A PROSPECTIVE CLINICO-PATHOLOGICAL STUDY OF PREGNANCY AND LEPROSY IN ETHIOPIA

by

MARGARET ELIZABETH DUNCAN
M.B., Ch.B.

Thesis presented for the degree of
Doctor of Medicine, University of Edinburgh

December 1982

BIBL. UNIV. EDINBURGENSIS

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This thesis is dedicated to the mothers who took part in the study, that the knowledge gained may be to the benefit of mothers with leprosy and their children

and to

my own Mother

whose life-long interest in leprosy was heightened by her personal experience of immune complex disease, and who gave me constant encouragement.

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ABSTRACT

One hundred and fourteen women with leprosy and 33 healthy controls were studied through 120 and 36 pregnancies respectively and followed up with their babies during lactation. Sixty-one mothers showed deterioration of their leprosy status (overt leprosy, relapse in cured cases, and deterioration in those on treatment, 28 with suspected drug resistance); 31 showed relapse during the third trimestre of pregnancy, 21 as a transient phenomenon. Reversal reaction, in contrast, occurred immediately after delivery, while erythema nodosum leprosum showed the highest incidence in the first trimestre; both reactions persisted/recurred well into the second year of lactation. The most serious effect of these reactions was nerve damage. Nearly half of the leprosy patients showed loss of sensory and/or motor nerve function during a single pregnancy/lactation: all mothers were at risk. Silent neuritis seen more frequently than overt neuritis (48 : 37 episodes) was a particularly dangerous and hitherto undescribed risk of pregnancy.

Placental function, normal in healthy controls, showed a falling trend across the leprosy spectrum to lowest in mothers with lepromatous leprosy: babies' birth weights, placental weights and placental coefficients followed the same trend. No morphological abnormality was detected in any of the placentae. Placentae of lepromatous mothers were small because of reduced cytoplasmic mass; very few acid-fast bacilli or debris were found, even in placentae from women with very active lepromatous leprosy. Milk studies showed no Mycobacterium leprae and no significant differences between the different groups of mothers in terms of total protein or defence factors. Babies of mothers with leprosy grew more slowly, were more susceptible to infections and had a higher infant mortality rate than babies of healthy mothers: this was most marked in babies of mothers with lepromatous leprosy. Two children developed overt leprosy, with histological confirmation and spontaneous healing.

Evidence for the transplacental transmission of Mycobacterium leprae antigen/whole Mycobacterium leprae are: i) increase in IgA in cord sera; ii) increased antibodies against Mycobacterium leprae antigen 7 during the first 18 months of life; and iii) specific IgA, IgG and IgM antibodies against Mycobacterium leprae during the first two years of life, in babies of mothers with lepromatous leprosy.



Plate 1. "Study of ..... environmental factors in association with pregnancy is one of the fascinating ....."

PREFACE

Study of medical disease and environmental factors in association with pregnancy and lactation, and the neonate after birth is one of the most fascinating aspects of medicine. Such observations led to the "discovery" of corticosteroid therapy for rheumatoid arthritis. By observing the transient form of the disease in the neonate, the role of immunological factors in Hashimoto's disease, idiopathic thrombocytopœnic purpura and myasthenia gravis were first appreciated. Work on haemolytic disease of the newborn led to the understanding and prevention of Rhesus sensitisation. These observations and many others have broadened our knowledge of the disease(s) concerned and have had wide application outwith the limited confines of obstetrical medicine.

In the case of leprosy, while many leprologists consider that pregnancy has an adverse effect on leprosy in terms of exacerbation of the disease and occurrence of reactions, this "adverse effect" has never been quantified by a prospective study (with the possible exception of a study in Saigon, the report of which is not available). Reports on the association of pregnancy and leprosy consist largely of retrospective reviews and isolated case reports. The effect of the lactational period on leprosy has not been studied, largely because of the generally accepted idea that while leprosy bacilli had been observed in the placenta by a few workers (in the pre-antibiotic era), transmission of the infection from mother to child could be prevented by separation of the two immediately after birth. Studies of the defence factors in the milk of mothers with leprosy have not been undertaken. General observations on the growth, development and progress of children of mothers with leprosy have not been recorded except by one physician, at the turn of the century, who observed that children of mothers with lepromatous leprosy were frequently small at birth and died of "athrepsie" within a few months of birth.

A considerable amount of detailed epidemiological work was done, mostly in the Philippines from 1920 - mid 1950's, on the development of the infection in children of leprous parents, with special emphasis on the diagnosis and evolution of the disease. Because of the long incubation period for leprosy and lack of modern immunological techniques, the study of "pre-leprosy" was not possible at that time.

As is frequently the case in research projects, the study reported here arose out of discussions on an unrelated topic. I had been working for 3 years on aspects of sexually transmitted diseases (STD) in Ethiopia. Investigation of the epidemiology, aetiology and clinical features of STD as seen in Ethiopian women had revealed disease patterns and response to infection very different from those recorded in the western world. I wondered whether this could be related to immunological factors. Dr. Harold Wheate, a senior leprologist at ALERT, suggested that I consult Dr. Ross Barnetson and Dr. Gunnar Bjune regarding the possibility of carrying out a clinical and immunological study of STD in women with leprosy and healthy controls. These two researchers felt that an obstetrical study of immune responses in mothers and babies would be of more interest than the proposed gynaecological survey. A small pilot study was then carried out.

Using the in vitro technique of lymphocyte transformation, 5 out of 10 healthy mothers and their babies at birth were shown to have lymphocytes sensitised to Mycobacterium leprae. Was in utero sensitisation of lymphocytes to M. leprae due to a soluble lymphocyte factor transferable from mother to child? (Barnetson, Bjune and Duncan, 1976). While the lymphocytes of mothers with active lepromatous leprosy and their babies did not show sensitisation to M. leprae, it was observed that women with lepromatous leprosy gave birth to babies who were small for gestational age. Clearly, further investigation was indicated.

To pursue these observations, a prospective collaborative clinical and immunological study was established by the Medical Research Council Leprosy Project in Ethiopia and the Armauer Hansen Research Institute (AHRI).

The clinical aspects of the study were carried out by myself. The collection and immediate processing of specimens was done by myself, or under my personal supervision. Routine laboratory investigations were done by the hospital laboratory; investigations for leprosy were done by the Staff of the MRC unit; investigations requiring special techniques were to be carried out by MRC or other collaborative laboratories in the UK.

The immunological aspects of the study were undertaken by the AHRI staff. Dr. Gunnar Bjune, whose chief interest was lymphocyte transformation, supervised these tests for the first 8 months of the study, before returning to Oslo. His successor, Dr. Reidar Melsom, was more interested in aspects of humoral immunity. Owing to political developments within the country, many ex-patriots with young children were advised to leave and thus, of necessity, after a relatively short stay in Ethiopia, Dr. Melsom returned to the laboratory of Professor M. Harboe in Oslo. He developed the techniques for detecting and quantitating antibodies to M. leprae, and later used these techniques in the examination of sera from the mothers and babies in the study.

During two and a half years at the Leprosy Hospital it became clear to me that the mother with leprosy and her child needed to be studied as a unit. In a third world country with no social security, healthy children are an essential investment for the future. They alone can be relied upon to support financially and care for their sick, crippled or aged parents (especially mother). The women, with whom I worked, knew well that pregnancy made their leprosy worse and in many cases also caused neuritis. Many of them volunteered the information that they wished no more than one or two pregnancies. At the same time it became apparent that the children, especially those of lepromatous mothers, were disadvantaged in terms of low birth weight, slow growth rate and undue susceptibility to infections from which many of them died. Furthermore, immunisation did not appear to confer lasting immunity. With the demise of one child, the lepromatous mother would then undergo the hazards of another pregnancy in the hopes of bearing and rearing a healthy child in the stead of the first.

This thesis presents different aspects of the interactions of pregnancy, leprosy and lactation as observed in Ethiopian women and their children.

Some of the work included in this thesis is the result of co-operative studies with the Armauer Hansen Research Institute, Addis Ababa. These are :-

1. The sections dealing with lymphocyte transformation tests in the assessment of cell mediated immunity in collaboration with Dr. R.St.C. Barnetson (MRC Leprosy Project), Dr. G. Bjune and Dr. R. Melsom (AHRI).

2. The sections relating to studies of humoral immunity which were the result of co-operative studies with Dr. R. Melsom. The techniques were developed in the laboratory of Professor M. Harboe in Oslo and the tests were carried out there. Although much of the immunological work has been done in Oslo or analysed there, contact has been maintained throughout the study by regular two-way correspondence and meeting from time to time.

3. The sections concerning the development of leprosy and the growth and development of children of leprous mothers include references to Phase II. This was a follow up assessment of mothers and children carried out in March and April 1980 by Dr. Suzanne Menzel.

The results of these co-operative studies are not dealt with in full but are presented in summary form with reference to appropriate publications.

The work recorded in the sections concerning the effect of pregnancy on leprosy and the effect of leprosy on pregnancy, lactation and child rearing was performed by the author.

The thesis was composed solely by myself.

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It is a pleasure to acknowledge the assistance and co-operation of all those who have made this study possible. I am grateful to Dr. R.J.W. Rees, Head of the Laboratory for Leprosy and Mycobacterial Research, National Institute for Medical Research, London, for inviting me to undertake this prospective study and for co-ordinating the study in the U.K. during the time I was working in Ethiopia. Dr. J.M.H. Pearson, Head of the Medical Research Council Leprosy Project, Addis Ababa, not only taught me the essentials of clinical leprosy, but was always ready with helpful suggestions, and took a keen interest in the development of the study and shared my enthusiasm as trends became apparent. He has also provided constructive criticism during the analysis of the results and writing up of the study. Dr. R.St.C. Barnetson and Dr. Gunnar Bjune advised in formulating the initial study and followed its progress with interest and helpful criticism thereafter.

I wish to thank those who provided assistance during the clinical phase of the study in Ethiopia :

Independent classification of the mothers was provided by Dr. J.M.H. Pearson.

Independent assessment of babies suspected of developing leprosy was made by Dr. J.M.H. Pearson, Dr. J. Warndorff and Dr. H.W. Wheate.

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Sensory skin tests and voluntary muscle tests were done by Miss J. Watson and Mr. W. Brandsma and staff of the Physiotherapy Department.

Nerve conduction velocity was measured in selected patients by Dr. Ben Naafs.

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Routine laboratory tests and chest X-rays were done in the Addis Ababa Leprosy Hospital.

The Sisters and staff of Ward 3 Addis Ababa Leprosy Hospital provided splendid in-patient care. They collected the urine for oestriol assay, did an occasional emergency delivery, looked after sick mothers and babies, and supervised feeding in babies who failed to thrive.

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Miss Mary Amalia, Mrs. Pamela Bahiru, Dr. P.H. Huguenin and Ato Tshoma Kassahun were available at the critical time and

helped me to sort, list, cross-check and pack the 5,000 specimens which were shipped in batches to the U.K., mostly on solid-phase CO_2.

Mrs. O.P. Wheate typed innumerable lists and specifications of specimens, in quintuplicate, and provided invaluable secretarial assistance.

My thanks go to the mothers who so willingly participated in this study and so faithfully brought their babies for assessment.

I am most grateful to Dr. Reidar Melsom for all the work he has done in Oslo in developing techniques for accurate quantitation of IgA in cord blood, for detection of IgG and IgM antibodies to M. leprae antigen 7 by RIA, and detection of IgA, IgM and IgG antibodies to sonicated M. leprae by sRIA, and for carrying out these assays on the sera of mothers and babies in this study.

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Finally, my thanks are due to those of my family and friends who prayed for the safe conduct of this study in Ethiopia during a time of civil unrest, for safe transfer of specimens and data to the U.K. when international transport was liable to sudden disruption, and for completion of the study in the U.K. "More things are wrought by prayer than this world dreams of."

The results of some of the work incorporated in this thesis have already been published. The relevant references are :

Barnetson, R.St.C., Bjune, G. and Duncan, M.E. (1976).
Evidence for a soluble lymphocyte factor in the transplacental transmission of T-lymphocyte responses to Mycobacterium leprae.
Nature, 260, 150-151.

Bjune, G., Duncan, M.E., Barnetson, R.St.C. and Melsom, R. (1978).
In vitro modulation of lymphocyte responses to phytohaemagglutinin by plasma in mother and baby at the time of birth.
Clinical and Experimental Immunology, 32, 517-522.

Duncan, M.E. (1980).
Babies of Mothers with Leprosy have small placentae, low birth weights and grow slowly.
British Journal of Obstetrics and Gynaecology, 87, 471-479.

Melsom, R., Duncan, M.E. and Bjune, G. (1980).
Immunoglobulin concentration in mothers with leprosy and in healthy controls and their babies at the time of birth.
Leprosy Review, 51, 19-28.

Melsom, R. and Duncan, M.E. (1980).
Demonstration of antibodies against Mycobacterium leprae both in immunoglobulin G and M in sera from pregnant and non-pregnant lepromatous leprosy patients.
Leprosy Review, 51, 125-135.

Melsom, R., Duncan, M.E., Harboe, M. and Bjune, G. (1980).
Antibodies against Mycobacterium leprae antigen 7 from birth to 18 months of age: an indicator of intra-uterine infection in leprosy.
Clinical and Experimental Immunology, 42, 107-113.

Duncan, M.E., Melsom, R., Pearson, J.M.H. and Ridley, D.S. (1981).
The Association of Pregnancy and Leprosy. I. New cases, relapse of cured patients and deterioration in patients on treatment during pregnancy and lactation - Results of a prospective study of 154 pregnancies in 147 Ethiopian women.
Leprosy Review, 52, 245-262.

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The Association of Pregnancy and Leprosy. II. Pregnancy in dapsone resistant leprosy.
Leprosy Review, 52, 263-270.

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IgA and IgM antibodies against M. leprae in cord sera and in patients with leprosy: An indicator of intrauterine infection in leprosy.
Scandinavian Journal of Immunology, 14, 343-352.

Duncan, M.E. and Oakey, R.E. (1982).
Estrogen excretion in pregnant women with leprosy: Evidence of diminished feto-placental function.
Obstetrics and Gynecology, 60, 82-86.

Duncan, M.E. and Pearson, J.M.H. (1982).
Neuritis in Pregnancy and Lactation.
International Journal of Leprosy, 50, 31-38.

Duncan, M.E., Pearson, J.M.H., Ridley, D.S., Melsom, R. and Bjune, G. (1982).*
Pregnancy and Leprosy: The consequences of alterations of cell mediated and humoral immunity during pregnancy and lactation.
International Journal of Leprosy, 50

Melsom, R., Duncan, M.E. and Harboe, M. (1982).
IgA, IgM and IgG anti-M. leprae antibodies during the first two years of life from babies of leprosy mothers.
Clinical and Experimental Immunology, 49, 532-542.

Duncan, M.E. and Oakey, R.E. *
Reduced oestrogen excretion due to clofazimine?
International Journal of Leprosy, in press.

Duncan, M.E., Melsom, R., Pearson, J.M.H., Menzel, S. and Barnetson, R.St.C. *
A clinical and immunological study of four babies of mothers with lepromatous leprosy, two of whom developed leprosy in infancy.
International Journal of Leprosy, in press.

Reprints of these papers, except those marked *, are bound in the Appendix.

INTRODUCTION

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HISTORICAL ASPECTS

Leprosy is an ancient disease. Engel (1903) claimed Egypt as the cradle of leprosy, long before the Christian era while Muir (1939) recorded :

> "Africa is credited with being the birthplace of leprosy, the first described being slaves from the Sudan some 1350 years B.C. This is, however, only a tradition, and from our present-day knowledge of leprosy, it would not be difficult to imagine that leprosy had originally been a widely distributed mild disease, capable of showing itself in a severe form under certain circumstances."

The earliest indubitable references to leprosy, however, come from India, and go back to c.600 B.C. (Lowe, 1947). Earliest descriptions of the disease from India and China are suprisingly accurate, and clearly describe the disease which is known today as Leprosy and is caused by the Mycobacterium leprae. Both skin and nerve signs of the disease were recognised and chaulmoogra oil is mentioned as a treatment (Browne, 1979). It is likely that leprosy was brought to Europe and the Mediterranean basin by the armies of Alexander the Great after his Indian campaign 327-326 B.C. Thus leprosy in the days of Jesus Christ may well have been "modern" leprosy. Leprosy referred to in the Old Testament is, however, unlikely to be what we understand as leprosy, but is probably a collection of infectious diseases and conditions affecting the skin, clothing of wool, linen and leather, and the walls of dwellings (A.V. 1611a). The Hebrew word translated as leprosy comes from the same root as "stricken by God". Thus leprosy came to be regarded as a curse or judgement by God, as seen in the story of Miriam, Moses' sister (A.V. 1611b) and Gehazi, Elisha's servant (A.V. 1611c). However, while Biblical references to leprosy (A.V. 1611d) have done much to instil the idea of the unclean-ness, incurability and communicability of the disease, Biblical teaching, in particular the words of Jesus Christ "Heal the sick ..... cleanse lepers" (A.V.1611e) has prompted and inspired a tremendous volume of philanthropy which has been to the benefit of victims of true leprosy (Lie, 1938).

True leprosy, known then as elephantiasis Graecorum, spread gradually into and across Europe. Spread of the disease was associated with movement of troops from endemic areas, Roman and Saracen in particular (Rogers and Muir, 1946a). By the seventh century it had

reached the British Isles (Newman, 1895a); from Scotland spread was by sea routes to the Shetlands, Scandinavia, Faroes and Iceland. During the years of the Crusades, in the eleventh to thirteenth centuries, leprosy became more widespread reaching epidemic proportions. While leprosy has been known in North East Africa for centuries, resulting possibly from an early extension of the disease from the Nile Valley in the second century A.D. (Violato, 1937), spread of leprosy in South Africa was associated with migration of tribes, in particular the Hottentots and Bushmen as the continent was opened up by Dutch and British colonialists (Impey, 1895a). Migration of Chinese labourers in search of work resulted in the spread of leprosy across the Pacific to the Western seaboard of the United States of America, to Australia, across Malaya, the Archipelago and Oceania where it was widely known as the Chinese disease (Thomson, 1897a; Cantlie, 1897a). Aboriginal American Indians were free of leprosy until the Spanish and Portuguese conquerors and colonists brought the disease with them. The problem was increased dramatically by the enormous numbers of Negro slaves imported from the great tropical African leprous centre, especially after emancipation, and was supplemented later by infected Chinese and East Indian immigrant labourers (Rogers and Muir, 1946b).

Leprosy is now a worldwide disease with the number of sufferers estimated at between 15 and 20 million, of whom only 1 in 5 is receiving treatment. Most of the world's leprosy sufferers live in tropical and subtropical developing countries where financial resources and medical facilities and personnel are limited, where communications present major problems, and where internal strife, civil war, or revolution, cause such political instability that effective leprosy control seems beset with insurmountable difficulties.

Causation and transmission of leprosy

From earliest records two themes run side by side: leprosy was highly infectious and leprosy could result from incurring the anger of supernatural powers as has been referred to earlier and as is seen in the story of Troylus and Cresseid. Cresseid attributes her misfortunes in love to the fickleness and pranks of the gods and, thus, incurs their wrath :

"Thy cristall ene minglit with blude I mak,
Thy voice sae clear, unpleasand hoar and hace,
Thy lustie lyre owerspred with spottis blak,
And lumpis haw appearand in thy face.
Where thou cumis, ilk man sall flee the place.
Thus sall thou go begging frae hous to hous
With cup and clapper like ane Lazarous."

.....

"Therefore in secreit wyse ye let me gang
Into yone hospitall at the tounis end."

.....

"Then in ane mantill and ane baver hat,
With cup and clapper wonder privily,
He opnit ane secreit yett, and out thereat
Convoyit her, that nae man suld espy,
Into ane village half ane mile thereby,
Deliverit her in at the spitaail hous,
And daylie sent her part of his almous."

(Henryson, c.1420–1490).

The latter idea had been developed in Judaio–Christian circles that leprosy was God's judgement for sin (A.V. 1611f) and that this judgement could be extended to the third and fourth generation (A.V. 1611g). However, these attitudes were not confined to the centres of Judaio- Christian teaching, as in Chinese tradition leprosy has been thought of as punishment for sexual misdemeanour and that it is transmissible within the family to the third and fourth generations (Report R.C.P. 1867a; Newman, 1895b; Cantlie, 1897b; Skinsnes, 1964). The children born of the fourth generation are considered healthy and can return to society (Hobson, 1861).

Sexual transmission of leprosy

The idea of venereal transmission of leprosy was prevalent in England in the Middle Ages and prompted some of the rules of the leper houses (Newman, 1895c). Richter (cited by Danielssen and Boeck, 1848a) maintained that leprosy was transmitted by sexual contact. In China, women with leprosy believed they could be cured of their disease if they had sexual connection with a healthy male, thus transferring the disease to him. Thus the practice of "selling leprosy" was developed. The dread of this scourge exerted a great influence on promiscuous intercourse in China and on the general moral conduct of the people (Report R.C.P. 1867a; Cantlie, 1897b). Amongst the natives of Mysore

it was a common belief that leprosy was a form of venereal disease (Report R.C.P. 1867b).

After the advent of syphilis to Europe in the sixteenth century, there was undoubtedly some confusion between the clinical features of syphilis and leprosy, caused in part by ulcers, sores, lymphadenopathy and destruction of nasal bones, and the term 'syphilitic leprosy' came into use. The observation that syphilis is transmitted by sexual contact may have given additional support to the theory of sexual transmission of leprosy.

Hereditary transmission

The Greek and Arabian physicians had a universal belief in hereditary transmission of leprosy "as all body fluids were affected" (Simpson, 1842a). It is likely that such a belief influenced laws regarding marriage and divorce in Europe dating from the seventh century and the practice, in Europe, of castration of lepers, and the burial alive of leprous mother and child.

Schilling (1778a) observed that leprous parents could give birth to healthy babies but such babies could not remain free from leprosy unless they were separated from their parents at the time of birth and brought up in a healthy environment, with wholesome feeding. Leloir maintained that children separated from leprous parents early, "si on les sépare tôt de leurs parents", had a very good chance of escaping leprosy, especially if they were sent to a non-infected district, but if they remained with their parents there was very slight chance of escape (Leloir, 1886a).

Danielssen and Boeck, who were the chief protagonists of the theory of hereditary transmission, virtually denying the possibility of leprosy being contagious, stated that a few cases of leprosy could occur spontaneously (1848b). The strongest case for hereditary transmission of leprosy seems to be that of the leprosy victims in the Shetlands of the eighteenth century who were all from a few families (Simpson, 1841a). However, if hereditary transmission was so important, one is faced with the question as to why leprosy in the Shetlands died out when the victims of the disease were effectively isolated on the Island of Papastour.

G. G. SCHILLINGII

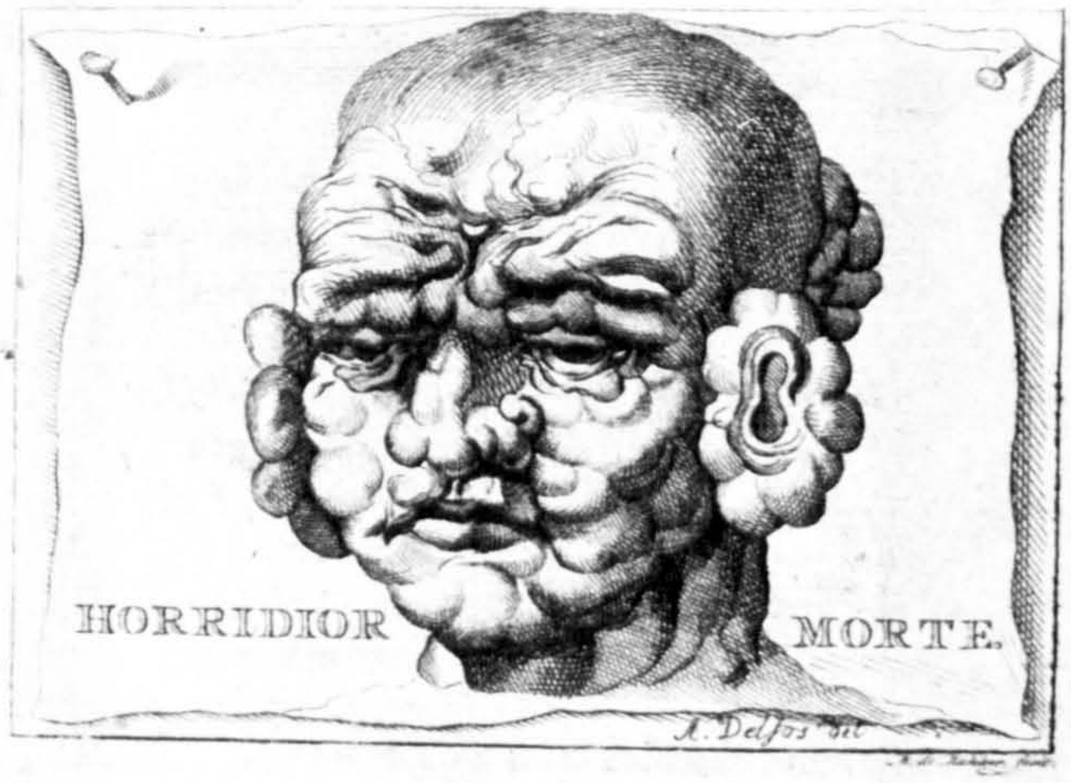
DE

LEPRA

COMMENTATIONES.

RECENSUIT

J. D. HAHN.



LUGDUNI BATAVORUM,
APUD SAM. ET JOAN. LUCHTMANS,
TRAJECTI AD RHENUM,
APUD ABR. VAN PADDENBURG,
MDCCLXXVIII.

Plate 2. Horridior morte .. "I am horrified by death" or "by a more dreadful death", depicts the fear of leprosy current in the eighteenth century. Schilling was one of the first to observe that leprous parents could give birth to healthy babies, but such babies could not remain free from leprosy unless separated from their parents at the time of birth and brought up in a healthy environment.

(By Courtesy, Library of the Royal College of Surgeons, Edinburgh.)

The belief in hereditary transmission of leprosy was so deeply grounded in the minds of Punjabees generally that they were in the habit of burying alive not only the leper himself, but also his relations and friends lest in multiplying their kind, the disease would be communicated to distant generations (Report R.C.P. 1867c).

In Iceland leprosy was considered to be both an inheritable and a very infectious disease (Ehlers, 1895a). The Lolos, natives of Szechuan province of China, the Siamese and Javanese all believed in hereditary transmission of leprosy (Cantlie, 1897c). The Annamites (amongst whom leprosy was very prevalent) declared "leper parents always give birth to leper children although, on the other hand, the malady does not declare itself before the tenth, eleventh or twentieth year" (Cantlie, 1897d). However, Impey (1896a) considered that there was no proof of hereditary transmission of leprosy and that hereditary predisposition occurred only in a small number of cases, and Choksy (1902 c.) in Bombay, observed that only 5% of leprosy was due to hereditary transmission, although 11% had a family trait.

The theory of hereditary transmission was not universally held - Adams (1807)maintained that as reproductive capacity of both men and women was impaired, leprosy should be a self-limiting disease if hereditary transmission was important. This view was challenged by Danielssen and Boeck (1848c) on the grounds of their interviews with patients and autopsy findings. The case for transmission of hereditary predisposition of the disease rather than hereditary transmission of leprosy was put forward by Hjaltelin (1841) and supported by Tache and Roose (1890). Roose who had observed the occurrence of leprosy in 4 children aged 4-6 years felt that these children, who possibly had inherited a predisposition to leprosy, had been infected by their leprous parents after birth by contagion.

Hjaltelin's hypothesis was further modified by Baumgarten (Hansen and Looft, 1895a) who suggested that the bacilli of both tuberculosis and leprosy can be transferred from leprous parents to child, that the bacilli can be dormant, and be passed subsequently to a third generation before the disease is seen.

Hansen and Looft argued against heredity on the grounds that the bacillus is a parasite and not a heredity factor with anatomical and physiological peculiarities. They did, however, suggest that

"hereditary transmission" be replaced by the term "hypothesis of latent infection" (1895b).

The question "Is the contagion of leprosy transferable by way of intrauterine infection?" was asked by Neisser (1885a). As proof was lacking, he felt the hypothesis of inheritable disposition was more easily defended.

Leprosy as an infectious disease/
Transmission of leprosy by contagion

That leprosy was regarded as an infectious disease and was treated as such is clear from events of history. The spread of leprosy along the routes of invading, and retreating armies, and along trade routes by land and sea; the epidemic of leprosy coinciding with the return of the Crusaders, the establishment of leper hospitals, outside the towns, the laws forbidding lepers entry to towns, are all evidence. Guy de Chauliac, practising in the fourteenth century, not only provides an unequivocal description of leprosy but gives, in great detail, instructions regarding the examination of one suspected of having leprosy - as he observes :

> " ..... in the examination and judgement of lepers there must be much circumspection, because the injury is very great, whether we thus submit to confinement those that ought not to be confined, or allow lepers to mix with the people, seeing the disease is contagious and infectious."

(Cited by Simpson, 1841b).

The infectiousness of leprosy is suggested by Donne :

> "By thee the silly amorous sucks his death [sic: seely]
> By drawing in a leprous harlot's breath."

(Donne, 1573-1631), but whether he considered the transmission to be by skin contact, sexual contact, or by infected breath, Donne does not say.

A leper was considered to be able to transmit infection by touch (touching children or food), by washing in a common fountain, and "by his pestilent breath" (Simpson, 1842b). The clothes of a leper were also considered as potentially infectious and were burned if they were found within the town.

Cullen (1772) in his "Nosology" stated the disease was contagious, Schilling (1778b) stated that leprosy could be transmitted by the pus from abscesses, also by respiration and from a leprous wet nurse to a suckling infant.

In Iceland, where segregation of lepers was not practised, but where lepers were looked after for a year at a time in one vicinity before moving on, sometimes sharing not just a room but a bed - leprosy spread - with the migration of the lepers (Ehlers, 1895b).

In some parts of China corpses of lepers were burned rather than buried for fear of contagion (Newman, 1895d). In other parts of China, infection by transient contact was not thought to be of importance, as shown by the lack of fear in medical students examining leprous patients. However, prolonged contact was considered important in the spread of leprosy (Cantlie, 1897e). Three other modes of transmission of leprosy are known in Chinese thinking : Chinese folk medicine often incorporated children's urine, when advertently urine from a leprous child was used, this was thought to cause leprosy. A further source of infection was considered to be "wrong lymph" - or the serum of an infectious person. Arm to arm vaccination against smallpox was frequently practised (Cantlie, 1897f), and following one such campaign there was a great increase of leprosy in children (Cantlie, 1897g). MacNamara (1893a) cites two such cases in non-Chinese children, one a doctor's son, the other the child of the ship's captain who subsequently developed leprosy. The third and most interesting theory, and that most nearly approaching that of transmission of infection by a leprosy bacillus is an ancient Chinese belief that leprosy "is caused by a microscopic animal that flies unseen" (Cantlie, 1897f).

The dispute regarding hereditary transmission and contagion in the spread of leprosy raged throughout the nineteenth century.

