major criteria will rarely be met.

Under the heading 'minor epidemiologic criteria', Rathbun has included 'Mother an untreated contact to lesion syphilis during pregnancy'. Presumably this is added to cover the lag period of 4-8 weeks after infection during which the mother's serology may be negative (Felman and Nikitas 1980).

2.4.3 Criteria of Hira et al (1985)

These authors have also published guidelines for the diagnosis of congenital syphilis (see Appendix 1, Table A.1.1).

In general, the format is similar to that of Kaufman et al 1977), although there are some changes. The radiological features have been moved to the section of minor clinical criteria. This seems reasonable in view of the large numbner of false positives (35%) obtained in normal infants (Shopfner 1966) In addition, the 'haemolytic anaemia' listed by Kaufman et al as a minor clinical criterion has been replaced with jaundice and anaemia (listed as 2 separate criteria). Again, this has some merit as the anaemia of congenital syphilis is not always haemolytic (Sartain 1965).

On the other hand, jaundice is common amongst newborns without congenital syphilis and, as suggested by Ingall and Musher (1983 pg 347) it is when the jaundice persists or appears after the first week that the diagnosis should be considered.

One of the major criticisms of the criteria of (Hira et al 1985) is that the probable and possible cases are identical with respect to the clinical signs. This makes it pointless to call the radiologic changes 'minor' rather than 'major'. The authors have suggested that in Zambia the minor clinical criteria are much commoner in syphilitic infants than noninfected controls and hence have been given the same weight as the major clincal criteria. This finding cannot necessarily be extrapolated to other communities where congenital syphilis is less prevalent.

It would seem that the criteria have no real advantage over those of Kaufman et al although they could serve to alert workers in Zambia to the possibility of congenital syphilis.

2.4.4 Criteria of Mascola *et al* (1985)

Mascola *et al* (1985) published a very similar set of criteria to those of Kaufman *et al* (see Appendix A.1, Table A.1.2). The only change was in the category of 'possible' congenital syphilis where serological criterion A or B with only one minor criterion was added. Although Kaufman did not explicitly state this, for practical purposes the criteria are the same in these 2 papers.

2.4.5 Criteria outlined by the Centers for Disease Control

More recently, the Centers for Disease Control (CDC 1988c) have suggested a different set of diagnostic classifications for congenital syphilis (Table 2.3).

Cases formerly known as 'probable' or 'possible' are now regarded as 'compatible'. This may be useful in deciding who needs treatment. Clinically and prognostically, however, it does not distinguish for example an infant who has gross signs of disease from one who is completely well but whose mother had only 2 doses of benzathine penicillin.

Stillborn infants with a reactive STS are also placed in the 'compatible' category. This may be useful for surveillance but from a clinical point of view may give one a false sense of security about the diagnosis. Some authors have regarded infection as 'proven' only when spirochaetes have been demonstrated in the infant or placenta (Dippel 1944; Naeye 1977). Once again, where maternal syphilis is prevalent, an adequately treated mother could have a stillborn infant with a positive STS who died from an unrelated cause.

It is apparent that none of the proposed criteria are perfect. The more recently suggested guidelines do not appear to have any significant advantages over those of Kaufman *et al* (1977). Those of Rathbun (1983) may be too broad for a developing country where intrauterine growth retardation and incomplete maternal therapy are common. The resultant groups of patients obtained with the criteria of Kaufman and co-workers (i.e. definite, probable, possible, unlikely) appear to be more clear-

cut than those obtained with the Centers for Disease Control criteria (confirmed, compatible, unlikely).

The Centers for Disease Control criteria also have implications for treatment which need to be considered in a developing country such as South Africa. As previously mentioned, 5-8% of antenatal clinic attenders have positive serology for syphilis. According to Manning et al (1985) 90% of pregnant women with syphilis at King Edward VIII Hospital in Durban did not receive the therapy as prescribed. These women would be regarded as not having received adequate treatment. The Centers for Disease Control would recommend treating all the infants of these mothers with a 10 day course of parenteral penicillin. In the light of the load on the health care system, this would probably not be feasible.

Rather than treat all cases various authors (e.g. Stokes et al 1945 pg 1097-1099; Kaufman et al 1977; Ingall and Musher 1983 pg 366) have suggested a period of follow-up and surveillance (usually lasting 3-4 months). Again, this poses a problem when one considers the numbers involved.

For the purposes of this study consistent criteria for the diagnosis of congenital syphilis were considered to be essential. The guidelines of Kaufman et al (1977) were followed with the exception that 6 months was taken as the limit for nontreponemal tests to revert to negative.

2.5 SUMMARY

The history of the pregnancy and the results of the STS for syphilis performed on the mother and/or baby may indicate that the infant is at risk for congenital syphilis.

In this situation, the demonstration of T.pallidum is the method of choice for definitive diagnosis of congenital syphilis, but is not often possible.

Clinical and radiological changes and a positive FTA-ABS (IgM) test are helpful if present but their absence does not exclude the diagnosis. In practice, monitoring serial VDRL titres

combined with clinical surveillance is often the most certain method of diagnosis. Ideally, one would like a test (or tests) which could be done prior to the infant's discharge and following which definite decisions as to whether the infant is infected or not could be made. Work from Alford et al (1969a) suggests that nontreponemal IgM may be produced before disease-specific IgM and Reimer et al (1975) have noted production of relatively large amounts of RF and small amounts of specific IgM. Thus, testing for RF to identify infected infants appears to be worth further evaluation. Likewise, the use of elevated IgM levels as a test for congenital syphilis has not been extensively studied and no large prospective study has been reported.

Furthermore, the clinical findings in infants with congenital infection may be similar to those produced by other conditions. Would testing for RF help clarify which infants have congenital syphilis and those who do not?

Finally, the specificity of the FTA-ABS (IgM) has been in doubt following the work of Reimer et al (1975). Methods now exist for the rapid removal of RF. What effect would this have on the usefulness of FTA-ABS (IgM) test?

These questions merit further investigation.

TABLE 2.1 : *CRITERIA FOR THE DIAGNOSIS OF NEONATAL AND EARLY CONGENITAL SYPHILIS (KAUFMAN ET AL 1977)

Diagnostic Criteria

Clinical

Absolute

(1) Specimen from lesions showing presence of T.pallidum by dark-field examination, or by histologic examination

Major

(2) Condyloma lata
(3) Osteochondritis, periostitis
(4) Snuffles or hemorrhagic rhinitis

Minor

(5) Fissures of lips
(6) Cutaneous lesions
(7) Mucous patches
(8) Hepatomegaly, splenomegaly
(9) Generalized lymphadenopathy
(10) Central nervous system signs
(11) Hemolytic anemia
(12) Elevated cells or protein in cerebrospinal fluid

Serologic

A. Reactive STS (VDRL or FTA-ABS)
B. Reactive FTA-ABS (IgM) Test
C. Nonreactive STS (VDRL and FTA-ABS)
D. Reactive STS (VDRL or FTA-ABS) that does not revert to nonreactive within 4 months
E. Rising VDRL titer over 3 months

Maternal History

AA Documented history of adequately treated syphilis during pregnancy

Certainty of Diagnosis

Definite - Absolute clinical criterion

Probable - Any of the following: (1) Serologic criterion E; (2) Serologic criterion D; (3) One major clinical criterion and serologic criterion A or B; (4) Two or more minor clinical criterion and serologic criterion A or B; (5) One major and one minor clinical criterion.

Possible - Serologic criterion A or B without clinical criterion

Unlikely - (1) Serologic criterion C with any other criterion; (2) Serologic criterion A or B with maternal history AA.

STS - Serologic Test for Syphilis
VDRL - Venereal Disease Research Laboratory Test
FTA-ABS - Fluorescent Treponemal Antibody-Absorption Test
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TABLE 2.2: *CRITERIA FOR THE DIAGNOSIS OF EARLY CONGENITAL SYPHILIS (PATIENT YOUNGER THAN TWO YEARS) - RATHBUN (1983b)

Diagnosis Should Be Made When:

1. The absolute criterion is met.
2. The patient meets one major criterion and one major or minor criterion in another category.
3. The patient meets at least one minor criterion in each of the three categories.

Clinical Criteria:

Absolute:

1. Specimen from lesions showing Treponema pallidum on dark-field of histologic examination

Major:

2. Positive reagin test of cerebrospinal fluid
3. Condyloma lata
4. Osteochondritis, periostitis
5. Snuffles, hemorrhagic rhinitis
6. Bullous lesions, palmar/plantar rash

Minor:

7. Mucous patches
8. Hepatomegaly, splenomegaly
9. Generalized lymphadenopathy
10. Central nervous system signs
11. Hemolytic anemia, diffuse intravascular coagulation
12. Elevated cell count or protein in cerebrospinal fluid
13. Pneumonitis
14. Edema, ascites
15. Placental villitis or vasculitis
16. Intrauterine growth retardation

Serologic Criteria:

Major:

1. Fourfold rise in reagin titer and positive treponemal antibody test
2. Development of a positive treponemal antibody test after birth

Minor:

3. Positive reagin test after4 months of age
4. Positive treponemal antibody test after 1 year of age

Epidemiologic Criteria:

Major:

1. Untreated early syphilis in the mother within 4 weeks of delivery

Minor:

2. Untreated late latent syphilis in the mother
3. Early syphilis in the mother within 3 months of child's birth
4. Mother an untreated contact to lesion syphilis during pregnancy
5. Mother treated for syphilis during pregnancy with a drug other than penicillin
6. Mother treated for syphilis during pregnancy and not followed to delivery

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TABLE 2.3
DIAGNOSTIC CLASSIFICATIONS OF CONGENITAL SYPHILIS - CENTERS FOR DISEASE CONTROL (CDC 1988c)

Confirmed case

Identification of T.pallidum by dark field microscopy, fluorescent antibody, or other specific stains in specimens from lesions, autopsy material, placenta, or umbilical cord.

Compatible (formerly, "probable" or "possible") case

a reactive STS in a stillborn infant

OR

a reactive STS in an infant whose mother had syphilis during pregnancy and was not adequately treated, regardless of symptoms in the infant

OR

a reactive Venereal Disease Research Laboratory (VDRL) test of cerebrospinal fluid

OR

a reactive STS in an infant with any of the following signs: snuffles, condyloma lata, osteitis, periostitis or osteochondritis, ascites, skin and mucous membrane lesions, hepatitis, hepatomegaly, splenomegaly, nephrosis, nephritis, or hemolytic anemia

OR

fourfold or greater rise in titres of nontreponemal tests (VDRL or rapid plasma reagin (RPR)) and a confirmed fluorescent treponemal antibody absorption (FTA-ABS) or microhemagglutination assay for antibody to T.pallidum (MHA-TP) over a 3-month period

OR

a reactive treponemal test or nontreponemal test that does not revert to nonreactive in 6 months

Unlikely case

nonreactive STS

OR

treponemal tests revert to nonreactive within 6 months

OR

no symptoms in live-born infant whose mother, treated for syphilis during pregnancy, had a fourfold or greater fall in titer and the infant's STS is also fourfold or lower than the maternal titer was at the time of treatment

CHAPTER 3

RHEUMATOID FACTOR AND THE IMMUNE RESPONSE

3.1 INTRODUCTION

As described in Chapter 2, preliminary studies indicate that a significant percentage of infants with congenital syphilis have RF in their sera (Reimer et al 1975; Meyer and Malan 1987). This raises the question of what part RF plays in the immune response in congenital syphilis.

It is apparent that the host's inflammatory reaction is important in the development of the lesions of congenital syphilis (Benirschke 1974). Furthermore, congenital syphilis is the most severe form of syphilis (Handsfield and Lukehart 1984), with a high case fatality rate (see Section 1.2). It is of interest to note that the finding of RF in acquired syphilis is less common than in the congenital disease (Bartfeld 1969; Reimer et al 1975; Müller et al 1987; Meyer and Malan 1987). This difference may simply reflect the fact that there are more RF-producing cells in early life (Hardy et al 1987), and be unrelated to the severity of the disease. Higher concentrations of RF have, however, been associated with a more serious outcome in patients with e.g. rheumatoid arthritis (RA) (Gordon et al 1973).

To date, the role, if any, of RF in the pathogenesis of congenital syphilis has not been explored. There is, however, a large amount of literature describing the effects of RF in adults and experimental animals.

A summary of this literature is presented as a background against which further research on RF in congenital syphilis was undertaken.

3.2 HISTORY OF THE DISCOVERY OF RHEUMATOID FACTOR

Rheumatoid factors are antibodies reactive against a portion of immunoglobulin G and are so named because of their frequent association with RA (Davis 1979 pg 77; Carson 1982 pg 114).

Meyer in 1922 described the agglutination of red blood cells which had been 'sensitized' (or coated) with antibody. Serum from 8-10 patients (one of whom had cirrhosis and another with chronic bronchitis) resulted in erythrocyte agglutination. In retrospect this phenomenon was almost certainly due to RF.

Cecil et al (1930) found that serum from patients with RA agglutinated certain streptococci. This reaction was thought to lend support to the idea that streptococci were important in the pathogenesis of RA, although Wallis (1946a) doubted this. He showed that agglutination was not limited to streptocci but could also be obtained with pneumococci (Wallis 1946b). Lamont-Havers (1955) correctly surmised that a component of normal serum (later found to be IgG) attached to the bacteria and the agglutination was completed by a 'specific rheumatoid arthritis factor' from the serum of patients with RA.

Waaler also performed some of the early work on RF. He presented his work on the 'agglutinating activating (AA)' factor at a congress in 1939 and published his work in 1940. He found that sensitized sheep red blood cells were agglutinated with serum from a patient with RA. (Interestingly, the patient was initially thought to have syphilis).

Similar observations were made by Rose, Ragan, Pearce and Lipman (1948). Pearce who had RA was working on rickettsial disease. She contracted rickettsial pox and her serum was found to agglutinate sensitized sheep red cells. Rose suggested that the agglutination might be due to the RA. He found that the same reaction occurred in other patients with RA and the work was published without reference to Waaler's findings. Ragan (1961) later noted that Lipman had heard Waaler's earlier report at a microbiology congress. Hence, the sensitized sheep cell agglutination test has been known as the Waaler-Rose test and, in 1949, Pike et al called the factor responsible for the agglutination reaction 'rheumatoid factor'.

Modifications of the original Waaler-Rose test for RF were proposed by Heller et al (1949).

Heller et al later reported (1954) that the Waaler-Rose test was inhibited by prior treatment of the serum with Cohn Fraction II, suggesting that the RF was bound by gammaglobulin. Likewise, Cohn Fraction II together with tannic acid was successfully used to sensitize sheep red cells in place of the rabbit antibodies. This was called the FII tanned sheep cell agglutination test.

Singer and Plotz (1956) then successfully utilized the latex test system to demonstrate RF. Cohn Fraction II was used to coat latex particles which acted as an indicator instead of red cells. Modifications of the original method were proposed by Rheins et al (1957) and the test is still widely used today.

Epstein et al (1956) showed that an indicator system was not always necessary - the serum of patients with RA could react directly with Cohn Fraction II to form a precipitate.

A review of the literature indicates that while a large number of tests have become available to detect RF, the interpretation of test results has lagged far behind (Williams 1983).

Nevertheless, work was carried out on the immunological reactions involved in the various tests and in the structure of RF itself.

Franklin et al (1957) showed that RF was a gammaglobulin of the 19S type. These workers also showed that RF in the serum was present in a complex which could be separated into 19S and 7S components by ultracentrifugation.

Mellors et al (1959) then found that the presence of RF was not limited to the serum. These authors used aggregated gammaglobulin labelled with fluorescein as a marker for RF. They demonstrated that plasma cells in the synovium of patients with RA were actively producing RF. This suggested that RF was not merely an immunological curiosity but was in some way linked to the joint pathology. Active research into the structure, biological activity and measurement of RF has continued with the publication of some 3000 articles on the subject since the publication of Rose and co-workers in 1948 (Fraser 1988).

3.3 CONDITIONS ASSOCIATED WITH THE PRESENCE OF RHEUMATOID FACTOR

The presence of RF was thought to be specific for RA with some 'uncommon exceptions' (Ziff 1957). Singer (1961) questioned whether RF was part of the immunological reaction present in RA and allied conditions or whether it was part of a more nonspecific 'host reaction'. The latter possibility seems more likely in view of the fact that RF has been found in a large number of unrelated conditions, some of which are shown in Table 3.1.

Rheumatoid factor has also been found in normal individuals and is more common with increasing age (Mikkelson et al 1967; Hooper et al 1972, Landau and Plavnic 1982 pg 179-182).

It has, therefore, been necessary to define a level of RF to distinguish 'seronegative' from 'seropositive' patients.

TABLE 3.1

DISORDERS ASSOCIATED WITH THE PRESENCE OF RHEUMATOID FACTOR

Connective tissue diseases: rheumatoid arthritis, systemic lupus erythematosis, sclerodema, Sjogren's syndrome

Infectious diseases:

Viral - infectious mononucleosis, influenza, infectious hepatitis

Bacterial - syphilis, tuberculosis, leprosy, brucellosis, subacute bacterial endocarditis

Parasitic - malaria, kala-azar, trichinosis, toxoplasmosis

Neoplastic conditions: Leukaemia, myeloma, various malignancies

Other Conditions: Cirrhosis, asthma, sarcoidosis, myocardial infarction, psychiatric disturbances, rheumatic fever

(Data from : de Forest et al 1956; Epstein et al 1957; Rothermich and Philips 1957; Ziff 1957; Dresner and Trombly 1959; Peltier and Christian 1959; Bartfeld 1969; Hooper et al 1972; Reimer et al 1975; Carson 1982 pg 116-117)

3.4 STRUCTURE AND SPECIFICITIES OF RHEUMATOID FACTORS

In as much as the conditions associated with RF have proved to be diverse, so RFs of different structure and specificities have been described.

Rheumatoid factor activity has been demonstrated among immunoglobulin classes M, G, A and E (Carson 1982 pg 116).

IgM RF

IgM RF has a pentameric structure similar to other 19S antibodies. However, monomeric IgM RF has also been described (Theofilopoulos et al 1974).

The valency of IgM RF is 5, although it would be expected to be 10 as each IgM subunit has a valency of 2. It may be that steric hindrances prevent further IgG molecules from binding.

In order to characterize RF make-up further, the idiotypes (or antigenic determinants of the antibodies) have been extensively studied. Chen et al (1988) have recently reviewed this subject. They noted the heterogeneity of RFs in terms of idiotypic structure and suggested that several V (variable region) genes were involved in RF production.

IgG RF

The structure of IgG RF is similar to that of other IgG molecules although in the serum and synovial fluid IgG RF tends to exist in a complexed form. This is because IgG RF has a valency of 2 and has an antibody specificity to antigens in the Fc region of IgG. Thus, dimers or larger polymers of IgG RF may be formed (Pope et al 1974).

IgA RF

This usually exists in the form of dimers and has been found in sera from patients with RA, Sjogrens syndrome and systemic lupus erythematosis (Dunne et al 1979)

Specificity of rheumatoid factor

Polyclonal RFs have been shown to have specificity for antigenic determinants (epitopes) on the Fc region of IgG (Fudenberg and Kunkel 1961; Franklin and Fudenberg 1965). The epitopes have been localized to the second and third domains (CH_2 and CH_3) of the constant region of IgG. Interestingly, this is the same section of IgG to which staphylococcal protein A binds (Mannik and Nardella 1985).

The most important of the antigenic determinants is possibly the Ga antigen found in IgG subclasses, 1, 2 and 4 (Allen and Kunkel 1966; Holborow and Swannell 1983 pg 247). Rheumatoid factors react poorly with IgG subclass 3 (Natvig et al 1972). Some RFs show specificity toward the genetic marker Gm, found in the Fc fragment of IgG (Gaarder and Natvig 1970). Binding can be more pronounced toward human IgG (autologous or isologous) or may be heterologous against animal IgG (Carson 1981 pg 684).

Binding of RFs to IgG in immune complexes has been shown to be greater than binding to native IgG (Coulie and Van Snick 1985).

Rheumatoid factors may also react with various non-immunoglobulin antigens e.g. deoxyribonucleic acid (DNA) and nucleosomes (Williams 1979).

The association constants (or affinities) of RFs for their antigens are low in comparison to other antigen-antibody reactions (Williams 1979; Carson 1982 pg 123). The RF-IgG complexes are thus easily dissociated (Allen and Kunkel 1966).

3.5 INDUCTION OF RHEUMATOID FACTOR SYNTHESIS

It has already been mentioned that widely different diseases give rise to RF. It is not surprising, therefore, to find that there are several known stimuli for RF production.

These stimuli have been reviewed by Egeland and Munthe (1983), Carson (1985 pg 669-672) and Chen et al (1988).

3.5.1 Polyclonal B cell activators

Rheumatoid factors are immunoglobulins produced by B-lymphocytes. It follows that non-specific activation of different B-cell clones can result in RF formation. Known polyclonal stimuli such as hyperimmunization, tetanus toxoid, components of bacterial cell walls (e.g. lipopolysaccharide), pokeweed mitogen and Ebstein Barr virus have been studied for their RF-inducing capabilities. Work done *in vitro* and *in vivo* with laboratory animals and *in vitro* using human peripheral mononuclear cells from normal individuals, has shown that all these stimuli can result in RF production (Bokisch *et al* 1972; Slaughter *et al* 1978; Koopman and Schrohenloher 1980; Olsen *et al* 1982; Dziarski 1982; Welch *et al* 1983; Nemazee and Sato 1983; Coulie and Van Snick 1983). Coulie and Van Snick (1983) found that immunization with a variety of protein antigens could lead to RF synthesis in mice. *In vitro* RF production by spleen cells was not seen if the animal had only received a primary injection before sacrifice. The RF response was only observed as part of the secondary response in immune animals. The *in vitro* presence of RF was found to be short-lived; RF production was first demonstrated on day 2, it peaked on day 3 and ended on day 4. Interestingly, serum RF levels were not increased in the mice, possibly because of RF consumption by the immune complexes.

Nemazee and Sato (1983) noted that the RF response in mice ended when the foreign antigen had been cleared. Most of the RF produced was IgM and there was little immunoglobulin class switching. As pointed out by the authors, this pattern is similar to that found when B-cells produce immunoglobulin independently of T-cells.

3.5.2 Immune complexes

Immune complexes have been found in a wide range of diseases. In some circumstances, these complexes lead to induction of RF production. Evidence for this has been obtained in animal models. Thus, Abruzzo and Christian (1961) found that rabbits repeatedly immunized intravenously with killed bacteria (*Escherichia coli* and *Bacillus subtilis*) produced RF-like substances. The authors were of the opinion that the prolonged

immunization resulted in immune complex formation which in turn stimulated RF production. There was little direct evidence for this postulate, however, as immune complexes were not measured.

Williams and Kunkel (1963) described a similar response in rabbits immunized with horse spleen ferritin. Higher levels of RF could be obtained by injecting complexes containing ferritin and antibody to ferritin. The RF antibodies were shown to have definite specificity for individual rabbit gammaglobulins. This may indicate that the antibody response was not polyclonal. It was also noted that immunization with autologous gammaglobulin that had been enzymatically split with papain, resulted in greater RF synthesis than using unsplit gammaglobulin. The authors used this evidence to suggest that altered gammaglobulin in immune complexes may act as an antigen for the formation of RF. This postulate is still held today (e.g. Nemazee and Sato 1982).

Nemazee and Sato (1983), working with mice, were also able to show that RF was produced in response to immunization. They found it was necessary to give more than one injection of the antigen. The antigen used was keyhole limpet haemocyanin and the absence of RF induction following direct antigen exposure was thought to be unlike that seen with polyclonal B-cell activators. It was hypothesized that the RF response depended on the prior formation of immune complexes. Again there was no definite support for this. Nemazee published further observations in 1985. Injecting mice with antigen intraperitoneally and preformed antibody intravenously resulted in a brisk RF response (as determined using a plaque-forming assay for RF with spleen cells). Antigen or antibody alone did not produce this effect. The RF formed had the same IgG subclass specificity as that contained in the immune complex giving rise to it. Thus, complexes containing IgG_1 led to anti-IgG_1 production. Likewise, complexes with IgG_{2a} antigen resulted in the RF being anti-IgG_{2a} specific. These findings extended those of Williams and Kunkel (1963) and strongly suggested that immune complexes acted on their own and not as polyclonal B-stimulators to cause RF manufacture. If the latter

were the stimulus for RF synthesis one would not expect subclass specificity to be present.

Coulie and Van Snick (1985) reached similar conclusions to those of Nemazee (1985). Preformed immune complexes were given to irradiated mice. The mice received syngeneic spleen cells from donor mice that had been primed with exposure to the antigen present in the immune complexes. A considerable RF response ensued. Neither antigen nor antibody alone caused this reaction. The specificity of the RF produced could be demonstrated. The authors were also able to show that the antigen-antibody ratio in the immune complex was important in determining the effectiveness of the stimulus. Complexes formed in the presence of large antigen excess were shown to be poor RF inducers. Although no reason for this was suggested, it was shown that RF was only produced if there were Fc antibody fragments in the complexes. Perhaps the complexes formed in marked antigen excess had fewer Fc regions exposed.

The experiments performed by Coulie and Van Snick (1985) and Nemazee (1985) were also useful in elucidating the lymphocyte interactions involved in RF formation in mice. Coulie and Van Snick showed that if T-cells were removed from the donor spleen cells given to irradiated mice there was no RF response. Nemazee, using genetically athymic mice, also showed a lack of RF production in the absence of thymus-derived lymphocytes.

Further evidence indicating the need for T-cells came from Nemazee's work (1985) using CBA/N mice with the **xid** genetic defect. These mice, which respond poorly to T-independent antigens, were able to make RF following immune complex administration. This suggested that immune complexes stimulated RF-producing cells in the absence of T-cell help.

It was apparent that not all stimuli leading to RF formation were T-cell-dependent as athymic mice given lipopolysaccharide, a polyclonal B-cell stimulator, made RF. Nemazee (1985), therefore noted that there were at least two independent mechanisms for RF synthesis in mice, one involving immune complexes, the other polyclonal B-cell stimuli.

The relevance of these findings to human disease is uncertain. In vitro experiments, however, using mononuclear cells from human peripheral blood have demonstrated RF production. Pisko et al (1982) used heat-aggregated IgG (HaIgG) to simulate immune complexes. Normal human peripheral mononuclear cells were able to make RF after exposure to high levels of HaIgG. The response was a polyclonal B-cell one, however, as non-RF IgM was also produced. A much more specific RF response was obtained using amounts of HaIgG 1-10 000 times lower (Tao et al 1984). In this system, mononuclear cells from patients with RA produced RF without an associated rise in other types of IgM. The stimulus was not effective in lymphocytes from normal individuals. These experiments indicated that lymphocytes from normal individuals could make RF in response to simulated immune complexes and that lymphocytes from RA patients had been specifically primed to produce RF.

Evidence that immune complexes may give rise to RF antibodies in vivo comes from work in patients with subacute bacterial endocarditis. Thus, Carson et al (1978) found that on average, the levels of immune complexes peaked 9 days before the zenith of RF levels.

3.5.3 Antibodies mimicking the Fc portion of IgG

Molecular mimicry may be an important mechanism in the generation of certain diseases. The antigenic determinants (or epitopes) of a foreign substance may also be present in host tissue. The immune response directed towards the foreign antigen then reacts with the same epitopes in the host. In this way an autoimmune response to self-antigens may be invoked (reviewed by Zwillich and Lipsky 1987).

Interestingly, various bacterial and viral antigens bind specifically with human IgG.

These antigens include staphylococcal protein A, streptococcal Fc receptor and viral capsid (Reis et al 1984; Nardella et al 1985; Inman et al 1987). Mouritsen (1986) has suggested that antibody to these antigens may mimic the Fc portion of IgG.

Anti-idiotypic antibody could then bind to the initial antibody as well as to the Fc epitope of IgG. This anti-idiotypic antibody would in fact be RF.

3.5.4 Cross-reactivity between the Fc region of IgG and other antigens.

Cross reactivity with IgG has been demonstrated for example with nuclear antigens (Darwin et al 1986), DNA-histone (Agnello et al 1980) and cystoskeletal components (Rubin et al 1984). Rheumatoid factor is also able to cross react with these antigens, suggesting that they may trigger RF production.

3.5.5 Altered control mechanisms

Rheumatoid factor production has been shown to be subject to control by various means including suppressor T-cells and anti-idiotypic antibodies (Koopman 1981; Koopman et al 1983; Fong et al 1984; Abe et al 1984; Takeuchi et al 1985). It has been proposed that alterations in these components may be responsible for excessive RF synthesis, particularly in disease (Olsen and Jasin 1985). Recently prostaglandin E_2 has also been shown to exert a controlling role on in vitro RF production (Alvarellos et al 1988).

3.6 CELLULAR PRODUCTION OF RHEUMATOID FACTOR

Although in vitro experiments have shown that circulating mononuclear cells can make RF, these cells are not the major source of RF in RA (Egeland and Munthe 1983).

In patients with RA, cells in the synovial tissue, bone marrow, lymph nodes and spleen appear to be the main sites of RF production (Mellors et al 1959; Munthe and Natvig 1972; Vaughan et al 1976; Fehr et al 1981). The same localities may be active in other diseases associated with the presence of RF (Egeland and Munthe 1983), although there are few studies on this.

Until recently little has been written about the B-cell subsets giving rise to RF although it has several important functions and is present in many disease states. Hardy et al (1987)

were able to show that B-cells which were CD5 positive (Leu-1) were responsible for RF production. These cells comprised 20-30% of B cells in adults and 75% of B-cells in cord blood. It has also been shown that there is an expansion of this B-cell population in patients with RA (Plater-Zyberk et al 1985).

3.7 PHYSIOLOGICAL ROLE

The wide range of stimuli giving rise to RF led several authorities to the conclusion that RF plays a role in the normal immune response. The possible physiological effects of RF are discussed below.

3.7.1. Clearance of immune complexes

Immune complex handling by the body appears to be a complex process. Nevertheless, a model has been proposed whereby some immune complexes attach to erythrocytes. This prevents tissue binding and damage (reviewed by Kimberly 1987). Clearance of immune complexes from the circulation normally occurs when the complexes are stripped from the erythrocytes by the mononuclear phagocyte system (reviewed by Mannik 1982).

Van Snick et al (1978) performed comprehensive in vitro studies on the effect of RF on immune complex uptake by macrophages. The macrophages were derived from mouse peritoneal cavity, mouse lung and human mononuclear blood cells. The effect of purified IgM RF on removal of immune complexes was studied. The complexes were formed either with transferrin and anti-transferrin antibodies or HaIgG. To monitor the fate of the immune complexes, the antigens were radiolabelled with ^{125}I. Uptake of immune complexes was assessed by measuring the radioactivity associated with purified macrophages together with the radioactivity present in the supernatant culture fluids. The latter was thought to indicate processing of immune complexes by the cells and release of degradation products.

It was found that:

i. IgM RF resulted in a 20-fold increase in uptake or processing of the HaIgG by macrophages.

ii. RF was responsible for a rise in the uptake of antigen-antibody complexes by phagocytes. The ratio of antigen to antibody in the complexes influenced the RF effect. A maximal stimulation was obtained when the antigen concentration was 10 times greater than that of the antibody.

iii. Complement appeared to produce a separate effect to that of RF and could even abolish the latter's opsonic potential. The complement effects are discussed elsewhere.

The in vivo effect of RF was assessed by Van Snick and co-workers by injecting radiolabelled antigen-antibody complexes into the peritoneal cavity of mice. It was found that addition of IgM RF to the complexes formed in 10 times antigen excess, resulted in a 14-fold increase in the radioactivity of cells obtained by washing the peritoneal cavity.

Van Snick et al (1978) found that different RFs varied in their effects on immune complex uptake.

Hogben and Devey (1986) confirmed some of the in vivo findings of Van Snick et al (1978) using the mouse as an experimental animal. Rheumatoid factor was produced in the mice by intraperitoneal innoculation of bacterial lipopolysaccharide (LPS). Preformed immune complexes consisting of radiolabelled human serum albumin and antibodies to it were injected into the animals. Varying antigen-antibody ratios were used and immune complex clearance from the circulation measured. Findings were:

i. Immune complexes formed in antibody excess (shown to be large complexes) were cleared more rapidly in mice with LPS-induced RF.

ii. Immune complexes formed in antigen excess were cleared more slowly in mice with RF and in controls. Although complexes with 2 to 5 times antigen excess were removed more quickly in the presence of RF, complexes with 10 times antigen excess disappeared more slowly in the mice with RF than those without.

These last results differed from those of Van Snick et al (1978) but the antigen-antibody complexes were different. Also Van Snick's group used heterologous RF and clearance of complexes from the peritoneal cavity may not be the same as that from the circulation. Hogben and Devey (1986) also point out that LPS can affect phagocytic function apart from its action on RF.

Nevertheless, the in vivo findings of the latter authors appear to correlate with some of the in vitro effects observed by Van Snick et al (1978). Thus, larger complexes formed in slight antigen excess were removed more quickly in the presence of RF in both studies. The differential effect seen in vivo on clearance of small complexes formed in antigen excess may be explained by complement interactions. Presumably less complement would have been available in the peritoneal cavity than in the serum. This complement effect is discussed more fully elsewhere.

Further work on the effect of RF on immune complex clearance was carried out in rabbits. Adding IgM RF to immune complexes formed from antibody and double stranded DNA increased the clearance rate in rabbits (Taylor et al 1986). This effect may have been partly due to the action of RF in improving binding of immune complexes to rabbit platelets. This complement-dependent process is similar to the one which occurs in man when immune complexes bind to erythrocyte receptors (Cornacoff et al 1983). The latter reaction is also enhanced by IgM RF (Taylor et al 1986). In man, the immune complexes are then removed by phagocytes, mainly in the liver (Mannik 1982). This stripping could be aided by RF which can increase the uptake of immune complexes by macrophages in vitro (Van Snick et al 1978).

3.7.2 Resistance to infection

Another reason for finding RF in diverse situations may be that it aids the host defence mechanism in its response to foreign antigens. For example, RF may stabilize weak antigen-antibody interactions and this would enable it to serve as a first line defence system until sufficient antigen-specific antibody is produced (Welch et al 1983).

This 'amplifying' or 'enhancing' effect of RF has also been described by other authors (Nemazee and Sato 1982; Nemazee and Sato 1983; Coulie and Van Snick 1983). Nemazee and Sato (1982) suggest that enhancing antibodies (like RF) may improve complement fixation by immune complexes and thus trigger cytolysis of cellular antigens. It is also conceivable that antigen recognition may be improved by greater binding of antigen to the surface immunoglobulin of B-cells.

Specific examples of the RF effect on infections have been described. Notkins (1971) showed that RF attached to infectious virus-antibody complexes was not able to neutralize the virus but made the complexes more susceptible to neutralisation by complement.

Almeida and Griffith (1980) found that when there was little specific antibody to a virus, RF could cause lightly coated virus particles to be linked together. This may aid their removal. Similarly, RF may enhance the clearance of plasmodia (Green and Packer 1984). IgM RF has also been shown to protect suckling rat pups from Trypanosoma lewisi infection. The RF which was transferred in colostrum, amplified the effect of specific IgG against the organisms (Clarkson et al 1981).

3.7.3 Regulatory function

It has been suggested that RF may have a regulatory role in the immune system. The Fab fragment of RF is directed against IgG and Chen et al (1988) have proposed that RF may serve as a precursor of anti-idiotypic antibodies. These latter antibodies may bring immune responses against antigens to an end and in this way have a regulatory effect (Burdette and Schwartz 1987). Furthermore RF could assist clearance of idiotypic-anti-idiotypic complexes (Morgan et al 1979; Fong et al 1985).

It has been shown that new antigens are expressed when antigen-antibody complexes form. Antigenic sites on the Fc fragment may be revealed in this way. As RF is an antibody directed against Fc fragments and helps to clear immune complexes, it may exert an indirect modulating effect (Nemazee and Sato 1982). Finally,

it has been postulated that B-cells containing RF actually take up immune complexes, process the antigen and present it to regulatory T-cells (Chen et al 1988), although there is, as yet, little direct evidence to support this.

3.7.4 Complement activation

There are contradictory reports regarding the relationship between complement and RF, both of which bind to immune complexes (Davis and Bollet 1964). It was shown that IgM RF was able to fix and activate complement under certain conditions, e.g. when bound to IgG coated-erythrocytes (Zwaifler and Schur 1968; Tanimoto et al 1975; Taylor-Upsahl et al 1977). In these experiments it was found to be necessary to render the IgG used for coating incapable of its own complement activation by reduction and alkylation.

The interaction between IgM RF, immune complexes and complement activation is also controversial. Tesar and Schmid (1970), for example, found that RF increased complement activation by soluble immune complexes and complexes formed in antigen excess. Doekes et al (1985) and Shingu et al (1988), however, noted that IgM RF suppressed the complement-activating effect of immune complexes. Doekes and co-workers have pointed out that only the 'staple' form of bound IgM activates complement; the star-like form does not. IgM RF bound to different complexes may assume different configurations and have different effects on complement activation. Indeed, Tesar and Schmid (1973) found that different RF-containing sera varied widely in their complement-activating potential.

Shingu et al (1988) have suggested that IgG RF enhances the complement activation by immune complexes. However, using a different method to determine complement activity, Taylor-Upsahl et al (1977) were of the opinion that IgG RF was not involved in complement-mediated cell lysis.

It is, therefore, unclear whether IgM RF is functioning in a protective manner to reduce the complement-activating behavior of immune complexes or is actually increasing complement

activation in some situations. In addition, the effect of RF on complement activation in local tissues, for example the synovium, may be different to that in the circulation (Doekes et al 1985). Indeed, Ford (1983) has noted that there is no in vivo evidence to show that RF protects from immune complex mediated damage.

3.8 PATHOLOGICAL EFFECTS OF RHEUMATOID FACTOR

It is equally difficult to establish whether RF is behaving in a physiological or pathological manner in disease states (Levinson and Martin 1988).

The presence of RF in normal individuals as well as in a wide range of diseases suggests that RF production may be part of the normal immune response.

However, there is evidence that RF has a role in the pathogenesis of RA and possibly in other conditions as well.

3.8.1 In rheumatoid arthritis

Increased production of RF: The levels of RF are higher in RA than other diseases and tend to be more persistently elevated (Williams 1983). Adults with the disease are more likely to have RF detected in their sera than children with the same condition. This is particularly evident when conventional tests are used for RF, as children may have hidden RF complexed to circulating IgG (Moore et al 1988)

Enhanced synthesis of RF has been observed in vitro with peripheral blood mononuclear cells from patients with RA. Spontaneous IgM RF production occurred in patients with RA but not in normals. The patients demonstrating this had very active disease or vasculitis (Vaughan et al 1976). In addition, polyclonal stimulation by pokeweed mitogen resulted in a higher proportion of the IgM being IgM RF in supernatants from RA patients (Koopman and Schrohenloher 1980; Olsen et al 1982).

It has also been shown that immune complexes (in the form of HaIgG), stimulated IgM RF production by mononuclear cells in RA but not normals (Tao et al 1984).

Association with disease activity

The relationship between IgM RF concentrations and activity of the disease is controversial. Various workers have shown that the presence of IgM RF is associated with more severe, progressive and disabling forms of joint disease (Franco and Schur 1971; Panush et al 1971; Holborow and Swannell 1983 pg 247). Ernst et al (1988) found a correlation between disease activity and IgM RF levels in a group of patients with highly active RA. Other workers, however, have not found such a relationship (Withrington et al 1984; Lemm et al 1988), but have suggested that IgG RF levels may relate more closely to activity.

Nevertheless, a reduction in IgM and IgG RF was shown to accompany clinical improvement in patients receiving non-steroidal anti-inflammatory agents or gold therapy. IgM RF levels also decreased in patients treated with D penicillamine (Pope et al 1983). Olsen et al (1984) showed a fall in IgM RF production in vitro (which closely accompanied clinical improvement) following gold or D penicillamine therapy.

Rheumatoid factors in synovial tissues and synovial fluid

The presence of plasma cells containing IgM RFs in the synovia of patients with RA has been demonstrated in several studies (Mellors et al 1959; Munthe and Natvig 1972; Fehr et al 1981).

Prior pepsin digestion of synovial membranes has demonstrated the frequent presence of IgG RF. Intercellular and intracellular immune complex deposits containing IgG, IgM, C_3 and RF have also been shown (Fehr et al 1981).

IgM and IgG RFs have been isolated from synovial fluid (Panush et al 1971). IgG RF in synovial fluid appears to exist in self-associating complexes (Winchester et al 1971; Mannik and Nardella 1985). Formation of large complexes in serum may be

prevented by the presence of normal IgG to which the IgG RFs attach (Mannik and Nardella 1985). The synovial fluid complexes, however, may be large and are able to activate complement, although less efficiently than IgM RF (Sabharwal et al 1982). Indeed, low levels of complement have been detected in the synovial fluid of patients with RA who have raised serum RF levels (Ruddy et al 1969; Winchester et al 1970). Furthermore, Nardella et al (1983) have shown that IgG RFs indirectly cause synovial cells to release collagenase and prostaglandin. Thus IgG RFs may be responsible for tissue damage in RA synovial tissues. The role of IgM RF in synovial inflammation is less certain. It appears that IgM RF is necessary for complement fixation and phagocytosis of IgG complexes by neutrophils (Hurd et al 1970). This finding is supported by the fact that neutrophils from seronegative patients do not contain IgG and C_3 inclusions (Britton and Schur 1971).

Effects in tissues apart from the synovial membrane in rheumatoid arthritis

There is a well known association between high titres of RF and extra-articular manifestations of RA such as vasculitis, multiple subcutaneous nodules and systemic organ involvement (Singer and Plotz 1956; Kunkel and Tan 1964; Mongan et al 1969; Holborow and Swannell 1983 pg 250; Gioud-Paquet et al 1987). IgG RF and a low molecular weight IgM RF appear to be involved in the development of vasculitis (Theofilopoulos et al 1974). In addition, Elson et al (1983) found that the presence of circulating immune complexes containing RF, IgG and complement showed a better correlation with the development of vasculitis than synovitis in RA. The discovery of IgM RF in the lungs of patients with RA and pulmonary involvement led DeHoratius et al (1972) to suggest that RF played a role in the pathogenesis of rheumatoid lung.

Overall, these findings suggested that the presence of RFs including IgM RF may contribute to immune complex formation, complement fixation and chronic tissue damage in RA (Carson 1987 pg 1205).

3.8.2 Rheumatoid factor in other inflammatory conditions

There is work indicating that RF is associated with disease activity in a number of inflammatory conditions, both experimentally and in human disease.

Liver Disease

Dresner and Trombly (1959) detected RF in 71% of 96 cases of hepatocellular disease. Remission of the liver disease resulted in the test becoming negative. Similarly, 19 cirrhotics with positive RF tests were studied. In 10 cases whose RF tests became negative there was clinical remission of disease. The remaining 9 cases whose tests stayed positive did not improve. Another patient in remission initially had a negative RF test. The patient relapsed and the RF test became positive prior to the development of clinical signs. From their work, Dresner and Trombly concluded that the measurement of RF levels appeared to reflect the patient's clinical condition more accurately than other tests of liver function.

One possible drawback of the study, however, was that RF was measured using the euglobulin inhibition test. This test makes use of the fact that normal serum inhibits the agglutination of positive rheumatoid serum. Globulin (or Cohn Fraction II) from RF-containing serum does not, however, inhibit the agglutination (Ziff et al 1956). The problem with the method is not that it does not measure RF (indeed the test presumably detects hidden RF as well) but that it is technically difficult to perform and is not used today. The work has not been repeated by other investigators.

Subacute bacterial endocarditis (SBE)

Williams and Kunkel (1962) found RF in just over 50% of 51 patients with SBE. Many patients showed a marked fall in the amount of RF following treatment (RF was quantitated using latex and sheep cell tests as well as precipitin curves obtained with heat-treated sera and aggregated gammaglobulin as antigen). The decrease in RF levels after treatment suggested that the

antigenic stimulus had been removed. Immune complexes were thought to be one such stimulus.

The fact that not all patients with SBE produced RF was unexplained, although tests for 'hidden RF' were not in use at that time. Also unexplained was the fact that a few patients still had detectable RF after treatment (one such patient had chronic liver disease).

Messner et al (1968) was able to correlate the presence of RFs in SBE with the duration of infection and raised immunoglobulin levels.

In another study, Bacon et al (1974) confirmed the decline in RF levels (and other auto-antibodies) following cure of the disease.

Carson et al (1978) also measured IgM RF levels in patients with SBE. The RF levels peaked approximately 9 days after the immune complex levels, and levels of both IgM RF and immune complexes fell after treatment

Vasculitis

A number of associations between RF and vasculitis have been described. In fact, RF may be instrumental in the induction of vasculitis. This has been shown by the infusion of RF and immune complexes into the mesenteric vessels of the rat (Baum et al 1964). It appeared that immune complexes and the IgM RF-containing fractions from patients with RA caused thrombosis and haemorrhage in the vessels. The injection of immune complexes alone, however, did not produce vascular damage.

Likewise, IgM RF aggravated the vasculitis occurring as part of the Arthus reaction in rats. The Arthus reaction was produced by the intradermal administration of IgG antibody followed by intravenous antigen and was complement dependent (Floyd and Tesar 1979). The inflammatory reaction due to the antigen-antibody deposition was not only augmented by IgM RF but vascular permeability was also increased.

Skin biopsies in renal transplant patients with CMV infection have shown the presence of perivascular IgM deposits. The finding of these deposits appeared to correlate with the RF concentration (Baldwin et al 1987). Similarly, IgM accumulations in the skin blood vessels have been noted in association with circulating RF in patients with RA. The presence of IgM and C3 in the vessels were shown to be related to a perivasular mononuclear infiltrate (Westedt et al 1984). These results provide circumstantial evidence that IgM RF may be linked with vascular damage in man.

Lung disease

Experiments using rats with diffuse proliferative lung disease have demonstrated that IgM RF attaches to the linings of alveoli and arterioles in areas of inflammation and accentuates the tissue damage and vasculitis (DeHoratius and Williams 1972).

Renal disease

Rheumatoid factor has been implicated in the pathogenesis of renal disease. In rats with nephrotoxic nephritis, it has been shown that the injection of RF-containing serum from RA patients resulted in worsening of the proteinuria (McCormick et al 1969).

Deposits of RF have been found in the glomeruli in patients with glomerulonephritis. Rossen et al (1977) noted that RF was detected mainly in patients with severe glomerular damage. Rodriguez-Iturbe et al (1980) isolated IgG RF from the glomeruli in a patient with acute poststreptococcal glomerulonephritis.

In addition, elevated levels of serum RF have been detected in 90% of patients with the same condition, and appeared to be related to the duration of the nephritis (McIntosh et al 1979)

In rabbits with glomerulonephritis associated with SBE, the presence of RF in the glomeruli was closely related to the inflammatory changes (Sindrey et al 1981).

Using a mouse model, other workers have shown that RF can bind to immune complexes located in glomeruli and act as an

immunoabsorbent to bind further complexes (Ford and Kosatka 1982).

Finally, Ford (1983) has postulated that RF may contribute to chronicity in glomerulonephritis.

Lyme arthritis

IgM RF levels appear to be related to disease activity in this condition (Goebel et al 1988).

3.8.3 Summary

In summary, the studies in RA and other inflammatory conditions have found relationships between RF concentrations and

i. the age of the patient (onset of the disease in childhood is less often associated with the presence of RF - Moore et al 1988)

ii. the severity and extent of the condition (Holborow and Swannell 1983 pg 247; Gioud-Paquet et al 1987; Carson 1987 page 1205; Milgrom 1988)

iii. the duration of the illness (Messner et al 1968, McIntosh et al 1979)

iv. the disease pattern in terms of organ involvement (Singer and Plotz 1956, Holborow and Swannell 1983 pg 250)

v. the activity of the disease following therapy (Bacon et al 1974, Carson et al 1978,)

vi. immune complex levels (Carson et al 1978, Gioud-Paquet et al 1987).

These observations do not necessarily mean that RF has any causative role in the diseases mentioned. Nevertheless, the potential of RF to enhance tissue damage has been shown in a number of experimental situations.

3.9 RHEUMATOID FACTOR PRODUCTION IN CONGENITAL SYPHILIS

Rheumatoid factor production appears to be a consistent part of the immune response in congenital syphilis (Reimer et al 1975, Meyer and Malan 1987).

It is possible that induction of RF synthesis is related to the presence of immune complexes in congenital syphilis in a manner similar to that which was described in section 3.4.2.

It is, therefore, pertinent to discuss immune complex formation in congenital syphilis.

3.9.1 Immune complexes in congenital syphilis

Concurrent with the development of the host immune response, immune complexes appear in the circulation. This has been demonstrated in acquired syphilis (Sølling et al 1978; Engel and Diezel 1980) and in congenital syphilis. Kaplan et al (1972) and Wiggelinkhuizen et al (1973) showed that the nephropathy of congenital syphilis was an immune complex glomerulonephritis. Few studies, however, have measured circulating immune complexes in congenital syphilis. The work of Dobson et al (1988b) showed the presence of immune complexes in all six symptomatic infants with congenital syphilis. The complexes, which were predominantly IgM-containing complexes, were measured using a C1q solid phase assay.

Using a C1q binding assay, Samson et al (1990) also found raised levels of C1CS in 17 infants with congenital syphilis.

In their preliminary studies, Dobson et al (1988b) were able to show the presence of treponemal antigens and anti-treponemal antibody in the immune complexes in congenital syphilis. This work has been confirmed by S'anchez et al (1989).

In addition to finding treponemal antigens, endogenous host proteins in the form of creatine kinase and fibronectin degradation products were also detected in the immune complexes of infants with congenital syphilis (Dobson et al 1988b).

It is possible that the RF produced in congenital syphilis is induced by the immune complexes themselves. In particular, maternal IgG in the complexes may be altered, and the Fc region rendered immunogenic, resulting in RF production. A similar mechanism for RF synthesis in other conditions has been suggested by other workers (Williams and Kunkel 1963, Nemazee and Sato 1982).

Whilst there is evidence to suggest a relationship between immune complexes and RF levels in several conditions (see section 3.7) there is another possible mechanism leading to RF production in infectious diseases. This has been outlined in section 3.4.3 and has recently been reviewed by Williams (1988).

3.9.2 Antibodies mimicking the Fc portion of IgG

A number of bacterial, viral and parasitic organisms have Fc receptors for IgG. In the presence of infection, the immune response may result in the formation of antibodies to the Fc receptors. These antibodies would have combining sites which were the mirror image of the Fc receptor. An anti-idiotypic response to these antibodies would result in molecules with a cross-reactivity for the Fc portion of IgG ie. the anti-idiotypic antibody would be RF (Williams 1988).

Although there is no work to indicate that treponemes are endowed with Fc receptors, the hypothesis is attractive and could, conceivably, be responsible for RF formation in congenital syphilis.

Whatever the stimulus to RF induction in congenital syphilis, it is apparent that RF is found more often in the congenital than the acquired disease. Thus, in the former circumstance RF has been found in over 95% of cases in the 2 largest studies (Reimer et al 1975, Meyer and Malan 1987). Conversely, adults with syphilis have positive RF tests in 13-25% of cases, depending to some extent on the duration of the infection (Bartfeld 1969, Cerny et al 1985, Müller et al 1987).

What are the possible reasons for the difference in RF response?

3.9.3 Predisposition to RF production in early life

Very recently, attention has been focussed on the CD5 positive(+) subset of B cells. It has become apparent that these RF-producing cells make up a substantial proportion of B lymphocytes in early life. Thus, up of 50% fetal spleen B cells belong to the CD5+ subpopulation (Antin et al 1986), compared to 20% of adult spleen B lymphocytes (Gadol and Ault 1986). Furthermore, 75% of B cells in cord blood are CD5+ compared to 20-30% adult peripheral blood B cells (Hardy et al 1987).

Fong et al (1985) were able to demonstrate IgM RF production by B cells induced with Epstein-Barr virus in newborns. These workers found that the amount of IgM RF formed increased with age to young adulthood.

Levinson et al (1987) reported somewhat different results when cord blood lymphocytes were stimulated with Staphylococcus aureus Cowan I. Although both cord blood and normal adult peripheral blood mononuclear cells were shown to produce IgM RF following Staphylococcal activation, the cord blood response was over 3 times greater. The cord blood cells produced IgM class immunoglobulin only. This latter finding is in keeping with work which indicates that IgA and IgG-producing plasma cells are not readily inducible, and that the IgM response is dominant in early life (Steele et al 1977, Cooper 1987).

Nevertheless the results of Levinson et al appeared to differ from those of Fong et al in terms of the magnitude of RF production. The nature of the stimulus used was different, however, and further studies are needed to clarify these results.

In conclusion, the greater frequency with which RF is found in congenital (rather than acquired) syphilis may be due to larger numbers of readily inducible RF-producing B cells in newborns.

There are other theoretical reasons for increased IgM RF synthesis in congenital syphilis, and these relate to the regulatory T cells and macrophages.

3.9.4 Possible role of regulatory T cells and macrophages in increased RF production in congenital syphilis

Suppressor T cell function may be reduced in congenital syphilis. Evidence for this comes primarily from work in patients with acquired syphilis, although not all studies are in agreement (reviewed by Bos et al 1980).

A striking feature of some studies has been the demonstration of a depression of cell mediated immunity including delayed hypersensitivity reactions in acquired syphilis. Not only is there an absolute reduction in T lymphocyte numbers, but the numbers of suppressor T cells may also be decreased in secondary syphilis (Fulford and Brostoff 1972; From et al 1976; Bos et al 1980; Jensen and From 1982; Jensen et al 1982).

Jensen et al (1982) have postulated that immune complexes may be responsible for the immunosuppression.

If suppressor T cell function is decreased in congenital syphilis,it might mean that there is less control over production of RF and other immunoglobulins.

Macrophage function is depressed in newborns (reviewed by Lu and Unanue 1985). Chen et al (1988) have suggested that CD5+ cells may be able to process antigen-antibody complexes and present antigen to T cells. As this could occur independently of macrophages, CD5+ cells may have an advantage.

Reimer et al (1975) proposed another reason for the RF production in congenital syphilis.

3.9.5 RF formation against maternal IgG allotypes

Reimer et al have correctly pointed out that anti-immunoglobulins are often made against maternal IgG in the first year of life. These anti-immunoglobulins are of the IgM class and may be directed against the Gm (genetic marker) antigen on the foreign maternal IgG molecules (Steinberg and Wilson 1963).

It follows that this mode of response may be triggered early by the immune complexes containing foreign IgG in congenital syphilis.

Nevertheless, the reasons for finding relatively more IgM RF in syphilis in early life remain speculative at present.

What effects is this RF likely to have?

3.9.6 Effects of IgM RF in congenital syphilis

Unfortunately, the effects of these antibodies in congenital syphilis are also hypothetical.

Any or all of the physiological effects of RF described in section 3.6 may be operative. In particular, it is worth stressing the anti-infective properties of the antibody , which have been documented in trypanosomal infection in vivo and plasmodium infection in vitro (Clarkson et al 1981; Green and Packer 1984).

In addition, the "enhancing antibody " function of RF may be important in early life as antigen responsiveness is impaired (Roper and Cooper 1983 pg 19).

In spite of these potentially useful effects, there is evidence that RF plays a pathological role in RA and some inflammatory conditions (see section 3.8).

Whilst untoward consequences due to the presence of RF have not been documented in congenital syphilis, it is clear that the overall immune response plays an important role in the pathogenesis of the condition.

3.9.7 The effects of the immune response in congenital syphilis

Initial work in support of this came from pathological studies. It was found that in the rare instances where spirochaetes were present in the fetus prior to 5 months gestation there were no inflammatory lesions and there was no evidence of a cytopathic effect due to the organisms (Stowens 1959 pg 187; Silverstein 1962; Harter and Benirischke 1976).

Silverstein and Lukes (1962) noted that when the infected fetus was able to mount an immune response, plasma cells, inflammatory infiltrates and tissue damage were found in sites where the pathogen was located. Such sites included lung, pancreas and periosteum.

Certain lesions of early congenital syphilis may have an immunological pathogenesis. These include glomerulonephritis, placental vasculitis and haemolytic anaemia.

In addition, the stigmata of late congenital syphilis such as interstitial keratitis, Clutton's joints and eighth nerve deafness appear to be mediated by a hypersensitivity response (King and Nicol 1975 pg 117, 120). These maninfestations do not respond to penicillin treatment but may be improved by steroid+ therapy. Indeed, Olansky (1971 pg 736) has suggested that these features of late congenital syphilis may be due to an autoimmune response which is triggered by T.pallidum.

There is as yet little direct evidence of an autoimmune response in congenital syphilis. Recent data is available, however, to show that auto-antibodies are present in acquired syphilis and that autoimmune phenomena may be involved in the pathogenesis of some of the syphilitic lesions. Baughn et al (1986) have found immune complexes from patients with secondary syphilis to contain not only treponemal antigens, but also host proteins. These proteins were identified as fibronectin degradation products and anti-fibronectin antibodies. Immune complexes, of which these proteins are a part, have been demonstrated in renal and cutaneous lesions in patients with secondary syphilis (Tourville et al 1976; Jorizzo et al 1986).

Experimental animals also appear to make auto-antibodies when infected with T.pallidum. Thus, antibodies to creatine kinase have been demonstrated in sera from infected rabbits. The antibodies were absent from pre-infection sera and creatine kinase was not present in sonicated or whole treponemal organisms (Strugnell et al 1986). These authors postulated that tissue destruction led to release of creatine kinase from damaged mitochondria. The same workers reported finding auto-

antibodies reactive with a soluble fraction of skeletal muscle in human syphilis.

Similarly, guinea pigs infected with T.pallidum produce antibodies to treponemal antigens, creatine kinase and fibronectin. Auto-antibodies to the latter may be formed as a result of T.pallidum binding to fibronectin and inducing conformational changes leading to the molecule becoming immunogenic (Baughn et al 1987).

The presence of other auto-antibodies in acquired syphilis has been proposed. In fact the nontreponemal antibody tests are reactive against cardiolipin which is a component of normal mitochondrial membranes. However, cardiolipin is also a component of T.pallidum (Matthews et al 1979). Treponemes may incorporate mammalian cardiolipin (Ingall and Musher 1983 pg 337) and/or the VDRL may detect antibodies cross-reactive between T.pallidum and host cardiolipin (Doniach 1976 pg 210-214). That similar phenomena may be involved in congenital syphilis has been suggested by the very recent finding of creatine kinase and fibronectin degradation products in immune complexes in congenital syphilis (Dobson et al 1988b).

3.9.8 Overview of RF production in congenital syphilis

The fundamental question raised by the various findings described is 'Does RF have a pathological role in congenital syphilis?' It is tempting to suggest that it may, in the light of the observations that:

i. congenital syphilis is the most severe form of syphilis (Handsfield and Lukehart 1984)

ii. the host immune response takes part in the pathogenesis of the lesions of congenital syphilis

iii. there is a propensity for patients with early congenital syphilis to form RF

iv. RF may have pathological effects in other conditions.

However, essentially the same question has been investigated with regard to RA for almost 50 years and, as recently reviewed by Levinson and Martin (1988), is still unanswered.

Nevertheless, it does appear that RF production in congenital syphilis merits further study. Potential benefits would include a better understanding of the immune response in the condition. This may ultimately lead to a reduction in the mortality rate. Furthermore, recent work has indicated that the RF autoantibody is encoded by germ line genes and that newborn cord blood cells are capable of producing more RF than adult peripheral blood mononuclears (Levinson et al 1987). It seems reasonable to expect that the study of RF antibodies early in the development of the immune system may help clarify their role in later life.

Which aspects of RF induction in congenital syphilis should be investigated to establish a platform from which further research might be launched?

The classes of RF antibody produced could be determined and the percentage of IgM which is RF studied.

In addition, it would be useful to know if the factors associated with RF production in congenital syphilis are the same as those operative in other conditions (see Section 3.8.3). If similar determinants were identified, this would facilitate comparisons between the diseases with the added advantage that information from an earlier stage of ontogenesis would be available.

In some of the conditions where RF has been detected, it has been possible to show its presence at the site of the inflammatory lesions (in the synovium in RA and in the glomeruli in glomerulonephritis). Such findings have suggested at least a close association between the autoantibody and tissue damage, if not a direct relationship. It may be expedient to extend these observations to congenital syphilis where there is possibly a predisposition to RF formation.

It follows that, to investigate the RF response in congenital syphilis, reliable methods of measuring RF concentrations are needed. Some of the tests available are reviewed in Section 3.10.

3.10 DETECTION OF RHEUMATOID FACTOR

There are numerous tests described for the detection of RFs of the various immunoglobulin classes. The early development of assays for RF has already been described with the history of the discovery of RF.

Generally RFs in nonrheumatoid conditions are more reactive against human than rabbit IgG (Holborow and Swannell 1983 pg 247). Tests employing human IgG are more sensitive for detection of RF but are less specific for RA than those using rabbit IgG (e.g. the Waaler-Rose test - Carson 1981 pg 677). It needs to be borne in mind that IgM RF activity can be hidden because of the tendency of IgM RF to form complexes with autologous IgG in the serum. Dissociation of these complexes (e.g. by acidic treatment) may be necessary before IgM RF can be detected (Allen and Kunkel 1966).

3.10.1 Agglutination reactions

These tests detect mainly IgM RF owing to the latter's polyvalency; IgG RFs are poor agglutinators (Stage and Mannik 1972-73; Holborow and Swannell 1983 pg 248).

Latex agglutination test

The latex test is the most widely used test for the detection of IgM RF (Mannik 1979 pg 509). Singer and Plotz (1956) originally described a tube latex fixation test. The latex particles coated with Cohn fraction II human immunoglobulin were agglutinated in the presence of RF.

The latex slide agglutination test which is simpler and quicker than the tube method is often used as a screening test for the presence of RF. Waller (1969) found that the slide and tube test for RF had equal sensitivity and specificity in the

diagnosis of RA. Singer (1973-74), however, noted that the slide tests were less sensitive and reproducible when compared to the tube test. The poorer performance of the slide tests could be partly attributable to a lack of standardization.

Nevertheless, Cathcart (1975 pg 121) stated that the slide tests were useful, particularly when done in conjunction with a more reliable assay. False positive and false negative slide tests have been described. One possible cause of a false positive latex test is the presence of Clq component of complement. Latex particles coated with aggregated gammaglobulin can fix complement (Bernhard et al 1962) and this may result in non-specific agglutination (Stage and Mannik 1973). The problem can be overcome by heat treatment (Stage and Mannik 1972-73) or by dilution of the serum (Singer 1973-74). Although the end point titre gives an indication of the amount of RF present, the latex tests are not truly quantitative (Mannik 1979 pg 509).

Bentonite flocculation test

This test is similar to the latex test but uses clay particles instead (Bozicevich et al 1958). The test is slightly more complicated to perform than the latex test but the titration end point can be more objectively read (Cathcart 1975 pg 115).

Waaler-Rose test

This is also known as the sensitized sheep cell agglutination test (SCAT) and has been previously described. The test is more difficult to carry out than those mentioned above but is more specific for RA (Waller 1969; Cathcart 1975 pg 119). Heterophile antibodies need to be absorbed prior to the test (Stage and Mannik 1972-73). For this reason the use of human O cells as an indicator system has been proposed (Cathcart 1975 pg 119).

Tests using Cohn fraction II

These include the FII sensitized sheep cell agglutination test, and the FII inhibition test (or euglobulin inhibition test), and have already been described.

The tests have an increased sensitivity but decreased specificity for RA (Waller 1969; Cathcart 1975 pg 107).

Sensitized human D-Cell agglutination test

In this test human red blood cells are coated with incomplete anti-Rhesus (anti-Rh) antibodies. The system can be used to detect complement-fixing and agglutinating IgM RF (Waller and Vaughan 1956). One of the difficulties is in obtaining sufficient anti-Rh sera. The sensitivity and specificity of the test are similar to that of the latex and bentonite methods (Cathcart 1975 pg 121).

However, Mannik (1979 pg 509, 510) has pointed out that in the sensitized human D-cell agglutination test the IgG does not come from pooled sera so that some subclasses of IgG may not be represented. (This is in contrast to the latex test which uses pooled IgG).

3.10.2 Complement fixation methods

Rheumatoid factors can fix complement when reacting with IgG (Zwaifler and Schur 1968). This was shown by prior reduction and alkylation of the IgG to prevent its own complement activating effect. Tanimoto et al (1975) and Taylor-Upsahl et al (1977) described haemolytic assay systems making use of this property. As stated by the latter authors, the test is subject to too many variables to be of use for routine purposes but does demonstrate the potential complement fixing ability of RF.

3.10.3 Precipitation reactions

The work of Epstein et al (1956) has already been mentioned. Winchester and Agnello (1970) quantified anti-immunoglobulins using a radial immunodiffusion method. Rheumatoid factors produced a precipitate in a gel containing IgG aggregates.

The zone of precipitation was proportional to the RF concentration. Subsequently, Theofilopoulos et al (1974) have used the method to measure IgG and IgM RFs in RA patients with vasculitis.

3.10.4 Laser nephelometry

This technique has recently been modified for the detection of IgM RF. The method is based on the formation of complexes between HaIgG and IgM RF. The complexes scatter light in accordance with the amount of antibody present (Prentice et al 1987). The place of the test in clinical practice has not yet been established, although in the prospective study of Prentice et al, it was less sensitive for RF detection than the more conventional latex or haemagglutination tests.

3.10.5 Immunosorbent procedures

The basis of these procedures is that RF is removed from serum by absorption to IgG and subsequently eluted. IgG or gamma-globulin is insolubilized by cross-linking to glutaraldehyde or a similar substance. The preparation is washed several times to remove unbound immunoglobulin. Serum is added and incubated and unattached proteins are removed with the effluent. Absorbed proteins are eluted, usually with acidic treatment. The eluted fraction can then be analysed for immunoglobulins of different classes using a radial immunodiffusion method. Torrigiani and Roitt (1967) applied the method to patients with RA. Various workers have also detected anti-gammaglobulins (particularly in the IgG and IgA classes) in a high percentage of normal individuals (Panush et al 1971; Bianco et al 1974).

Turner (1976 pg 417-420) found that a significantly greater percentage of patients with RA had antiglobulins than normals. However, the statistical difference was no longer apparent when corrected for the immunoglobulin levels in the patient's serum. Turner also demonstrated the likelihood of nonspecific protein uptake by the insolublized IgG since repeated absorptions resulted in some further immunoglobulin binding. Albumin was also shown to bind to the IgG. Nardella and Mannik (1978) confirmed the nonspecific binding of IgG in immunoabsorbent columns.

Wernick et al (1981) used the method to prepare IgM and IgG RF standards. They found that 86% of the IgM RF was removed but no IgM was obtained from normal serum.

Other potential drawbacks include denaturation of the RF and a lack of specificity of radial immunodiffusion methods in detecting the RF classes e.g. when measuring IgG RF, IgM RF can form a precipitin zone with the anti-IgG present in the agar (Lea and Ward 1972).

In addition, IgG RF is likely to polymerize making radial immunodiffusion quantification difficult (Mannik and Nardella 1985).

Carson et al 1977 showed that normal IgG bound to IgM RF may be estimated as IgG RF, so that it may be necessary to remove the IgM before estimating IgG RF. A further problem with immunosorbent techniques is that RF (of all classes) may be lost during washing steps, leading to underestimation of the RF present (Mannik 1979 pg 510).

Nevertheless, not all the problems associated with the assay of IgG RF are specific to the immunoabsorbent method.

3.10.6 Enzyme-Linked Immunosorbent Assay (ELISA)

In general ELISAs offer advantages over radioimmunoasssays (RIAs) in that they are cheaper, do not require complex equipment and are not medically hazardous. They provide a highly sensitive quantifiable method for serological investigations, although their results are not usually as precise as RIA (Voller et al 1976).

The principle of RF ELISAs is that IgG is passively absorbed to a solid surface (e.g. polystyrene). Test serum is incubated with the antigen (IgG) and unbound substances are washed off. A class specific antibody (e.g. rabbit anti-human IgM) conjugated to a suitable enzyme (e.g. horseradish peroxidase) is added to the system. Bound RF (e.g. IgM RF) is detected by the anti-human immunoglobulin (e.g. anti-human IgM). The enzyme coupled to the latter then reacts with a substrate to produce a colour change which can be measured spectrophotometrically. Willems and Klaassen de Kort (1978) found good correlation between the IgM RF ELISA and the Waaler-Rose test, but that the ELISA method was more sensitive. The correlation with the latex-fixation test

was less good (84%); the authors suggested that the latex test was less specific. Maiolini et al (1978) also found good agreement between IgM RF ELISA results and the Waaler-Rose test. Gripenberg et al (1979) noted good correlation between results of an ELISA for IgM RF and the latex-fixation and Waaler-Rose tests. These authors noted a significant association between RF titres using the agglutination tests and the RF ELISA readings.

Methods have also been developed for measuring IgG and IgA RFs by ELISA (Palosuo and Milgrom 1981; Faith et al 1982; Procaccia et al 1987). In the IgG RF ELISA described by Faith et al (1982) microtitre plates were coated with rabbit IgG. Pepsin-treated sera were then added and bound anti-globulins detected by rabbit anti-human Fab conjugated with alkaline phosphatase. Bampton et al 1985 used a similar method. The pepsin digestion step was utilized to destroy IgM RF which may interfere in the assay. IgG RF was converted to F(ab')2 fragments by pepsin treatment but was still able to bind to the Fc fragment (Osterland et al 1963). The F(ab')2 fragments of IgG RF are then detected by the rabbit anti-human Fab. Employing this method, Faith et al (1982) found that the IgG RF assay gave results which were reproducible, although less so that the IgM RF ELISA. A number of authors have described a somewhat simpler assay system where the antiserum used is an anti-human IgG rather than the anti-human Fab (Gioud-Paquet et al 1987; Stone et al 1987). The effect of this alteration has not been determined. Mannik and Nardella (1985) have noted, however, that the antiserum chosen must be highly specific and cross-reactivity eliminated. Indeed the latter authors report that nonspecific binding of rabbit IgG to the solid phase may occur.

The effect of IgA RF was not taken into account in the above method. Palosuo and Milgrom (1981) also developed a similar ELISA for IgG RF. Differences from the method of Faith et al (1982) were that immune complexes containing IgG were used as a solid-phase antigen. IgG RF was measured using a goat antihuman IgG. Interference from IgM RF was eliminated using dithiothreitol.

Proccacia et al (1987) utilized a similar method to that of Faith et al (1982) to detect IgA RF. Pepsin-digested sera were incubated with rabbit IgG. F(ab')2 fragments of IgA RF were detected using a goat anti-human IgA conjugated to alkaline phosphatase. Potential problems with this assay are:

i. pepsin digestion coverts variable amounts (from 6-40%) of IgA to F(ab')2 fragments (LoSpalluto 1974; Calvanico and Tomasi 1974)

ii. F(ab')2 fragments of the antiserum should probably be used (Elkon et al 1981), although Faith et al (1982) found this was unnecessary (possibly because IgM RF had been destroyed by the pepsin treatment).

Measurement of the RF classes by ELISA has become popular. There is, however, a lack of standardization in terms of the type of antigen used, the antiserum employed and the method of eliminating interference from IgM RF when measuring RFs of other classes (Kleveland et al 1988, Ruschen et al 1988, Grunnet and Espersen 1988). In fact, Milgrom (1988) has expressed the opinion that measuring IgG RF by ELISA is of limited diagnostic value.

3.10.7 Radioimmunoassay (RIA)

The basis of the RIA tests for RF is as follows: IgG (human or rabbit) is bound to plastic tubes. Test serum is added and, after a period of incubation, unattached substances are washed off. Rheumatoid factors of different classes are detected by class-specific radiolabelled antibodies. The amount of radioactivity present is used to quantitatively estimate the RF level (Franchimont and Suteanu 1969).

Hay et al (1975) found IgM and IgG RF in patients with RA using this method. Carson et al (1977) and Mannik and Nardella (1985) have pointed out some limitations of the method including:

i. Underestimation of IgG RF

ii. IgM RF binding to the Fc portion of the anti-human IgG would be estimated as IgG RF

iii. IgG assayed may not be RF but simply IgG bound to the IgM RF.

Carson and colleagues (1977) therefore used the Fc portion of human IgG to coat plastic tubes. Following a blocking step, serum was added (for measurement of IgG RF pepsin-treated sera were used). Rabbit antibody to the F(ab')2 portion of human IgG were subjected to pepsin digestion and radiolabelled to measure the F(ab')2 portion of IgG RF. IgG RF is still underestimated by this method (Mannik and Nardella 1985). IgM RF was measured with the F(ab')2 segment of a rabbit anti-human IgM.

Dunne et al (1979) and Elkon et al (1981) have measured IgA RFs using an extension of the technique of Carson et al (1977).

While RIA methods have been developed for each of the RF classes, they are expensive and entail the use of radioactivity (Egeland and Munthe 1983). Therefore, they are not often used routinely (Holborow and Swannell 1983 p 248).

3.10.8 Other methods

The mixed reversed (solid-phase) passive anti-globulin haemadherence test (MRS PAH test) was described by March et al (1981). Using this test IgG and IgA RFs were found in many normal subjects although the levels were lower than in patients with RA. The test was subject to the same limitations as those mentioned in the RIA test of Hay et al (1975).

Waller and Richard (1976) separated IgM and IgG by chromatography and assayed IgG RF using a sensitized D-cell assay system.

A rheumatoid rosette assay and immunofluorescence techniques have also been described but are frequently associated with false positive reactions (Egeland and Munthe 1983).

Recently, a haemagglutination method using sheep erythrocytes to which IgG is covalently bound has been described (Assimeh and Johnson 1980).

Quin et al (1980) have developed a radioactive binding assay for detection of RF. Radiolabelled HaIgG and test sera are incubated. RF and radioactive IgG are precipitated using polyethylene glycol and the radioactivity estimated. The test can be used for IgG RF determination.

3.10.9 Immunocytochemical methods

Immunocytochemical methods have been used to demonstrate RF and RF-producing cells in the tissues and the usual technique is based on the work of Mellors et al (1959) which has already been described.

In 1963, Pernis et al used immunofluorescence to demonstrate RF in vascular lesions in patients with RA.

Munthe and Natvig (1972) determined the proportions of IgM and IgG RF producing plasma cells in rheumatoid synovial tissue. Cells and extracellular deposits containing RF were demonstrated with fluorescein-labelled HaIgG. Cells with free RF activity always produced RF of IgM class, whilst pepsin digestion of tissues enabled plasma cells containing IgG RF to be more readily detected.

3.10.10 Summary

The fact that there are so many tests to measure serum RF indicates, perhaps, that the ideal method has not been developed. Nevertheless, the trend appears to be to use agglutination tests, ELISAs, and, more recently, nephelometric techniques to identify and quantitate RF.

To detect RF in congenital syphilis, the RF latex test would appear to be suitable as a screening test and the RF ELISA may be an appropriate method for measuring RF classes, in particular IgM RF.

PART II

RHEUMATOID FACTOR AND TESTING FOR CONGENITAL SYPHILIS

CHAPTER 4 : INTRODUCTION AND AIMS

4.1 INTRODUCTION

It is possible to prevent congenital syphilis (Brown and Moore 1963). However, there is a high incidence of congenital syphilis in developing countries (Larsson and Larsson 1970; Hira et al 1982). This suggests that failure of primary prevention is not infrequent. Early detection and treatment of congenital syphilis are therefore important if the effects of the disease are to be minimized.

In general, there are 2 situations in which early diagnosis is attempted. The first of these is where the newborn infant is identified as being at risk of the disease due to the presence of maternal syphilis (Scotti et al 1969). The second instance is that where an infant demonstrates clinical signs compatible with the diagnosis of congenital syphilis (Pickering 1985).

The diagnosis of congenital syphilis in these 2 settings usually requires the use of serological tests (Borobio et al 1980). The limitations of existing serological tests in the case of high risk newborn infants have been pointed out. In summary, however, tests such as the FTA-ABS (IgM) test lack sensitivity in asymptomatic patients (Kaufman et al 1974). Other tests, such as the VDRL test, require a 3-4 months follow-up before final appraisal (Breasette 1979). Newer tests are still being evaluated and require laboratory-intensive methods. Tests in this category include Western blotting techniques (Dobson et al 1988a).

The usefulness of testing for RF (by means of the RF latex test) in situations where the newborn infant is at high risk of congenital syphilis has not been investigated. In particular, the sensitivity of the test would be the major consideration. If the sensitivity of the RF latex test in this context is established potential advantages would include its rapidity and

simplicity (Cathcart 1975 pg 121). A lack of specificity could be predicted from the studies of Bartfeld (1969) and Reimer et al (1975). This would, however, be of lesser importance than a high sensitivity, as the consequences of overdiagnosis are likely to be far less serious than those of underdiagnosis (Gonin 1985).

The use of serological tests for congenital syphilis in symptomatic infants is more certain (Rathbun 1983a). The VDRL test and FTA-ABS (IgM) test are often useful in this regard (Borobio et al 1980). However, in practice there is usually a delay of several days before the results of these tests are available. A test, which could be performed as a simple side-room investigation would be useful, particularly in view of the fact that congenital syphilis may mimic a large variety of conditions (Alford et al 1975).

It has been found that a high percentage of symptomatic infants with congenital syphilis have a positive RF latex test (Reimer et al 1975; Meyer and Malan 1987). However, there are no published studies that have investigated whether the RF latex test can distinguish between infants with congenital syphilis and those with similar clinical signs. If this is established, the characteristics of the RF latex test such as its rapidity and simplicity would clearly be advantageous.

As discussed above, testing for RF may have an unrealized value in the diagnosis of congenital syphilis. The presence of RF in congenital syphilis has, in the past, been regarded as an inconvenience, particularly in regard to the FTA-ABS (IgM) test (Ingall and Musher 1983 pg 359). Steps are usually taken to prevent interference by RF (Cerny et al 1985; Muller 1986). It is noteworthy, however, that the effect of abolishing RF activity has not been evaluated with respect to the FTA-ABS (IgM) test for congenital syphilis.

4.2 AIMS

The aims of the studies comprising Part Two were, therefore, to investigate these possibilities as follows.

Firstly to determine the usefulness of the RF latex test in the diagnosis of congenital syphilis where the newborn infant is identified as being at risk of the disease.

Secondly, to ascertain whether the RF latex test could distinguish between symptomatic infants with congenital syphilis and those whose clinical signs were due to other conditions.

Thirdly, to demonstrate the effect on the FTA-ABS (IgM) test of RF removal.

As these studies were to be carried out in the Peninsula Maternity and Neonatal Service in Cape Town some background information regarding this service is provided.

4.3 THE PENINSULA MATERNITY AND NEONATAL SERVICE (PMNS)

There are 5 maternity hospitals (Groote Schuur Hospital, Mowbray Maternity Hospital, Peninsula Maternity Hospital, Somerset Hospital, St Monica's Hospital) and 5 midwife obstetric units (Hanover Park, Heideveld, Khayelitsha, Mitchell's Plain and Retreat). High risk pregnancies are usually referred to the maternity hospitals for delivery.

Altogether 25615 newborns were delivered in 1987. Forty percent of the women delivering in the PMNS were Africans, 59% were Cape Coloureds and 1% were of caucasian extraction (van Coeverden de Groot and van der Elst 1987).

Of these patients, 94% received antenatal care prior to delivery (so called booked cases). According to hospital policy, serological tests for syphilis (STS) were performed on all patients who booked. The VDRL test was used as a screening test and the TPHA as a confirmatory test. A previous study found that 7.6% of booked mothers had serological evidence of active syphilis (Gonin 1985).

The prevalence of syphilis is higher amongst unbooked pregnant women. These patients have STS performed on arrival to the labour ward and the percentage with positive VDRL and TPHA tests

was reported by Meyer and Woods (1989). It was found that 15% of 631 such women had positive tests.

Once serological evidence of syphilis is obtained, treatment with benzathine penicillin 2,4 MU weekly for 3 weeks is commenced.

The number of pregnant women who complete this therapy prior to delivery is unknown. A pilot study from Khayelitsha Midwife Obstetric Unit (MOU) was undertaken by Subotsky and Delport (1988). Of 52 pregnant women with syphilis who presented in labour and whose treatment status could be ascertained, 22 (42%) were fully treated whilst 30 (58%) were untreated or incompletely treated. A study from King Edward VIII Hospital in Durban indicated that fewer than 10% of patients received treatment in the prescribed manner (Manning et al 1985).

The figures presented for the PMNS indicate that there are a large number of infants at risk of congenital syphilis in that they are born to mothers who are unbooked or inadequately treated.

How were these infants managed at the time the study was undertaken?

Infants with definite clinical signs who had serological and/or radiological evidence of congenital syphilis were treated with intramuscular procaine penicillin 50 000 units per kilogram daily for 10 days. Newborn infants who were asymptomatic had a total IgM estimation performed on blood obtained from a heel-prick. If the result was elevated, further investigations, in the form of the FTA-ABS (IgM) test and roentgenology of the long bones were carried out. If one or more of these tests were positive the infant was treated as already described. If the tests were negative a period of follow-up was arranged during which time the VDRL or RPR titre was monitored. Infants whose tests reverted to negative were discharged after 3 months. Infants whose tests showed rising titres or did not become negative during a 3-6 month follow-up were treated.

The overall prevalence of congenital syphilis in the PMNS is unknown, although Delport et al (1989) estimated it to be 0,05%. This is almost certainly an underestimation as stillbirths and asymptomatic cases were not included (Meyer 1990)

Compared to congenital syphilis, clinical signs due to other congenitally acquired infections are relatively rare amongst infants in the PMNS.

Congenital cytomegalovirus infection (CMV) was proven in only 5 cases between 1972 and 1982. There were 11 'possible' cases during this same period (Keen 1989). The prevalence of congenital CMV in the PMNS is not known. In similar populations elsewhere, however, 1.5% of infants are infected (Keen 1989). Of these only 5% will have the typical clinical signs at birth (Peckham et al, 1983; Stagno et al 1984 pg 108).

Congenital rubella was diagnosed in 6 cases between 1983 and 1985 (Keen 1989).

There were no cases of perinatally acquired human immunodeficiency virus (HIV) infection during the period in which the study on congenital syphilis was performed (February 1987 to May 1988). Likewise a small pilot study of 1762 antenatal sera in 1988 showed no positive tests for HIV (Keen 1989, Spracklen 1989).

There is little information regarding the prevalence of congenital toxoplasmosis in the PMNS. However, Jacobs (1978) obtained a figure of 0,003% in Johannesburg.

Of the congenital infections described above, congenital syphilis is readily preventable by good antenatal care (Fiumara 1951). Failing this the effects of the disease can be minimized by early diagnosis and treatment (Budell 1982 pg 936). The usefulness of the RF latex test in facilitating early diagnosis was evaluated in the studies which follow.

CHAPTER 5

AN EVALUATION OF THE RHEUMATOID FACTOR LATEX TEST IN NEWBORN INFANTS AT HIGH-RISK OF CONGENITAL SYPHILIS

The aim of this section was to determine the value of testing for RF in newborn infants at high-risk of congenital syphilis due to the presence of untreated or inadequately treated maternal syphilis.

The value of measuring RF by means of a latex test was assessed. The sensitivity, specificity, and positive and negative predictive values of the test were determined.

In addition, the usefulness of other diagnostic tests, which were also performed at birth, were compared to that of the RF latex test.

5.1 STUDY DESIGN AND METHODS

In essence, a group of newborn infants at high-risk of congenital syphilis were prospectively selected. A variety of tests, including the RF latex test were performed at birth. The infants were then followed-up until they could either be pronounced free from infection with Treponema pallidum or they fulfilled a set of criteria, enabling the diagnosis of congenital syphilis to be made.

5.1.1 Patient selection

Groups of high-risk newborns and controls were studied.

High-risk newborn infants

Newborns were regarded as being at high-risk for contracting congenital syphilis if their mothers had positive reagin (e.g. VDRL) and treponemal (e.g. TPHA) tests for syphilis and if their mothers were:

i. untreated or

ii. treated in the last month of pregnancy or

iii. treated with erythromycin

That infants in these categories are at high-risk of congenital syphilis has been described in Chapter 2 (Fiumara 1951; Fiumara et al 1952; Woody et al 1964, South et al 1964).

The method of patient selection was as follows.

The labour ward delivery book at Groote Schuur Hospital was examined daily (Monday to Friday). Mothers with positive VDRL and TPHA tests were interviewed and their hospital folders inspected. Information was obtained regarding:

i. the presence of signs or symptoms of syphilis in the mother

ii. the date on which the VDRL and TPHA were done

iii. the VDRL titres

iv. treatment given during current and previous pregnancies.

Details relating to treatment were also obtained from the cards of the local authority clinic responsible for treatment of sexually transmitted diseases. In uncertain cases the local authority clinic was contacted.

Mothers who had previously been treated for syphilis and who had stable, low reagin antibody titres were excluded from the study. Mothers whose serology had been performed before the last trimester of pregnancy had repeat VDRL and TPHA tests at delivery.

The mothers who were unbooked underwent serological testing on arrival to labour ward. Close liaison with the Microbiology Department at the University of Cape Town, Medical School enabled results to be obtained within 1 to 2 days of delivery. Mothers whose VDRL and TPHA tests were positive were considered for the study.

The infants were then examined clinically. Those who were symptomatic and fulfilled the diagnostic criteria described by Kaufman et al (1977) entered the study and were treated.

A set of diagnostic criteria were used since there is no 'gold standard' test available for the diagnosis of congenital syphilis (Kaufman et al 1974). The reasons for chosing the criteria of Kaufman et al (1977) are discussed in Chapter 2.

In addition to symptomatic patients, infants who were asymptomatic were included:

i. if their mothers were to be resident at a contactable Cape Town address for at least four months after delivery

ii. provided they did not receive treatment with antibiotics for coincidental reasons e.g. amniotic fluid infection syndrome.

A nursing sister, with community health experience, visited the mothers and infants soon after discharge to check their addresses.

An attempt was made to select 2 suitable cases per week.

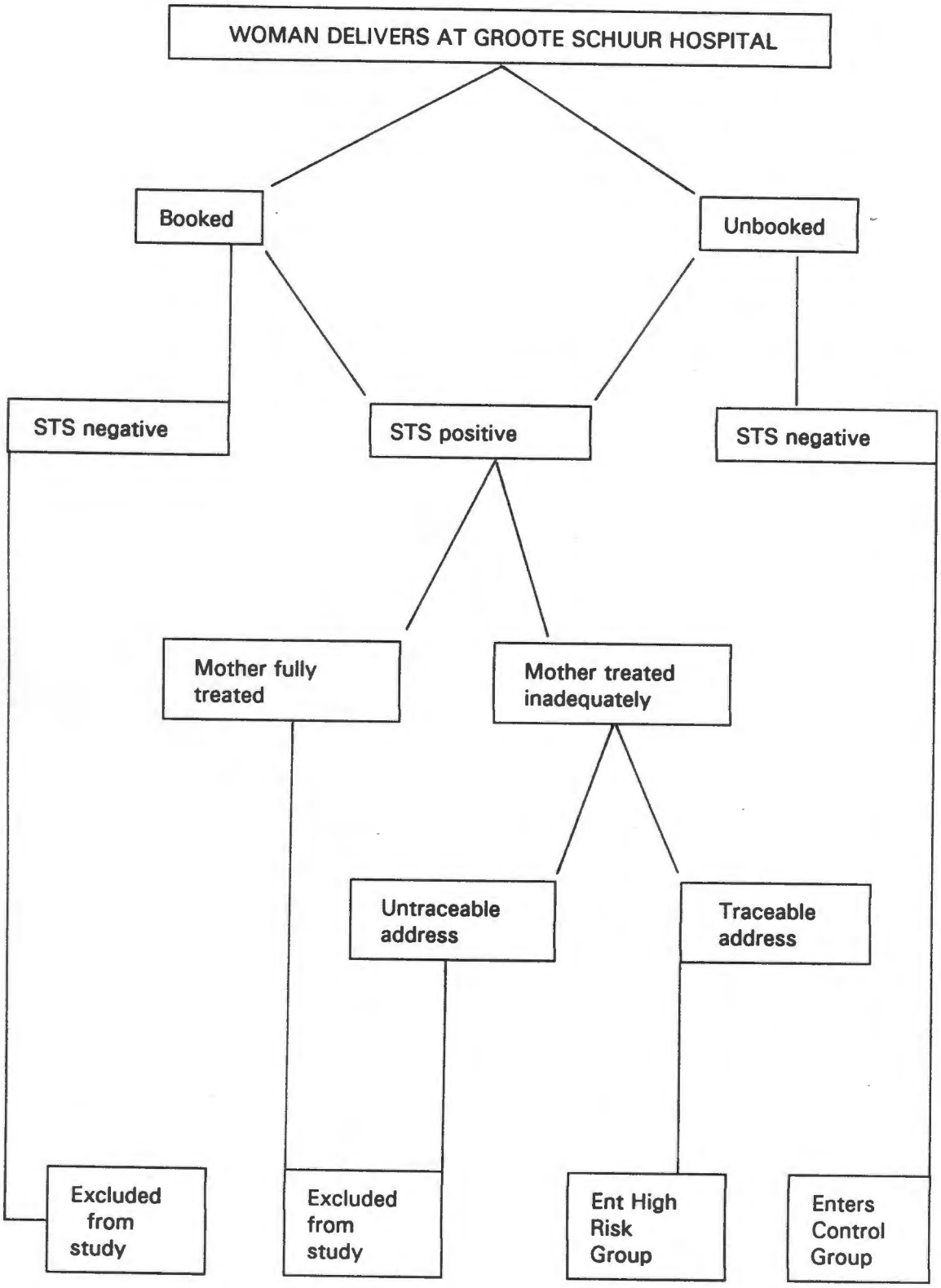
If two patients were not available at Groote Schuur Hospital in any one week then the labour ward registers in other parts of the PMNS were studied. The other units were contacted in the following order:

Heideveld MOU, Khayelitsha MOU, Peninsula Maternity Hospital, New Somerset Hospital.

The aim was to include 20 infants with congenital syphilis in the high-risk group. From the data quoted by Fiumara (1975) one would expect there to be 20 infants with congenital syphilis amongst 50 infants born to mothers with untreated early latent syphilis.

The flow chart (Figure 5.1) summarizes the selection of infants for the study from the time that an expectant woman delivers in hospital.

Figure 5.1: Flow chart of patient entry into study



Controls

It was anticipated that not all the high-risk infants would actually have congenital syphilis. The ones in whom the disease could be excluded would act as controls.

In addition, however, a group of infants with negative serology for syphilis were also studied. Newborn infants whose mothers were unbooked on arrival to labour ward were considered for this group.

Only mothers and their babies whose VDRL and TPHA tests were negative were included. An attempt was made to select 2 such newborn infants at Groote Schuur Hospital per week. The first 2 suitable controls who presented after midnight on Sunday of any given week were entered into the study.

As the aim was to compare the usefulness of the tests at birth the controls with negative STS were not followed-up.

5.1.2 Ethics

There is little doubt that symptomatic cases of congenital syphilis require appropriate therapy as soon as possible and this was adhered to throughout the study. At the time of commencement of the study (1987) the recommendations of the Centers for Disease Control (USA) and the WHO regarding the management of asymptomatic newborn infants considered to be at high-risk of the disease, were similar (Kaufman et al 1977 and WHO 1982). Both authorities suggested that a period of clinical and serological surveillance was justified provided that adequate follow-up could be ensured.

With regard to the present study asymptomatic patients were only included if they were from traceable addresses; hence follow-up was likely to be complete.

Parents were informed as to the nature of the study and their consent was obtained to perform the serological tests on the infants.

Approval for the project was obtained from the Ethics and Research Committee of the University of Cape Town.

5.1.3 Procedure at birth

The infants were examined clinically. Approximately 3 ml of venous blood was drawn into sterile plain glass tubes and allowed to clot at room temperature. The serum was removed and either tested immediately or aliquoted and frozen at -70°C.

The following serological investigations were performed on all infants: RF latex test, total IgM level, VDRL and TPHA.

Sera from infants born to mothers with positive STS were subjected to the FTA-ABS (IgM) test.

Radiological examination of the long bones was also performed on the infants whose mothers had positive STS.

The tests listed above were chosen for comparison with the RF latex test because they were felt to be the most likely to be helpful in making the diagnosis of congenital syphilis (Kaufman et al 1974; Borobio et al 1980; Pickering 1985). Many authors recommend performing a CSF examination (e.g. Taber 1982; Rathbun 1983a). However, Srinivasan et al (1983) could not demonstrate any benefit from carrying out the test in asymptomatic newborn infants and the Centers for Disease Control (CDC 1988c) recognizes that the use of CSF examination in diagnosing congenital syphilis is debatable.

All tests were done within the first 2 days of life.

Infants with congenital syphilis were treated with procaine penicillin 50 000 u/kg daily for 10 days.

Mothers were treated with benzathine penicillin and referred to the local authority clinic for further therapy and tracing of contacts.

5.1.4 Follow-up procedure

Mothers with positive serology whose infants were asymptomatic were given follow-up appointments for 6 weeks of age. A letter was attached to the infant's 'Road to Health' card. The letter stated that the infant was to be seen at Groote Schuur Hospital for follow-up. Health care personnel were requested not to treat the infant without first discussing the case with the researcher.

The community worker visited the family prior to the follow-up appointment.

At the 6 week visit the mother was questioned with regard to:

i. health of the infant

ii. whether the infant had received any medications or injections

iii. whether she and her consort had received treatment.

Where possible, clinic cards were checked to substantiate the history.

The infant was examined. Venous blood was taken for repeat VDRL and RF latex titres.

A return visit was scheduled for 4 months of age. Bus fare was provided.

The procedure at the 4 month visit was the same as that described above.

Any infant who developed features of congenital syphilis was treated as outlined previously. The diagnosis of congenital syphilis at follow-up was made according to the criteria of Kaufman et al (1977), except that, as discussed in Section 2.4.1, 6 months was regarded as the limit by which passively transferred reagin antibodies should have disappeared in uninfected infants.

5.1.5 Serological tests

Rheumatoid factor

The presence of RF was determined using an RF latex slide agglutination test ('Ortho RA Test' produced by Ortho Diagnostic Systems, Belgium). Sera were tested at the Red Cross War Memorial Children's Hospital, Cape Town. The technologist performing the tests was not aware of the clinical or serological status of the patients.

Sera, which had been collected from high-risk patients and controls, were either tested immediately or stored at -70°C. Frozen samples were thawed slowly at room temperature; they were then thoroughly mixed.

The test procedure described in the diagnostic kit was followed, except that a serum dilution of 1:5 was used in addition to the recommended 1:40 dilution.

This manipulation would enable the detection of 4 IU of RF/ml. (The sensitivity of the RF latex test was 0.8 IU/ml).

Further details about performing the test, and aspects of quality control are discussed in Appendix 2.

Total serum IgM levels

These were measured using a commercially available radial immunodiffusion assay (Immuno-plate IV, Hyland Diagnostics, USA). A 'Low level human IgM Test Kit' (catalogue number 085-403) was used for the neonatal sera and a 'Human IgM test kit' (catalogue number 085-404) for the sera obtained at follow-up visits. Details of the actual procedure followed and the quality control determinations are given in Appendix 2. The upper limit of normal for this study was regarded as 42 IU/ml. This was the level 2 standard deviations above the mean obtained by testing 408 newborn infants who did not have congenital syphilis (see Appendix 2 and Figure A.2.1 for further information)

Venereal Disease Research Laboratory (VDRL) test

The VDRL antigen was obtained from Wellcome Diagnostics, England (catalogue number VD 02-03). The VDRL tube flocculation test (Harris et al 1948) was carried out by the Department of Medical Microbiology, University of Cape Town.

Treponema Pallidum Haemagglutination (TPHA) test

TPHA test kits were obtained from Fujirebio Incorporated, Japan (catalogue number FD 101). The test was performed according to the manufacturers instructions and was carried out in the same laboratory as the VDRL test.

FTA-ABS (IgM) test

The antigen used was the FTA Treponema pallidum suspension, procured from Behring, West Germany (catalogue number ORMK 04/05). The anti-human IgM was a product of Wellcome Diagnostics, England (catalogue number MF 04).

According to the manufacturer's recommendations possible interference from RF was eliminated using a sheep anti-human IgG (RF Absorbent, Behring, West Germany, catalogue number OUCG 14/15).

Serum was diluted 1:5 in FTA absorption medium from the FTA-ABS DS kit (Zeus Scientific, USA) and further diluted by a factor of 2 in the RF absorbent. This gave a final serum dilution of 1:10.

Further details of the test are recorded in Appendix 2. Testing was performed in the laboratory described above (c and d).

Radiography

The long bones of the upper and lower extremities were subjected to standard radiological techniques. The tests were performed by the Radiology Department at Groote Schuur Hospital. Once again, the radiologist giving the report did not know the clinical status of the patients; all request forms simply stated that there was a maternal history of syphilis.

5.1.6 Statistical methods

Statistical analysis was performed using the Student's t test, Chi-square test, the median test and Fisher's exact test (Bourke et al 1985 pg 113-122). The calculations were carried out using a computer programme (Epistat 1986).

5.1.7 Costs

The approximate costs of materials used to perform the various tests were obtained from the relevant hospital departments concerned. Technologist's time and use of laboratory equipment were not taken into account.

5.2 RESULTS

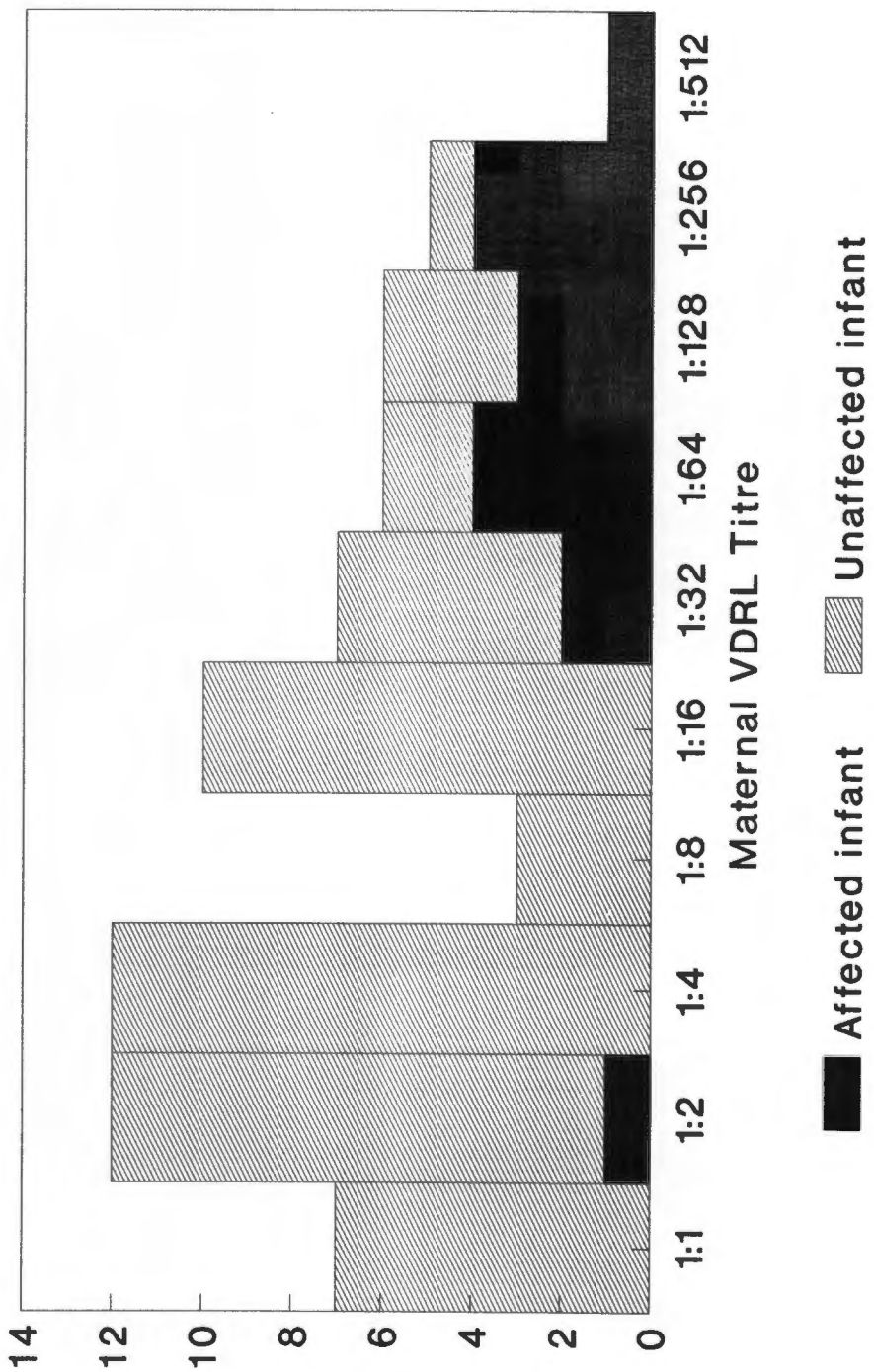
5.2.1 Mothers

There were a total of 69 mothers of whom 42 (61%) were Blacks and 27 (39%) were Cape Coloureds. None of the mothers had symptoms of syphilis either at antenatal clinic visits or at delivery. Sixty nine percent had received some antenatal care. The 69 mothers gave birth to a total of 15 infants with congenital syphilis (see Figure 5.2 and 5.3)

Overall, 50 (72%) of the mothers had not received any anti-syphilitic therapy prior to delivery. This group of mothers with untreated syphilis gave birth to 14 infants with congenital syphilis. The majority of mothers (13/14) with infected children had VDRL titres above 1:16.

Nineteen mothers (28%) had obtained treatment for syphilis. Fourteen received benzathine penicillin in the last month of pregnancy, while 5 had been treated with erythromycin. The infant of one mother (no. 5.6) in the treated group had radiological features of congenital syphilis. The mother had received one injection of benzathine penicillin 11 days prior to delivery. None of the other infants of this group of mothers developed congenital syphilis.

Figure 5.2: Frequency distribution of maternal VDRL titres in the last trimester of pregnancy



The maternal VDRL titres were obtained in the last trimester of pregnancy and in 64/69 cases in the last month. The mothers of infants who developed congenital syphilis had higher VDRL titres (median 1:128; range 1:2-1:512) than the mothers of infants that were not affected (median 1:4; range 1:1-1:32). This difference was statistically significant as determined using the median test ($p = 0.00017$).

Figure 5.2 shows the frequency distribution of the mothers' VDRL results in the treated and untreated groups. The number of affected infants is also shown.

Further information concerning the mothers is given in the tables describing the results (see Tables 5.1 and 5.2)

5.2.2 Infants

Of the original 69 infants, one was lost to 4 month follow-up (no. 5.47). When she was seen at 3 months, however, her VDRL titre was declining (1:1) and she was clinically well. Two infants (nos 5.26 and 5.53) were treated for congenital syphilis by attendant doctors because of the maternal history although there were no clinical signs or serological changes compatible with the disease. The overall outcome of the infants is summarized in Figure 5.3, and the results are detailed in Tables 5.1 and 5.2.

Infants with congenital syphilis: There were 15 infants in whom a diagnosis of probable congenital syphilis was made on the basis of the criteria of Kaufman et al (1977). As soon as an infant fulfilled the criteria, therapy with procaine penicillin, 50 000 u per kilogram per day for 10 days was commenced. Seven of the infants were treated at birth and the other 8 after a period of follow-up. The 7 cases treated at birth could be subdivided into 2 groups: those with clinical signs and those without. The infants without abnormal clinical findings at birth fulfilled the diagnostic criteria by virtue of having radiological abnormalities of the long bones. Had it not been for this investigative procedure the diagnosis would only have become apparent at a later stage.

Figure 5.3: Schematic diagram: Outcome of infants at risk for congenital syphilis

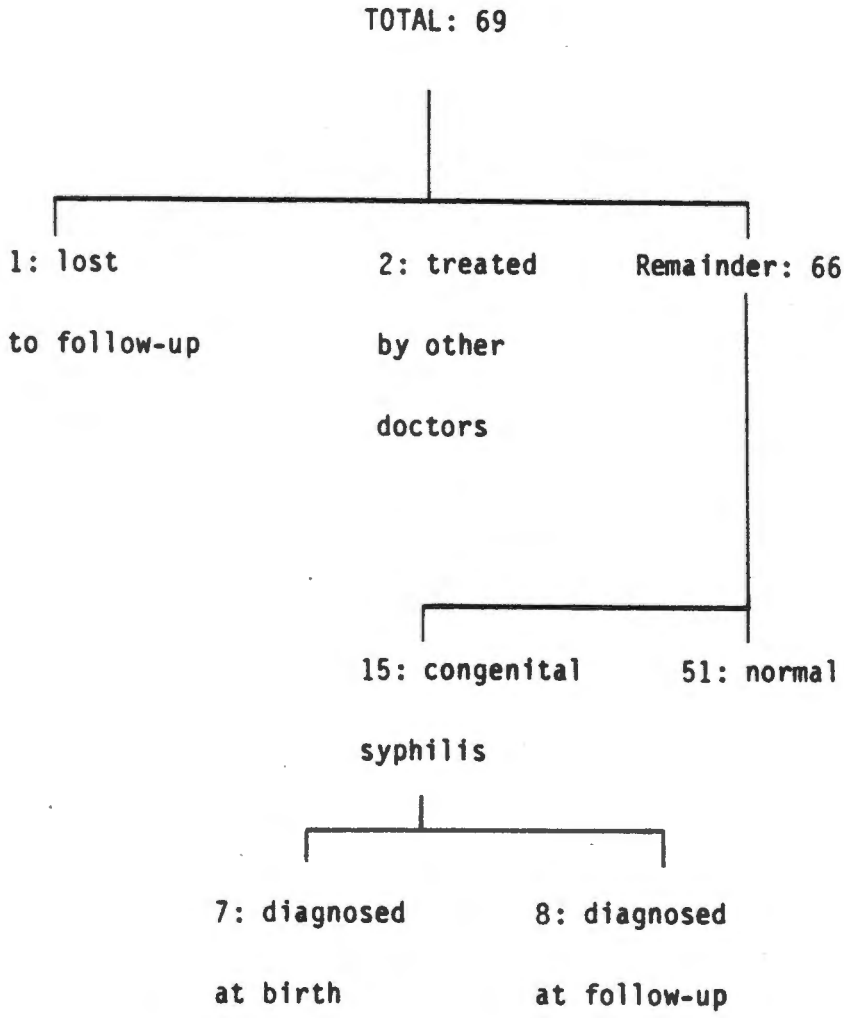


TABLE 5.1: CLINICAL FINDINGS AND RESULTS OF LABORATORY INVESTIGATIONS FOR INFANTS WITH CONGENITAL SYPHILIS

* NO.	MATERNAL DATA		INFANT DATA AT BIRTH									INFANT DATA AT FOLLOW-UP
	** VDRL TITRE	**** TREATED YES/NO	BIRTH WEIGHT (kg)	*** GESTATIONAL AGE	CLINICAL FINDINGS	SEX	RADIOLOGICAL CHANGES	TOTAL IgM IU/ml	RF LATEX TITRE	VDRL TITRE	FTA-ABS (IgM) TEST	COMMENTS
5.1	1:64	No	1,0	37	SGA, peeling skin of palms & soles hepatomegaly.	M	Yes	301	1:160	1:16	+ve	Treated at birth
5.2	1:256	No	1,25	30	Preterm, pallor, ascites, hydrocoeles, hepatosplenomegaly, pneumonia.	M	No	97	1:160	1:256	-ve	Packed cell volume 15. Died 4 hours after birth. PM findings: EMH, pneumonia alba, pancreatic fibrosis.
5.3	1:64	No	2,2	36	Preterm, jaundice, oedema, hepatosplenomegaly.	F	Yes	525	-ve	1:64	+ve	Conjugated hyperbilirubinaemia, hepatitis, thrombocytopaenia. Treated at birth.

Table 5.1 continued

* NO	MATERNAL DATA		INFANT DATA AT BIRTH								INFANT DATA AT FOLLOW-UP
	** VDRL TITRE	**** TREATED YES/NO	BIRTH WEIGHT (kg)	*** GESTATIONAL AGE	CLINICAL FINDINGS	SEX	RADIOLOGICAL CHANGES	TOTAL IgM IU/ml	RF LATEX TITRE	VDRL TITRE	FTA-ABS (IgM) TEST	COMMENTS
5.4	1:256	No	2,7	40	Snuffles	M	No	126	1:160	1:64	-ve	Treated at birth. Developed positive FTA-ABS IgM at follow-up.
5.5	1:256	No	2,7	40	Well	M	Yes	424	1:80	-ve	+ve	Treated in first few days of life.
5.6	1:512	Benzathine x1 11 days before delivery	3,3	40	Well	F	Yes	186	1:20	1:64	+ve	Treated in first few days of life.
5.7	1:32	No	3,3	42	Well	M	Yes	21	-ve	1:64	-ve	Treated in first few days of life.

Table 5.1 continued

* NO	MATERNAL DATA		INFANT DATA AT BIRTH								INFANT DATA AT FOLLOW-UP	
	** VDRL TITRE	**** TREATED YES/NO	BIRTH WEIGHT (kg)	*** GESTATIONAL AGE	CLINICAL FINDINGS	SEX	RADIOLOGICAL CHANGES	TOTAL IgM IU/ml	RF LATEX TITRE	VDRL TITRE	FTA-ABS (IgM) TEST	AGE	COMMENTS
5.8	1:64	No	3,0	40	Well	M	No	22	-ve	1:16	-ve	7w 18w	Well VDRL 1:2. RF latex -ve. Symmetrical rash on trunk, peeling palms and soles, snuffles, splenomegaly. VDRL 1:256, RF 1:80.
5.9	1:64	No	3,2	40	Well	F	No	33	1:40	1:16	-ve	6w 10w	Well. VDRL 1:16. RF 1:20. Snuffles, hepato-splenomegaly. VDRL 1:64. Periosteal elevation.
5.10	1:128	No	3,2	40	Well	F	No	172	1:20	1:16	-ve	6w	Snuffles, hepato-splenomegaly. VDRL 1:64. RF 1:40.

Table 5.1 continued

* NO	MATERNAL DATA		INFANT DATA AT BIRTH								INFANT DATA AT FOLLOW-UP	
	** VDRL TITRE	**** TREATED YES/NO	BIRTH WEIGHT (kg)	*** GESTATIONAL AGE	CLINICAL FINDINGS	SEX	RADIOLOGICAL CHANGES	TOTAL IgM IU/ml	RF LATEX TITRE	VDRL TITRE	FTA-ABS (IgM) TEST	AGE	COMMENTS
5.11	1:256	No	2,9	40	Well	F	No	14	-ve	1:32	-ve	6w	Snuffles, macular depigmented rash, hepatosplenomegaly VDRL 1:128. RF -ve
5.12	1:128	No	1,2	32	Preterm, SGA	F	No	14	-ve	1:8	-ve	6w 3mo	Thriving. VDRL 1:4 RF -ve. Snuffles, macular rash, splenomegaly. VDRL 1:256. RF 1:320
5.13	1:2	No	2,1	36	Preterm, SGA	M	No	16	-ve	-ve	-ve	6w 4mo	Well. VDRL -ve. RF -ve. Well. VDRL 1:8. TPHA +ve. RF -ve. Completed 10 days treatment immediately prior to follow-up.
5.14	1:32	No	3,0	40	Well	M	No	14	-ve	1:2	-ve	6w	Well. VDRL 1:8 RF 1:5.

Table 5.1 continued

* NO	MATERNAL DATA		INFANT DATA AT BIRTH									INFANT DATA AT FOLLOW-UP	
	** VDRL TITRE	**** TREATED YES/NO	BIRTH WEIGHT (kg)	*** GESTA-TIONAL AGE	CLINICAL FINDINGS	SEX	RADIO-LOGICAL CHANGES	TOTAL IgM IU/ml	RF LATEX TITRE	VDRL TITRE	FTA-ABS (IgM) TEST	AGE	COMMENTS
5.15	1:128	No	3,2	40	Well	M	No	31	-ve	-ve	-ve	6w	Well. VDRL 1:64. RF 1:10.

* Patient Code No
** Maternal VDRL titre in last trimester or at delivery
*** Gestational age in completed weeks
****Refers to any treatment given in the last month of pregnancy

Abbreviations:

EMH	-	extramedullary haematopoeisis
F	-	Female
M	-	Male
mo	-	months
N/A	-	Not applicable
PM	-	Postmortem
SGA	-	Small for gestational age
-ve	-	Negative
+ve	-	Positive
w	-	weeks

Infants with definite clinical signs of congenital syphilis at birth

Three of the 4 infants in this category had evidence of widespread disease (see Table 5.1). Case no. 5.2 died on day 1. The postmortem findings were consistent with the diagnosis of congenital syphilis although spirochaetes were not identified.

Most of the infants mounted an immunological response. Three had positive RF latex tests while 4 had elevated total IgM levels. Only 2 patients had positive FTA-ABS (IgM) tests. Case no. 5.3 showed evidence of having produced IgM (total and anti-treponemal IgM levels elevated; no IgM RF). The absence of free RF was confirmed with the RF ELISA. Techniques to detect hidden RF were also negative (see Chapter 9).

None of the infants had significantly elevated VDRL titres i.e. 4 times greater than those of their mothers (Ingall and Musher 1983 pg 358).

Infants with congenital syphilis who did not have definite clinical signs at birth

Three infants (nos. 5.5-5.7) had metaphyseal dystrophy of the long bones demonstrated on roentgenology soon after birth. All three were term, appropriately grown infants. Two were noted to have minimal pretibial oedema. Two of these 3 infants had positive RF latex tests, elevated total IgM levels and positive FTA-ABS (IgM) tests. In the third infant these test results were not abnormal. The 3 infants were treated soon after birth.

Eight infants (nos. 5.8-5.15) had no evidence of congenital syphilis at birth but features of disease became apparent during follow-up.

Six of the 8 were term infants of normal birth weight; 2 who had low birth weights were preterm and small for gestational age (SGA)*. Two infants had positive RF latex tests at birth and 1 had a raised total IgM level. The other tests (FTA-ABS (IgM)

* SGA - birthweight less than tenth percentile on the charts of Lubchenco et al (1966)

and roentgenograms of the long bones) were negative at birth in all 8 infants.

Five of the 8 infants who were not treated at birth developed clinical signs of congenital syphilis at follow-up (nos. 5.8-5.12). In addition the VDRL titres became diagnostically elevated (i.e. showed a four-fold rise in titre over the birth result). The other 3 infants (nos. 5.13-5.15 were well but had diagnostically raised VDRL titres.

The RF latex results at follow-up are also given in Table 5.1. Of the 8 cases diagnosed at follow-up 1 was treated prior to the follow-up date. Six of the remaining 7 had positive RF latex tests at or prior to the time congenital syphilis was diagnosed.

Infants not affected

There were 51 infants who was asymptomatic at birth and who did not fulfil the diagnostic criteria for congenital syphilis during follow-up. Table 5.2 shows the details of each patient in this group.

Most of the infants were full-term with birth weights appropriate for gestational age. Sixteen infants were of low birth weight: 7 were term and SGA; 7 were preterm; 2 were preterm and SGA. The proportion of low birth weight infants was similar to that of the infants with congenital syphilis ($p > 0,05$ using Fisher's exact test).

TABLE 5.2: CHARACTERISTICS OF HIGH-RISK INFANTS WHO WERE NOT AFFECTED

* CASE NO	MATERNAL DATA		INFANT DATA AT BIRTH						INFANT DATA AT FOLLOW-UP			
	** VDRL TITRE	**** TREATED YES/NO	BIRTH WEIGHT (kg)	*** GESTA-TIONAL AGE	CLINICAL FINDINGS	SEX	TOTAL IgM IU/ml	VDRL TITRE	AGE	VDRL TITRE	RF LATEX TITRE	COMMENT
5.16	1:2	No	1,8	34	Preterm No compli-cations	F	20	-ve	6w 4mo	-ve -ve	-ve -ve	Well Well
5.17	1:4	No	1,6	34	Preterm No compli-cations	M	20	-ve	6w 4mo	-ve -ve	-ve -ve	Well Well
5.18	1:8	No	3,3	40	Well	F	45	-ve	7w 4mo	-ve -ve	-ve -ve	Papular urticaria Well
5.19	1:16	Yes	2,5	40	SGA	F	35	1:32	6w 4mo	ND -ve	-ve -ve	Well Lower respiratory tract infection
5.20	1:1	No	2,1	36	Preterm SGA. Fetal alcohol syndrome	F	28	-ve	6w 4mo	-ve -ve	-ve -ve	Failure to thrive

Table 5.2 continued

* CASE NO	MATERNAL DATA		INFANT DATA AT BIRTH						INFANT DATA AT FOLLOW-UP			
	** VDRL TITRE	**** TREATED YES/NO	BIRTH WEIGHT (kg)	*** GESTA-TIONAL AGE	CLINICAL FINDINGS	SEX	TOTAL IgM IU/ml	VDRL TITRE	AGE	VDRL TITRE	RF LATEX TITRE	COMMENT
5.21	1:2	No	2,6	40	Well	M	21	1:1	3mo 6mo	-ve -ve	-ve -ve	Difficult to motivate to attend follow up
5.22	1:2	No	2,8	40	Well	M	23	-ve	6w 4mo	-ve -ve	-ve -ve	Well Well
5.23	1:4	Yes	2,8	40	Well	F	32	1:1	6w 4mo	-ve -ve	-ve -ve	Well Well
5.24	1:4	Yes	3,7	40	Well	M	26	1:8	7w 4mo	-ve -ve	1:40 -ve	Well Bronchitis
5.25	1:32	Yes	1,2	30	Preterm	M	9	1:2	5w 4mo	-ve -ve	-ve -ve	Thriving Well
5.26	1:16	No	2,7	40	Well	F	16	-ve	6w 4mo	-ve -ve	-ve -ve	Well Treated at local clinic because of maternal history

Table 5.2 continued

* CASE NO	MATERNAL DATA		INFANT DATA AT BIRTH						INFANT DATA AT FOLLOW-UP			
	** VDRL TITRE	**** TREATED YES/NO	BIRTH WEIGHT (kg)	*** GESTA-TIONAL AGE	CLINICAL FINDINGS	SEX	TOTAL IgM IU/ml	VDRL TITRE	AGE	VDRL TITRE	RF LATEX TITRE	COMMENT
5.27	1:16	No	2,4	37	Well	F	33	-ve	6w 4mo	-ve -ve	-ve -ve	Well Well
5.28	1:1	No	3,2	40	Well	M	16	-ve	10w 4mo	-ve -ve	-ve -ve	Defaulted initial follow-up at 6w Well
5.29	1:2	No	2,6	40	Well	M	26	1:1	8w 4mo	ND -ve	-ve -ve	Well, TPHA -ve Well
5.30	1:128	No	3,4	40	Well	M	35	-ve	6w 4mo	ND -ve	ND -ve	Well on home visit Defaulted follow-up Presumed viral infection with transient rash lasting 1 week
5.31	1:32	Yes	2,9	40	Well	M	27	1:8	6w 4mo	1:8 -ve	-ve -ve	Well Upper respiratory tract infection

Table 5.2 continued

* CASE NO	MATERNAL DATA		INFANT DATA AT BIRTH						INFANT DATA AT FOLLOW-UP			
	** VDRL TITRE	**** TREATED YES/NO	BIRTH WEIGHT (kg)	*** GESTA-TIONAL AGE	CLINICAL FINDINGS	SEX	TOTAL IgM IU/ml	VDRL TITRE	AGE	VDRL TITRE	RF LATEX TITRE	COMMENT
5.32	1:2	Yes	1,3	35	Preterm SGA	M	27	1:1	6w	ND	ND	Defaulted hospital visit
									4mo	-ve	-ve	Thriving
5.33	1:16	Yes	3,3	40	Lethargic	M	222	1:4	6w	1:2	-ve	Well
									4mo	-ve	-ve	Well
5.34	1:1	No	3,1	40	Well	F	16	-ve	7w	-ve	-ve	Well
									3mo	-ve	-ve	Well
5.35	1:16	No	1,5	34	Preterm	M	22	1:4	6w	1:4	-ve	Well
									4mo	-ve	-ve	Well
5.36	1:16	No	2,3	37	Well	M	9	1:2	6w	1:1	-ve	Upper respiratory tract infection
									4mo	-ve	-ve	Well
5.37	1:1	No	3,2	40	Well	F	16	-ve	6w	-ve	-ve	Well
									4mo	-ve	-ve	Well

Table 5.2 continued

* CASE NO	MATERNAL DATA		INFANT DATA AT BIRTH						INFANT DATA AT FOLLOW-UP			
	** VDRL TITRE	**** TREATED YES/NO	BIRTH WEIGHT (kg)	*** GESTA-TIONAL AGE	CLINICAL FINDINGS	SEX	TOTAL IgM IU/ml	VDRL TITRE	AGE	VDRL TITRE	RF LATEX TITRE	COMMENT
5.38	1:4	No	2,9	40	Well	M	33	-ve	6w 4mo	-ve -ve	-ve -ve	Well Well
5.39	1:2	No	4,1	40	Well	M	17	-ve	6w 3mo	-ve -ve	1:160 1:160	Upper respiratory tract infection 3cm hepatomegaly Well
5.40	1:2	Yes	3,3	40	Well	F	36	+ve (Titre not done)	6w 4mo	-ve -ve	-ve -ve	Bronchitis Well
5.41	1:1	No	2,9	40	Well	F	61	1:1	6w 4m	-ve -ve	-ve -ve	Well Cough, diarrhoea
5.42	1:4	No	2,1	40	SGA	M	10	1:1	6w 4mo 12mo	1:1 1:2 -ve	-ve -ve -ve	TPHA positive TPHA negative Well

* CASE NO	MATERNAL DATA		INFANT DATA AT BIRTH						INFANT DATA AT FOLLOW-UP			
	** VDRL TITRE	**** TREATED YES/NO	BIRTH WEIGHT (kg)	*** GESTA-TIONAL AGE	CLINICAL FINDINGS	SEX	TOTAL IgM IU/ml	VDRL TITRE	AGE	VDRL TITRE	RF LATEX TITRE	COMMENT
5.43	1:4	No	2,0	35	Preterm	F	28	-ve	6w	ND	ND	Well, but did not attend hospital follow-up
									4mo	-ve	-ve	Thriving
5.44	1:2	No	3,5	40	Well	F	28	-ve	6w	-ve	-ve	Well
									4mo	-ve	-ve	Well
5.45	1:1	No	3,0	40	Well	F	22	-ve	6w	-ve	-ve	Well
									3mo	-ve	-ve	Intertriginous rash
									5mo	-ve	-ve	Well
5.46	1:4	No	2,5	40	SGA	F	21	1:2	4w	-ve	-ve	Well
									4mo	-ve	1:80	Well
5.47	1:4	Yes	2,5	40	SGA	F	31	1:4	7w	1:2	-ve	Well
									3mo	1:1	-ve	Well. Unable to trace for further visits.

Table 5.2 continued

* CASE NO	MATERNAL DATA		INFANT DATA AT BIRTH						INFANT DATA AT FOLLOW-UP			
	** VDRL TITRE	**** TREATED YES/NO	BIRTH WEIGHT (kg)	*** GESTA-TIONAL AGE	CLINICAL FINDINGS	SEX	TOTAL IgM IU/ml	VDRL TITRE	AGE	VDRL TITRE	RF LATEX TITRE	COMMENT
5.48	1:4	No	3,4	40	Well	M	37	-ve	6w	ND	ND	Well. Did not attend hospital follow-up.
									4mo	1:1	-ve	Coughing.
									5mo	-ve	-ve	Well.
5.49	1:16	Yes	3,3	40	Well	M	21	1:1	6w	1:1	-ve	Well
									4mo	-ve	-ve	Well
5.50	1:4	No	1,9	37	SGA	F	29	1:1	4w	1:1	-ve	Good weight gain
									4mo	-ve	-ve	Upper respiratory tract infection
5.51	1:8	No	3,5	40	Well	F	32	1:1	6w	-ve	-ve	Well
									4mo	-ve	-ve	Well
5.52	1:16	Yes	3,1	40	Well	M	14	ND Lip-aemic	6w	-ve	-ve	Well
									4mo	-ve	-ve	Well

Table 5.2 continued

* CASE NO	MATERNAL DATA		INFANT DATA AT BIRTH						INFANT DATA AT FOLLOW-UP			
	** VDRL TITRE	**** TREATED YES/NO	BIRTH WEIGHT (kg)	*** GESTA-TIONAL AGE	CLINICAL FINDINGS	SEX	TOTAL IgM IU/ml	VDRL TITRE	AGE	VDRL TITRE	RF LATEX TITRE	COMMENT
5.53	1:32	No	1,1	32	Preterm SGA	M	26	1:64	6w 3mo	1:4 ND	-ve ND	Thriving Well. Treated by attendant doctor because of maternal history.
5.54	1:16	No	3,0	40	Well	F	16	-ve	6w 5mo	-ve -ve	-ve -ve	Well Well
5.55	1:16	Yes	3,6	40	Well	M	32	1:8	6w 4mo	1:4 -ve	-ve -ve	Desquamating rash. Gastroenteritis Hypoproteinaemia
5.56	1:2	No	2,9	40	Well	F	64	-ve	6w 4mo	ND -ve	ND -ve	Well. Defaulted hospital visit. Well
5.57	1:32	No	3,5	40	Well	F	13	1:4	4w 5mo	-ve -ve	-ve -ve	Well Well
5.58	1:64	No	1,1	30	Preterm	F	13	1:4	6w 4mo	1:2 -ve	-ve -ve	No complications Gaining well

Table 5.2 continued

* CASE NO	MATERNAL DATA		INFANT DATA AT BIRTH						INFANT DATA AT FOLLOW-UP			
	** VDRL TITRE	**** TREATED YES/NO	BIRTH WEIGHT (kg)	*** GESTA-TIONAL AGE	CLINICAL FINDINGS	SEX	TOTAL IgM IU/ml	VDRL TITRE	AGE	VDRL TITRE	RF LATEX TITRE	COMMENT
5.59	1:8	Yes	3,1	40	Well	M	28	-ve	2mo 4mo	1:1 -ve	-ve -ve	Well Well
5.60	1:2	Yes	3,5	40	Well	M	16	1:1	6w 4mo	-ve -ve	-ve -ve	Well Well
5.61	1:64	Yes	3,3	40	Well	F	<5	-ve	6w 4mo	1:1 -ve	-ve -ve	Well Well
5.62	1:256	Yes	2,2	40	SGA	F	29	1:128	1mo 2mo 4mo 6mo	1:16 1:4 -ve ND	-ve -ve -ve ND	Well Well Presumed viral ill-ness with lymphad-denopathy, rash Well
5.63	1:32	Yes	1.4	32	Preterm	F	22	1:4	2mo 4mo 8mo	-ve ND -ve	1:80 ND -ve	Gaining weight Defaulted hospital visit Well

* CASE NO	MATERNAL DATA		INFANT DATA AT BIRTH						INFANT DATA AT FOLLOW-UP			
	** VDRL TITRE	**** TREATED YES/NO	BIRTH WEIGHT (kg)	*** GESTA-TIONAL AGE	CLINICAL FINDINGS	SEX	TOTAL IgM IU/ml	VDRL TITRE	AGE	VDRL TITRE	RF LATEX TITRE	COMMENT
5.64	1:1	No	2.8	40	Well	M	43	1:8	6w 4mo	1:2 -ve	1:20 1:40	Well Had chicken pox 2 weeks prior to visit
5.65	1:4	No	2.7	40	Well	M	28	1:1	6w 4mo	-ve -ve	-ve -ve	Well Well
5.66	1:2	Yes	2.8	40	Well	M	13	1:2	6w 4mo	-ve -ve	-ve -ve	Well Well
5.67	1:128	No	2.2	40	SGA	M	32	1:16	2mo 5mo	1:2 -ve	-ve -ve	Well Well
5.68	1:128	No	2.5	40	SGA	F	5	1:2	2mo 5mo	1:1 -ve	-ve -ve	Well Well

Table 5.2 continued

* CASE NO	MATERNAL DATA		INFANT DATA AT BIRTH						INFANT DATA AT FOLLOW-UP			
	** VDRL TITRE	**** TREATED YES/NO	BIRTH WEIGHT (kg)	*** GESTA-TIONAL AGE	CLINICAL FINDINGS	SEX	TOTAL IgM IU/ml	VDRL TITRE	AGE	VDRL TITRE	RF LATEX TITRE	COMMENT
5.69	1:4	No	3.6	40	Well	M	36	-ve	6w 4mo	-ve -ve	-ve -ve	Well Well

* Patient code number

** Maternal VDRL titre in last trimester of pregancy or at delivery

*** Gestion age in completed weeks

**** Refers to any treatment given in the last month of pregnancy

ND Not done
SGA Small for gestational age
F Female
M Male
w Weeks
mo Months
-ve Negative
+ve Positive

The RF latex test was negative at birth in all 51 infants.

Twenty nine of the infants had a positive VDRL test (in 1 patient serum was insufficient for a titre). Twenty one patients had negative tests (in 1 infant the specimen was lipaemic and the test could not be done). The median VDRL titre in the unaffected infants was 1:1 (range 0-1:128), which was lower than in the infants with congenital syphilis (median 1:16; range 0-1:256). This difference in VDRL titres was statistically significant ($p=0.033$ using the median test). The TPHA was positive in 49 infants, doubtful in 1 and nonspecific in another (the FTA-ABS IgG test was positive in these latter 2 infants).

Forty nine of the infants had a normal total IgM level while 5 had elevated levels (> 42 IU/ml). Of the 5 infants, 4 (nos. 5.18, 5.41, 5.56 and 5.64) were well, appropriately grown infants, whose mothers had not received therapy for syphilis. The fifth infant (no. 5.33) was born at term but was somewhat lethargic on day 1. His mother had been treated for syphilis with erythromycin during pregnancy. Blood cultures and a CSF examination were negative and by day 3 he appeared well and was discharged without treatment. Urine cultures for CMV were negative. All 5 infants had negative IgM antibody tests for toxoplasmosis, rubella and cytomegalovirus infections. Repeat serum samples tested for CMV IgM remained negative at the 6 week and 3 month follow-up. The cause for these elevated IgM levels was not determined.

None of the 51 infants had abnormalities detected on radiography of the long bones and all had negative FTA-ABS (IgM) tests.

Considering all the asymptomatic infants at birth i.e. those shown to be unaffected as well as those who went on to develop features of congenital syphilis, it can be seen from Table 5.3 that the RF latex test, radiography and the FTA-ABS (IgM) test were able to identify a proportion of the infected infants. The total IgM test and the VDRL were less useful in this regard as positive results were also obtained in unaffected infants.

Results at follow-up

During the follow-up period several infants had minor ailments such as upper respiratory tract infections and diarrhoeal disease (see Table 5.2). As far as could be ascertained from the mothers, none of the infants were given antibiotics. Case no. 5.20 had features of fetal alcohol syndrome and no. 5.55 developed diarrhoeal disease with hypoalbuminaemia requiring admission at 4 months of age. Three other infants, nos. 5.30, 5.39 and 5.62, had probable intercurrent viral infections, the clinical signs of which resolved spontaneously.

Five patients developed a positive RF latex test on follow-up. In 4 cases (nos. 5.24, 5.39, 5.63 and 5.64) the test was positive between 4-8 weeks. Case No. 5.46 had developed a positive test by 4 months. One infant had an upper respirtory tract infection at the time and another had recently had chicken pox. The remainder were well.

Of the 30 infants who had a known positive VDRL titre at birth, 12 patients had negative tests by 6 weeks, the majority of the remainder were negative by 4 months.

Control infants

Eighty five normal infants whose mothers had negative VDRL and TPHA tests were studied. The infants had no signs of congenital infection. Their findings are shown in Appendix 3. Fifteen were term and SGA, 4 preterm and SGA and 12 were preterm.

The RF latex test was negative in all cases at birth. The median total IgM level was 21 IU/ml with a range of 5-291 IU/ml.

In 3 cases, the total IgM level was elevated. The cause of this elevation was not apparent; the IgM antibody tests for rubella, toxoplamosis and cytomegalovirus were negative in all cases and the urine culture for cytomegalovirus, which was performed in 1 case, was negative.

5.2.3 Comparison of test results

Usefulness of the various diagnostic tests

A summary of the results obtained in the infants with congenital syphilis is shown below (Table 5.3).

TABLE 5.3:

SUMMARY OF TEST RESULTS AT BIRTH FOR INFANTS AT RISK OF CONGENITAL SYPHILIS

	Infant Affected		Infant not Affected
	Clinical signs present	No clinical signs	
No.	4	11	51
+ve RF latex test	3	4	0
Total IgM (> 42 IU/ml)	4	3	5
X-ray abnormality	2	3	0
+ve FTA-ABS (IgM) test	2	2	0
+ve VDRL test	4	8	29

The sensitivity, specificity and the positive and negative predictive values of the various tests in the diagnosis of congenital syphilis can be calculated.

RF Latex Test

A 2 x 2 contingency table (Table 5.4) to calculate the usefulness of the RF latex test in the diagnosis of congenital syphilis is shown below.

TABLE 5.4

USE OF THE RF LATEX TEST IN THE DIAGNOSIS OF CONGENITAL SYPHILIS

	Infant Affected		Infant Not Affected		
RF Latex Test:					
Positive	7	a	b	0	7 a+b
Negative	8	c	d	51	59 c+d
	15	a+c	b+d	51	66 a+b+c+d

The sensitivity $\frac{a}{(a + c)}$ x 100% was 46.7%

The specificity $\frac{d}{(d + b)}$ x 100% was 100%

The positive predictive value $\frac{a}{(a + b)}$ x 100% was 100%

The negative predictive value $\frac{d}{(d + c)}$ x 100% was 86.9%

Similarly, the values for infants with congenital syphilis who were asymptomatic at birth were determined. The 2 x 2 contingency table is not shown but the results appear in Table 5.5. It was apparent that the sensitivity of the RF latex test was lower (36.4%) amongst this group of patients.

Total IgM, FTA-ABS (IgM), X-Rays, qualitative VDRL

The values for these tests also appear in Table 5.5. The sensitivity of the RF latex test was less than that of the VDRL, while the former was more sensitive than the FTA-ABS (IgM) test or radiology of the long bones in the diagnosis of congenital syphilis. These differences were not statistically significant ($p > 0,05$ using Fisher's exact test).

TABLE 5.5: EVALUATION OF VARIOUS TESTS (PERFORMED AT BIRTH) IN THE DIAGNOSIS OF CONGENITAL SYPHILIS

	RF LATEX TEST	TOTAL IgM	FTA-ABS (IgM)	X-RAY LONG BONES	POSITIVE VDRL TEST
Sensitivity (%)	46.7 (36.4)	46.7 (27)	26.6 (18)	33.3 (27)	80 (73)
Specificity (%)	100 (100)	91.7 (90)	100 (100)	100 (100)	42 (42)
Positive predictive value (%)	100 (100)	58.3 (37)	100 (100)	100 (100)	29.3 (22)
Negative predictive value (%)	86.4 (88)	85.2 (85)	82.3 (85)	83.6 (86)	87.5 (88)

(Figures in brackets refer to the performance of the tests in patients who were asymptomatic at birth)

The specificities of the various tests were similar, with the exception of the VDRL which was significantly less specific than the RF latex test in the diagnosis of congenital syphilis ($p < 0,01$ using the Chi-square test).