A case for hereditary predisposition to leprosy was argued by Kierulf (1853) who noted that the spontaneous development of the disease was always in endemic areas and never where it was unknown. The observation that 11 parents of children with leprosy developed the disease after the birth of the children was made by Holmsen (1857) who, along with Kierulf, believed in the existence of a specific virus for leprosy. Believing that heredity played a subordinate role in the

transmission (Hjort, 1857), claimed that the Norwegian government, misled by Danienssen and Boeck, had instituted useless measures for control of the disease. Drognat-Landré, however was the first to seriously analyse the problem, prompted by seeing, in Holland, 10 Dutch patients with leprosy contracted in the colonies. After making observations on leprosy in the native and ex-patriot communities, on the appearance of leprosy in children of leprous parents and a critical review of the history of the disease in Surinam, Drognat-Landré (1868) concluded that contagion was the sole means of propagation of leprosy. His monograph, published at a time when leprologists in many countries were in hot pursuit of the "virus of leprosy", was eclipsed by Hansen's discovery of the bacillus of leprosy and fell into obscurity for 70 years (Jeanselme, 1933a).

Evidence for the contagion theory was based on :

1. the spread of leprosy in the Sandwich Islands where up to 1859 isolated cases only were seen. In 1859 the first two cases, both Chinese immigrants, were seen on the Islands themselves. By 1866, 400 cases had been diagnosed and were transferred to Molokai Island. By 1881 there were 800 lepers on Molokai. The latest statistics (prior to 1885) showed 1/10 of the natives, or 4,500 out of a population of 45,000 had leprosy, and 2,000 lepers had died between 1870–85. Spread of leprosy had occurred despite good physique of the natives, good food, and a healthy climate (Neisser, 1885b);

2. the report of Dr. Emerson (1888) who showed that of 66 healthy workers at Molokai, 39 had developed leprosy, 11 were suspects and only 16 were still non-leprous (MacNamara, 1893b);

3. the case of Father Damien, a Belgian priest of a non-leprous family who went in 1873 to work amongst the lepers of Molokai settlement. In 1882 he showed the first sign of leprosy in his foot and in 1889 he died of the disease (MacNamara, 1893c).

4. the steady reduction of cases of leprosy in Norway as effective segregation of lepers was practised (Hansen and Looft, 1895c).

Furthermore, against the heredity theory Hansen and Looft (1895c) refer to the case of 170 leprous Norweigian emigrants who went to the U.S.A. where they had adequate spacious accommodation, clean surroundings, good food and where none of their children developed leprosy.

After Hansen's discovery of the leprosy bacillus in 1873, search was made for the bacillus in various body tissues, fluids, secretions and excretions. Further possible routes for spread of infection, including transmission by inoculation by insect vectors, were suggested. Thus flies and biting insects were incriminated (Impey, 1896b).

Although leprosy was considered to be contagious, susceptibility to the development of the disease was not universal (Roose, 1890c), but might be affected by impaired health (Report R.C.P. 1867d; Roose, 1890d). Furthermore, the infection might lie dormant for some time, becoming overt leprosy in response to some other unfavourable factor such as debility, unsanitory surroundings, or an acute illness (Impey, 1896b).

Social attitudes and laws in relation to leprosy, marriage, divorce and procreation

In the seventh century, Rothan, King of Lombards, made laws to prevent marriage of lepers (Newman, 1895e). A hundred years later, in 757, the parliament of Pepin , King of France, passed a law in which leprosy was regarded as a cause of separation; the healthy partner of the marriage was allowed to remarry (Simpson, 1841c). In 789 Charlemagne promulgated laws forbidding the marriage of lepers (Thin, 1891a). A similar edict was made about 950 by the Welsh King Hoel Dha; however, at this time the term "leprosy" covered various skin diseases (Simpson, 1841c). In 1186, Pope Urban III allowed that subsequent leprosy was a sufficient reason why a betrothed couple should not be compelled to marry (Robertson, 1872).

Scots law, prior to the days of King Malcolm Canmore, in its practice of hygienic measures to control disease, ordered castration of epileptics, insane or carriers of diseases transmissible from father to son; at the same time, to prevent the spread of leprosy, it banished any woman sufferer from the company of men, with the penalty of burial

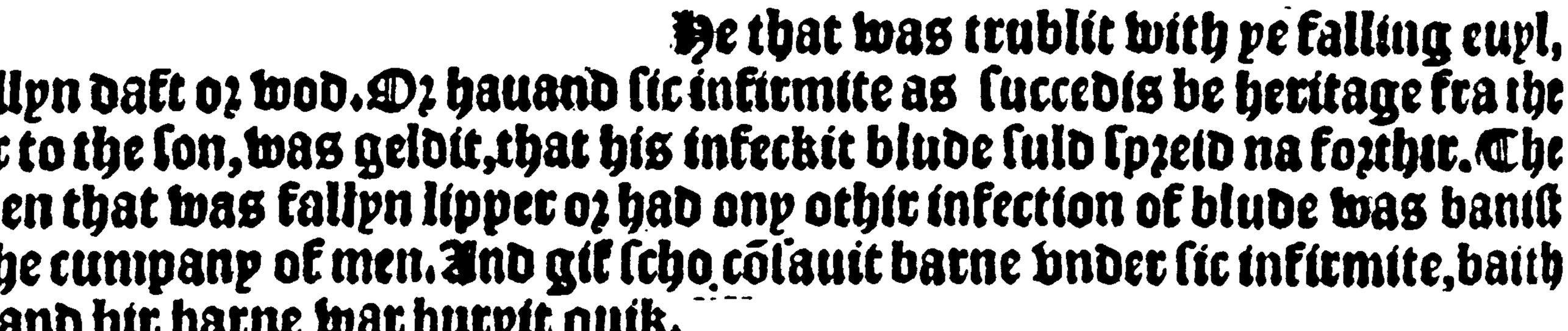

Ye that was trublit with ye falling euyl,
or fallyn daft or wod. Or hauand sic infirmite as succedis be heritage fra the
fader to the son, was geldit, that his infeckit blude suld spreid na forthir. The
wemen that was fallyn lipper or had ony othir infection of blude was baniſt
fra the cumpany of men. And gif scho cõlauit barne vnder sic infirmite, baith
scho and hir barne war burȳit quik.

Plate 3. One of the earliest references to the woman with leprosy and her child.
From: History and Croniklis of Scotland be Maister Hector Boece,
Translatit be Maister Johne Bellenden, 1535. Fol D i v.

(By Courtesy, Edinburgh University Library.)

alive, with her child, should she give birth while suffering from leprosy (Boece, 1535). The practice of castration of lepers was apparently widely practised in the Middle Ages (Hostalrich, 1912).

Segregation of lepers from healthy persons in the Middle Ages was followed by separation of the sexes, as evidenced by the rules of the leper houses. Rules for St. Julian's Hospital, thirteenth century, stated that those admitted were to be single: if they were married they were to part by consent and vow chastity (Newman, 1895c). Sometimes the lepers' wives lived with them, as was the case at the Edinburgh Greenside Hospital in 1591, where to enforce complete segregation of the lepers, one of the wives (Janet Galt by name) was allowed out to go to the markets .....

> "That the said Jonet Galt onelie cum to the merkats for bying of sic viveris as is necessar to the saidis persouns, and presume to gang to na other pairt nor place in hir cuming and returning to and fra the saidis merkats, under the payne (of hanging) aforesaid."

(Extracts from the records of the Burgh of Edinburgh, 1589-1603). The lepers took it in turn to sit and beg alms at the hospital door.

In 1757 in France, leprosy was a valid cause for divorce. In Great Britain at the same time there were laws which forbad co-habitation if either husband or wife was a leper; the leper in these circumstances being considered as dead (Thin, 1891a). These laws which were not Acts of Parliament as Thin states (1891) were regional laws, statutes or canons but none the less binding (Creighton, 1891).

Icelandic law in 1776 forbad the marriage of lepers (Ehlers, 1895c), while Norweigian law in 1781 allowed divorce of lepers and remarriage of the healthy partner (Danielssen and Boeck, 1848e). In 1790, in Norway, a second law was passed allowing husbands whose wives were placed in the leper hospital at Bergen to remarry, the woman being declared to be civilly dead (Thin, 1891a).

In 1874, Bishop of Crete found it necessary to recommend to the priests not to sanction marriages with or among lepers (Thin, 1891a).

In China, leprosy was regarded as legal grounds for annulment of promise of marriage contract (Drognat-Landré, 1868d), or divorce (Newman, 1895f). In Kwantung province of China, in the nineteenth century, in arranging child betrothals, great care was sought in

8 ITEM þat na lippir folk nothir man nor womā fra thyn furth entº na cum ī to na burgħ of þe realm̃e bot thriſ ī þe wolk þat is to ſay ilk monūday ilk weddȳis day ꝛ ilk friday fra teñ hourꝭ to twa eftº none Ande quhar farꝭ ꝛ mcatꝭ fallꝭ on þai dais at þai leif thare entꝭe ī the borowis ꝛ gang oñ þe morñ to get þare leving

ITEM at na lipouſ folk ſit to thig nothir in kirk nor ī kirk garde na ī nane vthir place w'in þe borowis bot at þare awin hoſpitale ande at þe porte of þe toune ꝛ vthir placꝭ outewith þe borowis

ITEM at þe biſchoppis officiallis ꝛ denys inquire diligently ī þare viſitacioñis of ilk piche kirk gif ony be ſmyttit with lippir ande gif ony ſic be fundyn ſwa ſmyttit at þai be denūcit to þe king gif þai be ſecularꝭ ande gif þai be clerkꝭ to þare biſchoppis Ande þat þe burgeſ ger keip þis ſtatute vndº þe payñ cōtenit in þe ſtatute of beggarꝭ Ande quhat lipouſ at keipis noꝰ þis ſtatute þat he be banyſt for eū of þat burgħ quharº he diſobeyis And in lik wyſ to landwart

Plate 4. Laws governing persons afflicted with leprosy (Acta Parliamentorum Jacobi I, 1427, Capita 8).

(By Courtesy, Library of the Royal College of Surgeons of Edinburgh.)

ascertaining the absence of leper trait in the other party: despite this they were willing to hire lepers to care for their children! In Cochin China where leprosy was very prevalent, the Annamite leper did not marry: if leprosy declared itself after marriage, the husband avoided his wife's bed for fear of giving her the disease (Cantlie, 1897h).

In the nineteenth century, regulations regarding marriage within leper asylums varied considerably. On the one hand in South Africa, where leprosy was considered to be spread by contagion rather than hereditary transmission, conjugal intercourse was discouraged between lepers until they were past the child bearing age, and was not permitted at all between lepers and healthy persons (Impey, 1896a). On the other hand in India, where marriages amongst lepers were not prolific (Choksy, 1902c), we find that marriage was permitted for mutual care rather than enjoyment of sexual relations (Choksy, 1902a). It was observed that of 1600 inmates of Matunga leper asylum, Bombay, in 9 years only 7 children were born. A similar observation was made in Hawaii where in a colony of 2,864 lepers, only 26 children were born (Choksy, 1902a), and in the Maracaibo Island leper colony where marriage was permitted, only 2 children were born in 15 years (Choksy, 1902c).

In Canton Province of China, marriage between lepers was only permitted with those having the same type or grade of the disease (Report R.C.P. 1867e).

In Panama, at the Palo Seco asylum, marriages of lepers were allowed only after sterilisation of the male on his written request (Rogers and Muir, 1946c).

Reduced fertility amongst lepers, however, was not always to rule. In Indo-China, where birth rate amongst lepers was high and hereditary transmission of leprosy was considered most important, a strong case was made for sterilisation of leprosy patients of both sexes (Hostalrich, 1912).

In Korea, the segregation of sexes practised in leper hospitals resulted not only in sexual perversion, but also in patients leaving the leper hospital. Such patients formed, in some cases, transient attachments with those of the opposite sex and joined leper camps - children born in such circumstances not only had a precarious home life

but, if they remained with their parents, half of them became infected with leprosy. A system of arranged marriages, arranged adoptions (in accordance with local customs) together with voluntary sterilisation was found to be effective in providing for the needs of segregated lepers (Wilson, 1935).

Leprosy in relation to child bearing

In the pre-sulphone ere, leprosy was associated with subfertility if not frank infertility (Roose, 1890e; Choksy, 1902a; Le Dentu, 1910). This was attributed to frigidity by Zambaco (1897), to "decreasing sexual instinct" with progression of the disease (Danielssen and Boeck, 1848f). Adams (1807e) and Roose (1890e) observed testicular atrophy and Hansen and Looft (1895d) observed destruction of the testicle by scarring with connective tissue resulting in azospermia.

Leprosy occurring prior to puberty resulted in primary amenorrhoea (Leloir, 1886b), while leprosy occurring after puberty was observed to cause menstrual irregularity progressing to secondary amenorrhoea (Danielssen and Boeck, 1848g; Leloir 1886b; Roose, 1890e). The secondary amenorrhoea was attributed to infection of the Fallopian tubes, ovaries or uterus, based on observations of two autopsies out of 17 in which tubercles were seen on these organs (Danielssen and Boeck, 1848h). However, as a number of their patients also had tuberculosis, one wonders whether the tubercles seen were lesions of tuberculosis rather than leprosy.

The few pregnancies which did occur were observed to be normal, with in many cases babies appearing healthy at birth and with no gross abnormality of the placenta (Le Dentu, 1910). However, Zambaco (1897) then observed a large number of abortions in women with leprosy. These he attributed to septicaemia with M. leprae, resulting in placental infection leading to foetal infection and abortion. The reason for these abortions not being diagnosed as due to foetal leprosy was that as in many cases of abortion, both foetus and placenta were discarded without being properly examined, an observation repeated later by Montero (1927). The only placentae he examined personally had no evidence of leprosy bacilli (1897a). A particularly interesting observation was that many of the children born to women with leprosy



Plate 5. "A particularly interesting observation was that many of the children born to women with leprosy were remarkably small for the period of gestation ..."

were remarkably small for the period of gestation and appeared like "old men" or "an abortion at term" (1897b).

The adverse effect of pregnancy on leprosy appears to have been first observed by Zambaco (1897c). He cites the cases of 4 women who developed overt leprosy in connection with pregnancy, 3 immediately postpartum and one during the third trimestre. He observed reaction in the skin occurring in two cases postpartum until 3 months of lactation, and in one patient he observed silent neuritis with the sudden development postpartum of "main en griffe" in two successive pregnancies in one patient. He described his observations as follows :

> "et à ce propos, je dirai en passant que l'influence de la grossesse est funeste chez les lépreuses. La maladie se réveille et marche avec rapidité. La parturition imprime à son tour, une grande gravité à l'évolution de la maladie, en tout comparable à celle de la tuberculose, dans les même circonstances."

(Zambaco, 1897d).

The effect of puberty on the incidence of leprosy with a sudden increase in the number of girls in proportion to boys has been noted by Rodrigues (1926), Blenska (1966) and Richardson (1936). The appearance of overt leprosy in association with pregnancy or exacerbation of the existing disease has been noted by Neff (1926), Montero (1927b), Jeanselme (1933b), Muir (1930), Money (1945) and King and Marks (1958) who observed that deterioration of leprosy patients was more obvious in those who were not receiving treatment (18 out of 23) than in those who were on treatment with sulphones (5 out of 23). Davey and Schenck (1964) observed that "although exacerbation of leprosy does arise during pregnancy, it is much more common during the puerperium and the early months of lactation".

Reaction due to leprosy was observed postpartum by Neff (1926), while Rodrigues (1926) observed 1/6 of the women with leprosy suffered from "lepra fever" during pregnancy.

Most of the observations on the association of leprosy and pregnancy and the effect one on the other are contained in individual case reports or relatively small retrospective surveys. No record of any clearly defined <u>prospective</u> study could be traced in the available world literature.

LEPROSY IN YOUNG CHILDREN

Danielssen and Boeck believed that leprosy attacks the foetus, and recorded cases of infants "in earliest years" with skin tubercles. They substantiated their belief with reports from parents who claimed that leprous blisters were seen on the extremities of their babies from "earliest months". Unfortunately, they gave no details of these very early cases. The youngest seen by them personally was an 8 year old with anaesthetic leprosy. From hospital records they quote the onset of leprosy: 7 out of 188 cases of nodular leprosy had onset of the disease under the age of 5, and 3 out of 85 cases of anaesthetic leprosy had onset under the age of 5 (1848i). In a much later report, Danielssen is cited as having never seen a case of congenital leprosy, although on one occasion he had seen a case develop at one year of age (Zambaco, 1897e).

Rogers from Barbados (1867) observed that in children of leprous parents, leprosy appeared to be latent until 7, 8 and 9 years of age when it manifested itself by cutaneous appearances. He noted that a cachectic state of constitution preceded the appearance of leprosy (Report R.C.P. 1867d).

Leprosy was seen in very young children only in isolated cases: "an infant in arms" (Dr. Day of Madras), one 2 year old (Abercrombie of Cape of Good Hope) and a 3 year old (Manget of Guiana); usually leprosy was seen after puberty (Report R.C.P. 1867f).

In reporting observations of the conditions under which leprosy occurred in China and the Far East, Cantlie stated that cases of congenital leprosy had never been seen, leprosy was never found in children under the age of 2, and the youngest child to be seen with leprosy was a 3 year old in Hong Kong, to which he added that there might be younger children affected in some of the leprosy asylums (1897i).

Choksy (1902b), reporting from Bombay, stated that no case of congenital leprosy had been observed, and that the disease had hardly ever been notified in a child under the age of 2.

Congenital leprosy

Two cases of congenital leprosy were described by Navarro (1890). The first one was of a male baby "remarkably wasted" at birth who was observed to have numerous "leprous spots" all over the body. This baby was born to a mother who developed clinical evidence of leprosy shortly after delivery, the baby at the age of 2 months had "leprous tubercles" on the face, elbows and knees. His sister aged 8 developed clinical signs of leprosy at this stage. The baby, mother and sister all died of leprosy 2 years later. No bacteriological or histological investigations of the lesions were made. The second case was of a female child born in 1848 to a woman with advanced lepromatous leprosy, the baby had numerous leprous spots all over and a well developed tubercle on the left ear. No investigations were carried out, the diagnosis of leprosy was made purely on clinical grounds.

Three cases of congenital leprosy were described by Zambaco (1897). Two were observed by himself personally, the first was born to a lepromatous leprous mother whose husband was healthy. The second child was born to a healthy mother and a leprous father. In both cases there were numerous macules typical of leprosy. The first child had steady progression of the disease and died at the age of 4. The second child had several successive eruptions of lesions which faded without completely disappearing. The third case was that of a colleague, Dr. Zambounis. The baby was born to a leprous mother and at birth was covered with patches and tubercles. No follow up observations are reported. In none of these cases was there bacteriological or histological confirmation.

In addition to these cases, Zambaco recorded details of 5 more children developing leprosy at a very early age. One child born to a mother with tuberculoid leprosy, developed macules "like the mother's" at the age of a few days. The lesions disappeared and reappeared and then became permanent. The second child, sibling to the first, developed an eruption of macules at the age of 3 months. At the age of 12 months a few small tubercles had disappeared. The baby died at the age of 16 months. The third child, born at term but very small for gestational age, "like an abortion", developed numerous macules at the age of 15 days. This child's mother was healthy but the father had lepromatous leprosy. The baby's lesions became more marked and

invasive and the child died of cachexia at the age of 1 year. The fourth child, born to a mother with tuberculoid leprosy and a healthy father, developed macules at the age of 3 months. The baby was observed to have slow progress of the disease. The fifth child, born to a mother with lepromatous leprosy, developed macules at the age of 5 months. No follow up is reported. These cases unfortunately had no bacteriological or histological confirmation.

Leprosy in a child of 5 months, born to parents both of whom were leprous, was described by Tardieu (1916). Suspicious lesions were present from the age of 1 month; at the age of 5 months these nodules were confirmed as being due to leprosy when bacilli were found in the nose. The disease progressed rapidly in this child.

Crozier and Cochrane (1929) described a baby born to a bacilliferous lepromatous father and mother with tuberculoid leprosy of 13 years' duration. The baby was born with a suspicious indolent ulcer on the heel which healed after injections of hydnocarpus oil. The baby remained under observation until the age of 5 months and was brought back to the hospital at the age of 12 months with a typical discoloured "patch" on the left scapula. The ulcer on the heel was negative for M. leprae. The patch on the shoulder was not biopsied nor examined bacteriologically, but the lesion healed after 2 months' treatment with hydnocarpus oil. In view of the very early suspicious lesions in these two children, Tardieu and Crozier and Cochrane raised the possibility of these being authentic cases of pre-natal infection.

Nakayo (1914) reported the case of a 3 month old child whose parents were lepers, sections of the skin showed typical leprous infiltrate and lepra bacilli. The umbilical and placental blood had been examined when the child was born, but no bacilli were found.

Case reports of leprosy in very young children have appeared sporadically in literature since that time. Despite these reports, standard teaching at the beginning of the 20th century :

> "leprosy is manifested very exceptionally as early as the third year, rarely before the fifth or sixth year, which would correspond to the classical period of incubation of the acquired disease" (Morrow, 1899),

> "leprosy is extremely rare before the fifth or sixth year" (Manson, 1914),

"leprosy rarely appears before the third or fifth year"
(Scheube, 1903),

"the youngest cases are rarely under 3 or 4 years of age"
(Osler, 1912),

has only undergone slight modification in 70 years :

"leprosy can occur at any age but is rare in infants"
(Bryceson and Pfaltzgraff, 1979a).

Meirelles (1922; 1923) stated that leprosy was very rare in the first 4 years of life, and that the few early cases seen were almost certainly due to contagion and could be avoided by separating children from their parents at birth.

Early lesions and the development and incidence of leprosy in the children of leprous parents

The first serious study of early lesions of leprosy in the children of leprous parents was undertaken at the Culion Island Leprosarium in the Philippine Islands. The population of the colony was approximately 5,000 in the early 1920's, and on account of inter-marriage amongst the patients, the number of births in the colony had steadily increased. A limited number of children were isolated in a separate building known as 'the negative children's house' located away from the leprous population and a smaller number were sent away from the Island and adopted by relatives elsewhere. On account of the inadequate facilities for isolation, the majority of children born of leprous parents were permitted to live with them, thus mingling with the other patients. The infant mortality rate of 42% was comparable to that of children of healthy parents in the Philippines.

Gomez, Basa and Nicolas (1922) carried out the first investigation of 308 children whose ages ranged from 1 - 13 years. Twenty-four out of 308 (7.8%) had bacteriologically positive leprosy; 78 (25%) were diagnosed clinically as having definite or suspicious lesions which were bacteriologically negative. There were no proven cases of children under 2 years, although 6 had suspicious lesions, and of children under 5 years, 4 were bacteriologically positive, 8 clinically diagnosed, and 34 suspicious. The youngest child with positive leprosy was aged 3; the youngest child bacteriologically negative with clinical lesions was aged 2, and the youngest suspect was 1 year. The most frequent

recognisable site of the early lesion of leprosy was the skin, not the nose; infection via this route was considered most likely on account of the great prevalence of skin diseases amongst the children, one-third of whom had some form of itch. The most frequent recognisable early lesion of leprosy was a macule, usually hypopigmented, which initially was negative for the leprosy bacillus. Sensory changes were observed only in the older children. The hypopigmented macules observed in the skin of many children were considered as either leprous or precursors of definite leprous manifestations.

Within the short study period spontaneous healing and disappearance of the lesions was observed in several cases, even those which were bacteriologically positive. Leprosy was observed to affect both sexes equally.

From bacteriological investigation of nasal mucosa and skin lesions in young children, Solis and Wade (1925) showed the lowest incidence of nasal tests positive for M. leprae was in the youngest children. Nasal tests were only positive if the skin was positive: 60% of children showed positive results from the skin only. From these findings, they suggested that nasal mucus invasion by M. leprae is secondary to the skin infection in young children.

Continuing the work of Gomez et al (1922), Rodriguez (1926) investigated, in particular, the children who had been found to have suspicious lesions. These lesions he observed were changeable and evanescent in nature: in 2 years of observations, of 58 suspicious lesions, 24 became negative without treatment, 19 became clinically leprous, and 14 were still suspicious. Of the children classified initially as negative, one-third had developed suspicious lesions. An average of 5 months' delay occurred between the diagnosis of suspicious and confirmed leprosy. (It should be noted Rodriguez only regarded those bacteriologically psotive as leprous.) He found two children under the age of 2 years to be bacteriologically positive, one had the first suspicious lesion observed at 8 months, and at 18 months this was confirmed as positive; the other was suspicious at 17 months and confirmed as positive at 22 months. Fifty percent of confirmed positive cases occurred in children between the ages of 3 and 6 years. Rodriguez considered the incubation period for leprosy in children to be 3 years and 9 months, a figure very similar to that calculated by

Rogers and Muir. He therefore recommended, for children separated at the age of 6 months from their parents, a minimum observation period of 5 years before the child could be pronounced healthy.

Despite his observation that 6 children developed suspicious lesions between the ages of 3 and 6 months (3 of these children becoming bacteriologically positive at the age of 2-3 years), and the observations of other workers that acid-fast bacilli had been found in the placenta (Sugai and Monobe; San Juan; and Pineda) or in neonates (Sugai and Monobe, 1913), Rodriguez stated that no congenital case of leprosy was seen or reported in 871 children born at Culion up to 1924. He went on to state :

> "the lepra bacillus present in the tissues of the leprous mother reaches the foetal circulation in a considerable percentage of cases, but the evidence is conclusive that transmission by this route very rarely takes place."

This view was supported by the observation of Hasseltine (1924) that only 1 out of 121 children developed leprosy in 15 years at Molokai where there was a policy of segregation of the baby from the mother at birth. This was the view held by most leprologists for the next 50 years. Infection of the young child was thought to be largely due to contagion; skin parasites, in particular acarus scabeii, were blamed for providing a portal of entry for leprosy. It was observed that the incidence of leprosy in children was highest where both parents had lepromatous leprosy, and it was lowest where the parents had neural leprosy. The initial lesion, as already observed, was in 75% of cases a single hypopigmented macule, usually situated on the back, thighs or cheeks.

Chiyuto (1933) reported early leprotic changes in 39 children born to couples in which both father and mother suffered from leprosy. All children had lived with their parents for a varying length of time from 7 months to several years. Fourteen of the children were diagnosed as having leprosy by the age of 5 years, the youngest being $2\frac{1}{2}$ years. Skin lesions were accompanied by peripheral nerve and lymph gland enlargement. The earliest lesions were hazy, depigmented areas with borders merging gradually with normal skin. However, the margins of these lesions became well defined when they were observed at an appropriate distance and with the correct angle and lighting. An eruption of minute papulo-vesicles simulating goose flesh was often the

first skin lesion observed; gradually this healed with flattening of the lesions to give yellowish tinted central healing and pinkish circinate raised margins simulating "ringworm", a process taking approximately 3 months. With further healing, complete depigmentation occurred, the lesions eventually fading to hazy, depigmented areas, hardly distinguishable from other depigmented areas of the skin, such as could occur following non-leprous skin infections.

Histologically, the early lesions were characterised by the perivascular round cell infiltration of Manalang (1931), sometimes showing the picture of tuberculoid leprosy, although that picture was usually seen when the skin lesions were more definite and accompanied by anaesthesia. No cases showed M. leprae in skin smears or biopsy: in one case with biopsy-proven leprosy, 8 bacteriological smear tests were negative. The lepromin test was always negative in children under the age of 1 year. This was thought to be due to constant contact, and hence infection of young children in infancy, and was considered as indicating lack of resistance to the infection.

While Chiyuto's work drew attention to the importance of the hazy hypopigmented macule,or the "hazy, pale area" as it was later known, in oonjunction with peripheral nerve enlargement and lymphadenopathy as an early leprotic change in young children, Lara and de Vera (1935a) reported that many children born outwith the Culion Leper Colony were found to have similar hazy depigmented lesions of the skin which were not due to leprosy but were due to the healing scars of common skin infections which were widely prevalent. They remarked that perivascular round cell infiltration could be regarded as diagnostic of leprosy as it was frequently seen in non-specific dermatitis. They pointed out that, while many of the early unstriking changes seen in children of lepers were probably of leprotic nature, not all such lesions could be regarded as diagnostic of the disease. Lara and de Vera drew attention to a previously unrecognised skin manifestation of early leprosy in very young children, namely what they termed a shiny papule. This was found to be present in 6 children aged from 15-23 months and in 4 out of 6 was bacteriologically positive for M. leprae. A 3 month old child with a bacteriologically negative lepromatous macule developed, at the same site, a bacteriologically positive papule 14 months later.

In a further report, Lara and de Vera (1935b) noted that of 10

consecutive cases of very early leprosy in children, 7 cases presented with characteristic shiny, red papules and only one case with a depigmented macule. In 7 cases the definite lesion was preceded by one or more indefinite lesions, which were usually of the "hazy, pale area" type. They observed a tendency of these early lesions, even those which were bacteriologically positive, to spontaneous healing. They remarked, however, that clinical and bacteriological resolution was faster in the cases treated with hydnocarpus oil injections.

In a report on the progress of children separated from their parents at birth or soon thereafter, in Hawaii, in a 3 year follow up study during which children were examined several times a year, Wayson (1936) observed that 35 children showed suspicious signs of leprosy. By suspicious signs he referred to skin lesions, usually with some peripheral nerve enlargement. Twenty-five of these children aged from a few months to 16 years did not show any evidence of sensory disturbance on neurological testing, their lesions were transient and therefore were not biopsied, and from this Wayson concluded that these children did not have leprosy! One wonders whether his comtemporaries working in the Philippines would have agreed with his diagnosis. Nine out of the 10 remaining children, however, showed neurological changes. These were mostly in the older children, and what is of particular interest is that some of these children (no definite numbers are given) showed not only spontaneous disappearance of the skin lesions but definite regression or disappearance of the neurological findings. This seems to be the first report of spontaneous recovery of neurological damage in early cases of leprosy in children.

Doull et al (1936) in an epidemiological survey in Cordova in the Philippines found 104 new cases, of whom only one was aged less than 9. They observed that the peak incidence in this group of patients was in both males and females in the 10–15 year group which they attributed to the effect of puberty; however, they noted that 3 children, all boys, had developed the earliest signs of leprosy before the age of 5.

Meanwhile, in India considerable interest was being taken in the children of leprous parents, and similar observations were made to those already reported from the Philippines and Hawaii: namely that of children separated from their parents at an early age but not at birth, 4 out of 46 children developed leprosy all with anaesthetic macules

which healed spontaneously (Richardson, 1936). The concept of juvenile leprosy as seen in India was described by Muir (1936). The child received a massive exposure to M. leprae before his lymphocytes were competent to deal with the infection; clinical findings could be slight, intermittent or even absent, with skin lesions usually hypopigmented macules appearing and disappearing; there was a tendency in this type of leprosy for the child to develop the infected multi-bacillary leprosy of adulthood. The youngest case personally observed by Muir was aged 3 months.

Cochrane (1936) and Cochrane, de Simon and Fernando (1937), in observations on children with leprosy in Ceylon, observed spontaneous self-healing of lesions, usually of the macular type, in children classified as having a very mild form of neural leprosy (N1). They were so impressed with self-healing that they advocated a trial of non-treatment for children with this form of the disease. They observed, however, that the cases which had progressive disease either had a close household contact with an infected case of multi-bacillary leprosy, or were in a poor, general state of health.