The positive predictive values of the total IgM test and the VDRL were less than that of the RF latex test although only the latter difference was statistically significant ($p < 0,05$ with Fisher's exact test).

The different tests had comparable negative predictive values.

When the tests were evaluated in the asymptomatic infants, the sensitivities were lower and the positive predictive values of the total IgM test and the VDRL were also reduced (Table 5.5).

VDRL titre results

The mothers' VDRL titres were generally higher in those infants who developed congenital syphilis (Figure 5.2).

In the group of infants whose outcome is known the diagnostic value of a maternal VDRL titre of 1:32 or above can be construed from the following 2 x 2 table (Table 5.6).

TABLE 5.6

DIAGNOSTIC VALUE OF MATERNAL VDRL TITRE IN CONGENITAL SYPHILIS

	Infant Affected		Infant not Affected		
VDRL 1/32 or more	14	a	b	12	26 a+b
VDRL 1/16 or less	1	c	d	41	42 c+d
	15	a+c	b+d	53	68 a+b+c+d

The sensitivity was 93.3%, specificity 80.4%, positive predictive value 58.3% and negative predictive value 97.6%.

Similarly, for infants with VDRL titres of 1:16 or more, the results are shown in Table 5.7. (The total number of infants is 64 as not all infants had known VDRL titres at birth)

TABLE 5.7

DIAGNOSTIC VALUE OF INFANTS VDRL TITRE IN CONGENITAL SYPHILIS

	Infant Affected		Infant not Affected		
VDRL 1:16 or more	10	a	b	3	13 a+b
VDRL 1:8 or below	5	c	d	46	51 c+d
	15	a+c	b+d	49	64 a+b+c+d

The sensitivity was 66.7%, specificity 93.9%, positive predictive value 76.9% and negative predictive value 90.2%

Costs

The costs of the various tests were as follows:

RF latex test	R0.67
Total IgM test	R4.66
Radiography of long bones	R0.75
FTA-ABS (IgM) test	R5.00
VDRL test	R0.01

5.3 DISCUSSION

In this prospective study various tests used in the diagnosis of congenital syphilis were compared. The aim was to ascertain the usefulness of the RF latex test in a group of high-risk newborns.

5.3.1 Usefulness of the RF Latex Test

Sensitivity

The overall sensitivity of the RF latex test (defined as its ability to be positive in persons with the disease) was 46.7% (see Table 5.4)

Symptomatic infants

Considering only the 4 symptomatic cases, the sensitivity was 75%. This figure is within the range reported for other studies. Reimer et al (1975) detected RF in 26 (96.3%) of 27 cases, while Meyer and Malan (1987) reported positive RF latex tests in all 14 infants with clinical signs of the disease. Dobson et al (1988a) found RF in 3 (50%) of 6 symptomatic infants and Pedersen et al (1989) detected RF in 7 of 9 such patients. Whilst all the studies report on infants with congenital syphilis, not all the patients were newborns. The ages of the subjects and therefore the results may not be comparable. Evidence in support of age-related changes in RF levels is presented in Chapter 9.

Nevertheless, it is interesting to note that one of the symptomatic infants in the present study had no detectable RF at the time of diagnosis. This is in contrast to the 100% positivity found in the pilot study (Meyer and Malan 1987). The reason(s) for the negative result are unknown. Hidden RF (i.e. RF complexed to serum IgG and therefore not demonstrable in the latex test) was not detected (see Chapter 9). Overwhelming infection has also been suggested as an explanation for the lack of an IgM response in infants with congenital syphilis (Ackerman 1969). The case in question, however, did not have severe life threatening disease and had an elevated total IgM level. Another explanation is that RF was produced, but became deposited in the tissues along with immune complexes. Tissue deposition of RF has been shown to occur in the kidney (Ford and Kosatka 1982), and it has been postulated that it could deplete the serum of free RF (Rossen et al 1977). These aspects of the RF response in infants with congenital syphilis are further discussed in Chapter 10.

Asymptomatic cases

The RF latex test was positive in only 4 of the 11 infants without definite clinical signs of congenital syphilis (sensitivity 36.4%).

Except for the VDRL tests, however, the other tests did not have a better sensitivity. This is disturbing as it is in the group of infants without clinical signs of disease that a sensitive diagnostic test is most needed (Kaufman et al 1974). The reason(s) for the low sensitivity of the RF latex test in this setting are unknown. It is likely, however, that some of these infants were infected late in pregnancy and that their disease was therefore of short duration. The absence of radiographic features of disease in 8 of the infants who lacked definite signs of congenital syphilis would be in keeping with this as bony lesions are usually apparent after approximately 5 weeks (Ingraham 1936). Two infants had negative VDRL tests at birth and this also suggests late infection (Boissiere 1967; Sparling 1971).

It has even been described that infants may become infected during delivery (Stokes et al 1945 pg 1094). This is a rare event, however, and was considered unlikely in view of the absence of a primary site of innoculation being found in the asymptomatic cases in this study.

Rheumatoid factor production can occur within 2 weeks in certain infectious diseases (Barfeld 1968), although work in patients with subacute bacterial endocarditis has shown that RF is more likely to be present the longer the duration of infection (Messner et al 1968; Bacon et al 1974). In additon, RF levels appear to be directly related to the antigenic load (Carson et al 1978). The explanation for the absence of RF in some of the clinically well infants with congenital syphilis may, therefore, be recent infection and/or the presence of a low antigen load.

Attempts were made to improve the sensitivity of the tests used in the present study to detect RF. The manufacturers recommend that a dilution of 1:40 be used for the RF latex test. In this

study, the test was performed at dilutions of 1:5 and 1:40 (at a dilution of 1:5, a serum concentration of 4 IU/ml of RF would be detectable). There was no loss of specificity using the 1:5 dilution when carrying out the tests on newborns. In addition, 2 cases (nos. 5.6 and 5.10) had positive RF latex tests at a dilution of 1:20 but negative tests at 1:40. The recommended cut-off levels for adults may therefore not apply in newborns.

Borobio et al (1980), using a commercial latex kit at a dilution of 1:25 reported negative RF tests in all 9 patients with suspected congenital syphilis. Of the cases described 2 were definitely asymptomatic and another 2 presumably asymptomatic at the time of testing. It would be interesting to know if the latex tests were repeated at other dilutions.

Of the 26 patients with congenital syphilis described by Reimer et al (1975) who had positive RF latex tests, only 12/26 (46%) had positive tests at a dilution greater than 1:32.

The rationale for performing the RF latex test at a dilution of 1:40 as well as at 1:5 in the present study was to eliminate any prozone effect.

The RF ELISA described in Chapter 8 was able to detect 0,025 - 0,05 IU/ml of RF. Using this more sensitive test, RF was detected in 9 of the 15 infants with congenital syphilis (sensitivity 60%). These results are presented in more detail in Chapter 9. The apparent improvement in sensitivity was not statistically significant (Fisher's exact test $p > 0,05$) and was at the expense of using a more complicated test. Workers in other fields have not found a meaningful advantage when using the RF ELISA to detect RF compared to the more conventional RF latex test (Stone et al 1987). This is further discussed at the end of this Chapter.

Specificity

The specificity of the RF latex test performed at birth was 100%. That is, all the infants in the high-risk group who did not have congenital syphilis had negative RF tests at birth. No

previously reported study has determined the specificity of the RF latex test in congenital syphilis. It is known, however, that RF is produced in other congenital infections such as rubella, CMV and toxoplasmosis (Reimer et al 1975; Hyde et al 1975; Stagno et al 1980). One would expect, therefore, that if a large enough population were tested, congenital infections other than syphilis would be detected using the RF latex test. Although the incidence of congenital CMV in the population studied is unknown, similar populations elsewhere have a 1.5% incidence (Keen 1989).

Forty two percent of infants with congenital CMV have been shown to have positive RF latex tests (Griffiths et al 1982). Therefore, the 100% specificity found in the present study is almost certainly an overestimation due to the relatively small size of the high-risk group.

Indeed, in a developed country, such as Britain, there is little congenital syphilis and proportionately more CMV (Peckham et al 1983; Adler 1984). Using the RF latex test to diagnose congenital syphilis in such a situation would be likely to lead to many false positives.

Less is known about the presence of RF in other congenital infections such as that caused by HIV.

The cases with positive RF latex tests in the study had other strong supporting evidence for congenital syphilis e.g. positive maternal serology, characteristic radiological abnormalities, positive FTA-ABS (IgM) tests after RF removal or diagnostically rising VDRL titres. It is highly unlikely that any of the infants did not have congenital syphilis.

Positive predictive value

The positive predictive value of the RF latex test was also 100% in the study. For reasons similar to those mentioned concerning the specificity of the test, the positive predictive value would be expected to be lower if infants with other congenital infections were present in the population studied.

Nevertheless, it appears that the false-positive rate of the RF latex test in well infants at birth is low. In this study 53 high-risk newborns and all 85 controls had negative tests. Stagno et al (1980) found negative tests in 676 well newborns. In the study reported by Meyer and Malan (1987), 80 neonatal controls had negative tests.

In the current study, however, RF was detected in well infants outside the neonatal period. Thus 5/51 (9.8%) of the infants who did not have congenital syphilis developed positive RF tests at follow-up between 4 weeks and 4 months. Similarly, Stagno et al (1980) found that 12-14% of well infants had positive RF latex test between 1 and 3 months of age. Reimer et al (1975) found positive tests in 6 (4.3% of 42) well infants; their age is not stated. The possible reasons for the presence of RF outside the neonatal period are discussed in Chapter 9.

Negative predictive value

The negative predictive value of the RF latex test was 86.6%. This value would change depending on the prevalence of congenital syphilis in the population studied. The intention in the present study was to apply the test to a defined group of high-risk newborn infants. In this group the prevalence of disease was 22.7%. Studying a similar group, Larsson and Larsson (1970) obtained a prevalence of 21%, whilst Srinivasan et al (1983) reported a figure of 12,7%. The prevalence of congenital syphilis in the present study and in the studies quoted above was lower than the figure of 40% for infants of mothers with untreated early latent syphilis reported by Fiumara (1975).

If the RF latex test was applied to a group of infants with a lower prevalence of disease the negative predictive value would increase. For example, Delport et al (1989) reported that the prevalence of congenital syphilis in Cape Town was 0.05%. It is known that 7.6% of antenatal clinic attenders had positive serological tests for syphilis (Gonin 1985) and that there were 25 030 live births in 1987 (van Coeverden de Groot and van der Elst 1987). If one assumes that 12 infants with congenital

syphilis (0.05% of 25 030) were born to the 1902 (7.6% of 25 030) expectant mothers with positive serology the negative predictive value of the test is:

$$\frac{1902 \times 100}{(1902 + 6)} = 99.7\%$$

This calculation assumes that the sensitivity of RF latex test is unchanged and that there is no increase in false positives. The calculation is in fact incorrect because:

i. the group of mothers at risk is increased by the 5% or so who are unbooked

ii. the incidence of congenital syphilis may be higher than that quoted as asyptomatic infants were not followed-up to determine outcome (Meyer 1990).

5.3.2 Total IgM

Sensitivity

The overall sensitivity of the total IgM test (46.7%) was the same as that of the RF latex test.

Symptomatic infants

Measuring total IgM levels was more sensitive in identifying infants with clinical signs of congenital syphilis 4/4 (100%), than those without signs (3/11 or 27.3%).

Other studies report similar figures for symptomatic infants e.g
Tan (1973) found raised levels in 5/6 cases: 83.3%

Srinivasan *et al* (1983) found raised levels in 4/4 cases: 100%

Ewing *et al* (1985) found raised levels in 3/3 cases: 100%

Malan (1987 pg 628) found raised levels in 17/18 cases: 94.4%

Meyer & Malan (1987) found raised levels in 10/12 cases: 83.3%.

Asymptomatic cases

The sensitivity of the total IgM estimation in asymptomatic infants was low. It is difficult to compare this figure to those of other studies reporting IgM levels in asymptomatic infants because the final diagnosis in these studies was not based on an acceptable 'gold standard' (Mamunes et al 1970, Borobio et al 1980; Srinivasan et al 1983) . In these studies infants were treated before the outcome was known and therefore the value of the test could not be determined. Alford et al (1969b) found normal IgM levels at birth in 2 infants who subsequently developed congenital syphilis.

Specificity

The specificity of the total IgM test in the diagnosis of congenital syphilis was 92%. This was somewhat lower than that of the RF latex test (100%). The presence of raised IgM levels in 5/53 (9.4%) of the group without congenital syphilis was responsible for the lower specificity.

Increased IgM levels were also present in 3/85 (3.5%) of the controls. None of the total of 8 infants with elevated levels had congenital syphilis although in the case of one infant (no. 5.33) the mother had been treated, so it is possible that the infant had been treated in utero and the raised IgM level was part of the immune response. This seems unlikely, however, as there were no other features of infection and the radiological examination of the long bones was normal.

The presence of other infections in the infants with raised IgM levels could not be totally excluded. All 8 infants had negative IgM antibody tests for rubella, toxoplasmosis and CMV. The presence of negative tests does not completely exclude these diagnoses, however (Stagno et al 1980; Griffiths et al 1982; Keen 1985).

Virus isolation from urine culture is a more useful method for the diagnosis of CMV (reviewed by Keen 1985) and negative results were obtained in 3 patients. Attempts were made to

obtain urine samples in the other 5 patients, but problems were encountered. Owing to bed shortages in the hospital, these well babies had been discharged by the time the IgM result was obtained. Several patients did not attend early follow-up as requested. Urine was not always obtainable after a reasonable wait during the outpatient visit. Urine leakage and faecal contamination were also encountered.

One of the infants with an elevated IgM level (no. 5.33) had transient non-specific signs of an infection but the work-up did not demonstrate any organisms.

Other studies have also demonstrated raised IgM levels in apparently well infants. Stiehm et al (1966) found increased levels in 7 (5.4%) of 129 cord sera from newborns whose mothers had had normal pregnancies. McCracken et al (1969) detected elevated cord IgM levels in 10% of 2600 controls. Stagno et al (1980) tested 676 cord sera whilst screening for sub-clinical CMV infection. Twenty one (3.1%) of the unaffected infants had raised concentrations of IgM.

The fact that positive RF tests were not obtained in the 8 cases with high amounts of IgM in the current study may reflect the absence of infection.

On the other hand, if early infection were present there may have been time to produce IgM (which can appear after 5 days - Alford et al 1967) but not RF.

Positive predictive value

The positive predictive value of the total IgM test (58%) was lower than that of the RF latex test, although this difference was not statistically significant ($p > 0.05$ using Fisher's exact test) The finding of elevated levels of IgM in infants who did not have congenital syphilis was responsible for this. Five of 51 controls had raised total IgM concentrations compared to none with positive RF latex tests. The higher number of false positive IgM tests was not, however, statistically significant ($p > 0,05$ using Fisher's exact test).

Negative predictive value

The negative predictive value of the total IgM test (85.7%) was similar to that of the RF latex test (86.9%).

5.3.3 Fluorescent Treponemal Antibody Absorption Test

The performance of the FTA-ABS (IgM) test will now be considered.

Sensitivity

The overall sensitivity of the test was 26.6%. This was apparently lower than that of the RF latex test (46.7%) but this difference was not statistically significant ($p > 0.05$ using Fisher's exact test). Considering symptomatic cases only, the sensitivity of the fluorescent IgM test was 50%. Kaufman et al (1974) pooled the data from several studies. They found the sensitivity of the test in symptomatic infants to be 88.4%. However, infants were included if they developed signs of disease at any time within the first month of life. Thus, the age of the infants studied may not be comparable to those of the present study. Furthermore, the largest study considered by Kaufman (that of Scotti et al 1969), does not supply information as to when the symptoms became apparent.

Rosen and Richardson (1975) found positive FTA-ABS (IgM) tests in all 33 infants who were symptomatic with congenital syphilis within the first week of life.

Borobio et al (1980) reported positive tests in 5/5 symptomatic newborns at birth. In a previous study from Groote Schuur Hospital, Meyer and Malan (1987) determined that sera from 12/12 infected newborns were reactive in the FTA-ABS (IgM) test.

The sensitivity of the FTA-ABS (IgM) test in the asymptomatic infants in the present study (2/11 or 18.2%) was also considerably lower than the 65% sensitivity for cases with delayed onset disease reported by Kaufman et al (1974).

Rosen and Richardson (1975) found positive tests in 3/3 newborns in a similar category.

One possible reason for the apparently low sensitivity in the present study is that the method of performing the tests differed.

Subsequent to the work cited above, it has become commonplace for steps to be taken to prevent RF interference prior to testing for specific treponemal IgM (Cerny et al 1985; Muller et al 1987). Rheumatoid factor may, in fact, have been responsible for some of the positive FTA-ABS (IgM) tests (Reimer et al 1975). Therefore, RF removal could have reduced the sensitivity of the test. This hypothesis was investigated in Chapter 7. It was found that if measures to remove RF were not employed, the FTA-ABS (IgM) test was positive in 60% of asymptomatic infants.

The specificity of the FTA-ABS (IgM) test was 100% in the study. That is to say, there were no false positive tests. Kaufman et al (1974) reported 10% false positives. This difference could be attributed to the presence of RF. The present study considered only newborn infants; there were no positive RF latex tests in the newborn controls. Conversely, approximately 10% of the older controls had positive RF latex tests at follow-up. As the studies reported by Kaufman et al (1974) considered older infants, RF may have been present in uninfected infants and have resulted in a positive specific IgM test (Reimer et al 1975).

The positive predictive value of the FTA-ABS (IgM) test was 100%. This would imply that the investigation may be a useful confirmatory test. Indeed, Kaufman et al (1974) suggested this but noted that there had been few studies which have looked at results in infants with similar clinical signs who did not have congenital syphilis. This is further explored in Chapter 6.

The negative predictive value (82.8%) was similar to that of the other tests evaluated.

5.3.4 Radiography

Radiological examination of the long bones was less sensitive (33.3%) than the RF latex test (46.7%). However, this difference was not statistically significant ($p > 0.05$ using Fisher's exact test). As with the other tests, symptomatic infants were more likely to have abnormal films (2/4 or 50%) than asymptomatic ones (3/11 or 27.3%). Higher sensitivity of radiological examination in infants with clinical signs has been previously reported e.g. Platou 1949 and Hira et al (1985) found sensitivities of 88 and 95% respectively. However, the sensitivity in asymptomatic infants has not been previously reported.

The specificity of the roentgenograms was high (100%). All the infants with bony involvement had metaphyseal dystrophy or an 'osteitis-like' dystrophy. Rarely, other infections, including septicaemia have been noted to give similar radiological changes. However, septicaemic infants without signs of congenital syphilis were not included in the high-risk group as they received antibiotics at birth and were excluded from the study. The presence of a periosteal reaction alone would be expected to have a lower specificity as such changes have been reported in normal infants (Malmberg 1944; Shopfner 1966).

The positive and negative predictive values of the radiological examination of long bones did not differ significantly from those of the RF latex test.

5.3.5 VDRL

The usefulness of the VDRL test to diagnose congenital syphilis was also determined in the study.

Qualitative results

Considering merely qualitative results, the sensitivity of a positive test in infants with congenital syphilis was higher than that of the RF latex test (80% compared to 46,7%). This difference was not statistically significant ($p > 0,05$ using Fisher's exact test).

The Centers for Disease Control (CDC 1986) have also reported a high sensitivity for the VDRL test in congenital syphilis (95%).

The specificity of a positive VDRL result at birth was low (41.5%). This is presumably because of passive transfer of reagin antibodies from the mother to the fetus in the absence of infection. For similar reasons, the positive predictive value of the VDRL was only 28%. The specificity and positive predictive values of the RF latex test were significantly better than those of the VDRL ($p < 0,05$ using the Chi-square test and Fisher's exact test respectively). These results confirm the well-documented problem with the VDRL test, namely its inability to distinguish between active infection and passive transfer of maternal antibodies (Stokes et al 1945 pg 1096-1099; Brown and Moore 1963). It was for this reason that better tests were sought in the first place (Scotti and Logan 1968). The negative predictive value was 87.5%, confirming that a negative test cannot completely exclude congenital syphilis (Ingall and Musher 1983 pg 365).

Quantitative results

Infants' VDRL titres

The median VDRL titre of infants in the infected group in the study (1:16; range 0-1:256) was significantly higher than that from the non-infected group (1:1; range 0-1:128). The p value was 0.033 using the median test.

Srinivasan et al (1983) also found higher reagin antibody titres amongst infected infants. Fifty eight of 61 unaffected infants born to mothers with untreated or inadequately treated syphilis had RPR titres below 1:4. Conversely the 4 infants who definitely had congenital syphilis had RPR titres of 1:8 or above.

Mascola et al (1985) found that 66% of infected infants had a VDRL titre of 1:16 or above. Unfortunately, there was no control group with which to compare these titres. Three of 5 infants with congenital syphilis described by Alford et al

(1969a) had VDRL titres at birth of 1:16 or above.

Maternal VDRL titres

From this data, it seems that infants born to mothers with syphilis with higher VDRL titres may be more likely to have congenital syphilis. A possible explanation for this is that the maternal VDRL titre in untreated cases rises to a peak at 1 year after infection and then gradually declines (Hart 1986). Although high titres do not always indicate early infection (Felman and Nikitas 1980), they may be found in the stage of secondary syphilis, a period during which the disease is highly infectious for the infant (Fiumara et al 1952).

Consideration of the maternal VDRL titres in the current study would appear to support this. Figure 5.2 shows that 14/15 of the mothers with affected babies had VDRL titres above 1:16. Indeed, the sensitivity of a maternal VDRL titre of this level was 93.3% with a negative predictive value of 97.6%. The sensitivity was significantly higher than that of the RF latex test ($p < 0,05$ with Fisher's exact test).

Mascola et al (1985) found that the mean maternal VDRL titre amongst 50 mothers with infected babies was 1:64.

Srinivasan et al (1983) do not give the maternal RPR titres in the group of high-risk unaffected infants they studied. However, 3/4 mothers whose infants had congenital syphilis had RPR titres of 1:32 more. In another study the VDRL titres were known in 4 mothers whose infants were infected (Alford et al 1969a). All 4 mothers had titres of 1:16 or above.

Therefore, there appears to be an increased risk of congenital syphilis with higher VDRL titres. However, in the current study as well as those cited not all mothers whose infants developed the disease had high titres of reagin antibodies. In addition, it should be noted that serological testing in the mothers was performed in the last trimester and often at the time of delivery. Whether a VDRL titre obtained earlier in pregnancy would be as useful is unknown. Finally, two-fold changes in end

point dilutions are accepted as within the limits of reproducibility of the VDRL test (Ingall and Musher 1983 pg 358).

The data does suggest, however, that measuring the VDRL titre is useful in the diagnosis of congenital syphilis and that other quantitative methods of detecting reaginic antibodies, such as a VDRL ELISA, would be worth evaluating.

5.4 RECOMMENDATIONS AND CONCLUSIONS

Some final comments can be made on the basis of the data obtained.

None of the tests performed on high-risk infants or their mothers have sufficient sensitivity at birth to completely rule out a diagnosis of congenital syphilis. The fact that the disease is treatable means that a test with 100% sensitivity is needed. As adverse effects would be unlikely if unaffected infants were treated, a lower test specificity would be acceptable.

The failure of the tests evaluated to meet these requirements means that in the absence of complete maternal treatment, the infant must either be followed-up for a period of time or treated. The latter option is suggested by the Centers for Disease Control (CDC 1988c).

If one considers the implications of this for a city such as Cape Town, one realizes that there are logistical problems. A study by Gonin (1985) indicated that 7.6% of pregnant women attending the antenatal clinics of the PMNS had positive serological tests for syphilis. Applying this percentage to the 25 030 live-born infants in 1987 one obtains a figure of 1902 (i.e. 1902 infants were born to mothers with positive STS). Previous studies indicate that only a percentage (approximately 40% at most) of these mothers will have been fully treated for syphilis antenatally (Manning et al 1985, Subotsky and Delport 1988). This means that 60% of the newborns (1141 in the example above) will need treatment with 10 days of procaine penicillin.

Most of these patients would be treated as outpatients which could be problematic as, in all likelihood, infants with congenital syphilis would default treatment, necessitating time-consuming patient tracing. This state of affairs would best be avoided in the local situation where health facilities are already faced with coping with high morbidity and mortality rates due to conditions such as diarrhoeal disease, tuberculosis and measles (Yach et al 1989; Molteno et al 1989). Admittedly, however, the latter diseases are preventable and the circumstances may improve in time.

The option of following-up the high-risk cases also needs to be considered. The feasibility and cost of this procedure are difficult to estimate. It is noteworthy, however, that 72% of seropositive infants defaulted a follow-up programme in Durban (Naicker et al 1983). This implies that tracing of patients is likely to be necessary. In the present study the cost of this (in terms of the salary and travelling expenses of a community worker) was approximately R12 000 for 69 patients (or R173 per patient). Applying this figure to the 1141 newborns who may require treatment, the cost of follow-up may be R197 393. In fact, this could be an underestimate as the mothers in the present study were selected on the basis of residence at contactable Cape Town addresses. An unselected population would presumably be more difficult to trace.

The maternal VDRL titre appeared to have the highest sensitivity of the tests evaluated and is relatively inexpensive. How useful would the test be in practice?

There are a number of points to be considered:

i. The VDRL titres were obtained in the last trimester of pregnancy; in over 90% of cases in the last month. This means that, in most cases, the test would need to be performed between the mother's admission to labour ward and the infant's discharge.

ii. The VDRL tube test was used in the present study. This test requires that VDRL antigen is prepared fresh daily, that prior heat-inactivation of the serum is performed and that strict quality control measures are practiced (Larsen et al 1984 pg 876-880).Such tests are best carried out in a central reference laboratory (WHO 1986). This may be particularly important as patient management would depend on the reagin titre.

The main disadvantage of measuring the maternal VDRL titre is that testing at a central laboratory is necessary. Now, populations where syphilis is common often have less well developed health-care systems (Ratnam et al 1982). In such situations there is likely to be a demand on hospital beds for maternity patients. Infants at risk of having congenital syphilis (e.g. those born to unbooked mothers or whose mothers have untreated syphilis) may be discharged within 1 or 2 days of delivery. Serological results may be unavailable by this time. A further disadvantage is the relatively low positive predictive value of the maternal VDRL titre (58%).

Other measures of reaginic antibody with sensitivities and specificities similar to those of the VDRL flocculation tests are available (Larsen et al 1984 pg 876). Of these the RPR card test, although more expensive than the VDRL test (R0.75 compared to R0.01), is suitable for use in peripheral laboratories (WHO 1986). The mechanical rotator necessary for the test would need to be obtained (approximate cost R2000). The main problem with using the card test as a side-room or peripheral laboratory test is that the actual reagin titre is required and not simply a positive or negative result. Wide variations in titre have been reported between peripheral and central laboratories (Delport 1988).More accurate methods of quantitating the reagin response of the mother (e.g. the VDRL ELISA described by Pedersen et al 1987) would appear to be desirable.

Apart from the delay in obtaining results, measurement of the maternal VDRL titre may be important in the management of high-risk infants. Infants born to mothers with VDRL titres of 1:32

or above could be treated or followed-up. The risk of missing affected infants would only be 7% if this were done. The advantage would be that the number of infants requiring further management would be greatly decreased. This can be seen from the fact that 26% of antenatal patients with positive VDRL tests had titres of 1:32 or more (Gonin 1985). Assuming that 40% of these patients were treated (Manning et al 1985; Subotsky and Delport 1988), a total of 179 infants would require follow-up and/or treatment (compared to 1141 if some form of selection were not attempted). These observations apply to the maternal VDRL titre. Somewhat similar interpretations are possible considering the VDRL titre in the infant. Nevertheless, the sensitivity of the latter test in the infants was lower (67%) and the 1-2ml of blood required for VDRL testing is more easily obtained from the mother.

As the RF latex test is simple, can be rapidly performed and has a high positive predictive value (100%), it may have a role to play in patient management. Rheumatoid factor testing could be done as a side-room or peripheral laboratory test prior to discharge. Serum from a heel-prick could be used (Meyer and Malan 1987). The test is relatively cheap (it would cost R764 to test the high-risk cases in the PMNS in the example cited). A positive RF latex test would indicate the need for particularly close evaluation of the infant prior to discharge. The high positive predictive value of the RF latex test obtained in the population studied, means that most of the infants with positive tests would be likely to have congenital syphilis. In this way nearly 50% of the affected infants would be detected prior to discharge.

A negative RF latex test, however, would not exclude the diagnosis of congenital syphilis. In fact, when testing at-risk infants the RF latex test would most often be negative. This can be deduced as follows: the prevalence of congenital syphilis in the high-risk group was 23%. Of the cases of congenital syphilis, 53% would have negative tests. Therefore 77% plus 12% (or a total of 89%) of the high-risk infants would be RF latex test negative.The problem of the further management of these

infants remains if the RF latex test alone is used. The concomitant use of the maternal VDRL titre, however, would be helpful if infants of mothers with VDRL titres of 1:32 or more were followed-up or treated. This would enable the vast majority of the remaining infected infants to be accounted for.

Other tests, such as detecting IgM antibody to a 47 kD antigen by Western blotting are being developed (Dobson et al 1988a; S'anchez et al 1989). Whether these techniques will detect all asymptomatic infants with disease and whether they will be cost-effective tests, remains to be seen.

In the meantime, the only definite way of not missing patients with congenital syphilis is to follow-up or treat those at risk. The load, this places on the community health services and the attendant risk of failure could be reduced by doing 2 relatively cheap and simple tests at the time of birth. A positive RF latex test would select out nearly half of the infants who need treatment. The VDRL titre obtained on the mothers of at-risk patients with negative RF latex tests would indicate which infants should undergo further follow-up or treatment.

CHAPTER 6

THE VALUE OF THE RF LATEX TEST IN THE DIAGNOSIS OF CONGENITAL SYPHILIS IN SYMPTOMATIC INFANTS

The diagnosis of congenital syphilis in infants with classical clinical signs may not be difficult, provided one is aware of the possibility (Kaufman et al 1974; Mascola et al 1985). Owing to the varied manifestations of the disease, however, serological testing is employed to confirm or refute the diagnosis (Borobio et al 1980; Krugman and Katz 1981 pg 398; Rathbun 1983a). As stated in the criteria of Kaufman et al (1977), the diagnosis of congenital syphilis is unlikely in the presence of negative serological tests for syphilis. In fact, these workers never intended their criteria to be used in the absence of serological testing. Most of the tests employed take several days before the results are available. The RF latex test, however, can be rapidly performed on a small volume of serum and positive tests have been obtained in a high percentage of symptomatic infants with congenital syphilis (Reimer et al 1975; Meyer and Malan 1987).

The aim of this part of the study was to determine if the RF latex test could be used to distinguish infants with congenital syphilis from those presenting with similar clinical signs.

6.1 METHODS

6.1.1 Study design

The clinical signs of congenital syphilis are many (Krugman and Katz 1981 pg 396). However, the diagnosis is likely to be considered when certain physical findings are present (Alford et al 1975). Kaufman et al (1977) described clinical features which were useful pointers to the presence of congenital syphilis. These findings were:

- condylomata lata
- osteochondritis, periostitis

- snuffles or haemorrhagic rhinitis
- fissures of lips
- cutaneous lesions
- mucous patches
- hepatomegaly, splenomegaly
- generalized lymphadenopathy
- central nervous system signs
- haemolytic anaemia
- elevated cells or protein in the CSF.

The bony abnormalities associated with congenital syphilis are usually detected radiologically and not clinically (Narbarro 1951; Wilkinson and Heller 1971). Nevertheless, most authors describing criteria for the diagnosis of congenital syphilis have considered roentgenographic findings amongst the clinical criteria (Rathbun 1983b; Mascola et al 1985; CDC 1988c). This categorization was also used for this study.

Kaufman et al (1977) did not specify the exact cutaneous lesions referred to. For the purposes of this study, typical dermatological manifestations were regarded as being:

i. vesicular bullous eruptions

ii. superficial desquamation of the palms and soles

iii. an oval, symmetrical, hypopigmented maculopapular eruption affecting the buttocks, back, genitalia and extremities (King and Nicol 1975 pg 109; Chawla et al 1988).

Hepatomegaly was regarded as an enlargement of the organ of more than 2 cm below the right subcostal margin in the mid-clavicular line in newborns and 1 cm below the rib margin in older infants (Nelson et al 1979 pg 1110). Splenomegaly was said to be present if the inferior margin was more than 1 cm below the left

subcostal margin (Gandy and Robertson 1987 pg 56).

The central nervous system signs were not elaborated by Kaufman et al (1977). As reviewed by Ingall and Musher (1983 pg 348), however, pathological features in the central nervouse system in infants with early congenital syphilis are:

i. acute syphilitic leptomeningitis which presents with signs of meningitis and/or raised intracranial pressure with an abnormal cerebrospinal fluid, and a positive serological test for syphilis

ii. chronic meningovascular syphilis with hydrocephalus, cranial nerve involvement and vascular lesions

iii. cerebral infarction with acute hemiplegia and convulsions.

In considering the usefulness of these features in the diagnosis of congenital syphilis, Ingall and Musher (1983 pg 347) stated that other common conditions need to be exclude. For practical purposes infants presenting with only a CSF picture of aseptic meningitis (Nelson et al 1979 pg 727) or isolated CSF changes were not considered for the study unless the STS were known to be positive.

Haemolytic anaemia in newborns is often due to blood group incompatibility (Oski 1981 pg 551). Congenital syphilis usually forms part of the differential diagnosis when a patient has unexplained haemolytic anaemia (Ingall and Musher 1983 pg 347). For this reason, cases with haemolytic anaemia where the cause was readily apparent were not considered for the study.

Infants with one or more of the clinical criteria listed below were included in the study:

- condylomata lata
- metaphyseal dystrophy, periosteal dystrophy
- snuffles or haemorrhagic rhinitis
- fissures of lips

- cutaneous lesions
- mucous patches
- hepatomegaly, splenomegaly
- generalized lymphadenopathy
- unexplained hydrocephalus with cranial nerve involvement
- unexplained cerebral infarction with acute hemiplegia
- unexplained haemolytic anaemia.

Study infants consisted of newborn infants delivered in the PMNS and infants presenting to the Red Cross War Memorial Children's Hospital (RCWMCH) under the age of 4 months.

Doctors working in the PMNS or the admitting room at the RCWMCH were asked to inform the author of the arrival of patients meeting the criteria previously laid down. Frequent, regular contact helped to ensure that as many cases as possible were included. The ward admission records were also frequently checked.

Infants were entered into the study before the final diagnosis was known. They were examined by the author and their findings recorded.

The diagnosis of congenital syphilis was based on the criteria suggested by Kaufman et al (1977). As previously stated on page 48, however, these criteria require that serological testing be carried out. This point should be stressed as the diagnosis of probable congenital syphilis on the basis of 1 'major' and 1 'minor' clinical sign can only be made in the presence of positive serological tests for syphilis and not in isolation (see point 5 in the section 'Certainty of diagnosis' in Table 2.1 on page 54).

The clinical case study and the study of high-risk infants described in Chapter 5 were carried out at the same time. To avoid confusion, infants from Groote Schuur Hospital (GSH) were

not considered for the clinical case study whilst the study of high-risk cases was in progress.

6.1.2 Controls

Infants who did not have congenital syphilis but who were included in the high risk study (Chapter 5) because their mothers had positive serological tests for syphilis were used as controls. As described in Chapter 5, the infants were seen at birth, 6 weeks and 4 months.

Apart from serological tests for syphilis, RF and total IgM were determined. Radiological examination of the long bones was also undertaken at birth.

6.1.3 Investigations

After obtaining informed parental consent, the following investigations were performed.

Infants with clinical signs

Venous blood was drawn within 48 hours of presentation. The blood was collected in sterile glass tubes and allowed to clot at room temperature. The serum obtained was either tested immediately or aliquoted and stored at -70°C. The following tests were performed on each infant: VDRL, TPHA, RF latex test, RF ELISA and total IgM levels. Roentgenograms of the long bones were obtained.

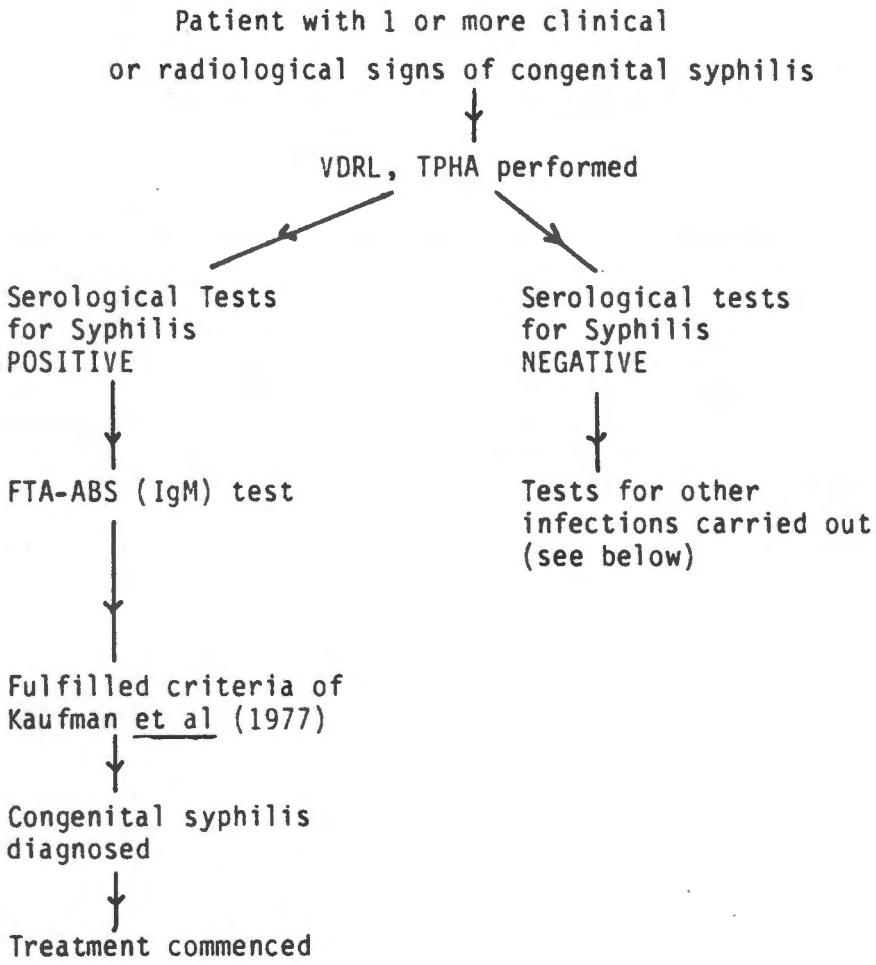
The sequence of investigations was as outlined in Figure 6.1.

The tests for other infections carried out in the cases where STSs were negative, were those that were appropriate for making a diagnosis (e.g. blood culture, serological tests for other intrauterine infections, viral isolation from urine samples).

Control infants

As described in Chapter 5, the VDRL and TPHA tests, RF latex test, total IgM test and radiological examination of long bones

Figure 6.1: Procedure in infants with clinical signs of congenital syphilis



were undertaken at birth in these infants. In addition, with the exception of the roentgenology, the investigations were repeated at the follow-up visits.

Mothers

Serological tests for syphilis (VDRL and TPHA) were performed on the mother's serum obtained as near to the date of delivery as possible.

6.2 RESULTS

A total of 49 patients with one or more clinical signs compatible with congenital syphilis entered the study over an 18 month period. Thirty four patients fulfilled the criteria of Kaufman et al (1977) and were diagnosed as having probable congenital syphilis. A further 15 patients had one or more clinical signs suggestive of congenital syphilis but the serological tests for syphilis (VDRL and TPHA) were negative. Of these patients, 7 had suspected or proven septicaemia, 5 had CMV infection, 1 rubella, 1 hereditary spherocytosis and 1 trisomy 13.

The details for each patient are given in the tables which follow. Tables 6.1 and 6.2 show the data for infants with probable congenital syphilis while Table 6.3 gives the details for patients with other conditions.

TABLE 6.1: CLINICAL AND LABORATORY FINDINGS IN NEWBORN INFANTS WITH CONGENITAL SYPHILIS

*NO.	MATERNAL DATA		INFANT DATA AT BIRTH										
	VDRL TITRE	BOOKED YES/NO	BIRTH WEIGHT (kg)	**GESTATIONAL AGE	CLINICAL FINDINGS	SEX	X-RAY CHANGES	RF LATEX TITRE	RF ELISA IU/ml	TOTAL IgM IU/ml	VDRL TITRE	FTA-ABS (IgM)	COMMENTS, LABORATORY FINDINGS
6.1	1:256	Yes	2.0	36	Preterm, pallor, petechial rash, hepatosplenomegaly, pneumonitis	F	Met	1:320	152	764	1:32	+ve	Died on day 1. Hepatitis, obstructive jaundice, meningitis. PM showed hepatic and pancreatic fibrosis with extra medullary haematopoeisis
6.2	1:256	Yes	2.3	40	SGA, oedema, ruptured bullae palms & soles, hepatosplenomegaly	M	Met	1:80	205	1500	1:32	+ve	Died on day 2. PM not done.
6.3	1:256	No	1:3	33	Preterm, SGA, hepatosplenomegaly, pneumonitis	M	Met	1:320	156	600	1:1024	+ve	Thrombocytopaenia
6.4	1:128	No	1.6	37	SGA, hepatomegaly	M	Met & Per	1:80	90	284	1:512	+ve	

Table 6.1 continued

*NO.	MATERNAL DATA		INFANT DATA AT BIRTH										
	VDRL TITRE	BOOKED YES/NO	BIRTH WEIGHT (kg)	**GESTATIONAL AGE	CLINICAL FINDINGS	SEX	X-RAY CHANGES	RF LATEX TITRE	RF ELISA IU/ml	TOTAL IgM IU/ml	VDRL TITRE	FTA-ABS (IgM)	COMMENTS, LABORATORY FINDINGS
6.5	1:128	No	1.8	36	Preterm, SGA, peeling of palms and soles, hepatosplenomegaly, pneumonitis	M	Met	1:160	92	726	1:256	-ve	Obstructive jaundice, hepatitis. Died aged 3 3 months. PM showed: chronic hepatitis, cerebral infarction, pancreatic fibrosis.
6.6	1:256	Yes	2.0	36	Preterm, SGA, jaundice, hepatosplenomegaly	F	Per	-ve	50	348	1:128	+ve	Obstructive jaundice.
6.7	1:128	No	2.6	40	Well	F	Met	1:80	15	255	1:128	+ve	
6.8	1:128	No	2.2	34	Preterm, jaundice, peeling of skin on palms and soles, hepatosplenomegaly, pneumonitis	F	Met	1:640	27	1124	1:64	-ve	Obstrucive jaundice, hepatitis, thrombocytopaenia.

Table 6.1 continued

*NO.	MATERNAL DATA		INFANT DATA AT BIRTH										
	VDRL TITRE	BOOKED YES/NO	BIRTH WEIGHT (kg)	**GESTATIONAL AGE	CLINICAL FINDINGS	SEX	X-RAY CHANGES	RF LATEX TITRE	RF ELISA IU/ml	TOTAL IgM IU/ml	VDRL TITRE	FTA-ABS (IgM)	COMMENTS, LABORATORY FINDINGS
6.9	1:64	Yes	2.4	35	Preterm, oedema, jaundice, peeling skin palms and soles, hepato-splenomegaly	M	Met	1:80	165	633	1:256	+ve	Obstructive jaundice, TPHA non-specific, FTA-ABS IgG positive.
6.10	1:256	Yes	1.1	29	Preterm, pallor, pneumonitis	M	Met	1:320	32	195	1:8	+ve	Died on day 2. PM: not done.
6.11	1:256	Yes	1.2	33	Preterm, SGA, jaundice, ruptured bullae palms and soles hepatosplenomegaly	M	Met & Per	1:80	11	484	1:128	+ve	Obstructive jaundice, hepatitis, dark-field microscopy of exudate from bullae negative.
6:12	1.128	Yes	2.3	40	SGA, jaundice, peeling rash palms & soles, hepatosplenomegaly	F	Met	1:1280	788	1200	1:128	+ve	Obstructive jaundice, hepatitis, thrombocytopaenia.

Table 6.1 continued

*NO.	MATERNAL DATA		INFANT DATA AT BIRTH										
	VDRL TITRE	BOOKED YES/NO	BIRTH WEIGHT (kg)	**GESTATIONAL AGE	CLINICAL FINDINGS	SEX	X-RAY CHANGES	RF LATEX TITRE	RF ELISA IU/ml	TOTAL IgM IU/ml	VDRL TITRE	FTA-ABS (IgM)	COMMENTS, LABORATORY FINDINGS
6.13	1:256	No	1.5	34	Preterm, SGA, oedema marked, ascites	M	Met	1:160	34	504	1:16	-ve	
6.14	1:128	Yes	1.5	34	Preterm, SGA, jaundice, oedema, peel-skin of palms & soles, hepatosplenomegaly	F	Met	1:160	165	1080	1:2048	+ve	Obstructive jaundice, hepatosplenomegaly, thrombocytopenia.
6.15	1:512	Yes	1.5	34	Preterm,oedema, peeling skin of palms & soles hepatosplenomegaly	F	Met & Per	1:40	21	732	1:256	+ve	Died day 8. Obstructive jaundice, superimposed E.coli septicaemia with meningitis At PM: hepatitis, extramedullary haematopoiesis, pneumonitis.
6.16	1:128	Yes	2.2	37	SGA, oedema hepatosplenomegaly, pneumonia	M	Met	1:320	850	735	1:128	+ve	Thrombocytopaenia, nephritis.

Table 6.1 continued

*NO.	MATERNAL DATA		INFANT DATA AT BIRTH										
	VDRL TITRE	BOOKED YES/NO	BIRTH WEIGHT (kg)	**GESTATIONAL AGE	CLINICAL FINDINGS	SEX	X-RAY CHANGES	RF LATEX TITRE	RF ELISA IU/ml	TOTAL IgM IU/ml	VDRL TITRE	FTA-ABS (IgM)	COMMENTS, LABORATORY FINDINGS
6.17	1:256	Yes	1.4	32	Preterm, petechial rash, hepatosplenomegaly	F	Nil	1:320	3	156	1:16	-ve	Died day 3. Haemolytic anaemia, thrombocytopaenia. No PM done.
6.18	1:32	Yes	2:8	40	Hepatomegaly	M	Met	1:640	24	375	1:128	+ve	
6.19	1:128	No	2.0	35	Preterm, oedema jaundice, pallor, hepatosplenomegaly, ascites	F	Nil	1:80	6	935	1:512	+ve	Obstructive jaundice, thrombocytopaenia, haemolytic anaemia.

* Patient code number
** Gestational age in completed weeks

Abbreviations

F - Female
M - Male
-ve negative
+ve positive
Met - metaphyseal dystrophy
Per - periosteal dystrophy
PM - postmortem
SGA - small for gestational age
w - weeks

TABLE 6.2: CLINICAL AND LABORATORY FINDINGS IN OLDER INFANTS WITH CONGENITAL SYPHILIS

*NO.	MATERNAL DATA		INFANT DATA AT PRESENTATION									
	VDRL TITRE	BOOKED YES/NO	AGE AT PRESENTATION	CLINICAL FINDINGS	SEX	X-RAY CHANGES TITRE	RF LATEX IU/ml	RF ELISA IU/ml	TOTAL IgM	VDRL TITRE (IgM)	FTA-ABS	COMMENTS, LABORATORY FINDINGS
6.20	1:128	Yes	6 w	Marked pallor, hepatosplenomegaly	M	Per	1:80	38	270	1:64	+ve	Thrombocytopaenia
6.21	ND	No	10 w	Arthritis of wrist	F	Per	1:160	30	785	1:512	+ve	Obstructive jaundice, hepatitis, anaemia
6.22	1:16	No	8 w	SGA, failure to thrive, marked pallor, serosanguinous nasal discharge, peeling palms and soles, generalized lymphadenopathy, hepatosplenomegaly, CCF.	F	Met & Per	1:160	520	1380	1:256	+ve	Thrombocytopaenia.
6.23	ND	No	8 w	Snuffles, hepatosplenomegaly	M	Per	-ve	0	340	1:1	+ve	Anaemia. TPHA remained positive for over 1 year following treatment

Table 6.2 continued

*NO.	MATERNAL DATA		INFANT DATA AT PRESENTATION									
	VDRL TITRE	BOOKED YES/NO	AGE AT PRESENTATION	CLINICAL FINDINGS	SEX	X-RAY CHANGES	RF LATEX	RF ELISA	TOTAL IgM	VDRL TITRE	FTA-ABS IgM	COMMENTS, LABORATORY FINDINGS
6.24	1:256	Yes	2 w	SGA, jaundice, hepatomegaly	M	Met	1:160	32	471	1:2048	+ve	Obstructive jaundice, hepatitis, thrombocyto-paenia
6.25	1:32	No	1 w	SGA, oedema, jaundice, hepatomegaly	M	Met	1:160	238	1490	1:256	+ve	Obstructive jaundice, TPHA non-specific, FTA-ABS (IgG) positive.
6.26	1:128	No	16 w	Oedema, eczematous rash, hepatosplenomegaly	M	Met & Per	1:160	500	1117	1:512	+ve	Anaemia, nephrotic syndrome. Renal biopsy: membranous glomerulonephritis with crescents.
6.27	1:8	No	6 w	Pallor, oedema, skin rash, generalized lymphadenopathy, pseudoparesis, hepatospleno megaly.	M	Met & Per	1:80	80	521	1:4	-ve	Obstructive jaundice

Table 6.2 continued

*NO.	MATERNAL DATA		INFANT DATA AT PRESENTATION									
	VDRL TITRE	BOOKED YES/NO	AGE AT PRESENTATION	CLINICAL FINDINGS	SEX	X-RAY CHANGES TITRE	RF LATEX IU/ml	RF ELISA IU/ml	TOTAL IgM	VDRL TITRE (IgM)	FTA-ABS	COMMENTS, LABORATORY FINDINGS
6.28	ND	No	9w	Oedema, pallor, peeling rash palms & soles, hepatosplenomegaly	M	Met & Per	1:160	160	1000	1:256	+ve	
6.29	1:1024	No	4w	Oedema, pallor, symmetrical hypopigmented macular rash, hepatosplenomegaly	F	Nil	1:160	118	1210	1:256	+ve	Obstructive jaundice, hepatitis, thrombocytopaenia
6.30	1:128	No	12w	Snuffles, peeling palms & soles, macular hypopigmented rash, hepatosplenomegaly	M	Per	1:80	38	525	1:256	+ve	Anaemia

Table 6.2 continued

*NO.	MATERNAL DATA		INFANT DATA AT PRESENTATION									
	VDRL TITRE	BOOKED YES/NO	AGE AT PRESENTATION	CLINICAL FINDINGS	SEX	X-RAY CHANGES TITRE	RF LATEX IU/ml	RF ELISA	TOTAL IgM	VDRL TITRE (IgM)	FTA-ABS	COMMENTS, LABORATORY FINDINGS
6.31	1:64	No	6 w	Peeling rash palms & soles, macular hypo-pigmented rash, hepatosplenomegaly	M	Met & Per	1:160	54	227	1:64	+ve	Anaemia
6.32	1:32	No	11 w	Peeling rash palms & soles, macular hypo-pigmented rash, splenomegaly.	M	Met & Per	1:80	6	520	1:16	+ve	Hepatitis, anaemia
6.33	1:128	Yes	2 w	Preterm, SGA, oedema, jaundice peeling of palms & soles, hepato-splenomegaly	M	Met	1:160	96	255	1:128	-ve	Obstructive jaundice, thrombocytopaenia.
6.34	1:4	No	8 w	Bony swelling of forearm, hepato-splenomegaly.	F	Per	1:20	14	675	1:128	+ve	

* - Patient code number

Abbreviations

F - Female
M - Male
CCF - congestive cardiac failure
Met - metaphyseal dystrophy
Per - periosteal dystrophy
PM - postmortem
SGA - small for gestational age
w - weeks

TABLE 6.3: CLINICAL AND LABORATORY FINDINGS IN INFANTS WITH CONDITIONS OTHER THAN CONGENITAL SYPHILIS

*NO	AGE	CLINICAL FINDINGS	X-RAY OF LONG BONES	RF LATEX TITRE	TOTAL IgM (IU/ml)	COMMENTS AND LABORATORY FINDINGS	FINAL DIAGNOSIS
6.35	Birth	Jaundice, petechial rash, microcephaly, hepato-splenomegaly	Metaphyseal dystrophy	1:10	300	Obstructive jaundice CMV isolated from urine	Congenital CMV
6.36	Birth	SGA, splenomegaly	Normal	-ve	223	CMV isolated from urine	Congenital CMV
6.37	Birth	SGA, purpuric rash, cataracts	Metaphyseal dystrophy	1:10	21	Rubella IgM ELISA positive	Congenital rubella
6.38	Birth	Preterm, purpura, hepato-splenomegaly, microce-phaly.	Normal	-ve	22	11 ribs noted on CXR. IgM tests for toxo-plasmosis, rubella, CMV negative. Chromosome analysis abnormal PM. extramedullary hae-atopoeisis, cardiac defect	Trisomy 13
6.39	Birth	Preterm, jaundice, hepatosplenomegaly	Metaphyseal dystrophy	-ve	9	Eschericia coli isolated from blood culture	Septicaemia

Table 6.3 continued

*NO	AGE	CLINICAL FINDINGS	X-RAY OF LONG BONES	RF LATEX TITRE	TOTAL IgM (IU/ml)	COMMENTS AND LABORATORY FINDINGS	FINAL DIAGNOSIS
6.40	Birth	Preterm, hepatosplenomegaly	Normal	-ve	16	Staphylococcus aureus isolated from blood cultures	Septicaemia
6.41	Birth	Oedema, lethargy	Metaphyseal dystrophy	-ve	36	Blood and CSF cultures negative. Tests for IgM antibody to toxoplasmosis, rubella and cytomegalovirus negative. Marked clinical improvement in response to antibiotics	Suspected septicaemia
6.42	Birth	SGA, conjunctivitis, hepatomegaly	Normal	-ve	65	Gonococcal ophthalmia, IgM tests for CMV, rubella, toxoplasmosis, HIV negative, urine CMV negative. Signs resolved after antibiotics.	Suspected septicaemia (gonococcal).
6.43	Birth	Petechial rash, bleeding tendency, splenomegaly	Metaphyseal dystrophy	-ve	126	Thrombocytopaenia and disseminated intravascular coagulation. Eschericia coli isolated from blood cultures.	Septicaemia

Table 6.3 continued

*NO	AGE	CLINICAL FINDINGS	X-RAY OF LONG BONES	RF LATEX TITRE	TOTAL IgM (IU/ml)	COMMENTS AND LABORATORY FINDINGS	FINAL DIAGNOSIS
6.44	5 wks	Hepatosplenomegaly	Normal	-ve	223	Urinary tract infection. Tests for toxoplasmosis, rubella, cytomegalovirus negative. Eschericia coli isolated from blood cultures.	Septicaemia
6.45	6 wks	Eczematous rash. Irritable.	Metaphyseal dystrophy Periosteal reaction	-ve	126	Thrombocytopaenia. IgM tests for CMV, toxoplasmosis, rubella negative. Blood cultures negative. Clinical improvement and resolution of low platelet count on antibiotics.	Suspected septicaemia
6.46	6 wks	Hepatosplenomegaly	Normal	-ve	89	Thrombocytopaenia. CMV isolated from urine.	CMV infection.
6.47	4 mo	Pallor, hepatosplenomegaly	Periosteal dystrophy	1.80	ND	CMV isolated from urine.	CMV infection
6.48	4 mo	Hepatosplenomegaly	Periosteal dystrophy	-ve	305	IgM ELISA for CMV positive. Tests for rubella, toxoplasmosis negative.	CMV infection

Table 6.3 continued

*NO	AGE	CLINICAL FINDINGS	X-RAY OF LONG BONES	RF LATEX TITRE	TOTAL IgM (IU/ml)	COMMENTS AND LABORATORY FINDINGS	FINAL DIAGNOSIS
6.49	3 mo	Pallor. Hepatospleno-megaly.	Periosteal dystrophy	-ve	120	Haemolytic anaemia Osmotic fragility increased.	Hereditary spherocytosis

* Patient code no

Abbreviations

CMV - Cytomegaloviurs
ELISA - Enzyme-linked immunosorbent assay
ND - Not done
SGA - Small for gestational age
-ve - Negative

6.2.1 Infants with congenital syphilis

Maternal characteristics

The mothers of 31 of the 34 infants in this group had positive STSs for syphilis (the remaining 3 delivered in outlying areas and did not accompany their infants to hospital). The serological tests of the mothers were obtained either at the time of presentation or within the last trimester of pregnancy. None of the women were symptomatic. Twenty eight (90%) of the 31 mothers with known VDRL results had titres of 1:32 or above.

Nineteen (56%) of the women whose infants had probable congenital syphilis were unbooked or the results of their serological tests were not available at presentation.

None of the mothers had received any treatment for syphilis prior to delivery. Fourteen of the 15 booked cases did not return to the antenatal clinics after the initial visit. One patient was referred to a clinic for sexually transmitted diseases but did not attend for treatment.

Infants with congenital syphilis - clinical data

Nineteen (56%) of the 34 patients with probable congenital syphilis presented in the first 3 days of life (these were recorded as presenting at 'birth' in the tables), the remaining 15 patients were older. Three days was taken as the dividing line because the total IgM levels start to rise soon after birth (Alford 1971), making it difficult to evaluate the usefulness of this test without establishing different control values.

Of the 34 patients 7 were preterm, 7 were preterm and SGA and 7 were SGA (3 infants had unknown birth weights and gestational ages).

Thirteen patients were Cape Coloureds and 21 were Blacks.

Overall, 15 of the mothers were booked and had VDRL titres obtained antenatally. Therefore, when these mothers presented to hospital with their infants the maternal serology result was available from the 'Road to health card'.According to the

criteria of Kaufman et al (1977), a diagnosis of probable congenital syphilis can be based on the presence of 1 'major' clinical sign and positive maternal serology or 2 'minor' clinical signs with positive serology. At presentation to hospital an immediate or pretest diagnosis could have been made in 15/34 or 44% of cases (presuming the mothers brought the 'Road to health card' with them).

Once the result of the STS was obtained from the laboratory the diagnosis could be made in all 34 patients. Thirty one of these patients had features (including abnormal radiological appearances of the long bones) compatible with the 'major' clinical signs of congenital syphilis listed by Kaufman et al (1977). Apart from the bony abnormalities, only 3 patients had 'major' clinical features in the form of snuffles or haemorrhagic rhinitis.

Two or more of the clinical signs described as 'minor' by Kaufman et al were present in 19 of the infants with probable congenital syphilis.

Infants with congenital syphilis - serological results

Thirty two (94%) of the 34 infants with probable congenital syphilis had positive RF latex tests at a dilution of 1:5 (31 tests were positive at 1:40). Eighteen of 19 newborns and 14 of 15 older infants had positive RF latex tests. Total IgM levels were elevated in all 19 newborns presenting with probable congenital syphilis at birth and in 14 of 15 infants who presented later.

The VDRL results were positive in all 34 cases. Six of the infants diagnosed at birth as having probable congenital syphilis had a VDRL titre which was at least 4 times greater than that of the mother. In addition all infants had positive treponemal tests for syphilis (TPHA or FTA-ABS IgG). The FTA-ABS (IgM) results were positive in 28 (82%) of 34 cases at presentation. Fifteen of the patients with positive tests were newborns and 13 were older infants.

6.2.2 Infants with clinical signs who did not have congenital syphilis

There were 15 infants in this group. Overall, 7 infants had septicaemia, 5 had CMV infection, 1 had congenital rubella, 1 had hereditary spherocytosis and 1 had trisomy 13.

The clinical and laboratory features of each infant as shown in the Table 6.3 will be described in more detail. However, it should be noted that each of the infants had negative nontreponemal and treponemal tests for syphilis.

Septicaemia

Altogether 7 patients (nos. 6.39-6.45) were diagnosed as having septicaemia. In 4 the blood cultures were confirmatory and yielded Escherichia coli in 3 cases and Staphylococcus aureus in 1 case. Another patient (no. 6.42) had gonococcal ophthalmia and hepatomegaly. The blood culture was negative but the symptoms resolved following antibiotic treatment. Serological tests for CMV, toxoplasmosis, rubella and a urine test for CMV were negative. This patient may have had systemic gonococcal disease (Fanaroff and Martin 1987 pg 790).

Patient no. 6.41 presented soon after birth with lethargy and oedema which were thought to represent non-specific signs of infection. No organisms were isolated from cultures of the blood or cerebrospinal fluid. Serological tests for chronic intrauterine infections were negative. The symptoms rapidly disappeared on antibiotic therapy.

The other patient with a final diagnosis of suspected septicaemia had a diffuse eczematous rash, irritability and thrombocytopenia. Blood and CSF cultures were negative but the irritability and low platelet count resolved following treatment with antibiotics.

All these patients had negative RF latex tests while 1 had a raised total IgM level at birth.

Cytomegalovirus infection

Five patients (nos. 6.35, 6.36, 6.46, 6.47, 6.48) had

cytomegalovirus infection. In 4 patients the virus was isolated from the urine. In a fifth patient (no. 6.48) the CMV ELISA for IgM was positive. Two of these patients had positive RF latex tests and 2 had elevated total IgM levels. A lack of serum prevented the IgM concentration from being determined in 1 case (no. 6.47).

Other conditions

One patient (no. 6.37) had congenital rubella. This was suggested by the spectrum of clinical signs and the rubella IgM ELISA which was positive.

Case no. 6.38 had some clinical features of a chronic intrauterine infection but in addition had a cleft palate. At postmortem additional findings were those of congenital heart disease and the cause of the hepatosplenomegaly was found to be extramedullary haematopoiesis. The final diagnosis of trisomy 13 was confirmed by chromosomal analysis.

The presenting features of pallor and hepatosplenomegaly in case no. 6.49 were subsequently ascribed to hereditary spherocytosis.

6.2.3 Controls

These consisted of the 51 infants who were at risk of congenital syphilis but who were shown to be unaffected (see Chapter 5).

The mothers of these infants had positive STS for syphilis and were either untreated or inadequately treated. The infants were examined at birth and followed up at the ages of approximately 6 weeks and 4 months.

The details of each case are given in Table 5.2.

The important results for this Chapter are as follows. None of the infants had congenital syphilis, as judged by the absence of clinical signs of disease as well as the negative serology for syphilis at 3 to 4 months of age.

Rheumatoid factor latex tests were negative at birth in all cases. Results of RF latex tests were available in 46 cases at

follow-up between 4 - 8 weeks and in 50 cases at 3 to 4 months. Altogether 5 patients (approximately 10%), had positive RF latex tests at some stage during follow-up.

In contrast to the negative RF latex results at birth, 5 patients (9.8%) had elevated total IgM levels at birth but did not have congenital syphilis.

From the sera obtained at follow-up, the distribution of the total IgM levels was determined. At 4-6 weeks the mean was 100 $^{+}$ 104 IU/ml and at 3-4 months 175 $^{+}$ 180 IU/ml (see Appendix 4)

6.2.4 Analysis of results

To determine the usefulness of the RF latex test in the diagnosis of congenital syphilis when a patient presented with clinical signs of the disease, Table 6.4 was constructed.

From the results obtained in the infants with clinical signs the sensitivity, specificity and positive and negative predictive values of the RF latex test and the total IgM test were determined. The calculations were performed as described in Chapter 5. Results are summarized in Table 6.5

The overall performance of each test is shown, and in addition, a separate column shows the values at birth.

There were no statistically significant differences between these tests ($p > 0.05$, Fisher's exact test).

6.3 DISCUSSION

The aim of the study was to ascertain whether the RF latex test gave a useful initial indication that an infant's clinical signs were due to congenital syphilis, whilst the results of other investigations were awaited.

Overall, relatively small numbers of patients with clinical signs due to conditions other than congenital syphilis were included in the study. There are several possible reasons for this:

TABLE 6.4

THE USEFULNESS OF THE RF LATEX TEST AND THE TOTAL IgM LEVEL IN INFANTS WITH CLINICAL SIGNS SUGGESTIVE OF CONGENITAL SYPHILIS

FINAL DIAGNOSIS	RF LATEX TEST		TOTAL IgM LEVEL	
	Positive	Negative	Elevated	Normal
Congenital syphilis	32	2	33	1
Other condition (with similar clinical signs)	3	12	4	11

TABLE 6.5:

SUMMARY OF THE PERFORMANCE OF THE RF LATEX TEST AND TOTAL IgM TEST IN THE DIAGNOSIS OF CONGENITAL SYPHILIS IN SYMPTOMATIC INFANTS

	RF LATEX TEST		TOTAL IgM TEST	
	Overall (%)	At birth (%)	Overall (%)	At birth (%)
Sensitivity	94.1	94.7	97	100
Specificity	80	77.8	73.3	55.6
Positive predictive value	91.4	90	89.2	82.6
Negative predictive value	85.7	97.8	91.7	100

i. the clinical signs elaborated in the study design led to the selection of infants with congenital syphilis

ii. other congenital infections are relatively rare in the population studied (see Chapter 4).

iii. It is possible that more cases of congenital syphilis were referred to the author than cases with other diagnoses. Frequent communication with colleagues was used in an attempt to overcome this. The fact that over 50% of cases of congenital syphilis had insufficient criteria to make such a diagnosis at the time of entry into the study, makes this possibility unlikely.

The sensitivity of the RF latex test at birth (94.7%) as well as amongst infants aged 0-4 months (94.1%) was high. Reimer et al (1975) and Meyer and Malan (1987) noted similar results, although only 50% of the patients described by Dobson et al (1988a) had positive RF latex tests.

It should be considered, however, that a pre-test diagnosis of congenital syphilis could be made in 44% of cases in the current study. The pre-test diagnosis could be achieved by considering the known results of the maternal VDRL (done at the antenatal booking visit) and the clinical presentation of the infant.

The specificity of the RF latex test performed on the infants with clinical signs of congenital syphilis was 80%. Positive RF latex tests were obtained in 2 (40%) of the 5 patients with CMV. This is similar to the 7/20 (35%) incidence of positive tests in CMV noted by Stagno et al (1980) but lower than the 13/16 (81%) reported by Reimer et al (1975).

The one patient in the study with congenital rubella also had a positive RF latex test. Reimer et al (1975) found positive RF latex tests in 4/6 (67%) of infants with congenital rubella.

None of the other patients who did not have congenital syphilis had positive RF latex tests. The majority of these infants had suspected or confirmed septicaemia.

The results obtained in the controls demonstrated that asymptomatic infants did not have positive RF latex tests at birth. However, 5/46 (10.9%) had positive tests between 1 and 4 months. This indicates that if larger numbers of infants were tested outside the neonatal period more false positive tests may be obtained and the specificity of the RF latex test could be lower.

The positive and negative predictive values of the RF latex test (91.4% and 85.7% respectively) were high. One needs to bear in mind, however, that these figures would change depending on the prevalence of congenital syphilis in the study group. Congenital syphilis was relatively common in the group selected. Studying a different population, e.g. that of a developed country where congenital syphilis is rare and congenital CMV with clinical signs commoner, the positive predictive value would probably be lower. However, the prevalence of congenital syphilis is generally much higher in developing countries (Hira et al 1982) and the test is likely to be more valuable in such situations.

The total IgM test gave similar results to that of the RF latex test although the sensitivity was slightly higher as one of the infants with congenital syphilis had a raised total IgM level but a negative RF latex test. In addition, another patient with presumed septicaemia had an elevated IgM level. The RF latex test was negative in this case and this may simply reflect that the total IgM rises within a few days of the onset of infection whilst RF takes several weeks to appear (Bartfeld 1969, Jawetz et al 1980 pg 162).

The total IgM was measured by radial immunodiffusion. Results were obtainable after 24 hours which was a considerable disadvantage when considering the test as a side-room investigation. The RF latex test result was available in a matter of minutes. Total IgM can also be measured by a latex test (Tympner and Neuhaus 1972). This is likely to give rapid results but this method was not evaluated in the study.

The radiological changes in the long bones were used as a

criterion for entry into the study, so that their diagnostic value could not be independently determined. It is noteworthy, however, that the changes of periosteal dystrophy and metaphyseal dystrophy were not specific for congenitally infected infants but were also present in 3 infants with septicaemia and 1 with haemolytic anaemia. Caffey (1939), reported similar findings, while Shopfner (1966) noted that periosteal reactions could be found in up to 35% of normal term infants.

The VDRL test enabled the diagnosis of congenital syphilis to be made in all the affected infants. At the same time the test was negative in all the infants without the disease. As the VDRL tube test is inexpensive, does the RF latex test have any advantage? The usefulness of the latter test lies in the fact that it can be performed as a side-room investigation. The VDRL test, however, is best carried out in a central laboratory (WHO 1986). As has been discussed elsewhere, this is because of the need for fresh antigen on a daily basis as well as the fact that heat inactivation of the serum is required. In addition, strict quality control is advisable (Larsen et al 1984 pg 875-879). The RPR test is recommended for peripheral laboratories (WHO 1986) and may also be suitable as a side-room test. The cost of this test, however, is approximately R0.75. This is similar to that of the RF latex test (R0.67). The disadvantage of the RPR is that a mechanical rotator (at a cost of R2000) is needed at each locality where the test is to be offered. The RF latex test would appear to be technically easier to carry out and requires a minimum of equipment.

In summary, all the patients with positive RF latex tests at birth had a congenital infection. The RF latex test was not able to distinguish, however, between the various types of chronic intrauterine infection at birth. Therefore, the prevalence of other congenital infections in the population influences the value of the test for congenital syphilis.

In a developing country such as South Africa, where maternal syhphilis is still relatively common, a positive RF latex test appears to provide useful confirmatory evidence of a diagnosis

of congenital syphilis in a symptomatic infant. Even in more developed communities it is of value to indicate that symptoms in a neonate are likely to be due to a congenital infection.

Measuring the total IgM levels gave similar results, the advantages of the RF latex test being its rapidity and lower cost (see Chapter 5).

In the older age group (4 days to 4 months) positive RF latex tests were encountered in well infants in the control group (see Chapter 5). As suggested by Stagno et al (1980), this phenomenon may occur predominantly outside the neonatal period. This reduces the value of a positive test in older patients although the results of the more specific serological tests would indicate that the RF latex test result was positive in the absence of congenital infection.

In conclusion, the sensitivity of the RF latex test in the diagnosis of congenital syphilis was found to be high in infants presenting with one or more clinical signs of the disease. In the setting in which it was investigated, the technique would appear to have value as a rapid side-room test for congenital syphilis.

CHAPTER 7

RHEUMATOID FACTOR AND THE FTA-ABS (IGM) TEST FOR CONGENITAL SYPHILIS

It has been suggested that the presence of serum RF may result in the FTA-ABS (IgM) test being non-specific for congenital syphilis (Reimer et al 1975).

It follows, therefore, that RF interference should be eliminated prior to the test being performed. Muller and Sinzig (1982) separated the IgM and IgG fractions and carried out the FTA-ABS (IgM) test on the former fraction. Removal of the IgG prevents any RF interaction, although fractionation procedures do not always remove all the IgG and can be time consuming and complicated (Sever 1969; Muller et al 1987). A more convenient method has been found to be that of removing the IgG by immunoprecipitation (Gispen et al 1975). This not only abolishes the effect of RF, but has also been shown to improve the sensitivity of testing for anti-treponemal IgM (Cerny et al 1985).

Before this technique can be recommended, however, it is important that the effect on the FTA-ABS (IgM) test of eradicating RF interference be determined.

7.1 METHODS

7.1.1 Patient selection

Sera from patients described in Chapters 5 and 6 were used. The study groups are outlined below:

i. Infants having congenital syphilis with clinical signs at presentation

ii. Infants with congenital syphilis without clinical signs at presentation

iii. Infants without congenital syphilis but with similar clinical signs at presentation

iv. Control infants: newborn infants at high-risk of congenital syphilis who did not, in fact, develop the disease were considered as controls. The features which resulted in the infants being high-risk are described in Chapter 5. In brief, however, the mothers had positive serological tests for syphilis and were either untreated or inadequately treated.

Sera was obtained from the controls as follows:

i. within 3 days of birth

ii. at follow-up visits in the first 4 months of life.

The older control group was considered necessary since not all the patients with congenital syphilis were newborn infants. Each of the 15 older patients with congenital syphilis was matched with a randomly selected control infant of a similar age.

7.1.2 Serological tests

The FTA-ABS (IgM) test was performed as described in Appendix 2. All sera were pretreated with sorbent to remove antibodies to nonpathogenic treponemes and tested at a final dilution of 1:10. Rheumatoid factor activity was removed by precipitating the IgG with a sheep anti-human IgG (Behring, West Germany, catalogue number OUCG 14/15). For this procedure 10 ul of serum was added to 40 ul of the anti-human IgG. After being allowed to stand for 15 minutes at room temperature the precipitate was removed by centrifugation at 300 G for 10 minutes.

Completeness of RF removal was checked by means of an RF ELISA (see Chapter 8). Ten microlitres of serum in 40 ul of PBS pH 7,2 was mixed with 50 ul of anti-human IgG. After incubation and removal of the precipitate PBS pH 7,2 was added to reach a final dilution of 1:200.

Radial immunodiffusion plates (LC Partigen immunodiffusion plates, Behring, West Germany, catalogue number OTCR 03) were

used to measure the IgG concentration following treatment of the sera with the anti-human IgG.

The FTA-ABS (IgM) test was carried out before and after the IgG immunoprecipitation step.

7.2 RESULTS

7.2.1 Patients having congenital syphilis with clinical signs at presentation

There were 37 cases in this category, 22 of whom presented in the first few days of life (see Table 7.1) and 15 presented later (Table 7.2). The RF ELISA which was positive in 35 of 37 cases prior to IgG removal became negative in 32 (IgG was not detected in any of the sera following treatment with the anti-human IgG).

The FTA-ABS (IgM) test was positive in 34 (92%) of the 37 cases prior to abolishing RF interference and in 29 (78,4%) of the 37 cases afterwards. This difference was not statistically significant (Chi-square test $p > 0.05$).

The immunofluorescence was brighter in 8 cases before treatment with the anti-human IgG and brighter after removal of IgG in one case.

These results are summarized in Tables 7.1 and 7.2.

7.2.2 Patients with congenital syphilis without clinical signs at presentation

Three newborn infants (nos. 5.5 - 5.7) (as described in Chapter 5) had no definite clinical signs of congenital syphilis but the diagnosis could be made on the basis of the serological and radiological features. A further 8 newborn infants developed features of congenital syphilis during follow-up (cases 5.8 - 5.15 reported in Chapter 5). These 11 infants were considered together with 1 infant from the study group in Chapter 6 (no. 6.7) who had no abnormal clinical signs but metaphyseal dysplasia was noted on radiographic examination of the long bones.

TABLE 7.1

EFFECT OF RF REMOVAL ON THE FTA-ABS (IgM) TEST IN NEWBORN INFANTS WITH CLINICAL SIGNS OF CONGENITAL SYPHILIS

		BEFORE TREATMENT WITH ANTI-IgG		AFTER TREATMENT WITH ANTI-IgG	
PATIENT CODE NO.	AGE AT PRESENTATION	RF LEVEL IU/ml	FTA-ABS (IgM) *	RF LEVEL IU/ml	FTA-ABS (IgM) **
5.1	Birth	24	+ ve	0	+ ve
5.2	Birth	4	+ ve	0	- ve
5.3	Birth	0	+ (brighter)	0	+ ve
5.4	Birth	21	+ ve	0	- ve
6.1	Birth	152	+ (brighter)	0	+ ve
6.2	Birth	205	+ ve	0	+ ve
6.3	Birth	156	+ (brighter)	0	+ ve
6.4	Birth	90	+ ve	0	+ ve
6.5	Birth	92	+ ve	6	- ve
6.6	Birth	50	+ ve	0	+ ve
6.8	Birth	27	+ ve	0	- ve
6.9	Birth	165	+ ve	0	+ ve
6.10	Birth	32	+ ve	0	+ ve
6.11	Birth	11	+ (brighter)	0	+ ve
6.12	Birth	788	+ ve	60	+ ve
6.13	Birth	34	+ ve	0	- ve
6.14	Birth	165	+ ve	0	+ ve
6.15	Birth	21	+ (brighter)	0	+ ve
6.16	Birth	850	+ ve	34	+ ve
6.17	Birth	3	- ve	0	- ve
6.18	Birth	24	+ ve	0	+ ve
6.19	Birth	6	+ ve	0	+ ve
No.of positive tests		21/22 (95,5%)		16/22 (72,7%)	

*)
**) Tests performed at 1:10 dilution

TABLE 7.2

EFFECT OF RF REMOVAL ON THE FTA-ABS (IgM) TEST IN OLDER INFANTS WITH CONGENITAL SYPHILIS

		BEFORE TREATMENT WITH ANTI-IgG		AFTER TREATMENT WITH ANTI-IgG	
PATIENT CODE NO	AGE AT PRESENTATION	RF LEVEL IU/ml	* FTA-ABS (IgM)	RF LEVEL IU/ml	** FTA-ABS (IgM)
6.20	6 w	38	+ (brighter)	0	+ ve
6.21	2 mo	30	+ (brighter)	0	+ ve
6.22	10 w	520	+ (brighter)	7	+ ve
6.23	3 mo	0	- ve	0	+ ve
6.24	2 w	32	+ ve	0	+ ve
6.25	2 w	238	+ ve	0	+ ve
6.26	4 mo	500	+ ve	5	+ ve
6.27	6 w	80	- ve	0	- ve
6.28	2 mo	160	+ ve	0	+ ve
6.29	1 mo	118	+ ve	0	+ ve (brighter)
6.30	3 mo	38	+ ve	0	+ ve
6.31	10 w	54	+ ve	0	+ ve
6.32	14 w	6	+ ve	0	+ ve
6.33	2 w	96	+ ve	0	- ve
6.34	2 mo	14	+ ve	0	+ ve
No.of positive tests		13/15 (86,7%)			13/15 (86,7%)

*) Tests performed at 1:10 dilution
**)

Using the sera obtained at birth, RF was removed from 7 cases by the addition of the anti-human IgG. Results are shown in Table 7.3.

The FTA-ABS (IgM) test was positive in 10 of the 12 cases prior to RF removal and in 3 cases afterwards. This difference was statistically significant (Fisher's exact test $p < 0,05$).

7.2.3 Patients without congenital syphilis but with similar clinical signs at presentation

There were 15 cases in this category who were described in Chapter 6. There was sufficient serum for FTA-ABS (IgM) testing in all but 1 patient (no. 6.43). Three of the infants (nos. 6.35, 6.37 and 6.47) had positive tests for RF. The RF activity was eliminated by IgG removal. All 14 cases had negative FTA-ABS (IgM) tests before and after the steps to eradicate RF.

7.2.4 Control infants

At birth: all 51 newborn infants had negative tests for RF using the latex and ELISA methods (see Chapters 5 and 9). One of the infants (no. 5.65) had a positive FTA-ABS (IgM) test prior to IgG removal. None of the control patients had a positive test after steps to eliminate IgG.

Older infants: of the 15 age-matched older controls, 2 had positive FTA-ABS (IgM) tests prior to the use of the anti-human IgG (see Table 7.4). The RF ELISA demonstrated the presence of RF in 4 cases altogether, 1 of whom (no. 5.49) was FTA-ABS (IgM) positive.

Following the addition of the anti-human IgG, the FTA-ABS (IgM) test was negative in all cases.

TABLE 7.3:

EFFECT OF RF REMOVAL ON FTA-ABS (IgM) TEST (PERFORMED AT BIRTH) IN INFANTS WITH CONGENITAL SYPHILIS WHO DID NOT HAVE CLINICAL SIGNS AT BIRTH

			BEFORE TREATMENT WITH ANTI-IgG		AFTER TREATMENT WITH ANTI-IgG	
PATIENT CODE NO.	AGE AT INITIAL TESTING	AGE AT DIAGNOSIS	RF LEVEL IU/ml	FTA-ABS (IgM) *	RF LEVEL IU/ml	FTA-ABS (IgM) **
5.5	Birth	Perinatal	35	+ ve	0	+ ve
5.6	Birth	Perinatal	27	+ ve	0	+ ve
5.7	Birth	Perinatal	0	+ ve	0	- ve
5.8	Birth	4 mo	12	+ ve	0	- ve
5.9	Birth	10 w	20	+ ve	0	- ve
5.10	Birth	6 w	19	+ ve	0	- ve
5.11	Birth	6 w	3	+ ve	0	- ve
5.12	Birth	3 mo	0	+ ve	0	- ve
5.13	Birth	4 mo	0	- ve	0	- ve
5.14	Birth	6 w	0	+ ve	0	- ve
5.15	Birth	6 w	0	- ve	0	- ve
6.7	Birth	Perinatal	15	+ ve	0	+ ve
Totals with positive tests (%)			10/12 (83.3%)		3/13 (25%)	

*) Samples tested at a dilution of 1:10

**)

TABLE 7.4

EFFECT OF RF REMOVAL ON FTA-ABS (IgM) TEST IN CONTROL INFANTS BETWEEN 1 MONTH AND 4 MONTHS OF AGE

PATIENT CODE NO	RF CONCENTRATION (IU/ml)	FTA-ABS (IgM) TEST	
		*BEFORE	**AFTER
5.19	0	-ve	-ve
5.25	0	-ve	-ve
5.28	0	-ve	-ve
5.34	0	-ve	-ve
5.36	0	-ve	-ve
5.40	0	-ve	-ve
5.46	7,6	-ve	-ve
5.49	4,2	+ve	-ve
5.50	0	-ve	-ve
5.58	0	-ve	-ve
5.63	7,5	-ve	-ve
5.65	2,8	-ve	-ve
5.67	0	-ve	-ve
5.68	0	+ve	-ve
5.69	0	-ve	-ve

*) samples tested before and after elimination of RF

**) effect at 1:10 dilution

7.3 DISCUSSION

The aim of this study was to determine the effect on the sensitivity and specificity of the FTA-ABS (IgM) test of eliminating RF interference. The method chosen to prevent the latter was that of IgG removal by precipitation. As IgM RF in the serum is associated with IgG (Egeland and Munthe 1983), it follows that precipitation of the latter should extract the RF (Gispen et al 1975). In addition, as IgM RF binds more avidly to aggregated than native IgG (Chen et al 1987), it is likely that the IgG-antiIgG complexes form a suitable substrate for RF attachment. The technique of RF removal proved to be simple and effective. Rheumatoid factor activity was not detected in 89% of RF positive sera following the procedure. The treated sera with residual RF had no IgG antibody indicating that RF interference could no longer take place. The effects of the steps to prevent RF activity are discussed below.

7.3.1 Effect of RF removal on the sensitivity of the FTA-ABS (IgM) test in the diagnosis of congenital syphilis

The sensitivity of a test has been defined as its ability to be positive in patients with the disease (Jaffe 1975). Two groups of patients with congenital syphilis were studied.

Symptomatic patients with congenital syphilis

In symptomatic infants in the present study, the sensitivities of the test before (92%) and after (78.4%) RF removal were not significantly different (Chi-square test $p > 0.05$). However, tests in 6 patients which were positive before RF removal became negative afterwards. Rheumatoid factor was demonstrated in the sera of these patients. It is possible that RF was responsible for the positive tests. This could occur in 2 ways:

i. The IgM RF may bind directly to the treponemes. The anti-human IgM conjugated antibody would then attach to the RF giving a positive test (which would revert to negative after RF extraction).

ii. The RF, by attaching to IgG bound to the treponemes, would be detected by the anti-human IgM conjugate in the same way

as specific IgM.

The first postulate appeared to be unlikely because a number of patients (e.g. numbers 6.17 and 6.27), had detectable IgM RF but a negative FTA-ABS (IgM) test. In addition, the IgM RF extracted from patient 6.12 (see Section 9.3) gave a negative result when tested in the FTA-ABS (IgM) test. These findings indicate that IgM RF does not attach to the spirochaetes directly. In contast to this, the addition of IgM RF to serum containing pathogen-specific IgG has been shown to give rise to falsely positive IgM immunofluorescence tests, suggesting that the second mechanism is the correct one (Shirodaria et al 1973).

Another patient (no. 6.23), whose serum tested negative for RF, had a positive FTA-ABS (IgM) test after IgG precipitation. This phenomenon may have been due to competitive inhibition between IgG and IgM treponemal antibodies. IgG extraction would then have eliminated the competition for binding sites (Cohen et al 1967).

Steps to remove IgG (and hence RF) from the sera could, therefore, have had different effects on the sensitivity of the FTA-ABS (IgM) test in different patients. It was apparent, however, that the sensitivity of the FTA-ABS (IgM) test after RF removal in the symptomatic infants in the present study (78,4%) was lower than that in various other studies. Thus, Kaufman et al (1974) quoted a sensitivity of approximately 96%. Similarly, Rosen and Richardson (1975) found that 100% of symptomatic infants with congenital syphilis had positive tests. A previous study from the PMNS (Meyer and Malan 1987) also demonstrated a high sensitivity (100%).

The FTA-ABS (IgM) in the study reported in 1987 was performed in exactly the same way as in the present study except that the anti-human IgG was not used. It appears that the use of the anti-human IgG to remove RF may have had the effect of reducing the sensitivity of the test.

Patients with congenital syphilis without clinical signs at presentation

Extraction of RF by means of IgG removal resulted in a significant reduction in the sensitivity of the FTA-ABS (IgM) test in this group of patients. The reason for this may be that, as suggested by Reimer et al (1975), infants with congenital syphilis produce relatively large amounts of IgM anti-IgG (RF) and less IgM directed against Treponema pallidum (specific IgM). This may be particularly true in early disease as nonspecific IgM (e.g.RF) is produced before specific IgM (Alford et al 1969).

These findings could explain the observation in the present study that RF removal had a greater effect on the sensitivity of the FTA-ABS (IgM) test in the asymptomatic patients than in the symptomatic ones. The former group probably had earlier disease and could have produced more IgM RF than specific IgM. The IgM RF may have been largely responsible for the reactive FTA-ABS (IgM) results as indicated by the fact that most of the tests became negative after RF removal. In the symptomatic group with more advanced disease, however, the FTA-ABS (IgM) test may have been positive because of the presence of specific IgM; the extraction of RF would not have altered this result.

The presence of RF alone, however, does not explain the positive FTA-ABS (IgM) tests before IgG removal in all cases. Patients 5.12 and 5.14 had negative RF tests. This means that antiglobulins could not have caused these results unless hidden RF was present. (This is RF that is not detected by the usual tests because it is bound to IgG - Allen and Kunkel 1966). Tests to detect hidden RF were carried out as described in Chapter 9, but were negative.

Another explanation for the different FTA-ABS (IgM) results in the patients without RF, is that treatment of the sera with the anti-human IgG may have removed some of the specific IgM. This

hypothesis could not be directly tested but was thought to be unlikely because of results obtained in 5 control patients who were RF negative. In these patients the total IgM concentrations before and after IgG precipitation gave almost identical results (see Appendix 5).

Finally, it is possible that the RF was so closely associated with immune complexes in the patients with congenital syphilis that its removal resulted in precipitation and loss of the immune complexes (and therefore of the specific IgM also).

The sensitivity of the FTA-ABS (IgM) test before IgG removal (88%) was greater than the 65% described by Kaufman et al (1974) for a similar group of patients. The latter authors, however, considered delayed onset cases only. If patients without clinical signs who had abnormal roentgenograms are excluded from the analysis of the present study, the sensitivity becomes 75% (6/8).

7.3.2 Specificity

The specificity of a test is defined as its ability to be negative in persons without the disease (Jaffe 1975). Several groups of patients without congenital syphilis were studied, and the effect of RF removal on the specificity of the FTA-ABS (IgM) test investigated.

Patients without congenital syphilis but with similar clinical signs at presentation

None of the patients with conditions mimicking congenital syphilis had false-positive FTA-ABS (IgM) tests. Reimer et al (1975) argued that RF in the sera of such patients may make the FTA-ABS (IgM) test nonspecific. The reason put forward was that IgM RF would be detected as specific IgM. The events would be as follows: maternal IgG directed towards T.pallidum would bind to treponemal antigens. IgM RF would then bind to the maternal IgG; the anti-human IgM fluorescein-labelled conjugate would then identify the bound RF in the same way as anti-treponemal IgM.

Studies have demonstrated that this occurs, for example, when testing for IgM antibodies to rubella, toxoplamosis and CMV (Gispen et al 1975; Hyde et al 1975; Stagno et al 1980). Reimer et al (1975) demonstrated that the phenomenon could occur in congenital syphilis. It appeared that both anti- treponemal IgG and IgM RF were necessary to obtain a false-positive test. This leads one to question the likelihood of fulfilling these 2 conditions in infants with diseases other than congenital syphilis.

Apart from congenital syphilis, the most likely reason for a symptomatic infant to have RF present in the serum is probably CMV infection.

The probability of a false positive FTA-ABS (IgM) test due to symptomatic CMV can be calculated.

Approximately 8 percent of expectant women had positive treponemal IgG antibody tests in the PMNS (Gonin 1985) Therefore, if one studied 100/8 = 12 cases of CMV, each of whom had produced RF, one could expect one false positive FTA-ABS (IgM) test. Considering that:

i. the prevalence of symptomatic CMV may be approximately 0.08% (see Chapter 4)

ii. 42% of infants with CMV produce RF (Griffiths et al 1982)

iii. 26 000 newborn infants are delivered annually in the PMNS (see Chapter 4).

One could expect 9 cases of symptomatic CMV with positive RF tests every year.

The 12 cases of CMV would, therefore, be seen over 15 months. It is therefore, perhaps not surprising, that none of the symptomatic patients in the present study had false-positive FTA-ABS (IgM) tests due to RF activity. A larger study would be necessary to demonstrate enhanced FTA-ABS (IgM) specificity following RF removal in symptomatic infants with other

congenital infections (the commonest of which would presumably be CMV).

Newborn infants at high risk of congenital syphilis who were unaffected

These infants had positive treponemal tests for syphilis but all had negative tests for RF. Only 1 patient was found to have a positive FTA-ABS (IgM) test prior to IgG removal. It is possible that a lack of conjugate specificity was responsible for the result (Alford et al 1969a).

There was no significant increase in FTA-ABS (IgM) specificity observed following steps to eliminate RF interference. Newborn infants have not been shown to make RF in the absence of a congenital infection (Stagno et al 1980, Meyer and Malan 1987). The probability of a mother having anti-treponemal IgG and the infant having RF due to a congenital infection other than congenital syphilis is likely to be low as previously discussed. On the other hand a high percentage of asymptomatic mothers will have IgG antibodies to rubella and CMV (Chonmaitree et al 1982; Keen 1985). If congenital syphilis develops in this setting and the infant produces RF, the IgM antibody tests for rubella or CMV may be falsely positive (Gispen et al 1975; Stagno et al 1980).

Specificity in older infants outside the neonatal period

Here a different situation prevails. More infants are likely to be RF positive although they are otherwise well. Percentages range from 10-15% (Reimer et al 1975; Stagno et al 1980). Therefore, the older infants who had passively transferred maternal anti-treponemal IgG could be expected to have a higher incidence of false positive FTA-ABS (IgM) tests due to the effect of RF.

The results of the present study confirmed the higher incidence of false positive FTA-ABS (IgM) tests prior to IgG removal (2 tests were positive before and all were negative afterwards). Surprisingly, the improved specificity was not always due to RF removal as one patient (no. 5.68) had negative RF tests. Hidden

RF may have played a part. Although this substance was not directly sought, it was not found in a group of infants with congenital syphilis (see Chapter 9), Furthermore, 2 other patients who had RF present had negative FTA-ABS (IgM) tests.

It appears that RF is not the only cause of the false positive FTA-ABS (IgM) tests seen prior to IgG removal.

As an alternative explanation, it is possible that the conjugate specificity is deficient. Removing the IgG from the serum would then remove anti-treponemal IgG which, if the postulate is correct, could be identified as anti-treponemal IgM.

This hypothesis could be tested by removing the IgM RF but not the anti-treponemal IgG (e.g. by mercaptoethanol or pepsin treatment) and repeating the FTA-ABS (IgM) test on the group of high-risk controls.

That anti-treponemal IgG is necessary for a false positive test to occur is suggested by the lack of false positives in the group of infants who were symptomatic but who did not have congenital syphilis. These infants all had negative anti-treponemal IgG tests (see Chapter 6).

7.4 SUMMARY

In summary, the following points can be made.

i. The use of the anti-human IgG preparation to eliminate RF interference in the FTA-ABS (IgM) test was simple and effective.

ii. There was no observed increase in the sensitivity of the FTA-ABS (IgM) test following steps to remove RF from the sera to be tested. There was, in fact, a reduction in sensitivity of the test for both symptomatic and asymptomatic infants with congenital syphilis. The loss in sensitivity was more marked in asymptomatic infants, the very group where a sensitive test is most needed (Kaufman et al 1974).

iii. The specificity of the FTA-ABS (IgM) test appeared to be somewhat improved following IgG precipitation. This was noted mostly for infants outside the neonatal period. The cause of the reduced specificity was unclear but did not appear to be entirely due to the presence of RF.

7.5 CONCLUSIONS AND RECOMMENDATIONS

The recommendations to be made apply solely to the population studied and are not necessarily applicable to communities where other congenital infections e.g. CMV are commoner than congenital syphilis.

In newborn infants the prevention of RF interference reduced the sensitivity of the FTA-ABS (IgM) test. This finding was particularly marked in the asymptomatic cases where a sensitive test is essential. It seems important not to extract RF in these patients.

A low incidence of false positives (2%) was found amongst high-risk newborns and false positive FTA-ABS (IgM) tests were not seen amongst a group of infants with clinical signs who did not have congenital syphilis (even though some had positive RF tests). Prevention of RF interference did not seem to be of value in this group either.

The older high-risk infants had a greater incidence of false positive FTA-ABS (IgM) tests. The danger of over-diagnosis, however, is much less than the problem of under-diagnosis in congenital syphilis.

The findings suggest that,in the context in which the FTA-ABS (IgM) test was studied, it is immaterial whether the test was positive due to the presence of specific IgM or IgM RF. In both instances the patient is likely to have congenital syphilis. Hence it seems advisable that no steps be undertaken to prevent RF interference; this is most important when testing newborns.

PART III

RHEUMATOID FACTOR AND THE IMMUNE RESPONSE IN CONGENITAL SYPHILIS

As pointed out in Chapter 3, there are a number of aspects of RF production in congenital syphilis which merit further study.

i. How much IgM RF is produced and are other RF classes present?

ii. What factors are associated with RF synthesis in congenital syphilis?

iii. Is tissue-bound RF present at sites of inflammation in congenital syphilis?

CHAPTER 8

MEASUREMENT OF RHEUMATOID FACTOR CONCENTRATIONS USING AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

8.1 INTRODUCTION

In order to determine if there were associations between the RF levels and various clinical and laboratory findings in congenital syphilis it was necessary to measure the RF concentration.It would, for example, be possible to compare the RF levels with the severity and extent of the disease. A similar approach in patients with RA has demonstrated an association between the RF titre, clinical activity and extra-articular manifestations of the disease as well as a higher mortality (Gordon et al 1973; Holborow and Swannell 1983 pg 247; Gioud-Paquet et al 1987; Carson 1987 pg 1204).

The ELISA technique provides a more sensitive estimate of the serum RF concentration than the conventional tests (such as the latex agglutination tests), which are difficult to quantify (Bampton et al 1985; Gioud-Paquet et al 1987). It also allows specific classes of RFs to be measured and this has provided useful information regarding the pathogenesis of rheumatoid vasculitis (Stone et al 1987).

The quantitation of RF by means of an ELISA has been described by several workers (Willems and Klaassen de Kort 1978, Gripenberg et al 1979, Faith et al 1982). These methods were adopted with minor modifications.

The establishment of the ELISA is described in:

Section 8.2: reagents used and technique followed.

Section 8.3: evaluation of the RF ELISA.

8.2 MATERIALS AND METHODS

8.2.1 Materials

Carrier surface

The assays were performed on 96 well, flat bottomed polystyrene microtitre plates. Greiner (West Germany, catalogue number 655001) and Nunc (Denmark, catalogue number 4-42404) plates were evaluated.

IgG

Human IgG was used to coat the plates (some workers have used rabbit IgG; human IgG was chosen here because binding of RF from non-RA patients appears to be better - Bartfeld 1969; Egeland and Munthe 1983.

Purified IgG was obtained from ICN Immunobiologicals (USA, catalogue number 64-145). Immunodiffusion was carried out by the manufacturers to demonstrate the presence of IgG and the absence of IgM and IgA. The IgG was heat-aggregated by heating it to 63°C for 20 minutes in a waterbath (Gripenberg et al 1979)

Peroxidase-conjugated antibody, enzyme and substrate

The conjugated detection antibody was a goat anti-human IgM coupled to peroxidase. The F(ab')2 fragment, which was u-chain specific, was used (obtained from Cappel Products, USA. catalogue number 33010201).

The substrate was 2,2'-azinobis (3-ethylbenzthiazoline sulphonic acid diammonium salt) acquired from the Sigma Chemical Company, USA (catalogue number A1888). One hundred microlitres of a 40 mM solution of 2,2'-azinobis in 10 ml of 0.05 M citric acid at pH 4 was used. One hundred microlitres of 3% hydrogen peroxide was added to 10 ml of substrate just before use.

Buffers

The antigen coating buffer used was a carbonate-bicarbonate buffer at pH 9.6. Sera were diluted in Dulbecco's phosphate-buffered saline (PBS) pH 7.2 obtained from Highveld Biologicals

Plates were washed with a PBS-Tween solution (0.05% Tween-20) at pH 7.2. The Tween-20 was supplied by Merck, West Germany.

Blocking of nonspecific binding sites

These were blocked with a solution of either i) 1% goat serum with 1% bovine serum albumin (BSA, from Boehringer Mannheim, West Germany) and 0,75 g glycine in 100 ml PBS pH 7,2 or ii) the same solution containing 2,5% BSA. The effectiveness of these 2 solutions was compared by assaying 5 sera in duplicate with each of the blockers.

Measurement of optical density

This was performed using an SLT 210 photometer (SLT-Labinstruments, Austria) at a wavelength of 405 nm and using a reference wavelength of 620 nm.

8.2.2 Serum samples and methods

During the establishment of the RF ELISA serum samples were obtained as follows.

Positive control sera

i. an external (or primary) standard, the International Reference Preparation of RA serum obtained from the WHO. The characteristics of this preparation have been reported by Anderson et al (1970).

ii. an internal (or in-house) standard obtained from an adult patient with RA. This patient had a positive RF latex test (titre 1:160) and, apart from being the internal standard, the serum was also used as a high-titre positive control serum during the establishment of the RF ELISA.

iii. sera from 2 infants with congenital syphilis who had positive RF latex tests.These sera were used as mid- and low-titre positive control sera.

Negative control serum

This was obtained from a normal term newborn infant whose serum was RF latex test negative.

Heat inactivation of the sera was not carried out (Carson et al 1977; Gripenberg et al 1979).

All sera were aliquoted in 100 ul amounts and stored at -70°C.

Method for the RF ELISA

Checkerboard titrations were performed to ascertain the optimum concentrations of reagents (Voller et al 1980 pg 367-371). The plates were coated with 100 ul of HaIgG at concentrations ranging from 0.25 - 2.0 mg/100 ml in coating buffer. Rows A, C, E and G of each plate were coated with antigen whilst rows B, D F and H received coating buffer only. After being left overnight at 4°C, the plates were washed once with PBS pH 7,2.

The plates were then blocked with the solutions previously described. Greiner plates were blocked with 250 ul per well and the Nunc plates with 450 ul per well. After 1 hour the plates were washed with PBS pH 7,2.

The sera were assayed in duplicate (100 ul serum per well). Serum dilutions of 1:100 to 1:4000 in PBS-Tween were added to antigen-coated wells and to uncoated wells. Four wells on each plate had no sera added. After overnight incubation at 4°C, the plates were washed 6 times with PBS-Tween.

Conjugate dilutions of 1:800 to 1:3200 in PBS-Tween were tested to determine the optimum conjugate concentration. One hundred microlitres of conjugate was placed in each well and left for 1 hour at room temperature. Following this the plates were washed 10 times with PBS-Tween and 100 ul of substrate added. The optical density was read at 405 nm after 1 hour.

8.2.3 Results of checkerboard titrations

Effect of different polystyrene plates

The use of the Greiner and Nunc plates were compared with respect to nonspecific binding and the strength of the antibody signal obtained.

Optical density readings were measured in each experiment to determine nonspecific (background) binding. The background readings were obtained from wells in which either the coating antigen (IgG) and/or the test serum were omitted.

The background values obtained from wells with antigen but no serum were very similar to those obtained with no antigen and no serum. The effect of the antigen on the background optical density readings obtained with both plates was of the order of 0.003 - 0.007. The major portion of the background measurements was due to non-specific binding of serum to the wells. Consequently, in the results which follow only the backgrounds due to the serum were taken into account.

During the establishment of the ELISA, the mean optical density of 200 background values due to nonspecific binding of serum to the Greiner plates was 0.075 with a standard deviation of 0.029.

The Nunc plates gave lower background readings (mean of 200 tests 0.055 with a standard deviation of 0.019).

In Figure 8.1, graphs 1-8 (obtained with Greiner plates) can be compared with graphs 9-12 (obtained with Nunc plates). It can be seen that, apart from the background values, the positive and negative control sera gave similar results on the different plates.

Blocking buffers

The background values obtained when the solution containing 1% BSA was used were very similar to those observed with the 2.5% solution. The difference in the mean backgrounds obtained was 0.001; the 2.5% solution giving slightly lower results. Bovine

serum albumin at a concentration of 1% was used in the subsequent assays.

IgG coating antigen concentration

As shown in Figure 8.1 graphs 1-4 and 9 and 10, IgG concentrations below 0.5 mg 100 ml resulted in a fall in the optical density readings measured with the positive control sera. Higher IgG concentrations did not have a marked effect on the values obtained with these sera, although the backgrounds were slightly higher (backgrounds here were calculated from wells coated with antigen but to which no serum was added). A coating antigen concentration of 0.5 mg/100 ml was therefore chosen for future assays.

Peroxidase-conjugated antibody dilutions

Graphs 5-8 and 11 and 12 in Figure 8.1 show the effect of varying conjugate and serum dilutions. Although the highest optical density readings were obtained with the more concentrated conjugates, the background values were also highest.

An acceptable distinction between positive and negative control sera and a reduction in background readings could be achieved with conjugate dilutions between 1:1600 and 1:3200. A final conjugate dilution of 1:2000 was used (Figure 8.1, graphs 11 and 12).

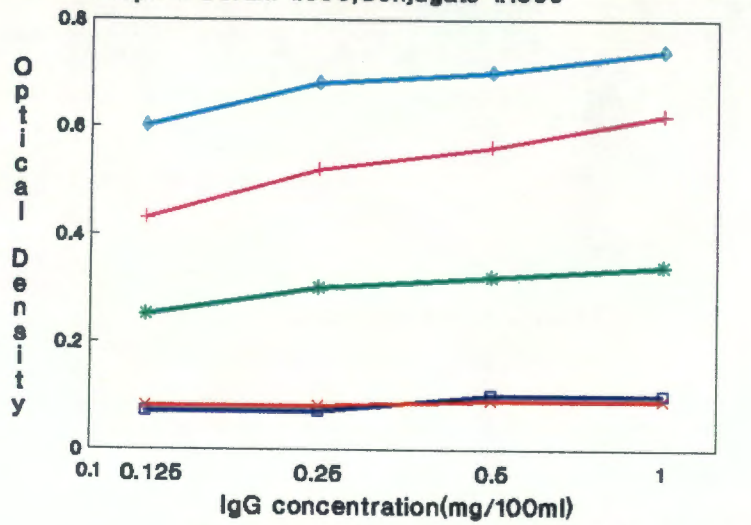
Serum Concentrations

Figure 8.1, graph 11 shows the effect of using different serum concentrations whilst maintaining the IgG strength of 0.5 mg/100 ml and the conjugate strength at 1:2000. Again, higher backgrounds were a feature of sera tested at higher concentrations (e.g. 1:100). A serum dilution of 1:500 allowed a good distinction to be made between positive control sera containing high, intermediate and low amounts of RF.

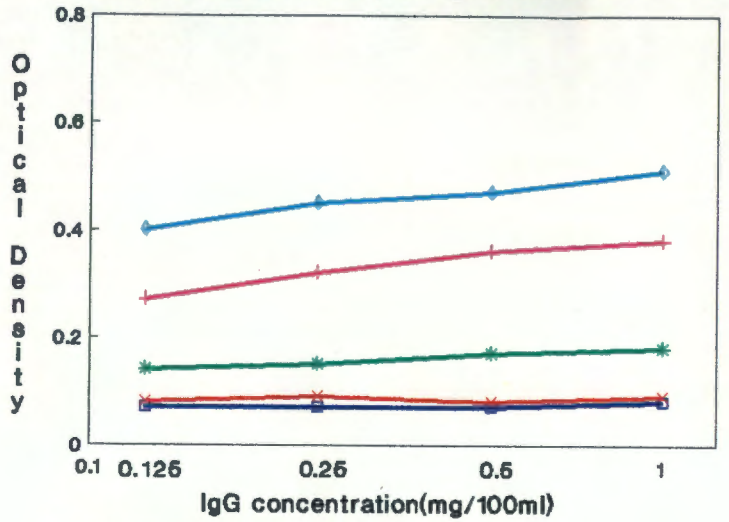
Figure 8:1 Graphs 1-12 showing the results of checkerboard titrations obtained during the establishment of the IgM RF ELISA

GRAPHS 1-4: CHECKERBOARD TITRATIONS USING GREINER PLATES

Graph 1. Serum 1:500;Conjugate 1:1600

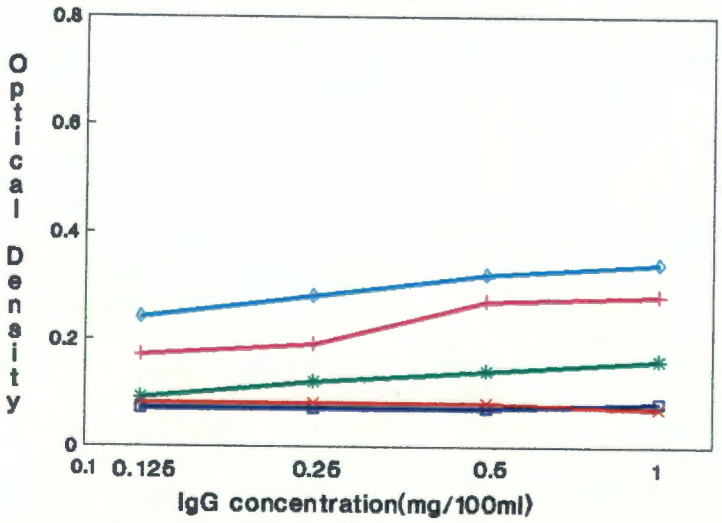


Graph 2. Serum 1:1000;Conjugate 1:1600

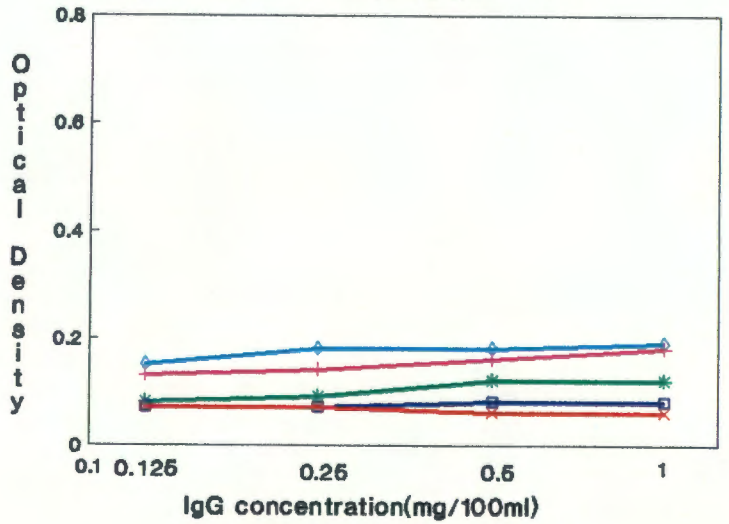


- High titre positive control serum
- Mid titre positive control serum
- Low titre positive control serum
- Negative control serum
- Background value

Graph 3. Serum 1:2000;Conjugate 1:1600

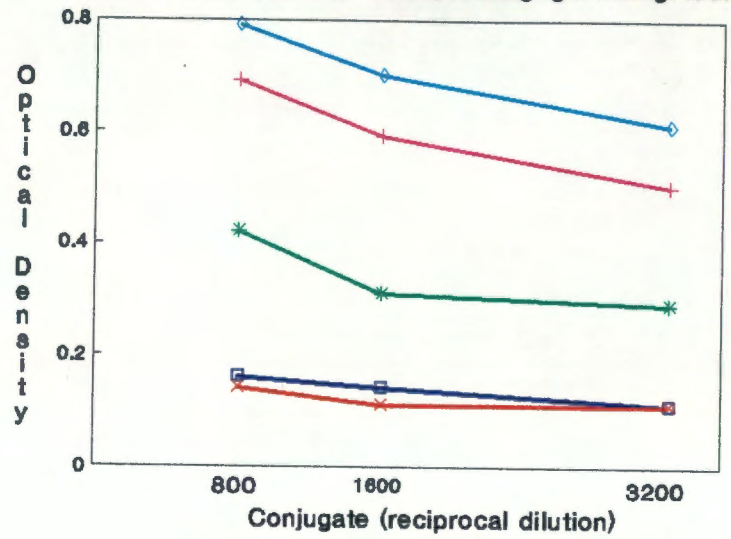


Graph 4. Serum 1:4000;Conjugate 1:1600

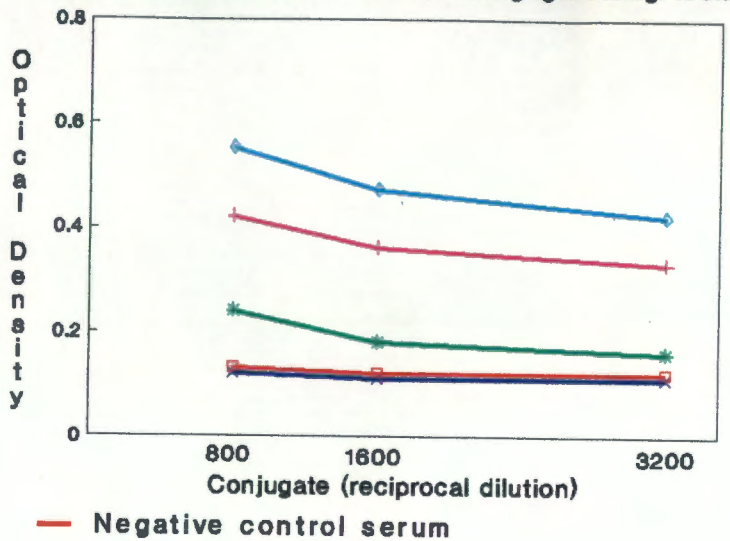


GRAPHS 5-8: CHECKERBOARD TITRATIONS USING GREINER PLATES

Graph 5. Serum dilution 1:500;Coating IgG 0.5mg/100ml

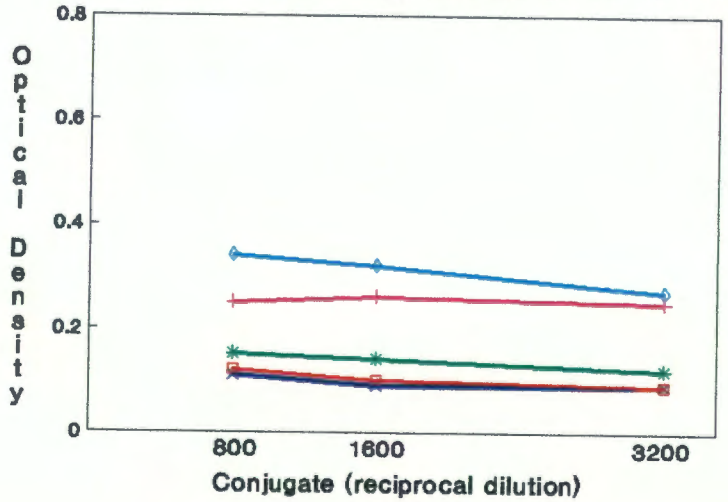


Graph 6. Serum dilution 1:1000;Coating IgG 0.5mg/100ml

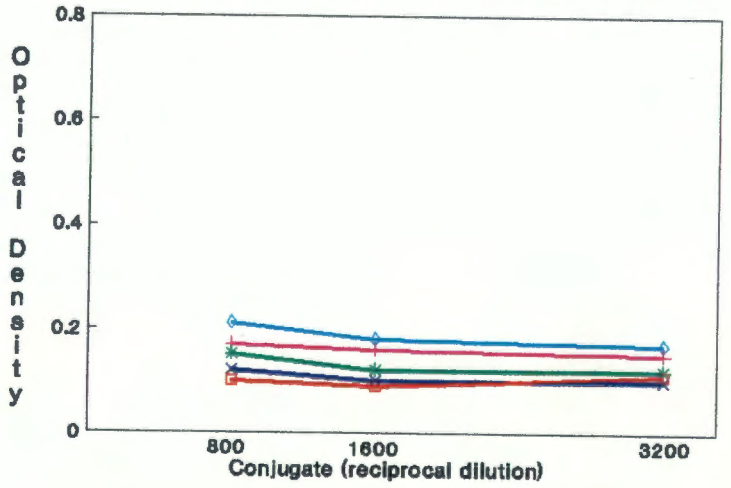


High titre positive control serum
Mid titre positive control serum
Low titre positive control serum
Negative control serum
Background value

Graph 7. Serum dilution 1:2000;Coating IgG 0.5mg/100ml

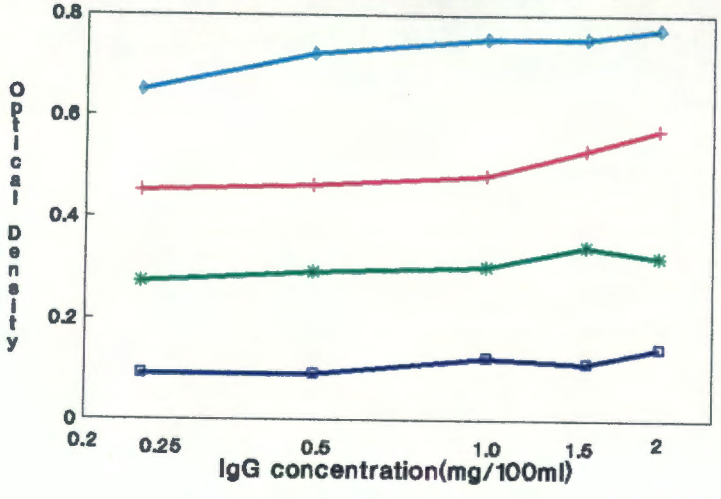


Graph 8. Serum dilution 1:4000;Coating IgG 0.5mg/100ml

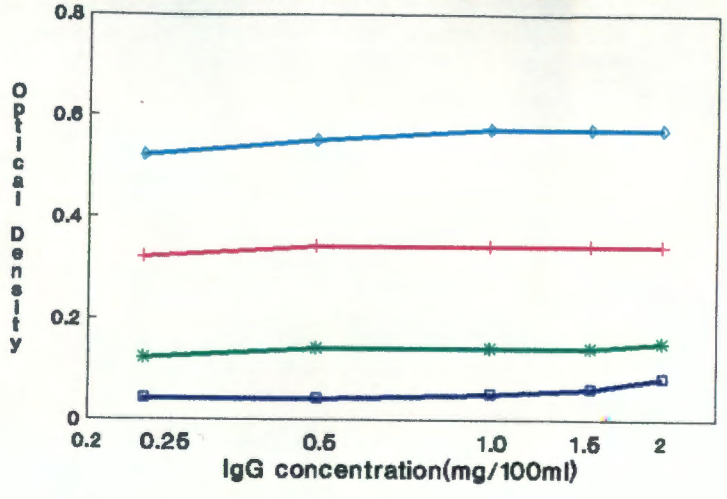


GRAPHS 9-12 : CHECKERBOARD TITRATIONS USING NUNC PLATES

Graph 9. Serum 1:500;Conjugate 1:2000

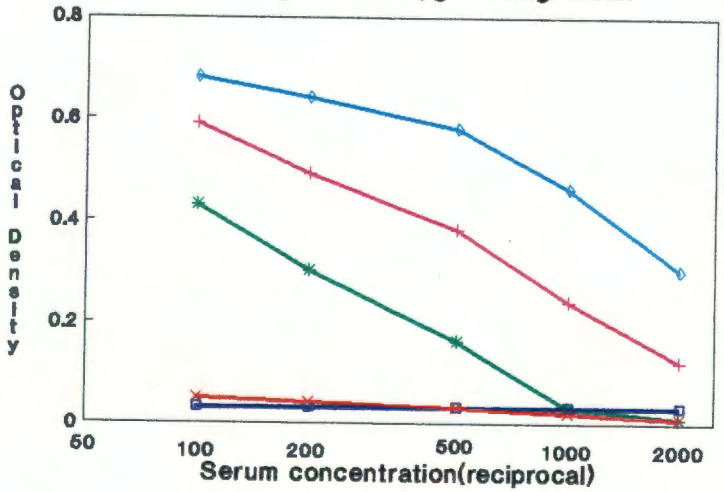


Graph 10. Serum 1:1000;Conjugate 1:2000

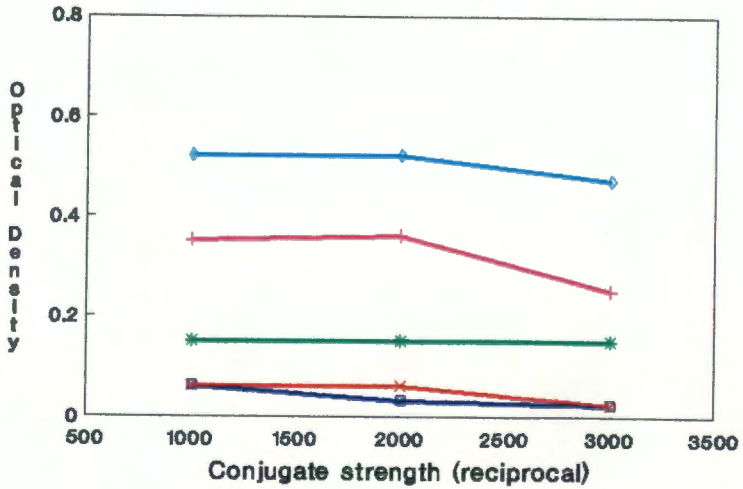


- High titre positive control serum
- Mid titre positive control serum
- Low titre positive control serum
- Negative control serum
- Background value

Graph 11. Conjugate 1:2000;IgG 0.5mg/100ml



Graph 12. Serum 1:1000;IgG 0.5mg/100ml



8.3 EVALUATION OF THE RF ELISA

Once the working strengths of the reagents had been determined the following steps were carried out to validate the ELISA.

8.3.1 Determination of the specificity of the RF ELISA

The specificity of the ELISA for RF

This was checked by comparing the results of the RF ELISA and the RF latex tests and by investigating the effect of RF removal.

Comparison of RF ELISA and RF latex test results

The RF ELISA was performed on the 34 patients with congenital syphilis who had positive RF latex tests and were described in Chapter 6 (patients 6.1 to 6.34). The RF ELISA results are recorded in Chapter 9. The important results for this section are that all the patients who had positive latex tests had a positive RF ELISA reading. Two patients were RF latex test negative, 1 of these (no. 6.6) gave a positive result with the RF ELISA, the other (no. 6.23) was negative.

Similar results were obtained with the two tests in the control patients at birth. All these patients were RF latex test negative (see Chapter 5) and, as described in Chapter 9, their sera gave insignificant readings with the RF ELISA.

Effect of RF removal

Rheumatoid factor was removed by 2 methods.

i. by addition of a sheep anti-human IgG: this precipitated IgG and RF with it (further described in Chapter 7) . Ten microlitres of serum in 40 ul of PBS pH 7.2 were added to 50 ul of sheep anti-human IgG. After incubating for 15 minutes at room temperature the solution was centrifuged at 300 G for 10 minutes. The supernatants obtained from 2 sera which were RF latex positive were tested in the RF ELISA at a dilution of 1:200.

ii. by addition of a HaIgG absorbent prepared from Cohn Fraction II by sodium sulphate precipitation using the method of Christian (1958). This technique is outlined in Chapter 9. Rheumatoid factor was removed by adding 80 ul of this sodium sulphate fraction 1 (containing 1.26 mg of protein per ml) to 250 ul of serum. After incubation for 30 minutes at room temperature the precipitate was removed by centrifugation at 1500 G for 10 minutes. Two sera, which were RF latex positive (from different patients to those above) were treated with the HaIgG and tested in the RF ELISA at dilutions of 1:4000 and 1:500 respectively.

When RF was removed from the 2 sera by means of IgG immunoprecipitation the results shown in Table 8.1 were obtained.

TABLE 8.1

SPECIFICITY OF THE RF ELISA AS DEMONSTRATED BY RF REMOVAL USING IgG IMMUNOPRECIPITATION

SERUM**	OPTICAL DENSITY READING*	
	Before IgG Immunoprecipitation	After IgG Immunoprecipitation
1	0.479	0.05
2	0.138	0.010

* the mean of duplicates after subtraction of backgrounds

** serum was diluted 1:200

The optical density values were shown to be markedly lower after the immunoprecipitation technique to eliminate RF.

Extracting RF by adding HaIgG also reduced the optical density readings in the RF ELISA. The details are shown in Table 8.2

TABLE 8.2

SPECIFICITY OF THE RF ELISA DEMONSTRATED BY RF REMOVAL USING HEAT-AGGREGATED IgG

SERUM**	OPTICAL DENSITY READING*	
	Before addition of heat-aggregated IgG	After addition of heat-aggregated IgG
3	0.21	0.10
4	0.24	0.01

* mean of duplicates after subtraction of backgrounds

** serum no. 3 was diluted 1:4000

serum no. 4 was diluted 1:500

Specificity of the ELISA for IgM RF

The ELISA method described is likely to detect mainly IgM RF (Faith et al 1982). The specificity of the ELISA for IgM class antibodies depends on the specificity of the goat anti-human IgM conjugate. The specificity of the conjugate was determined by an ELISA similar to that reported by Faith et al (1982). The procedure for the ELISA was the same as that detailed previously with some modifications.

The coating antigens were anti-human IgM, anti-human IgG and anti-human IgA. The first 2 antibodies were obtained from Dakopatts, Denmark (catalogue numbers A091, A090) and the anti-human IgA from Behring, West Germany (catalogue number ORCI 04,05). One hundred microlitres of the coating antigen at a

dilution of 1:100 in coating buffer was added and left at 4°C overnight. The plate was washed and blocked as previously described. Human serum from a normal adult control was added after being diluted 1:500 in PBS-Tween and the plate was again incubated at 4°C overnight. Following a washing step the goat anti-human IgM conjugate at a dilution of 1:500 in PBS-Tween was applied (for 1 hour). The plates were washed and substrate added for 1 hour after which the optical density was read at 405 nm.

The results are shown in Table 8.3

TABLE 8.3

THE SPECIFICITY OF THE GOAT ANTI-HUMAN IgM PEROXIDASE-LABELLED CONJUGATE FOR IgM CLASS ANTIBODIES

COATING ANTIGEN*	OPTICAL DENSITY READING**
Anti-human IgM	0.51
Anti-human IgG	0.025
Anti-human IgA	0.025

* Coating antigen used at 1:100 dilution

** Mean of duplicates after background values subtracted

It was demonstrated that very low readings were obtained with the anti-human IgG and anti-human IgA coating antigens.

To further examine the specificity of the RF ELISA for IgM class antibodies a pepsin digestion step was carried out. Pepsin digestion destroys IgM RF (Theofilopoulos et al 1974). The method of pepsin digestion is outlined in Chapter 9. The RF ELISA was performed on 5 RF latex positive sera (obtained from

infants with congenital syphilis) before and after treatment of the sera with pepsin. The final serum dilution after pepsin digestion was 1:200. Table 8.4 shows that pepsin digestion of the sera practically abolished the readings obtained in the ELISA.

TABLE 8.4

THE EFFECT OF PEPSIN DIGESTION ON THE OPTICAL DENSITY READINGS OBTAINED WITH THE RF ELISA

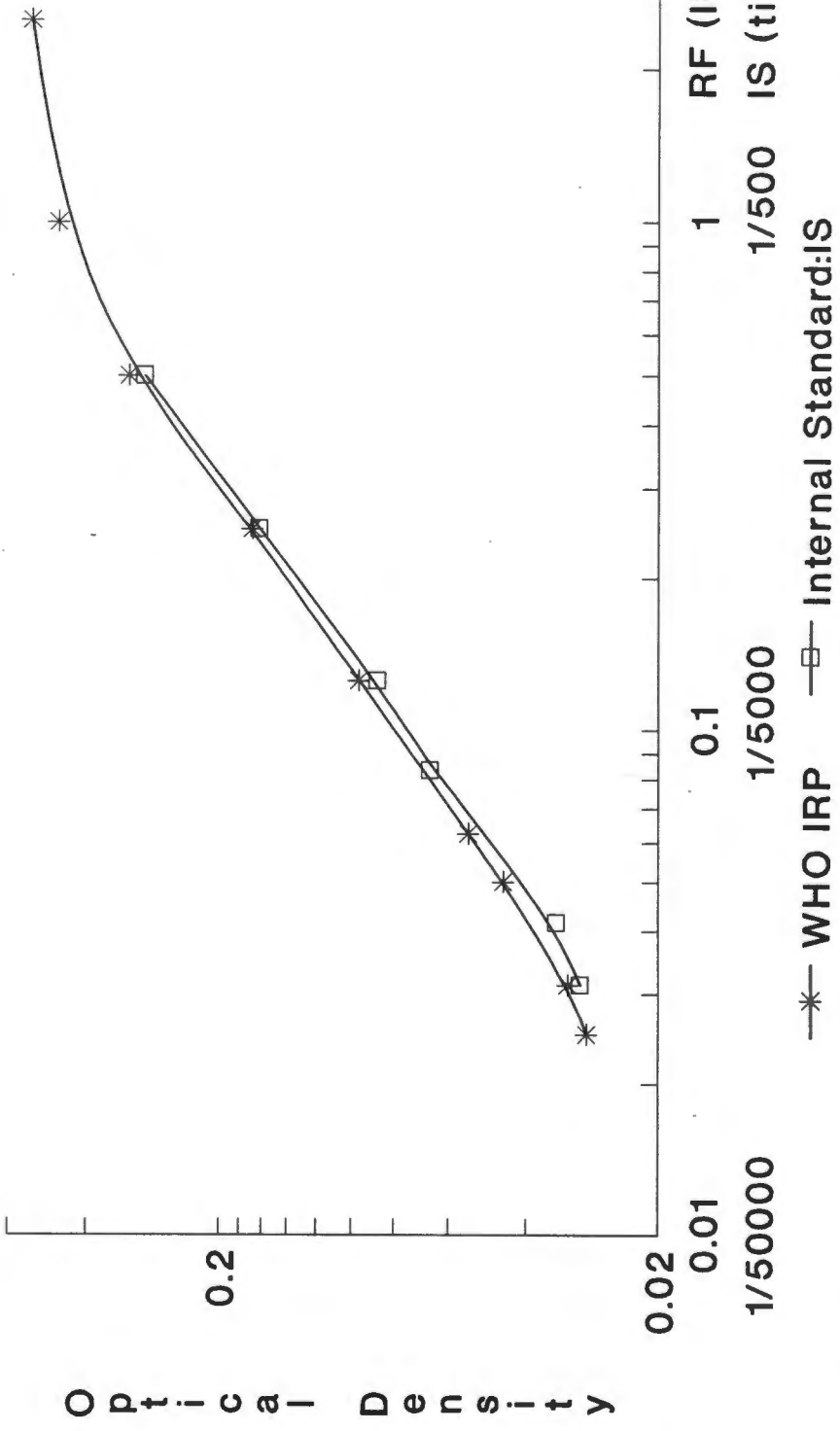
SERUM	BEFORE PEPSIN DIGESTION		AFTER PEPSIN	
	SERUM DILUTION	OPTICAL DENSITY READING*	SERUM DILUTION	OPTICAL DENSITY READING*
1	1:200	0.177	1:200	0.018
2	1:200	0.100	1:200	0.030
3	1:1000	0.180	1:200	0.009
4	1:2000	0.441	1:200	0.013
5	1:1000	0.400	1:200	0.008

* Mean of duplicates after background values subtracted

8.3.2 Standardization of the RF ELISA

This was done using the external (WHO) reference serum for RF and the internal standard (see Section 8.1). The ELISA was performed on the standards at dilutions from 1:100 - 1:32000. The graph in Figure 8.2 is representative of the results

Figure 8.2: Dose-Response Curves for the Rheumatoid Factor ELISA



WHO IRP:International Reference Preparation of Rheumatoid Factor

obtained. The 2 standards gave very similar results over a range of dilutions.

For standardization of the RF ELISA it was also necessary to show that parallelism existed between the test sera and the standard (Hamilton and Adkinson 1988 pg 66-69). This was achieved by assaying 2 sera over a range of different dilutions. The results showed that below a reading of 0.6, the optical densities of the test sera were parallel to those of the internal standard (see Figure 8.2).

8.3.3 Determining the cut-off values for the RF ELISA

The upper limit was determined by measuring dilutions of the external standard from 1:200 to 1:500. The point at which the graph of optical density versus concentration demonstrated a plateau effect was regarded as the upper limit of the ELISA.

It can be seen that above an optical density of 0.4 (background reading subtracted) the graph became definitely sigmoid in shape (see Figure 8.2).

The lower cut-off was also determined. The lowest optical density reading which was on the linear portion of the graph was determined using serial dilutions of the WHO standard.

The results obtained in 4 assays are shown in Table 8.5. It can be seen that very low concentrations of RF could be detected. The lowest optical density readings on the linear portion of the curve were between 0.032 and 0.053 with a mean of 0.042 (background values subtracted).

For practical purposes the lower cut-off value was regarded as an optical density of 0.05.

8.3.4 Reproducibility of the RF ELISA

Inter-assay variation

This was determined by measuring the RF content of 3 sera on different occasions. The sera chosen had high, intermediate and low RF concentrations respectively.

TABLE 8.5

THE LOWEST OPTICAL DENSITY READINGS FALLING ON THE LINEAR PORTION OF THE GRAPH OBTAINED BY PLOTTING OPTICAL DENSITY VERSUS SERIAL DILUTIONS OF THE WHO STANDARD

ASSAY	RF CONTENT OF WHO STANDARD (IU/ml)	LOWEST OPTICAL DENSITY ON LINEAR PORTION OF GRAPH*
1	0.05	0.045
2	0.031	0.032
3	0.05	0.038
4	0.024	0.053

* Optical density after background values subtracted

The inter-assay variation was as follows (values given are the means of duplicate samples).

High-titre serum: Values (in IU/ml) of 390, 360, 400, 380, 340, 370, 390, 360, 375 were obtained in different assays. The mean was 374 with a standard deviation of 18.7. The coefficient of variation was 5%.

Mid-titre serum: The concentration of RF (in IU/ml) obtained in different tests was 160, 150, 190, 160, 155, 140, 180, 200, 150, 190, 165, 155.

The mean was 167.3 with a standard deviation of 19.67. The coefficient of variation was 11.75%.

Low-titre serum: The amount of RF (in IU/ml) present in this serum when it was assayed on different occasions was 14, 15.3,

12.5, 12.5. The mean was 13.56 IU/ml with a standard deviation of 1.35. The coefficient of variation was 9.9%.

Intra-assay variation

This was measured by assaying the same serum on 12 occasions. The intra-assay variation was obtained for a high, intermediate and low titred sera.

The intra-assay variation is given below (results expressed in IU/ml).

High-titre serum: The results obtained in the same ELISA were 360, 400, 370, 380, 340, 370, 375, 390, 360, 400, 360, 390. The mean was 374.6 The standard deviation was 18.27 with a coefficient of variation of 4.87%.

Mid-titre serum: The RF concentrations measured when the sample was analysed 12 times were 156, 160, 156, 146, 142, 155, 150, 144, 152, 146, 134, 147. The mean was 149 with a standard deviation of 7.3 and a coefficient of variation of 4.9%.

Low-titre serum: When the serum was assayed 12 times the RF concentrations were 11.6, 12.6, 12.0, 11.5, 13.0, 12.6, 11.6, 14.6, 12.6, 12.6, 12.5, 11.3.

The mean was 12.36 with a standard deviation of 0.873 and a coefficient of variation of 7%.

8.3.5 Effect of storage of serum

Fresh WHO reference serum was obtained 1 year after the initial batch and the RF concentrations of the internal standard re-determined.

The mean RF concentration was 360 IU/ml compared to 370 IU/ml after storage. This difference was within the limits of the inter-assay variation for the ELISA.

Similarly, mean values of 147 IU/ml and 155 IU/ml after storage for 1 year (at -70°C) were obtained with another serum sample.

8.4 DISCUSSION

The RF ELISA was established with the aim of providing a sensitive, reproducible and quantitative test for RF. A number of variables which could affect the assay were examined.

8.4.1 Reagents and methods

Carrier surface

The Nunc and Greiner microtitre plates appeared to have a similar sensitivity for the detection of RF. However, the background readings were considerably lower with the Nunc plates. Nunc plates were used for subsequent assays.

Bampton et al (1985) compared the use of various microtitre plates for the detection of IgM RF in patients with RA. Although no significant differences were found with regard to the overall sensitivity, Linbro plates (Flow Laboratories Ltd, Scotland) were chosen as they were cheaper than the other plates (including Nunc plates).

Likewise, Stone et al (1987) evaluated the use of different types of plates (Linbro, Nunc, Falcon and Dynotec). These workers also chose Linbro plates, having found that background values were higher and/or reproducibility between wells was lower with other plates. The authors do not specify which plates were responsible for the problems, or the magnitude of the difficulty encountered.

Although Linbro plates were not tested in the present study, neither high backgrounds nor a lack of reproducibility were observed.

IgG

Human IgG was used for the RF ELISA. Whilst IgG from different sources has been studied, most workers have used human or rabbit material. Gripenberg et al (1979) compared human and rabbit IgG as coating antigen for an RF ELISA in patients with RA. Stronger reactions were found with the human IgG. In addition,

Bartfeld (1969) stated that binding of RFs from patients with nonrheumatoid conditions was more pronounced with human IgG.

Heat aggregation of the IgG increases the avidity of the latter for IgM RF (Egeland and Munthe 1983). Hence this procedure was employed in the assay. The human IgG was a pooled preparation, the reason being that this source would be likely to contain many of the IgG antigens recognized by RFs (Egeland and Munthe 1983).