Campos (1937) reported that leprosy was very rare in children under one year old in Brazil, with only one case being known. In a study of 28 children all born to parents with contagious leprosy, he observed that although all the children had received a massive infection from their parents, they all demonstrated a form of leprosy with a higher degree of immunity, namely leprosy with tuberculoid histology, and a positive lepromin skin test. Of 9 children under the age of 2½ years, 5 had nodular lesions, 1 a papuloid lesion, and 3 had annular lesions with raised edges (the sarcoïde lesions of Boeck). Campos suggested that the absence of clinical signs within the first year of childhood was due to the first early infection being overcome by congenital immunity, probably in the form of increased antibodies, hence the form of leprosy appearing in the second and subsequent years of life was characteristic of that seen in an immunised organism. The natural history of leprosy in these children demonstrated the self-healing of depigmented macules and tuberculoid forms of leprosy in children without progression to the lepromatous form.

Cochrane and Rajogopalan (1938) elaborated on the characteristics of lesions of leprosy in children under the age of 14. From their

observations of a school survey in Saidapet (the first centre for study of leprosy in children in India), they found 108 out of 1,671 children of leprous parents had evidence of leprosy. The incidence was 2.5% of children with definite leprosy; this was increased to 6.4% if those with "probable" leprosy were included. They listed 10 characteristics of the lesions :

> 1) they were always observed in children; 2) the lesions were multiple, hypopigmented or slightly erythematous; 3) the edge of the lesion was indistinct; 4) it was observed better in oblique than direct light; 5) there was no sensory loss detected on examining the lesions; 6) there was no nerve enlargement or anaesthesia of the extremities; 7) bacilli could not be found in the lesions by the usual methods; 8) the lepromin test was always negative; 9) history of contact with an infectious case was almost invariable; 10) black and white photography was unsatisfactory for demonstrating the lesions which, however, showed clearly with colour photography.

The higher incidence in boys was due chiefly to the difficulties experienced in examining girls apart from their hands and feet: for cultural reasons no female child could expose her trunk, thighs or upper arms. The lesions were considered as precursors of cutaneous (lepromatous) leprosy and indicated a massive infection with a poor immune response on the part of the child.

Nolasco and Lara (1940) reported the histopathological features of early lesions in 14 children of leprous parents, the children's ages ranging from 19½ months to 3 years and 4 months; the duration of contact with the parents ranged from 5 to 18 months. In this prospective study, the lesions were biopsied in part only so that comparable repeat biopsies could be carried out for control. They stressed the point made by Lara and de Vera (1935b) that early lesions of childhood leprosy were inconspicuous and unless special efforts were made to find them, they would be missed. They also emphasised that great patience and diligence was required in searching for acid-fast bacilli in the biopsies; sometimes they had to spend a whole day searching in serial biopsies from one case. The rarity of M. leprae in the biopsies was noted by the fact that in one case only one acid-fast bacillus was found, in the centre of a perivascular round cell infiltration of the skin. Of the 14 children, 4 had easily seen acid-fast bacilli, 7 had only scanty or very scanty acid-fast bacilli, only one or two being found in many sections examined, 2 had doubtful acid-

fast bacilli and 1 no acid-fast bacilli. In these 3 cases the histological features were diagnostic (2 cases) or consistent with leprosy (1 case). On histological examination, 11 out of 14 had definite epithelioid or tuberculoid changes, 2 had early epithelioid changes, 1 had only perivascular round cell infiltration, but an acid-fast bacillus was found. The tuberculoid lesions all involved skin appendages, hair follicles, smooth muscle, sweat glands and/or dermal nerves. On examination of the dermal nerves, 11 out of 14 had clear nerve involvement with tuberculoid or epithelioid lesions. In only 3 cases histology showed no nerve involvement.

In a further publication of Nolasco and Lara (1948), the progress of 13 of the 14 children previously described (1 child had died) was reported. All the primary lesions had healed. The children at the onset of their leprosy had been between the ages of 11½ and 17 months (6 cases), and 19 and 27 months (7 cases). All the early thickened, papular or wheal-like bacteriologically positive lesions had healed in less than 3 years. Eleven of the 13 children were lepromin strongly positive (3+), 2 were moderately strongly positive (2+). The clinical evolution of the lesion had averaged 6-7 months, the duration from onset to start of the regression of the lesion averaged 10.3 months, the duration of resolution of the lesion averaged 3.7 months. They concluded that the primary lesion of leprosy in very young children underwent spontaneous resolution through natural processes, leaving scars histologically similar to scars from other skin infections such as scabies. The clinical, bacteriological and histological findings suggested very strong evidence of a high degree of natural or individual resistance to leprosy in very young children. This was supported by the observation that 12 of the 13 children were still living with their parents 8 years after the initial examinations and had no sign of active leprosy.

Nolasco and Lara raised the question - "had the children sterilised their systems of leprosy bacilli, or had the bacilli gone into hiding, e.g. in lymphnodes, nerve or testes or other body tissue, to reappear later?"

The results of a study of approximately 260 cases of early leprosy in infancy in childhood in children born to leprous parents were reported by Lara (1948). These children had been studied from birth

with assessments approximately every 2 months. A high incidence of leprosy was observed in infants and children who were permitted close contact with their parents for a minimum of 5 months and maximum of 5 years. This was in contrast to the 600 children of healthy, non-leprous employees at Culion, none of whom had developed leprosy in 25 years despite the proximity of their living quarters to the hospital and occasional contact with bacilliferous patients. There was a slight difference in the sex prevalence, the boys outnumbering the girls by 6%; transmission was considered to be chiefly by direct contact with bacilliferous infectious patients; other modes of transmission such as intrauterine or by breast feeding required further evaluation, and transmission by indirect contact was regarded as having a minor role. A precipitating factor was a measles epidemic which was followed by an unusual increase in new cases and reactivation in existing or healed cases.

The age of onset was defined as the age at which the first recognisable signs of the disease were present; for 196 cases this was between the ages of 11 and 37 months, with an average of 20.4 months for children under the age of 5. Only 4 children had undoubted lesions by 11 or 12 months. The site of the lesions corresponded to the exposed parts with direct pressure contact as occurred in carrying young children. Spontaneous healing was observed in approximately 60% of the lesions; the clinical, bacteriological and natural evolution of the lesions is shown in the accompanying Table.

The histology of the bacillated lesions (0-3+) showed in 3 out of 4 cases predominantly tuberculoid pathology. The lesions which were only slightly bacillated (0-1+) showed, in the majority, round cell histology. The children with these lesions were thought to have some defect in immune response, resulting in later lepromatous progression, despite the general histological similarity. The lesions in which no bacilli were demonstrable (77 cases) showed in 80.5% distinct tuberculoid histology, in 11.7% round cell collections, in 5.2% undifferentiated histology, and in 2.6% healed or healing lesions. The flat, hypo-pigmented lesions relatively free of bacilli were found in older children. The bacillated, thick lesions found especially in the very young children, were those which showed the maximal tendency to spontaneous healing.

TABLE 1

Clinical features of lesions of leprosy in children (Lara, 1948)

	Clinical Appearance	Size	BI	Duration	Number	Frequency and Outcome	Spontaneous Healing
i)	Flat wheal-like papule	3-5 mm ⩾10 mm	⅔, 1-4+ *(mod. bac.)	Transient 2-6 months	More numerous	45% - wider distribution tend to spread before fading	77.6% (no relapse seen)
ii)	Raised reddish and hypo-pigmented macule		50%, 1+ (slightly bac.)	Become larger, then fade, 50% persist		20%	44.2%
iii)	Papulo-nodule	3-5 mm ⩾10 mm	3-4 ++ (mod. bac.)	Become soft and depressed, fading in 1-2 years or more	Few, rarely 6	12.7% - In 2 years becomes the second commonest lesion seen (21%)	88% (85% showed no relapse in 1-13 years)
iv)	Hazy, flat depigmented areas **	Usually at least 10 mm (increasing in size)	70% BI -ve	A few fade; most persist to become lepromatous later		12% - commonest form in 3-6 year old group (48%)	25.8%
v)	Pebbled micro-papulate		85% BI -ve	Very few persist 2 years		5.4% - in older children 12.5% of all types	78.5%
vi)	Indurated scar		60% BI +ve (mod. bac.)	Only seen in younger children		3.5% - probably is the "fading" stage of v) undiagnosed earlier	80%

* mod. bac. = moderately bacillated.

** iv) is similar to Muir and Cochrane's "Juvenile" Leprosy in India and is a pre-lepromatous form.

With the advent of sulphones as a specific chemotherapeutic agent for leprosy, the picture of leprosy in young children changed (Lara and Ignacio, 1956). The overall prevalence of leprosy in children of leprous parents (prevalence 20.9% in the pre-sulphone era; 19.9% in the sulphone era) did not alter significantly - this is thought to be due to failure of compliance by the parents for fear that their leprosy might be healed and they would then be requested to leave the relative sanctuary of the Culion Leprosy Hospital - but certain changes were apparent. Prior to the advent of sulphones, of children developing leprosy by the age of 5, 95% had developed it within the first 3 years of life. Following the advent of sulphones, only 57% of those developing leprosy by the age of 5 had developed it by the age of 3. The average age at onset had increased from 20.7 months to 33.5 months in the sulphone era. The morphology of the lesions was very much the same, but there was a considerable decrease in the thickened lesions, papular nodules and wheal-like lesions which had been characteristic of leprosy in very young children, so that less than 50% of all lesions showed these clinical features, while the macular lesions which were present in 25% prior to the advent of sulphones were present in 40% after the advent of sulphones. The bacteriological picture was much the same as before, but the histological picture showed very few typical tuberculoid or predominantly epithelioid changes.

Reporting on 1,142 cases of leprosy in children under the age of 14, out of a total of 9,510 cases, Nuñez (1949) showed that 35 were under the age of 4 years (3% of the childhood cases: 0.37% of all cases). He observed the same clinical features,in Mexico, as Lara had done in the Philippines, with the commonest early signs being papulo-urticarial or papulo-nodular lesions. He noted that the lesions came and went over long periods of time and approximately 10% had a relapse or recurrence 1-3 years after apparent cure.

In Africa, Wallace (1944) noted the commonest lesions in children to be macules; the typical (as seen in India) pre-cutaneous leprosy lesions were not common in East Africans, neither apparently were the early tuberculoid lesions reported by Lara. Blenska (1966) reporting on leprosy in children in Uganda, noted that the copper-coloured macule, typically seen first on the forehead or cheek, was usually the earliest lesion. However, diagnosis was often not easy because of concomitant skin infection usually of mycotic origin. While these lesions might

undergo self-healing, treatment with dapsone was recommended as without it, many of the children progressed to lepromatous leprosy later.

Gehr (1954 and 1957) reported in detail leprosy in the child of a mother with lepromatous leprosy. At 9 months of age the baby had positive smears from the nasal mucosa, but skin smears were negative 7 times between 9 and 13 months, when the baby developed on the cheek a nodule which was positive for acid-fast bacilli. By that time nasal mucosa was negative, the ears were positive, and the lepromin test was negative. Gehr (1957), commenting on his case report, stated that he had reported it wrongly as being a lepromatous lesion, as it followed the same pattern as the nodules of early leprosy in very young children reported by Lara et al, with spontaneous healing at the age of 20 months. In 3 years following the first case report, he had seen 10 more similar cases and noted :

> "It is not impossible that the condition is quite common here, but leprosy is rarely suspected or else parents pay scanty attention to the often quite insignificant lesions. Spontaneous regression and cure is probably quite frequent."

In a survey of 601 children with leprosy, 89 (14%) were 5 years or younger when first examined; 72 had tuberculoid leprosy, of which 7 were of the nodular type; 8 were indeterminate, and 7 were lepromatous.

Reporting 1,147 cases of leprosy in 16 years at Saidapet, Ramanujam (1980) observed that the early forms described by Lara were not seen in Indian children: 83% of the children had tuberculoid leprosy, and of the benign forms 71.4% showed spontaneous healing. Only one lepromatous case was seen in a child under 4 years, 20 were in the 5-9 year old group, and 76 in the 10-14 year old group.

Source of infection

Having accepted the fact that children usually acquire leprosy from their parents by contagion, Rodriguez (1926) investigated the effect of leprosy in the parents on the children and compared his results with those already published by Sand (1911-1912) and Lie (1918). The incidence of leprosy was highest in marriages where both father and mother had leprosy, next highest where the mother had leprosy but the father was non-leprous, and lowest where the father was leprous but the mother non-leprous. The type of leprosy and stage of the disease was

then considered: the incidence of leprosy was highest (35.3%) among the children in contact with mothers with lepromatous leprosy; in mothers with the mixed (dimorphous) type, 13.5% of the children became infected, while in the case of mothers with tuberculoid leprosy only 9.1% of the children were infected. The degree of infection in the mother did not influence the incidence of leprosy in her children. The incidence of leprosy in the children according to the type and stage of the father's leprosy showed 25% of children developing leprosy where the father had lepromatous leprosy, but considerably lower (15%) where the father had mixed or tuberculoid leprosy. In Sand's cases there was a higher incidence of leprosy in the offspring of parents with lepromatous leprosy than among the children of those with tuberculoid leprosy, the difference being especially marked when the mothers were lepromatous.

In an epidemiological analysis of families and contacts of children with leprosy, Cochrane and Rajagopalan (1943) observed that of children registered at the clinic as having leprosy, more than half who had been exposed to infection in the family had already shown signs of leprosy. There seemed to be no correlation between the percentage of children infected and which relatives or co-tenants of the house were infected. Cochrane et al took this to mean that there was no proof of family susceptibility to leprosy, rather that it was the infectious contact who was important in spreading the infection. They noted an increase in the percentage of children with leprosy with increasing age, thus 36% of children aged up to 4 years had leprosy, while 67.2% were in the 10-14 year old group.

The highest incidence of leprosy in children in families was observed where the mother was leprous, then when a daughter was leprous, and then when either the father or a son had the infection (Plantilla, 1935, cited by Lara, 1948). Related field studies in the Philippines had lent support to the opinion that bacteriologically negative parents could transmit leprosy to their children, but the ability was of a lower order than in bacteriologically positive cases (Lara, 1948).

Montestruc (1953) and Montestruc et al (1954) reported 3 puzzling cases of leprosy developing in a very young child newborn to healthy parents. The first was a three month old infant frequently looked after by her paternal aunt who had bacilliferous lepromatous leprosy.

The child was found to have numerous acid-fast bacilli in smears from two extensive macules. The second case was of a 6 month old infant cared for irregularly by an aunt with lepromatous leprosy from the age of 2-6 months. The child developed a nodule which was full of acid-fast bacilli when she was 6 months old. The third case was of a 3 week old infant cared for on several occasions by a lepromatous baby minder. This child developed macules on her face which were positive for acid-fast bacilli. There were many cases of leprosy in this child's family.

Wayson (1936) and Worth (1960) reporting experience at Molokai noted that, despite the separation of children from their parents at birth, between 1903 and 1929, 69 children (12-13%) developed leprosy. This was attributed to the fact that they were cared for by the non-leprous wives of leprosy patients, who had contact with infectious cases within the leprosy hospital. After 1930, children separated from their infectious parents were kept under much stricter segregation: not one out of 77 developed leprosy.

Browne (1970) observed :

> "The ubiquity of leprosy infection is such that when the prevalence of the disease reaches 5 per 1,000 in any community, all the population must be regarded as exposed."

In his foreword to "Leprosy in Children", Browne (1976) noted that persons with supposed "closed" (non-contagious) forms of leprosy may be the source of infection. "A child with hazy patches which are teaming with M. leprae is indicative of a serious epidemic." The rate of infection among children exposed to leprosy cases at home varied according to the infectivity of the index case. The risk of infection to contacts of lepromatous patients was, roughly estimated, 4 times that of contacts of tuberculoid patients (Noussitou, Sansarricq and Walter, 1976).

Route of infection

Prior to birth:

Neisser (1885) had asked: "Is the contagion of leprosy transferable by way of intrauterine infection?" At that time proof was lacking. However, in 1913 Sugai and Monobe found acid-fast bacilli in 9 out of 12 placentae and in 10 out of 12 blood samples from newborn babies, and

in one placenta they found typical leprous lesions on histology.

Acid-fast bacilli were found in the heart blood of a 6 month foetus, at autopsy of a lepromatous pregnant woman (Rabinowitsch, 1913). Other reports of positive findings in placentae and cord blood followed (for review, see p.62). While there are a number of reports of negative findings in placentae, umbilical cord, membranes and foetal blood at birth, there is ample evidence that M. leprae can cross the placenta.

There are a number of puzzling cases reported by leprologists of repute of very young children born to healthy parents or, in some cases, a healthy mother while the father was noted to have lepromatous leprosy. In attempting to explain these cases, a quotation from Browne (1976) is apt :

> "The observant and thoughtful clinician embarking on a serious study of leprosy in children ..... will be forced willy nilly to use all his professional wits in the search for 'index cases' and 'viable bacilli' and portals of 'exit'",

to which I would add 'portals of entry'. Is there perhaps some truth in the statement that leprosy may be transmitted as a venereal disease? It has been shown (Naeye and Blanc, 1970; Naeye, Dellinger and Blanc, 1971) that in women of low socio-economic status who suffer from dietary deficiency, particularly that of zinc, non-pathogenic organisms can cross intact membranes as an ascending infection from the vagina to cause an intrauterine infection and, in particular, intrauterine pneumonia. It is well known that male patients with advanced lepromatous leprosy carry a considerable number of M. leprae on the skin of the genital organs. Furthermore, a case of leprous urethritis has been reported by Jeanselme (1914). It is not, therefore, unreasonable to suggest that M. leprae deposited in the female genital tract during coitus could ascend and cross intact membranes to infect the baby without the mother herself having leprosy. In cases where the father himself did not have leprosy, the possibility exists that the mother had more than one sexual partner.

A second possible explanation for these very early cases might be that the woman was infected with leprosy and incubating the disease, but that it had not become overt. During pregnancy, with suppression of cell mediated immunity, the bacilli would have the opportunity to

multiply in sufficient numbers to cause a bacteraemia without a skin lesion becoming manifest. With the recovery of cell mediated immunity postpartum, most of the bacilli would be destroyed by the mother's defence mechanisms and her leprosy might not become overt during the period of observation.

After birth :

The risk to the child of suckling an infectious mother was first noted by Schilling (1778a), who advocated removal of the child at birth from the mother, so that it could be brought up in surroundings where there was no risk of infection. He also pointed out the possibility of a suckling infant acquiring leprosy from a leprous wet nurse, a risk reiterated by Drognat-Landré (1868e).

Babes and Kalindero (1895) found acid-fast bacilli in milk from women with active lepromatous leprosy, as did Sugai and Monobe (1912), Pedley (1967 and 1968) and Saha, Sharma and Siddiqui (1982). However, Rodriguez (1926) noted that they were unable to isolate M. leprae from 45 samples of milk from women with active leprosy. Furthermore, when he investigated the incidence of leprosy in children with regard to the mode of feeding while they were babies, he noted that of 138 children who were breast fed by their mothers, 11.6% developed leprosy, of 65 children who were partly breast fed and partly bottle fed, 16.9% developed leprosy, and of 54 children who were bottle fed only, 24% developed leprosy. He suggested from his findings that the presence or absence of M. leprae in milk was not an important factor in transmission of the disease, rather that the increased incidence of leprosy in bottle fed babies implied a lack of defence factors received by these children because they were not breast fed.

In advanced cases of lepromatous leprosy, there could be lepromatous infiltration involving the breast and nipple, and thus a risk of bacilli being introduced directly into the mouth of the baby should there be any crack or breach of the skin covering of the lesions (Jeanselme, 1910). However, Rodriguez (1926) pointed out that while he had observed involvement of breasts and nipples in middle-aged nulliparous women with advanced lepromatous leprosy, it was very rare in parous middle-aged women. He attributed this to lack of function of the breast and in conclusion noted that involvement of the nipple in patients with lepromatous leprosy was more common in men than in women.

While Jeanselme (1910) considered it safe for a woman with tuberculoid leprosy to breast feed her baby, he pointed out the very considerable risks of leprosy being transmitted from a wet nurse to the suckling child in countries where leprosy is endemic (1933c). Clearly, as Lara said (1948), the whole question of possible transmission of leprosy through the mother's milk has to be evaluated in the context of :

i) there being other possible routes of infection;

ii) the risk of depriving the baby of appropriate defence factors;

iii) the 50% probability of the baby dying of gastro-enteritis, should it have to be bottle fed, in a country where preparation of sterilised feeds is virtually impossible.

Transmission of M. leprae by droplet is of significance to the newborn child where the mother or child minder is not only bacilliferous but shedding M. leprae from her nose during the period of close contact while suckling, feeding and talking to the child. The infecting dose of M. leprae falling on to the child's mouth and face could be considerable.

Probably the most important route of transmission to the child is by prolonged skin to skin contact (Lara, 1948), and here skin infections may provide an important role of entry. The frequency of skin diseases in young children reaches a peak at the age of 12 months (Lara et al, 1935a). Scabies, in particular, may be transmitted to the child as a mother with lepromatous leprosy and skin sensory loss is liable to have a heavy scabies infection. Subsequent scratching by the child will break the integrity of the skin surface. Thereafter, either skin to skin contact with bacilliferous lesions with bacteria on the skin, or transmission by means of clothing laden with M. leprae from droplet spread, or pus from abscesses (Schilling, 1778b) could result in infection of the child. Clothes may have a protective function, as Nolasco et al (1940) observed a rarity of lesions on the trunk which they attributed to the wearing of clothes. Similarly, the hair may prevent infection of the child's scalp, as has been pointed out by Manalang (1948) that Japanese children who had their heads shaved at 100 days after birth are liable to leprous infection of the scalp.

The possible role of other biting insects carrying infection from an infected person to the child, while theoretically possible, must



Plate 6. " ... the whole question of possible transmission of leprosy through the mother's milk has to be evaluated in the context of there being other routes of infection, the risk of depriving the baby of appropriate defence factors together with the 50 per cent probability of the baby dying of gastro-enteritis should it have to be fed in a country where preparation of sterilised foods is virtually impossible."

remain of relatively minor importance as the children living at Culion, outside but near to the leprosy hospital, did not develop leprosy although they were subject to the same biting insects as the children living within the leprosy hospital (Lara, 1948).

LEPROSY - THE BACILLUS, TRANSMISSION, CLASSIFICATION AND CLINICAL MANIFESTATION

Leprosy is a classical example of a chronic disease where the clinical manifestations of the disease are due not to the bacillus which is virtually non-toxic (Godal *et al*, 1974), but to the immune response of the host.

Mycobacterium leprae, the causative organism of the disease, is an obligate intracellular acid-fast bacillus which has an affinity for skin, particularly in the cool areas of the body, and a unique ability to invade peripheral nerves. It is found in the skin predominantly in macrophages, and in peripheral and dermal nerves in Schwann cells. It has to date never been cultivated successfully *in vitro* but will multiply in the footpads of mice. More widespread infection occurs in animals thymectomized at birth, emphasising the role of thymus-mediated immunity in this infection (Rees *et al*, 1967).

M. leprae has a multiplication time of 12 days (Shepard and McRae, 1965). The incubation time of the infection is long, on average 2 to 5 years, but periods of up to 27 years have been recorded (Thin, 1891b). The host may harbour up to 10^{13} bacilli in body tissues (Shepard, Levy and Fasal, 1968) and have a bacteraemia of 10^{5}/ml without signs or symptoms of septicaemia (Drutz, Chen and Lu, 1972). The host may thus remain active, with no evidence of being unwell. Indeed, it has been stated that leprosy *per se*, even in cases of advanced infection, is rarely the cause of death (MacNamara, 1866; Roose, 1890f; Hansen and Looft, 1895e; Mitsuda, 1924; Manson-Bahr, 1946).

The prediliction of *M. leprae* for skin in cooler areas of the body led to experimental multiplication of the organism in the mouse foot-pad (Shepard, 1960) and, more recently, in the 9-banded armadillo (Kircheimer and Storrs, 1971). The armadillo is not only an animal model for lepromatous leprosy, but is providing very large numbers of bacilli for experimental work and the manufacture of a vaccine (Webb, Mims and Turk, 1979; Smelt, Rees and Liew, 1981). The European hedgehog, which also has a low body temperature, is now being investigated as an alternative to the armadillo for experimental work in Britain and Europe (Rees, Lowe and McDougall, 1979; McDougall, Rees and Lowe, 1979).

Contrary to previous belief, M. leprae is an organism of high infectivity. In a study of staff contacts in the Addis Ababa Leprosy Hospital, Godal and Negassi (1973) demonstrated that the staff contacts developed high responses in the lymphocyte transformation test (LTT) using M. leprae as antigen, and suggested that most developed sub-clinical infection, a situation analogous to that in tuberculosis. The prevalence, however, of clinical leprosy in Ethiopia is 0.5%, or 1 in 2,000.

Mode of spread of M. leprae/transmission of leprosy

After the discovery of M. leprae by Hansen, the staining properties of the bacillus and its characteristics were confirmed by Neisser (1879). Thereafter, acid-fast bacilli showing the characteristics of M. leprae were identified, not only in the skin and nerves (Thin, 1883; Neisser, 1885c; Cornil and Babes, 1886a; Thin, 1891c), the nose (Leloir, 1886c; Cornil and Babes, 1886b), and pus and ulcerating lesions (Leloir, 1886d), but also in virtually every form of human secretion and excretion: saliva, milk and seminal fluid (Babes and Kalindero, 1888), urine (Lagane, 1912, 1913), faeces (Merian, 1913), and also in the placenta and cord blood of babies. M. leprae were isolated from the blood (Thin, 1883; Leloir, 1886e) and in experimental studies, from blood-sucking insects (Long, 1911; Stelwagon, 1919).

It is now generally accepted that the most important means of spread is by droplet transmission from infections lepromatous patients; a number of studies have recently shown that the most likely spread of the organism is from the nose (Shepard, 1962; Goodwin, 1967; Pedley, 1973; Davey and Rees, 1974; Rees and Meade, 1974), thus confirming work of almost 100 years ago by Leloir (1886f), Jeanselme and Laurens (1897), Schäffer (1897) and Sticker (1897). The "nose blow" is now used as a method of assessing the infectivity of patients. Oral transmission of the disease has recently received attention (Hubscher, Girdhar and Desikan, 1979).

Skin-to-skin transmission is of relevance, chiefly in the transmission of leprosy from mother to child (Noussitou et al, 1976). The role of breast milk in the transmission of infection to babies has not been fully investigated. Although M. leprae have been isolated from milk of women with untreated leprosy (Babes et al, 1888; Sugai

et al, 1912; Pedley, 1967), reports from Guadeloup and Nigeria suggest that where full aseptic techniques, with wearing of gowns and masks, are used, the risk of infecting young children is very low (Le Dentu, 1910; Preventoria, 1945). The risk of transplacental infection has hitherto been considered negligible, as :

i) with the exception of the earlier reports, congenital leprosy has never been established;

ii) leprosy is exceedingly rare in children under the age of 2 years (Noussitou et al, 1976); and

iii) children separated from their mothers at birth had a greatly reduced incidence of leprosy, although a considerable percentage died as a result of gastro-enteritis (Worth, 1960; Wallace, 1944; Preventoria, 1945).

Classification of leprosy

Danielssen and Boeck (1848) and Hansen and Looft (1895), in their classical descriptions of leprosy, described two distinct forms of leprosy - "tuberosa", subsequently termed "lepromatous", and "anaesthetic", subsequently termed "tuberculoid". They observed that a mixed form of tuberculoid and lepromatous leprosy could exist in one patient and considered this as a transformation or progression from one form to the other during the course of the disease (Danielssen and Boeck, 1848j; Hansen and Looft, 1895f). The modern concept of "borderline" leprosy was not formulated, however, until the middle of this century, nearly 100 years after the initial description of tuberculoid and lepromatous leprosy. A comprehensive system of classification which included borderline leprosy was adopted by the Sixth International Congress of Leprology in Madrid 1953, using a suggested scheme proposed by Wade (1952). Ridley and Jopling (1966) introduced a five-group classification as follows :

Tuberculoid leprosy	(TT)
Borderline tuberculoid leprosy	(BT)
Borderline leprosy	(BB)
Borderline lepromatous leprosy	(BL)
Lepromatous leprosy	(LL)

A later modification by Ridley and Waters (1969) defined a group between BL and LL which they termed indefinite leproma (LI), and later Ridley renamed LL as LL_p (polar lepromatous) and LI as LL_s (sub-polar lepromatous) (Ridley, 1974). The Ridley and Jopling (1966), Ridley and Waters (1969) and Ridley (1974) classifications are those currently used for research purposes and in clinical leprosy.

The clinical manifestations of uncomplicated leprosy

The clinical manifestations of the disease are due to the host's immunological response to the bacillus rather than variability in the properties of the bacillus. Where there is a powerful cell-mediated immune response, the lesions are localised and there are very few bacilli, found chiefly in nerves: this is tuberculoid leprosy (TT). Where cell mediated immunity is low, probably because of a specific defect of T-lymphocyte reactivity to M. leprae (Godal et al, 1971), the disease is diffuse and generalised: this is lepromatous leprosy (LL). Between these two polar forms of the disease lie the immunologically unstable borderline forms of the disease exhibiting features of both tuberculoid and lepromatous leprosy. A spectrum of the disease has been defined in terms of bacteriological load, immunological response/status, histology and clinical features (Myrvang et al, 1973).

For the clinical features of leprosy reactions, see pp. 41 - 43.

IMMUNOLOGY OF LEPROSY

Cell mediated immunity underlies the immune response in leprosy. The organisms are killed within macrophages activated by lymphocyte products generated in the immune response to the bacilli. The delayed hypersensitivity inflammatory reactions found in leprosy are a manifestation of the cell mediated response and involve a local accumulation of mononuclear cells. The wide spectrum of leprosy seen clinically and histologically is due to the level of cell mediated immunity and/or delayed hypersensitivity (DH) possessed by the individual patient (Myrvang et al, 1973).

In the mid 1970s the current view was that polar lepromatous leprosy was characterised by lack of cell mediated immunity towards M. leprae, apparent uninhibited growth of the bacilli, and a high serum content of antibodies against various mycobacterial antigens. In the highly resistant form, tuberculoid leprosy, the reverse was the case : cellular immunity and hypersensitivity were well developed, whereas antibodies to mycobacterial antigens occurred infrequently and in low concentration (Figure 1). With advances in immunology of leprosy, this view has been somewhat modified. The present status of immunological aspects of leprosy is reviewed by Godal (1978), Bullock (1979) and Turk (1979).