The working concentration of IgG employed as a coating antigen was 0.5 mg/100 ml. Gripenberg et al (1979), Faith et al (1982), Procaccia et al (1987) and Moore et al (1988) all used the same IgG concentration.

Peroxidase-conjugated antibody, enzyme and substrate

The goat anti-human IgM conjugate utilized was found to be highly specific for IgM. Although Faith et al (1982) and Procaccia et al (1987) determined the specificity of the conjugate used, few other authors describing RF ELISAs state that this was done. For example, Gripenberg et al (1979), Bampton et al (1985), Gioud-Paquet et al (1987) and Stone et al (1987) do not mention that conjugate specificity was checked.

The F(ab')2 fragment of the conjugate was used in the present study. This was to prevent non-specific binding of RFs to the Fc portion of the conjugate (Carson et al 1977).

The conjugate strength chosen was 1:2000 which provided a low background value but a reasonable signal and was the most economical in terms of the use of reagents. The enzyme used in the ELISA was peroxidase. Peroxidase was chosen in preference to alkaline phosphatase as it was cheaper and readily available (Voller et al 1976).

The substrate employed was 2.2' azinobis. Bampton et al (1985) compared 2.2' azinobis and orthophenyl-diamine as substrates. It was found that the latter substance was more sensitive. However, the former substrate was selected for the present study because of local availability and experience with its use.

Buffers

In order to block nonspecific antibody absorption after the IgG coating step, solutions containing 1% and 2.5% BSA were evaluated. There was no significant difference observed in the background readings and a 1% solution was chosen. Stone et al (1987) also used 1% BSA. Bampton et al (1985), however, found that the blocking step could be omitted altogether. This may be worth further study because of the potential savings in terms of time and cost.

Incubation times

Voller et al (1980 pg 370) recommended 18 hour incubation periods at 4°C for both antigen coating and conjugate binding. For convenience, these time periods were chosen for the present ELISA.

Few workers have reported the effects of different incubation times on the RF ELISA. However, Bampton et al (1985) found that maximal binding of rabbit IgG to the polystyrene plates occurred after overnight incubation at 37°C. Whether the improved binding altered the sensitivity of the assay is not stated.

Heat inactivation of sera

This appears to be unnecessary (Carson et al 1977; Gripenberg et al 1979). With the exception of Bampton et al (1985) none of the authors quoted in this chapter used heat inactivated sera.

8.4.2 Evaluation of the RF ELISA

Having established the protocol for the RF ELISA the technique was evaluated and the results of this will be discussed.

Specificity of the ELISA for RF

The RF ELISA appeared to be measuring RF. This was concluded from the following findings:

i. the linear dose-response curve obtained with the external (WHO) standard

ii. the fact that all the patients with positive RF latex tests had a positive RF ELISA and vice versa.

iii. the reduction in optical density readings obtained with the ELISA following removal of RF.

Gripenberg et al (1979) and Faith et al (1982) reached similar conclusions in studies designed to test points ii) and iii) above. Neither of these authors used an external standard, however.

Specificity of the ELISA for IgM RF

That the RF measured was of the IgM class was suggested by a number of findings.

i. The peroxidase-conjugate was highly specific for IgM. This, however, only provides indirect evidence that the ELISA is measuring IgM RF.

ii. RF activity was lost following pepsin digestion. This step is known to destroy IgM RFs but not IgG or IgA RFs to the same extent (Theofilopoulos et al 1974).

iii. Tests employed to detect other classes of RF were consistently negative. These tests are described in Chapter 9 and included an immunoabsorbent column and an IgA ELISA.

iv. The RF latex test detects IgM RF predominantly (Stage and Mannik 1972-73).

As noted previously, there was a close association between the results of the latex tests and the RF ELISA results.

Faith et al (1982), Procaccia et al (1987), Stone et al (1987) and others have employed a similar RF ELISA to detect IgM RF.

Standardization of the RF ELISA

This was achieved using the WHO reference preparation. Subsequent ELISA results were expressed in terms of IU/ml. Klein and Janssens (1987) have reported on the use of the WHO

standard and have demonstrated that the use of international units improves the comparison of data between laboratories.

The internal standard chosen was shown to reflect the RF concentration over a wide range of dilutions.

Furthermore, it could be shown that the linear portion of the curves obtained with the internal standard and the sera of 2 positive control patients, were parallel. This is important as it means that the sera could be tested at any dilution within the upper and lower ranges of the ELISA (Hamilton and Adkinson 1988 pg 66-69).

Upper and lower cut-off values of RF ELISA

The upper limit was chosen as an optical density of 0.4 and the lower cut-off limit as 0.05 (after the background readings were subtracted). Other workers do not appear to have reported the upper and lower cut-off limits. Nevertheless, the ELISA evaluated in the present study was able to detect less than 0.05 IU/ml of RF. This can be contrasted to the sensitivity of the RF latex test which is 0.7 IU/ml (Orthodiagnostics package insert). The ELISA has previously been reported to be a more sensitive test for the measurement of RF than the latex test (Gioud-Paquet et al 1987).

Reproducibility of the RF ELISA

The results obtained in the present study were compared to those obtained by other workers. Gripenberg et al (1979) found an inter-assay coefficient of variation of 20% and an intra-assay coefficient of variation of 11% at a serum dilution of 1:500. Faith et al (1982) reported that the inter-assay coefficient of variation (15 sera tested on 2 occasions) was 'less than 10%'. A similar value was obtained for the intra-assay coefficient of variation (sera tested in triplicate). Stone et al (1987) reported inter and intra-assay coefficients of variation of 8% and 6% respectively. Procaccia et al (1987) do not state the reproducibility of their RF ELISA. Kemeny and Chantler (1988 pg 22) recommended that the upper, middle and lower ranges of the curve be tested. Unfortunately, none of the authors quoted

state which portion of the curve was tested. This makes if difficult to compare results. Nevertheless, the inter-assay coefficient of variation of between 5% and 11.7% and the intra-assay coefficient of variation between 4.9% and 7% obtained in the current study were thought to be acceptable.

Effect of storage of serum

The effect of the RF concentration of storage for one year at -70°C was demonstrated, by means of the use of fresh external standard, to be minimal. This does assume that the external (WHO) standard maintains its potency. Some workers have questioned this (Klein and Janssens 1987). Nevertheless, Wernick et al (1981) showed that repeated freezing and thawing of sera (on 4 occasions) had no effect on the RF levels.

8.5 CONCLUSIONS

This chapter describes the establishment of an RF ELISA. It was shown that the technique was specific for RF of the IgM class and gave reproducible results. In addition, the test sensitivity was higher than that expected for the RF latex test (according to the package insert). In the work which follows, the ELISA was used to quantitate RF concentrations in patients with congenital syphilis and to establish a normal range amongst controls.

CHAPTER 9

RHEUMATOID FACTORS IN CONGENITAL SYPHILIS

QUANTITATION AND CHARACTERIZATION

Infants with congenital syphilis frequently have RF in their sera as demonstrated by the use of the RF latex test (Reimer et al 1975; Meyer and Malan 1987).

The results of studies described in Chapters 5 and 6 support these findings. The IgM RF response in infants with congenital syphilis was further investigated by performing a number of experiments:

i. RF was quantitated more accurately using the IgM RF ELISA which was described in Chapter 8. The IgM RF concentrations in patients with congenital syphilis were compared to those of controls.

ii. The presence of hidden IgM RFs was sought.

iii. The contribution made by IgM RF to the total IgM response was determined.

In addition, techniques were used to measure RFs of the IgA and IgG immunoglobulin classes.

9.1 IGM RF CONCENTRATIONS IN INFANTS WITH CONGENITAL SYPHILIS AND CONTROLS

9.1.1 Study outline

Three groups of patients and controls were considered. The groupings were made on the basis of age because IgM anti-IgG is more likely to be found in the serum of normal infants as they grow older (Wilson and Steinberg 1965; Speiser 1966).

The age categories were:

i. within 3 days of birth

ii. between the ages of 4 days and 2 months

iii. older than 2 months but less than 4 months of age.

These groups wee chosen because the control infants (previously described in Chapter 5) were followed-up at these ages.

9.1.2 Materials and methods

Serum samples were obtained as follows:

Sera from infants within 3 days of birth

There were 26 newborns with congenital syphilis in this group. These infants have previously been described in Chapter 5 (nos. 5.1-5.7) and Chapter 6 (nos. 6.1-6.19).

Controls: The sera from 96 normal newborn infants were randomly selected using random number tables. The infants were selected from those described in Chapter 5. They did not have congenital syphilis. This was demonstrated either by the results of extended follow-up or by the fact that the STS in mother and baby were negative at birth.

Sera from infants aged 4 days - 2 months

There were 9 infants with congenital syphilis in this age group (nos 6.20 - 6.29). Their clinical features have been described in Chapter 6.

Controls: Sera from 39 infants described in Chapter 5 were used as controls. These infants had all been followed-up between the ages of 1 and 2 months and did not have congenital syphilis.

Sera from infants over the age of 2 months but less than 4 months old

Six of the infants with congenital syphilis were in this age group at presentation (nos 6.30 - 6.36).

Controls: Forty-one of the infants followed-up between the ages of 3 and 4 months were selected as controls.

The sera from infants in the groups above were thawed by allowing them to come to room temperature. They were then vigorously mixed and tested in duplicate in the IgM RF ELISA. Sera from patients with congenital syphilis were initially tested at a dilution of 1:500. Samples from the controls and from infants with congenital syphilis whose ELISA was negative at 1:500 were re-tested at a dilution of 1:100.

9.1.3 Results

Within 3 days of birth

The IgM RF concentrations in the patients with congenital syphilis in this age group are shown in Appendix 6 (Table A.6.1). The median RF concentration was 29.5 IU/ml with a range of 0-850 IU/ml.

When the 96 sera from the control patients were tested in the RF ELISA the results shown in Appendix 6 (Table A.6.2) were obtained. It was found that the optical density readings (after the background values were subtracted) were below the lower limit of sensitivity for the RF ELISA. This was apparent even when the sera were analysed at a dilution of 1:10. For this reason the optical density readings are shown rather than the RF concentration in IU/ml.

The median optical density was 0.016 and the 95th percentile was 0.039 which, at a dilution of 1:100, corresponded to an RF concentration of 2.5 IU/ml.

Infants aged 4 days - 2 months

The RF concentrations in the sera of the 9 infants with congenital syphilis in this age group are also shown in the Appendix 6 (Table A.6.3). The median RF level was 80 IU/ml with a range of 14-238 IU/ml.

When the RF concentration was measured in the 39 controls the median was 3.2 IU/ml and the 95th percentile was 17.5 IU/ml.

Infants over the age of 2 months but less than 4 months old

The 6 infants in this group with congenital syphilis had a median serum RF value of 46 IU/ml; the range was 0-520 IU/ml. The median RF concentration in the 41 control sera was 5.0 IU/ml and the 95th percentile was 29.5 IU/ml (see Appendix 6, Table A.6.4).

The results are summarized in Figure 9.1. Between birth and 3 days of age, 24 (92%) of the 26 infants had RF levels above the 95th percentile of the controls. Infants with congenital syphilis aged 4 days - 2 months had elevated RF levels in 8 out of 9 (89%) of cases. In the oldest group (over 2 months - 4 months) 4 of the 6 infants had RF levels which were raised with respect to the controls.

It can also be seen from Figure 9.1 that the upper limit of normal increased with increasing age of the controls.

9.1.4 Discussion

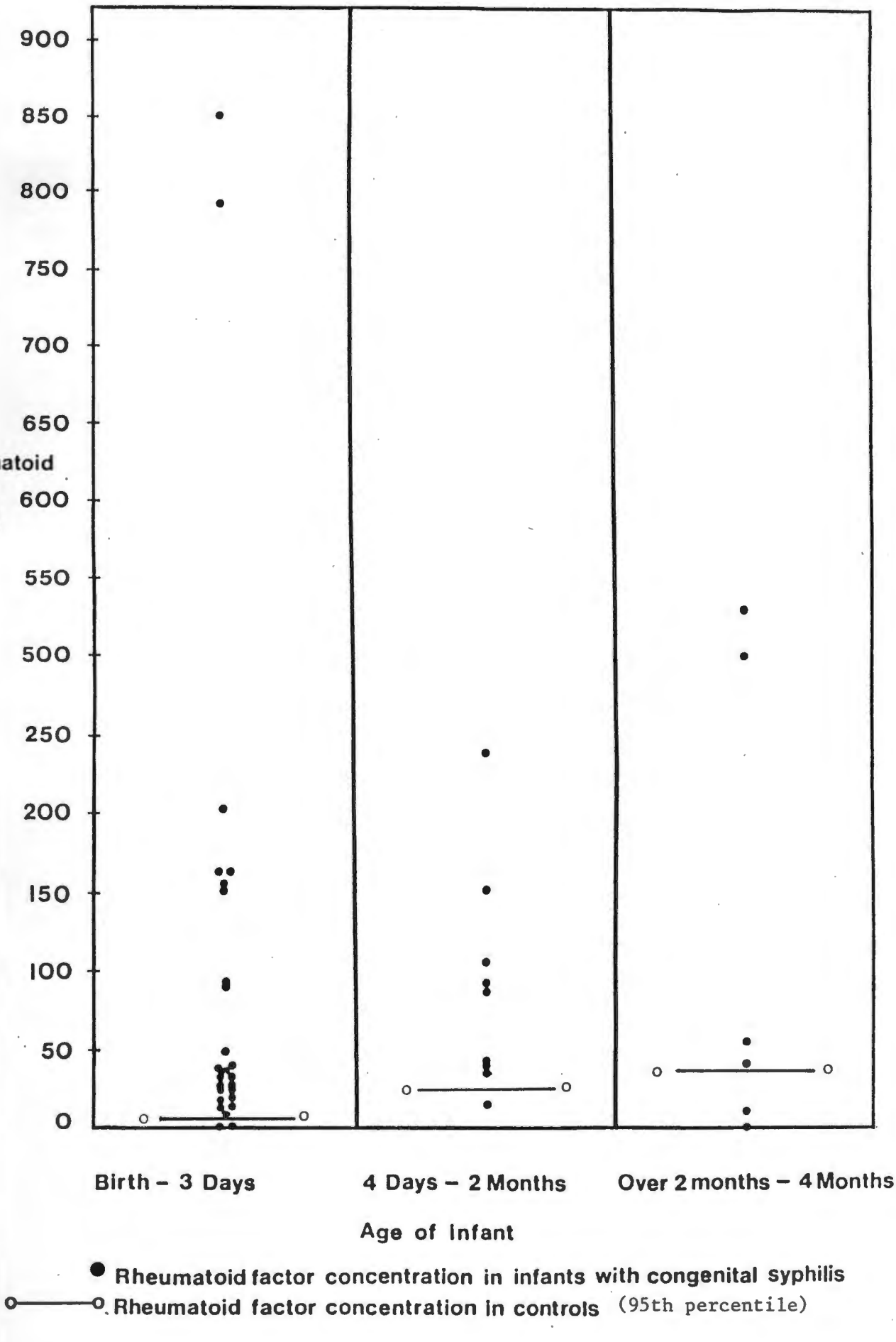
Patients with congenital syphilis

Most of the patients with congenital syphilis described in this chapter had serum RF concentrations which were above the upper limit of age-matched control infants.

This trend was particularly noticeable among the newborn infants with congenital syphilis. Of those presenting at birth 24/26 had elevated IgM RF levels as measured with the IgM RF ELISA. All except one (no. 6.6) of the 24 with elevated RF concentrations had positive RF latex tests. The 2 patients who had no detectable RF by ELISA were also latex test negative.

Of the older patients with congenital syphilis, 14 out of 15 had IgM RF detectable with the ELISA. However, 2 of the 14 (nos. 6.32, 6.34) had a RF level within the normal range for age. The RF latex test was positive in the 14 infants where RF was detected by ELISA. It should be noted, however, that the RF latex test was positive in 10% of control infants who were tested between 1 and 4 months of age (see Chapter 5)

Figure 9·1 IgM Rheumatoid factor levels in infants with congenital syphilis at different ages compared to the upper limits of normal



It is interesting to speculate why serum RF was not detected in some patients.

It is possible, as suggested by Rossen et al (1977) that the RF was deposited in tissues. Another explanation may be that low levels of immune complexes were present in these patients. This was established to be the case in 2 instances (patients 5.3 and 5.7 had levels of 6% and 0% respectively). There was insufficient serum to measure immune complex levels in patient 6.23 (see Chapter 10 for details of immune complex measurements).

Controls

Using the RF ELISA different normal ranges were obtained at various ages. The newborn infants had the lowest values and infants from 2 - 4 months the highest. However, the fact that the control infants were sampled at 2 distinct ages prevents a true longitudinal assessment from being made.

Stagno et al (1980) have described that the prevalence of positive RF latex tests increases with age in early infancy. They found negative tests in all 676 normal newborns studied but positive tests in 1 out of 7 (14%) infants at 1 month and in 4 out of 32 (12%) at 3 months.

The reason for the normal infants having demonstrable RF (or IgM anti-IgG antibody) is unclear. However, Steinberg and Wilson (1963) and Wilson and Steinberg (1965) have described that normal infants may produce IgM antibodies against maternal IgG allotypes. Allotypic markers on IgG may be of the Gm specificity and these Gm markers are genetically determined (Egeland and Munthe 1983). Presumably the infant may recognize the maternal allotypes as foreign. Speiser (1966) reported that anti-Gm antibodies appear at 6 months of age and peak at 12-18 months.

Rheumatoid factors may be directed against Gm antigens on IgG (Mannik 1979 pg 504). Therefore as suggested by Reimer et al (1975) the IgM RF found in some normal infants after birth may

represent the presence of anti-allotypic antibodies.

If this is so, the present study indicates that these antibodies may be measurable before 6 months of age. Indeed, Steinberg and Wilson (1963) reported anti-allotypic antibodies in an infant aged 7 weeks and suggested that the antibody may be present from birth but undetectable by the methods then in use.

The demonstrated rise in IgM RF levels between birth and 4 months in the present study may be in keeping with the rise in anti-Gm antibodies noted by others (e.g. Wilson and Steinberg 1965, Speiser 1966), the earlier onset in this study reflecting the use of the more sensitive ELISA detection system.

Whatever the cause of the raised IgM RF levels in normals, their presence limits the usefulness of measuring RF levels by ELISA to diagnose congenital syphilis beyond the first 2 months of life. Regarding the diagnosis of congenital syphilis in the infants described, it was apparent that the ELISA did not have substantial advantages over the simpler RF latex test. The lack of significant diagnostic benefit achieved using the RF ELISA instead of the RF latex test has also been reported in RA (Stone et al 1987).

The purpose of accurately quantitating the IgM RF levels in the infants with congenital syphilis was to determine if associations existed between RF levels and clinical and laboratory findings. This is further investigated in Chapter 10.

9.2 TESTING FOR HIDDEN IGM RF IN INFANTS WITH CONGENITAL SYPHILIS

The reactivity of serum IgM RF may be concealed by the formation of complexes with circulating IgG. The conventional methods used to detect RF may then be inadequate (Allen and Kunkel 1966). This has been shown to occur in juvenile chronic arthritis, for example, where only 5-10% of patients have positive RF latex tests but over 60% are reported to have hidden RF (Moore et al 1988).

The aim of this section was to determine the concentration of

hidden RF amongst infants with congenital syphilis. It would then be apparent if steps to measure hidden RFs were necessary to obtain an accurate estimate of the total serum RF concentration.

Allen and Kunkel (1966) described a method of acid treatment and exclusion chromatography to detect hidden RF. However, this is time consuming. Dorner et al (1983) found that ion exchange chromatography gave comparable results. The latter method was therefore chosen for this study.

9.2.1 Materials and methods

Sera

Latex test negative sera: Sera from 9 patients with congenital syphilis whose RF latex tests were negative were studied. The patient code numbers are shown in Table 9.1. In addition, sera from 2 normal newborns (5.68 and 5.69) were used as negative controls.

Latex test positive sera: Sera from 7 infants with congenital syphilis whose RF latex tests were positive were also tested for hidden RF. The first 7 patients with sufficient serum (200 ul) were chosen.

Methods

Commercially prepared ion exchange columns (Quick-Sep, System II, obtained from Isolab, USA) were used. These columns were designed to separate IgM from IgG (Johnson and Libby 1980) and were found to be suitable for detecting hidden RF (Dorner et al 1983). The manufacturers instructions were followed. Briefly, the serum was diluted in the IgG wash buffer and applied to the column. Further wash buffer was added to remove unbound material. An IgM elution buffer was added and the IgM eluate (at a dilution of 1:20) was collected.

The RF latex test and RF ELISA were used to detect RF in the sera and IgM eluate. The RF ELISA was carried out at a final dilution of 1:100 and the latex test at a dilution of 1:20.

TABLE 9.1

RHEUMATOID FACTOR LEVELS IN SERA AND IgM FRACTIONS IN INFANTS WITH CONGENITAL SYPHILIS

	SERUM		IgM FRACTION	
PATIENT CODE NO.	RF LATEX TITRE	RF ELISA (IU/ml)	RF LATEX TITRE	RF ELISA (IU/ml)
5.1	1:160	24	1:80	0
5.3	<1:5	0	<1:20	0
5.7	<1:5	0	<1:20	0
5.8	<1:5	12	<1:20	0
5.11	<1:5	3	<1:20	0
5.12	<1:5	0	<1:20	0
5.13	<1:5	0	<1:20	0
5.14	<1:5	0	1:80	0
5.15	<1:5	0	<1:20	0
5.68	<1:5	0	<1:20	0
5.69	<1:5	0	<1:20	0
6.6	<1.5	50	<1:20	5
6.9	1:80	165	<1:20	76
6.13	1:160	34	1:80	3
6.17	1:320	3	1:20	0
6.20	1:80	38	1:80	10
6.26	1:160	500	ND*	106
6.33	1:160	96	1:80	22

*ND - not done

In addition, the RF ELISA was used to determine the RF concentration in the IgG fraction obtained from 2 sera. This was done to obtain an estimate of RF losses in this fraction.

9.2.2 Results

The table shows the RF values in the sera and the IgM fractions.

It can be seen that the RF concentrations obtained with the ELISA were generally lower following passage through the ion exchange columns.

It could be shown that RF was lost in the IgG fraction. The RF concentrations in this fraction of patients 6.9 and 6.26 were 8 and 15 IU/ml respectively.

Hidden RF was only detected in the IgM fraction using the RF latex test in one case (5.14). The RF ELISA in this case remained negative following the ion exchange procedure.

9.2.3 Discussion

The method was easy to carry out. It was apparent, however, that substantial losses of RF occurred. Some of the RF was present in the IgG fraction. Presumably the remainder was not eluted from the column.

Dorner et al (1983) were able to recover over 60% of the total serum IgM in the IgM fraction. However, it was not shown that RF recovery was similar. Moore et al (1988) used an RF ELISA to measure RF concentrations in the IgM fractions obtained from 30 patients with juvenile chronic arthritis using an ion exchange column. In 15 cases lower RF values were noted in the IgM fraction following the procedure. In 10 cases the RF values were the same while in 5 cases they were higher. It is apparent, therefore, that RF losses were also experienced by these workers.

Hidden IgM RF was only detected in 1 case in the present study. One of the limitations of the method was that the RF latex test had to be carried out at a dilution of 1:20 on the IgM fraction. Nevertheless, even the more sensitive RF ELISA did not detect hidden RF in the IgM fraction.

It was evident that no significant benefit was experienced using the ion exchange method to measure hidden RF. The procedure was

not carried out in subsequent tests. Indeed, Moore et al (1988) have proposed that the IgM RF ELISA measures hidden and classical RF without the need for IgM separation.

9.3 RHEUMATOID FACTOR LEVELS AND THE TOTAL IGM RESPONSE

It is known that infants with congenital syphilis may have elevated levels of serum IgM (Alford et al 1967). It is not known, however, what proportion of the total IgM response in congenital syphilis is IgM RF.

Results of in vitro experiments showed that newborn umbilical cord blood cells were capable of producing large amounts of IgM RF when stimulated with Staphylococcus aureus Cowan 1 (Levinson et al 1987). It could be hypothesized, therefore, that a high percentage of the IgM produced in congenital syphilis would be IgM RF.

The most direct way of determining the amount of IgM that was RF would be to measure RF concentrations using the RF ELISA and then to relate these to the total IgM levels, However, the WHO standard measures RF in IU/ml (Anderson et al 1970). This cannot be directly converted to IU/ml of IgM. Wernick et al (1981) overcame this problem by preparing a purified RF standard. This work cannot be extrapolated to other studies, however, as these workers did not compare their standard with an international standard.

In order to repeat this work a similar method was used to isolate IgM RF from the serum of an infant with congenital syphilis. The RF and IgM concentrations of this purified standard were then determined simultaneously.

9.3.1 Materials and Methods

An immunoabsorbent method, based on the batchwise procedure described by Avrameas and Ternynck (1969), was followed.

Insolubilized gammaglobulin cross-linked to glutaraldehyde was used to absorb RF from test sera. The RF was subsequently

eluted under acidic conditions (Torrigiani and Roitt 1967; Wernick et al 1981).

Preparation of insolubilized gammaglobulin

The method of Avrameas and Ternyck (1969) was used to cross-link gammaglobulin and glutaraldehyde. Human Cohn Fraction II (1.5 g in 30 ml 0.1 M phosphate buffer pH 7) was added to glutaraldehyde (6 ml of a 2.5% solution). The gammaglobulin (obtained from ICN Immunologicals, Lot no 50) was added dropwise to the glutaraldehyde (obtained from BDH Chemicals, England). The resulting gel was allowed to stand for 3 hours at room temperature. The gel was then mixed with 400 ml of 0.2 M phosphate buffer pH 7.3 and homogenized.

The mixture was placed in a beaker, stirred, and allowed to settle at 4°C overnight. The supernatant was decanted and the precipitate mixed with 100 ml of Tris-saline pH 7.4. After a period of 30 minutes the supernatant was again discarded. This procedure was repeated 4 times with the intervals between stirring and decanting being decreased to 15 minutes, 10 minutes, 5 minutes and 2 minutes. In this way fine particles were removed. The remaining insolubilized protein was then washed twice in 200 ml of 0.1 M glycine-HCl buffer. The immunoabsorbent was finally washed twice with Tris-saline pH 7.4 and the optical density of the supernatant was found to be 0 at 280 nm (a Beckman spectrophotometer, model 25, was used).

Serum samples: these were obtained from:

i. A patient with congenital syphilis whose RF latex test and IgM RF ELISA tests were positive. The patient has been described in Chapter 6 (patient code number 6.12). The serum RF concentration was 788 IU/ml and the serum was shown to contain only IgM RF (see Sections 9.4, 9.5 and 9.6).

ii. A control patient (no. 5.120) whose RF latex and RF IgM ELISA tests were negative.

Prior to immunoabsorption, the sera (2 ml from each patient) were heated to 56°C for 30 minutes to inactivate complement.

Batchwise procedure of immunoabsorption

Avrameas and Ternynck (1969) used approximately 60 mg of insolublized protein per ml of serum. The immunoabsorbent prepared as described above contained 41 mg of gammaglobulin per ml. Therefore, 1.5 ml of immunoabsorbent was used per ml of serum.

The immunoabsorbent was placed in glass test tubes (12 x 75 mm) and serum added. The samples were incubated for 1 hour at 37°C in a waterbath (a mechanical shaker was used to ensure thorough mixing). They were then left at 4°C overnight (Wernick et al 1981). They were allowed to return to room temperature (Nardella and Mannik 1979 noted less non-immunospecific protein-protein binding at this temperature) and centrifuged at 350 G for 15 minutes. The supernatant was carefully removed, and analysed with the RF ELISA to detect unbound RF. The immunoabsorbent and bound material were then washed with Tris-saline pH 7.4 (Wernick et al 1981). Repeated washing followed by centrifugation at 350 G for 5 minutes was carried out until the optical density of the supernatant at 280 nm was less than 0.035.

Elution

The immunoabsorbed protein was then eluted with 0.1 M glycine HCl at pH 2.8. One millilitre of the latter was added to the immunoabsorbent and the solution agitated with a vortex mixer. Following centrifugation at 350 G for 5 minutes the eluate was obtained. The procedure was repeated following the addition of 1 ml of Tris-saline 7.4. This buffer was used rather than glycine -HCl to obtain a second eluate as more than 1 exposure to the latter has been shown to destroy RF (Wernick et al 1981). The eluates were neutralized with 1 or 2 drops of 0.1 M sodium hydroxide to obtain a pH of 7.2 - 7.4.

Analysis of eluates

The eluates were concentrated approximately 20 times using a Centricon 30 microconcentrator (Amicon, USA, catalogue number 4208). The IgM RF levels of the eluates were measured before and after concentration to assess whether RF was lost during this step.

The IgM and IgM RF concentrations were then measured and the means obtained. IgM levels were determined on radial immunodiffusion plates (Immuno-plate IV, Hyland Diagnostics, USA). The IgM RF levels were measured using the RF ELISA, the preparation being tested at a dilution of 1:50.

9.3.2 Results

The supernatant obtained following exposure of the serum containing RF to the immunoabsorbent contained 65 IU/ml of RF. This indicated incomplete absorption.

The concentration step did not result in further loss of RF, however. This was demonstrated by finding 3.8 IU/ml of RF in the eluate before concentration and a mean of 77.5 IU/ml afterwards.

When the concentrated eluate from the patient with congenital syphilis was assayed with the IgM RF ELISA the following RF concentrations were obtained: 72.5, 72.5, 85, 80 IU/ml. The mean was 77.5 IU/ml with a standard deviation of 6.1. The IgM levels were 19.0, 19.0, 20.5 IU/ml. The mean was 19.5 IU/ml with a standard deviation of 0.87. It was evident, therefore, that 1 IU of IgM corresponded to 4 IU of RF.

The eluates from the control patient contained no RF or IgM.

Percentage of IgM which is RF in infants with congenital syphilis

Using the conversion factor obtained from the purified IgM RF standard, the percentage of the IgM response which was RF was

calculated for each of the patients with congenital syphilis who were described in section 9.1.

The data is shown in the table in Appendix 6 (Table A.6.5) and summarized in Figure 9.2. The mean amount of IgM which was IgM RF was 4% with a range 0 - 29% and a median value of 3%.

9.3.3 Discussion

Preparation of the IgM RF standard

It was demonstrated that not all the IgM RF was removed from the test serum following exposure to the immunoabsorbent. Other workers have obtained similar results (Lea and Ward 1972; Wernick et al 1981), although Torrigiani and Roitt (1967) reported minimal RF losses during this step.

The reason for the variable RF absorption is unknown, but may be explained by the fact that different RFs have different affinities for IgG (Egeland and Munthe 1983). Thus, higher affinity RFs would tend to be absorbed whilst lower affinity ones may not attach to the immunoabsorbent.

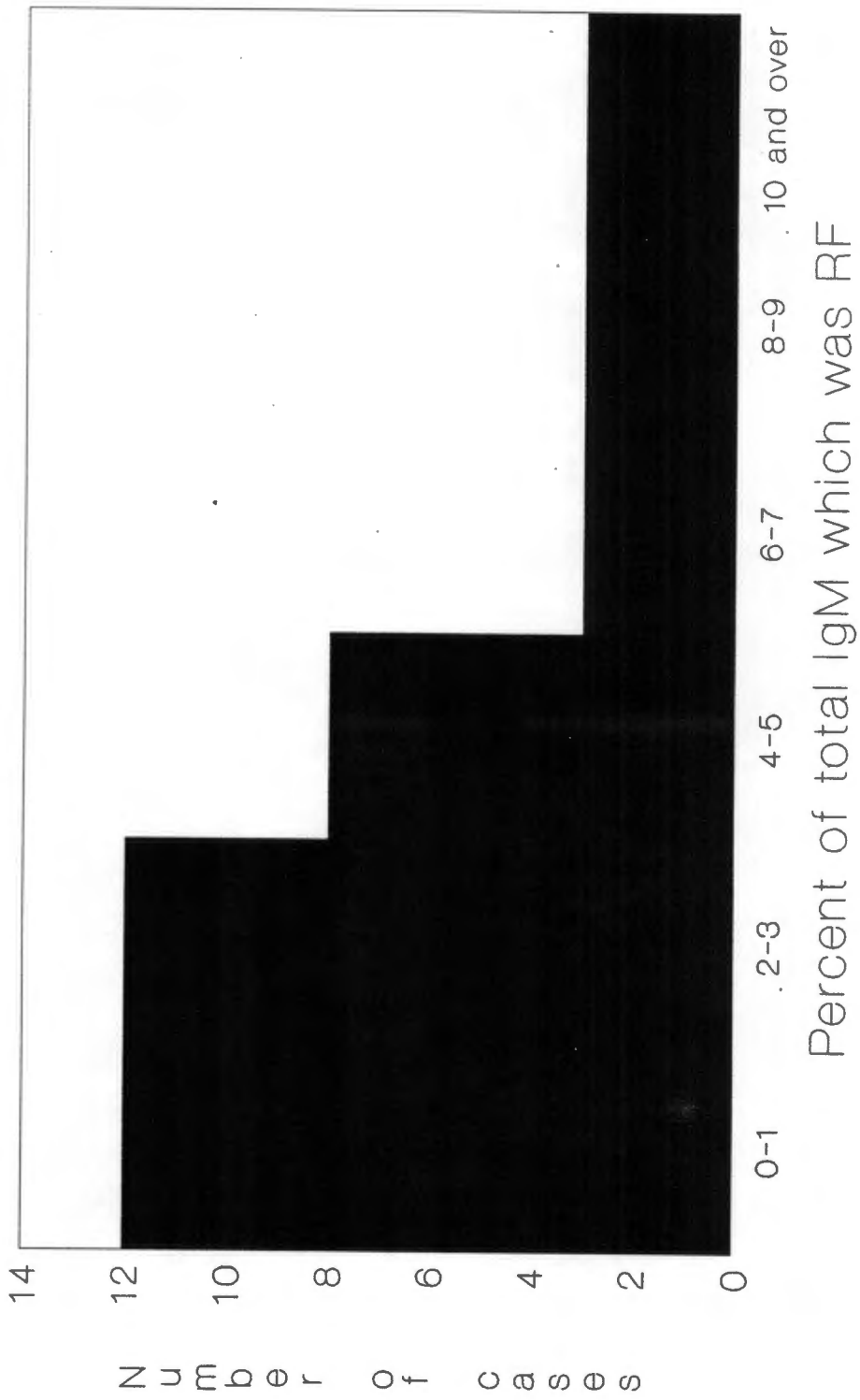
This is one of the disadvantages of the method in that the RFs in the sample chosen to make a standard may have stronger or weaker binding constants than the RFs in the test sera.

This would effect the estimation of IgM RF levels. Wernick et al (1981) appear to have ignored this, as Mannik and Nardella (1985) have pointed out. To overcome this problem in future, another method of standardizing the RF ELISA to allow quantitation of the amount of IgM which is RF, would be needed.

Relationship between IgM RF and total IgM levels

The circulating IgM RF accounted for a variable amount of the IgM response in the infants with congenital syphilis with a mean of 4%. Although, in some cases, a high percentage of the IgM was RF (up to 29%), the median value was 3%. There was a statistically significant correlation between the total IgM levels and the amount of IgM which was RF. Spearman's Rank

Fig. 9.2: Percentage of total IgM which was IgM RF in infants with congenital syphilis



Correlation Coefficient was 0.66 which was significant at the 0,1% level ($p < 0.001$).

It might have been predicted that a greater amount of the IgM would be RF. Possible reasons for this being:

i. up to 75% of the B lymphocytes in cord blood belong to the Leu 1^+ subset which are the RF producing cells (Hardy et al 1987).

ii. the IgM RF secreted by cord mononuclear cells in response to stimulation with Staphylococcus aureus Cowan I accounted for 16,7% of the total IgM produced (Levinson et al 1987).

There are several explanations for the seemingly small percentage of IgM RF in the infants with congenital syphilis.

Firstly, the results of Levinson et al (1987) were obtained in vitro, not in vivo. It is conceivable that the infants with congenital syphilis did, in fact, produce larger amounts of RF but the latter may have been deposited in the tissues. Indeed, Ford and Kosatka (1982) have shown experimentally that RF attaches to the tissue-bound immune complexes. Such complexes have been demonstrated in the kidney and placenta in congenital syphilis (Wiggelinkhuizen et al 1973, Samson 1989) and may be present at other sites.

Secondly, different agents may have differing abilities to induce RF synthesis. For example, when stimulated with Ebstein-Barr virus, few RF producing cells were found in neonates (Fong et al 1985). The stimulatory effect of immune complexes containing treponemal antigens may, therefore, be less than that of the Staphylococcus aureus used by Levinson et al (1987).

Thirdly, as discussed previously, the standard used in the present study to determine the amount of IgM which was RF may have had an affinity for IgG which was different to that of the other RFs in the patients with congenital syphilis. Indeed, the same criticism could be applied to the work of Levinson et al (1987). These authors used a monoclonal RF from a patient with Waldenstrom's macroglobulinaemia and assumed that the RFs found

in the cultures from the cord blood cells all had the same affinity for IgG.

Interestingly, in 17 adult patients with RA, Wernick et al (1981) found that an average of 6% of the total IgM was RF. Patients with RA also have large numbers of Leu-1$^+$ B cells (Plater-Zyberk et al 1985). There are, therefore, similarities in the IgM RF responses seen in RA and congenital syphilis.

What of the remainder of the IgM produced in congenital syphilis? A percentage is undoubtedly IgM directed against T.pallidum itself (Scotti and Logan 1968). However, Reimer et al (1975) found that some infants with congenital syphilis did not make a significant amount of IgM against the spirochaete, but made more IgM RF. However, these workers did not directly measure the concentrations of either of these IgM antibodies. Therefore, an effort was made in the present study, to measure the amount of anti-treponemal (or specific) IgM using the method described by Altemeier et al (1969). The amount of specific IgM would then be compared with the concentration of IgM RF.

A treponemal suspension (Behring, catalogue no. ORMK 04/05) was incubated with serum from which RF and IgG had been removed using sheep anti-human IgG (as described in Chapter 7). After overnight incubation at 37°C the suspension was centrifuged and the supernatant tested for the presence of specific IgM using the FTA-ABS (IgM) test. The purpose was to measure total IgM levels before and after removal of specific IgM. However, in spite of repeated attempts made under a variety of conditions and with different sera, the specific IgM could not be removed.

The reason for this failure to remove the IgM in what was presumably an excess amount of antigen, is unknown. However, there is still much to be learnt regarding antigen-antibody interactions in syphilis (reviewed by Musher 1984 pg 296).

It is clear, however, that the IgM response in congenital syphilis has different components. This indicates that the RF production in congenital syphilis is part of a polyclonal B lymphocyte response. The stimulus for this response may be the

presence of immune complexes as has been shown by Pisko et al (1982). Using mononuclear cells from healthy adults these workers were able to show that under certain conditions, immune complexes composed of HaIgG led to a polyclonal IgM response which included IgM RF production in vitro.

9.4 IgG AND IgA RFS IN CONGENITAL SYPHILIS

Rheumatoid factors in these immunoglobulin classes have been detected in low concentration in normal individuals but high levels occur in patients with a wide variety of infections and autoimmune diseases (Gharavi et al 1985).

High levels of IgG RF have been associated with vasculitis, subcutaneous nodules and more severe forms of RA (Scott et al 1981).

Concentrations of IgA RF have been found to correlate with the severity and activity of the disease and also with the presence of systemic symptoms in patients with RA (Gioud-Paquet et al 1987). In addition, the presence of bone erosions have been linked to the finding of IgA RF in patients with rheumatic diseases (Arnason et al 1987).

Whether IgG or IgA RFs are present in congenital syphilis has not been extensively investigated, although Dobson et al (1988a) reported the absence of IgG RF in 5 infants with congenital syphilis.

9.4.1 Measurement of IgG RF using radial immunodiffusion

There are many techniques described for the measurement of IgG RF. Mannik and Nardella (1985) have pointed out the pitfalls associated with some of the methods. In their review, the authors favour the use of radioimmunoassay or an ELISA based on similar principles. Because of the cost of the reagents and the difficulty of obtaining highly purified anti-human Fab, another method, based on reverse radial immunodiffusion on agar plates impregnated with aggregated gammaglobin, was used. This procedure has been described by Theofilopoulos et al (1974).

9.4.2 Materials and methods

Sera from patients with congenital syphilis

Five infants with congenital syphilis were studied. They were selected using random number tables; patients being included if they had sufficient serum available (300 - 500 ul). The infants are described in more detail elsewhere and included the patients coded 6.6, 6.12, 6.18, 6.28 and 11.5. All these infants had elevated IgM RF levels as measured using the RF ELISA.

Sera from patients with rheumatoid arthritis

The sera of 4 adults with RA were tested for IgG RF in an effort to find a positive control serum. These patients attended the outpatient RA clinic at Groote Schuur Hospital and had active disease with positive RF latex tests.

Normal newborn serum

Serum from a normal newborn was used as a negative control. The serum tested negative for RF using both the RF latex test and the RF ELISA. The patient code number was 5.120.

Pepsin digestion of sera

This was carried out to destroy IgM RF (Theofilopoulos et al 1974) and was necessary because IgM RF may give a false positive reaction for IgG RF (Lea and Ward 1972).

To each serum an equal volume of 0.1 M sodium acetate buffer (pH 4.1) was added. (The pH of the mixture was checked and found to be below 4.5). Pepsin obtained from the Sigma Chemical Company was used. A freshly prepared solution containing 10 mg/ml in the sodium acetate buffer was then added (0.2 ul of pepsin per ul of serum). The sera were allowed to digest overnight in a waterbath at 37°C. Mixing was facilitated by means of a mechanical shaker. The samples were then dialysed overnight against 3 changes of Tris-buffered saline pH 7.5 (0.05 M Tris in 0.2 M sodium chloride). To determine the completeness of digestion, the dialysates were then added to agar immunodiffusion plates (made according to the method described

by Rose and Friedman 1980 pg 5-6). Fifteen microlitres of anti-human IgM (obtained from Dakopatts, Denmark, catalogue number A.091) was placed in the centre well. Ten microlitres of the pepsin-digested sera were then added to the surrounding wells. After an incubation period of 48 hours at room temperature, the immunodiffusion plates were examined for precipitin lines. Sera which reacted with the anti-human IgM were re-treated with pepsin and used only after demonstrating the absence of IgM.

Preparation of immunodiffusion plates for IgG RF detection

The technique of reverse immunodiffusion was used (Theofilopoulos et al 1974). The antigen used was heat-aggregated Cohn Fraction II (ICN Immunologicals) which was prepared according to the method of Christian (1958). Two grams of gammaglobulin were dissolved in 100 ml of Tris-buffered saline pH 8.0 and aggregated by heating to 63°C for 10 minutes in a waterbath. Twenty ml of 2.18 M sodium sulphate was then added and the cloudy suspension obtained centrifuged for 20 minutes at 1000 g. The precipitate was re-suspended in 5 ml of buffer at pH 8.0 and dialysed at 4°C overnight against 3 changes of Tris-buffered saline (0.05 M Tris HCl with 0.2 M sodium chloride pH 7.5). The protein concentration of the dialysate was determined by measuring the optical density at 260 and 280 nm and applying a standard formula (Richterich 1969)

Three ml of a solution containing 1.3 mg of gammaglobulin per ml was then added to 9 ml of molten 1% agar at 60°C. Agar plates were prepared and wells 4 mm in diameter and 1 cm apart were cut.

The sera which had previously been digested with pepsin were added to the wells (20 ul of serum per well) and read at 24 hours and again at 48 hours.

9.4.3 Results

None of the 10 sera tested in which IgM was removed by pepsin digestion and IgG RF measured by radial immunodiffusion showed any precipitation rings.

9.4.4 Discussion

The technique described by Theofilopoulos et al (1974) has not been used to measure IgG RF in congenital syphilis by other workers. Whilst this study was in progress, the results of work by Dobson et al (1988a) became available. Using a radioimmunoassay they were unable to detect IgG RF in the serum of 5 infants with congenital syphilis.

The apparent failure of infants with congenital syphilis to make IgG RF is not unexpected. This is because, although class-switching is likely to occur in RF-producing plasma cells in adults (Gharavi et al 1985), B cells synthesizing IgG and IgA are not readily inducible in infants (Roper and Cooper 1983 pg 19). IgM antibodies are the predominant antibody response early in life (Alford 1971) and therefore IgM RF is likely to be the major RF class produced in infants with congenital syphilis.

Nevertheless, the technique used by Theofilopoulos et al (1974) has been regarded by some workers as being relatively insensitive for the detection of IgG RF (Wernick et al 1981). For this reason an immunoabsorbent method was also set up in the present study. This method was used to detect RFs of the IgG and IgA classes simultaneously.

9.5 DETECTION OF IgG AND IgA RFS USING AN IMMUNOABSORBENT METHOD

The batchwise procedure of immunoabsorption as described by Avrameas and Ternynck (1969) was used. This method was outlined in the section dealing with the preparation of an IgM RF standard. Essentially, the only difference was that pepsin-digested sera were used (Wernick et al 1981, Faith et al 1982, Procaccia et al 1987). The reason for this was that pepsin digestion destroys IgM RF (Theofilopoulos et al 1974). IgM RF,

if present in the test sera, can result in the isolation of normal IgG from the immunoabsorbent (Mannik and Nardella 1985).

9.5.1 Materials and methods

Serum samples

Sera from the patients with congenital syphilis that were tested for the presence of IgG RF using the reverse radial immunodiffusion technique were used. The patient code numbers were 6.6, 6.12, 6.18 and 6.28.

The serum of a patient with seropositive RA was used as a positive control and that of a normal newborn infant (no. 5.120) as a negative control.

Pepsin digestion of sera

This was carried out as previously described (Section 9.4.2). The IgM RF ELISA was performed on the sera before and after exposure to the pepsin to ensure complete destruction of IgM RF.

Batchwise procedure of immunoabsorption

The method has been previously elaborated (Section 9.3.1).

Analysis of eluates

The neutralized eluates were concentrated as described (Section 9.3.1). Levels of IgM, IgG and IgA were then measured on radial immunodiffusion plates.

Plates obtained from Hyland Diagnostics, USA (Immuno-plate IV) were used to quantitate IgM. IgG and IgA concentrations were determined on plates supplied by Behring (LC Partigen immunodiffusion plates OTCR 03 and OTCP 03).

In addition, the eluates were tested for IgM, IgG and IgA using turbidimetry. This was performed in the Chemical Pathology laboratory, Red Cross War Memorial Children's Hospital.

The reason for including turbidimetry in addition to radial immunodiffusion was that the latter may underestimate IgG RF

because of the tendency of this substance to undergo self-polymerization (Mannik and Nardella 1985).

9.5.2 Results

The results are shown in Table 9.2 below. The IgM RF ELISA values before and after pepsin digestion are given. Sera had no measurable IgM after pepsin digestion. The results shown are for the pooled first and second eluates.

TABLE 9.2

IgG AND IgA RHEUMATOID FACTORS ISOLATED USING AN IMMUNOABSORBENT METHOD

PATIENT NO.	IgM RF ELISA IU/ml		*ELUATE IMMUNOGLOBULIN LEVELS		
	BEFORE PEPSIN DIGESTION	AFTER PEPSIN DIGESTION	IgM IU/ml	IgG IU/ml	IgA IU/ml
6.6	50	0	0	0	0
6.12	788	0	0	0	0
6.18	24	0	0	0	0
6.28	160	0	0	0	0
**RA	380	0	0	0	2.3
5.120	0	0	0	0	0

* Measured by radial immunodiffusion

** Adult patient with rheumatoid arthritis

None of the sera had detectable IgG RF levels as measured with either immunodiffusion or turbidimetry. The patient with RA had measurable IgA in the eluted fraction.

9.5.3 Discussion

The results were similar to those obtained with the reverse immunodiffusion method in that IgG RF was not isolated.

The use of an immunoabsorbent technique to measure IgG RF has been subject to some criticism. One of the reported problems is that of nonspecific protein-protein interaction (Turner 1976 pg 417-420). This non-specific binding was noted to be less at room temperature (Nardella and Mannik 1978). When the immunoabsorbent method was carried out at this temperature the phenomenon was not observed in the present study as indicated by the absence of IgG in the eluate from the normal newborn infant (no. 5.120).

The use of the pepsin digestion step to destroy IgM RF prevented isolation of normal IgG from the immunoabsorbent, thus circumventing another problem cited by Mannik and Nardella (1985)

The possible pitfalls of quantitating RFs using immunodiffusion have been noted by Lea and Ward (1972) and Mannik and Nardella (1985). Hence the technique of turbidimetry was employed in addition to that of immunodiffusion, although without noticeably affecting the results.

When the negative results obtained are considered with those of Dobson et al (1988a) it appears that IgG RF is not present. This work could be confirmed with larger numbers of infants with congenital syphilis using an IgG RF ELISA or radio-immunoassay method. The IgG RF ELISA was not used in the present study. This was because of the expense and difficulty of obtaining highly purified rabbit anti-human Fab conjugate used as a second antibody (Faith et al 1982, Bampton et al 1985).

The immunoabsorbent method detected IgA RF in the serum of an adult with RA but not in the infants with congenital syphilis.

To verify these negative results in the infants an IgA RF ELISA was established. Compared to the IgG RF ELISA, the second antibody conjugate used for this procedure (a goat anti-human IgA which was alpha-chain specific) was readily available.

9.6 IGA RF ELISA

The method of Procaccia et al (1987) was used with minor modifications.

9.6.1 Materials and Methods

The same materials described for the IgM RF ELISA were used, with the some exceptions.

Peroxidase-conjugated antibody

A peroxidase-labelled goat anti-human IgA was used. The F(ab')2 fragment of this alpha chain specific antibody was obtained from Cappel Products, USA (catalogue number 33010021).

TABLE 9.3:

THE SPECIFICITY OF THE GOAT ANTI-HUMAN IgA ANTIBODY FOR IgA CLASS IMMUNOGLOBULINS

COATING ANTIGEN	*OPTICAL DENSITY
Anti-human IgA	0.63
Anti-human IgG	0.25
Anti-human IgM	0.32

* Mean of duplicates after background values subtracted

Specificity of the conjugate

This was determined in the same way as that of the conjugated antibody used in the IgM RF ELISA (see Chapter 8).

It was shown that, although the conjugate detected mainly IgA class antibodies, there was considerable cross-reactivity for other immunoglobulin classes. In an effort to improve conjugate specificity, the latter was absorbed with human IgG. (Although the conjugate demonstrated a lack of specificity for IgM in addition to IgG it was not absorbed with the former because the pepsin digestion step destroyed IgM antibodies)

Absorption of the anti-human IgA conjugate

The absorption was carried out in a polystyrene Falcon tube. Five hundred microlitres of purified human IgG (from ICN Immunobiologicals) at a concentration of 4 mg/ml in coating buffer was added. The IgG was allowed to bind to the tube at 4°C overnight. The tube was then washed 3 times with PBS pH 7.2. Ten percent foetal calf serum (from Centrolab, Cape Town) in PBS pH 7.2 was added for 2 hours at room temperature as a blocking agent.

After 3 washes with PBS pH 7.2, 500 ul of the anti-IgA conjugate at a dilution of 1:50 in PBS was added. This was incubated at 37°C for 30 minutes and then overnight at 4°C. The absorbance of the conjugate as measured at 280 nm was 0.89 before addition to the plastic tube and 0.89 afterwards.

This indicated that an insignificant amount of protein had been removed.

Serum samples

Infants with congenital syphilis: Sera from the same 4 infants used for the batchwise immunoabsorbent procedure were studied (patient code numbers 6.6, 6.12, 6.18. 6.28). In addition, sera from another 2 patients with congenital syphilis (patient code numbers 5.5 and 11.5 were tested in the IgA RF ELISA.

Positive control serum: the serum from the patient with RA which was shown to contain IgA RF using the immunoabsorbent procedure was used.

Negative control serum: this was obtained from a normal newborn infant (patient code number 5.120).

The sera were all subjected to pepsin digestion as previously described and contained no IgM. A serum dilution of 1:50 in PBS Tween was used in the ELISA.

Checkerboard titrations The optimum conditions for the assay were determined.

IgG concentration: concentrations from 0.5 - 3 mg per 100 ml were evaluated as the coating antigen.

Blocking solutions: to determine the optimum blocking conditions, different solutions were tested. One percent bovine serum albumin (BSA) with 1% goat serum, 4% BSA and 4% BSA with 0.25% BSA added to the serum and conjugate were assessed.

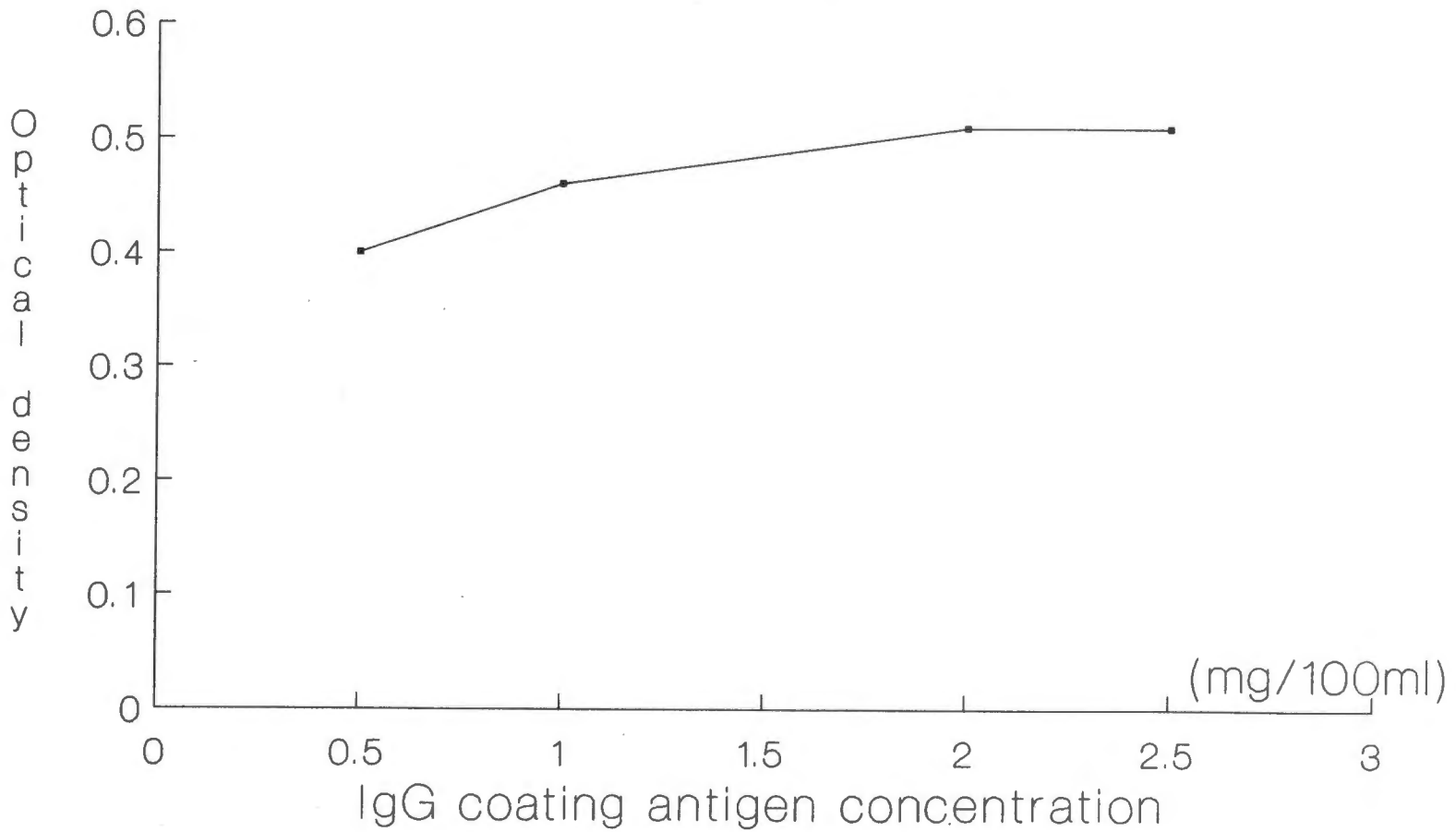
Peroxidase-conjugated antibody: the absorbed conjugate was employed at dilutions from 1:500 - 1:2000.

Incubation times: the conjugate was left on the plates for a period of 1 hour as for the IgM RF ELISA or 2 hours as suggested by Procaccia et al (1987).

9.6.3 Results

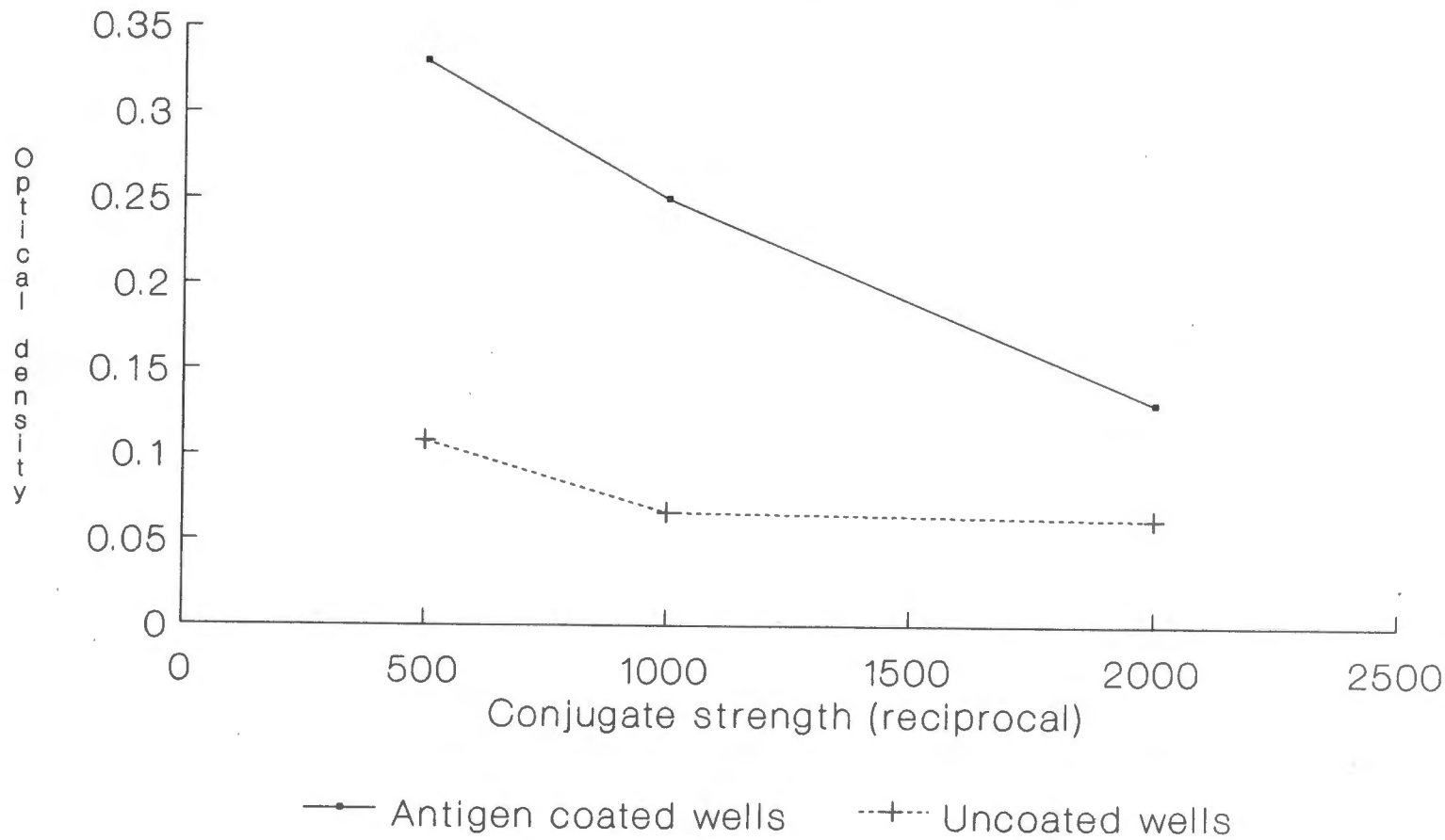
Checkerboard titrations: The results obtained during the establishment of the IgA RF ELISA are shown in Figure 9.3 and Figure 9.4. From Figure 9.3 it can be seen that even when no serum was added, the conjugated antibody bound to the IgG coating antigen. Several steps were taken in an effort to minimize this.

Figure 9.3 : Background optical density readings obtained in IgA RF ELISA from wells containing IgG antigen only



Conjugate strength 1:500

Figure 9.4 : Background optical density readings obtained in IgA RF ELISA at different conjugate strengths



IgG coating antigen 1mg/100ml

Reduction of nonspecific binding of peroxidase conjugate to IgG

i. Decreasing the strength of the IgG coating antigen lowered the background optical density readings obtained (Figure 9.3). The use of a solution containing 0.5 mg of IgG per 100 ml resulted in the lowest values.

ii. Using a more dilute conjugate resulted in less binding to the IgG. This is shown in Figure 9.4 where it can be seen that the lowest nonspecific conjugate reaction with the IgG was encountered at a conjugate strength of 1:2000.

iii. Applying the conjugate for 1 hour instead of 2 also reduced conjugate binding to the coating IgG. In one experiment, the optical density obtained from wells containing only coating antigen, conjugate and substrate was 0.60 after 2 hours, while after 1 hour it was 0.38.

In addition, during the development of the IgA RF ELISA it was evident that there was conjugate binding to the polystyrene plates.

Nonspecific binding of peroxidase conjugate to the polystyrene plates

That this binding occurred was shown from the background readings obtained from wells containing no coating IgG but to which conjugate and substrate were added. Figure 9.4 demonstrates that the reaction was lessened by using more dilute conjugates. Thus, an optical density of 0.062 was obtained at a conjugate strength of 1:2000.

The blocking solutions also had some effect on the binding of the conjugate to the polystyrene. The mean background value from 6 wells was 0.078 when 4% BSA was used, 0.078 with the 4% BSA with 0.25% BSA added to the serum and conjugate and 0.10 with the 1% BSA and 1% goat serum.

From these results, the final working strength of the reagents used were determined.

Optimum concentration of reagents

In the absence of positive control sera from patients with congenital syphilis, the conditions chosen for the performance of the IgA RF ELISA were those which gave the lowest background values. These were:

- a coating antigen concentration of 0.5 mg / 100 ml
- a conjugated antibody dilution of 1:2000
- a blocking step with 4% BSA
- applying the conjugated antibody to the polystyrene plates for 1 hour.

Serum samples

The samples were tested at a dilution of 1:50. The results were negative in all cases except for the adult patient with RA. The optical density obtained in this case after the background values were subtracted was 0.186.

9.6.3 Discussion

The method

The goat anti-human IgA conjugated antibody clearly lacked specificity for IgA: significant cross-reaction with IgM and IgG was noted. The IgM binding was considered unimportant because pepsin-digested sera were used, hence destroying the IgM (Theofilopoulos et al 1974). The IgG reactivity of the conjugate was not abolished by an absorption step. Nevertheless, by using low concentrations of the IgG coating antigen and the anti-human IgA conjugate, relatively low background values were obtained. Ideally, if the method were to be repeated the effect of using an affinity purified conjugate should be assessed.

The results

The immunoabsorbent method and the ELISA for the detection of IgA RF gave identical results, both in terms of the negative findings in the patients with congenital syphilis and the positive results in the patient with RA.

IgA RFs have not previously been studied in congenital syphilis. In general, IgA production occurs much later in childhood (Allansmith et al 1968).

Nevertheless, Tan (1973) has reported raised IgA levels in newborns with congenital syphilis. It is possible that these results were due to the presence of IgM RF which could produce a false positive result for IgA using radial immunodiffusion plates (Lea and Ward 1972).

IgA RFs have been reported in 88% of adults with active RA (Bampton et al 1985), so that it was not surprising to find this substance in the adult control patient.

9.7 SUMMARY AND CONCLUSIONS

The IgM RF levels were strikingly higher in most of the patients with congenital syphilis compared to the controls. There was some overlap, however, particularly in infants over the age of 2 months. The mean IgM RF levels increased in the control patients from birth to 4 months. Hidden RF was only detected in the serum of 1 patient with congenital syphilis. Measuring total IgM levels and the concentration of IgM RF showed that some infants with congenital syphilis had produced large amounts of IgM RF and that there was a significant correlation between the total IgM and IgM RF.

IgG and IgA RF were not found in the infants with congenital syphilis who were studied. This suggested that there was little or no class switching amongst RF-producing plasma cells in these patients.

Overall, the results of these studies are in keeping with observations on normal newborn infants which have shown that IgM

is the predominant immunoglobulin class produced. The IgG and IgA producing plasma cells appear to be less readily inducible (Cooper 1987). The IgM response is a polyclonal one, perhaps stimulated by the presence of immune complexes containing treponemal antigens and maternal IgG.

CHAPTER 10

ASSOCIATIONS BETWEEN THE SERUM RHEUMATOID FACTOR CONCENTRATION AND VARIOUS CLINICAL AND LABORATORY FINDINGS IN CONGENITAL SYPHILIS

10.1 INTRODUCTION

In the previous chapters the frequent occurrence of RF in patients with congenital syphilis has been demonstrated.

As described in Chapter 3, infants with congenital syphilis have a tendency to produce RF more often than adults with acquired syphilis.

This raises the question as to whether RF is implicated in the disease process and might even be partly responsible for the greater severity of congenital syphilis compared to the acquired form of the disease.

That RF plays a role in the pathogenesis of RA and other inflammatory conditions such as glomerulonephritis has been suggested (Ford 1983; Chen et al 1988). It has not been possible to determine for certain, however, that RF has a pathological rather than a physiological role in these conditions (Olsen and Jasin 1985; Levinson and Martin 1988). The same difficulty of separating cause from effect arises in congenital syphilis.

Whilst it may not be possible to ascribe a definite role to RF in congenital syphilis, it would appear that the variables associated with the presence of RF in congenital syphilis merit further study. In particular, it would be useful to ascertain whether factors similar to those which have been identified in RA and other inflammatory conditions, are related to RF levels in congenital syphilis. Not only would such investigation facilitate comparison between RF formation in different diseases but it would allow RF production to be investigated during an early stage in the development of the immune response.

In this study the factors considered were those outlined in Chapter 3, Section 3.7.3 and included:

i. the age of patient

ii. the severity and extent of disease

iii. the duration of the illness

iv. the disease pattern in terms of which organs were involved

v. the activity of the disease following therapy

vi. immune complex levels.

10.2 STUDY DESIGN AND METHODS

10.2.1 Patient selection

Patients with definite clinical and/or radiological signs of congenital syphilis whose disease was confirmed by the results of serological tests were studied. These infants all fulfilled the criteria of Kaufman et al (1977) and included:

a. patients outlined in Chapter 5 who presented and were diagnosed and treated at birth

b. the patients described in Chapter 6.

Control sera were obtained as outlined in Chapter 9, Section 9.1.

10.2.2 Quantitation of RF levels

This was achieved using the RF ELISA set up as described in Chapter 8. The RF levels were determined on the patients with congenital syphilis using sera obtained at the initial presentation. The RF levels obtained in control infants have already been presented in Chapter 9.

10.2.3 Factors influencing RF production

Age of patients

The RF levels in infants with congenital syphilis at different ages have already been compared (Chapter 9). In this section, the relationship between gestational age and RF concentration

was investigated.

The gestational age in completed weeks was assessed according to the method of Ballard et al (1979) and collated with the RF concentration. In addition, RF levels in preterm newborn infants (less than 37 weeks gestation) were contrasted with those of term newborns (37-42 weeks gestation). The RF levels of patients who presented after the age of 3 days were not considered in this analysis.