Cell mediated immunity

Lymphocyte transformation tests (LTT) have been used to measure lymphocyte reactivity in different forms of leprosy and have contributed very considerably to our understanding of the disease. Suppression of responses to mitogens and antigens have been shown to be more marked in patients with lepromatous leprosy compared to those with tuberculoid leprosy, especially in those with active disease (Dierks and Shepard, 1968; Bullock and Fasal, 1971). Cell mediated immunity (measured by LTT and leucocyte migration inhibition test (LMIT)) has been correlated with delayed hypersensitivity (assessed by the response to skin tests using lepromin, or more recently purified protein derived from armadillo-grown M. leprae). Myrvang et al (1973) using LTT, LMIT and early and late lepromin tests, showed a clear progression across the spectrum from high responsiveness in polar tuberculoid to low or

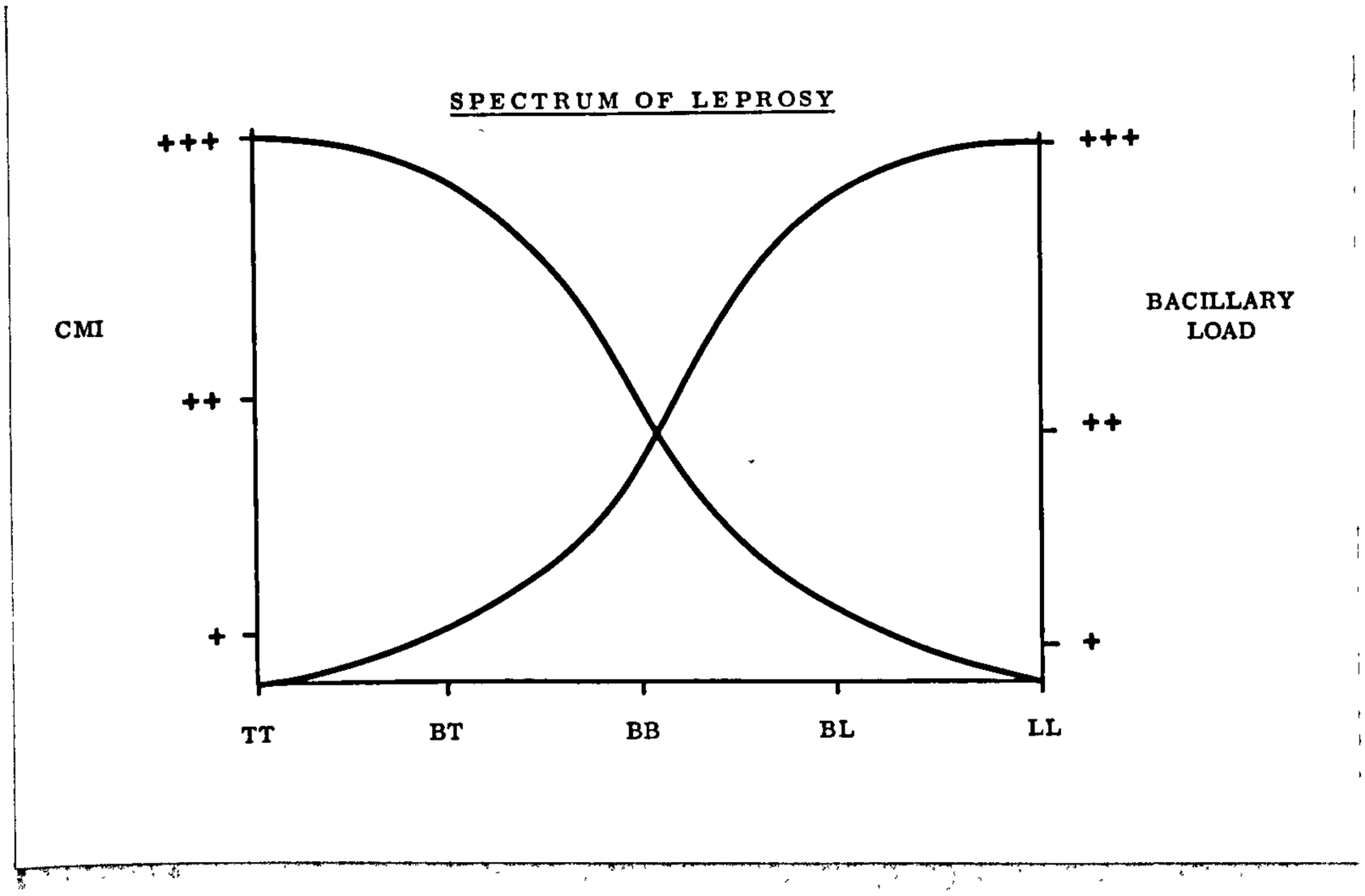


Figure 1. The spectrum of leprosy in terms of cell mediated immunity (CMI) and bacillary load.

non-responsiveness in polar lepromatous patients. These results were specific to M. leprae as the responses to BCG and PPD in LTT did not show the same decline across the spectrum of leprosy. The spectrum of immune responsiveness, shown by laboratory experiments, was confirmed by histological studies on the changes in lymphnodes, thus in TT leprosy the para-cortical areas were well populated with lymphocytes, while in LL_p patients the para-cortical areas were infiltrated with undifferentiated cells of the histiocyte and macrophage series which failed to eliminate M. leprae. This histological picture persisted despite anti-leprosy therapy of up to 10 years' duration (Turk and Waters, 1971). Recently it has been shown that there is a relative immunodeficiency, even in TT leprosy (Godal, 1978).

Non-responsiveness in lepromatous leprosy may be due to active suppressor cell mechanisms, but these are far from being completely understood and the mechanisms have still to be worked out. Some of the depression of CMI as measured by LTT appears to be due to the suppressive effect of plasma factors most marked in patients with lepromatous leprosy (Bullock and Fasal, 1971; Nelson et al, 1975). The suppressive effect is less marked in patients who have been treated.

Suppressive factors are thought to be more important towards the lepromatous end of the spectrum and may have a role in preventing reversal reaction (Bjune and Barnetson, 1976) or in preventing ENL. It is likely that plasma factors play a particularly important role in pregnancy.

Humoral Immunity

Although the chief defence against intracellular organisms such as M. leprae is through cell mediated immune mechanisms, humoral antibodies are produced against the organism.

B-cell function is probably normal in leprosy and the germinal centres of lymphnodes are well preserved across the spectrum of leprosy (Turk and Waters, 1971). There appears to be in fact an increase in the number of circulating B-cells and an inversion of the T-cell/B-cell ratio especially in lepromatous leprosy (Dwyer, Bullock and Fields, 1973; Kazimiera et al, 1973). Also, active immunisation of leprosy patients results in normal antibody levels across the spectrum.

The extent to which humoral immunity plays a protective role in the pathogenesis of leprosy is unknown (Bryceson, 1981). Despite this, recent developments indicate that understanding of humoral immunity in leprosy may provide the basis for diagnostic tests; furthermore, humoral immunity plays an important role in the pathogenesis of erythema nodosum leprosum (considered later).

Rees et al (1965) showed that patients with lepromatous leprosy had large numbers of mycobacteria in the skin and a high titre of circulating antibody to mycobacterial polysaccharide. Bullock, Ho and Chen (1970) found that only lepromatous leprosy patients had significantly raised levels of immunoglobulins IgA, IgG and IgM when compared with normal controls. Srivastava, Agarwal and Goedde (1975), in a study of leprosy patients in Ethiopia, showed increased levels of IgA, IgG and IgM in all leprosy patients. In some of the classifications, however, there was a wide scatter of results compared with normal controls, thus significantly higher levels of IgA, IgG, IgM and IgD were observed in lepromatous patients compared with healthy controls; in tuberculoid leprosy patients significantly higher levels were observed only in IgG, IgM and IgD; and in borderline lepromatous patients significantly higher levels were only observed in IgG. Barnetson et al (1976a) showed an increase of IgA, IgG and IgM in patients with borderline leprosy during reversal reaction: IgG and IgA levels subsequently fell below the baseline figures during the post reaction period. The rise in immunoglobulin levels was considered to be the effect of reversal reaction rather than a cause. It was postulated that this might be due to factor(s) produced by activated T-cells causing a non-specific stimulation of B-lymphocytes during reversal reaction.

Saha, Agarwal and Misra (1978) reported significantly raised levels of IgA, IgG and IgM in lepromatous patients, but noted that the secretory IgA of lepromatous patients in saliva, intestinal aspirate and breast milk was significantly reduced (Saha et al, 1982).

Antimycobacterial antibodies

The credit for advances in the methodology of assay of anti-mycobacterial antibodies must go to Professor Harboe and his team in Oslo. The development of various types of RIA and their application is reviewed by Harboe (1981).

REACTIONS IN LEPROSY

It has long been recognised that many patients with leprosy have acute episodes of inflammation, which are now recognised as being immunological reactions due to hypersensitivity to bacillary antigens. Two types of reactions are commonly seen :

i) Reversal reaction (RR), also called type 1 lepra reaction.
ii) Erythema nodosum leprosum (ENL), also known as type 2 lepra reaction.

The earliest description of these is given by Danielssen and Boeck who recognised two distinct forms of reaction in patients with leprosy. In lepromatous patients they observed eruptions of red nodules, mostly of lentil size, which were tender to touch, appeared for a few days at a time, recurred for periods varying from weeks to months, and were associated with high fever and sometimes reaction in the eye (1848k). In tuberculoid patients they observed reaction to take the form of fever, exacerbation of lesions, severe pain especially at night radiating to the extremities, lymphadenopathy, extension of anaesthesia, and sudden complete paralysis (1848l).

Hansen and Looft gave similar descriptions with additional details that the eruptions in lepromatous patients in some cases were associated with ulceration of the nodules, iridocyclitis, swollen and painful nerves, testicles or joints. They observed that amongst lepromatous patients outcome of the disease depended on the course of eruptions: patients with frequent episodes of eruptions developing nephritis or amyloid disease of kidneys, liver or spleen, progressed to early death (1895g). They observed, however, in the macular eruptions of tuberculoid leprosy, that with healing of the lesions the leprosy itself was healed (1895h).

i) Reversal Reaction

Reversal reaction is considered to be an example of delayed hypersensitivity reaction (Coombs and Gell Type IV hypersensitivity reaction) (Coombs and Gell, 1975). It is associated with an increase in cell mediated immunity as shown by increased responses to M. leprae in lymphocyte transformation tests (Godal et al, 1973). In addition to the skin or nerve lesions there may be "upgrading"

or shift along towards the tuberculoid end of the leprosy scale which may be detected clinically and histologically. Reversal reaction is particularly common in the immunologically unstable borderline zone of the leprosy spectrum, and its most serious consequence for the patient is development of nerve damage which may occur very suddenly but is reversible if treated quickly with adequate doses of corticosteroids (Weddell and Pearson, 1975).

Campos and de Souza (1954) described the skin lesions of reaction in tuberculoid leprosy, noting the abrupt onset with the possibility of nerve involvement. Skin lesions appeared more active and occasionally desquamated. The lepromin skin test, if negative before the reaction, became positive afterwards; histology was of tuberculoid granuloma with epitheloid cells. Tajiri (1955) describing reaction in borderline cases, noted that it was a benign development in the malign lepromatous form of leprosy, especially when the Mitsuda reaction became positive. Ridley (1969) observed early lymphocytic infiltrate in reversal reaction, with the formation of an epitheloid cell granuloma and diminution of numbers of bacilli as the reaction progressed.

In a prospective study, Barnetson et al (1975) not only demonstrated increase in cell mediated immunity as detected by the lymphocyte transformation test during periods of reaction, but showed that reaction in skin was associated with increased responsiveness to whole M. leprae (surface antigen), while reaction in nerve was associated with increased responsiveness to sonicated M. leprae (cytoplasmic antigens).

ii) Erythema Nodosum Leprosum

ENL is seen most commonly in lepromatous patients towards the end of the spectrum, with rather more than 50% developing ENL by the end of the first year of treatment (Waters, Rees and Sutherland, 1967).

Campos and de Souza (1954) describing ENL, attributed it to an allergy or sensitisation and observed histologically polymorphonuclear leucocyte infiltration and oedema superimposed on a regressive lepromatous lesion with granular bacilli. Tajiri (1955) observed that in ENL the Mitsuda reaction remained negative. The clinical and histological features of ENL were further elaborated by

Karat et al (1967), Ridley (1969) and Waters, Turk and Wemambu (1971).

Wemambu et al (1969) demonstrated by fluorescent microscopy granular deposits of immunogloublin and complement with, in some cases, soluble mycobacterial antigen in the dermis of lesions from patients with ENL. In a few patients serum levels of C3 were increased. They suggested ENL was a manifestation of the Arthus phenomenon. Their findings were confirmed by Waters et al (1971). Increased C1q binding in sera of leprosy patients, especially lepromatous patients with active ENL, was taken as further evidence for the immune complex aetiology of ENL (Moran et al, 1972; Gelber et al, 1974). Bjorvatn et al (1976) demonstrated that serum ^{125}I-C1q binding activity was increased in lepromatous patients with and without ENL and also in tuberculoid patients compared with healthy controls. They observed, however, that C3d, a product of metabolism of C3, was increased in patients with ENL but rarely in patients with uncomplicated lepromatous leprosy. This suggested that an activation of the complement system might be involved in the pathogenesis of ENL. The absence of a correlation between serum ^{125}I-C1q binding activity and C3d implied that extravascular immune complexes were involved.

In ENL the significance for the patient apart from the general malaise is two-fold. The more severe forms of ENL can on occasions present serious problems for which it is justifiable to give prolonged treatment with corticosteroids (Weddell and Pearson, 1975). Secondly, ENL may be associated with nerve damage: nerves may be painful and tender for prolonged periods, though they may only show mild loss of function (Pearson and Ross, 1975).

Recent investigations are now throwing new light on the pathogenesis of ENL. Lack of uniformity in the immunological and biochemical findings in patients with ENL suggests that it may in fact represent two forms of disease with different pathogeneses (Webb et al, 1979) :

1. Cutaneous ENL, usually due to extra-vascular immune complex formation.
2. Systemic ENL, due to deposition of circulating immune complexes.

TREATMENT OF LEPROSY

Until the 1940's, treatment for leprosy consisted of isolation of those afflicted with the disease, attempts to improve the general health of the patients, local applications to the skin, treatment of secondary skin infection, thermal baths, purges, and symptomatic treatment of other complaints. Chaulmoogra oil administered by injection (frequently into the lesions of leprosy) was found to have a mild antileprotic effect. It is the active principle of various herbal remedies used in India, Africa and South America (Nwude and Ebong, 1980), and some improvement was observed in cases where there was a tendency to self-healing, namely patients at the tuberculoid end of the spectrum. Surgical removal of isolated lesions was also practised and some early attempts at immunotherapy were made.

The Sulphones

Promin, a sulphone derivative which was not effective for tuberculosis but was effective in leprosy, was introduced in 1941, some 30 years after the first synthesis of sulphones. This brought new hope to leprosy patients and physicians alike. Dapsone (4'4 diamino diphenyl sulphone, DDS) was first used against leprosy in 1947 (Bushby, 1964) and became the standard treatment in about 1952. It is bacteriostatic, though low degree bactericidal activity can be demonstrated experimentally. It is cheap and almost free from side effects when used in orthodox dosage (50-100 mg daily in adults). It has a very high safety margin: after a dose of 100 mg the ratio of peak serum concentration to minimal inhibitory concentration (MIC) is about 500 (rifampicin, with the next highest, has a ratio of peak serum concentration/MIC of 30). Furthermore, the half life is about 1 day; thus the period in which the serum concentration exceeds the MIC is about a week for dapsone compared with 1 day for rifampicin. Thus even where patient compliance is poor and dapsone is swallowed erratically, effective dapsone levels can be achieved in the majority of patients (Ellard, Pearson and Haile, 1981).

The assessment of chemotherapy

Dapsone was in general use for a decade or so before the first demonstration of limited multiplication of *M. leprae* in the mouse foot-

pad (Shepard, 1960). Before that time, the death of M. leprae could be monitored only indirectly. Two methods in general use were assessment of the clinical condition and measuring the fall in the concentration of M. leprae in skin smears (the Bacterial index or BI: Ridley, 1958). Both these assessments, however, measured the disposal of killed M. leprae (a process that is not drug related) rather than killing. The BI shows little change in the first 18 months or so of treatment, and reaches zero only after 5-7 years of effective chemo-therapy.

The observations that the bacillus of leprosy was solid staining when viable but upon death acquired a granular appearance before it finally disappeared was first made by Neisser (1885d). The appearance of M. leprae in skin smears, using this observation, which was subsequently confirmed by others, is now used for following the response to treatment in patients receiving antileprotic drugs. This qualitative assessment or "Morphological Index" (MI) gives the percentage of uniformly or solid staining bacilli in the skin smear (Waters and Rees, 1962). It is expected to fall close to 0 during the first 6 months of treatment with dapsone. In general, suspensions of M. leprae containing only granular bacilli fail to multiply when injected into the mouse foot-pad.

Dapsone, however, did not kill all the bacilli as persistent, solid staining, viable bacilli were observed after 9 to 18 months of treatment (Waters and Rees, 1962). These "persisters" or "dormant" bacilli were observed usually in muscle (Leiker, 1971) or in nerves (Boddingius and Stolz, 1981) where it was thought they were "hidden" from host defence mechanisms.

Dapsone resistant leprosy

Growth of M. leprae in the foot-pads of mice (Shepard, 1960) was used to prove dapsone resistance for the first time in 1964 (Pettit and Rees). The problem of dapsone resistance in Malaysian patients was described by Pearson, Rees and Waters (1975) with most patients having been on treatment for 15-16 years prior to developing resistance. Clinical evidence of dapsone resistance is the rise of BI and MI in patients who are known to be taking their drugs, in association with subsequent appearance of new lesions which frequently assume the form of very

sharply defined nodules ("histoid" lesions: Wade, 1963). Laboratory proof depends on the multiplication of resistant bacilli in the foot-pads of mice. Studies from both Malaysia and Ethiopia have revealed a good correlation between the clinical and laboratory methods (Waters, 1977; Pearson et al, 1977a).

When patients relapse due to the emergence of dapsone resistant leprosy, M. leprae recovered from their lesions can show different grades of resistance. Low grade is defined as resistant to the equivalent of dapsone 1 mg daily (when tested in the mouse foot-pad) but inhibited at 10 mg daily. High grade is resistant to the equivalent of 100 mg daily. The more regular the treatment and higher the dosage, the more likely is the resistance to be high grade. Also, patients with low grade resistance are likely to show clinical improvement for several years if they receive regular treatment with dapsone alone in full dosage.

Dapsone resistance was first suspected in Ethiopia in the early 1970's, and by 1977 some 3% of the patients at risk in Addis Ababa were developing dapsone resistance each year (Pearson et al, 1977a). The majority relapsed after about 10 years of dapsone monotherapy (Pearson et al, 1979). In addition to the problem of secondary dapsone resistance, primary dapsone resistance is now a significant problem in Ethiopia (Pearson, Haile and Rees, 1977).

The causes of treatment failure

The problem of dapsone resistance, both primary and secondary, is now world-wide (Pearson, 1981a). Relapse, however, may not just be due to dapsone resistance, it may be due to inadequate treatment, with subsequent re-activation of dormant bacilli, failure of patient compliance (Low and Pearson, 1974), initial wrong classification at the diagnostic clinic (Touw-Langendijk and Naafs, 1979), or possibly due to re-infection with dapsone-resistant bacilli in patients with no/low host resistance (British Medical Journal Editorial, 1977).

The problems of compliance in leprosy with self-administration of dapsone are similar to those experienced in general medicine in the Western world and in third-world countries with tuberculosis (Meade, 1977). Additional problems in the third world include problems of access of patients to treatment due to lack of communications, civil

disturbance, geographical features such as mountains and deserts, and monsoon-type rainfall which may effectively block all transport by land for 3-9 months of the year. A further problem is that of "drug peddling" whereby patients seek to make a living by selling their dapsone and prednisolone to unregistered patients.

Treatment of dapsone resistance

In order to deal with the problems of relapse and resistance, alternative drugs have been tried, singly or in combination. These include clofazimine, a bacteriostatic agent, rifampicin, a bacteriocidal drug, thiambutosine, methimazole ethionamide, and thiacetazone. Dormant bacilli have been observed in patients receiving thiambutosine, clofazimine and rifampicin (Leiker, 1971; Waters et al, 1978), and drug resistance both to rifampicin (Jacobson and Hastings, 1976) and thiambutosine (Waters, 1969) has been reported. Planned regimes using triple therapy - rifampicin, clofazimine and dapsone, for multibacillary leprosy and dual therapy - rifampicin and dapsone, for paucibacillary leprosy given for short courses under supervision are now recommended (World Health Organisation, 1982). It is hoped that this will lessen the risks associated with the poor compliance experienced in third world countries (Ellard, 1981).

Drugs used in the present study

Almost all the patients in the present study were receiving dapsone throughout the study period. In addition, many patients received clofazimine and/or prednisolone for the management of reactions, and a considerable number were on clofazimine for the treatment of dapsone resistant leprosy. Dapsone is not known to have adverse effects on mother or foetus; clofazimine and prednisolone, however, can affect both.

Clofazimine

This drug acts directly against M. leprae, causing fall in MI to 0 in 6 months (a similar fall to dapsone) and BI fall of 1 unit per annum, also similar to dapsone. It also has a definite anti-inflammatory action and has been found particularly useful in the treatment of ENL. The side effects are discolouration of the skin due to deposits of red pigment in the fat layer chiefly in macrophages (Desikan and Balakrishnan, 1976). Gastro-intestinal upset may be a problem at

first, particularly in high dose treatment, but can usually be regulated by administering clofazimine with the main meal of the day. Deposition of crystals in the kidneys may occur with transient microscopic haematuria.

In experimental studies on rats, clofazimine in doses rather higher than those used in human leprosy was observed to cause abortion (Stenger et al, 1970). In human pregnancy clofazimine has not been widely used, but the effect of clofazimine in 18 pregnancies has been recorded (Waters, 1969; de las Aguas, 1971; Schultz, 1972; Karat, 1975; Plock and Leiker, 1976; Farb, West and Pedvis-Leftick, 1982). It is clear that clofazimine passes the placenta as in most cases the baby was hyperpigmented at birth; however, more clofazimine is considered to pass through the blood-milk barrier than across the placental barrier and "pink milk" has been recorded (Waters, 1969). No complication due to clofazimine has been reported but the manufacturers recommend that it should not be prescribed within the first 3 months of pregnancy unless the drug's use is urgently indicated. However,

> "in treating pregnant women with clofazimine, the unknown risk to the embryo must be balanced against the indications for giving the drug, especially the serious nature of the acute reactions of leprosy that occur during pregnancy. Thus, in erythema nodosum leprosum, the value of giving this anti-inflammatory, anti-bacterial drug,must be weighed against the increased risk of abortion or of still-birth in uncontrolled, or steroid-controlled reaction, together with the near certainty of severe exacerbation of the reaction at the end of the first week or the puerperium." (Waters, 1969)

Prednisolone

Prednisolone is the drug of choice for neuritis in reversal reaction and should be used in conjunction with appropriate anti-leprotic therapy, starting with 30-40 mg daily, reducing the dose to 20-30 mg daily in the second month, and after that according to the nerve deficit index (Naafs, Pearson and Wheate, 1979). The use of prednisolone for both reversal reaction and ENL has been reviewed by Pearson (1981b). Treatment for reversal reaction is as indicated by Naafs. Treatment for ENL in contrast and only for severe cases which cannot be controlled by any other means is in courses of 2-3 weeks' duration, starting with 30 mg, tapering off within 5-6 days, and repeated as necessary.

"QURTIMAT" (RHEUMATISM) AS A SYMPTOM IN LEPROSY

"Aches and pains" are not infrequently seen in patients with leprosy, and are a commonplace accompaniment of reactions, particularly ENL. Among patients in Ethiopia the Amharic word qurtimat is used to describe such pains. They are best understood (in western terms) as muscular aches and pains, and (as in the west) are more common in older people (Dr. Mesfin Demissie, personal communication).

However, Ethiopian leprosy patients commonly used the word to describe a type of pain in muscle and/or bone that was more specifically associated with leprosy itself. It was aching, nagging, or gnawing in character, usually widespread, but occasionally localised to a particular region. It could usually be differentiated from nerve pain, and often occurred in the absence of nerve tenderness. In a number of lactating patients, the pain was severe enough to make them weep, and a few of them were unable to carry their babies or even hold them for suckling because of the severity of their pain.

This symptom of leprosy in Ethiopians was first analysed by Barnetson (1977a), who used the term "limb pains" to translate qurtimat. In a prospective study of reactions in borderline leprosy, he observed that the majority of patients first presented because of limb pains, often in association with neuritis, and that the incidence of these limb pains decreased following treatment until, after 1-2 years, they occurred only in patients who had developed nerve reactions. That qurtimat is a symptom of active leprosy was confirmed by Naafs (Barnetson, 1977b), who observed that Ethiopian patients with "cured" tuberculoid leprosy tended to present with limb pains when they relapsed.

In a graphic description of severe ENL resulting in crippling nerve damage, Jeanselme (1933d) writes :

> "la névrite ne se traduisait que par des douleurs vagues occupant la face et les quatre membres. Leur signification fut d'abord méconnue et je les attribuai à un pseudo-rhumatisme lié à l'infection lépreuse."

He also records (1933e) "pseudo-rheumatism" occurring in a patient with "mixed" leprosy with marked generalised lymphadenopathy associated with a reactional process.

This type of pain in leprosy has received little attention in recent literature, and patients who complain of it tend merely to be dismissed with (or without) aspirin tablets. Nevertheless, it can be an important warning symptom, associated with relapse, "overt neuritis" (pain and/or tenderness of nerves) or "silent neuritis" (loss of nerve function without nerve pain or tenderness). The association of gurtimat with neuritis and reactivation of leprosy in patients with leprosy during pregnancy, the puerperium, and lactation is examined in the present study.

LEPROSY IN ETHIOPIA

Leprosy has been known in Ethiopia for centuries, possibly resulting from spread of the disease from the Nile valley in the second century A.D. (Violato, 1937). Strelcyn refers to the Abba Yohannes manuscript dating from "not later than the 16th century" (1968a) which describes the preparation of specific herbal remedies for leprosy (Strelcyn, 1968b). Leprosy spread from Ethiopia, with the slave trade, to the Caribbean (Drognat-Landré, 1868f).

The first serious description of leprosy in Ethiopia is given by Mérab (1912) who considered the prevalence of leprosy to be 1 - 2 per thousand. Many educated Ethiopians at that time thought the prevalence was nearer 5 - 10 per thousand, on the grounds that most cases remained hidden for shame or fear of ostracism (Mérab, 1912a). There was thought to be 1,000 cases of leprosy in Addis Ababa out of a population of 50,000 (Jadassohn, 1913). Health conditions in the capital deteriorated in the early 20th century with the prevalence of infectious diseases, including leprosy, increasing with the city's rapid population growth (Sandford, 1946).

Leprosy was recognised as a nationwide problem with an estimated prevalence of 150,000 in 1955, the highest prevalence being in the predominantly Christian central highlands (Price, 1969a). The differences of prevalence from one part of the country to another are clearly related to the religion of the predominant tribal group. The Moslems regard leprosy as infectious and thus practised segregation of leprosy patients (Pankhurst, 1961), while in most Christian areas leprosy sufferers enjoyed complete freedom of movement (Pankhurst, 1968a). Leprosy in general was not regarded as contagious, as was syphilis, but rather more as a familial disease; thus the leprosy sufferer was the son, grandson or great-grandson of a leprosy patient (Mérab, 1912b). The Ethiopian Orthodox Church has for many centuries, in the name of Gebre Christos, the patron saint of leprosy sufferers, given philanthropic help to those who lived around or within the churches and monasteries (Price, 1969b). Ras Makonnen encouraged the Capuchin missionaries to found the Harrar Leprosy Hospital in 1901 and subsequently took a keen interest in the work (Pankhurst, 1968b). Emperor Haile Sellassie I gave permission to the Sudan Interior Mission to build the Princess Zenebework Hospital in Addis Ababa in 1930 and

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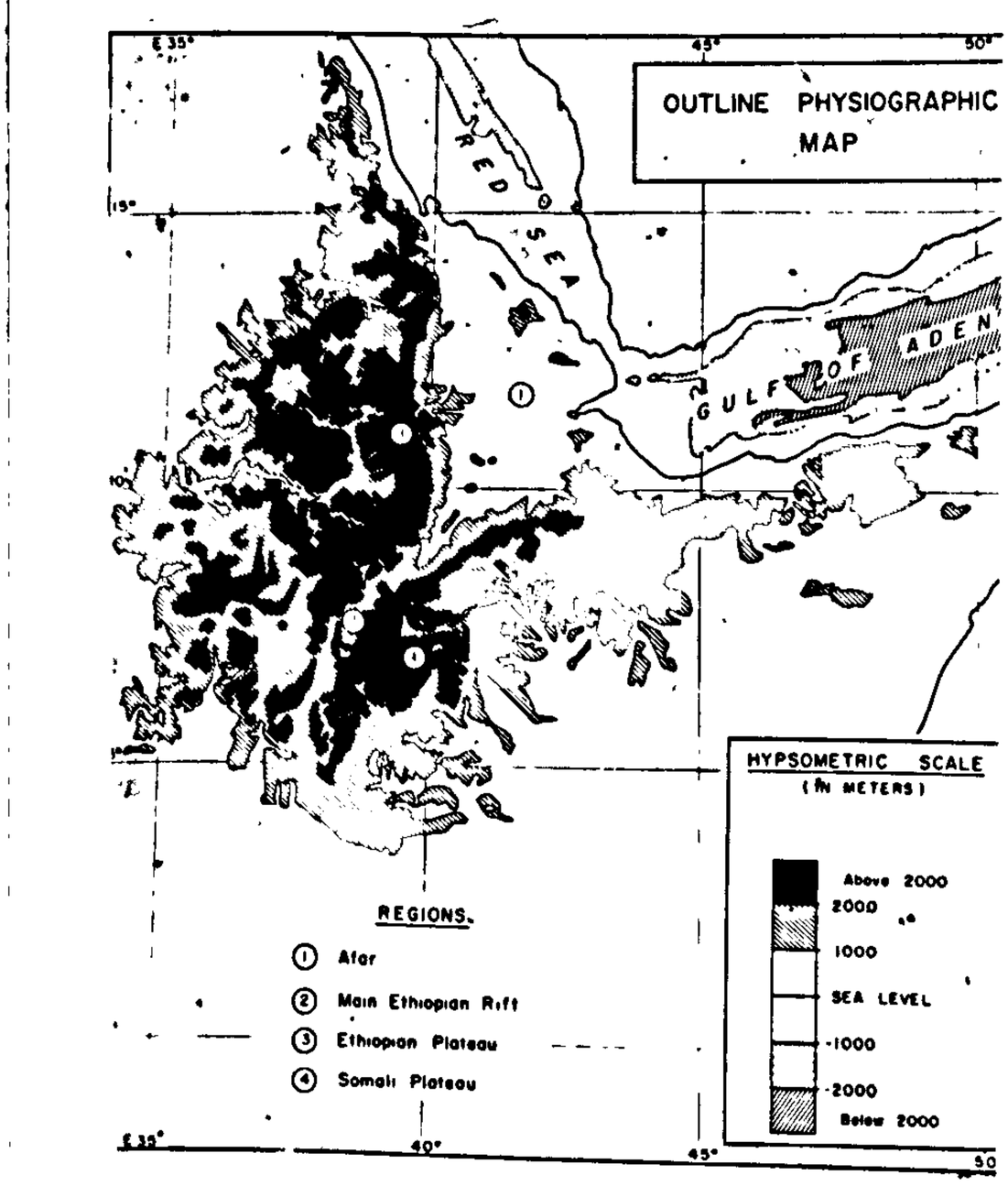


Plate 7. "Leprosy was recognised as a nationwide problem ... with the highest prevalence being in the predominantly Christian central highlands" or Ethiopian plateau (3). For centuries, this part of Ethiopia was cut off from explorers and colonisers by the malarial Rift Valley (2) to the south, the inhospitable Afar (1), Danakil Desert, to the East, and the rapids of the crocodile infested Blue Nile flowing out to the West. The bitter cold and rarified atmosphere of the Ethiopian Plateau guarded by tremendous escarpments deterred potential invaders.