Severity and extent of disease

Infants were divided into those who had severe disease and those who did not. The criteria for these groupings were based on reports in the literature outlining prognostic features for infants with congenital syphilis. Infants with widespread signs at or soon after birth have a worse prognosis than those whose illness starts over 1 month of age (Stokes et al 1945 pg 1100; Brown and Moore 1963; Ingall and Musher 1983 pg 362; Krugman and Katz 1981 pg 396; Chawla et al 1988).

In addition, newborn infants with non-immune hydrops are likely to have a poorer outcome (Tan 1973; Chawla et al 1988).

Renal involvement may, on occasion, be associated with severe renal damage (Wiggelinkhuizen et al 1973).

For the purposes of the study, severe disease was identified when:

i. there were 2 or more organ systems involved in the first month of life, or

ii. non-immune hydrops was noted, or

iii. life-threatening complications were present.

Milder disease was said to be present in infants who developed clinical signs after the first month of life or when the diagnosis was made earlier but there were fewer than 2 organ systems involved.

The systems considered for this analysis were the liver, reticulo-endothelial system, the haematological system, the musculoskeletal system, the kidneys, the skin, the respiratory tract and the central nervous system.

From the description of the severity it is apparent that this is partly related to the extent of the disease. Nevertheless, the latter was also analysed separately.

Extent of the disease The extent of the disease was scored according to the number of the following signs that were present:

- liver involvement
- reticulo-endothelial involvement
- haematological involvement
- bony disease
- skin signs
- oedema.

The maximum score was therefore 6. Involvement of other systems e.g. the renal tract were not added to the list because very few of the patients demonstrated disease in these systems. The score obtained was then correlated with the RF concentration. VDRL titres were also correlated with the severity and extent of the disease.

Duration of infection

As most women with syphilis in pregnancy are asymptomatic (Kaufman et al 1977; Wendel 1988), the timing of fetal infection is usually unknown.

Careful studies by Ingraham (1936) indicated that the characteristic metaphyseal dystrophy took approximately 5 weeks to develop, whilst the periosteal changes were evident by 16 weeks.

It follows that radiological evidence of a periosteal dystrophy may indicate that the disease has been present for at least 16 weeks.

The usefulness of the periosteal dystrophy in determining duration of infection is limited, however, by the finding of such changes in 47% of normal preterms (Malmberg 1944) and 35% of normal term infants (Shopfner 1966).

Another assessment of the duration of infection may be possible in some infants who develop clinical signs after birth. The clinical features indicating recent onset are reported to be snuffles and skin manifestations (Stokes et al 1945 pg 1100; Budell 1982 pg 924). On the other hand, renal involvement points to a longer period of infection.

For the purposes of this study, recent onset disease was said to be present in the following circumstances:

i. infants presenting at birth without evidence of periosteal dystrophy on radiography of the long bones.

ii. infants presenting after birth who had the early clinical features described above in the absence of periosteal changes on roentgenogram.

Tardy disease was diagnosed if a periosteal dystrophy was present at the time of diagnosis, or if renal disease was evident.

(The division of cases into those of recent onset and those with a tardier course should not be confused with 'early' and 'late' congenital syphilis. The latter categorization is based on the age of the patient, 'late' disease occurring over the age of 2 years).

Disease pattern

The presence of certain clinical signs or the involvement of certain organs may be associated with the induction of RF synthesis. For example, active liver disease was found to correlate with the presence of RF in adults with cirrhosis (Dresner and Trombly 1959).

For the purpose of the present study, the liver was regarded as being involved if there were clinical signs of liver disease (e.g. jaundice or hepatomegaly as defined in Chapter 6) and/or if there were abnormal liver function tests. The liver function tests were carried out by the Chemistry Laboratory at the Red Cross War Memorial Children's Hospital and consisted of the measurement of serum bilirubin, alkaline phosphatase and alanine aminotransferase (ALT) concentrations. The following normal ranges were adopted (Nelson et al 1979 pg 2076-2090):

Conjugated bilirubin	:	0 - 4 umol/L
Alkaline phosphatase	:	newborns 50 - 165 U/L
	:	older 20 - 150 U/l
ALT	:	5 - 28 U/L

The concentrations of ALT and conjugated bilirubin were compared to the RF levels.

The reticulo-endothelial system was considered to be affected if there was splenomegaly (as defined in Chapter 6.1.1) or generalized lymphadenopathy.

Haematological involvement was diagnosed if there was anaemia (haemoglobin level below the accepted normal for age (Oski 1981 pg 545-547)) or thrombocytopenia (platelet count below 150 000 per mm^3 (Nelson et al 1979 pg 2090)).

Bony involvement was considered to be present if there were typical roentogenographic findings (Cremin and Fisher 1970).

Skin lesions (including mucocutaneous manifestations) were evaluated separately. A petechial rash due to thrombocytopenia was assessed with the haematological manifestations.

The respiratory system was adjudged diseased if there was respiratory distress with radiological evidence of pneumonitis (Malan 1987 pg 627).

An indication of placental involvement in congenital syphilis is an increase in placental weight relative to that of the infant (Budell 1982 pg 923-924). A percentile chart for gross placental weight at various infant weights has been established (Malan et al 1990) and is shown in Appendix 8. Using this chart it was possible to calculate a ratio of observed to expected (50th percentile) placental weights. The ratio obtained was compared with the RF levels.

Generalized oedema was categorized separately. The aetiology of the oedema is multifactorial. Anaemia, liver disease and renal disease may be contributory (Tan 1973).

Evidence of other organ involvement was obtained in some instances (e.g. syphilitic meningitis, pancreatitis). As few infants demonstrated these findings they were not analysed separately.

Immune complexes The RF levels at the time of diagnosis were compared to the levels of circulating immune complexes (CIC).

The latter were measured by a Clq binding assay performed according to the method of Zubler et al (1976). The CIC assays were done in the Renal Laboratory , Groote Schuur Hospital. The normal range for this laboratory is 0-7%. To determine the CIC levels in infants with congenital syphilis the first 25 patients who had sufficient serum available (200 ul) were chosen. In addition, serum samples from 13 control infants (described in Chapter 9) were randomly selected using random number tables and the Clq binding assay was carried out.

Effect of treatment The effect of treatment was assessed by measuring RF levels before and at intervals after treatment. The RF Levels were compared with VDRL titres. A steady decline in the latter signifies successful therapy (Felman and Nikitas 1980).

A group of 10 infants with congenital syphilis whose families were resident at fixed addresses were selected. Serum was obtained at the start of treatment, on the day treatment was completed (day 10) and at monthly intervals for 3 months. The

RF concentration and VDRL titres in these infants were compared to those obtained at follow-up from the 51 infants at risk of congenital syphilis who were unaffected. Details of the control infants and their VDRL titres at follow-up are shown in Table 5.2. The RF concentrations in the controls at follow-up have been reported in Chapter 9.

10.2.4 Analysis of data

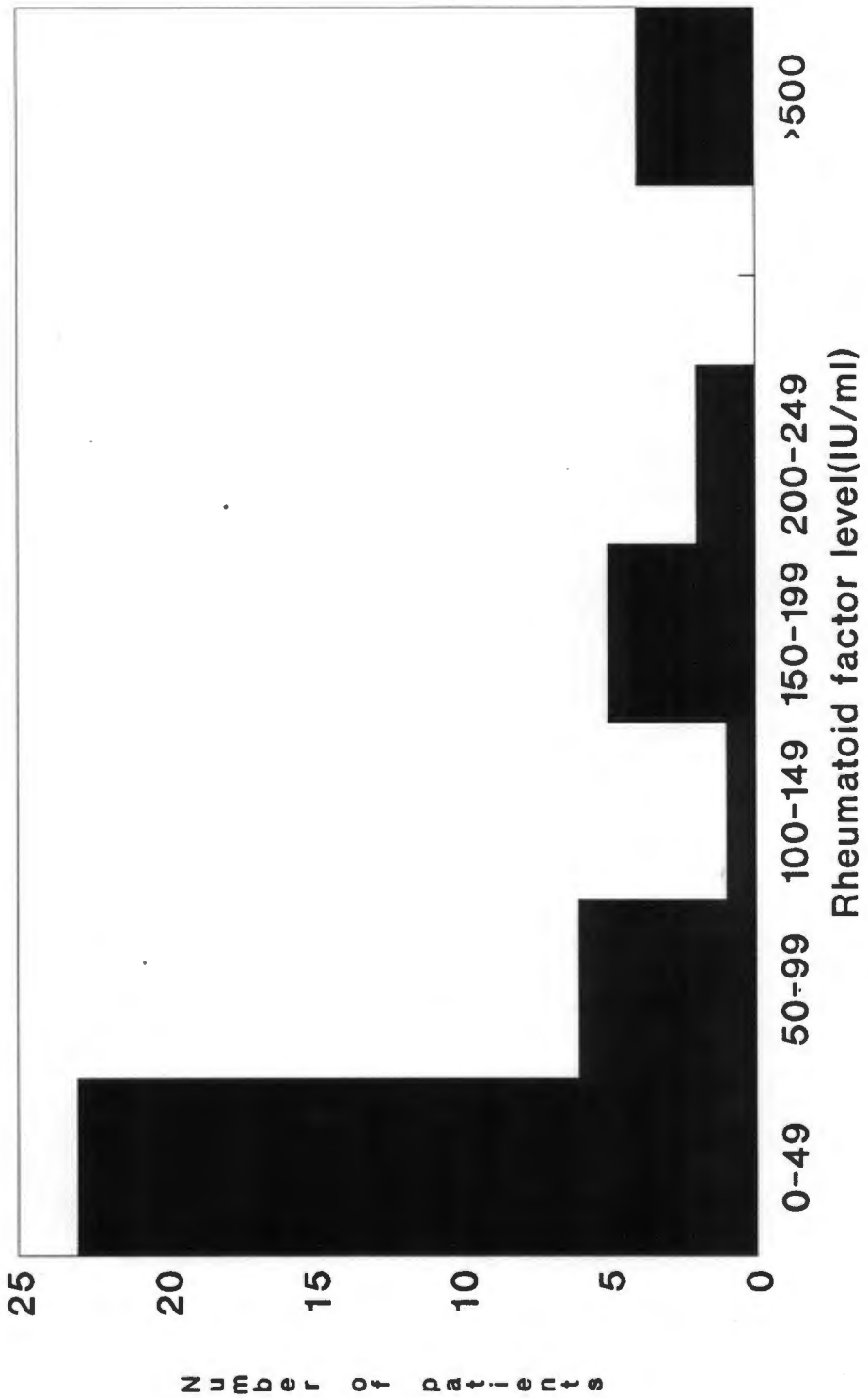
The RF values in the infants with congenital syphilis were analysed by non-parametric statistical tests involving ranking the data. This method was chosen in preference to the use of parametric tests because the RF values were not normally distributed (see Figure 10.1). The non-parametric tests used were the Mann-Whitney test and Spearman's Rank Order Correlation Coefficient (Conover 1971 pg 224-236; Bourke et al 1985 pg 156-157).

The principle behind the use of the Mann-Whitney test is illustrated in the example which follows.

According to the Null hypothesis one would assume that patients with evidence of liver involvement would have RF levels which were no different to those without liver disease. In other words, any arrangement of the patients with and without liver disease would be equally possible when their RF values were ranked.

The sample size of patients without liver disease was denoted n, and the sample size of infants with liver disease m. The RF values of all the patients were ranked. The patient with the lowest RF value was given rank 1. If the ranks were tied the average rank was assigned.

Figure 10.1: Frequency distribution of rheumatoid factor levels in patients with congenital syphilis



The test statistic, T, was determined as follows:

$$T = S - \frac{n\ (n + 1)}{2}$$

Where S is the score of the ranks assigned to n patients (the patients without liver disease in the example).

The critical regions at which T attained statistical significance (at the probability (p) value of 0.05), are available from standard tables.

To simplify the recording of results a 0 was used to denote patients who did not have a particular variable and a 1 to indicate those who did. If the variable was not applicable in a specific case, a blank was left. Table 10.1 summarizes the designation of the patients.

TABLE 10.1:

DESIGNATION OF PATIENTS INTO THOSE WITH OR WITHOUT DIFFERENT VARIABLES

DESIGNATION	VARIABLE			
	AGE	SEVERITY	DURATION	*DISEASE PATTERN
0	Preterm	Mild	Recent	Absent
1	Term	Severe	Tardy	Present

* Absence or presence of a particular finding

Spearman's Rank Correlation Coefficient was used to assess concordance, for example between the levels of RF and CIC.

Unless otherwise specified, 2-tailed tests were carried out. The Mann-Whitney results were calculated by hand, whereas Spearman's Rank Correlation Coefficient was determined using a computer programme (Epistat,1986).

10.3 RESULTS

The 41 patients included in the study are described in Chapters 5 and 6. Overall, 30 cases were less than 1 month of age at presentation.

Details of each patient are shown in Appendix 7 (Tables A.7.1, A.7.2, A.7.3) and the results are summarized in Figure 10.2. (The patient code numbers correspond to those in Chapters 5 and 6.)

The T values, together with the critical regions for each variable, are shown in Tables 10.2 and 10.3.

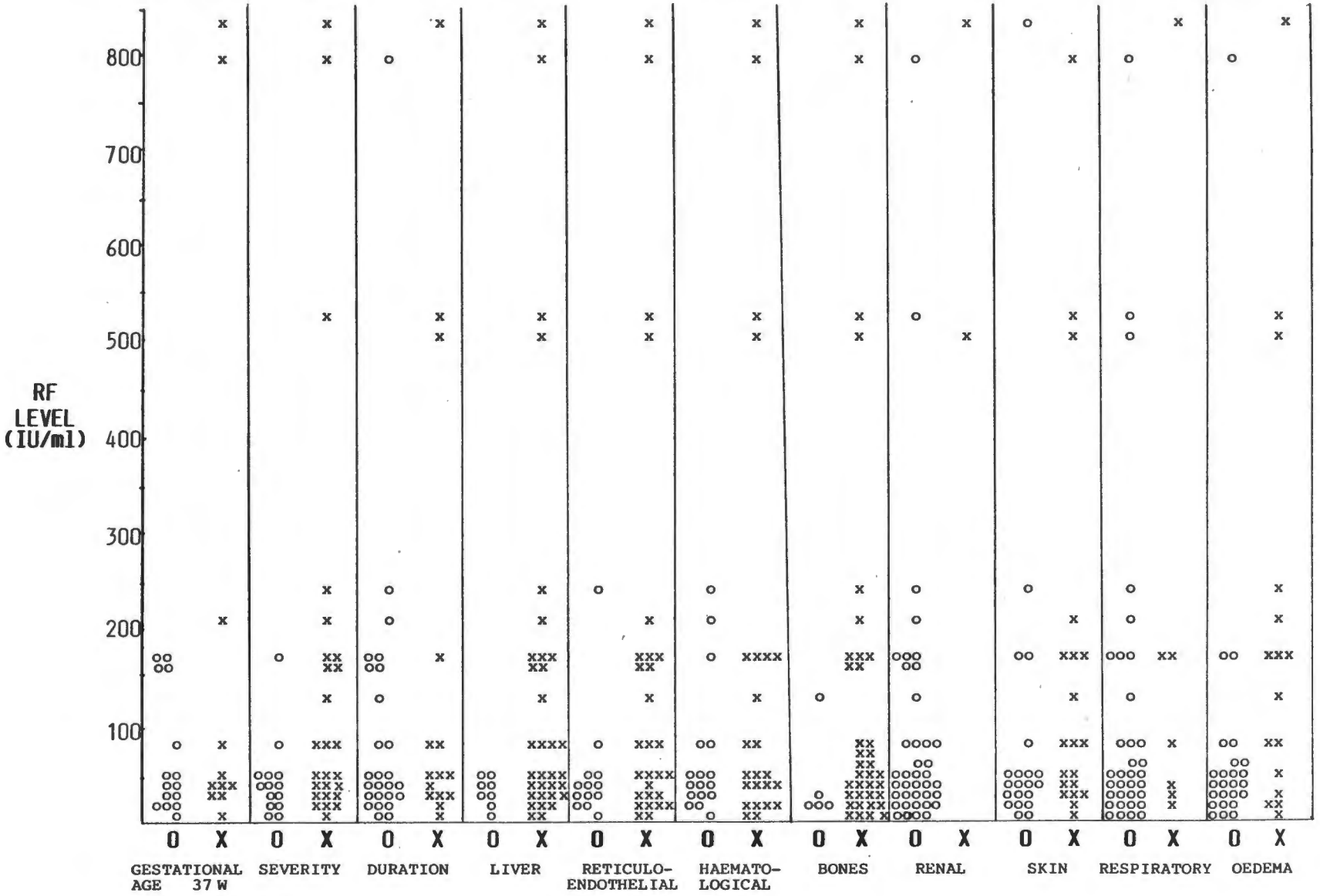
10.3.1 Age of patients

In Chapter 9 it was shown that in infants with congenital syphilis the RF concentrations of those presenting at birth were similar to those who presented between 2 and 4 months of age. The levels of RF in controls, however, increased between birth and 4 months of age.

The RF concentrations of infants with congenital syphilis were considered in relation to gestational age. The overall results are shown in the scatter diagram (Figure 10.3). A significant correlation between the variables was not found using Spearman's Rank Correlation Coefficient ($p > 0,05$).

In addition, comparing the RF levels of preterm infants (under 37 weeks gestation) with term infants (37 weeks and above) a statistically significant relationship between gestational age and RF levels was not apparent ($p > 0.05$).

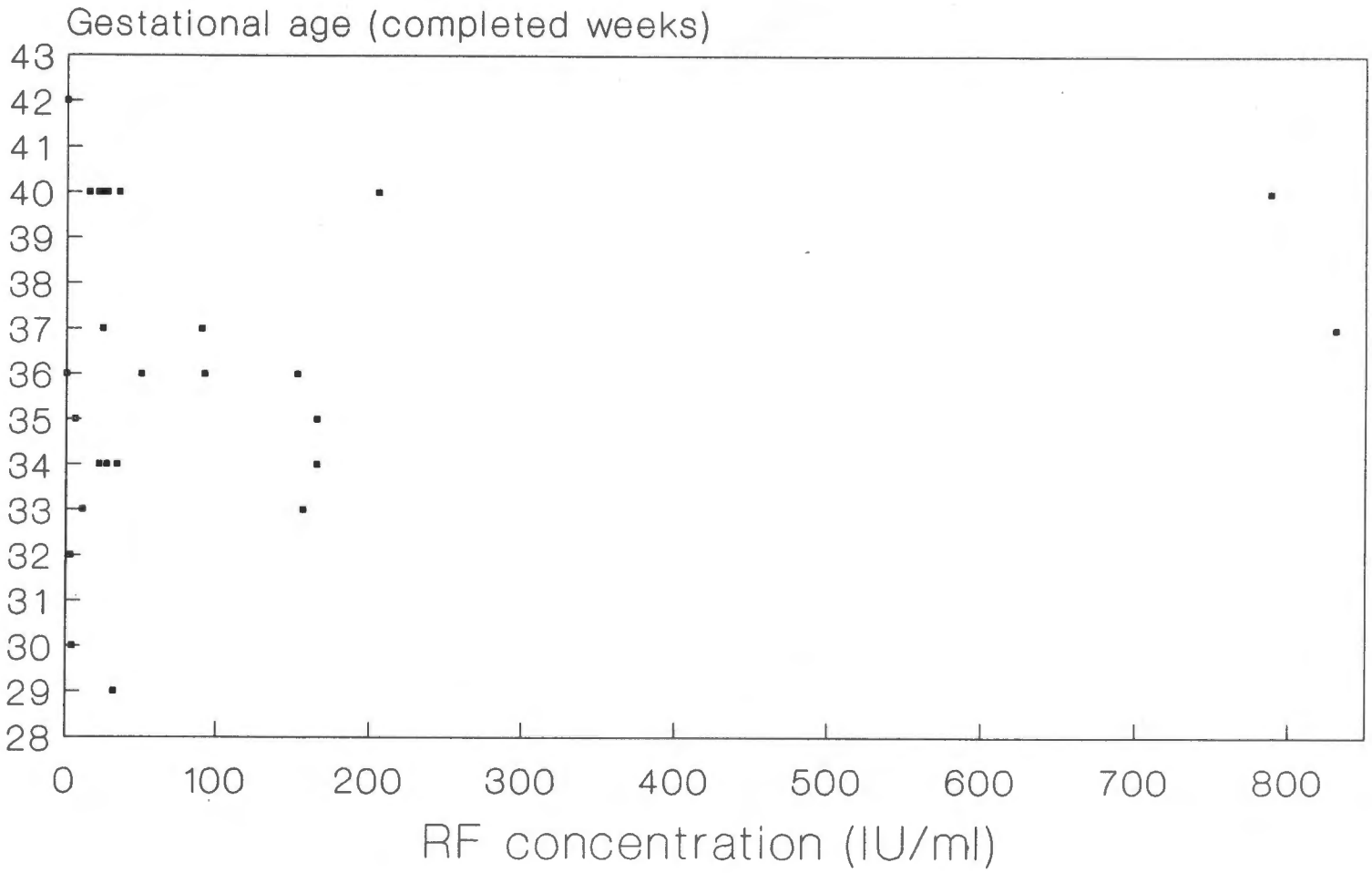
in congenital syphilis.(The upper limit of normal for RF concentration in newborn controls was 3 IU/ml).



0 - finding absent

X - finding present

Fig.10.3: Scatter diagram showing RF levels at various gestational ages in infants with congenital syphilis



10.3.2 Severity of disease

The severity of the disease was determined according to the criteria in the methods section. According to these criteria the severity could not be determined in 1 patient (no. 6.26).

He had late onset disease (usually associated with a good prognosis) but had renal involvement at the age of 4 months. Renal biopsy showed membranous glomerulonephritis with epithelial crescent formation. The reversibility of the changes was uncertain as some of the features were similar to those of a patient with the same condition who developed glomerulosclerosis with marked loss of glomeruli (Wiggelinkhuizen et al 1973). Because of the uncertainty of the outcome, the results of this patient were not considered in the analysis.

The calculated T value was statistically significant ($p < 0.05$), leading to the Null hypothesis being rejected. That is, an association between RF levels and the severity of the disease could be shown.

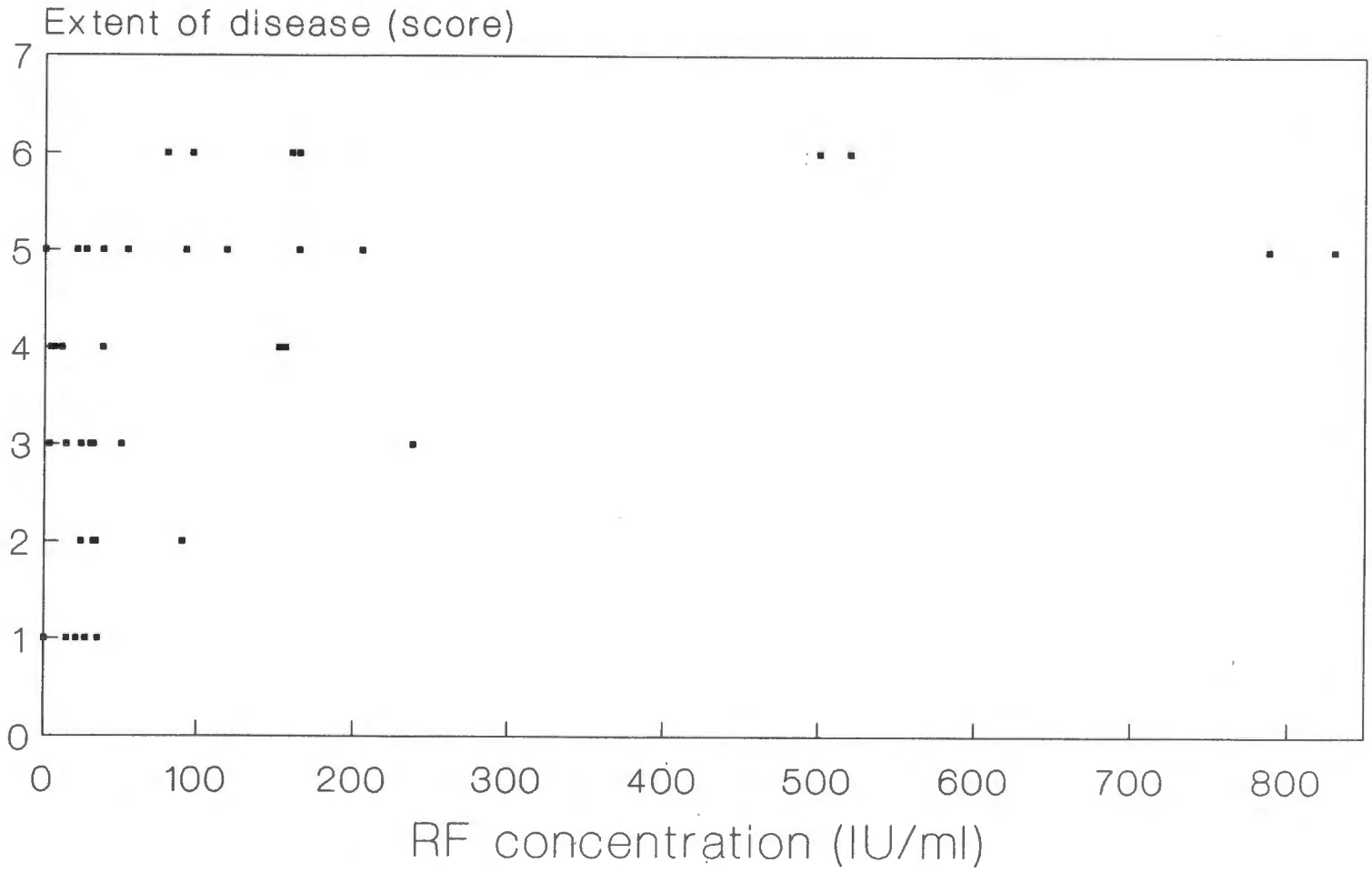
When the RF concentrations in the 5 newborns who died shortly after birth were compared with those who survived, however, there was no significant difference in the rankings ($p > 0.05$) using the Mann-Whitney test).

The VDRL titre did not correlate with the severity of the disease ($p > 0.05$ using the Mann-Whitney test).

10.3.3 Extent of the disease

When the extent of the disease was scored using the procedure described in the methods section, a statistically significant association between RF levels and higher scores was found ($p < 0.05$ using Spearman's Rank Correlation Coefficient). The results are shown in Figure 10.4. The score of the extent did not correlate with the VDRL titre (Spearman's $r = 0.26$; $p > 0.05$).

Fig.10.4: Scatter diagram showing score of extent of disease and RF levels in infants with congenital syphilis.



10.3.4 Duration of infection

A statistically significant relationship between the RF concentration and the chronicity of infection (as previously defined) was not demonstrated ($p > 0.05$).

10.3.5 Disease pattern

A statistically significant association ($p < 0.05$) was found between RF levels and the presence of the following:

- liver involvement,
- renal disease,
- oedema.

Statistically significant correlations ($p > 0.05$) were not observed with the other variables; these included:

- reticulo-endothelial system involvement,
- haematological involvement,
- bone involvement,
- skin involvement,
- respiratory system involvement.

Bone and skin involvement showed a trend towards being associated with higher RF concentrations. The results are summarized in Table 10.3.

The relative placental weight was statistically significantly higher in newborn infants with higher RF levels. The results are tabulated in Appendix 7 (Tables A.7.4) and shown in Figure 10.5. The p value for Spearman's Rank Correlation Coefficient was < 0.05 for a 1-tailed test.

The RF levels were compared to the ALT and conjugated bilirubin concentrations where the latter were available. A statistically significant correlation was not observed ($p > 0.05$ using Spearman's Rank Correlation Coefficient). The actual values are shown in Appendix 7 (Table A.7.5) and in Figures 10.6 and 10.7.

TABLE 10.2

THE TEST STATISTIC (T) AND THE CRITICAL REGIONS FOR THE MANN-WHITNEY TEST CALCULATED FOR DIFFERENT VARIABLES

VARIABLE	T	CRITICAL REGIONS		p VALUE
Gestational age (⩾ 37 completed weeks)	93	56	109	N/S*
Disease severity	257	113	251	< 0.05
Duration of infection	214	123	267	N/S

* N/S - p value not significant at 5% level

TABLE 10.3

THE TEST STATISTIC (T) AND THE CRITICAL REGIONS FOR THE MANN-WHITNEY TEST CALCULATED FOR DIFFERENT DISEASE PATTERNS

DISEASE PATTERN	T	CRITICIAL REGIONS		P VALUE
Liver involvement	196	72	192	< 0.05
Reticulo-endothelial involvement	230	113	251	N/S*
Haematological involvement	239	130	278	N/S
Bone involvement	138	41	139	N/S
Renal involvement	76	7	71	< 0.05
Skin involvement	273	135	285	N/S
Respiratory involvement	139	62	176	N/S
Oedema	289	127	273	< 0.05

* N/S - p value not significant at 5% level

10.3.6 Immune complexes

The RF levels showed a statistically significant correlation with the CIC levels ($p < 0.000001$ using Spearman's Rank Correlation Coefficient). The CIC levels measured in the normal infants had a mean value of 3.8% with a standard deviation of 1.2%. Figure 10.8 shows the results and the actual values are given in Appendix 7 (Table A.7.4).

10.3.7 Effect of treatment

When the median RF levels obtained at diagnosis, and then at monthly intervals for 3 months after treatment were compared with the VDRL titres done at the same time a statistically significant correlation was observed ($p < 0.001$) with Spearman's Rank Correlation Coefficient. In general, the RF levels had decreased one month following treatment. One patient (no. 5.3), however, had no detectable RF at delivery but the concentration increased in the first 2 months after treatment. In addition, the RF level in patient no. 6.34 was higher 1 month after treatment than at the commencement of therapy, but then declined steadily.

Figure 10.9 shows that changes in the median RF values in infants with congenital syphilis closely followed those of the VDRL titres. In addition, a peak in both levels was observed after completion of therapy on day 10. (The actual RF levels and VDRL titres obtained in each patient are shown in Appendix 7 (Table A.7.6) the RF concentration and VDRL titres in the control patients are shown in Figure 10.10.

10.4 DISCUSSION

As shown by the results obtained, associations between a number of variables and RF levels in congenital syphilis were demonstrated. Each of the variables will be considered in turn.

10.4.1 Age of the patient

It was shown in Chapter 9 that the RF concentrations in infants with congenital syphilis were similar at different ages from birth to 4 months. During this same period however, the RF levels in control infants increased. It was postulated that the anti-immunoglobulins detected might have Gm specificity.

The results analysed in this chapter indicated that the amount of RF in newborn infants with congenital syphilis did not appear to be related to the presence or absence of prematurity. It is apparent, however, that the CD 5+ subset of B lymphocytes responsible for RF production accounts for a substantial proportion of B lymphocytes from early fetal life (Antin et al 1986). The

present study would suggest that these cells are functionally capable of producing anti-gammaglobulins as early as 29 weeks gestation and that neither gestational age nor postnatal age affect the amount of RF induced by the infection.

10.4.2 Severity and extent of disease

Infants who were judged to have more severe congenital syphilis had significantly higher RF levels than those with a milder illness.

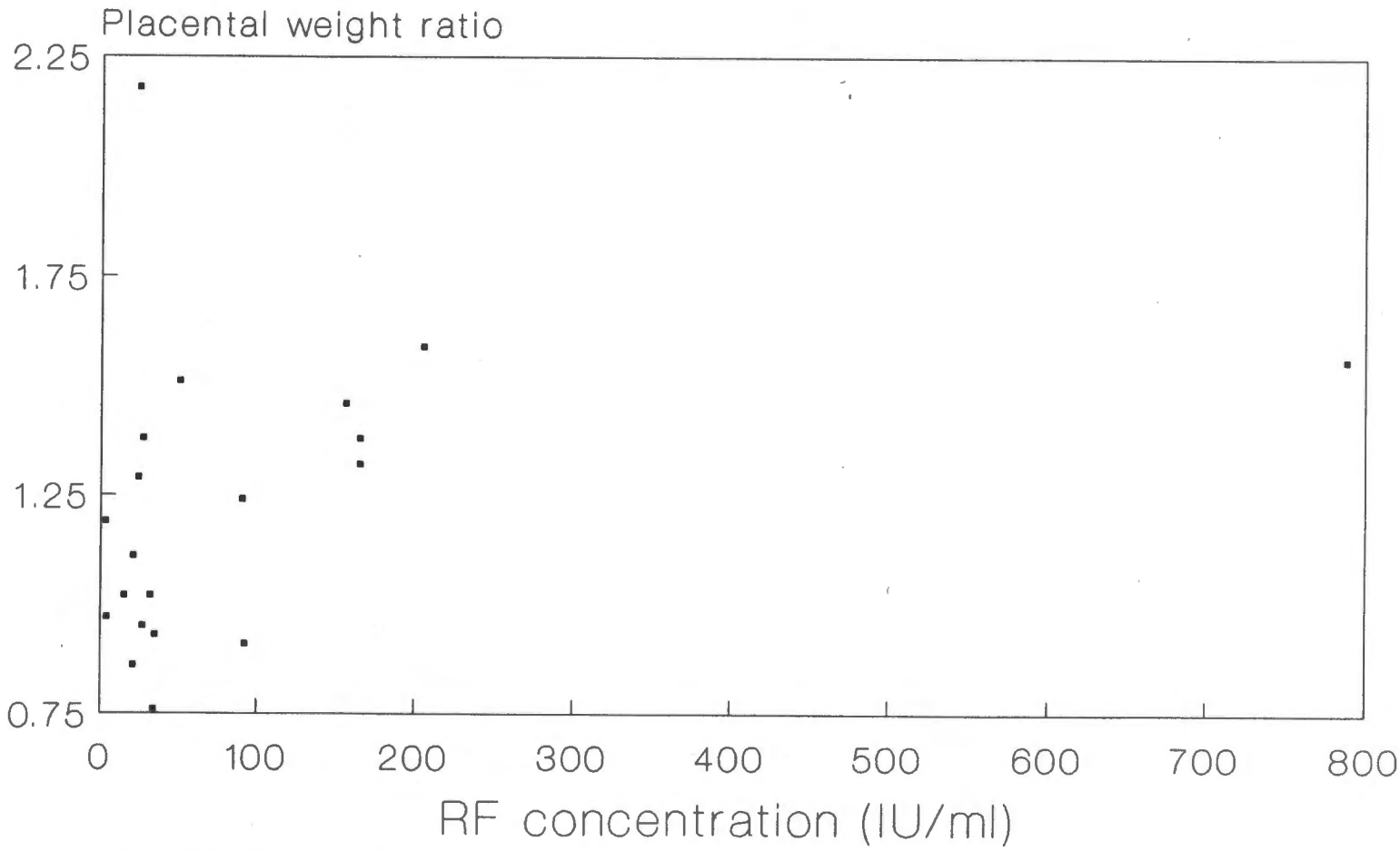
The assessment of the severity depended partly on the extent of the condition and, in addition, there was a direct correlation between the number of organ systems involved and the RF activity.

These findings have not previously been reported in congenital syphilis and, in fact, the severity of the disease has not been related to the results of serological tests.

Work in other conditions has demonstrated similar findings: e.g. higher RF levels are associated with more severe RA (Carson 1987 pg 1204; Milgrom 1988) as well as more severe vasculitis in experimental animals (Floyd and Tesar 1979). In addition, the presence of RF in renal tissue has been found to correlate with more severe kidney disease (Rossen et al 1977).

A possible explanation for the findings in congenital syphilis relates to the effects of immune complexes. If, as appears likely, the latter mediate some of the tissue damage in congenital syphilis, it is possible that there is a quantitative effect. Higher levels of immune complexes could then lead to more severe disease, extensive clinical signs and higher RF levels. In the light of experimental animal studies such as those of Baum et al (1964), McCormick et al (1969), and DeHoratius and Williams (1972), which have been reviewed in Section 3.8.2, it may be that RF actually exacerbates tissue damage in congenital syphilis.

Fig.10.5: Scatter diagram of RF levels and placental weight ratios # in infants with congenital syphilis



Ratio of observed/expected placental weight

Fig.10.6: Scatter diagram showing RF and alanine aminotransferase levels in 13 infants with congenital syphilis

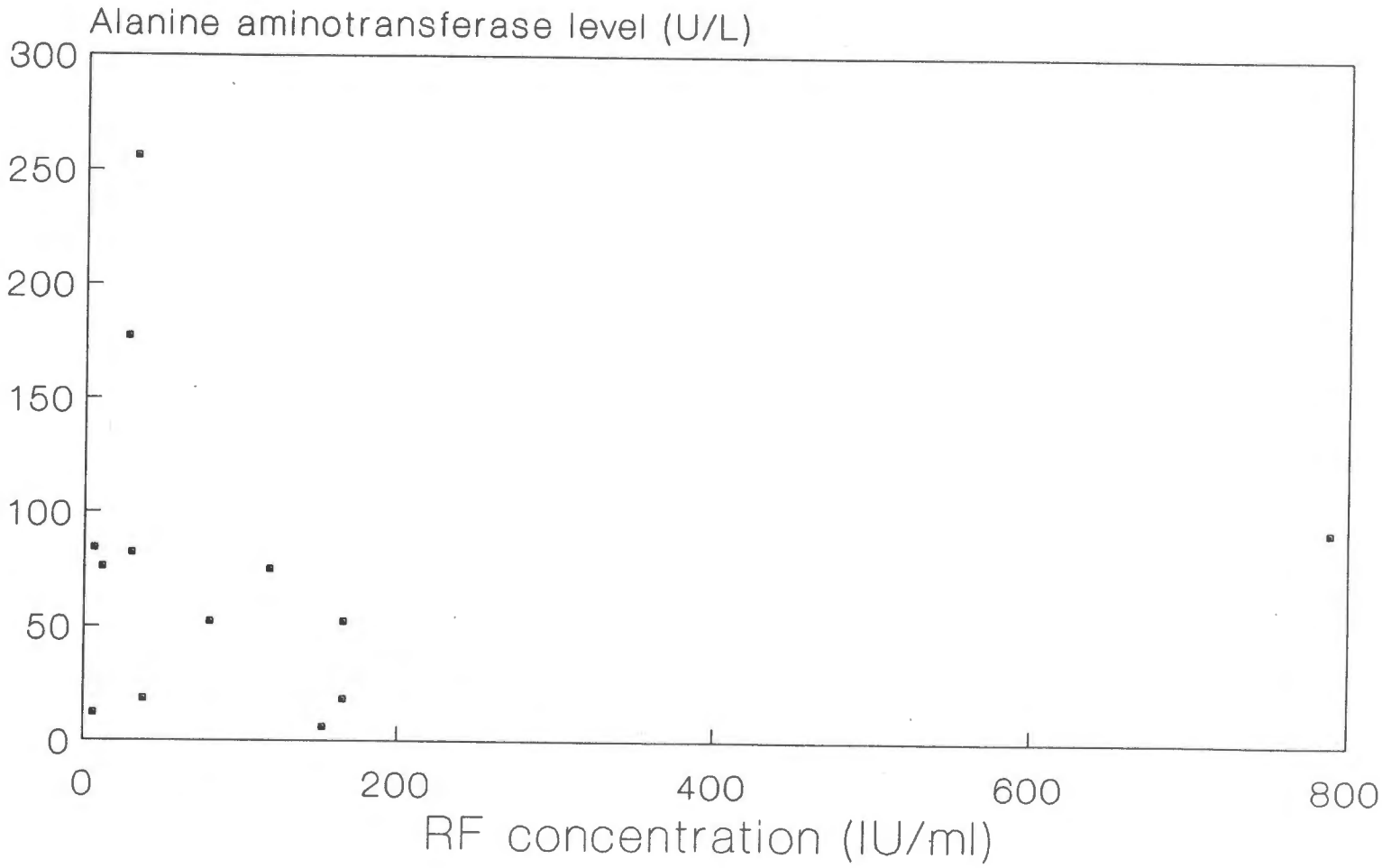


Fig.10.7: Scatter diagram showing RF and conjugated bilirubin levels in 16 infants with congenital syphilis

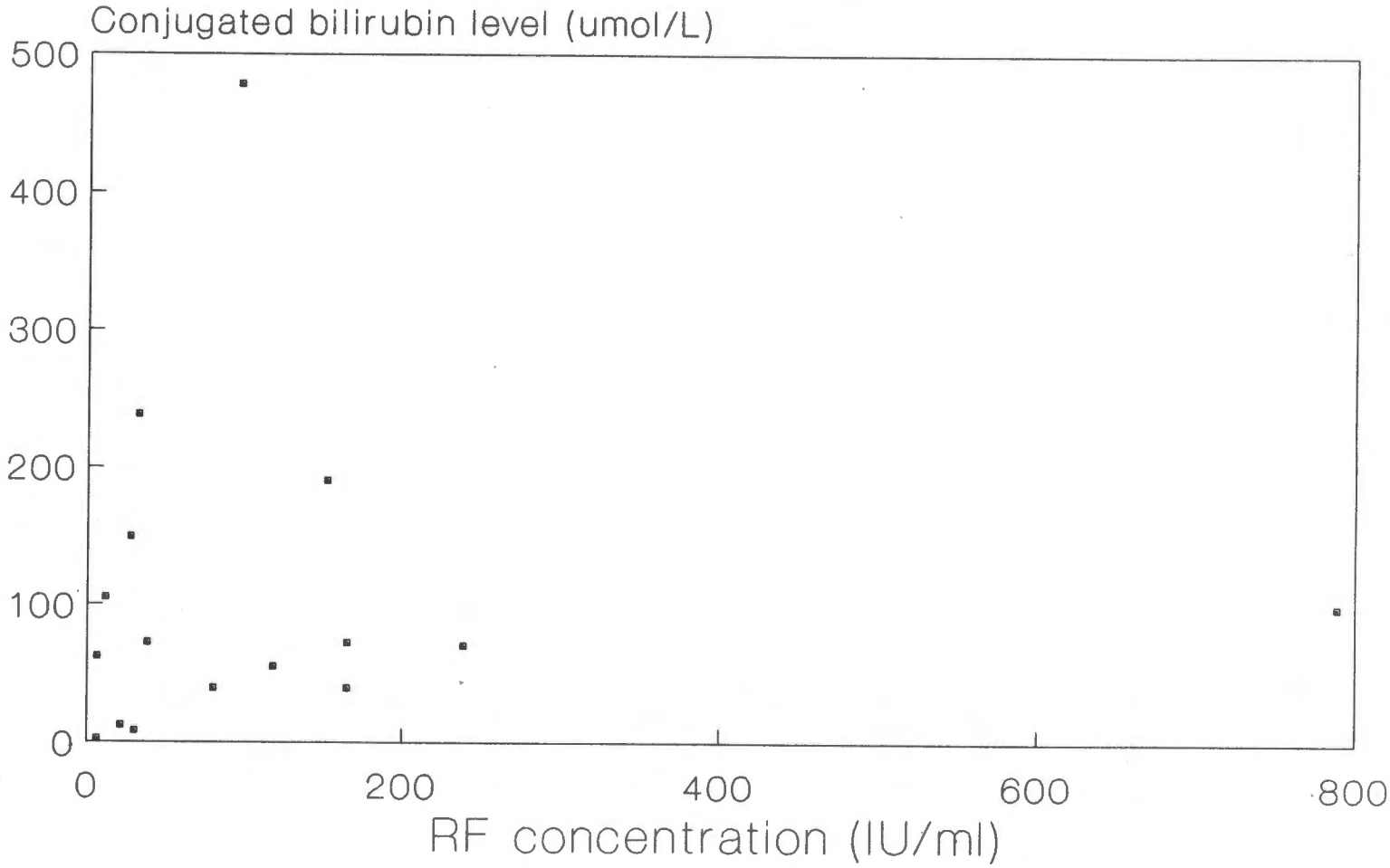


Fig.10.8: Scattergram of immune complex levels (% binding) and RF concentrations in infants with congenital syphilis

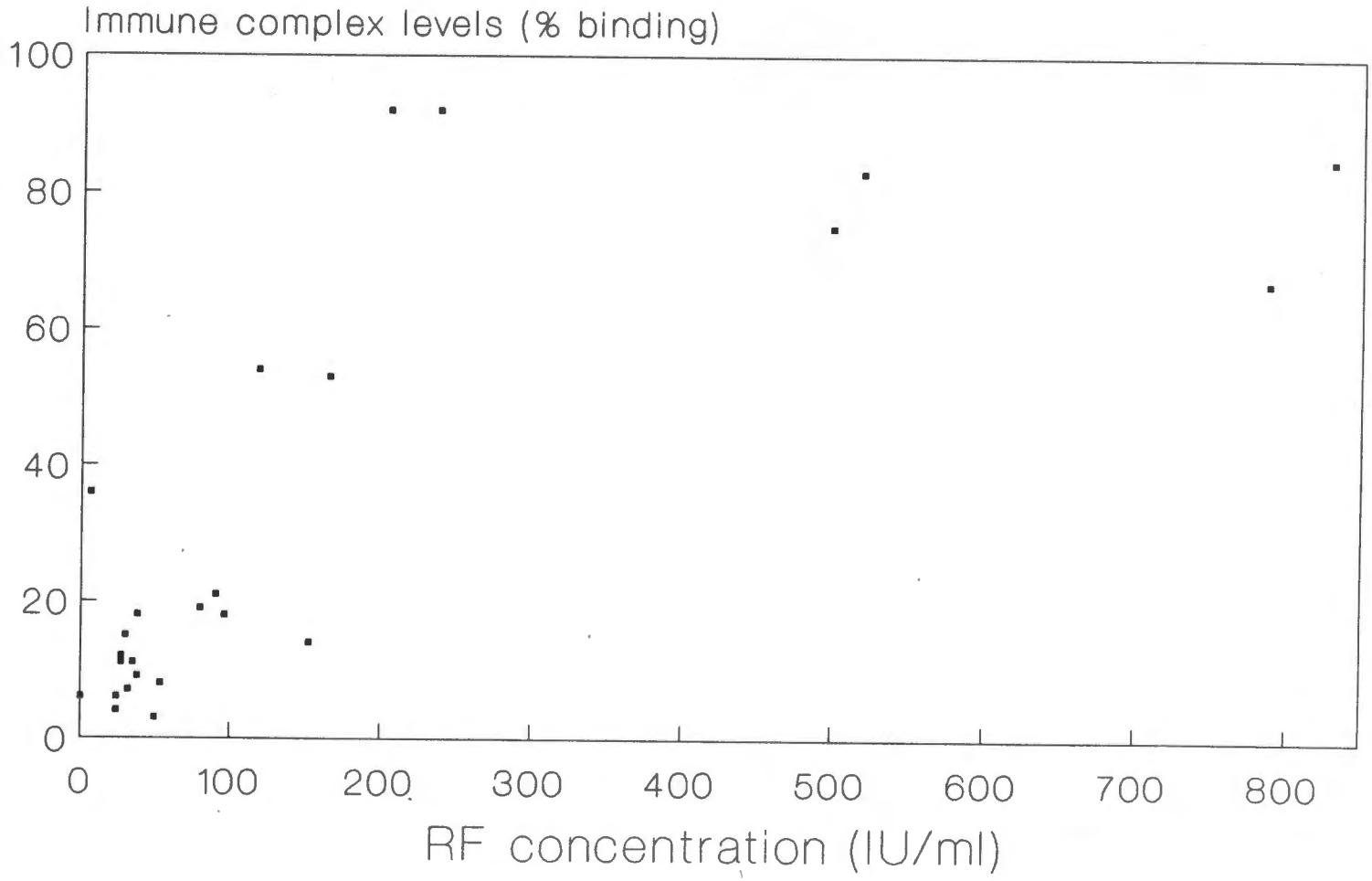


Fig. 10.9 Effect of treatment on median RF levels and VDRL titres in infants with congenital syphilis

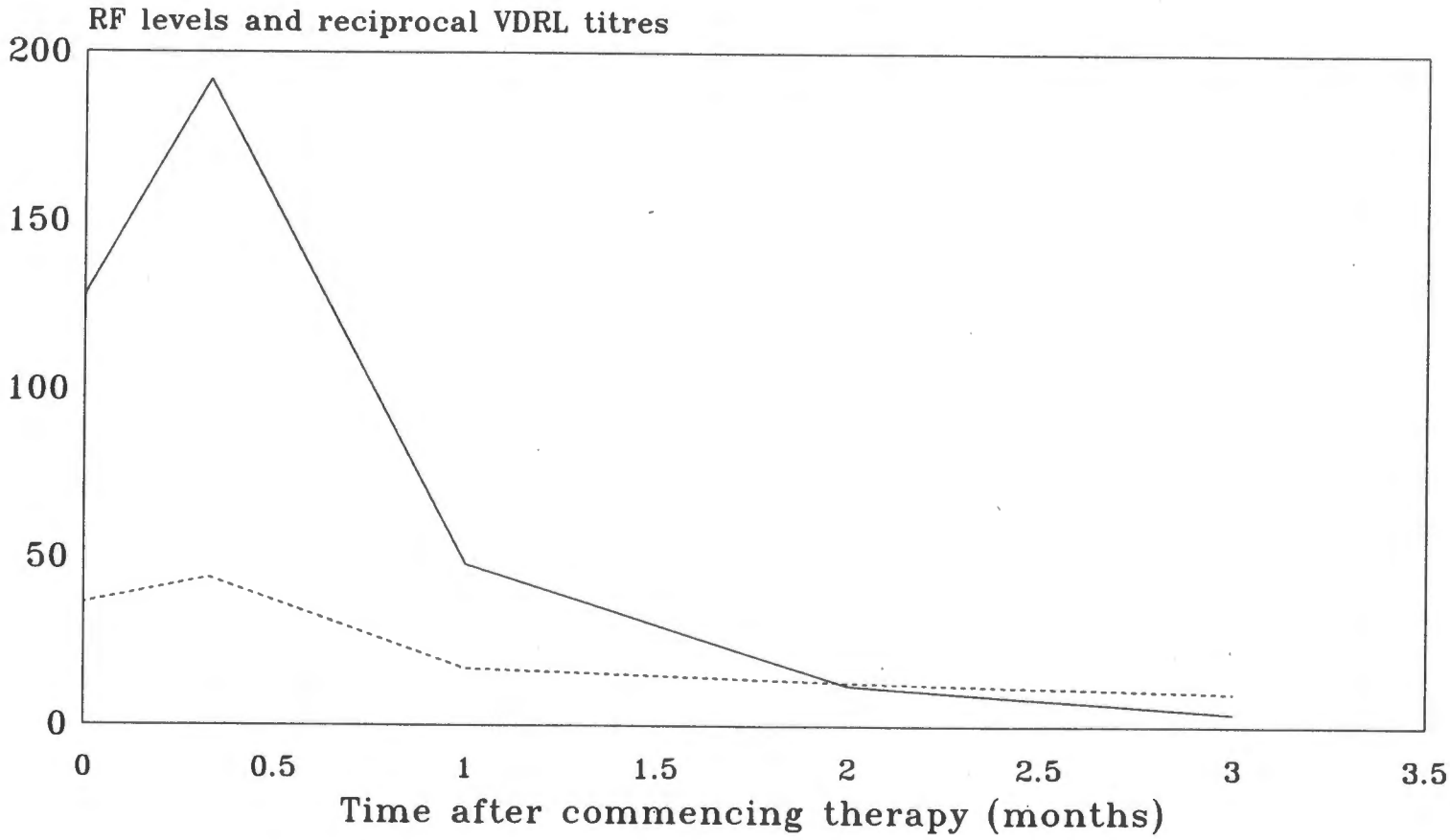
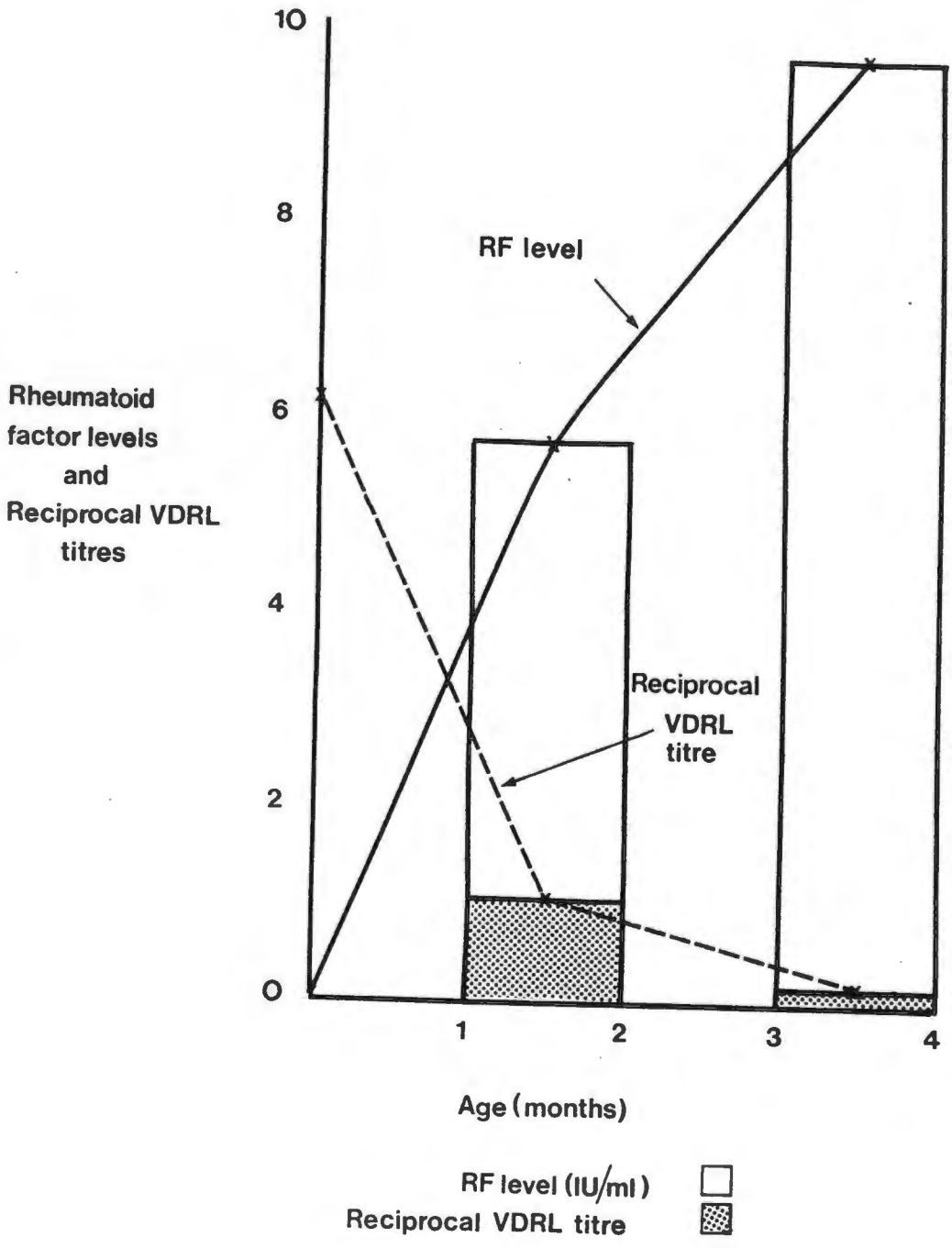


Fig 10.10 **Rheumatoid factor levels and VDRL titres in control infants**



The RF concentrations in the 5 newborns who died soon after birth were not higher than those of the newborns who survived. It was also evident that not all those who demised had the most extensive disease. These findings appeared to be contradictory to those just discussed and remain unexplained, although the numbers are probably too small to draw any conclusions.

There was no statistically significant relationship between VDRL titres and the severity or extent of the disease. A similar lack of relationship has also been noted in adult disease (Felman and Nikitas 1980).

10.4.3 Duration of infection

Messner et al (1968) and Bacon et al (1974) were able to show an association between RF levels and the duration of infection in SBE. Unfortunately, the chronicity of congenital syphilis is difficult to ascertain. It was thought that the simplest measure of duration would be the bony lesions, in particular the periosteal dystrophy (Ingraham 1936).

Using this criterion, there was no association between RF levels and the duration of infection. It is possible, however, that the older infants (who were more likely to have periosteal dystrophy Hira et al 1985) were also more likely to have milder disease (and lower RF levels). There were insufficient numbers of infants presenting after birth with early features of congenital syphilis (e.g. snuffles, skin rash) who did not also have involvement of other systems. On the other hand, renal involvement is thought to occur relatively late in the course of the disease (Ingall and Musher 1983 pg 348). The two infants with renal disease had very high RF levels. However, it is uncertain whether the elevated RF levels were associated with the renal pathology itself or the chronicity.

No other assessment of chronicity was possible, so that its role in determining RF levels in congenital syphilis remains uncertain, but from other literature cited, likely.

10.4.4 Disease pattern

Liver involvement: Infants with clinical or laboratory evidence of liver involvement had higher RF levels than those who did not ($p < 0.05$).

The effect of RF in aggravating inflammatory damage in the kidney and lung of experimental animals has been established (McCormick et al 1969, DeHoratius and Williams 1972). It remains a possibility, therefore, that RF had some potentiating effect on the liver damage. However, no correlation could be shown between the level of RF and the concentrations of ALT or conjugated bilirubin in the present study.

There are alternative explanations for the raised RF levels seen with liver disease. It is known that the liver is involved in the stripping of immune complexes from the circulation (Williams 1982). Liver disease could interfere with this process and result in higher levels of CICS which could in turn induce more RF synthesis (Nemazee 1985).

Another possibility is that the increase in RF parallels that of other gammaglobulins which are frequently elevated in chronic liver disease (Nelson 1979 et al pg 1149).

It is unknown if any of these mechanisms are responsible for the observed association between RF levels and liver disease.

Reticulo-endothelial involvement: The features of splenic and lymph node enlargement were not linked to higher RF levels ($p > 0.05$). Signs of RES involvement may be explained by lymphoid hyperplasia. Alternatively, extramedullary haematopoiesis related to anaemia and/or chronic hypoxic stress as well as prematurity may produce evidence of RES involvement (Fanaroff and Martin 1987 pg 836). Such involvement may, therefore, be allied to the haematological findings. Even when the variables of haematological and RES involvement were considered together, however, a significant association was not established.

Haematological system: The presence of anaemia and thrombocytopenia were not associated with higher RF levels in infants with congenital syphilis.

Anaemia in patients with RA has been related to the finding of RF in the serum (Harvey et al 1983). These workers put forward evidence that IgM antibodies and/or RF was capable of directly suppressing erythropoeisis.

Rheumatoid factor has also been proposed as a cause of haemolytic anaemia (Goldberg and Barnett 1969). In the present study only 2 infants with congenital syphilis (nos. 6.17 and 6.19) had definite evidence of haemolysis. These infants did not have RF levels which were significantly higher than those without haemolysis ($p > 0.05$).

The observations made in the study do not exclude any association between RF and anaemia or thrombocytopenia but simply indicate that a quantitative relationship was not demonstrated.

Bone disease: A statistically significant relationship between higher RF levels and bony lesions was not demonstrated in the present study ($p > 0.05$). The pathophysiology of the bony changes in congenital syphilis has not been established. In patients with RA a correlation has been noted between bony erosions and RF levels determined by agglutination tests, although a closer association has been shown with IgA RF (Arnason et al 1987). The periarticular lesions seen in RA have a different distribution to those of congenital syphilis. It seems unlikely, therefore, that RF is directly causitive of the symmetrical metaphyseal dysplasia of congenital syphilis.

Nevertheless, higher RF levels have been related to the presence of vasculitis in RA (Singer and Plotz 1956; Gordon et al 1973). It is conceivable that a vasculitis could affect the actively growing bones indirectly in congenital syphilis. This awaits further study.

Renal disease: Although only 2 infants with congenital syphilis had documented renal disease, the RF levels in these patients were significantly elevated compared to those who did not have renal disease ($p < 0.05$).

In general, the effect of RF in glomerulonephritis is unclear.

Rheumatoid factor has been demonstrated, however, in the blood and glomeruli in patients with post-streptococcal glomerulonephritis (McIntosh et al 1979; Rodriguez-Iturbe et al 1980)). In addition, RF was present in the glomeruli in transplant kidney rejection, systemic lupus erythematosis and polyarteritis nodosa (Rossen et al 1977). In these conditions the finding of RF was related to more severe renal damage.

However, the effect of the kidney damage itself on the RF levels is unknown. The concentrations of various proteins are altered in nephrotic syndrome; in general gammaglobulins are decreased but some macroglobulins e.g. $alpha_2$ globulin are increased (Pesce and First 1979 pg 38-39). IgM levels have been reported to be increased in 6/7 studies of children with minimal change nephrotic syndrome (Meadow et al 1981; Chan et al 1987). Two studies have reported persistently elevated IgM levels after remission in minimal change nephropathy and normal IgM concentrations in patients with nephrotic syndrome due to other causes (Giangiacomo et al 1975; Meadow et al 1981). A third, albeit smaller, study reported increased amounts of IgM in all patients with nephrotic syndrome. The IgM levels decreased on recovery (Chan et al 1987). The relationship between IgM levels and nephrotic syndrome is, therefore, unclear. In the present study the IgM levels in the patients with nephrotic syndrome due to congenital syphilis were not significantly higher than those without renal involvement ($p = 0.22$ using the Mann-Whitney test). These findings suggest that renal disease does not increase IgM concentrations in patients with congenital syphilis and, by inference, increased levels of IgM RF would not be expected in these cases.

In experimental animals, RF resulted in increased glomerular damage in nephrotoxic serum nephritis (McCormick et al 1969). Similarly, in the glomeruli of mice given intravenous immune complexes, RF not only attached to trapped complexes, but also allowed the binding of unrelated immune complexes. This immunoabsorbent effect of RF was thought to be related to its multivalency and could increase the pathogenicity of immune complexes (Ford and Kosatka 1982).

To demonstrate a direct pathological effect due to RF in congenital syphilis, however, an animal model would need to be found. In the meantime, other experimental work seems to support the concept that RF may augment renal damage due to immune complexes (Ford 1983).

Skin involvement: Concentrations of RF tended to be higher in patients with skin involvement but this relationship was not significant at the 5% level. Although the cause of skin lesions in congenital syphilis has not been documented, immune complexes have been associated with vasculitis and skin damage in acquired syphilis (Jorizzo et al 1986).

That RF may play a role in vasculitis in the skin has been suggested in transplant patients with CMV and in patients with RA (Westedt et al 1984; Baldwin et al 1987). The findings of the present study are not at variance with this hypothesis.

Lung involvement: This study did not find a statistically significant association between RF levels and lung disease in congenital syphilis ($p > 0.05$).

Rheumatoid factor has been demonstrated in the lungs of patients with RA who have rheumatoid lung (DeHoratius et al 1972). In addition, experimental animals develop more severe perigranulomatous pneumonitis and vasculitis in the presence of RF (DeHoratius and Williams 1972). On the basis of these experimental findings, an association between lung involvement in congenital syphilis and RF levels may have been expected. This was not the case, however.

The radiographic features used to define the presence of lung involvement in the infants are nonspecific (Malan 1987 pg 627). It is possible that the lung involvement in some of the infants with congenital syphilis was caused by other conditions e.g. chorioamnionitis. This may have masked any association between RF levels and lung disease.

Placental involvement: The enlargement of the placenta seen in congenital syphilis may be due to the presence of oedema (Budell 1982 pg 924). Congenital syphilis is associated with a

placental vasculitis (Russel and Altshuler 1974) and the latter may produce oedema. As RF can cause a vasculitis in experimental animals (Floyd and Tesar 1979) it was postulated that higher RF levels would be associated with a heavier, more oedematous placenta. The statistically significant correlation between RF levels and the placental weight ratio would provide some support for this postulate ($p < 0.05$ using Spearman's Rank Correlation Coefficient for a 1-tailed test).

Oedema: Infants with congenital syphilis with generalized oedema had higher RF levels than those who did not ($p < 0.05$). Oedema in congenital syphilis has been attributed to anaemia, cardiac failure, liver involvement and nephrotic syndrome (Ingall and Musher 1983 pg 345-349). A vasculitis, as observed in the placenta, could presumably occur in the skin and lead to oedema. It is also noteworthy that hydrops is associated with a poor prognosis (Tan 1973; Chawla et al 1988).

Both liver and kidney involvement have been shown to be related to RF levels in the present study. Furthermore, the severity appeared to correlate with RF levels. These factors may be the reason for the association demonstrated between RF levels and oedema.

Interrelationships between the variables, as implied above were considered likely. For this reason the statistical analysis was not extended to cover the large number of combinations that were possible.

10.4.5 Immune complexes

The demonstrated association between RF levels and levels of CICs was not unexpected. Experimental evidence exists which indicates that CICs give rise to RF production (Nemazee 1985; Coulie and Van Snick 1985).

Whilst Dobson et al (1988b) and Samson et al (1990) have reported finding elevated levels of CICs in infants with congenital syphilis there have been no published studies relating RF production in newborns with the presence of CICs. Several workers have compared RF levels and CIC levels in adults

with various conditions and using a number of CIC assays.

Thus, Carson et al (1978) showed a temporal relationship between CIC levels and RF concentrations in patients with SBE. The CIC levels did not correlate with the actual amount of RF (the Raji cell assay and conglutinin radioimmunoassay were used to assay CICs).

Elkon et al (1983), using a staphylococcal binding assay, were also unable to find an association between CIC levels and RF concentrations in SBE.

A similar lack of correlation between amounts of CIC and RF has been noted in acute post streptococcal glomerulonephritis (McIntosh et al 1979) and HIV infection (Procaccia et al 1987). The CIC levels in these 2 studies were determined using the C1q binding assay.

The findings in patients with RA appear to differ from those in the infectious diseases above in regard to CIC levels and RF concentrations. Thus, for example, both Pope et al (1981) and Gioud-Paquet et al (1987) found significant correlations between concentrations of CICs and RF in RA (C1q binding assays were used for detection of CICs).

The reason for this difference may be related to the fact that CICs in RA are composed primarily of IgG-IgG RF complexes or IgG-IgM RF complexes (Theofilopoulos and Dixon 1979). It follows, then, that CIC and RF concentrations would correlate.

It may be that a similar situation prevails in congenital syphilis (i.e. that the CICs are composed partly of RF). This has been suggested by Dobson et al (1988b) and the striking correlation observed between CIC levels and IgM RF concentrations would tend to support the idea.

It is also interesting that the CICs found in acquired syphilis in adults have a similar composition to those in infants with congenital syphilis (Baughn et al 1986; Dobson et al 1988b). Nevertheless, RF is found in only 13-25% of adults with acquired syphilis (Bartfeld 1969; Cerny et al 1985; Muller et al 1987)

compared to over 95% of infants with congenital infection (Reimer et al 1975; Meyer and Malan 1987).

These differences may indicate that infants produce RF more readily.This has been discussed in Sections 3.8.3.- 3.8.5.

10.4.6 Effect of treatment on RF levels and relationship to VDRL titres

The decline in the RF levels in the months following therapy in the infants with congenital syphilis was unlike the pattern observed in the controls.

The drop in RF levels in the infected infants was not unexpected, however. Similar observations have been made in SBE (Williams and Kunkel 1962; Bacon et al 1974). In addition, in RA effective therapy is followed by a decline in RF concentrations (Olsen and Jasin 1985).

A likely explanation for the findings in congenital syphilis is that treatment with penicillin reduced the levels of CICs by destroying the treponemal antigen.

It was interesting to note that the VDRL titre declined in a similar fashion to the RF concentration. Rheumatoid factor and anti-cardiolipin antibodies (which are measured by the VDRL test) are both auto-antibodies (Doniach 1976 pg 210). The close relationship between RF levels and the VDRL titres, and the fact that both declined following therapy, lends support to the idea that autoimmune phenomena may be involved in the pathogenesis of congenital syphilis.