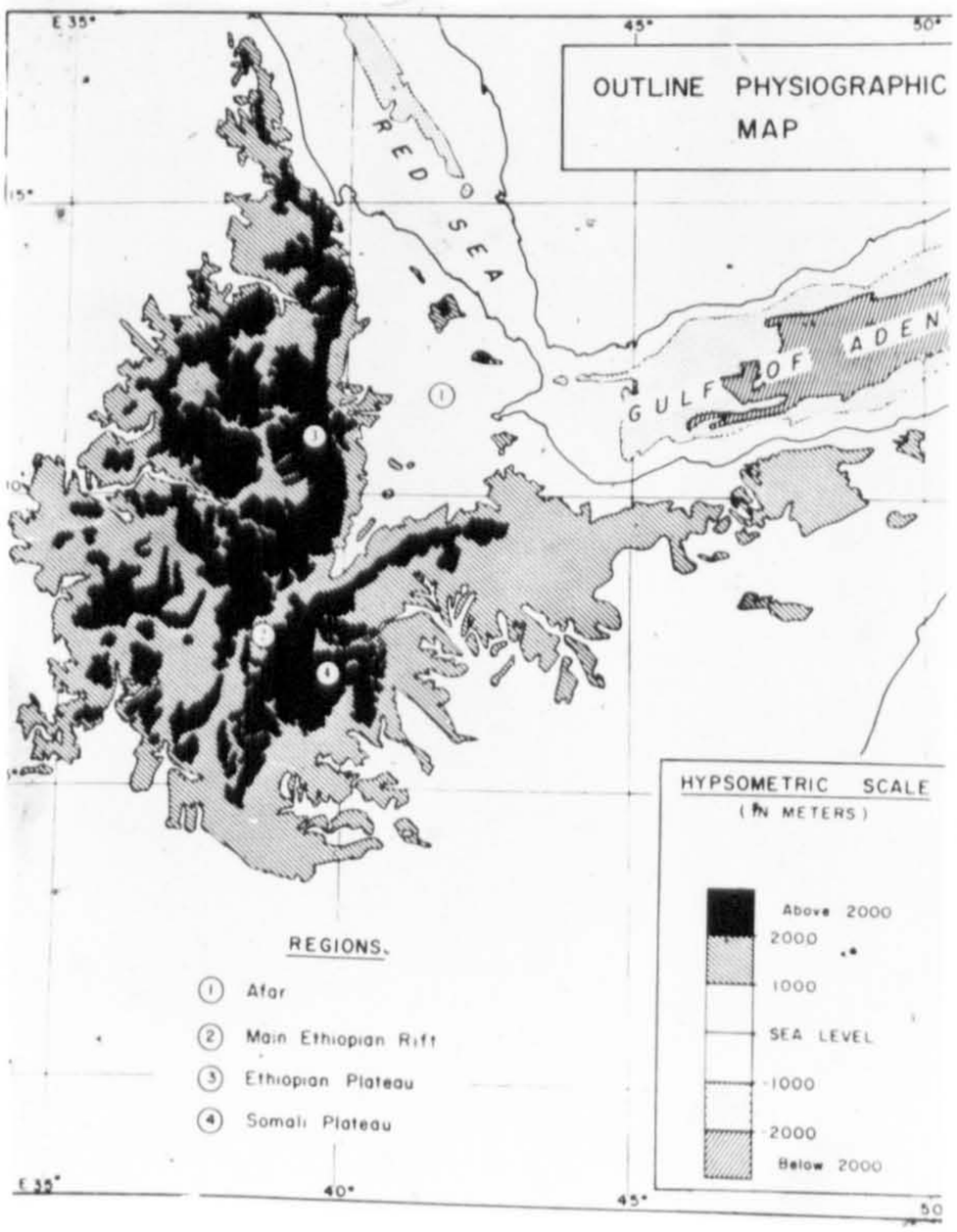


Plate 7. "Leprosy was recognised as a nationwide problem ... with the highest prevalence being in the predominantly Christian central highlands" or Ethiopian plateau (3). For centuries, this part of Ethiopia was cut off from explorers and colonisers by the malarial Rift Valley (2) to the south, the inhospitable Afar (1), Danakil Desert, to the East, and the rapids of the crocodile infested Blue Nile flowing out to the West. The bitter cold and rarified atmosphere of the Ethiopian Plateau guarded by tremendous escarpments deterred potential invaders.

encouraged the influx of various Christian missionary groups to undertake health care, including leprosy, throughout the empire.

Control and treatment of leprosy

While a major role had been played by voluntary agencies in the care of patients with leprosy throughout the country, an important advance was the establishment of the all African Leprosy Rehabilitation and Training Centre (ALERT) at the Princess Zenebework Hospital in 1965. The Armauer Hansen Research Institute (AHRI) was founded in 1970 to undertake immunological research, and clinical research was undertaken by the British Medical Research Council Leprosy Project. The National Leprosy Control Project of the Ministry of Public Health was also established in 1970 in order to co-ordinate the work of all agencies engaged in leprosy control into a coherent programme and to provide a service for those areas of the country which were not already catered for by the existing health units. The National Leprosy Control headquarters were adjacent to ALERT and the Princess Zenebework Hospital, later renamed the Addis Ababa Leprosy Hospital.

With the establishment of the Addis Ababa Leprosy Hospital as a centre for rehabilitation and training, the policy for treatment of patients changed. Institutional treatment ceased, except for those with severe reactions, those suspected of developing drug resistance, those requiring surgery and those with severe intercurrent infections. Many in-patients were repatriated to their own home towns in rural areas. Out-patient clinics were established under the supervision of Leprosy Control in cities, major towns, and in villages in rural areas where clinics were held on market days. Chemotherapeutic drugs were dispensed at weekly, fortnightly or monthly intervals, new patients were assessed, registered patients followed up and complications treated by the health worker or referred for specialist opinion.

In spite of the policies adopted, many patients elected to remain in Addis Ababa, or returned there shortly after being sent home, establishing large villages in the vicinity of the Leprosy Hospital. Of an estimated 160,000 people with leprosy, 80,000 are known to have the disease and are registered for treatment: the remaining 80,000 either have early forms of the infection and do not suspect that they have leprosy, or have no access to medical consultation, or are afraid

of the diagnosis and the ostracism which it may bring and deliberately hide their lesions.

Modus vivendi of Ethiopian leprosy patients

The way of life of leprosy patients living in Addis Ababa was similar to that of other Ethiopians of low socio-economic class. Houses were made of eucalyptus and daub, with thatched or corrugated iron roofs. Many houses were grossly overcrowded supplying shelter not only for the family, but relatives and friends coming to visit the city or the Leprosy Hospital from rural areas. Water was drawn from a standpipe in the village street, or carried from the local river. Except in the Hospital area, sanitation was non-existent. The city refuse tip was one mile away to leeward of the hospital. Flies, mosquitoes and biting insects were in abundance except for body lice, which were far less prevalent than in other areas of the city (Dr. MacConnell, unpublished observation). Wood, for domestic use,was carried from nearby forests.

The patients supported themselves by subsistence farming, working for the Hospital craft centre, making and serving locally made beer (talla) or mead (tej), carrying wood or water, domestic employment, or by begging. Begging is regarded as a profession by the Ethiopians as it is meritorious to give alms. Thus in many cases the beggars were the wealthiest in the community.

At the time of the study reported here, there was no form of state social welfare. The Hospital provided subsidised footwear for registered leprosy patients, as part of the programme for prevention of plantar ulcers. Milk powder for supplementary feeding was provided for babies of mothers requiring admission during the course of the study. Free infant-formula "faffa" (locally produced infant feeding from pulses and grains) was provided by some of the municipal mother and child welfare clinics as an incentive to mothers to take their babies for vaccination/immunisation programmes.

OBSTETRICAL AND PAEDIATRIC PRACTICE IN ETHIOPIA

In Ethiopian culture, women are delivered at home attended by a traditional midwife and women friends/relatives. The placenta must not be seen by anyone after it has been delivered and must be buried immediately, either inside or outside the house, according to tribal custum. The placenta is commonly known as the "ingida-lijd" or the visiting child (Mérab, 1912c). Such an attitude may have contributed to the insistence of some of the mothers in the study to deliver at home, thus ensuring we were unable to obtain placenta for examination. Help from trained medical personnel is sought in cases of prolonged labour, malpresentation, haemorrhage or eclamptic fits. In many cases consent from mother-in-law or mother has to be obtained before moving the patient.

In the early 1970's obstetric facilities in Ethiopia were restricted to Government hospitals and clinics in the cities and larger towns, and to mission hospitals or clinics in rural areas. When conditions were at their best, there were 400 doctors for the whole country, including 9 specialist obstetrician gynaecologists, 7 of whom were in Addis Ababa, and responsible for 15,000 deliveries per year. Specialist paediatric care was supplied by the Ethio-Swedish Paediatric Clinic, a University department which provided in and out-patient care, a neonatal unit, and with the University Department of Obstetrics had established mother and child welfare clinics (3 with delivery centres) throughout the city. The women in Addis Ababa had just began to appreciate and utilise, to the full, the facilities provided when the revolution began in 1974. With the closure of the University Medical Faculty in 1975, most ex-patriot specialist obstetrical and paediatric staff had to leave the country: the Obstetrics Department in particular was severely affected.

The Leprosy Hospital, which provided a 24 hour "general practitioner" type service for medical emergencies, did not have an obstetric service. While the community nurses would deliver women in advanced labour, other parturient women were taken by ambulance to the nearest hospital or delivery centre where there was a bed available. With the limitations of obstetric and paediatric facilities in Addis Ababa in 1975, it will be appreciated that the appointment of an

obstetrician to the Leprosy Hospital was a significant factor in obtaining patient co-operation for this study.

By 1977, the city of Addis Ababa had been divided into "kebele(s)" (housing association(s)) for local administration, provision of food-stuffs and provision of child care clinics. These clinics were run by Ethiopian community nurses who carried out vaccination programmes and also treated minor ailments.

MATERNAL RESPONSE TO PREGNANCY

In certain diseases natural remission occurs with advance of pregnancy followed by deterioration after delivery. Such diseases include rheumatoid arthritis (Hench, 1938 and 1949; Oka, 1953); ulcerative colitis (de Dombal et al, 1965); and sarcoidosis (Siltzbach, 1965). Systemic lupus erythematosus frequently presents for the first time immediately postpartum (Scott, 1977), as does rheumatoid arthritis (Oka, 1953). Transient hypothyroidism and transient hyperthyroidism have been observed following delivery of patients with Hashimoto's and Graves' diseases respectively (Amino et al, 1977a and 1977b). In poliomyelitis (Moloshok, 1965), tuberculosis (Rich, 1951; Turner, 1950) and leprosy (Hardas, Survey and Chakrawarti, 1972; Rose and MacDougall, 1975), the disease has been shown to become overt or to progress rapidly during pregnancy, especially the third trimestre and immediately postpartum; and the high fatality and unusual severity of viral hepatitis in malnourished women during late pregnancy is well known (Borhanmanesh et al, 1973).

Possible explanations for these observations have been :

1) Raised hormone levels during pregnancy

   (a) Increased levels of free cortisol and 17-hydroxycorticosteroid explain the remissions of rheumatoid arthritis and ulcerative colitis (Scott, 1977; de Dombal, 1965). Increase in endogenous corticosterone causing suppression of host resistance may in part explain the exacerbation of tuberculosis and leprosy during pregnancy. In mouse experiments no change was observed in the rate of multiplication of M. leprae, but a higher count of viable bacilli was achieved in footpads of mice fed on a diet containing hydrocortisone (Shepard and McRae, 1965). Similar results have been obtained in experiments using M. tuberculosis (Batten and McCune, 1957).

   (b) Treatment with stilbestrol and thyroid hormones has been considered a factor in the sudden appearance of skin lesions of leprosy (Symmers, 1951; Iswariah, 1944; Jopling, 1979, personal communication).

Conversely, treatment with drugs which have anti-thyroid activity has been successful in murine leprosy (Koh, Katz and Gallego-Correa, 1969).

2) Metabolic disturbances

General malnutrition and protein deficiency are probable aetiological factors in leprosy (Skinsnes , 1976; Irgens, 1980), and have been reported as common in pregnant women in Ethiopia (Gebre-Medhin and Gobezie, 1975). The rapid worsening of leprosy and disappearance of reactions under conditions of near starvation has been recorded (Ryrie, 1947). It is likely that depression of CMI associated with severe malnutrition (Chandra, 1974) is responsible for these phenomena.

3) Alterations in cell mediated immunity (CMI) during pregnancy

It is generally agreed there is some non-specific suppression of CMI during pregnancy probably due to serum factor(s), although lymphocyte factors may also be involved, in particular cell mediated suppressor mechanisms may cause an impaired response to PPD which reverts to normal shortly after delivery (reviewed by Duncan et al, 1981a; 1982).

Attempts to explain this suppression of CMI in terms of endocrine levels have been largely unsuccessful. Oestrogen dependent pregnancy associated 2-glycoprotein (PAG) has considerable immunosuppressive properties but other factors must also be involved (reviewed by Stimson, 1980). In leprosy, in particular, it is very likely that plasma from pregnant patients contains increased suppressive factors, as plasma from mothers with leprosy had a greater inhibitory effect on their babies' lymphocyte transformation than plasma from healthy mothers (Bjune et al, 1978).

Further evidence for depressed immune and phagocyte function during pregnancy is provided by the following observations :

i) Pregnancy serum inhibited the response of normal polymorphonuclear leucocytes (PMNs) to a chemo-attractant (Takeuchi and Persellin, 1980).

ii) Pregnancy serum inhibited phagocytosis of Staphylococcus aureus by normal PMNs (Persellin and Thoi, 1979).

iii) Mixed lymphocyte culture responsiveness was depressed by pregnancy serum and in particular pregnancy zone protein (Björksten et al, 1978).

The possible clinical significance of the laboratory findings of depressed immune and phagocyte functions is the activation of mycobacterial and certain viral diseases (Bjorksten, 1980).

It is of interest that Masson, Delire and Cambiaso (1977) and Levinsky, Stirrat and Redman (1978) have shown the presence of antigen antibody complexes in the serum of pregnant women, with increase toward the end of normal pregnancy. While the role of immune complexes in human pregnancy remains unknown, there is evidence that such complexes can block the cyto-toxic effect of killer lymphocytes, thus contributing to immunological enhancement and aiding in placentation (Fox, 1978a).

THE PLACENTA

1. Immunology of the placenta

The placenta, being a foetal organ, contains paternal-type antigens that are alien to the mother, and is therefore an allograft or transplant, which should be rejected. However, in most cases, there is failure of the maternal immune system to dispose of what has been termed "nature's transplant". Possible explanations for this phenomenon reviewed by Anderson (1971), Fox (1978a) and Faulk and Johnson (1980) include the following :

i) Antigenic immaturity of foetal tissues. While it is now proven beyond doubt that foetal tissues contain and can express histocompatibility antigens, the nature of these is still rather ill-defined (Billington, 1975).

ii) The uterus as an immunologically privileged site. While intrauterine allografts of parathyroid tissue (Poppa et al, 1964) and skin (Beer and Billingham, 1971) were promptly rejected, survival time was considerably prolonged when intrauterine skin grafts were made during the pre-implantation stage of pregnancy. The decidua may protect the allograft from rejection by blocking the afferent limb of the immune response (Beer, 1975).

iii) Absence of histocompatibility antigens from the trophoblast. The results of recent work suggest that placental histo-compatibility antigens are shielded from the maternal tissues by trophoblast which is, in the transplantation sense, antigenetically inert.

iv) Protection of trophoblast by a local mechanical or hormonal barrier. While electron microscopy has failed to show any constant separation of trophoblastic and maternal tissues, human chorionic gonadotrophin (HCG) produced by the trophoblast may mask trophoblastic antigens or inhibit an attack by maternal lymphocytes.

v) Non-specific depression of maternal immune response. See p.57.

vi) Specific reduction of maternal immune response by immunological enhancement. The term 'immunological enhancement' has been applied to the situation in which transplanted cells grow and flourish in a host who is normally capable of reacting to them. By definition, this phenomenon is mediated by blocking antibodies such as have been demonstrated by Faulk et al (1974) and Rocklin et al (1976).

vii) Suppression of maternal immune response by the foetus. It has been shown that foetal cord blood lymphocytes can inhibit the response of maternal lymphocytes to PHA and other mitogens. The significance in vivo of this foetal suppressive activity in vitro requires further evaluation.

2. Infection of the placenta

For practical purposes, the placenta may be regarded as being infected by two routes (Benirschke, 1960), either by an ascending infection from the genital tract, usually though not invariably occurring after rupture of the membranes, or by a hamatogenous spread from a focus in maternal tissues. In the first case chorioamnionitis occurs; in the second, a stromal infection with a localised or generalised villitis is seen.

Defence mechanisms against ascending infection include the physical barrier offered to organisms by intact membranes, the antibacterial action of the amniotic fluid possibly due to its content of zinc (Schlievert, Johnson and Galask, 1976), and the cervical mucus which acts not only as a physical barrier to bacterial ascent but is thought to exert an antibacterial effect (Enhörning, Huldt and Melén, 1970) and, possibly due to content of immunoglobulins, an immunological effect. A clear relationship has been shown between low socio-economic status and a high incidence of chorioamnionitis without prolonged membrane rupture (Naye et al, 1971).

Haematogenous infections

Haematogenous infection is always secondary to an overt or sub-clinical maternal infection by a pathogenic organism. Much of our

knowledge is derived from reports of isolated cases; in many such reports attention has been focussed on the foetus, and the placenta has been studied in only a perfunctory manner if at all (Warthin and Cowie, 1904; Fox, 1978b). Infection of the placenta may occur without the foetus being infected, as in tuberculosis and malaria; in viral infections, while the foetus may be adversely affected, the placenta quite often appears normal. The subject has been well reviewed by Fox (1978b).

While the placenta has frequently been considered as an obstacle to the free passage of organisms from the maternal circulation to the foetus, it is now thought to act only as a partial barrier to foetal infection.

Effects of haematogenous infection

The most important consequence of placental infection is the establishment of an inflammatory lesion in placental tissue which serves as a focus from which the foetus is later infected. Other and more controversial consequences are impairment of trophoblastic function with subsequent foetal growth retardation or death. Heavy malarial infestation of the placenta is often associated with an unduly small, but otherwise healthy baby (Jelliffe, 1967). In this case, the infection does not cause damage to or destruction of placental tissue: massive histiocytic infiltration of the inter-villous space is the cause of any defect of foetal oxygenation or nutrition that may occur.

Tuberculous infection of the placenta

Tuberculosis was one of the first placental infections to be described accurately (Schmorl and Kockel, 1894; Schmorl and Geipel, 1904; Warthin and Cowie, 1904). The lesions range from typical miliary tubercles to large confluent caseous foci. The tubercles may be found within the villi or in the perivillous fibrin. There is often a non-specific chronic deciduitis and granulomata may be seen at the junction of the decidua and the inter-villous space. Acid-fast bacilli have been isolated from the placenta and foetal tissues without there being any histological evidence of inflammatory response. There has, however, been no serious prospective study of tuberculous infection of the

placenta and the foetus utilising newly available immunological techniques, despite the fact that tuberculosis is still a major problem in the developing world.

Placental infection in leprosy

A few small series (le Dentu, 1910; Sugai and Monobe, 1912 and 1913a; Inaba, 1938; Cerruti and Bechelli, 1936; Trespalacious and Piñeyro, 1955; King and Marks, 1958; Davison and Bernard, 1975) and isolated case reports (Ferrari, 1887; Montero, 1927; Jeanselme, 1933b; Maurus, 1978) have been recorded, but there has been only one major study of placental morphology in pregnancy complicated by leprosy (Pineda, 1928) (Table 2). Moreover, some of the literature is only available in abstract form.

Sugai and Monobe (1913b) examined 12 placentae from women with leprosy (5 tuberculoid, 7 lepromatous). They were able to demonstrate numerous M. leprae in 3 out of 5 placentae from tuberculoid mothers and 6 out of 7 placentae from lepromatous mothers, the organisms being present both in the inter-villous space and the villous trophoblast; they remarked, however, on the almost complete lack of any inflammatory response to the mycobacteria as only one out of 12 of the placentae had typical leprous lesions on microscopic examination. Ten out of 12 of the cord blood samples were positive for acid-fast bacilli. The finding of numerous M. leprae in 3 out of 5 placentae from mothers with tuberculoid (pauci bacillary) leprosy is very odd and makes one wonder whether the classification of mothers was correct, and if so, whether the numerous AFB were indeed M. leprae.

Pineda (1928) found no lesions typical of leprosy on microscopic examination, although 57 out of 104 placentae were positive for acid-fast bacilli and 25 out of 104 cords were also positive. Trespalacious and Piñeyro (1955) and King and Marks (1958), studying 27 and 6 placentae respectively, found no histologic or bacteriological evidence of leprosy. Thus it appears, due to the slow multiplication time of M. leprae and the relatively short duration of pregnancy, that few if any leprosy bacilli multiply in the placenta, and there is not time for them to provoke an immunological cellular response. Furthermore, since the advent of antibiotics for leprosy, the number of potential cases in which

TABLE 2 PLACENTAL STUDIES IN LEPROSY

Author	Date	No. of Examinations				Type of examination and Results			
		Placenta	Umbilical Cord	Full term Infants	Pre-term foetus (age in months)	Macroscopic	Microscopic	Bacteriological	REMARKS
Ferrari	1887	1	-	-		Normal	No lesion typical of leprosy	No bacilli	Baby developed overt leprosy age 9/12
le Dentu	1910	7	-	-	-		Normal	No bacilli	
Jeanselme	1910 1933b	3	3	-	-		Normal	No bacilli	2 tuberculoid, 1 lepromatous
Sugai & Monobe	1912 1913	12	12 (cord blood)	12			1/12 typical leprous lesions	9/12 placentae positive 10/12 baby blood positive	3/5 tuberculoid, 6/7 lepromatous mothers
Montero	1927	1	1 (cord blood)	-	-			Both placenta and cord blood positive	
Pineda	1928	104	104	(in discussion refers to +ve M.leprae in SB's)	-		No lesions typical of leprosy	57/104 placentae positive 25/104 cords positive	
Cerruti & Bechelli	1936	5	7	3	1 (4/12)	Normal	No lesions typical of leprosy	1/5 placentae positive 1/7 cords positive	2 separate cases - both mothers lepromatous in reaction
Inaba	1938	13	-	-	-			5/13 placentae positive	
Trespalacios & Pineyro	1955	27	27	-	-		No lesions typical of leprosy	All negative	23 lepromatous, 4 tuberculoid mothers
King & Marks	1958	6	-	-	-		No lesions typical of leprosy	All negative	
Davison & Bernard (cited by Valla, 1976)	1975 *	50	-	-	1 (5/12)		No lesions typical of leprosy	3 placentae had AFB in macrophages	2 babies and and 1(5/12) foetus with +ve placentae, were all normal
Maurus	1978	1	-	-	-		Normal	No bacilli	

* Reference incorrectly indexed, unobtainable.

a massive bacteraemia could occur during pregnancy with subsequent infection of the placenta is considerably reduced. The most likely patients to have a significant bacteraemia during pregnancy must be those relapsing with dapsone resistant infection and those patients who are unable to obtain their medication for several months at a time.

3. Assessment of placental function

Assessment of the foetal growth *in utero* and of placental function, for convenience referred to as foeto-placental function, may be made :

i) on clinical grounds by accurate and frequent measurement of the height of the uterine fundus, preferably by the same observer and after the patient has emptied her bladder;

ii) by ultrasound measuring initially the crown/rump length and then the biparietal diameter of the foetal skull. This measurement should be carried out at regular intervals to assess the rate of growth of the foetus;

iii) biochemical assays, in particular the assessment of oestrogen excretion in the urine.

Oestriol assay in the assessment of foeto-placental function

The quantity of oestrogen excreted daily in the urine in late pregnancy is widely accepted as an accurate index of foeto-placental function and provides a valuable indicator for the prediction of impending intrauterine death from placental insufficiency (reviewed by Wilde and Oakey, 1975). Urinary oestrogen, predominantly oestriol, is critically dependent on production of precursors by the foetal adrenal glands and on the ability of the placenta to convert these precursors (androgen sulphates) to oestrogen through a complex series of reactions (reviewed by Oakey, 1970a). Corticosteroid therapy in excess of 75 mg cortisol daily has been shown to suppress oestriol production without prejudicing the outcome of the pregnancy, while subnormal oestriols in patients receiving less than 75 mg cortisol daily is indicative of the foetus failing to thrive *in utero* (Oakey, 1970b).

Maternal complications such as hypertension and pre-eclampsia are often associated with low oestrogen excretion, particularly when they disturb placental function sufficiently to lead to a small, growth-retarded foetus. Other maternal conditions, not induced by pregnancy, appear to have little influence on oestrogen excretion, although anaemia including thalassaemia, is associated with a high incidence of subnormal oestrogen excretion (Beischer et al, 1968). Poor maternal nutrition and high altitude have also been associated with low oestrogen excretion, possibly due to foetal undernutrition in these patients who lived in poor social and economic conditions (Iyengar, 1968; Sobrevilla et al, 1968).

It has generally been considered that a small foetus has a small placenta, but that the small placenta is rarely a cause of poor oestriol production (Thomson, Billewicz and Hytten, 1969). However, Coyle and Brown (1963) showed a correlation between the level of urinary oestriol and babies weighing more than 8 lbs or less than $6\frac{1}{2}$ lbs at birth. Elder (1971) showed a correlation between the oestriol levels at term and foetal and crude placental weight, and Jenkins, Farquhar and Oakey (1971),in a study of prolonged pregnancy, showed a correlation between levels of urinary oestriol and infant weight, crude placental weight and placental coefficient. Severe hypoplasia of the foetal adrenals, as seen in anencephaly, has been associated with very low oestriol excretion, thus establishing the role of the foetal adrenals in oestriol synthesis (Frandsen and Stakemann, 1961). Foetal hypoxia in utero also affects oestriol production as shown in the levels of oestriol before and after intrauterine transfusion in cases of severe Rhesus isoimmunisation (Michie and Robertson, 1971).

In this study urinary oestriol was used as a measure of foeto-placental function. The results were only known after the birth of the baby and hence in no way affected management.

IMMUNOGENESIS IN THE HUMAN FOETUS

This subject is reviewed by Solomon (1971), Porter and Knight (1972), Stiehm and Fulginiti (1973), Jones (1976) and Cooper and Dayton (1977). The ancestral undifferentiated cells giving rise to the immunological and haemopoietic systems in mammals originate in the yolk sac wall. Small lymphocytes appear in peripheral blood at about 7 weeks' gestation, developing further in the thymus to emerge as T-cells by 12 weeks, with well developed responsiveness to PHA by 14 weeks.

Precursors of B-cells are present in the foetal liver and adult marrow, but little is known about the factors influencing the differentiation of these cells. Later stages in the maturation of B-cells, including the transition to plasma cells, may be under the influence of other organs such as the spleen, lymphnodes and Peyer's patches. Identification of B-cells by the presence of immunoglobulins on their cell surfaces occurs only in the terminal (plasma cell) stages of B-cell maturation.

Humoral immunity in the foetus and neonate

The human foetus acquires passive immunity by the selective placental transfer of maternal IgG (Gitlin *et al*, 1964) which begins as early as 12 weeks (Gitlin and Biasucci, 1969), levels increasing with gestational age. The greater part of this increase is due to maternal IgG which can be differentiated from the small amount of endogenous IgG of foetal origin (Mårtensson and Fudenberg, 1965). After birth the IgG level decreases as a result of normal catabolism of passively transferred maternal IgG and a lag in the infant's own IgG synthesis (Figure 2).

The foetus is able to synthesise immunoglobulins by the beginning of the second trimestre. IgA and IgM do not cross the placenta but are synthesised by the foetus in small amounts. Levels of IgA above 11 mg/100 ml and IgM above 20 mg/100 ml are considered abnormal and presumptive evidence for infection *in utero* (Stiehm, Amman and Cherry, 1966).

Specific antibody production

A specific antibody response may be provoked in the foetus following maternal exposure to a variety of antigenic stimuli, even when these

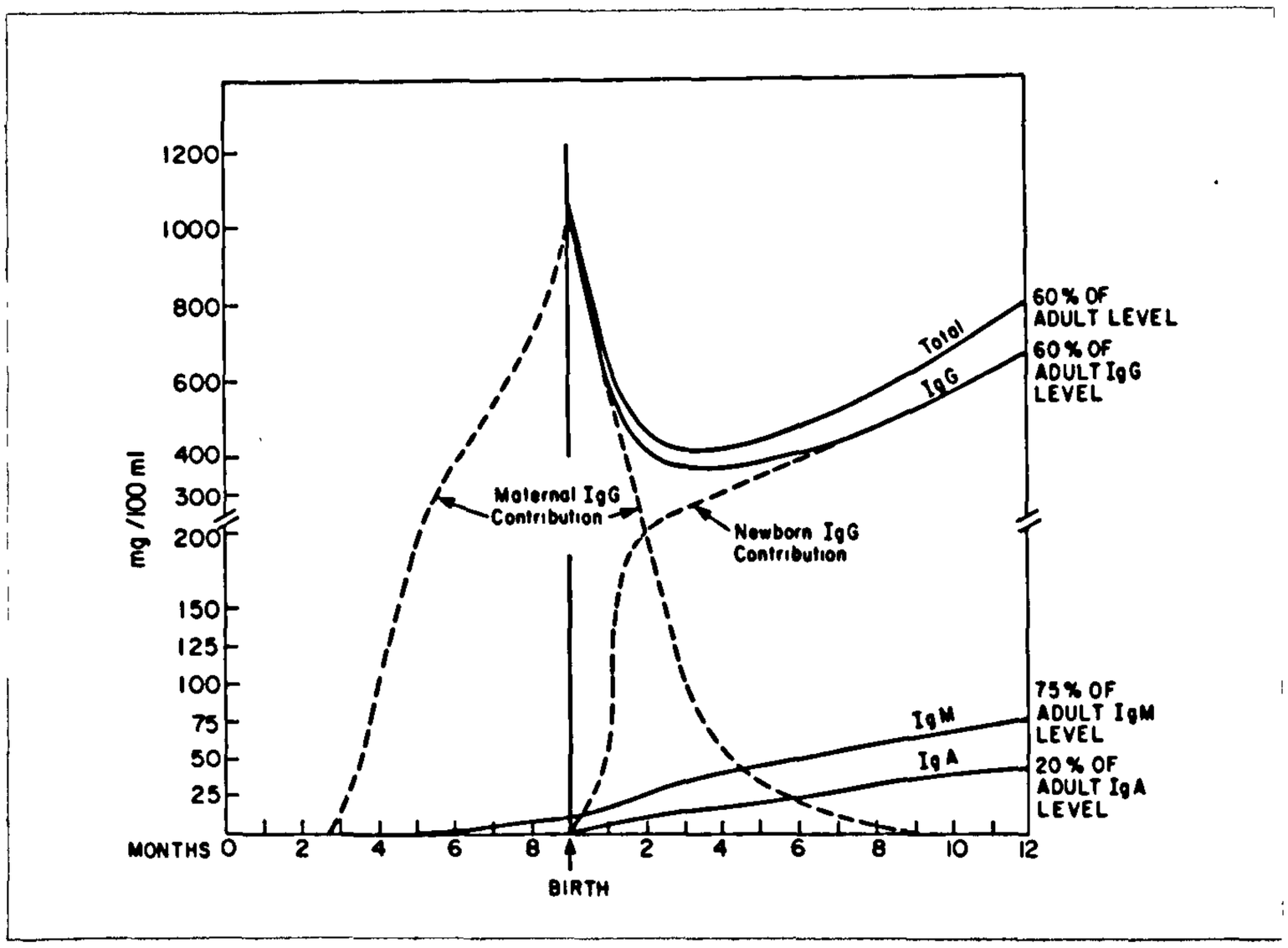


Figure 2. Immunoglobulin (IgG, IgM and IgA) levels in the foetus and infant in the first year of life. The IgG of the foetus and neonate is almost entirely of maternal origin. The maternal IgG disappears by the age of 9 months by which time endogenous synthesis of IgG by the infant is well established. The IgM and IgA of the neonate are entirely endogenously synthesised since maternal IgM and IgA do not cross the placenta. (From Stiehm and Fulginiti, 1973; By Courtesy, W.B. Saunders Co. Ltd.)