Another noteworthy feature was that the RF level and the VDRL titre both increased initially following treatment. This was reminiscent of the rise in liver enzymes seen immediately after therapy (Long et al 1984), and may be akin to the Jarisch-Herxheimer reaction . A possible explanation for these events is that death of treponemes released new antigens which in turn invoked antibody production and immune complex formation (Ingall and Musher 1983 pg 363). The latter could lead to enhanced RF synthesis and raised RF levels within 2 weeks (McIntosh et al 1979). Furthermore, as CICs are cleared by the liver (Williams

1982), damage to the organ could result and lead to raised liver enzymes. Finally, cardiolipin is one of the antigenic components of T.pallidum (Musher 1984 pg 294). Release of this substance may not only result in elevation of the VDRL titre but could cause immune complex formation (and in turn stimulate RF production).

The rise in RF concentration observed 1 month after completion of treatment in patient no. 5.3 was difficult to explain. It was also uncertain why the RF level at diagnosis was not increased. The levels of CICs in this patient, although elevated (6,0%), were not as high as in most of the other patients. Possibly the stimulus was insufficient to produce circulating RF (it is conceivable that RF was present but had been deposited in the tissues). The fact that RF was detected after treatment (and presumably after clearance of the treponemal antigen) may indicate that the RF had been formed in response to other causes such as the Gm antigens on maternal IgG (Steinberg and Wilson 1965).

10.5 SUMMARY

Various clinical and laboratory findings were correlated with RF levels in congenital syphilis. The ranked RF levels were found to be associated with the severity and extent of the disease, involvement of the liver and kidney and with the presence of oedema. In addition, a close relationship between RF concentrations and levels of CICs as well as the VDRL titres were found.

Essentially the relationship was between RF, CICs and the severity and extent of the disease. The separate relationship with liver and kidney disease may simply reflect that disease of these organs is part of the definition of the severity and extent of the condition. The oedema may be exacerbated by liver or renal disease.

Taken together, these findings suggest that RF is related in some way to the disease process. Work in experimental animals indicates that RF can exacerbate tissue damage (DeHoratius and

Williams 1972; Floyd and Tesar 1979). The results of this study would appear to support the suggestion that RF may assume a pathological role in certain circumstances.

Having demonstrated that similar factors are operative in the RF production in congenital syphilis and other conditions it would appear to be worth investigating congenital syphilis as a possible model for the induction of RF synthesis. This would have the advantage that a germ-line response could be studied early in life when the RF-manufacturing CD5+ cells are known to be plentiful (Hardy et al 1987; Levinson et al 1987).

CHAPTER 11

DEMONSTRATION OF TISSUE-BOUND RHEUMATOID FACTOR IN CONGENITAL SYPHILIS USING IMMUNOFLUORESCENCE

The demonstrated association between RF levels, the severity and extent of the disease and the concentrations of circulating immune complexes (CICs) in infants with congenital syphilis implies that RFs and CICs may be mediators of tissue inflammation and damage in this condition. This hypothesis would be strengthened by finding these entities at the sites of tissue damage.

Indeed, Samson (1989) has demonstrated components of immune complexes (i.e. IgG, IgM and complement) in the placentae of 5 infants with congenital syphilis. Similar complexes were not found in the placentae of controls. The presence of RF has not been documented in any of the organs of infants with congenital syphilis but RF has been detected by means of fluorescein-labelled IgG in other inflammatory conditions (Mellors et al 1959, Rossen et al 1977).

The aim of this section was to use immunocytochemistry to show the presence of RF in tissues from patients with congenital syphilis. At the same time it was planned to confirm that immune complexes were deposited in the same vicinity as the RF.

11.1 METHODS

11.1.1 Preparation of fluorescein-labelled IgG

Materials

The following materials were used:

IgG: this was a commercially obtained purified human IgG from a pooled source (ICN Immunobiologicals USA, catalogue no 64-145.

Fluorescein isothiocyanate (FITC) - this was supplied by Sigma Chemicals, USA.

Buffers and solutions: Phosphate buffered saline pH 7.2 was

made up as described in Chapter 8.

In addition, a solution of 0.25 M sodium carbonate pH 9.0 in 0.1 M sodium chloride was prepared.

Sephadex G-25 column: The column was 1.5 cm in diameter and 30 cm long (Biorad, Econo-column USA). The Sephadex G-25 was a product of Pharmacia Fine Chemicals, USA. Medium particle size (50 - 150 u) was used.

Spectrophotometer: A Beckman spectrophotometer, model 25 was used.

Methods:

The IgG was dissolved in the carbonate buffer (20 mg in 2 ml). The fluorescein was added at a concentration of 0.03 mg per mg of IgG (Johnson and Holborow 1986 pg 28.4). The mixture was left for 22 hours at 4°C.

The Sephadex column was used to separate bound from unbound fluorescein by means of gel filtration. The column was prepared according to the method described by Johnstone and Thorpe (1982). Air bubbles were removed from 75 ml of the swollen Sephadex particles in PBS pH 7.2 by applying a vacuum. The slurry was suspended in 250 ml of buffer and allowed to stand for 20 minutes. Fine particles were then aspirated and a final volume of approximately 100 ml obtained.

The column outlet was closed and 10 ml of PBS pH 7.2 added. The gel slurry was stirred and poured into the column until the latter was filled to within 2 cm of the top. The particles were allowed to settle for 2 hours. Excess buffer was passed through the column and the column was packed with the Sephadex gel. The fluorescein and IgG mixture was added to the column and a flow rate of 100 ml/hr was used. The column was eluted with PBS (pH 7.2)and the first coloured fraction was collected. The fluorescein/protein ratio was calculated after measuring the absorbance of the conjugated fraction at 280 and 495 nm.

The following formula was applied (Johnstone and Thorpe 1982 pg

259): Molar ratio =

$$\frac{2.87 \times \text{absorbance at 495 nm}}{\text{absorbance at 280 nm} - 0.35 \times \text{absorbance at 495 nm}}$$

The molar fluorescein/protein ratios were 2.33 - 2.89. The protein concentration of the fluorochrome IgG conjugate was measured according to the formula: mg/ml protein =

(1.55 x absorbance at 280 nm)-(0.76 x absorbance at 260 nm) (Richterich 1969 pg 241).

A portion of the FITC-labelled IgG was heat-aggregated (Ha) before use as this preparation has been shown to be more reactive (Mellors et al 1959). The heat-aggregation was carried out by heating in a waterbath to 63°C for 10 minutes (Gripenberg et al 1979, Fehr et al 1981).

11.1.2 Other fluorescein-labelled conjugates

Fluorescein-labelled anti-human IgG was acquired from Behring, West Germany (catalogue number OTKB04/05). Fluorescein-labelled rabbit anti-human IgM and anti-human C3 were obtained from the same source (catalogue numbers OTKC04/05 and OTKD 04/05)

11.1.3 Immunohistochemical method

Frozen tissue sections were air dried and then fixed with anhydrous acetone for 10 minutes at 4°C. The sections were washed with water followed by 0.05 M PBS pH 7.2. Excess water was removed from the slides and the tissues were then stained.

Protein concentrations of FITC-labelled IgG of 2.8 mg/ml, 1.4 mg/ml, 0.7 mg/ml and 0.35 mg/ml were used initially to determine the optimum dilution. Sufficient fluorochrome conjugate was placed on the slides to cover the tissue and the sections were stained for 1 hour at room temperature.

The specificity of the immunohistochemical procedure was checked by inhibition tests. These were carried out by reacting the tissues with non-labelled IgG for 30 minutes. This was followed

by washing with PBS pH 7.2, and addition of the FITC-labelled IgG for 1 hour.

Following the staining procedure, slides were washed with PBS pH 7.2 and cover-slips applied.

The staining procedure for the other fluorochromes was identical to that described above for the FITC-IgG. Protein concentrations of 1.2 mg/ml were used.

Fresh section were examined with a fluorescence microscope (Nikon Optiphot) which employed epi-fluorescent illumination. Microscopy was performed using objectives with magnifications of 10-60 times.

Photography was carried out with a Nikon camera attachment (FX-35A) Fujichrome and Konica film (400 ASA) were used.

11.1.4 Patient Selection

Patients with congenital syphilis: Tissues from patients with congenital syphilis were available as follows:

Postmortem Specimens: Specimens were obtained (with parental consent) from 3 newborn infants with congenital syphilis.

Patient 1: this patient was described in Chapter 6 (code no. 6.1). This postmortem interval was 2 days

Patient 2: This preterm infant has also been described in Chapter 6 (code no. 6.15). The cause of death was probably a secondary septicaemia (Escherichia coli) and the postmortem interval was also 2 days.

Patient 3: (code no. 11.1) This male infant, whose gestational age was 34 weeks died 20 minutes after birth. The infant was under weight for gestational age (1990 g), pale and oedematous. The placenta weighed 970 g and was pale. The infant's mother had a positive VDRL (titre 1: 2048) and TPHA. She had received 1 dose of 2.4 megaunits of long-acting benzathine penicillin 1 week prior to delivery.

Apart from the postmortem results to be described, other investigations were as follows: radiographs of the long bones demonstrated a marked metaphyseal dysplasia and periosteal reaction. The FTA-ABs (IgM) test was positive and the RF latex test was positive at a dilution of 1:40.

Placentae: In addition to the placentae from the 3 infants who died, placentae were available from 2 other infants with congenital syphilis. The placentae from patients 11.1,11.2 and 11.3 were obtained at birth and frozen immediately.

Patient 11.2: She was a preterm infant (30 weeks gestation) weighing 1100 g, who was found to have hepatoslenomegaly. The placenta weighed 300g and was friable and oedematous. The maternal VDRL was positive to a titre of 1:512 and the TPHA was positive. Radiographs of the long bones showed metaphyseal dystophy and the serum RF concentration was 24 IU/ml.

Patient 11.3: This infant was 30 weeks gestation and weighed 1450g at birth. The infant had clinical features of pallor, oedema and hepatosplenomegaly. The placenta weighed 700g and was pale. The maternal VDRL titre was 1:256 and the TPHA was positive. The patient demised shortly after birth.

Control patients

Postmortem specimens:

Patient no 11.4: This infant was a newborn of 33 weeks gestation. He died 6 hours after birth primarily as a result of hyaline membrane disease. The mother had negative serological tests for syphilis. The consent of the parents was obtained and sections of the liver and spleen were stained with the fluorescein-IgG conjugate. Tissue from this patient was used as a negative control for patient no. 11.1.

Placentae: Placental tissue was obtained from 4 control stillborn infants whose gestational age had been matched with that of the test patients. The mothers gave informed consent for the placentae to be examined. The stillbirths were fresh and the placentae had no macroscopical evidence of

chorioamnionitis. All 4 stillbirths were due to abruptio placentae. The gestational age was assessed by means of the maternal history, ultrasonic findings and the external characteristics of the infants. The tissue for microscopic examination was obtained from areas of the placenta which were not haemorrhagic and frozen immediately. Table 11.1 shows the clinical details for the control patients.

After obtaining the tissues they were frozen with liquid nitrogen and stored at -70°C until used.

11.2 RESULTS

11.2.1 Optimum FITC-IgG concentrations

The best contrast between tissue fluorescence and backgrounds was obtained with 0.35-0.7 mg/ml of the protein-conjugate.

11.2.2. Inhibition experiments

The use of aggregated-IgG at a concentration of 0.7-1.4 mg/ml was found to completely prevent binding of the FITC-labelled IgG.

11.2.3 Tissue sections

The tissues obtained from patients 1 and 2 were autolytic and unsuitable for further study. The results obtained with the remaining patients will be described under the headings of the organ studied.

Placenta: Placentae which were appropriate for histological study were obtained from 3 patients with congenital syphilis (11.1, 11.2 and 11,3).

The findings on light microscopy were those of a mild chronic inflammatory infiltrate in case no. 11.1 and blunting and enlargement of the villi in case no. 11.2. Case no. 11.3 showed the same features as 11.2 except that patchy inflammatory changes and a vasculitis were also present.

TABLE 11.1:

CLINICAL DETAILS OF PATIENTS WHOSE PLACENTAE WERE USED FOR CONTROL PURPOSES

NUMBER	BIRTHWEIGHT (g)	ESTIMATED GESTATIONAL AGE (COMPLETED WEEKS)	CLINICAL INFORMATION	PLACENTAL WEIGHT (g)	MATERNAL VDRL RESULT
11.5	800	30	Female, preterm, SGA	200	-ve
11.6	1700	36	Female, preterm	400	-ve
11.7	1050	30	Male, preterm	275	-ve
11.8	1500	36	Female, preterm	400	-ve

Using the Ha fluorescein-labelled IgG to detect RF, positive immunofluorescence was demonstrated in the placentae of all the infants with congenital syphilis.

The main regions of positivity were the inner lining of small blood vessels within the placental villi. As shown in the photographs which follow, many vessels appeared to be involved. The fluorescence was regular and the vessel lining was smooth. There were, in addition, granular deposits in the stroma of some of the villi. It was not possible to determine if these accumulations were intracellular (photographs 11a, 11c, 11e and 11m).

The positive staining was completely inhibited by the use of non-labelled IgG (photographs 11b, 11d and 11f).

The native i.e. non-heat aggregated IgG-fluorochrome conjugate produced far less fluorescence than the Ha preparation. Staining was present in the lining of some of the small blood vessels in patients with congenital syphilis.

Unstained placental tissue from the patients with congenital syphilis did not demonstrate autofluorescence

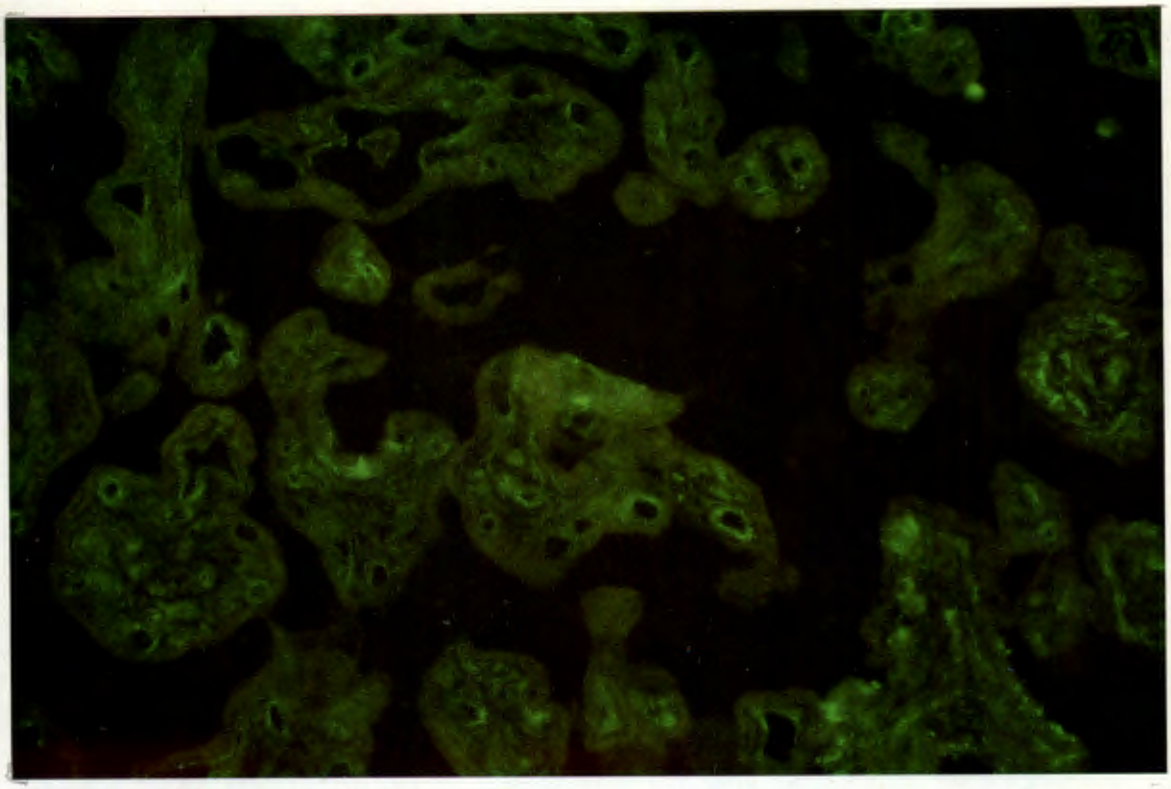
The immunohistochemical staining with fluorescein-labelled anti-human IgM, anti-human IgG and anti-C3 was also positive in the placentae of patients with congenital syphilis. Positivity was most marked along the course of small blood vessels. Photographs 11k, 11n and 11p demonstrate these findings.

The placentae of the 4 controls had a different histological appearance to that described above.

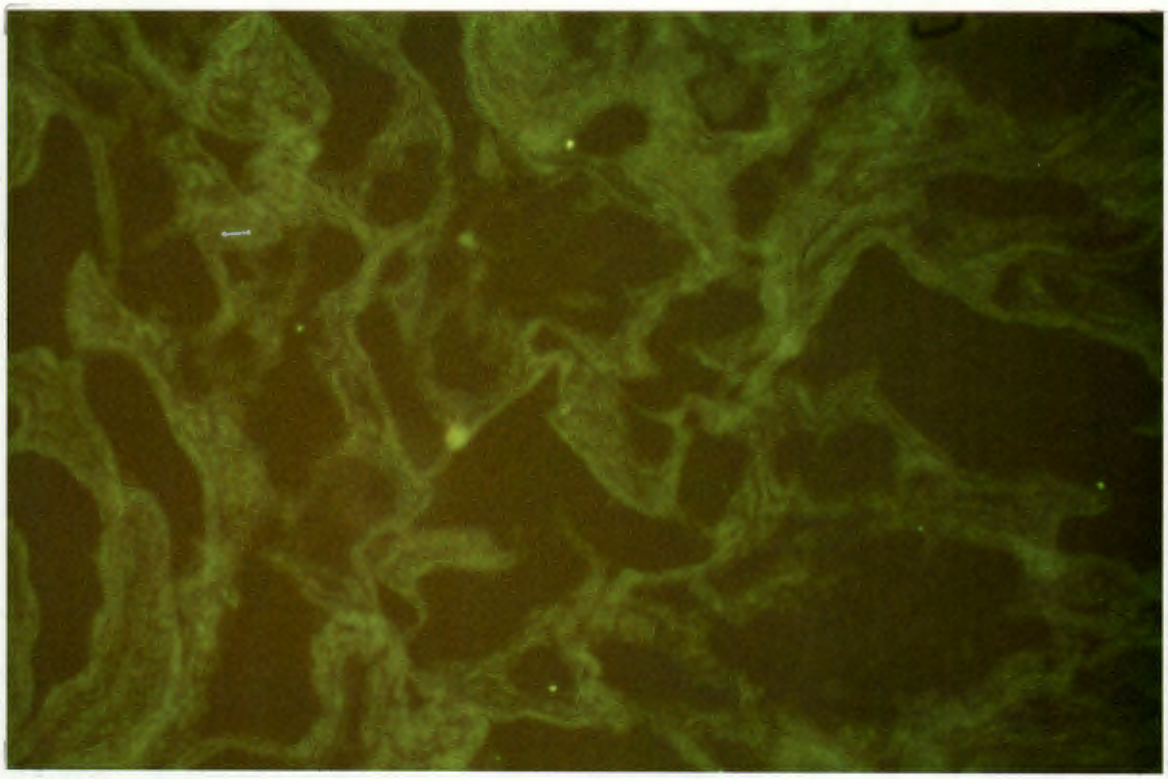
Light microscopy revealed that the placentae were normal; there was no inflammatory cell infiltrate.

There was minimal staining with the Ha fluorescein-labelled IgG (photographs 11g, 11h, 11l, 11o). No fluorescence was observed in control placentae using non-aggragated IgG.

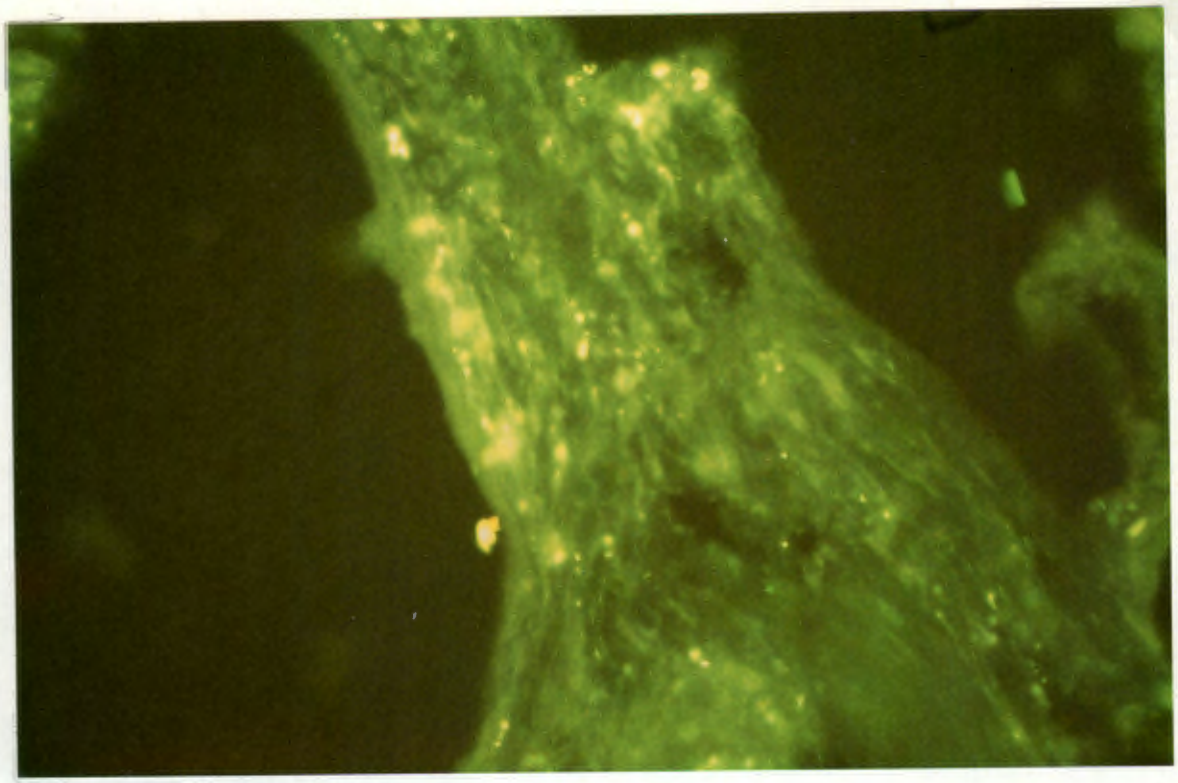
In addition, the use of the anti-human IgM, anti-human IgG and anti-C3 indicated that positive fluorescence was not present in



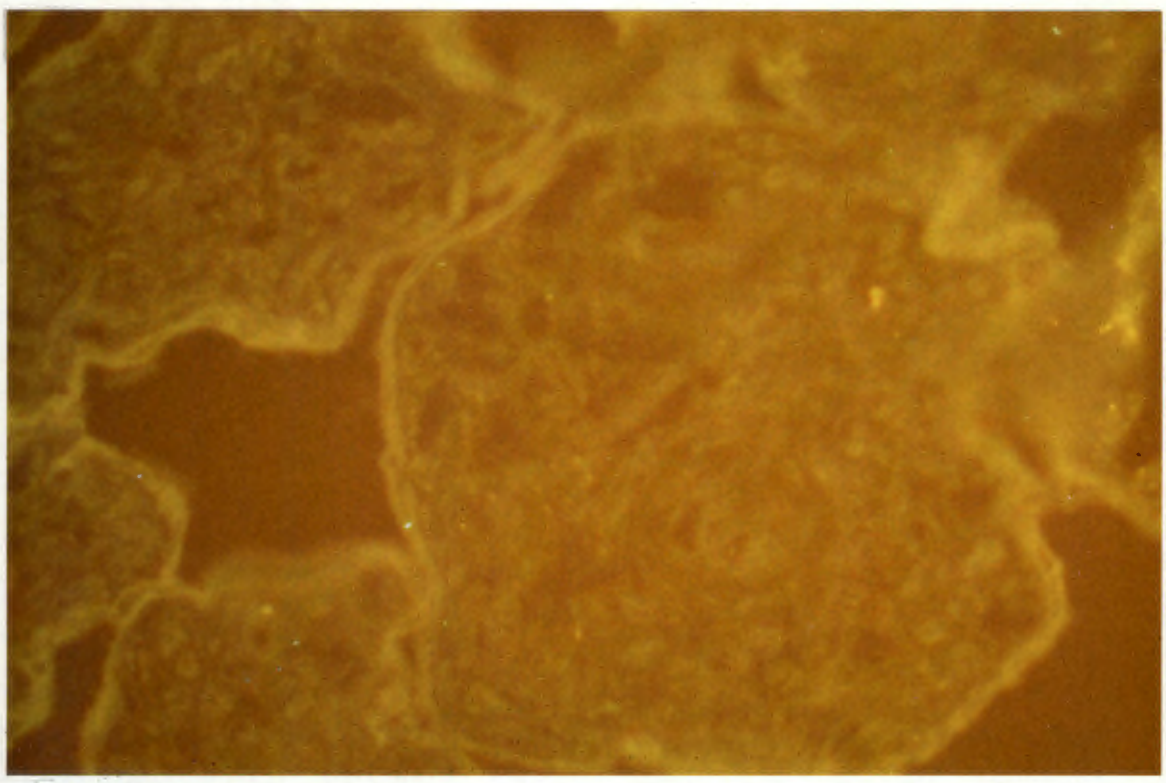
11a Placenta of pateint no 11.1 stained with FITC-IgG. (Magnification x20, Fuji film 400 ASA). Note RF deposits in blood vessels and villi.



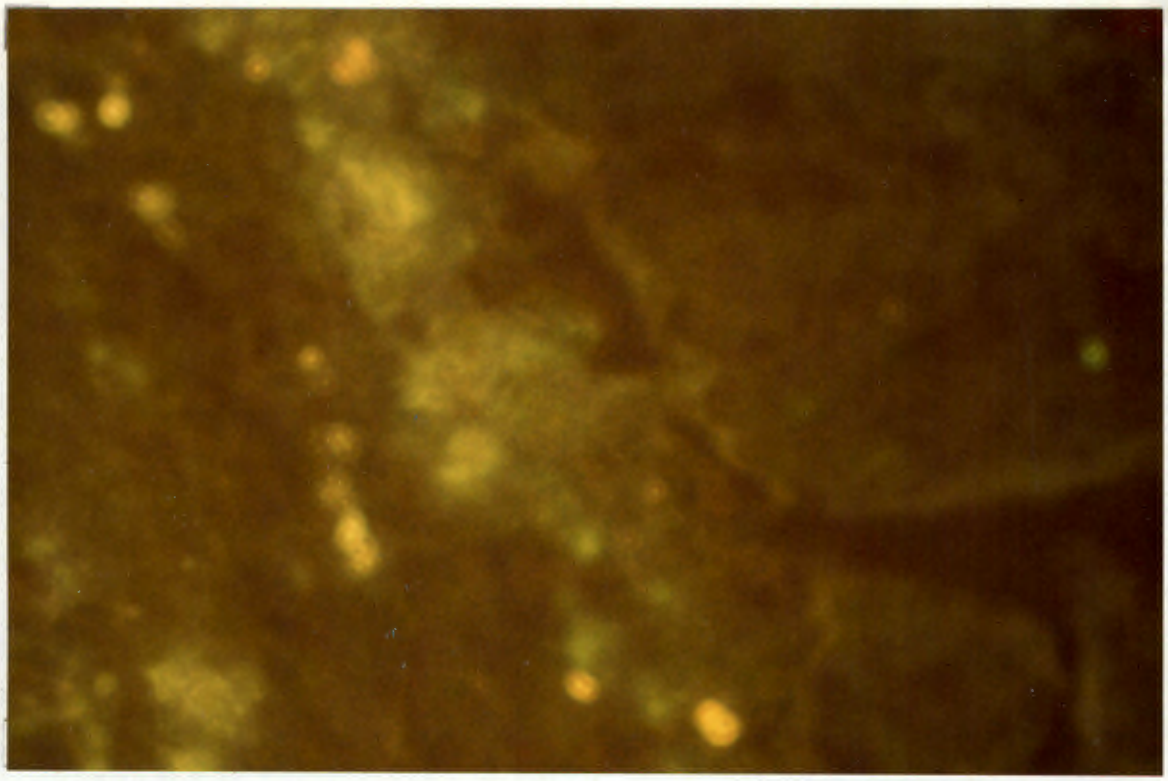
11b Same placenta stained with FITC-IgG after an inhibition step using non-labelled heat-aggregated IgG (Magnification x20, Fuji film 400 ASA, neutral density filter ND4). Note absence of fluorescence.



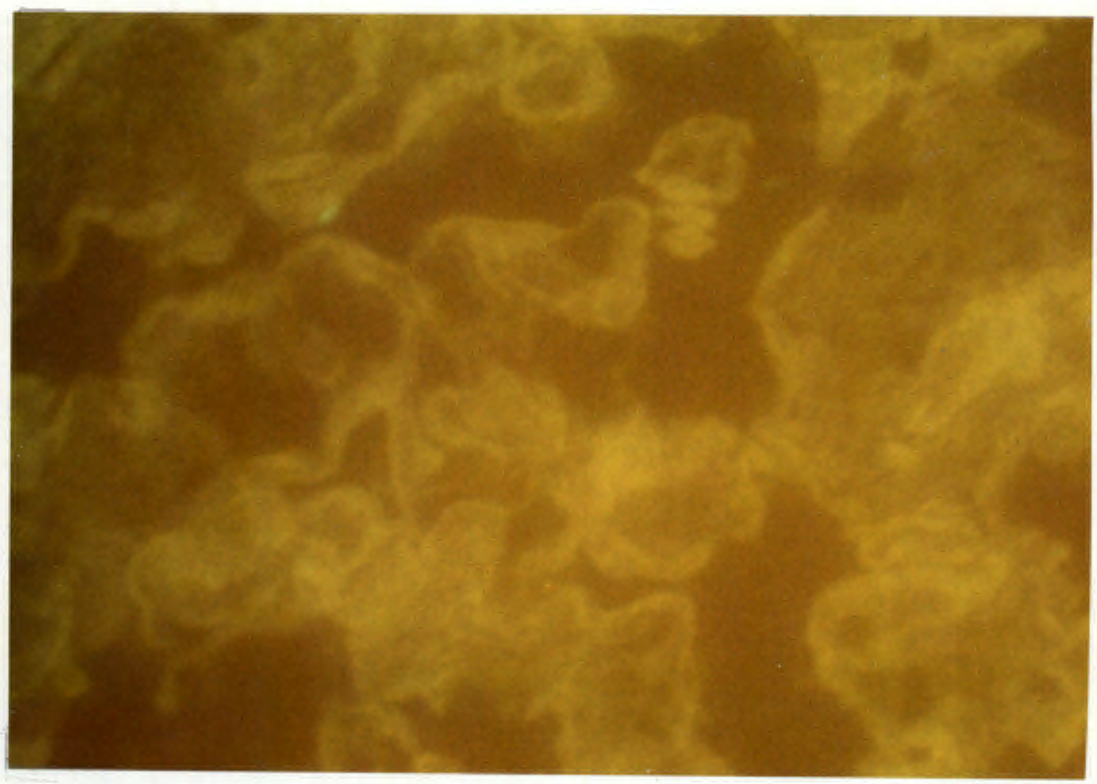
11c Placenta of patient no 11.2 stained with FITC-IgG. (Magnification x40, Fuji film 400 ASA, neutral density filter ND2). Granular deposits of RF along walls of small blood vessels and in the stroma of the villi.



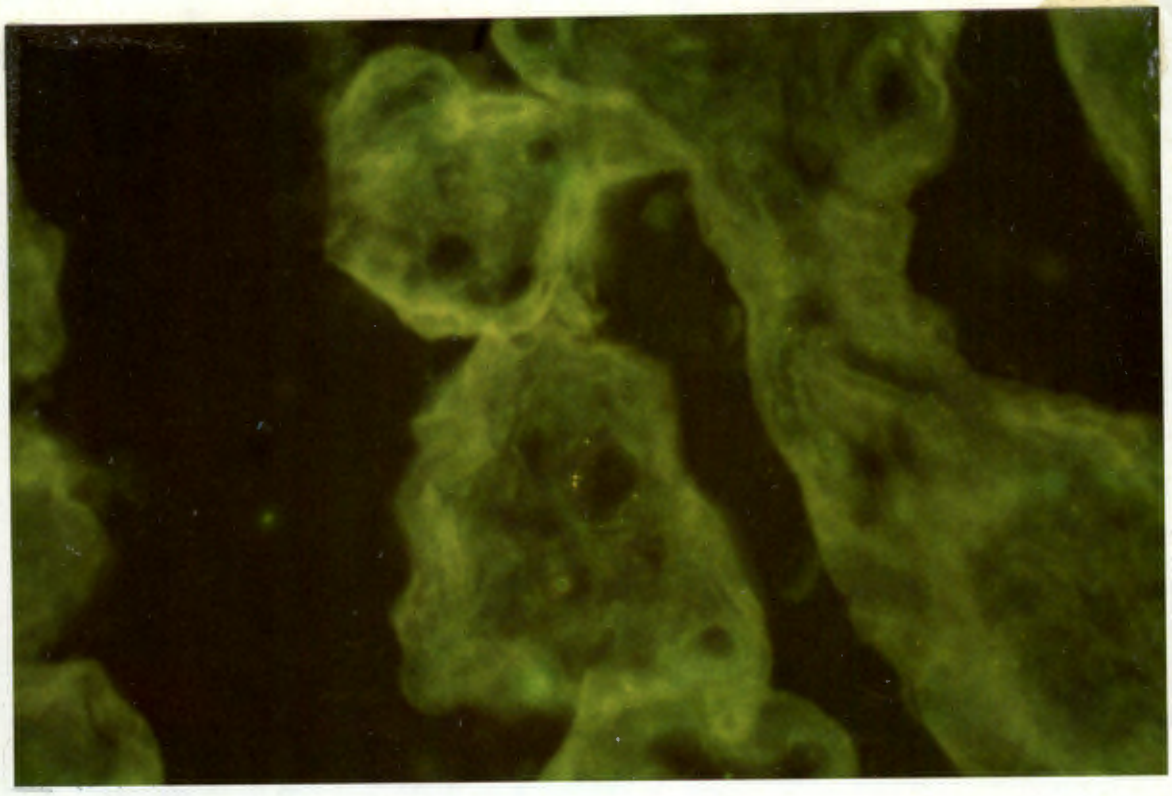
11d Placenta of patient no 11.2 stained with FITC-IgG after an inhibition step using non-labelled heat-aggregated IgG. (Magnification x40, Fuji film 400 ASA neutral density filter ND4). A lack of immunofluorescence is present.



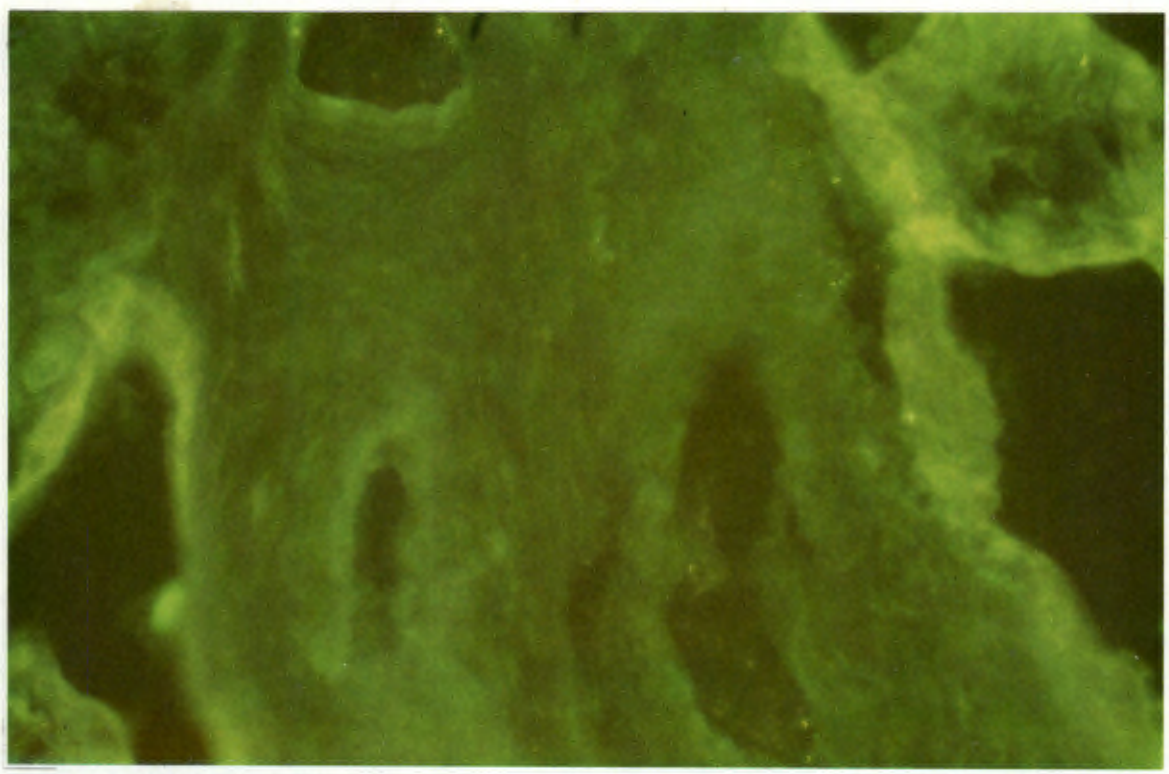
1e Placenta of patient no 11.3 stained with FITC-IgG. (Magnification x40, Fuji film, neutral density filter ND2). Fine and coarsely granular RF deposits in a placental villus. Some of the larger deposits may be intracellular.



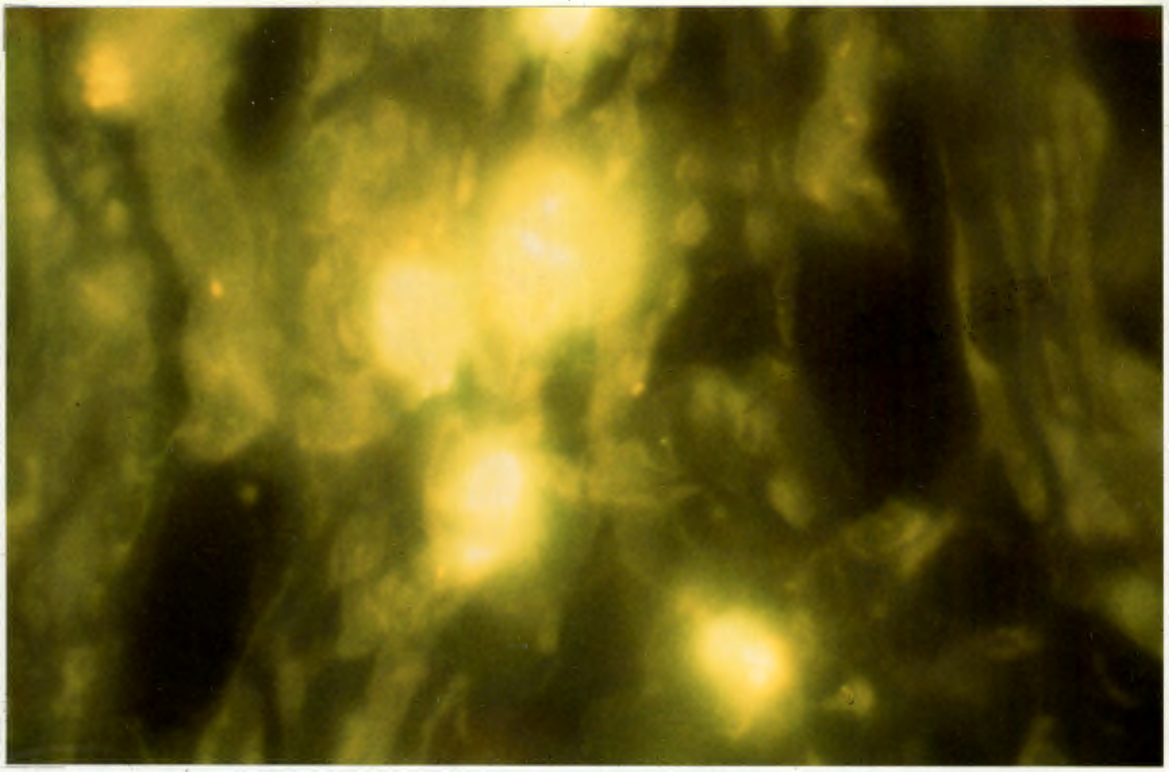
f Placenta of patient no 11.3 stained with FITC-IgG after an inhibition step using non-labelled heat-aggregated IgG. (Magnification 40x, Fuji film 400 ASA, neutral density filter ND4). Immunofluorescence is absent.



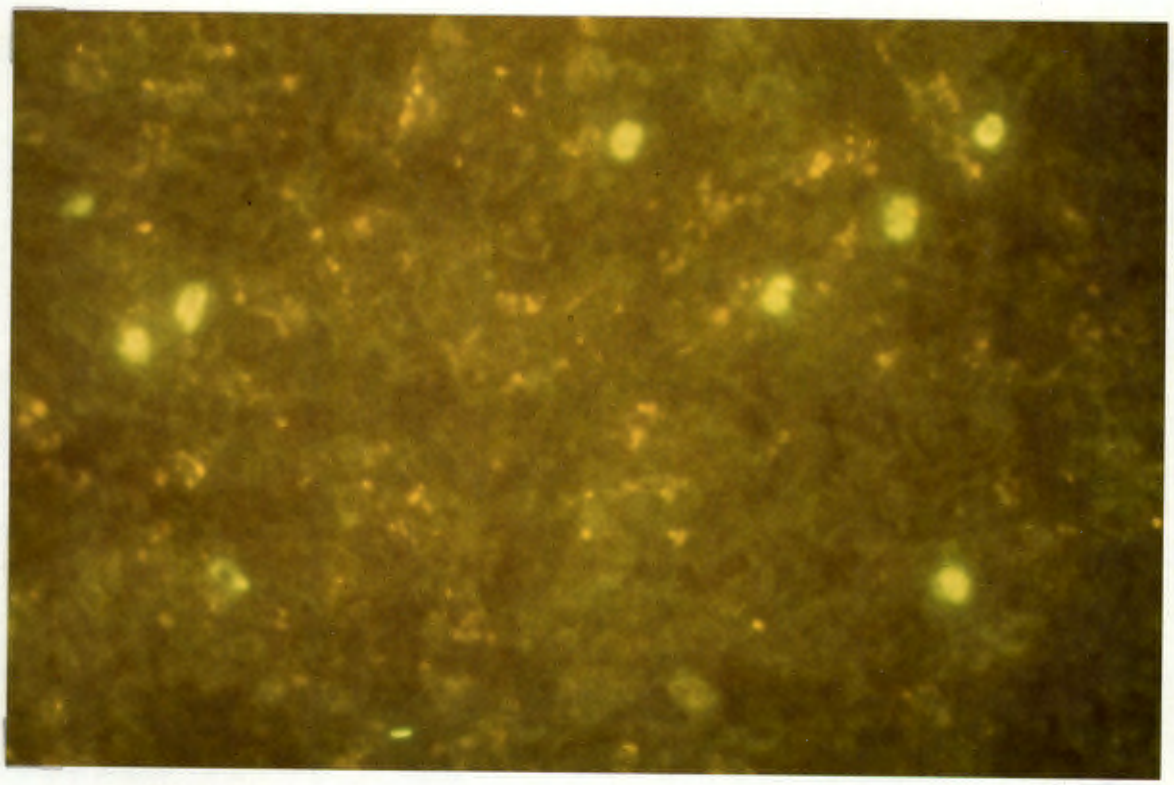
11g Placenta of control patient no 11.5 stained with FITC-IgG. (Magnification x20, Fuji film 400 ASA). No RF was demonstrated.



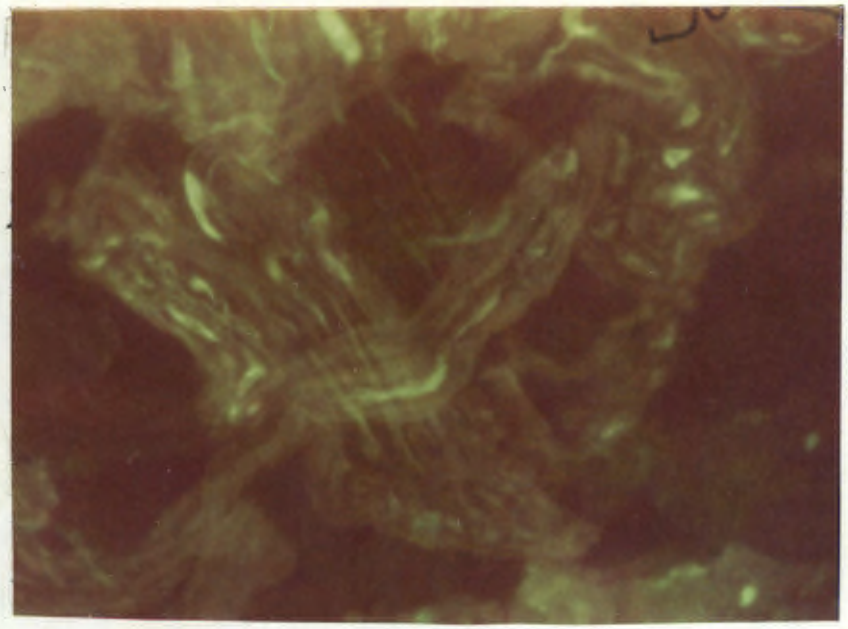
1h Placenta of control patient no 11.7 stained with FITC-IgG. (Magnification x40, Fuji film 400 ASA). No RF was localized.



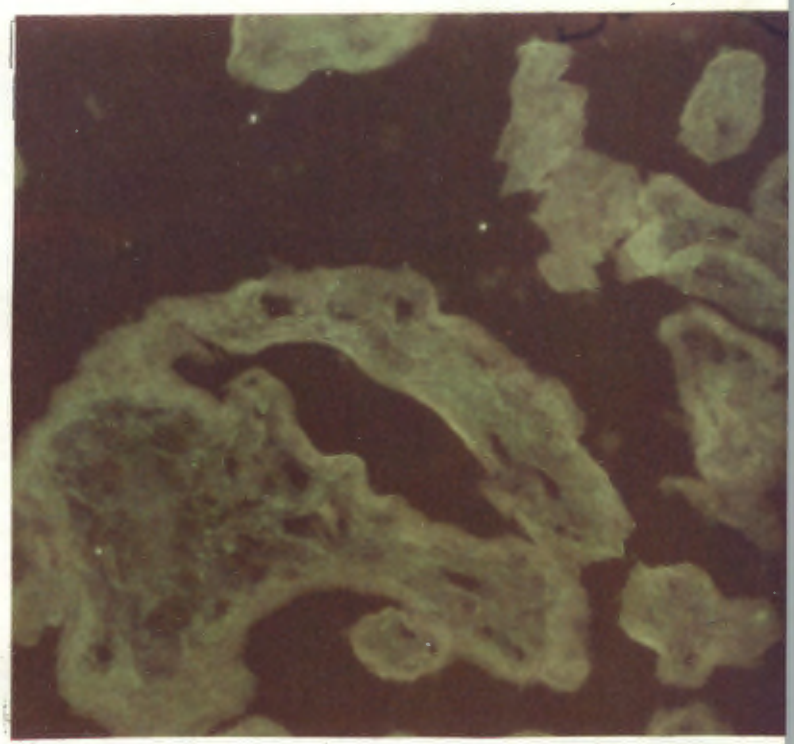
11i Liver of patient no 11.1 with congenital syphilis stained with FITC-IgG. Positive immunofluorescent staining in Kuppfer cells adjacent to a portal tract. (Magnification x40, Fuji film 400 ASA)



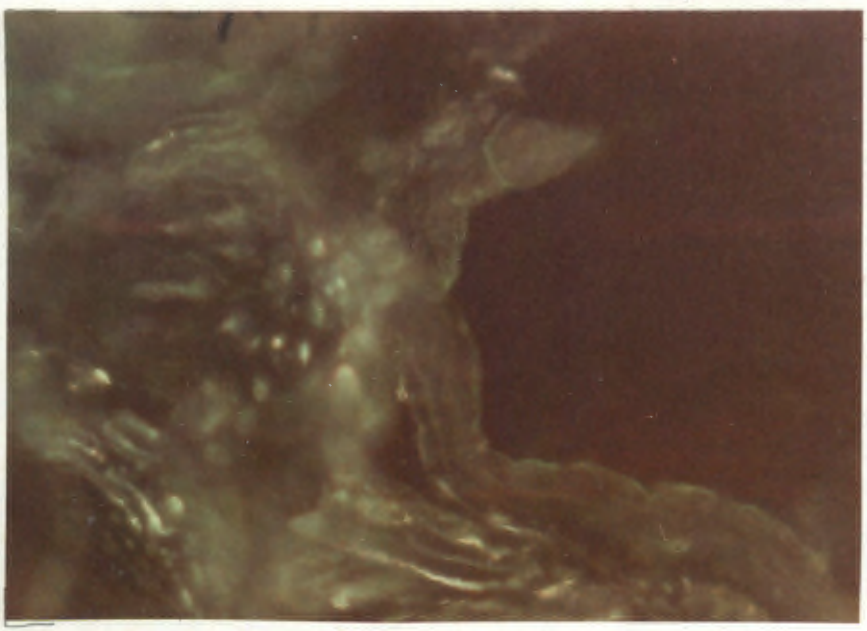
1j Spleen of patient no 11.1 with congenital syphilis stained with FITC-IgG. Bright immunofluorescence in macrophages, lesser fluorescence in cells amongst splenic pulp. (Magnification x40, Fuji film 400 ASA, neutral density filter ND4).



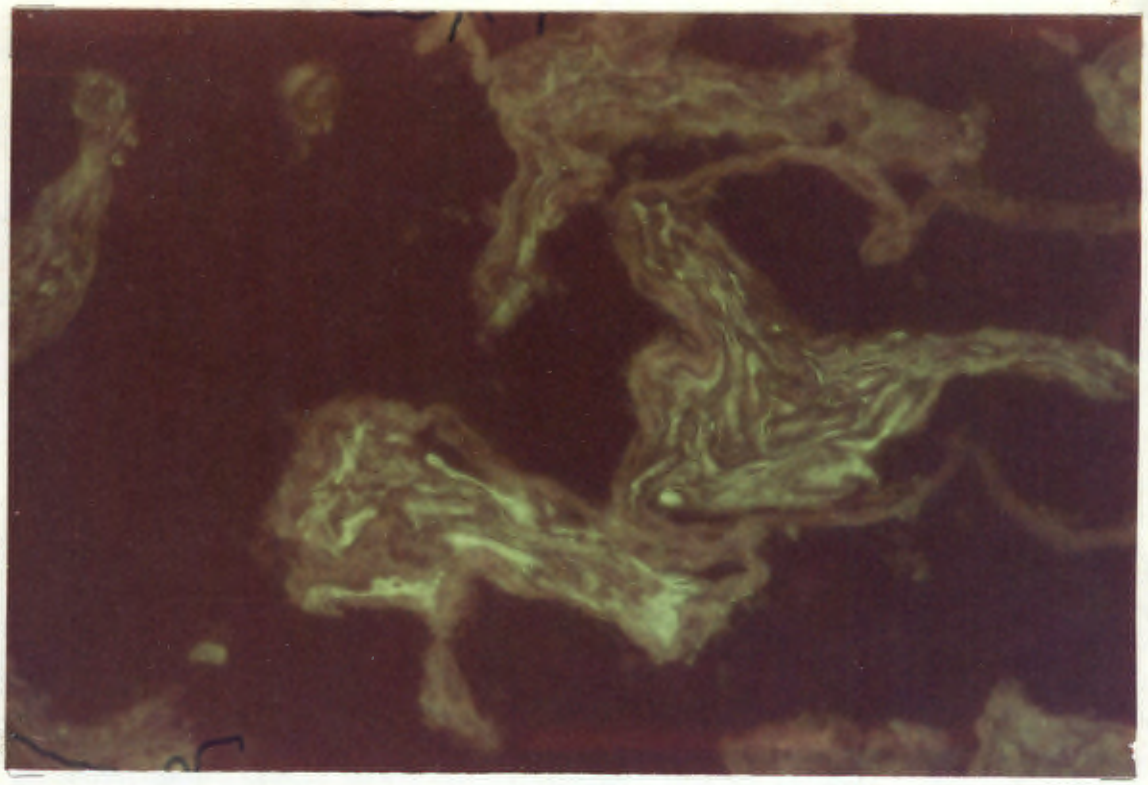
11k Placenta of patient no 11.1 with congenital syphilis stained with fluorescein-labelled rabbit anti-human IgM. Note the localization of IgM to the walls of blood vessels (Magnification x20 Konica film 400 ASA)



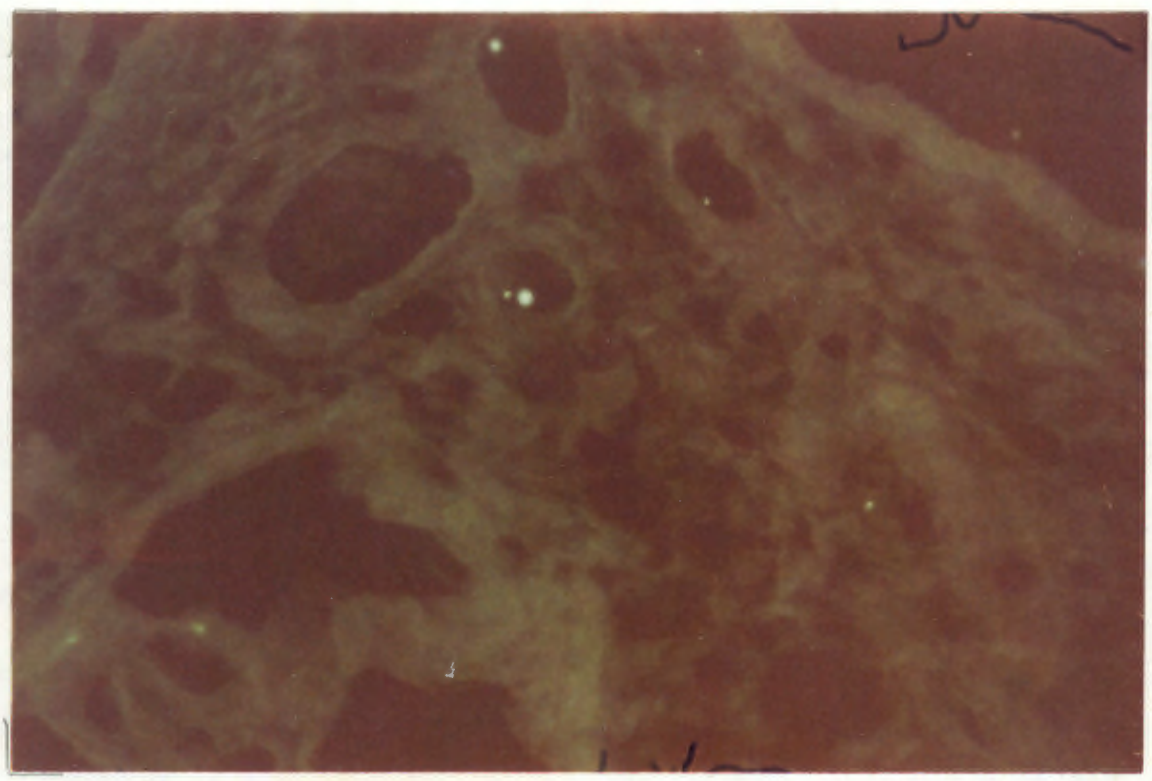
111 Placenta of control patient no 11.5 stained with fluorescein-labelled rabbit anti-human IgM. No IgM staining noted. (Magnification x20 Konica film 400 ASA).



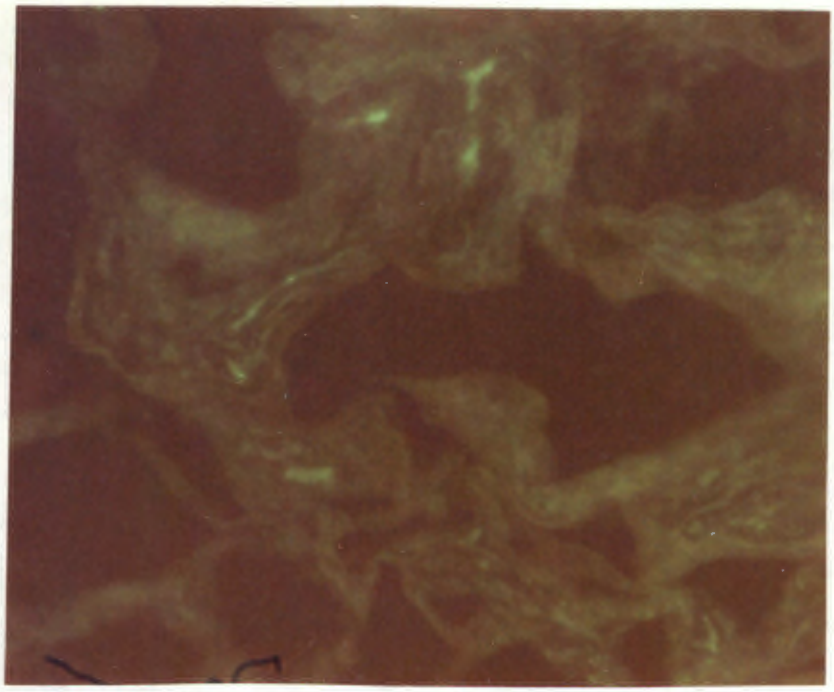
11m Placenta of patient no 11.1 with congenital syphilis stained with fluorescein-labelled IgG. Note the presence of RF in a similar location to the IgM in photograph 11k. In addition, extravascular RF is present. (Magnification x20, Konica film 400 ASA).



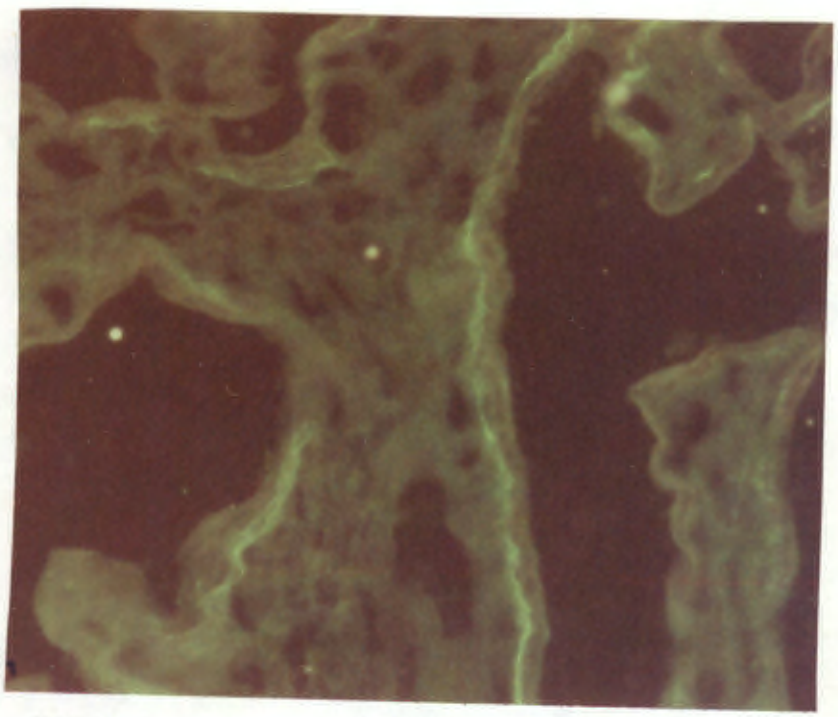
11n Placenta of patient no 11.2 with congenital syphilis stained with fluorescein-labelled rabbit anti-human IgG. Note extensive IgG depostis in walls of blood vessels. (Magnification x20, Konica film ASA 400)



11o Placenta of control patient no 11.5 stained with fluorescein-labelled rabbit anti-human IgG. No positive tissue fluorescence is seen. (Magnification x20, Konica film ASA 400).



11p Placenta of patient no 11.1 with congenital syphilis stained with fluorescein-labelled rabbit anti-human C3. Complement deposits are localized around blood vessels. (Magnification x20, Konica film ASA 400).



11q Placenta of control patient no 11.5 stained with fluorescein-labelled rabbit anti-human C3. Trophoblast-lining cells have taken up the stain. (Magnification x20, Konica film ASA 400).

the fetal blood vessels of the controls. There was, however, a linear deposit of C3 detected on the maternal surface of some villi (photograph 11q).

Liver: The light microscopic findings in patient no. 11.1 were those of a mild periportal inflammation and a mild inflammatory cell infiltrate extending into the parenchyma. Using Ha fluorescein-labelled IgG, positive fluorescence was demonstrated in the portal tracts (photograph 11i).

The fluorescence was most marked in Kuppfer cells and not blocked by the use of non-labelled IgG. Positive staining was not seen in chronic inflammatory cells or in the liver parenchyma.

The liver from the patient who served as a control (no. 11.4) was normal by light microscopy. The sections stained with Ha fluorescein-labelled IgG had a pattern of immunofluorescence similar to that described above (most marked in Kuppfer cells). Again, the fluorescence was not inhibited by the use of non-labelled IgG.

Spleen: The presence of an inflammatory infiltrate and congestion was noted on light microcopy in patient no. 11.1. Positive fluorescence was noted following staining with Hag fluorescein-labelled IgG. The fluorescence was not inhibited by non-labelled IgG and was observed in macrophages and cells in the splenic pulp (photograph 11j).

Similar findings were present in the spleen of the control patient (no. 11.4).

Kidneys: Tissue obtained from patient no. 11.1 at autopsy showed mild inflammatory changes only. Immunofluorescent staining was negative.

No abnormalities were demonstrated in the kidneys of the control patient.

Lungs: A marked inflammatory infiltrate was noted with the presence of plasma cells, lymphocytes and fibrosis in the lungs of the patient with congenital syphilis. In spite of numerous attempts, for technical reasons frozen sections of the lung could not be cut.

Other Findings: The patient with congenital syphilis (No. 11.1) had fibrotic changes in the pancreas and extramedullary haematopoiesis in many organs. The brain and pancreas were not further examined because of the presence of autolysis.

Summary of results

Positive immunofluorescence was observed using both non-aggregated and heat aggregated fluorescein-labelled IgG to detect RF in the placentae of all 3 patients with congenital syphilis studied. The changes were most marked in small blood vessels.

In addition, using fluorescein-labelled anti-human IgM, IgG and C3 fluorescence was noted in the placentae of these same patients. These phenomena were not seen in unstained placentae or to the same degree in the placentae of control infants.

Non-specific positive fluorescence was noted in the liver and spleen of both the patient with congenital syphilis and in the control patient.

11.3 DISCUSSION

There was a marked difference demonstrated in the staining of the placentae from patients with congenital syphilis and controls. The staining with heat aggregated FITC-IgG in the patients appeared to be specific for antibodies to IgG (i.e. RF) or Fc receptors in that it was inhibited by prior treatment with non-labelled IgG.

Using tissues derived from patients with RA, various workers (e.g. Mellors et al 1959; Hijmans et al 1969; Munthe and Natvig 1971; Fehr et al 1981) have put forward evidence that the use of fluorescein-labelled IgG detects RF. It may be that the

fluorescent deposits observed with the FITC-IgG in patients with congenital syphilis in this study were due to RF.

Nevertheless, the placenta contains Fc receptors which could bind the aggregated IgG (Burton and Gregory 1986 pg 11). Indeed, such receptors have been shown to be present on endothelial cells in the apical aspect of fetal stem vessels (Johnson et al 1976). In addition, IgG coated red blood cells have been successfully used as indicators to detect Fc receptors on trophoblastic tissue (Page Faulk and Johnson 1980 pg 12-14). Receptors in the latter site are not demonstrated when aggegated IgG is used (Johnson et al 1976).

Three types of Fc receptor are known to exist. Of these, only type II receptors (Fc γ RII) have been found in the placenta. Type II receptors bind aggregated IgG and immune complexes, but have no affinity for native IgG (Tax and Van der Winkel 1990).

In the light of these findings, the FITC-IgG staining observed in the placentae with the heat aggregated preparation could be due to Fc receptors. However, Fc γ RII have no affinity for non-aggregated IgG so that the use of this substance would not detect these receptors. Rheumatoid factors, although having greater affinity for aggregated IgG will bind to non-aggregated IgG (Rossen et al 1977). The demonstration of placental immunofluorescence using non-aggregated IgG in the present study is likely to be due to the presence of RF.

As both Fc receptors and RF recognize the same epitope(s) on IgG (Mouritsen 1986), blocking experiments would not be expected to allow differentiation between Fc γ RII and RF. In keeping with this, unlabelled heat aggregated IgG prevented all fluorochrome staining in the present study.

There was a striking difference between the fluorescence observed in the placentae of patients and controls using the labelled HaIgG. This could indicate increased numbers or affinity of the Fc receptors in response to disease, the presence of RF or both.

The effect of congenital syphilis and the immune complexes found therein on Fc receptor expression are unknown. In acute mastitis, however, immune complexes can block Fc receptors on phagocytic cells (Targowski and Klucinski 1985). In addition,in a number of diseases associated with the presence of immune complexes (e.g. systemic lupus erythematosis, Sjogren's syndrome and dermatitis herpetiformis) there is decreased clearance of IgG-coated erythrocytes. In the case of systemic lupus erythematosis, the clearance returns to normal with remission of the disease (Unkeless et al 1988). In other diseases such as malaria, there is an increased expression of monocyte Fc receptors (Ward et al 1984). Proteolysis, such as may occur in sites of inflammation, can increase the affinity of type II Fc receptors for IgG (Tax and Van der Winkel 1990). Although the effects of infection and inflammation on Fc receptor expression appear to be variable, the possibility of increased affinity in congenital syphilis cannot be excluded.

The positive staining of trophoblast observed with heat aggregated FITC-IgG in the placentae of patients with congenital syphilis is unlikely to be due to Fc receptors. In contrast to the endothelial cells Fcδ RII have not been demonstrated in trophoblastic tissue using fluorescein-labelled IgG (Johnson et al 1976). The positive staining in the present study may have been due to deposits of RF.

Utilizing other fluorochromes (anti-human IgG, IgM and C3) components of immune complexes, particularly IgM, were demonstrated in the placentae, thus confirming the work of Samson (1989). The pattern of immune complex localization appeared to be similar to that of heat aggregated FITC-IgG accumulation.

Taken together, these findings suggest the presence of RF in the placentae of infants with congenital syphilis.

While there have been no previous reports of RF deposits in the blood vessels of patients with congenital syphilis, a placental vasculitis has been well described (Russell and Altshuler 1974).

Similarly, other workers have shown a relationship between RF and vasculitis in various conditions apart from congenital syphilis. In RA there is an association between vasculitis (and other systemic manifestations of RA) and RF levels (Epstein and Engleman 1957; Gordon et al 1973; Theofilopoulos et al 1974; Gioud-Paquet et al 1987).

DeHoratius et al (1972) observed extensive pulmonary capillary RF deposition in patients with rheumatoid lung. In order to investigate the role of RF in the pulmonary lesions of RA, an animal model was developed (DeHoratius and Williams 1972). these workers found that after inducing a diffuse proliferative lung disease in rabbits, the intravenous injection of RF resulted in arteriolar RF deposition. In addition, some of the animals developed segmental haemorrhagic lung infarction following the injection. The RF was also deposited in alveolar walls and appeared to cause an increase in inflammation around existing granulomas.