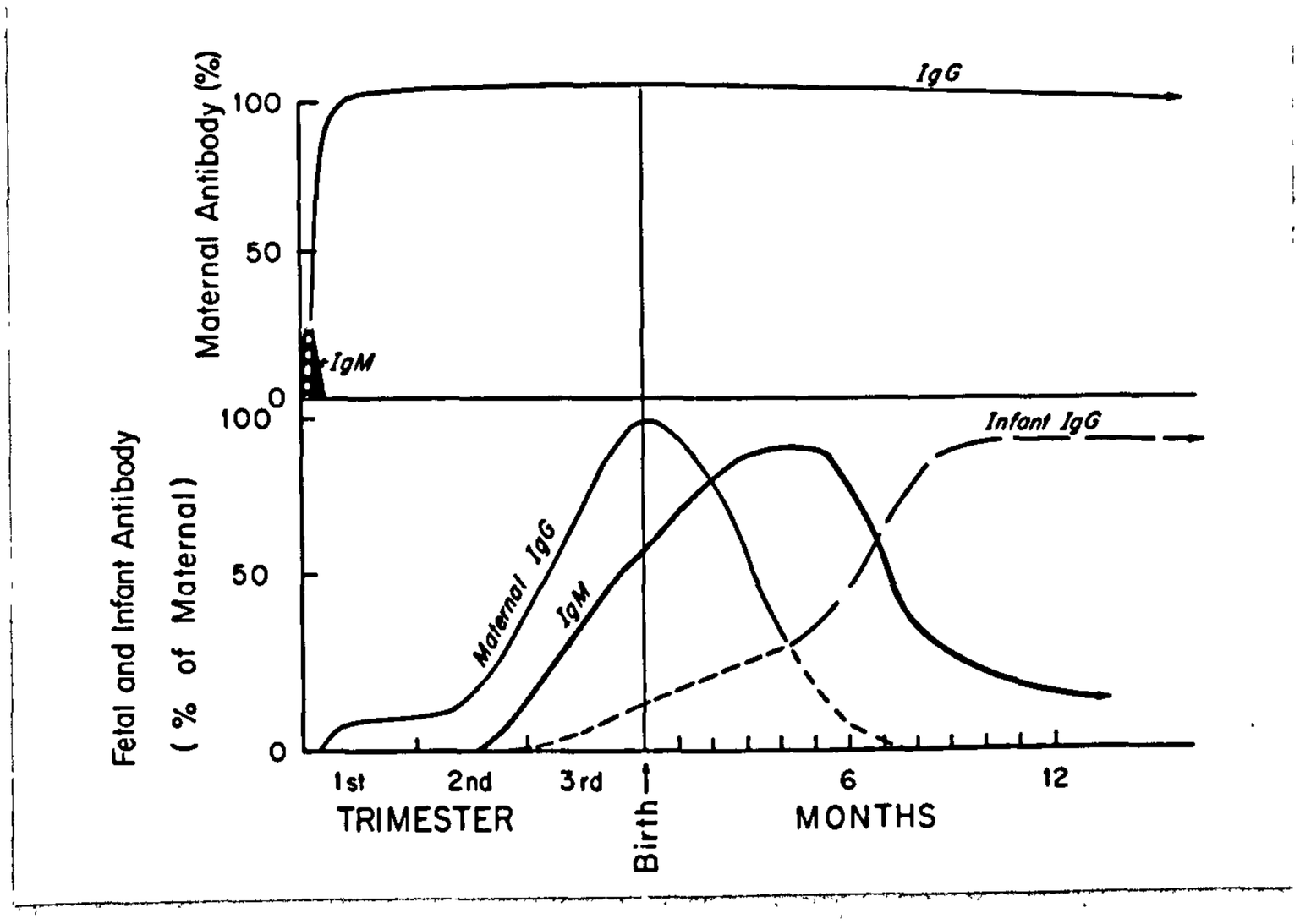


Figure 3. Maternal, foetal and infant antibodies of IgG class expressed as a percentage of maternal IgG antibody levels. (From Stiehm and Fulginiti, 1973; By Courtesy, W.B. Saunders Co. Ltd.)

occur at an early stage of gestation. Such stimuli include maternal coliform and viral infections, and evidence of active humoral immunity in an otherwise normal neonate in these circumstances is diagnostic of intrauterine infection (Cramer, Kunz and Gill, 1974; Jones, 1976).

Prior to the commencement of the present study of the association of leprosy and pregnancy, serological detection of specific antibodies to M. leprae was not available. Furthermore, there had been no serological study of babies born to mothers with leprosy to investigate the possibility of intrauterine infection having occurred.

The complement system

None of the complement proteins cross the placenta. The initial synthesis of complement components occurs at approximately 4 weeks of gestation. At birth, although the human neonate has in its serum only one-half to two-thirds of the normal concentration of complement and properdin components, for most individuals this is sufficient to deal with the multiplicity of infectious agents to which the newborn is exposed.

Immune complexes

It is generally considered that immune complexes of maternal origin do not pass the placental barrier. While the essential components are present in the foetus in several cases of foetal infection in utero, little is known of immune complex formation in the newborn except in the case of infants severely infected by cytomegalovirus. No study of immune complexes due to leprosy in the neonate has been carried out.

TRANSFER OF IMMUNOGLOBULINS/ANTIBODIES TO THE FOETUS/NEONATE

Transplacental transfer of antibodies is reviewed by Solomon (1971), Beer and Billingham (1976a) and Jones (1976), and transfer of specific antibodies through the colostrum and milk is reviewed by Beer and Billingham (1976b), McClelland (1977), Hanson et al (1979) and Lamm et al (1979). During the last month of pregnancy there is an active transfer of maternal antibodies so that the newborn baby has a higher level of IgG than the mother. The quality and quantity of antibody transmitted from mother to newborn will depend upon the antigenic stimulation which the mother has received from pathogens in her environment during the later stages of pregnancy. The neonate thus has acquired passively IgG antibody specific for the micro-organisms in the mother's environment. The duration of persistent, effective maternal antibody is, however, determined by the peak level of maternal antibody. In humans, the half life of IgG is about 20 days.

Specific IgA antibodies for the micro-organisms in the mother's environment are also transferred to the neonate in colostrum and early milk, and may affect the success rate of immunisation in the early days of extra-uterine life. The biological role of secretory IgA includes various antibacterial activities. The secretory IgA in milk is resistant to digestion by intestinal proteolytic enzymes and can be detected, apparently intact, in the faeces.

The IgA concentration in colostrum falls rapidly from extremely high initial levels (almost 60 times the mean normal serum level) on the first day of the puerperium to approximately the normal serum level in mature milk by 10 days postpartum. IgA is synthesised in the human breast probably in response to "homing" lymphocytes and under hormonal control. In contrast to the high levels of IgA, early samples of colostrum contain IgM in concentrations similar to those in serum, but the levels fall rapidly to 10% of the serum values, and in later samples IgM is undetectable. IgG mean concentrations are never greater than 3% of the normal serum level in early milks, and in later samples are undetectable (McClelland, McGrath and Samson, 1978).

MATERNO-FOETAL/NEONATAL CELLULAR TRANSFER

Transfer of cells across the placenta

The two-way transfer of cells across the placenta has been reviewed by Solomon (1971), Russell (1975), Mendenhall (1976) and Beer and Billingham (1976c). While there is evidence that two-way cellular transfer may occur during pregnancy, in most cases transplacental haemorrhage occurs only during labour at the time of delivery, and the volume is usually less than 5 ml. While evidence for maternally induced tolerance and maternal induction of runt disease has followed animal experiments, runt disease has been seen in human infants in the early days of intrauterine transfusion of packed red cells in the treatment of foetuses severely affected by Rhesus sensitisation, when no attempt was made to render the blood free of leucocytes before transfusion. It is not known, however, whether maternal lymphocytes can survive in normal infants.

Of perhaps more significance than the transfer of lymphocytes from mother to child is the transfer of maternal lymphocyte sensitisation (reviewed by Barnetson, Bjune and Duncan, 1976). To what extent the transfer of lymphocyte sensitisation is a protective mechanism for the foetus is as yet unknown and requires further investigation.

Cellular transmission in colostrum and breast milk

The possibility of a cell mediated immune function of human colostrum and milk has been largely overlooked, although breast secretions contain a variety of cells including those of the lymphocyte series. Various aspects of cellular immunity of milk are reviewed by Parmely and Williams (1979), Ogra and Ogra (1979) and Head and Beer (1979). Adverse effects of cellular transfer include various degrees of runt disease. The fact that transfer factor may be transmitted in breast milk and that transient sensitisation to PPD has been recorded, has considerable implications for the baby of the mother with leprosy, and clearly this subject requires further investigation.

IMMUNOLOGY OF LACTATION

Humoral defence factors in breast milk

In many developing countries breast feeding has a dual purpose. Human breast milk supplies the safest form of feeding for the infant, with reduced morbidity and mortality from infections (Goldman and Smith, 1973; Gerrard, 1974) and provides a culturally acceptable form of contraceptive. Protection against infection is mediated by several components in the milk, the most important being secretory IgA (Hanson and Winberg, 1972; Goldman and Smith, 1973; McClelland *et al*, 1978). Antibodies to many micro-organisms, bacteria and viruses have been demonstrated (Goldman and Smith, 1973). Specific IgA antibodies have been reported by Hanson and Brandzaeg (1973), Shearman, Parkin and McClelland (1972), Allardyce *et al* (1974) and Carlsson *et al* (1976b). Secretory IgA levels are not apparently affected by maternal malnutrition (Carlsson *et al*, 1976a).

The biological importance of lactoferrin is recognised in that the potentially important property is its bacteriostatic effect, which may be present in the neonate despite the presence of proteolytic enzymes (Samson, Mirtle and McClelland, 1980).

No overall differences were found between milk samples from privileged and underprivileged Ethiopian or Swedish mothers with regard to total daily nitrogen and amino acid content (Svanberg *et al*, 1977). No significant differences were found between the two Ethiopian groups in the milk volumes or total nitrogen, non protein nitrogen, lactose and individual milk proteins (lactoferrin, lactalbumin, serum albumin, IgG and IgM): both Ethiopian groups showed a significantly higher value for lactoferrin than did the Swedish mothers (Lönnerdal *et al*, 1976).

The diet of Ethiopian highland women of low socio-economic class was poor in first-class protein (meat was eaten by these women only 4 times a year on the chief feast days of the Church calendar), but rich in second-class protein. It consisted of *ingera*, a pancake made of fermented dough, and *wot*, a highly spiced stew of pulses and vegetables. *Tef*, an iron-rich grain, was the cereal most commonly used for making *ingera*, although as *tef* became more difficult to obtain (mid-1977 onwards), maize or wheat was used as a substitute

From studies in India and Pakistan, it has been shown that there is a flattening out of the baby's growth curve at 3-4 months of age, paralleled by a reduction in the mother's milk volume output per day (Gopalan, 1958; Mehta and Kala, 1971). Svanberg et al (1977) suggested that the total volume of milk ingested by the infant required further study, and also that factors other than nutritional could be held accountable for the growth failure in breast-fed infants in developing countries.

Ethiopian children of mothers with leprosy have been reported as growing more slowly than babies of healthy mothers and being unusually susceptible to infections which are not normally seen in breast-fed infants. These observations were most marked in the babies of mothers with lepromatous leprosy (Duncan, 1980). The present study includes investigation of the humoral defence factors and protein levels in milk of Ethiopian mothers with leprosy and of healthy controls, and compares the results with those obtained from healthy Scottish women.

HEPATITIS B INFECTION IN ETHIOPIAN MOTHERS WITH LEPROSY AND THEIR BABIES

Carriage rates for Hepatitis B surface antigen (HB_sAg) vary from 5-10% in Africa, but there has been little published information available for Ethiopia, although infectious hepatitis is an important cause of both morbidity and mortality. A high prevalence of HB_sAg presents a potential hazard to laboratory workers because of the risk of spread by contaminated serum. Few workers in countries where leprosy is endemic have facilities immediately available for the newly introduced immunological techniques, hence, increasingly, sera from leprosy patients is having to be sent to laboratories in the "developed world" where the risk of acquiring Hepatitis B from imported sera is not acceptable. For the safety of laboratory workers, all imported sera require to be tested for HB_sAg before they can be processed in the United Kingdom. Furthermore, as HB_sAg may be transmitted through breast milk and across the placenta, specimens of milk and placenta from HB_sAg sero-positive women must be regarded as potentially infectious.

Hepatitis B infection has been of great interest to leprologists who have used the carriage rate of HB_sAg as an indirect indicator of impaired immune responsiveness in patients with leprosy. It has been claimed that the impaired immune responsiveness of patients with lepromatous leprosy predisposes them to Hepatitis B infection and to become carriers (Serjeantson and Woodfield, 1978; Nuti et al, 1977).

The testing of serum from the mothers and babies of this study for HB_sAg and anti-HBs would not only comply with laboratory regulations, but would establish the prevalence of Hepatitis B infection in Ethiopian women across the spectrum of leprosy, and the risk of transmission of Hepatitis B from mother to child.

PATIENTS, MATERIALS AND METHODS

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M O T H E R S

Selection of mothers

Initially the patients studied were those with active tuberculoid leprosy, active lepromatous leprosy with positive skin smears, and healthy controls; later the study group was broadened to include women with 'cured' tuberculoid leprosy who had stopped treatment and women with chronic, quiescent lepromatous leprosy with negative skin smears. Selection of the patients within the above general classification was based on their willingness to participate in the study, to deliver their babies in hospital rather than at home, and to be seen with their babies for regular assessment, including blood tests, for a period of up to 2 years during lactation.

One hundred and forty-seven Ethiopian women were studied during 156 pregnancies. There were 114 women with leprosy (120 pregnancies) and 32 women without leprosy (healthy controls: HC, with 36 pregnancies). The women who were all from the low socio-economic class lived, for the most part, in the villages surrounding the Addis Ababa Leprosy Hospital. They were first seen, for this study, when they presented themselves at the Hospital ante-natal clinic which supplied ante-natal care for registered leprosy patients, wives of leprosy patients and members of staff.

Classification and treatment of mothers

The 114 women with leprosy were classified initially as follows using the scale of Ridley and Jopling (1966) :

Cured tuberculoid and borderline tuberculoid leprosy (released from control)	(TT and BT/RFC)	... 25 (25 pregnancies)
Active tuberculoid and borderline tuberculoid leprosy	(TT and BT)	... 17 (18 pregnancies)
Borderline lepromatous leprosy	(BL)	... 40 (41 pregnancies)
Lepromatous leprosy	(LL)	... 32 (36 pregnancies)

For simplicity in classification of women according to treatment regime, each pregnancy is considered as one patient. Thus of 120 patients, 87 were receiving dapsone monotherapy (50–100 mg daily) but 26 patients (1 BL, the rest BT or TT) were believed cured, had stopped treatment and had been "released from control (RFC, see below). Six patients (2 BL, 4 LL) had already developed dapsone resistant leprosy, as defined by Pearson et al (1979) and were receiving clofazimine (4 patients, all LL, 5 pregnancies) or rifampicin plus thiambutosine and dapsone (2 patients both BL).

Treatment and supervision of these patients was carried out through the hospital outpatient clinics. Eighty-two patients receiving dapsone monotherapy (18 TT and BT; 36 BL; 28 LL) were supplied with dapsone tablets on a weekly or fortnightly basis by paramedical leprosy workers at hospital or municipality clinics, were referred to hospital clinics for treatment of reactions or other complications of leprosy, and were assessed by a doctor at the hospital "Review Clinic" every 6 months when routine slit skin smears were examined. Five patients (2 BL, 3 LL) receiving dapsone monotherapy 100 mg daily in a chocolate coated tablet for suspected dapsone resistance (Pearson et al, 1979), and 7 patients (2 BL, 5 LL) with proven dapsone resistance were seen every 6 months at a special clinic for the treatment of drug resistant leprosy; routine slit skin smears were done every 6 months and biopsies were taken annually.

Patients released from control (RFC)

At the start of the study, the practice in the hospital for stopping treatment of leprosy was as follows : TT patients were RFC after 2–3 years of treatment with dapsone 50–100 mg daily; BT patients were RFC after 4 or more years of treatment with dapsone 50 mg daily (300 mg weekly) when there had been no clinical evidence of active leprosy for at least 2 years; BL patients were RFC when they had received treatment for 15–20 years and had been skin-smear (BI) negative with no clinical evidence of active leprosy for 10 years; LL patients continued on treatment for life and hence were not RFC.

The 25 patients classified as TT and BT/RFC were originally classified as TT or BT at the hospital new case clinic. Diagnosis had been made on clinical grounds supported by negative BI but without

histological confirmation. Seventeen patients had been diagnosed at the Addis Ababa Leprosy Hospital and 8 at rural clinics or hospitals where they received their initial treatment before being transferred to Addis Ababa. One patient seen first in Addis Ababa and one patient coming from a rural clinic had had doubts raised regarding classification of leprosy and had been recorded as being 'LI' and 'BB' respectively on one occasion; on subsequent assessment by a senior hospital leprologist both were recorded as 'BT' on clinical grounds. The duration of stopping treatment ranged from 3 months to 10 years (mean 2.6 years).

Entry of women to the study: Timing and assessment

At the time of entry to the study (Table 3) in addition to full obstetrical assessment, a general physical examination was made and the patient's leprosy status assessed clinically; skin smears were taken from leprosy patients and a biopsy for histological classification, if it had not already been taken. Subsequent detailed leprosy assessments were made as indicated by the patient's symptoms and clinical state.

The first group of women admitted to the study were taken in during the third trimestre, several of them at or after 36 weeks' gestation. After the first 3 months of the study it was apparent that leprosy deteriorated during pregnancy, thereafter whenever possible, patients were admitted to the study during the first or second trimestre with detailed leprosy assessment at the time of entry and during each following trimestre and at 6 month intervals during lactation, more frequently if indicated.

TABLE 3

TIME OF ENTRY TO STUDY

Histological Classification of Leprosy	Number of Women Entering the Study			Total Pregnancies
	1st Trimestre	2nd Trimestre	3rd Trimestre	
HC	2	11	23 (7)*	36
TT and BT/RFC	-	13	12 (2)	25
TT and BT/Active	1	6	11 (3)	18
BL	4	19	18 (4)	41
LL	4	14	18 (4)	36

* Number ( ) indicates the number of women entering the study at or after 36 weeks' gestation.

HC = Healthy Contacts
RFC = Released from Control.

ROUTINE ASSESSMENT OF STUDY PATIENTS

I. LEPROSY ASSESSMENT

Women in this study were seen for routine ante-natal care at monthly intervals until 28 weeks' gestation, every 2 weeks until 34 weeks and weekly thereafter. In addition to receiving the routine ante-natal care, their leprosy status was assessed clinically, complications were recorded, and additional investigations arranged as indicated. They were admitted to hospital for 24-hour collections of urine for oestriol analysis and also for medical, obstetrical or social reasons as necessary. LL patients in particular were often admitted to hospital for several weeks prior to delivery to prevent foetal wastage by precipitate delivery at home. As in-patients they received routine ante-natal surveillance but stopped attending out-patient ante-natal clinics (ANC). This factor accounts largely for the reduced attendance at ANC by LL patients (Table 4).

At detailed leprosy assessment the patient's complaints, state of health and drug treatment were recorded. The patient was then examined in a well-lit room, with inspection and palpation of the skin, peripheral nerves and regional lymph nodes. Clinical drawings were made of the skin lesions, slit-skin smears were taken from 6 sites (both ears and 4 smears from active lesions, or from both ears, elbows and knees when no active lesions were seen; smears were taken from the same sites on subsequent occasions unless new lesions had appeared (in which case smears were taken from them) for BI and MI.

Biopsies were taken for diagnosis and classification from active lesions or, when the disease was quiescent, from the buttocks. The biopsies were divided in two and read by two independent leprologists. Patients who were deemed healthy contacts were assessed in the same way as leprosy patients with the exception of the skin biopsy, which was only done if there was a suspicious lesion or nerve enlargement. Clinical classification was undertaken by two independent observers. When a patient was suspected of having developed dapsone-resistant leprosy, a biopsy of an active nodule with a positive morphological index was taken and, when possible, tested in mouse foot pads for dapsone resistance (Rees, 1967).

TABLE 4.

FREQUENCY AND TIMING OF ASSESSMENT DURING PREGNANCY

Classification of leprosy	No. of Women	No. of Pregnancies	No. of † Attendances at ANC Mean ± SEM	FREQUENCY OF LEPROSY ASSESSMENTS ‡ X1	X2	X3	X4	X5	X>5	FREQUENCY OF LABORATORY INVESTIGATIONS # X1	X2	X3	X4	X5	X>5	Puerperal*	Lactation**	Not Done	No. of Admissions for Obstetrical/ Medical/Social Reasons	No. of Admissions for "Control" Urine Collections
HC	33	36	5.9 ± 0.5	32 (31)	4 (5)	-	-	-	-	14 (14)	-	-	-	-	-	1 (1)	8 (8)	13 (13)	4	22
TT and BT/RFC	25	25	6.2 ± 0.5	19 (17)	5 (6)	1 (1)	-	-	- (1)	11 (11)	5 (4)	-	-	-	- (1)	3 (3)	6 (6)	-	3	18
TT and BT/Active	17	18	5.1 ± 0.6	15 (3)	3 (5)	- (8)	-	-	- (2)	13 (12)	3 (3)	-	-	- (2)	-	-	2 (1)	-	4	11
BL	40	41	5.9 ± 0.5	32 (7)	9 (16)	- (13)	- (3)	- (1)	- (1)	19 (17)	15 (14)	2 (3)	- (2)	-	-	3 (3)	-	2 (2)	4	29
LL	32	36	4.5 ± 0.6	17 (4)	19 (17)	- (9)	- (5)	- (1)	-	16 (13)	14 (13)	3 (3)	- (1)	- (1)	- (2)	1 (1)	1 (1)	-	9	30

† Assessment by Doctor (M.E.D.)

‡ First number = the number of patient-assessments during pregnancy for this special study only; number ( ) = the number of patient-assessments for the special study together with the routine "full clinical assessments" at hospital review clinics and clinics monitoring suspected dapsone resistance.

HC = Healthy Contacts (without leprosy); TT and BT/RFC = Tuberculoid and borderline tuberculoid leprosy "Released from control", i.e. "cured"; TT and BT/Active = Tuberculoid and borderline tuberculoid leprosy, active; BL = Borderline lepromatous leprosy; LL = Lepromatous Leprosy

ANC = Ante-natal clinic.

First number = frequency of laboratory investigations (skin smear for BI & MI/Biopsy for histology/Biopsy for mouse foot pad tests), singly or in combination; number ( ) = frequency of laboratory investigations and additional tests (VMT with ST/DNC/skin test with A6), singly or in combination.

BI = Bacteriological Index. MI = Morphological Index.

* When it was not possible to perform skin smears for BI or Biopsy during pregnancy some were done immediately after delivery, during the puerperium.

** When skin smears for BI or Biopsy had not been done before, some were done for the first time in the study during lactation at follow-up assessments.

Voluntary muscle power tests (VMT) were performed by standard methods, and the muscle power graded on a 0-5 scale (Goodwin, 1968). Sensory skin tests (SST) were performed on palms of hands (using a stiff nylon bristle) and soles of feet (using a ball point pen tip); sufficient pressure was applied to indent the skin slightly. Standard sites (at least 5 for the area of distribution of each nerve) were stimulated, and the result of each recorded as "felt" or "not felt".

Motor nerve conduction velocity (NCV) was measured in a few patients to determine whether nerve damage was long standing or of recent onset.

Skin testing using a standardised purified protein of M. leprae grown in armadillos (A6) was done during lactation instead of lepromin testing.

The patient's hospital records were reviewed periodically and additional data regarding the initial diagnosis and treatment of leprosy, routine leprosy assessments, complications of leprosy and special investigations not obtained at the study assessments, was abstracted and used in the final analysis of results. The frequency of assessment and of laboratory tests and other investigations carried out during pregnancy and lactation is shown in Tables 4 and 5. The total number of special investigations is not shown as tests carried out at the same time, regardless of number, are recorded as one time of testing.

Diagnosis of deterioration of leprosy status

i) New 'overt' cases of leprosy were diagnosed clinically and confirmed by skin smears and biopsy.

ii) Relapse in RFC patients was diagnosed clinically, by the appearance of new lesions and/or new nerve damage or by positive BI or biopsy showing active leprosy.

iii) Deterioration in leprosy status of patients receiving treatment was defined as the occurrence of one or more of the following : conversion from negative to positive or rise in the patients BI or MI, appearance of new lesions, extension of existing lesions, erythema and oedema of margins of tuberculoid lesions (with no histological

TABLE 5

FREQUENCY OF LEPROSY ASSESSMENTS AND INVESTIGATIONS DURING LACTATION

Classification of leprosy	No. of Women	FREQUENCY OF LEPROSY ASSESSMENTS † X1	X2	X3	X4	X5	X>5	Seen with baby, asymptomatic, no leprosy assessment	FREQUENCY OF INVESTIGATIONS ‡ Not Seen	X1	X2	X3	X4	X5	X>5	Not Done
HC	36	11 (11)	3 (3)	-	2 (1)	- (1)	-	7 (7)	13 (13)	12 (11)	2 (4)	-	-	1 -	- (1)	21 (20)
TT and BT/RFC	25	9 (5)	5 (11)	3 (2)	-	-	- (1)	2 (1)	6 (5)	9 (6)	8 (8)	1 (3)	- (1)	-	-	7 (7)
TT and BT/Active	18	9 (4)	3 (5)	1 (1)	1 (2)	- (1)	- (3)	-	4 (2)	8 (3)	3 (5)	2 (2)	- (3)	-	- (1)	5 (4)
BL	41	8 (7)	6 (3)	6 (7)	6 (3)	2 (5)	1 (11)	6 (1)	6 (4)	10 (9)	14 (8)	4 (4)	1 (5)	2 (4)	2 (3)	8 (8)
LL	36	10 (3)	10 (8)	5 (10)	2 (5)	1 (3)	1 (5)	5 (2)	2 -	11 (7)	17 (13)	2 (6)	2 (2)	1 (1)	- (3)	3 (3)

† First number = the number of patient-assessments during lactation for this special study only; number ( ) = the number of patient-assessments for this special study together with the routine "full clinical assessments" at hospital review clinics and special clinics monitoring suspected dapsone resistance.

‡ First number = frequency of laboratory investigations (skin smears for BI & MI/Biopsy for histology/Biopsy for mouse foot pad test) singly or in combination; number ( ) = frequency of laboratory investigations and additional tests (VMT with ST/EMG /Skin test with "A6") singly or in combination.

evidence of reaction) or increased activity of the lesion as diagnosed by histology.

Definition of neuritis and assessment of nerve function

Neuritis in leprosy is usually defined as "pain and/or tenderness of nerves" (Pearson and Ross, 1975). In this study 'overt neuritis' is defined as pain and/or tenderness of nerve; all patients with overt neuritis except 9 also showed evidence of simultaneous impairment of nerve function. 'Silent neuritis' is defined as impairment of sensory and/or motor function without nerve pain or tenderness (Duncan and Pearson, 1982).

Motor function was recorded as impaired if there was loss of 2 or more points (on the 0-5 scale) in 2 separate muscles within the same nerve distribution. (In 3 episodes of neuritis nerve damage was recorded when the motor deficit, though only one point, included almost all the tested muscles.) In the presence of obvious clinical changes (such as facial paralysis or 'tic' of the facial muscles, wasting of the intrinsic muscles of the hand, 'curving' of the fourth and fifth fingers, claw hand or foot drop), formal VMTs were sometimes omitted. These are reported as "clinical assessments".

Impairment of sensory function was recorded if loss of sensation had occurred in at least two test sites within the distribution of a single nerve. In a few patients with extensive anaesthesia with anhidrosis, formal SSTs were omitted; they are reported as "clinical assessments".

Diagnosis of Reaction

i) Reversal Reaction (type 1 lepra reaction) was diagnosed in patients with borderline leprosy by the occurrence of one or more of the following : erythema and oedema (sometimes with ulceration) of skin lesions; tender enlargement of nerves with or without loss of nerve function and often of abrupt onset; loss of nerve function without tender nerves ('silent neuritis') (Pearson and Ross, 1975; Duncan and Pearson, 1982); tenosinovitis especially of the extensor tendons over the back of the wrist (Wheate, 1962) or histopathologically (Ridley, 1977).

ii) Erythema nodosum leprosum (ENL) (type 2 lepra reaction) was diagnosed in lepromatous patients by the occurrence of one or more of the following : the appearance in the skin of crops of shiny, painful red nodules, either superficial or deep, and lasting from 3-5 days (these were frequently accompanied by a systemic upset with fever, malaise, lymphadenopathy and tender enlarged peripheral nerves); iridocyclitis; dactylitis; or histopathologically (Ridley, 1977).

When silent neuritis occurred in a lepromatous patient (BL or LL) who had ENL either at the same time or previously, the silent neuritis is regarded as being due to ENL.

"Qurtimat" (rheumatism) as a symptom

Many of the ante-natal and more particularly the lactating patients complained of qurtimat during history taking; those who did not mention it were asked specifically about it; without exception they all denied having experienced it. Qurtimat was usually a symptom which brought the sufferer to hospital for treatment. Patients complaining of qurtimat were assessed fully as previously described. Nerve enlargement was recorded as associated with qurtimat only if it was a new finding; lymphadenopathy was considered as significant only in the absence of local infection and trophic ulcers and when the VDRL was negative.

In this study "rheumatism" has been used to translate qurtimat (Tekle Wolde, Destá, 1970).

Additional data from hospital case records: Baseline assessment

The patients' hospital case records were examined and data were abstracted regarding leprosy status (clinical relapse, slit skin smear results and biopsy reports), frequency and type of reaction prior to admission to the study, and objective measurement of sensory and motor function. Particular attention was paid to the three month period immediately preceding pregnancy: this was expected to provide a baseline figure for frequency of complication in Ethiopian women of childbearing age.

Duration of dapsone monotherapy prior to onset of dapsone resistance in Ethiopian patients

Duration of dapsone monotherapy prior to onset of clinical dapsone resistance in Ethiopian patients has already been reported by Pearson et al (1979). This information applied to both male and female patients. In order to assess the possible effect of childbirth on the emergence of dapsone resistance in female patients, the hospital outpatient and inpatient records were reviewed retrospectively and information regarding patient's age at the onset of the disease, the date of start of treatment, the response to treatment in terms of BI and MI, and evidence of relapse in terms of new nodules or rise of BI or MI were extracted. Observations regarding changes of dosage of dapsone and introduction of alternative therapy, the occurrence of reactions and the use of clofazimine for recurrent, persistent, troublesome ENL was also recorded. The patients were then interviewed and an obstetric history obtained from them. They were then asked whether they had observed any effect of pregnancy on the course of their leprosy and of the timing of appearance of new nodules and symptoms of reaction, and in particular of neuritis. Finally, each woman was asked her opinion regarding the effect of pregnancy on leprosy, whether it was beneficial or detrimental, and what her attitude was to repeated childbearing according to Ethiopian tradition.

II. OBSTETRICAL ASSESSMENT AND CARE

Ante-natal care

On reporting their pregnancy at the ante-natal clinic, the women participating in the study were examined, and the following examinations performed :

General and ante-natal

Complete physical examination, height, weight, skinfold thickness, blood pressure, uterine fundal height, urine analysis, haemoglobin, blood group, serological test for syphilis (VDRL); chest X-ray was normally delayed until the puerperium.

Patients were then seen every two weeks until Week 34 and weekly thereafter. At each visit the weight, blood pressure, fundal height, presentation of the foetus, presence of foetal heart sounds and results of urine analysis were recorded. The mothers were admitted to the Addis Ababa Leprosy Hospital for a day or two for collection of urine for oestriol assay, and as necessary for complications of leprosy for intercurrent illness.

Delivery of study patients

All women who had consented to participate in the study were issued with special delivery cards. These cards entitled them to a "priority" obstetric bed at a delivery centre, usually Lidetta Clinic, or the University Obstetric Unit in the Black Lion Hospital. Public transport (buses and taxis) stopped shortly after dark. None of the women had access to private transport. During the curfew only those with special passes (including doctors) were allowed to move in the streets. In the initial months of the study it became apparent that while healthy women and those with tuberculoid leprosy were usually able to get to the Leprosy Hospital in time for a supervised assisted delivery, women with lepromatous leprosy had a shorter labour and frequently delivered at home during the curfew. In order to ensure supervised delivery and collection of specimens, it became necessary to admit women with lepromatous leprosy to the MRC Ward of the Leprosy Hospital prior to delivery to await onset of labour.

Management of labour

The mothers were delivered in a nearby delivery centre (or occasionally in the Addis Ababa Leprosy Hospital itself) unless operative abdominal delivery was required, or paediatric intensive care was anticipated, in which case the mother was delivered in a hospital obstetric unit. Observations during labour, frequency and strength of contractions, and monitoring of the foetal heart and maternal pulse and blood pressure were carried out in accordance with the University practice in Addis Ababa.
Ethiopian women of the low socio-economic class in comparison with educated Ethiopian or ex-patriot women required very little analgesia during either first or second stages of labour.
Analgesics, including pethidine 100 mg and trichlorethylene, were given as required during labour; ergometrine maleate 0.5 mg was given intramuscularly with the delivery of the anterior shoulder or intravenously with the delivery of the head when operative vaginal delivery was required. The baby's Apgar score was recorded. Cord blood was collected after the cord was clamped, and swabbed with an antiseptic solution, before the delivery of the placenta. The baby was weighed immediately after birth, and its crown-heel length and head circumference measured. The placenta was weighed after membranes and cord had been trimmed, and samples were then taken for light and electron microscopy and immunohistology. Placental samples without fixative were also taken and stored at -20° C.

Whenever possible, the mother and baby were kept in the Addis Ababa Leprosy Hospital for 4-7 days postpartum until lactation was established, otherwise they went straight home, following local custom, within 6-12 hours after delivery.

Babies requiring intensive paediatric care were transferred immediately to the neonatal unit in the Black Lion Hospital. When the baby was well enough to be breast fed, the baby's mother was admitted to the neonatal unit and both were kept there until breast feeding was well established and the baby sufficiently well to be discharged home.

Post-natal examination

Routine post-natal examination was carried out 6 weeks after delivery.

The examination included measurement of blood pressure, weight, palpation of breasts, abdomen and legs, bimanual pelvic examination and urine analysis. Contraceptive advice was offered; this, however, was usually not accepted until towards the end of the first year of lactation, when one of the combined oestrogen/ progesterone pills was taken as the method of choice. Most women depended on prolonged suckling as their contraceptive.

ASSESSMENT OF PLACENTAL FUNCTION BY OESTRIOL ASSAY

Seventy-nine women with singleton pregnancies, essentially free from obstetric complications of pregnancy, were admitted to hospital for each 24-hour urine collection. Urine was collected over sodium azide (5 g), the 24 h volume was recorded and a portion stored at 4° C. The samples were heated at 65° C for 1 hour to destroy any pathogenic viruses and were transported by air and rail to Leeds, U.K., packed in solid CO_2. After thawing, urinary oestrogen was determined by a continuous flow method using a fluorimetric end point (Oakey, 1977). Measurements were made retrospectively and the results were not available for obstetric management.

The weights of infants and placenta were recorded for 58 of the women. The remaining 21 were unable to travel to hospital for delivery because of the curfew or preferred to deliver at home.

EXAMINATION OF THE PLACENTA

Collection of specimens

When the placenta was delivered it was examined along with the membranes routinely for completeness, and any obvious abnormality. The placenta was then trimmed of its membranes and cord, and weighed. Specimens were taken for :

i) light microscopy using Ridley's fixative (Formalin in 10% alcohol);

ii) electron microscopy, mincing the placenta very finely on a wax sheet with a new Gilette razor blade, and fixing the specimens in glutaraldehyde, transferring them to cacodylate buffer after 2 hours; the fixed tissue was embedded in resin before being despatched from Ethiopia (by airmail);

iii) immunohistology, by snap freezing specimens in either liquid nitrogen or carbon dioxide depending on availability: these specimens were then stored at -70° C and transported to the U.K., packed in solid phase CO_2, where again they were stored at -70° C;

iv) unfixed placenta was stored in scintillation counting vials at -20° C, transferred from Ethiopia to the U.K. packed in solid phase CO_2 and subsequently stored at -20° C; five gram aliquots of frozen placenta were used for placental enzyme studies and search for M. leprae by concentration techniques.

Search for M. leprae

i) Routine light microscopy. Sections of placenta, membranes and cord stained with haematoxylin and eisin, and Fite's stain were examined for presence of M. leprae. A 10 minute search was carried out routinely. Sections from patients considered "potentially infectious" were subjected to a 30 minute search using high dry and oil immersion lenses.

ii) Concentration methods. Five gram aliquots of placenta were homogenised and specially treated to allow the maximum concentration of the tissues to be examined for presence of AFB. Smears were made from the homogenates and examined for 10 minutes.

Placental morphology

Placentae from 78 women with leprosy and 17 healthy controls were examined by light and electron microscopy. The women were classified as follows :

	No. of mothers	No. of pregnancies
HC	17	18
TT and BT/RFC	11	11
TT and BT "active"	15	15
BL	34	34 *
LL	18	19 **

* 1 set twins, uniovular.
** 1 set twins, binovular.

In addition to examination of the placentae, sections of cord and membranes were also examined.

Immunohistology

Cubes of tissue were snap-frozen in liquid nitrogen and stored at -70° C.

Cryostat sections (3 μm) were reacted with an appropriate dilution of the rabbit antiserum under study (first antibody) at 4° C for 30 minutes and washed three times for 10 minutes in phosphate buffered saline (PBS)pH 7.4. The tissues were then reacted with a fluorescein isothiocyanate (FITC)-labelled sheep antiserum to rabbit immunoglobulin (second antibody) at 4° C for 30 minutes, washed three times and mounted in 50% glycerol in PBS, pH 8.6. Antisera to human IgG, IgA and IgM, to complement components C4, C3d and C9 and to fibrin/fibrinogen and plasmin/plasminogen were obtained from Dakopatts A/S, Copenhagen, Denmark. Antiserum to C1q was donated by Dr. P. Lachmann, Cambridge University, England. The specificity of these antisera and their optimal working dilutions (1:50 or greater in every case) were established by titration as previously described (Faulk and Johnson, 1977; Faulk et al, 1980). Controls for unwanted fluorescence were performed by substituting either PBS (conjugate control) or non-immune rabbit serum (serum control) for the first antibody, and the conjugate was absorbed against normal human serum in solid phase (Galbraith et al, 1978) to minimize unwanted fluorescence. Slides were examined using a Zeiss Univeral microscope equipped for transmitted illumination with a Tiyoda condenser and appropriate FITC filters, and photographs were taken using Kodak Tri-X film.

Placental enzyme studies

Forty samples of placenta were studied (10 HC, 10 TT and BT, 10 BL, 10 LL). Only those from women who were HBs negative were used. The placental samples were sterilised by irradiation before being used in any laboratory procedure.

Homogenates of placenta were prepared with an Ultra Turrax homogeniser (Janke and Kunkel, FRG) from about 1 g samples of placenta in 9 ml of buffer.

i) DNA was measured by a modification of the method of Burton (1956) as described by Simmonds et al (1982).

ii) Protein was estimated by the method of Lowry et al (1951) using albumin as a standard.

iii) Alkaline phosphatase was assayed by the method of Hausamen et al (1967) in 0.05 mmol/l tris buffer pH 7.6 with 1% (w/v)

Triton X-100 after extraction of the homogenate with an equal volume of n-butan-1-ol. Differential inhibition on samples from 4 patients was with 20 mmol/l homoarg, 2 mmol/l phe-ala-gly-gly and 5 mmol/l phe (Mulivor, Plotkin and Harris, 1978).

iv) β-glucuronidase was assayed by the automated fluorimetric method of Morgan, Vint and Ridout (1978) using a 4-methylumbelliferone coupled substrate.

v) Cytochrome oxidase was estimated spectrophotometrically (Cooperstein and Lazarow, 1951).

HUMORAL DEFENCE FACTORS IN THE BREAST MILK OF ETHIOPIAN WOMEN WITH LEPROSY AND HEALTHY CONTROLS

The milk study was initiated when the trend of slow growth in babies born to mothers with lepromatous leprosy became apparent. Thus it was only possible to obtain early samples of colostrum and milk from a small percentage of the women.

Collection of milk samples

Milk was collected after hand washing and cleansing of the mother's breast, areola and nipple with an antiseptic. The mother's head was averted and she was requested not to speak during the milk collection in order to minimise possible droplet contamination. Milk was obtained by digital expression of the areola and nipple, collected in a sterile container and placed immediately in the ward refrigerator (+ 4° C) until it was transferred to the laboratory storage freezer (- 20° C). No preservative was added. Two hundred and forty-four samples were transported to the U.K. in batches packed in solid phase CO_2 and kept at -40° C until they were processed).

The milk samples processed and included in this study are from women who were sero-negative for HB_sAg.

Laboratory methods

All samples were defatted by centrifugation at 15,000g for 30 minutes at 4° C and stored at -40° C until tested. Secretory IgA, lactoferrin and albumin were measured in all samples by radial immunodiffusion (Fahey and McKelvey, 1964), and total protein was measured by the Folin Lowry technique. IgG, IgM, β1A and β1E proteins were measured in the samples collected within 24 hours following parturition. The standard materials and antisera used in the radial immunodiffusion analyses have been described elsewhere (McClelland, McGrath and Samson, 1978). Analyses were carried out in duplicate. Comparison between the Ethiopian mothers was made by dividing the post-parturition samples into groups which consisted of day 1, day 2 to 7, day 8 to 1 month, 3 to 6 months, 7 to 12 months, and 13 months to 2 years. Samples from normal nursing mothers which had been collected from the Simpson Memorial Maternity Hospital, Edinburgh, during a previous study

(McClelland et al, 1978), were grouped where possible over the same time periods as the leprosy panel for comparison, i.e. day 1, day 2 to 7 and day 8 to 1 month. Analysis of variance using the unpaired 't' test was carried out between the Ethiopian groups according to classification of leprosy, and between the Edinburgh group and the cumulated results of the Ethiopian groups.



Plate 8. "The baby was carried on the mother's back, held in position by an ornamented cow hide or voluminous shawl ... until the next baby arrived".

B A B I E S

General care of babies of mothers attending the Addis Ababa Leprosy Hospital

Most of the babies were born in a nearby Mother and Child Health Delivery Centre. Within a few hours of birth, providing there had been no complications, the mother and baby were transferred back to the Leprosy Hospital where they remained in the MRC Leprosy Research Project Ward until breast feeding was established. During this time the baby was dressed in jacket, diaper and wrapper made from locally produced cotton material. The baby slept in the same bed as his/her mother and was breast fed "on demand", according to local custom. While general hygiene was encouraged, the mothers were not issued with special gowns or masks to wear when feeding their babies, nor was any attempt made to segregate the mothers or babies from their visitors. Food for the mother was supplied by the Hospital. Babies requiring special care were admitted to the neonatal ward of the University Paediatric Department of the Black Lion Hospital, to which the mother was also admitted once her baby was well enough to be breast fed.

After the mother and baby were discharged home, the baby was usually dressed according to local custom in a cotton dress, or knitted jersey which reached the level of the buttocks, and a knitted cap. Diapers were not usually used, although a few women followed the Hospital practice and made them of locally produced cotton: when the baby was small, he/she was usually swathed in a cotton wrapper. The baby was carried on the mother's back, held in position by an ornamented cow hide and thongs, or a voluminous shawl (shama). The child would be carried on the mother's back until the next baby arrived. Most mothers wore cotton dresses with high necklines, front opening and long sleeves over a short-sleeved petticoat. They usually slept in their petticoats and the traditional shama.

Feeding of babies and young children

Traditionally, the first feeding given to the baby is not the mother's milk or colostrum but butter and often rancid at that (Mérab, 1912d). This practice was still observed in Addis Ababa at the time of this study. Ethiopian women, in general, breast fed their babies until the arrival of the next, by which time the baby on the breast was about 2

years old. Supplementary feeding was introduced, when the baby was 6–12 months old, with tea and bread : occasionally cow's milk was given. Weaning was practised from about 1 year, the baby being introduced to potato, carrot and injera, a "pancake" made of fermented tef dough; wot, a spicey "stew" made with pulses, chillies and vegetables, was introduced towards the end of the second year. Fresh fruit with the exception of bananas was seldom given to young children, because of cost. Thanks to the influence of ex-patriot wives on the Leprosy Hospital compound, the idea of adding boiled cow's milk and an egg to the diet of toddlers was becoming accepted.

Dried milk powder and infant "formula", which were available in the stores at the time of the study, were too expensive for most Ethiopian women. In any case, because of the difficulties in sterilising feeding utensils, the risk to the babies from gastro–enteritis would have been very considerable. Reconstituted milk was supplied by the Leprosy Hospital for the babies of mothers admitted with reactions late in lactation.

"Wet nursing", practised widely in some parts of Africa, was not often observed in Ethiopia.

Faffa, a mixture of locally available grains and pulses, prepared for infant feeding, and easily made into "porridge" was available through the municipal mother and child health (MCH) clinics. Faffa, which was the product of research by the Ethiopian Nutrition Institute, not only contained a balanced diet for weaning and young children, but was available at a very reasonable cost. This was widely acceptable, the only limit to its use being availability, especially during the third and fourth years of the revolution (1977 and 1978).

Health status of fathers and other family contacts

Data regarding the health of other members of the family were obtained, but it was soon realised that not only family but extended family and friends, together with leprosy patients from the same village, could share the same small house. The occupants of the house comprised a shifting population, and hence not only did close control of contacts with leprosy become impossible, but the father's leprosy status became less relevant.

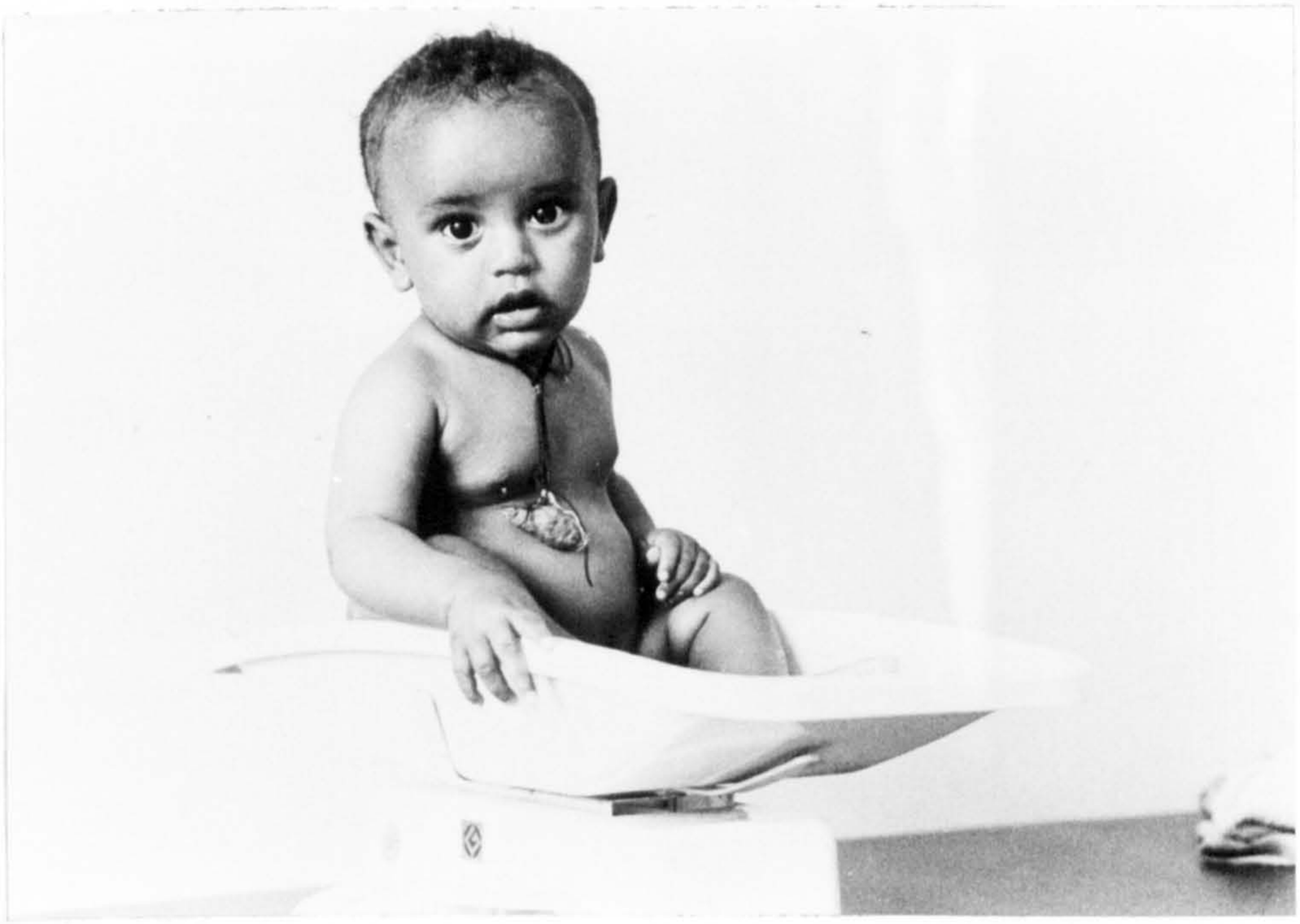


Plate 9. "Babies were completely undressed for weighing which is considered the simplest index of satisfactory growth ..."

Assessment of the babies: Phase I

Assessment of babies, skin testing and vaccination with BCG (Phase I) was done by myself from December 1975 until mid May 1978: Dr. Pearson did a few baby assessments during the next three months.

Assessment of the babies was carried out at birth, then monthly until 3 months and approximately every 3 months thereafter. Sick babies were brought to the special follow up clinics which were held twice or thrice weekly, or as emergencies for examination and treatment. Examinations were carried out in a well lit south-facing room, using daylight when possible. Babies were completely undressed for weighing, which is considered the simplest index of satisfactory growth (Morley, 1966), measuring of length and head circumference, and for examination. The mother was present throughout the examination and instructed regarding feeding, weaning and vaccination. Additional laboratory investigations were requested as indicated and intercurrent infections, including skin infections, were treated at the special follow up clinics.

The healthy mothers and those with cured TT and BT leprosy had less motivation to bring their babies for regular routine assessment, and seldom did so after the first 3 months of life, but their babies were weighed, measured and assessed if they attended a "sick baby clinic" usually because of an acute infection.

When any baby was found to have skin lesions suspected of being leprosy, measurements of the lesion(s) were made. Sensory loss could not be assessed using the traditional cotton wool test for light touch and pin prick was too crude. A pin held at an angle of 45^{o} to the skin, with sufficient pressure to indent the skin, was therefore used. Palpation of peripheral nerves and regional lymph nodes was also carried out. Three babies had biopsies of suspicious lesions under local anaesthesia. Routine slit skin smears were not made. Independent assessment was made by two senior leprologists (Dr. J.M.H. Pearson and Dr. H. Wheate or Dr. J. Warndorff).

Blood samples were collected, as far as possible, at 3 monthly intervals during the first year, and at 6 monthly intervals during the second year. Blood was collected usually by scalp venepuncture. Because of the Ethiopians' fear of blood tests (associated with their

traditional method of slaughter of beast, and occasionally foe, by cutting the jugular vein), blood was never collected from a sick baby except for essential diagnostic purposes, lest the mother should subsequently ascribe the sickness to a blood test. The request for blood was only once refused by a mother, who however agreed to the baby's blood test on the next visit to the clinic. The blood collected was used for immediate lymphocyte transformation tests (on approximately half the babies). The serum separated was stored at -70° C for shipment to the U.K. and subsequent testing in the U.K. and Norway.

Skin testing and vaccination/immunisation

Skin testing of babies to A6 (purified protein of M. leprae grown in armadillos) and PPD was carried out when the babies were 9-15 months old. BCG (0.1 ml) was given intradermally to all babies who were PPD negative and a certificate of vaccination was issued to the mothers. At approximately the same time, the Ministry of Public Health organised a vaccination campaign and well-baby clinic through the newly formed "kebele(s)". Despite maximal co-operation on our part, inevitably some of the mothers became confused by the two systems of follow up and dropped out of the special follow up clinics. Vaccination/immunisation given through the kebele well-baby clinics included BCG, smallpox, triple vaccine (DPT) and oral poliomyelitis, singly or in combination, according to supplies available.

Assessment of the babies: Phase II

Two years after the end of Phase I, when the children were aged 3-4 years, special follow up assessments were made of the mothers and children by Dr. Suzanne Menzel with help from Dr. Nsibambi and Dr. T. Warndorff. Assessment of the children included measurement of weight and height, full examination in a well lit room, slit skin smears, skin testing with AB22 (purified protein of M. leprae from infected armadillos) and PPD. A blood sample was obtained by venepuncture.

SEROLOGICAL TESTS

1. Measurement of serum bilirubin in cord blood

During the course of aliquotting the cord blood for immunological studies, it was observed that a number of cord blood specimens from babies of lepromatous mothers were unusually yellow. Serum bilirubin, therefore, was measured by spectrophotometry in 25 µl samples of undiluted cord sera from babies of mothers with lepromatous lerposy, together with samples from babies of mothers with tuberculoid leprosy and healthy mothers as controls.

2. Serological testing for Hepatitis B infection

Hepatitis B surface antigen

All serum samples collected were tested by a reverse passive haemagglutination assay (RHPA: Hepatest). Positive results were confirmed by a radioimmunoprecipitation (RIP) test (Burrell *et al*, 1973). Babies born to HBsAg positive mothers were also tested by RIP assay.

Anti-HBs

Samples from each of the mothers, and the follow-up samples of the babies born to carrier mothers, were tested by a precipitation assay (Burrell *et al*, 1974).

HBeAg and anti-HBe

Each of the HBsAg positive mothers was tested for HBeAg and anti-HBe by a solid phase radioimmunoassay (sRIA) developed in the laboratory of Edinburgh University Department of Virology. The immunoglobulin fraction from a human serum known to contain anti-HBe by gel diffusion was labelled with ^{125}I by the chloramine T method (Burrell *et al*, 1973). Human serum positive for HBeAg by gel diffusion was used as the antigen. The assay gave comparable results to a commercial system (Abbott) when tested in parallel against a panel of sera.

IMMUNOLOGICAL TESTS

The following tests were not performed by the author but in the AHRI laboratory, Addis Ababa, or by Dr. R. Melsom in Oslo. Therefore only an outline of the methodology is given with references. Most of the papers referred to are bound in the Appendix.

I. For cell mediated immunity

Lymphocyte transformation tests (LTT)

In this study LTT was performed by a micro method (Closs, 1975) with a few modifications. The antigens used were M. leprae whole and sonicated in concentrations standardised to 10^9, 10^8 and 10^7 bacilli/ml; BCG in concentrations 10^9, 10^8 and 10^7 bacilli per ml; PPD in concentrations 1.0 and 10 µg/ml; the mitogen used was PHA with a concentration of 10^2 (Barnetson, R.St.C., Bjune, G. and Duncan, M.E. Nature (1976), 260, 150-151; Bjune, G., Duncan, M.E., Barnetson, R.St.C. and Melsom, R. Clinical and Experimental Immunology (1978), 32, 517-522).

II. For humoral immunity

1. Measurement of concentration of immunoglobulins A, G and M

Immunoglobulin concentrations were determined by the single radial diffusion technique (Mancini et al, 1964; Fahey and McKelvey, 1965) with modifications to quantitate the very low levels of IgA and IgM in cord blood (Melsom, R., Duncan, M.E. and Bjune, G. Leprosy Review (1980), 51, 19-28).

2. Demonstration of antibodies against M. leprae antigen 7 in both IgG and IgM by radioimmunoassay (RIA)

Using M. leprae obtained from armadillo liver tissue, M. leprae antigen 7 was prepared, labelled with ^{125}I and tested by crossed immunoelectrophoresis and autoradiography. An RIA technique was developed to detect antibodies to this M. leprae antigen 7 preparation, and subsequently used in the testing of serial samples from mothers and babies, and pooled sera from 40 patients with active lepromatous leprosy who had been on treatment with dapsone for less than 6 months. The amount of antibodies against M. leprae antigen 7 in the baby sera was expressed as a percentage of antibody concentration in the

corresponding maternal serum taken at delivery (Melsom, R. and Duncan, M.E. Leprosy Review (1980), 51, 125-135; Melsom, R., Duncan, M.E., Harboe, M. and Bjune, G. Clinical and Experimental Immunology (1980), 42, 107-113).

3. IgA, IgM and IgG anti-M. leprae antibodies in babies of mothers with leprosy during the first 2 years of life

Using sonicated M. leprae purified from infected armadillo liver, a solid-phase radioimmunoassay (sRIA) was developed for measuring IgA, IgM and IgG antibodies against M. leprae in mothers with leprosy and their children up to the age of 2 years (Melsom, R., Harboe, M., Duncan, M.E. and Bergsvik, H. Scandinavian Journal of Immunology (1981), 14, 343-352; Melsom, R., Harboe, M. and Duncan, M.E. Clinical and Experimental Immunology (1982), 49, 532-542).

RESULTS

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Plate 10. Mothers and children of the Study together with Miss Liv Reitan (AHRI), Sister Almaz and Woizero Bekelech (MRC Ward Staff), Ato Moges and Ato Bekele (MRC Laboratory Staff) and the Author, May, 1978.

SELECTION OF PATIENTS FOR DIFFERENT ASPECTS OF THE CLINICAL STUDY

A. THE EFFECT OF PREGNANCY ON LEPROSY

As will be seen from the different parts of this section, there are minor discrepancies regarding numbers of mothers in the different classifications of leprosy. This is due to the fact that two patients admitted as healthy controls developed overt leprosy,and almost half of the cured tuberculoid patients relapsed with active leprosy or new nerve damage. Subsequently these patients were reclassified as having active tuberculoid or borderline lepromatous leprosy. The numbers of patients according to initial classification and reclassification are shown in Table 6.

Ideally, only the mothers who were followed throughout pregnancy and lactation should be included in the results. However, in the context in which the study was carried out, "ideal conditions" did not exist. Economic pressures were such that some mothers were unable to afford the time (effectively a full day away from work or begging) to attend the special clinics with their babies. Certain general observations regarding the practice of the patients attending the Addis Ababa Leprosy Hospital are a guide to interpreting the attendance of the mothers :

i) In general, leprosy patients within easy travelling distance of the hospital would always appear at a hospital clinic if any complication of leprosy occurs.
Development of acute reversal reaction, ENL or overt neuritis would result in an early appearance at a routine or "emergency" ("red medical") clinic.
Development of lesions indicative of relapse, on the other hand, would usually only be detected on their routine "review" clinic attendances at 6 monthly intervals, unless one of the leprosy workers at the weekly/monthly dapsone distribution clinics observed something untoward and requested an early referral to the "review" clinic.

ii) Patients with new "silent" neuritis were occasionally detected at the "Red Surgical" clinic when they attended for surgical

TABLE 6

Number of mothers and children in A9 Study: according to classification of mothers

Classification of mothers		On entry to study	After emergence of new cases and relapse	In follow up of children
HC	Mothers	33	31	32*
	Pregnancies	36	34	36*
TT & BT/RFC	Mothers	25	17ø	17
	Pregnancies	25	17	17
TT & BT/Active	Mothers	17	23	23
	Pregnancies	18	24	24
BL	Mothers	40øø	44øø	45**
	Pregnancies	41	45	46**
LL	Mothers	32øø	32øø	32
	Pregnancies	36	36	36
TOTAL	Mothers	147øøø	147	149
	Pregnancies	156øøø	156	159

ø Includes 1 mother who relapsed with active BT leprosy at 12 months' lactation after her last A9 assessment.