Other work in experimental animals appears to confirm the relationship between RF and vasculitis.

Experiments performed by Baum et al (1964) demonstrated that infusion of RF and immune complexes into rat mesenteric vessels caused vasculitis. Thrombosis was also observed. The pattern of RF deposition was linear in nature, similar to that found in the placentae of the 3 infants with congenital syphilis.

Floyd and Tesar (1979), using an experimental model, put forward evidence that RF was linked with inflammation and vasculitis. These workers studied the cutaneous Arthus reaction in rats. Injection of immune complexes and normal IgM resulted in only mild inflammatory changes. However, injecting IgM RF and immune complexes caused the Arthus reaction and a vasculitis to develop.

DeHoratius and Williams (1972) proposed that immune complex deposition resulted in the RF localization in the same area. Ford and Kosatka (1982) found that injecting RF into mice who had deposited immune complexes in their glomeruli resulted in RF

accumulation in the same site. The RF then acted as an immunoabsorbent for the deposition of further immune complexes. This could result in chronic ongoing damage.

These findings, together with those of this study, suggest that RF, together with immune complexes may induce a vasculitis in the placentae of infants with congenital syphilis. The experimental evidence cited above indicates that RF may aggravate the vascular and tissue damage.

No attempt was made in the present study to relate the serum RF levels to the degree of RF deposition in the placentae. Nevertheless, the association demonstrated between more severe and extensive disease and RF levels indicates that higher RF levels may cause more severe placental damage.

The increased placental size in congenital syphilis has been well described (McKelvey and Turner 1934, Russell and Altshuler 1974, Budell 1982 pg 923-924).

It is conceivable that RF together with immune complexes causes vasculitis, increased capillary permeability and oedema which in turn results in enlargement of the placenta. It may be possible to correlate the amount of oedema with the quantity of RF and immune complex deposition. This is an area for future research. Preliminary findings were presented in Chapter 10.2 where an association between RF levels and a greater placental weight was documented.

The heat-aggregated IgG appeared to bind avidly to the normal macrophages of the liver and spleen. The uptake by these cells was not prevented by the prior inhibition step. Macrophages, including Kuppfer cells are capable of removing circulating immune complexes (Mannik 1982). Heat-aggregated IgG has been used to simulate an immune complex (Pisko et al 1982) and this may explain the macrophage staining in the normal control as well as the patient with congenital syphilis.

Discrete RF deposits were not found in the liver or spleen. This is, perhaps, surprising but it may be that the Kuppfer cells and macrophages had ingested immune complexes (Mannik

1982).

The kidney did not appear to be affected in the patient 11.1 with congenital syphilis. Other authors have reported immune complex deposition in the glomeruli of infants with nephrotic syndrome due to congenital syphilis (Wiggelinkhuizen et al 1973). However, renal disease in congenital syphilis usually develops at 2-3 months of age (Ingall and Musher 1983 pg 349)

One of the limitations of the current study was a lack of suitable fresh tissue (apart from the placentae) for immunofluorescence. Delayed contact between parents and the medical staff meant that permission for a postmortem examination was not immediately forthcoming.

The findings are based on placentae from 3 patients and 4 controls, and the kidney, liver and spleen from 1 patient and 1 control. The conclusions should, therefore, be regarded as of a preliminary nature.

To extend this study a method of detecting RF in formalin-fixed tissue would allow use of specimens obtained over the years. It is conceivable that the peroxidase-antiperioxidase system could be used (Johnstone and Thorpe 1982 275-276). Whilst the method is attractive it does not appear to have been used to detect tissue RF and would need to be compared to the conventional method used in this study.

CHAPTER 12

CONCLUSIONS, RECOMMENDATIONS AND FUTURE RESEARCH

12.1 CONCLUSIONS AND RECOMMENDATIONS FROM PART II
Rheumatoid factor and testing for congenital syphilis

The RF latex test was shown to lack sensitivity in asymptomatic patients when applied as a screening test to a group of infants at high-risk of congenital syphilis i.e. a negative test could not be used to rule out the diagnosis. In busy and overcrowded newborn nurseries such as may be found in developing countries, the RF latex test may be of value. This is because its high predictive value and rapidity of performance on a serum sample collected in a capillary tube could help to identify a percentage of the infected newborns prior to hospital discharge. For example, a positive RF latex test in the presence of serological evidence of maternal syphilis is likely to be due to congenital syphilis. Early diagnosis would allow treatment to be quickly instituted without having to await the results of more complex investigations (e.g. the FTA-ABS (IgM) test). This would reduce the time of hospital admission and/or detect patients who should not be discharged before full evaluation.

Although the RF ELISA appeared to be somewhat more sensitive, the RF latex test was a far simpler and quicker test for the presence of RF. The total IgM test had similar sensitivity to the RF latex test, but a lower specificity. The sensitivities of the other tests performed on the infants were, unfortunately, no better. Therefore, one is left with the options of prolonged follow-up of high-risk infants or that of treating such cases. The selection of infants requiring further management could be made on the basis of those whose mothers have been inadequately treated and who have a VDRL titre of 1:32 or more in the last month of pregnancy.

In a different study it was found that the RF latex test was useful in determining which infants with one or more of the many signs and symptoms of congenital syphilis actually had the disease (sensitivity 94%, specificity 80%). The results were

rapidly available and the test could be used as a side-room screening test whilst awaiting the results of more definitive laboratory tests.

In the population attending the PMNS, the majority of symptomatic infants with positive RF latex tests had congenital syphilis (positive predictive value 91%). In other communities the value of the test would be determined by the prevalence of the disease.

The sensitivity of the fractionated FTA-ABS (IgM) test was shown to be adversely affected by IgG and RF removal. It would seem to be unnecessary and indeed deleterious to the sensitivity of the specific IgM test to attempt RF extraction in newborns. As pointed out, both anti-treponemal IgG and RF are needed to obtain a false positive FTA-ABS (IgM) test and these circumstances are unlikely to occur in the absence of congenital syphilis.

Eliminating RF interference in older infants appeared to reduce the number of false positive tests but at the expense of a reduced sensitivity. As the effect of unnecessary treatment is unlikely to be significant (Gonin 1985), it may be advisable to ignore the RF activity completely when using the FTA-ABS (IgM) test.

12.2 CONCLUSIONS FROM PART III - RHEUMATOID FACTOR AND THE IMMUNE RESPONSE IN CONGENITAL SYPHILIS

In summary, the following features were demonstrated:

1. Rheumatoid factor was present in the sera of a greater percentage of symptomatic infants with congenital syphilis (92%) than has been reported for adults with acquired syphilis (13-25% - Barfeld 1969; Muller et al (1987).

2. IgM RF was the only class of RF detected in the infants with congenital syphilis. It appeared that the IgM RF accounted for 4% of the total IgM produced (mean value).

3. An association could be demonstrated between RF levels and more severe and extensive disease ($p < 0.05$). The RF levels also correlated with liver and kidney involvement as well as oedema ($p < 0.05$). Levels of RF and CICs were closely related ($p < 0.000001$).

4. Using immunohistochemical methods, positive fluorescence likely to be due to RF, was found in the placentae of 3 infants with congenital syphilis, but not in controls. Deposition of RF was noted in blood vessels and extracellularly in placental villi.

5. Rheumatoid factor was not detected in the sera of newborn controls using the RF latex test and very low levels of RF were observed in these infants using the RF ELISA. It was apparent, however, that levels of anti-IgG increased in normal infants after birth.

These findings have already been discussed. Some conclusions will now be made under the following headings:

i. RF synthesis as part of the total IgM production in congenital syphilis

ii. RF and the pathogenesis of congenital syphilis.

The possible direction of future research will also be discussed.

12.2.1 RF synthesis as part of the total IgM production in congenital syphilis

It was evident that IgM RF and anti-treponemal (or specific) IgM were synthesized as part of the total IgM production. This indicated that a polyclonal response was in progress. It was found that 4% of the IgM produced was IgM RF; it should be borne in mind, however, that this figure reflects RF levels after tissue deposition. It was apparent that RF was found more often in congenital than acquired syphilis, suggesting that activation of CD5 + cells occurs more readily in the younger patients.

The elevated levels of anti-IgG noted in the older controls may be due to the production of IgM antibodies to foreign maternal Gm allotypes. The early onset of anti-IgG production demonstrated in the present study may reflect the use of a sensitive detection system.

That IgG and IgA RF were not detected implied that class-switching did not readily occur in the infants. This may be in keeping with observations that B lymphocytes produce predominantly IgM in early life (Steele et al 1977).

12.2.2 RF and the pathogenesis of congenital syphilis

The findings that RF levels were related to multisystem involvement and more severe disease and that RF may be deposited in the placental blood vessels suggested that RF may be involved in the pathogenesis of congenital syphilis.

It was likely that the presence of RF was related to the deposition of immune complexes. Whether the RF bound to immune complexes in the circulation or whether it attached to tissue-bound complexes was unknown, although its ability to do both has been established (Ford and Kosatka 1982).

Presumably RF may aggravate tissue damage by the following mechanisms:

i. by acting as an immunoabsorbent to bind more immune complexes

ii. complement activation: animal experiments have shown that complement is needed for the full effect of RF to be manifest (Floyd and Tesar 1979).

This leads one to question why a potentially physiological immunoglobulin (i.e. RF) should be associated with pathological effects?

Possibly there is excessive production of RF in congenital syphilis. Given suitable stimuli, RF manufacture, being part of a germ-line response, may be greater in early infancy. In

addition, it appears that in early life a mechanism exists whereby the normal infant begins to make anti-allotypic antibodies. As suggested by Reimer et al (1975) this could be exaggerated in infants with immune complex disease (e.g. congenital syphilis) because the IgG in the complexes is of maternal origin (and presumably allotypically different). The maternal IgG may assume a more antigenic nature because of configurational changes induced by the formation of immune complexes. The immune response of the young infant which is geared, for reasons previously set out, to make anti-immunoglobulin may produce large amounts of RF which exert a pathological effect.

12.3 DIRECTIONS OF FUTURE RESEARCH

12.3.1 Tests for the diagnosis of congenital syphilis

The results obtained in Chapter 5 indicated that the maternal VDRL titre near the time of delivery was a good predictor of whether an infant would develop congenital syphilis.

It may be worthwhile to establish an ELISA for VDRL antibody and determine if a reliable cut-off level can be identified. This would require a similar study to that described in Chapter 5.

Whilst there are a number of new techniques available to diagnose congenital syphilis it should be emphasized that none have been thoroughly tested in asymptomatic patients (Dobson et al 1988; S'anchez et al 1989; Pedersen et al 1989; Ijsselmuiden et al 1989). It would seem to be wise to await further studies before introducing these newer tests.

One of the reasons that establishing a diagnosis is so important in congenital syphilis is because of the prolonged nature of the treatment which is difficult to carry out in developing countries. It has been pointed out that the treatment regime was empirically derived. It is important that studies are performed to determine the efficacy of a shorter course of therapy. The possibility of using different agents (e.g. the newer cephalosporins) should be considered.

12.3.2 RF and the immune response in congenital syphilis

Could RF production in congenital syphilis be used as a model for other diseases? There are a number of reasons for suggesting this:

i. similar factors correlate with RF levels in congenital syphilis, RA, SBE and other conditions.

ii. RF synthesis is part of a germ-line response to and it may be advantageous to study the latter at an early stage of development.

Further studies on CD5+ cells in newborns in general and those with congenital syphilis in particular, appear to be warranted. The numbers of such cells could be quantitated and the stimulus for RF production in congenital syphilis sought (i.e. is the treponemal antigen important, or is complexed maternal IgG alone sufficient?).

The development of an animal model (e.g. the rabbit - Fitzgerald 1985 or the hamster - Kajdacsy-Balla et al 1987) would allow further investigation of the importance of RF in the development of the lesions of congenital syphilis. One would be able to compare, for example, the effects of injected immune complexes (containing treponemal antigens) with the same complexes and RF.

The study of Dresner and Trombly (1959) indicated that monitoring RF levels could be a useful way of assessing the activity of liver disease. This finding appears to be worthy of further study.

The above areas would seem to merit further research.

APPENDIX 1

TABLE A.1.1
GUIDELINES FOR DIAGNOSIS OF EARLY CONGENITAL SYPHILIS (FROM HIRA ET AL 1985)+

DIAGNOSTIC CRITERIA	CERTAINTY OF DIAGNOSIS	CRITERIA PRESENT
Definite criterion	Definite	1
1. Specimen from skin or genital mucosal surfaces demonstrates *Treponema pallidum* on dark-field examination	Probable	14, 15, or any combination of 2-9 with 10 and 12
	Possible	Any of 2-9 with 10 and 12 or 13
Major clinical criteria	Unlikely	10 and 16 with 12 or 13
2. Swelling of joints with pseudoparesis	Not possible (or very rare)	Any of 2-9 with 11 and 13
3. Skin rash including palmar/plantar bullous lesions		
4. Snuffles		
Minor clinical criteria		
5. Hepatosplenomegaly		
6. Jaundice		
7. Anaemia		
8. Radiologic changes in bones and joints		
9. Elevated cell count and/or rapid plasma reagin test reactivity of cerebrospinal fluid		
*Serologic criteria - mother		
10. Reactive tests		
11. Nonreactive tests		
Serologic criteria - infant		
12. Reactive tests		
13. Nonreactive tests		
14. Reactive tests not reverting to negativity within 4 months of birth		
15. Rising rapid plasma reagin test over 3 months		
Maternal History		
16. Documented history of adequately treated syphilis during pregnancy		

* The serologic tests for syphilis used in this study were the rapid plasma reagin (IgM) test and the fluorescent treponemal antibody-absorption (IgM) test.

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APPENDIX 1

TABLE A.1.2

***CRITERIA FOR THE DIAGNOSIS OF NEONATAL AND EARLY CONGENITAL SYPHILIS - MASCOLA ET AL 1985**

CLINICAL

Absolute

1. Specimen from lesions showing presence of Treponema pallidum by dark-field examination, or by histologic examination

Major

2. Condyloma lata
3. Osteochondritis, periostitis
4. Snuffles or hemorrhagic rhinitis

Minor

5. Fissures of lips
6. Cutaneous lesions
7. Mucous patches
8. Hepatomegaly, splenomegaly
9. Generalized lymhadenopathy
10. Central nervous system signs
11. Hemolytic anemia
12. Elevated no. of cells or protein level in cerebrospinal fluid

SEROLOGIC

A. Reactive STS (VDRL or FTA-ABS)
B. Reactive FTA-ABS (IgM) test
C. Nonreactive STS (VDRL or FTA-ABS)
D. Reactive STS (VDRL or FTA-ABS) that does not revert to nonreactive within 4 mo
E. Rising VDRL titer over 3 mo

MATERNAL HISTORY

AA-documented history of adequately treated syphilis during pregnancy

CERTAINTY OF DIAGNOSIS

Definite: Absolute clinical criterion

Probable: Any of the following: (1) serologic criterion E; (2) serologic criterion D; (3) one major clinical criterion and serologic criterion A or B; (4) two or more minor clinical criteria and serologic criterion A or B; (5) one major and one minor clinical criterion

Possible: Serologic criterion A or B with only one minor criterion, or no criterion

Unlikely: (1) serologic criterion C with any other criterion; (2) serologic criterion A or B with maternal history AA

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APPENDIX 2

TEST PROCEDURES AND QUALITY CONTROL

RHEUMATOID FACTOR LATEX TEST

The 'Ortho RA Test' produced by Ortho Diagnostics, Belgium was used.

Procedure

Twenty microlitres of serum was mixed with 80 ul of 'Ortho RA Glycine Buffer' in a 10 x 75 mm clean glass test tube. Twenty microlitres of this mixture was then transferred to a second test tube containing 140 ul of buffer to give a final dilution of 1:40.

The positive and negative control sera were diluted in the same way. The glass slide provided was placed on a bench-top beneath a white light source. Using a Pasteur pipette, 1 drop of each serum dilution was placed in the centre of a square on the glass slide.

One drop of 'Ortho RA latex antigen' was placed next to each serum to be tested. The antigen and serum were mixed with the plastic rod provided, taking care to cover the entire surface of the glass square. A timer was started after completion of the mixing and the slide tilted back and forth for one minute. If agglutination was present at 1 in 5, doubling dilutions were prepared as follows:

Tube	Composition	Dilution
1	20 ul serum + 80 ul buffer	1:5
2	20 ul tube I + 20 ul buffer	1:10
3	20 ul tube 2 + 20 ul buffer	1:20
4	20 ul tube 3 + 20 ul buffer	1:40

The highest dilution at which visible agglutination was detected was regarded as the end-point. As the Ortho RA test has been standardized against the WHO Rheumatoid Arthritis Reference Serum, an approximation of the RF concentration could be obtained.

Quality Control

In addition to using the positive and negative control sera provided, the within- and between-observer variation (Barker 1982 pg 19,20) were determined. Qualitative results only were considered because the RF concentrations were measured with the RF ELISA.

a. Within-observer variation

A random selection of 50 patients who formed part of the study groups of Chapters 5 and 6 was made using a random number table. The RF latex test, at a dilution of 1:5, was performed on two occasions (Run 1 and Run 2). The technologist was unaware of the results of Run 1 when performing Run 2. The results are shown in Tables A.2.1 and A.2.2.

TABLE A.2.1:
WITHIN-OBSERVER VARIATION USING THE RF LATEX TEST

PATIENT CODE NO.	RUN 1	RUN 2
5.2	+ve	+ve
5.3	-ve	-ve
5.7	-ve	-ve
5.15	-ve	-ve
5.16	-ve	-ve
5.23	-ve	-ve
5.25	-ve	-ve
5.30	-ve	-ve
5.35	-ve	-ve
5.36	-ve	-ve
5.42	-ve	-ve
5.44	-ve	-ve
5.50	-ve	-ve
5.53	-ve	-ve
5.54	-ve	-ve
5.59	-ve	-ve
5.60	-ve	-ve
5.72	-ve	-ve

Table A.2.1 continued

PATIENT CODE NO.	RUN 1	RUN 2
5.76	-ve	-ve
5.85	-ve	-ve
5.86	-ve	-ve
5.87	-ve	-ve
5.92	-ve	-ve
5.97	-ve	-ve
5.98	-ve	-ve
5.102	-ve	-ve
5.104	-ve	-ve
5.109	-ve	-ve
5.111	-ve	-ve
5.113	-ve	-ve
5.115	-ve	-ve
5.116	-ve	-ve
5.124	-ve	-ve
5.127	-ve	-ve
5.139	-ve	-ve
5.140	-ve	-ve
5.142	-ve	-ve
5.147	-ve	-ve
6.3	+ve	+ve
6.6	-ve	-ve
6.8	+ve	+ve
6.9	+ve	+ve
6.12	+ve	+ve
6.14	+ve	+ve
6.20	+ve	+ve
6.24	+ve	+ve
6.25	+ve	+ve
6.28	+ve	+ve
6.33	+ve	+ve

The within-observer repeatibility was calculated from Table A.2.2.

TABLE A.2.2

WITHIN-OBSERVER VARIATION WITH THE RF LATEX TEST

		RUN 1	
RUN 2		Positive	Negative
	Positive	12 a	b 0
	Negative	0 c	d 38

The within-observer repeatability was $\frac{a}{(a+b+c)} = 100\%$

b. Between-observer variation

A further 50 patients were randomly selected and the RF latex test was performed independently by 2 technologists. The results are given in Table A.2.3.

TABLE A.2.3:

BETWEEN-OBSERVER VARIATION USING THE RF LATEX TEST

PATIENT CODE NO.	TECHNOLOGIST 1	TECHNOLOGIST 2
5.6	- ve	- ve
5.9	- ve	- ve
5.12	- ve	- ve
5.13	- ve	- ve
5.16	- ve	- ve
5.18	- ve	- ve
5.20	- ve	- ve
5.21	- ve	- ve
5.22	- ve	- ve
5.27	- ve	- ve
5.30	- ve	- ve
5.32	- ve	- ve
5.36	- ve	- ve
5.37	- ve	- ve
5.38	- ve	- ve
5.40	- ve	- ve

Table A.2.3 continued

PATIENT CODE NO.	TECHNOLOGIST 1	TECHNOLOGIST 2
5.41	- ve	- ve
5.42	- ve	- ve
5.45	- ve	- ve
5.46	- ve	- ve
5.48	- ve	- ve
5.49	- ve	- ve
5.52	- ve	- ve
5.54	- ve	- ve
5.59	- ve	- ve
5.60	- ve	- ve
5.61	- ve	- ve
5.63	- ve	- ve
5.65	- ve	- ve
5.67	- ve	- ve
5.71	- ve	- ve
5.76	- ve	- ve
5.78	- ve	- ve
5.85	- ve	- ve
5.86	- ve	- ve
5.87	- ve	- ve
5.88	- ve	- ve
5.92	- ve	- ve
5.95	- ve	- ve
5.101	- ve	- ve
6.7	+ ve	+ ve
6.13	+ ve	+ ve
6.14	+ ve	+ ve
6.17	+ ve	+ ve
6.19	+ ve	+ ve
6.24	+ ve	+ ve
6.27	+ ve	+ ve
6.28	+ ve	+ ve
6.30	+ ve	+ ve
6.47	+ ve	+ ve

The between-observer repeatability was calculated from Table A.2.4.

TABLE A.2.4

BETWEEN-OBSERVER VARIATION WITH THE RF LATEX TEST

		Technologist 1	
		Positive	Negative
Technologist 2	Positive	10 a	b 0
	Negative	0 c	d 40

The between-observer repeatability was $\frac{a}{(a+b+c)} = 100\%$

TOTAL IgM TEST

The 'Low level human IgM Test Kit' and the 'Human IgM Test Kit' obtained from Hyland Diagnostics, USA (catalogue numbers 085-403 and 085-404) were used.

Procedure

A Hamilton micropipette was used to delivery 5 ul of serum to each well. Care was taken not to introduce bubbles or damage the walls of the well. The first 4 wells of one plate from each batch were used for the reference standards provided. Thereafter, only 1 reference serum was used per plate of the same lot number.

After the dilution of sera the plate cover was firmly replaced and the plate kept at room temperature for 24 hours. The diameter of the precipitin zone was measured using a calibrated measuring device. Reference curves were constructed and the IgM values of the samples determined.

The procedure for the 'Human IgM' kits was similar except that 3 reference sera were used.

Quality Control

The inter-assay variation for the 'Low level' plates was determined by measuring IgM concentrations on 3 known standards. The results are shown in Table A.2.5.

TABLE A.2.5

INTER-ASSAY VARIATION OF TOTAL IgM LEVELS

STANDARD	KNOWN IgM CONCENTRATION (IU/ml)	TEST RESULTS	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION (%)
1	65	65,65,64,69,61	64.8	2.86	4.4
2	42	44,44,48,44,40	44	2.8	6.4
3	32	32,29,28,28,25	28.4	2.5	8.8

The inter-assay variation of the 'Human IgM' kit was not determined because all the sera were stored and tested on one occasion.

The intra-assay variation was determined for the 'Low level' and 'Human IgM' plates. Two sera were tested with each plate. The results are shown in Table A.2.6.

TABLE A.2.6

INTRA-ASSAY VARIATION OF TOTAL IgM LEVELS

PLATE TYPE	SAMPLE	IgM RESULTS (IU/ml)	MEAN	STANDARD DEVIATION	COEFFICIENT VARIATION
'Low level'	1	38,38,38,38,38,36,41.5 40,36	38.2	1.73	4.5
	2	17,16,17,20,19,17,20, 16,17	17.7	1.58	8.9
'Human IgM'	3	112,108,112,112,120, 110,110,110,110,120	112.4	4.2	3.7
	4	252,252,252,252,252, 260,260,252,280,280	259	11.44	4.4

IgM Concentrations in 408 newborn infants

Total IgM levels were estimated by radial immunodiffusion in 408 normal newborn infants. The IgM determinations were performed as part of the Groote Schuur Hospital screening programme for congenital syphilis in

1985 and 1986. During this period, it was the hospital policy to test all infants born to unbooked mothers in this way. The VDRL and TPHA tests were also carried out on the mothers. From this information it was possible to analyze the IgM results of well babies born to mothers whose STS were negative at delivery. The results are shown in Figure A.2.1.

The mean IgM concentration was 20.18 IU/ml with a standard deviation of 11.12. The upper limit of normal was, therefore, regarded as 42 IU/ml (mean + 2 standard deviations).

PROCEDURE FOR THE FTA-ABS (IgM) TEST

The FTA-Treponema pallidum suspension (Behring catalogue number ORMK 04/05) was used to prepare antigen-coated glass slides. The slides were fixed in acetone for 10 minutes and air dried before use. Three wells were used for each test or control serum.

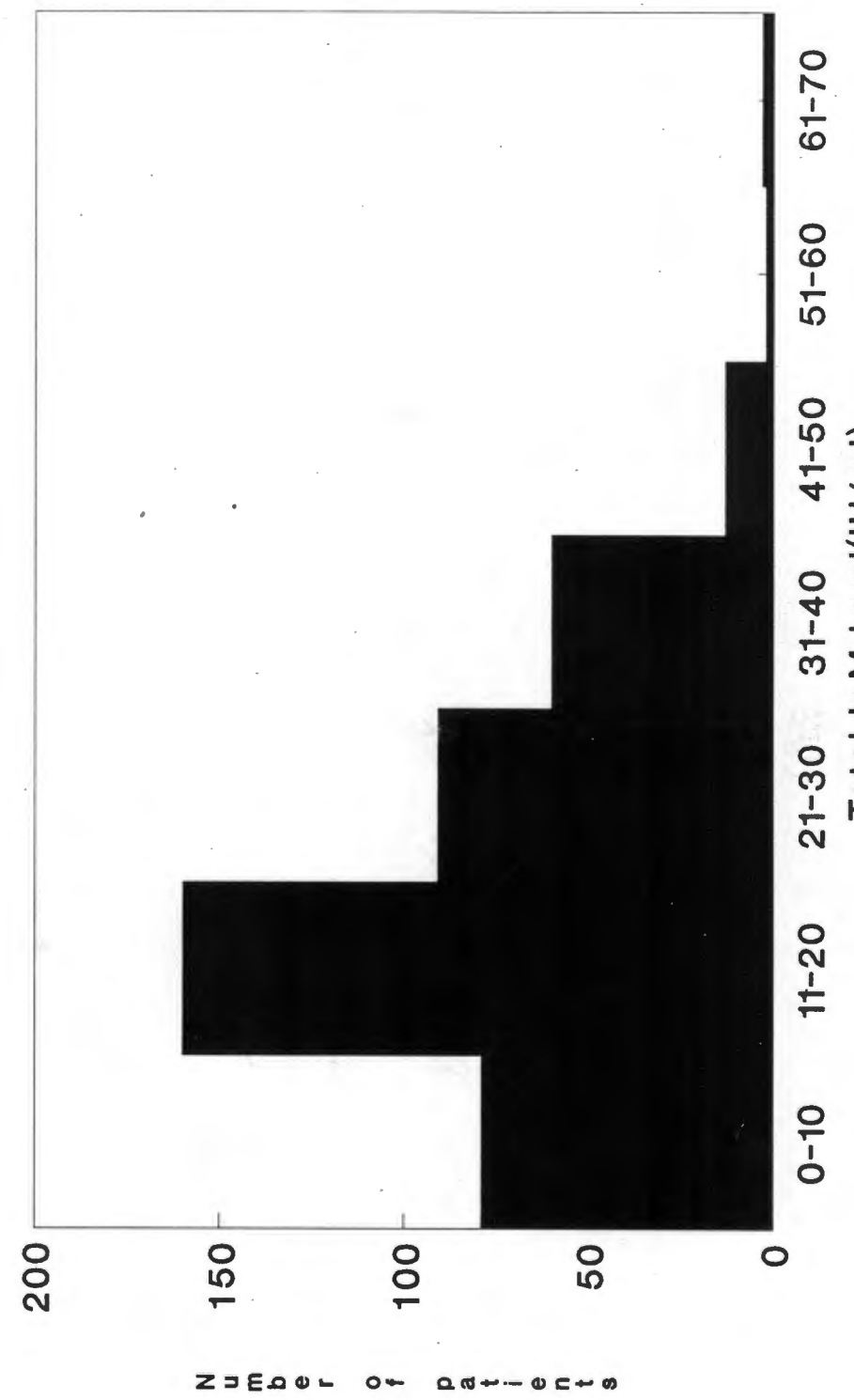
Ten microlitres of heat-inactivated serum was placed in a clean glass tube and 40 ul of FTA absorption medium from the FTA-ABS DS kit (Zeus Scientific, USA) was added. After thorough mixing, 10 ul of the solution was transferred to the first well of the slide for a pre-IgG precipitation FTA test. Forty microlitres of RF absorbent (Behring, West Germany, catalogue number OUCG 14/15) was added to the remaining 40 ul of test serum and absorption medium. Following incubation for 15 minutes at room temperature, the solution was centrifuged at 300 g for 10 minutes.

Ten microlitres of supernatant was then added to the second and third wells of the slide for post IgG precipitation FTA-ABS IgG and IgM testing. Following incubation in a moist chamber at 37°C for 3 hours, PBS (pH 7.6) was used to rinse and then soak the slides. After the slides had air dried, 10 ul of fluorescein-labelled anti-human IgG (Wellcome Diagnostics, England, catalogue number MF03) was added to the first and second wells and anti-human IgM (Wellcome Diagnostics, England, catalogue number MF04) to the third well.

The material was incubated for 40 minutes at 37°C, washed, dried and examined for fluorescence.

Positive and negative controls were set-up with each batch tested.

Figure A.2.1: Frequency distribution of total IgM levels at birth in 408 normal newborn infants



APPENDIX 3

TABLE A.3.1

CONTROL INFANTS WITH NEGATIVE SEROLOGICAL TESTS FOR SYPHILIS AT BIRTH

PATIENT CODE NUMBER	BIRTH WEIGHT (kg)	GESTATIONAL AGE (completed weeks)	SEX	RF LATEX TEST	TOTAL IgM IU/ml	CLINICAL FINDINGS
5.70	1.4	32	M	-ve	16	Preterm, HMD
5.71	3.1	40	F	-ve	14	Well
5.72	2.3	38	F	-ve	31	SGA
5.73	2.6	39	F	-ve	31	Well
5.74	3.2	40	F	-ve	14	Well
5.75	3.3	40	M	-ve	16	Well
5.76	3.6	40	F	-ve	8	Well
5.77	2.9	40	F	-ve	18	Well
5.78	2.5	37	F	-ve	14	Well
5.79	2.9	40	F	-ve	22	Well
5.80	3.8	40	M	-ve	30	Well
5.81	1.4	33	M	-ve	14	Preterm
5.82	2.3	38	M	-ve	29	SGA
5.83	3.4	40	M	-ve	31	Well
5.84	2.6	40	F	-ve	17	Well
5.85	2.2	34	M	-ve	16	Preterm

Table A.3.1 continued

PATIENT CODE NUMBER	BIRTH WEIGHT (kg)	GESTATIONAL AGE (completed weeks)	SEX	RF LATEX TEST	TOTAL IgM IU/ml	CLINICAL FINDINGS
5.86	2.9	40	M	-ve	16	Well
5.87	2.5	40	F	-ve	18	SGA
5.88	2.8	40	M	-ve	28	HIE
5.89	3.0	40	F	-ve	12	Well
5.90	1.5	30	F	-ve	31	Preterm
5.91	2.7	40	F	-ve	29	Well
5.92	3.5	40	F	-ve	24	Well
5.93	2.0	38	F	-ve	14	SGA
5.94	2.8	40	F	-ve	21	Well
5.95	2.8	36	F	-ve	35	Preterm
5.96	2.4	36	F	-ve	35	Preterm
5.97	2.1	37	F	-ve	18	SGA
5.98	1.4	38	F	-ve	35	SGA
5.99	2.4	40	F	-ve	27	SGA
5.100	2.3	40	M	-ve	26	SGA
5.101	1.6	33	M	-ve	10	Preterm
5.102	2.6	40	F	-ve	9	Well
5.103	2.3	40	M	-ve	30	SGA

Table A.3.1 continued

PATIENT CODE NUMBER	BIRTH WEIGHT (kg)	GESTATIONAL AGE (completed	SEX	RF LATEX TEST	TOTAL IgM IU/ml	CLINICAL FINDINGS
5.104	2.9	40	M	-ve	10	Well
5.105	2.6	40	M	-ve	28	Well
5.106	3.5	40	F	-ve	13	Well
5.107	2.8	40	F	-ve	13	Well
5.108	3.1	40	F	-ve	34	Well
5.109	2.4	37	F	-ve	5	Well
5.110	2.7	40	F	-ve	22	Well
5.111	2.5	40	F	-ve	14	SGA
5.112	2.8	40	M	-ve	35	Well
5.113	1.2	33	F	-ve	10	Preterm,SGA,HMD
5.114	2.8	40	F	-ve	291	Well
5.115	3.0	40	F	-ve	28	Well
5.116	1.9	36	F	-ve	40	Preterm
5.117	3.1	40	M	-ve	29	Well
5.118	3.5	40	M	-ve	17	AFIS
5.119	2.5	40	F	-ve	11	SGA
5.120	3.3	40	F	-ve	32	Well
5.121	1.1	30	M	-ve	7	Preterm, asphyxia, TTN

Table A.3.1 continued

PATIENT CODE NUMBER	BIRTH WEIGHT (kg)	GESTATIONAL AGE (completed weeks)	SEX	RF LATEX TEST	TOTAL IgM IU/ml	CLINICAL FINDINGS
5.122	2.8	40	M	-ve	12	Well
5.123	3.2	40	F	-ve	16	Well
5.124	3.2	40	M	-ve	12	Well
5.125	1.7	36	F	-ve	11	Preterm, SGA
5.126	2.4	36	M	-ve	28	Preterm
5.127	2.9	40	F	-ve	43	Well
5.128	2.0	40	F	-ve	16	SGA
5.129	2.5	40	M	-ve	30	SGA
5.130	3.2	40	M	-ve	21	Well
5.131	3.6	40	F	-ve	30	Well
5.132	3.5	40	M	-ve	37	Well
5.133	2.6	40	F	-ve	16	Well
5.134	2.8	40	F	-ve	22	Well
5.135	2.8	40	F	-ve	35	Well
5.136	3.0	40	M	-ve	5	Well
5.137	2.0	36	F	-ve	13	Preterm
5.138	1.5	36	F	-ve	13	Preterm, SGA, fetal alcohol syndrome
5.139	2.8	40	M	-ve	13	Well

Table A.3.1 continued

PATIENT CODE NUMBER	BIRTH WEIGHT (kg)	GESTATIONAL AGE (completed weeks)	SEX	RF LATEX TEST	TOTAL IgM IU/ml	CLINICAL FINDINGS
5.140	2.8	40	M	-ve	22	Well
5.141	2.9	40	F	-ve	16	Well
5.142	1.6	36	M	-ve	29	Preterm, SGA
5.143	1.6	34	F	-ve	28	Preterm
5.144	2.8	40	F	-ve	17	Well
5.145	2.3	37	F	-ve	33	Well
5.146	2.9	40	F	-ve	33	Well
5.147	3.3	40	F	-ve	18	Well
5.148	3.1	40	F	-ve	45	Well
5.149	3.5	40	M	-ve	9	Well
5.150	3.6	40	F	-ve	25	Well
5.151	2.7	40	F	-ve	32	Well
5.152	2.9	40	F	-ve	18	Well
5.153	2.0	40	M	-ve	16	SGA
5.154	2.5	40	F	-ve	29	SGA

ABBREVIATIONS:

AFIS - Amniotic fluid infection syndrome
F - Female
HIE - Hypoxic ischaemic encephalopathy
HMD - Hyaline membrane sisease
M - Male
SGA - Small for gestational age
TTN - Transient tachypnoea of the newborn
-ve - Negative

APPENDIX 4

TOTAL IgM LEVELS AT FOLLOW-UP

The total IgM concentration was measured in 43 infants without congenital syphilis who were between 4 and 6 weeks of age. The results are shown in Figure A.4.1, The mean IgM value was 100 IU/ml with a standard deviation of 64 IU/ml; median was 104 IU/ml (range 0-274 IU/ml).

Similarly, the mean IgM level of 45 infants between the ages of 3 to 4 months was 175 IU/ml with a standard deviation of 69 IU/ml; the median was 180 IU/ml (range 62-330 IU/ml). Figure A.4.2 shows the results.

Figure A.4.1: Frequency distribution of total IgM levels in 48 normal infants aged 1 to 2 months

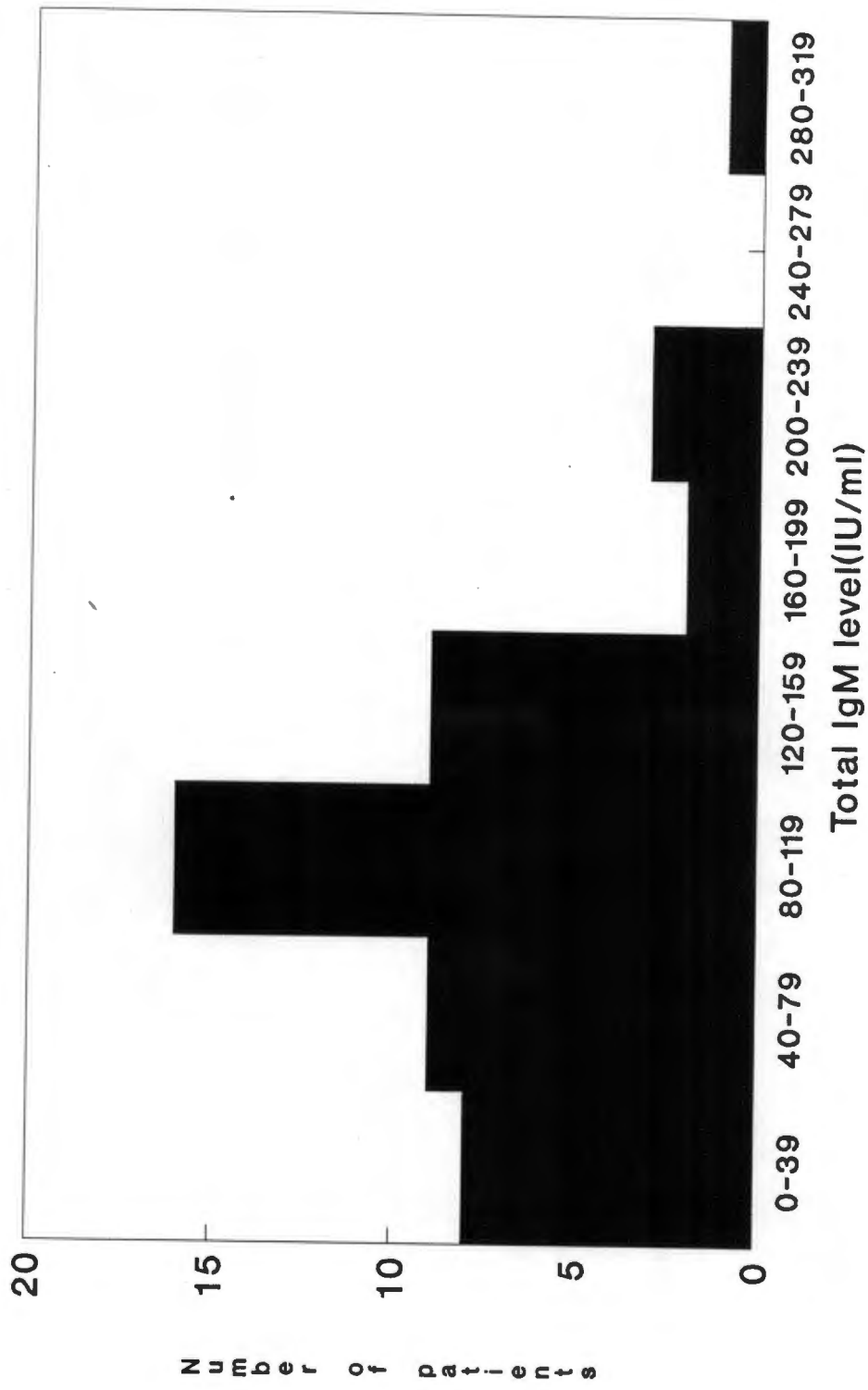
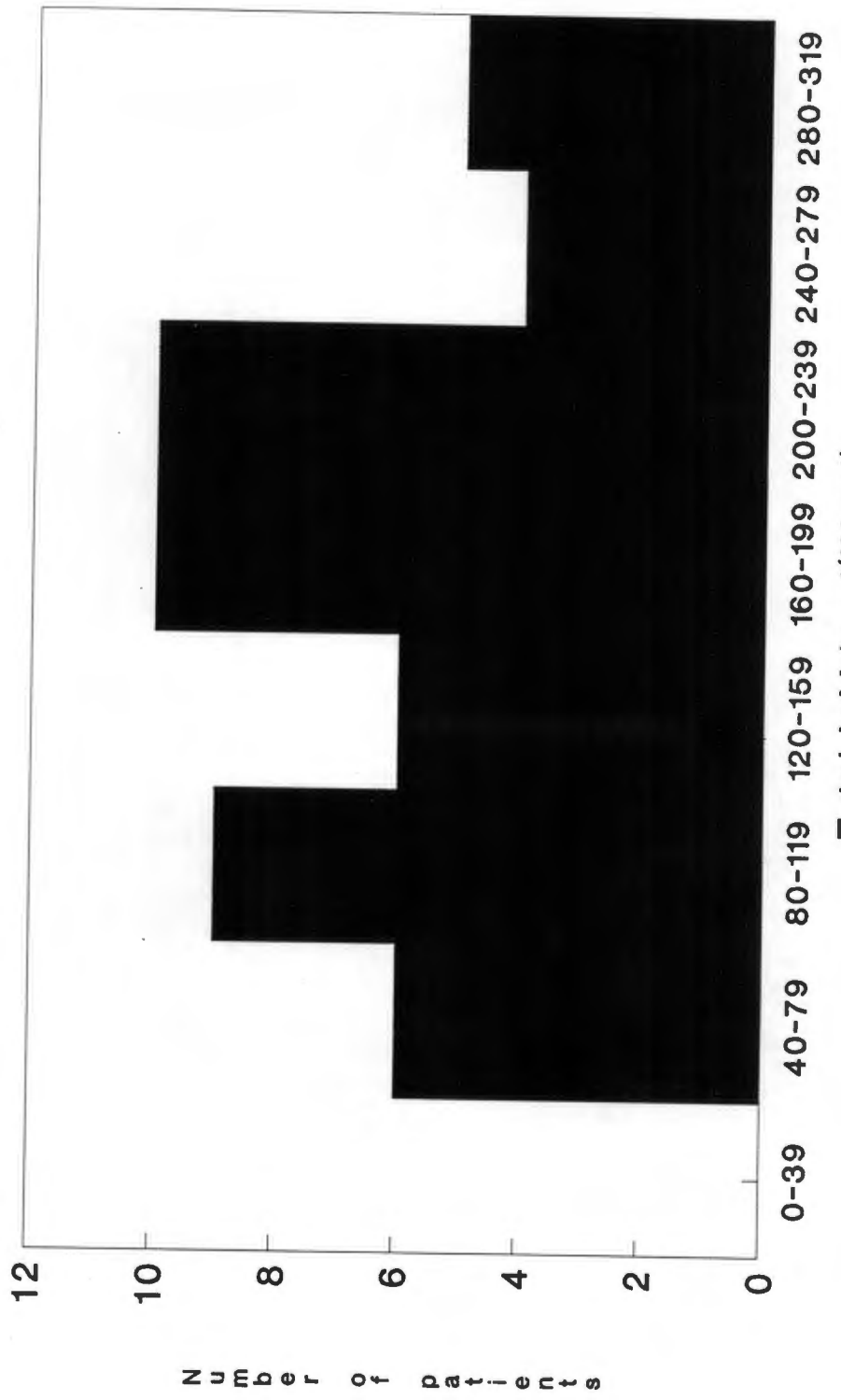


Figure A.4.2: Frequency distribution of total IgM levels in 50 normal infants aged 3 to 6 months



APPENDIX 5

THE EFFECT OF IgG PRECIPITATION ON THE TOTAL IgM LEVEL

To determine whether IgG precipitation resulted in a decrease in the total IgM concentration, 5 normal infants who were RF negative, were studied and their IgM levels measured.

Ten microlitres of serum was diluted with 40 ul of PBS pH 7.2 and 50 ul of the RF absorbent (anti-human IgG) was added.

After incubation (15 minutes at room temperature) and centrifugation (300 G for 10 minutes), total IgM levels were quantified using radial immunodiffusion plates (Immunoplate IV, Hyland Diagnostics, catalogue numbers 085-403 and 085-404). Taking into account the dilutions employed, the total IgM levels shown in Table A.5.1.

TABLE A.5.1
THE EFFECT OF IgG PRECIPITATION ON TOTAL IgM LEVELS

PATIENT CODE NO.	AGE (MONTHS)	TOTAL IgM VALUE (IU/ml) BEFORE IgG PRECIPITATION	AFTER IgG PRECIPITATION
5.22	4	274	263
5.25	4	160	160
5.41	4	180	185
5.55	4	270	268
5.62	4	201	204

It was observed that the IgG precipitation step did not alter the IgM concentration in these normal infants.

APPENDIX 6

TABLE A.6.1

SERUM RF CONCENTRATIONS IN INFANTS WITH CONGENITAL SYPHILIS WHO PRESENTED WITHIN 3 DAYS OF BIRTH

PATIENT CODE NUMBER	RF CONCENTRATION IU/ml	PATIENT CODE NUMBER	RF CONCENTRATION IU/ml
5.1	24	6.7	15
5.2	4	6.8	27
5.3	0	6.9	165
5.4	21	6.10	32
5.5	35	6.11	11
5.6	27	6.12	788
5.7	0	6.13	34
6.1	152	6.14	165
6.2	205	6.15	21
6.3	156	6.16	850
6.4	90	6.17	3
6.5	92	6.18	24
6.6	50	6.19	6

TABLE A.6.2

*OPTICAL DENSITY READINGS (BACKGROUND VALUES SUBTRACTED) OBTAINED WITH THE RF ELISA IN 96 NORMAL NEWBORN INFANTS

PATIENT CODE NO.	OPTICAL DENSITY	PATIENT CODE NO	OPTICAL DENSITY	PATIENT CODE NO	OPTICAL DENSITY
5.18	0.017	5.77	0.002	5.115	0.015
5.19	0.022	5.79	0.013	5.116	0.018
5.20	0.029	5.80	0.003	5.117	0.017
5.24	0.023	5.81	0.015	5.118	0.013
5.25	0.039	5.82	0.006	5.119	0.030
5.34	0	5.83	0.044	5.120	0
5.35	0	5.85	0	5.122	0.001
5.36	0.037	5.86	0.010	5.124	0.017
5.38	0.032	5.87	0.032	5.125	0.028
5.39	0.013	5.89	0.015	5.126	0.019
5.42	0	5.90	0.008	5.127	0.021
5.45	0.028	5.91	0.016	5.128	0.011
5.47	0	5.92	0.007	5.129	0
5.49	0.039	5.93	0.015	5.130	0.033
5.50	0.019	5.94	0	5.131	0
5.51	0.038	5.95	0.006	5.132	0.006
5.52	0.037	5.96	0.011	5.134	0.032
5.54	0.023	5.97	0.011	5.135	0.030
5.55	0.011	5.98	0.039	5.136	0.027

Table A.6.2 continued

PATIENT CODE NO	OPTICAL DENSITY	PATIENT CODE NO	OPTICAL DENSITY	PATIENT CODE NO	OPTICAL DENSITY
5.56	0.010	5.99	0.031	5.137	0.019
5.58	0.018	5.101	0.016	5.139	0.013
5.62	0.005	5.102	0.038	5.140	0.020
5.65	0.017	5.104	0	5.141	0.014
5.66	0	5.105	0.022	5.142	0.003
5.67	0.046	5.106	0	5.144	0.016
5.69	0	5.107	0.024	5.146	0.021
5.71	0.070	5.109	0.012	5.147	0.001
5.72	0.002	5.110	0.009	5.148	0.025
5.73	0.013	5.111	0.035	5.149	0.032
5.74	0.019	5.112	0.003	5.150	0.023
5.75	0.029	5.113	0.039	5.152	0.009
5.76	0.004	5.114	0.040	5.154	0.016

* Optical density reading after background value subtracted

TABLE A.6.3

RHEUMATOID FACTOR CONCENTRATIONS IN 39 NORMAL INFANTS AGED 4 DAYS - 2 MONTHS

PATIENT CODE NO	RF CONCENTRATION IU/ml	PATIENT CODE NO	RF CONCENTRATION IU/ml
5.16	3.3	5.47	6
5.17	10.3	5.48	5.1
5.18	6.2	5.49	4.2
5.19	0	5.50	0
5.20	3.8	5.52	3.4
5.22	3.2	5.53	0
5.23	5.1	5.54	0
5.24	18	5.55	9.2
5.25	0	5.57	0
5.26	0	5.58	0
5.27	0	5.59	3.3
5.33	4.5	5.60	0
5.35	0	5.62	3.7
5.36	0	5.63	7.5
5.37	2.9	5.64	17
5.38	4.2	5.65	0
5.39	78	5.67	0
5.41	0	5.68	0
5.42	5.1	5.69	0
5.46	7.6		

TABLE A.6.4

RHEUMATOID FACTOR CONCENTRATIONS IN INFANTS WITH CONGENITAL SYPHILIS OLDER THAN 4 DAYS BUT LESS THAN 2 MONTHS OF AGE

PATIENT CODE NO	RF CONCENTRATION IU/ml	PATIENT CODE NO	RF CONCENTRATION IU/ml
6.20	38	6.28	160
6.21	30	6.29	118
6.24	32	6.33	96
6.25	238	6.34	14
6.27	80		

TABLE A.6.5

RHEUMATOID FACTOR CONCENTRATIONS IN 41 NORMAL INFANTS OLDER THAN 2 MONTHS BUT LESS THAN 4 MONTHS OF AGE

PATIENT CODE NO	RF CONCENTRATION IU/ml	PATIENT CODE NO	RF CONCENTRATION IU/ml
5.16	4.8	5.43	0
5.17	4.5	5.44	6.4
5.19	14.5	5.46	22.5
5.20	3.9	5.47	0
5.22	6	5.48	3.3
5.23	5.1	5.49	5.8
5.24	0	5.50	4.2
5.25	0	5.51	3.2
5.26	5.8	5.52	13
5.27	9	5.54	4.6
5.32	10.5	5.55	12.5
5.33	5	5.57	6.4
5.34	0	5.58	7.2
5.35	12.8	5.59	4
5.36	0	5.60	2.8
5.37	25	5.62	0
5.38	6.4	5.64	35
5.39	115	5.65	2.8
5.40	0	5.68	3.2
5.41	9.1	5.69	0
5.42	5.6		

TABLE A.6.6

RHEUMATOID FACTOR CONCENTRATIONS IN INFANTS WITH CONGENITAL SYPHILIS OLDER THAN 2 MONTHS BUT LESS THAN 4 MONTHS OF AGE

PATIENT CODE NO	RF CONCENTRATION IU/ml	PATIENT CODE NO	RF CONCENTRATION IU/ml
6.22	520	6.31	54
6.23	0	6.32	6
6.26	500		
6.30	38		

TABLE A.6.7

SHOWING TOTAL IgM LEVELS, AMOUNT OF IgM WHICH IS RF AND PERCENTAGE OF TOTAL IgM WHICH IS RF IN INFANTS WITH CONGENITAL SYPHILIS

PATIENT CODE NO	TOTAL IgM (IU/ml)	*IgM WHICH IS RF (IU/ml)	**% IgM WHICH IS RF
5.1	301	6	2
5.2	97	1	1
5.3	0	0	0
5.4	126	5	4
5.5	426	9	2
5.6	186	7	3
5.7	0	0	0
6.1	764	38	5
6.2	1500	51	3
6.3	600	39	7
6.4	284	23	8
6.5	726	23	3
6.6	348	12	3
6.7	255	4	2
6.8	1124	7	1
6.9	633	41	6
6.10	195	8	4
6.11	484	4	1
6.12	1200	197	16
6.13	504	9	2

Table A.6.7 continued

PATIENT CODE NO	TOTAL IgM (IU/ml)	*IgM WHICH IS RF (IU/ml)	**% IgM WHICH IS RF
6.14	1080	41	4
6.15	732	5	1
6.16	732	213	29
6.17	156	1	1
6.18	375	6	2
6.19	935	2	0
6.20	270	10	4
6.21	785	8	1
6.22	1380	130	9
6.23	340	0	0
6.24	471	8	2
6.25	1490	58	4
6.26	1117	125	11
6.27	521	20	5
6.28	1000	40	4
6.29	1210	28	2
6.30	525	10	2
6.31	227	14	6
6.32	520	2	0
6.33	255	24	9
6.34	675	4	1

* to nearest IU/ml

** to nearest percent

APPENDIX 7

TABLE A.7.1

RHEUMATOID FACTOR LEVEL COMPARED TO GESTATIONAL AGE, SEVERITY OF DISEASE AND DURATION OF INFECTION IN PATIENTS WITH CONGENITAL SYPHILIS

PATIENT CODE NUMBER	RF LEVEL IU/ml	*GESTATIONAL AGE (NEWBORNS ONLY)	*GESTATIONAL AGE ⩾ 37 WEEKS (NEWBORNS ONLY)	DISEASE SEVERITY	DURATION OF INFECTION
6.16	830	37	1	1	1
6.12	788	40	1	1	0
6.22	520			1	1
6.26	500				1
6.25	238			1	0
6.2	205	40	1	1**	0
6.14	165	34	0	1	0
6.9	165	35	0	1	0
6.28	160			0	1
6.3	156	33	0	1	0
6.1	152	36	0	1**	0
6.29	118			1	0
6.33	96			1	0
6.5	92	36		1	0
6.4	90	37	1	1	1
6.27	80			0	1
6.31	54			0	1
6.6	50	36	0	1	1
6.30	38			0	1
6.20	38			0	1
5.5	35	40	1	0	0
6.13	34	34	0	1	0
6.24	32			1	0
6.10	32	29	0	1**	0
6.21	30			0	0
5.6	27	40	1	0	0
6.8	27	34	0	1	0

Table A.7.1 continued

PATIENT CODE NUMBER	RF LEVEL IU/ml	*GESTATIONAL AGE (NEWBORNS ONLY)	*GESTATIONAL AGE ⩾ 37 WEEKS (NEWBORNS ONLY)	DISEASE SEVERITY	DURATION OF INFECTION
5.1	24	37	1	1	0
6.18	24	40	1	1	0
6.15	22	34	0	1	1
5.4	21	40	1	0	0
6.7	15	40	1	0	0
6.34	14			0	1
6.11	11	33	0	1	1
6.19	6	35	0	1	0
6.32	6			0	1
5.2	4	30	0	1**	0
6.17	3	32	0	1**	0
5.3	0	36	0	1	0
5.7	0	42	1	0	0
6.23	0			0	1

* Gestational age in completed weeks

** Died shortly after birth

TABLE A.7.2

RHEUMATOID FACTOR LEVELS RELATED TO INVOLVEMENT OF THE LIVER, RETICULO-ENDOTHELIAL SYSTEM, HAEMATOLOGICAL SYSTEM AND RENAL SYSTEM

PATIENT'S CODE NUMBER	RF LEVEL IU/ml	INVOLVEMENT OF				
		LIVER	RES	HAEMATOLOGICAL SYSTEM	BONES	RENAL SYSTEM
6.16	830	1	1	1	1	1
6.12	788	1	1	1	1	0
6.22	520	1	1	1	1	0
6.26	500	1	1	1	1	1
6.25	238	1	0	0	1	0
6.2	205	1	1	0	1	0
6.14	165	1	1	1	1	0
6.9	165	1	1	0	1	0
6.28	160	1	1	1	1	0
6.3	156	1	1	1	1	0
6.1	152	1	1	1	1	0
6.29	118	1	1	1	0	0
6.33	96	1	1	1	1	0
6.5	92	1	1	0	1	0
6.4	90	1	0	0	1	0
6.27	80	1	1	1	1	0
6.31	54	1	1	1	1	0
6.6	50	1	1	0	1	0
6.30	38	1	1	1	1	0
6.20	38	1	1	1	1	0
5.5	35	0	0	0	1	0
6.13	34	0	0	0	1	0
6.24	32	1	0	1	1	0
6.10	32	0	0	0	1	0
6.21	30	1	0	1	1	0
5.6	27	0	0	0	1	0
6.8	27	1	1	1	1	0

Table A.7.2 continued

PATIENT'S CODE NUMBER	RF LEVEL IU/ml	INVOLVEMENT OF				
		LIVER	RES	HAEMATOLOGICAL SYSTEM	BONES	RENAL SYSTEM
5.1	24	1	0	0	1	0
6.18	24	1	0	0	1	0
6.15	21	1	1	0	1	0
5.4	21	0	0	0	0	0
6.7	15	0	0	0	1	0
6.34	14	1	1	0	1	0
6.11	11	1	1	0	1	0
6.19	6	1	1	1	0	0
6.32	6	0	1	1	1	0
5.2	4	1	1	1	0	0
6.17	3	1	1	1	0	0
5.3	0	1	1	1	1	0
5.7	0	0	0	0	1	0
6.23	0	1	1	1	1	0

TABLE A.7.3

RHEUMATOID FACTOR LEVELS RELATED TO INVOLVEMENT OF SKIN, RESPIRATORY SYSTEM AND OTHER SYSTEMS

PATIENT CODE NUMBER	RF LEVEL IU/ml	INVOLVEMENT OF			EXTENT OF DISEASE
		SKIN	RESPIRATORY	OEDEMA	
6.16	830	0	1	1	5
6.12	788	1	0	0	5
6.22	520	1	0	1	6
6.26	500	1	0	1	6
6.25	238	0	0	1	3
6.2	205	1	0	1	5
6.14	165	1	0	1	6
6.9	165	1	0	1	5
6.28	160	1	0	1	6
6.3	156	0	1	0	4
6.1	152	0	1	0	4
6.29	118	1	0	1	5
6.33	96	1	0	1	6
6.5	92	1	1	0	5
6.4	90	0	0	0	2
6.27	80	1	0	1	6
6.31	54	1	0	0	5
6.6	50	0	0	0	3
6.30	38	1	0	0	5
6.20	38	0	0	0	4
5.5	35	0	0	0	1
6.13	34	0	0	1	2
6.24	32	0	0	0	3
6.10	32	0	1	0	2
6.21	30	0	0	0	3
5.6	27	0	0	0	1
6.8	27	1	0	0	5
5.1	24	1	0	0	3
6.18	24	0	0	0	2
6.15	21	1	1	1	5

Table A.7.3 continued

PATIENT CODE NUMBER	RF LEVEL IU/ml	INVOLVEMENT OF			EXTENT OF DISEASE
		SKIN	RESPIRATORY	OEDEMA	
5.4	21	1	0	0	1
6.7	15	0	0	0	1
6.34	14	0	0	0	3
6.11	11	1	0	0	4
6.19	6	0	0	1	4
6.32	6	1	0	0	4
5.2	4	0	1	1	4
6.17	3	0	0	0	3
5.3	0	0	0	1	5
5.7	0	0	0	0	1
6.23	0	1	0	0	5

TABLE A.7.4

RHEUMATOID FACTOR LEVELS COMPARED TO PLACENTAL WEIGHT RATIO AND IMMUNE COMPLEX LEVELS

PATIENT CODE NUMBER	RF LEVEL IU/ml	INVOLVEMENT OF PLACENTA*	IMMUNE COMPLEX BINDING (%)
6.16	850		84.9
6.12	788	1.56	67
6.22	520		83.2
6.26	500		75
6.25	238		92.5
6.2	205	1.59	92
6.14	165	1.38	
6.9	165	1.32	53
6.28	160		
6.3	156	1.46	
6.1	152		14.1
6.29	118		54
6.33	96		18
6.5	92	0.91	
6.4	90	1.24	21
6.27	80		19.2
6.31	54		8
6.6	50	1.51	5.2
6.30	38		18
6.20	38		9.2
5.5	35	0.93	
6.13	34	0.76	11
6.24	32		6.7
6.10	32	1.02	
6.21	30		14.7
5.6	27	0.95	11.4
6.8	27	1.38	12.4
5.1	24	2.18	3.6
6.18	24	1.29	6.2
6.15	21	0.86	

Table A.7.4 continued

PATIENT CODE NUMBER	RF LEVEL IU/ml	INVOLVEMENT OF PLACENTA*	IMMUNE COMPLEX BINDING (%)
5.4	21	1.11	
6.7	15	1.02	
6.34	14		
6.11	11		
6.19	6		35.5
6.32	6		
5.2	4	0.97	
6.17	3	1.19	
5.3	0	1.01	6
5.7	0	1.16	
6.23	0		

* Ratio of observed:expected palcental weight

TABLE A.7.5

RHEUMATOID FACTOR LEVELS AND LIVER FUNCTION TESTS IN INFANTS WITH CONGENITAL SYPHILIS

PATIENT CODE NUMBER	RF LEVEL (IU/ml)	ALT (u/L)	CONJUGATED BILIRUBIN (umol/L)
6.12	788	93	99
6.25	238	ND	70
6.14	165	52	39
6.9	165	18	72
6.1	152	6	190
6.29	118	75	55
6.33	96	ND	478
6.27	80	52	39
6.20	38	18	72
6.24	32	256	238
6.21	30	82	8
6.8	27	177	149
6.15	21	ND	12
6.11	11	76	105
6.19	6	12	62
6.32	6	84	2

ND - not done

TABLE A.7.6

RHEUMATOID FACTOR LEVELS AND VDRL TEST RESULTS IN INFANTS WITH CONGENITAL SYPHILIS FOLLOWING THERAPY WITH PROCAINE PENICILLIN

PATIENT CODE NO.	TEST	TEST RESULTS				
		AT DIAGNOSIS	AT COMPLETION OF TREATMENT	AT 1 MO AFTER TREATMENT	AT 2 MO AFTER TREATMENT	AT 3 MO AFTER TREATMENT
5.3	*RF	0	0	14	68	14
	**VDRL	64	64	16	8	1
5.10	RF	33	30	8	9	11
	VDRL	64	128	16	4	4
6.9	RF	165	342	95	15	0
	VDRL	256	1024	1024	256	4
6.13	RF	35	29	4	0	14
	VDRL	16	16	16	16	8
6.18	RF	24	ND	5	ND	0
	VDRL	128	ND	32	ND	2
6.29	RF	118	320	240	113	36
	VDRL	256	256	256	128	16
6.30	RF	38	36	17	ND	10
	VDRL	256	256	64	ND	128
6.31	RF	54	52	17	6	10
	VDRL	128	256	64	8	4
6.33	RF	96	380	30	11	7
	VDRL	128	128	128	32	8
6.34	*RF	14	ND	43	33	0
	**VDRL	128	ND	8	2	0

MO - Month

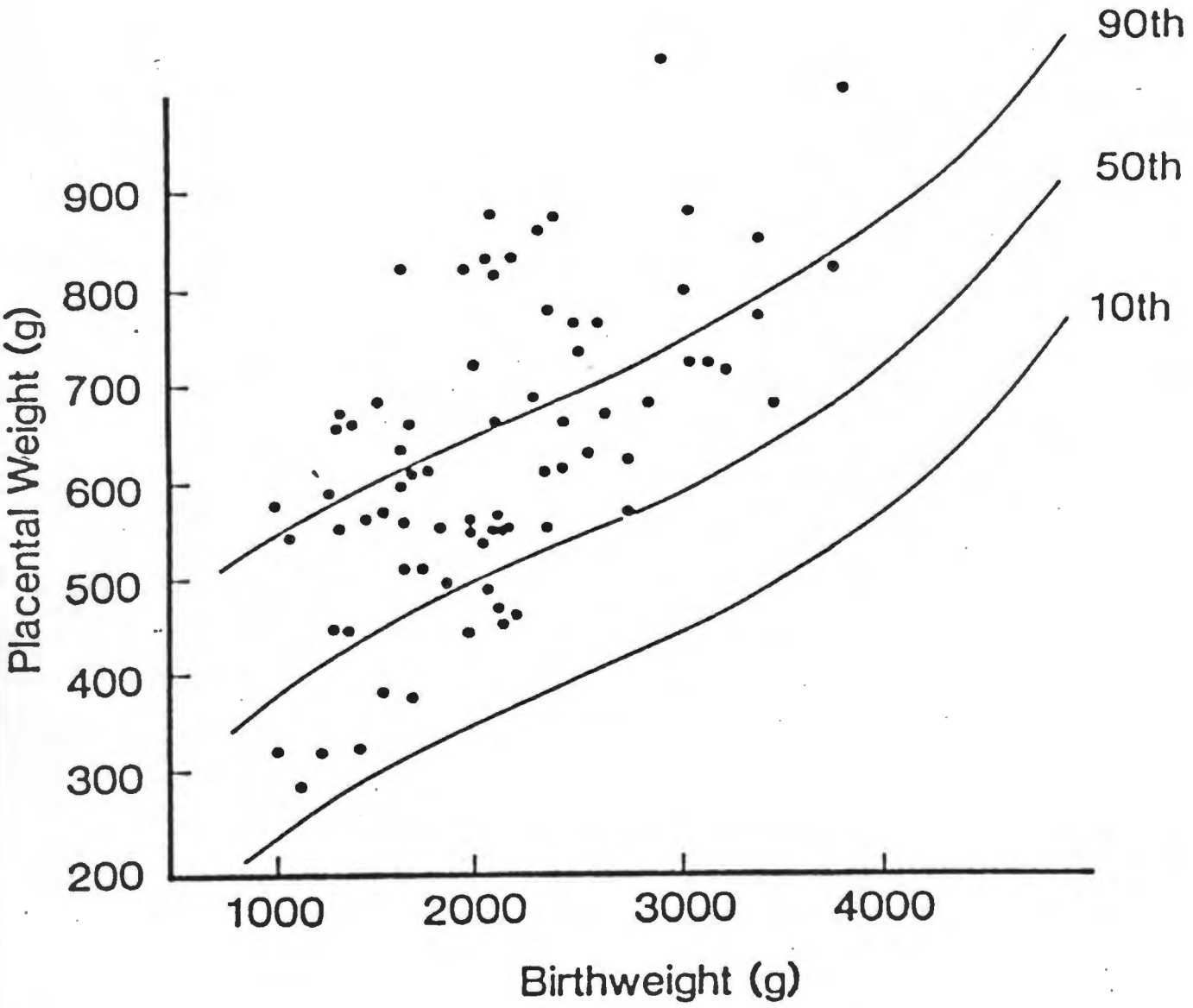
ND - not done

* RF level in IU/ml

** Reciprocal of VDRL titre

APPENDIX 8

Figure A.8.1 : Relative placental weight in congenital syphilis (from Malan et al (1990)*



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APPENDIX 9

The RF latex test was carried out by technologists (Mrs S Rousseau, and Mrs K Judge).

Total serum IgM estimates were performed by Mrs N Mallick and the author.

The VDRL, TPHA, FTA-ABS (IgM) and the RF removal for the FTA test were carried out by Mrs S Louw.

The optimal conditions for the RF ELISA, its validation, standardization and quality control were carried out by the author.

Measurement of the RF levels in patients and controls was performed by the author, Mrs S Rousseau and Mrs K Judge.

Hidden RF was detected by the author.

The amount of RF which was IgM was determined by the author.

IgG and IgA RFs were measured by the author.

The preparation of fluorochromes and the histological examination of the sections were performed by the author. Immunocytochemical staining was carried out by Mrs D Blake.

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