øø One mother was classified as BL for her first pregnancy (A9/27) and LL for her second pregnancy (A9/260) in the study. To avoid confusion in the Tables and text if she were listed as pregnancy 1 = BL; pregnancy 2 = LL, she is considered throughout the study as "2 mothers with one pregnancy each".

øøø In many places, in the text and Tables, for the sake of simplicity, each pregnancy is considered on its own and equivalent to one mother.

* Includes an additional 2 HC mothers (not in the initial Study intake) who were assessed and their babies followed up during lactation.

** Includes 1 additional BL mother with severe ENL and neuritis (not in the initial Study intake) who was referred for obstetric care. She was, however, excluded from the Study of the effect of pregnancy on leprosy on the grounds of being a selected patient.

treatment of trophic ulcers, and were referred back for urgent reassessment.

One can thus be almost certain that when a patient was attending the dapsone distribution clinics reasonably regularly without referral, all was well in terms of absence of leprosy reactions. If only the patients assessed at special clinics for this study were included, all those with reactions would have been included together with most of those with relapse, but many whose leprosy was being treated without complication would have been excluded. The rates of relapse and reaction would then have been unreasonably high. Thus, when it was realised that the relapse and reaction rates were so incredibly high in women during pregnancy and lactation, by comparison with earlier reports, the relapse/reaction rate was, if anything, "played down" by including as "non relapse and non reaction" those few patients attending the dapsone distribution clinics who escaped special assessment at the study clinics.

If patients had not been seen for several months, especially when in the case of "cured" patients there was suspicion or evidence of early relapse, recall requests were lodged with the social worker, and at the records office, dapsone distribution clinic and sometimes the local "kebele". Most of the truant patients living in Addis Ababa were thus traced.

Section 1 gives an overall view of the effect of pregnancy and the first 12 months of lactation - the period during which most mothers were still lactating - on leprosy in terms of relapse and reaction, comparing them with the "baseline" prevalence of relapse and reaction in non-pregnant Ethiopian women. Sections 2-6 then deal with different aspects of relapse and reaction in greater depth and for up to 2 years of lactation.

For Sections 1, 2, 5 and 6, all the patients in the study were included as per the initial classification of patients on entry to the study. While dapsone resistance is known to occur in patients with tuberculoid leprosy, and indeed was suspected in 3 of the TT and BT mothers in this study, it is only from the lepromatous patients that sufficient viable bacilli can easily be obtained for satisfactory mouse foot pad inoculation. Thus, for Section 3, the

study of dapsone resistant leprosy in pregnancy, only patients with lepromatous (BL and LL) leprosy were used as per the initial classification of patients. However, 5 BL cases were excluded, one because she was RFC and therefore not receiving dapsone, and 4 who were only seen during pregnancy and were not reassessed at special study clinics during lactation. For Section 4, the study of ENL in association with pregnancy, as ENL is usually only to be found at the lepromatous end of the spectrum, only those classified as BL or LL according to the revised classification were included.

B. THE EFFECT OF LEPROSY ON PREGNANCY

1. Oestrogen excretion in pregnant women with leprosy. All patients attending the ante-natal clinic were given cards requesting admission to Ward 3 for a minimum of three 24h collections of urine for oestriol assay. Admission was only possible when there were vacant beds, and thus it was not possible to collect urine for oestriol assay from all patients in the study. This was the only factor which determined selection of the patients for this part of the study.

2. Weights of babies and placentae. Only those babies delivered in hospital or delivery centre could be used for this part of the study. Because of the risk of travelling during the curfew in an unauthorised vehicle, a number of women going into labour during the night delivered at home.

3. The placenta in leprosy. The selection of mothers for this part of the study was determined by those who delivered in hospital or delivery centre.
Selection of patients for EM and immunohistological studies was determined largely by the time of day or night when the woman delivered and whether it was possible for me to be present at the delivery and to take the placental specimens to the leprosy hospital for snap freezing (in the case of immunohistology). A curfew pass was not always guarantee of safe conduct.

4. Humoral defence factors in breast milk. As stated in the text, this study was only initiated when the trend of slow growth in babies of mothers with lepromatous leprosy was

observed, hence it was only possible to obtain early specimens of colostrum and milk from a small percentage of mothers in the study. However, later specimens were obtained from all the mothers who were still lactating.

5. The outcome of pregnancies of women with leprosy and healthy controls. All the mothers, according to the initial classification, were included together with an additional 3 mothers and their babies (2 HC and 1 BL). The HC mothers were included when it was apparent that the follow up in that group was not as good as had been hoped; one of these mothers was thus in the study with two pregnancies. The BL mother was referred for special assessment and follow up during pregnancy and lactation on account of severe ENL and neuritis. She was, however, excluded from the study of the effect of pregnancy on leprosy on the grounds of being a selected patient.

6. A clinical and immunological study of 4 babies of mothers with lepromatous leprosy. The 4 babies selected for this study were the 4 who developed lesions suspected of being due to leprosy.

C. SOME ASPECTS OF CELL MEDIATED IMMUNITY IN MOTHERS WITH LEPROSY AND THEIR CHILDREN

1. Hepatitis B infection. No selection was observed at all in this study. Aliquots of all sera reaching the U.K. were tested for the presence of hepatitis B infection. The reason for certain mothers and babies not being tested was either that there was no serum left after the initial tests had been carried out in Addis Ababa, or the amount of serum available was all required for immunological testing in Oslo.

2. Evidence for a soluble lymphocytic factor in the transplacental transmission of T-lymphocyte responses to Mycobacterium leprae. Ten women with no clinical evidence of leprosy and no obstetrical complication, delivering at Lidetta Clinic, and their babies were used for this study. No special selection was observed except to ensure that delivery (and hence collection of specimens) occurred during normal laboratory working hours.

3. In vitro modulation of lymphocyte responses to PHA by plasma in mother and baby at the time of birth. No special selection of mothers and babies was observed for this section. All mother and baby pairs, where LTT was carried out on specimens collected at delivery, were included.

D. STUDIES OF HUMORAL IMMUNITY IN MOTHERS WITH LEPROSY AND THEIR BABIES

Selection of sera for these studies was based largely on availability and volumes of serum samples from mother and baby pairs at delivery and during the follow up period. False positive serological tests for syphilis have been reported in patients with lepromatous leprosy; furthermore, latent (sero-positive) syphilis is endemic in Ethiopia. While none of the mothers or babies in the study showed any of the clinical signs of syphilis, and any who were found to be VDRL positive were immediately treated with penicillin, serum samples which were VDRL positive were excluded from this study.

FOLLOW UP OF MOTHERS IN THE STUDY BY 3 MONTH PERIODS ACCORDING TO THE DATES OF FIRST AND LAST ASSESSMENTS FOR LEPROSY

The numbers of mothers seen, by 3 month periods throughout the study, are shown in Table 7.

Of 156 women entering the study, 16 were not seen after the third trimestre of pregnancy; of 140 women who were seen during the first 3 months of the study or at delivery, 95 (67%) were still in the study at 12 months and 27 were still in the study at 24 months after delivery.

Of the 16 women dropping out at the end of the third trimestre, 8 were healthy controls, 3 women with cured tuberculoid leprosy, 2 active tuberculoid patients and 3 BL mothers. The suspected reason for the high drop out rate in healthy mothers was that they had consented to be in the study for the sake of obtaining a prized study card which entitled them to delivery, with hospital transport provided, in a nearby delivery centre! Many of these women were wives of members of staff who had no intention of continuing in the study after delivery. A further 12 dropped out immediately after delivery (5 HC, 4 TT and BT/RFC, 1 TT/BT active, 1 BL/RFC and 1 LL). The strongest motivation for continuing in the study was seen amongst the women with lepromatous leprosy, particularly the LL group, who were concerned about the state of their own disease and also in case the child should be infected.

TABLE 7

Number of mothers in the study by 3 month periods according to the dates of the first and last assessment for leprosy

Classification of mother on entry to the study	Pregnancy (trimestre)			Lactation							
	1	2	3	0 - 3	4 - 6	7 - 9	10 - 12	13 - 15	16 - 18	19 - 21	22 - 24
HC	2	13	36	28	19	19	16	15	12	10	6
TT & BT/RFC	-	13	25	22	18	17	17	13	13	10	6
TT & BT on treatment	1	7	18	16	14	14	13	10	7	4	3
BL	4	23	41	38	34	30	25	23	19	12	8
LL	4	18	36	36	33	31	24	23	17	7	4
	11	74	156	140	118	111	95	84	68	43	27

Each mother = 1 pregnancy.

A. THE EFFECT OF PREGNANCY ON LEPROSY

1. Pregnancy and Leprosy: The consequences of alterations of cell mediated and humoral immunity during pregnancy and lactation

One hundred and forty-seven women were studied during 156 pregnancies. There were 114 women with leprosy (120 pregnancies) and 33 healthy women (healthy controls: HC, with 36 pregnancies). The women with leprosy were classified initially, on entry to the study, as follows using the scale of Ridley and Jopling (1966) :

Cured tuberculoid and borderline tuberculoid leprosy (TT and BT/"Cured)	...	25 (25 pregnancies)
Active tuberculoid and borderline tuberculoid leprosy (TT and BT/"Active")	...	17 (18 pregnancies)
Borderline lepromatous leprosy (BL)	...	40 (41 pregnancies)
Lepromatous leprosy (LL)	...	32 (36 pregnancies)

Eighty-three patients (87 pregnancies) were receiving dapsone monotherapy (50-100 mg daily); 26 patients (1 BL, the rest BT or TT) were believed to be cured and had stopped treatment. Six patients (2 BL, 4 LL) had developed dapsone resistant leprosy, and were receiving clofazimine (4 patients all LL, 5 pregnancies) or rifampicin plus thiambutosine and dapsone (2 patients both BL).

Assessment of the patients' leprosy was made during pregnancy and after delivery at 6 monthly intervals whenever possible. The assessment included full examination, clinical drawings, slit skin smears, biopsies for histology and mouse foot pad inoculation (Rees, 1967), sensory skin testing (Pearson and Weddell, 1971) and voluntary muscle testing (Goodwin, 1968).

Observations

i) Deterioration of the patient's leprosy status

Table A1(i) and Figure A1(i) show the number of cases and timing of the worsening of the leprosy status in the women in the study by 3 month intervals from 3 months prior to conception to 12 months postpartum. Two of the 156 women

TABLE A1(i) NUMBER OF PATIENTS SHOWING WORSENING OF LEPROSY STATUS.

Initial Classification of Leprosy Status	Number of Women Studied	Number of Pregnancies Studied	Number of patients with worsening of leprosy status								
			3 months preceding pregnancy	PREGNANCY			LACTATION				TOTAL
				1st Trimestre	2nd Trimestre	3rd Trimestre	0–3 Months	4–6 Months	7–9 Months	10–12 Months	
HC	33	36	–	–	–	1 (as BT)	1 (as BL)	–	–	–	2
TT and BT "Cured"	25	25	–	1 (as BT)	1 (as BT)	5 (2 as BT) (3 as BL)	–	–	–	1 (as BT)	8
TT and BT "Active"	17	18	–	–	–	5	–	1	1	–	7(5)
BL	40	41	1	3	3	8	3	0	–	–	18(5)
LL	32	36	1 *	2	4	12	1	–	–	1	22(11)
TOTALS	147	156	2	6 (3)	8 (2)	31 (13)	5 (1)	1 (1)	1	3 (1)	57 (21)

Figures in brackets ( ) indicate the number of patients with transient worsening of leprosy status.

* Further deterioration at 13 months postpartum.

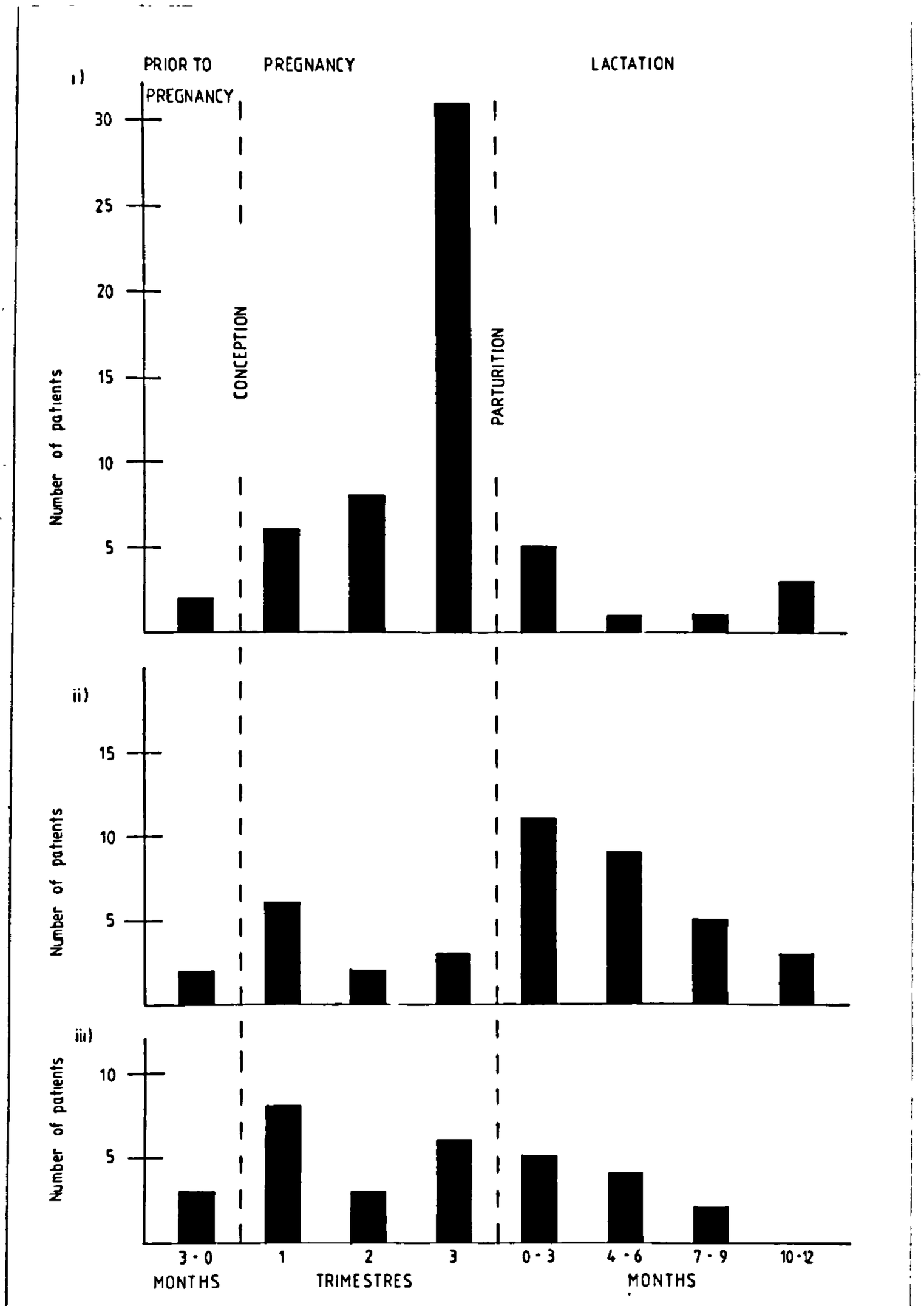


Figure A1(i). Time of first occurrence of complications of leprosy prior to conception and during pregnancy and lactation.

i. Worsening of leprosy status.
ii. Reversal (type 1 lepra) reaction.
iii. ENL (type 2 lepra) reaction.

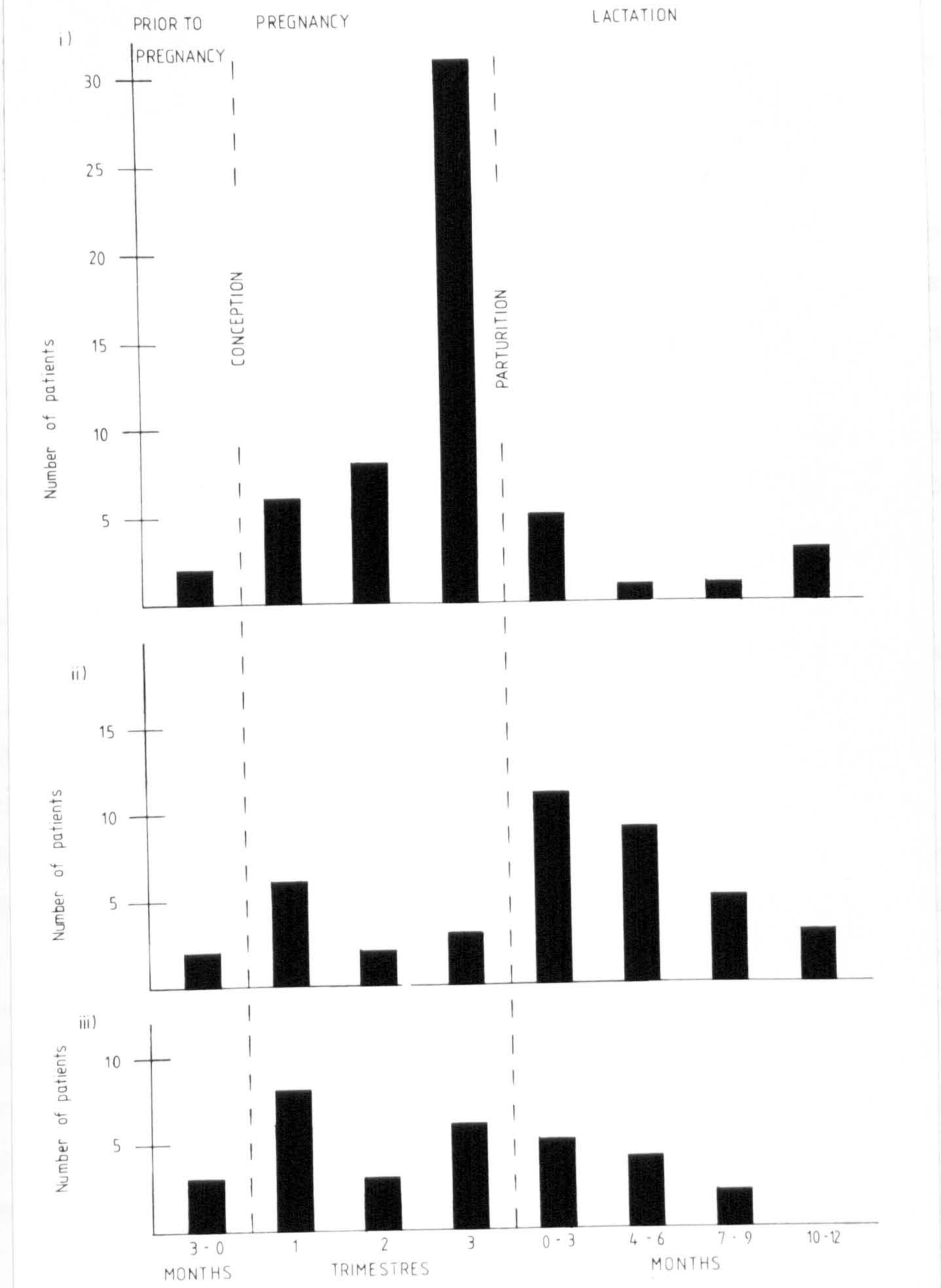


Figure A1(i). Time of first occurrence of complications of leprosy prior to conception and during pregnancy and lactation.

i. Worsening of leprosy status.
ii. Reversal (type 1 lepra) reaction.
iii. ENL (type 2 lepra) reaction.

(1 LL, 1 BL) had shown increased activity of slit skin smears prior to conception. By comparison, 55 (35.3%) showed worsening of their leprosy status in association with pregnancy or the first 12 months of lactation. In 43 of the 55 women the deterioration occurred during the second half of pregnancy or the first 3 months of lactation, most commonly (31 cases) during the third trimestre.

In 21 cases the deterioration was transient with increased activity (in skin lesions, slit skin smears or biopsy) most often in the third trimestre, which disappeared during lactation. However, in the remaining 34 cases (22% of those studied) the deterioration was significant and progressive. In many of the patients with lepromatous leprosy, deterioration was due to dapsone resistance, for details see pp. 110–114.

ii) Reversal (type 1 lepra) reaction

The number of patients who were diagnosed as having reversal reaction and the timing of the first episode in relation to pregnancy/lactation is shown in Table A1(ii) and Figure A1(i). Two women developed the reaction shortly after a previous pregnancy and within 3 months of the pregnancy under study. After an initial increase in the numbers of women with reaction in the first trimestre, the number of new cases dropped during the second and third trimestres and then increased sharply after delivery, decreasing only gradually within the first year of lactation. Reversal reaction often continued for many months. Figure A1(ii) shows the number of patients who showed evidence of reaction in each 3 month period, and demonstrates the magnitude of the problem during lactation. It should be noted, however, that this figure includes patients with "silent neuritis"; this is probably caused by reversal reaction, but histological proof of the aetiology has not as yet been obtained (Duncan and Pearson, 1982).

Site of reaction. Reaction in skin, or skin and nerve, was a feature of pregnancy and early lactation (especially with regard to the first occurrence); reaction in nerve alone was a marked feature of lactation and occurred in only 2 patients during the second half of pregnancy (Table A1(iii). Where reversal reaction occurred in skin during late lactation, it was always in association

TABLE A1(ii) INCIDENCE OF TYPE 1 AND TYPE 2 LEPRA REACTION ACCORDING TO CLASSIFICATION OF LEPROSY.

* Final Classification of Leprosy	Number of Patients *	Number of Pregnancies Studied *	Number of patients developing reactions		
			Type 1 lepra reaction	Type 2 lepra reaction	Type 1 and Type 2 Reaction Concurrently
TT and BT "Cured" and "Active"	40	41	11 (27%)	0	0
BL	44	45	19 (42%)	10 (22%)	3 (7%)
LL	32	35	10 (29%)	18 (51%)	2 (6%)

* The initial classification and grouping of patients has been revised to include new cases (previously HC) and relapse cases (previously TT and BT "Cured") under appropriate classifications.

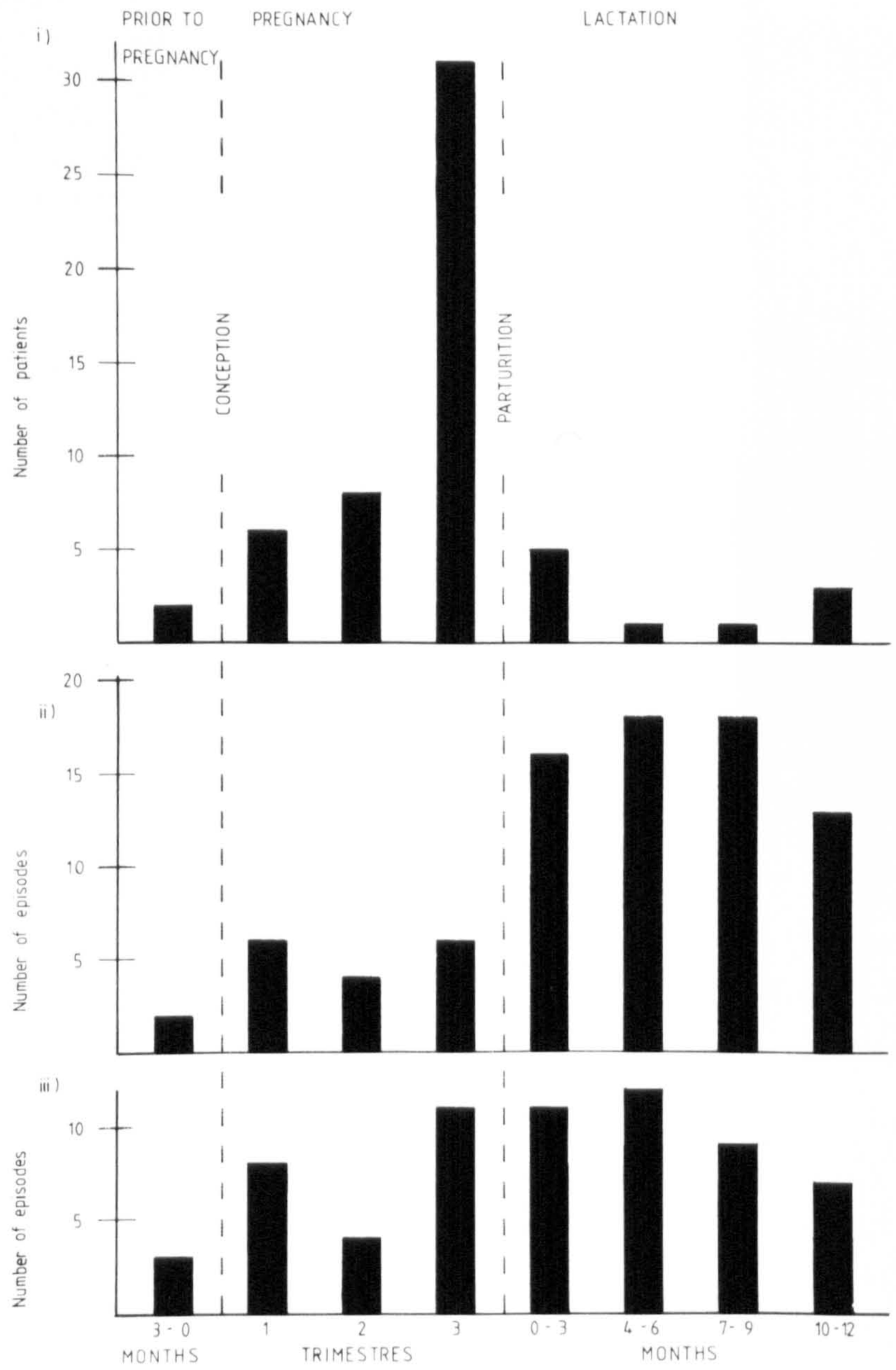


Figure A1(ii). Time of occurrence of complications of leprosy prior to conception and during pregnancy and lactation.

i. Worsening of leprosy status - first occurrence.

ii. Reversal (type 1 lepra) reaction - first, recurrent and continuing episodes.

iii. ENL (type 2 lepra) reaction - first, recurrent and continuing episodes.

TABLE A1(iii) NUMBER OF PATIENTS DEVELOPING TYPE 1 LEPRA REACTION IN PREGNANCY AND LACTATION - SITE OF REACTION .

	3 MONTHS PRECEDING PREGNANCY	PREGNANCY (TRIMESTRE)			LACTATION (MONTHS)				TOTAL PATIENTS	TOTAL EPISODES
		1	2	3	0-3	4-6	7-9	10-12		
SKIN ONLY		1	2 (1)	2 (1)	2 (2)	2 (1)	2 (2)	1 (1)	4	12
SKIN & NERVE		1	1 (1)	2 (1)	3 (1)	1	1 (1)	1 (1)	6	10
NERVE ONLY	2	4	1	2 (1)	11 (2)	14 (8)	14 (10)	11 (8)	28	59
UPGRADING *						1	1		2	2
TOTAL	2	6	4 (2)	6 (3)	16 (5)	18 (9)	18 (13)	13 (10)	40	83

Number in brackets ( ) denotes the number of patients developing recurrent episodes of type 1 reaction.

* Upgrading was diagnosed histologically with no clinical evidence of type 1 reaction.

with worsening of the patient's leprosy status. For full details of patients with neuritis, see pp. 119-122.

Downgrading and upgrading. These were diagnosed histologically in all cases. Downgrading from BL to LL occurred in 5 patients. In 3 the phenomenon was observed during the third trimestre, in one immediately postpartum and in one at 6 months postpartum in association with worsening of the leprosy status. Upgrading reaction was observed in 6 patients after delivery, in 4 of whom there was concomitant clinical evidence of reaction in skin or nerve.

iii) ENL (type 2 lepra reaction)

The number of patients diagnosed as having ENL and the timing of the first episode in relation to pregnancy/lactation are shown in Table A1(ii) and Figure A1(i) respectively. Three women had reaction during the 3 months preceding the pregnancy under investigation; in 2 cases the reaction followed a previous pregnancy. After a peak in the first trimestre, there was a rise in incidence of reaction in late pregnancy and the first 6 months of lactation. Figure A1(ii) shows the timing of the recurrent episodes of ENL: most of them occurred during the third trimestre and the first 9 months of lactation. Fuller details of these patients are given in pp. 115-118.

Mixed reactions

Reversal reaction and ENL occurred concurrently in 5 patients, all of whom were upgrading during lactation; one patient had previously downgraded during pregnancy. In 4 cases there was also some evidence of worsening of the leprosy status.

Illustrative Case Reports

1. This case illustrates relapse associated with downgrading during pregnancy, followed by active upgrading and reaction with a mixture of reversal reaction and ENL postpartum, and a further relapse with downgrading with suspected dapsone resistance.

Case Report A.D. A9/36

A.D., a primigravida, aged 31, with BL leprosy treated irregularly for 14 years with dapsone monotherapy, showed the first sign of relapse in the second trimestre (BI = 3.8, MI = 6%). She had

extensive skin infiltration but no evidence of new nodules. Biopsy during the third trimestre showed "LL hyperactive lesion with necrotising exacerbation reaction". Two weeks postpartum she developed a marked skin reaction with erythema and oedema of the skin lesions: biopsy of one such lesion was reported as "BL active with no evidence of reaction". Five months post-partum the skin reaction noted immediately postpartum had disappeared, but she now had extensive skin ENL, skin smears showed BI = 3.5, MI = 2%. A biopsy showed "BT; a strong upgrading reaction producing epitheliod and giant cell granuloma with necrotic foci and many polymorphs, much like ENL." A month later the biopsy was reported as "BT; there is a mild reaction but polymorphs are no longer present. Probably active upgrading." Twenty-four months after delivery A.D. had a further active relapse (associated, probably, with taking dapsone irregularly). Skin smears showed BI = 4.0, MI = 5%. Biopsy was reported as "LL, highly active".

2. This case shows relapse with dapsone resistance following pregnancy and then very marked upgrading while on effective chemotherapy following a subsequent pregnancy. The active reversal reaction was evident histologically for 2 years postpartum.

Case Report G.T. A9/214

G.T. developed leprosy at the age of 11, started treatment as BL on dapsone monotherapy at the age of 15, and because of "reaction" was treated irregularly and with low dose dapsone for 5 years. At the age of 21 she had a clinical relapse after her first pregnancy (BI = 3.3, MI = 2%). The following year the biopsy was reported as "LI active with histoid features", and at the age of 23 after a further rise in BI and MI, she was started on treatment with alternative therapy for dapsone resistant leprosy, proven by mouse foot pad tests. Her second pregnancy was uneventful but postpartum she complained of intermittent rheumatic pain, and during the next year in association with skin ENL, she showed histological evidence of a marked upgrading reaction with successive biopsies reported as follows: "LLs highly active", "BL in reaction", "BB - BT, apparently upgraded with mild reactional oedema" and "BT, active reversal reaction". A year later her biopsy was reported as "BT - BL, large lesions with much oedema suggests reversal reaction although no giant cells were seen", and 6 months later a biopsy showed "BL regressing".

3. This case history illustrates downgrading following delivery with ENL developing 3 years after the start of treatment and in association with lactation.

Case Report Z.B. A9/27 and A9/260

Z.B. developed leprosy at the age of 20 and 5 years later was diagnosed as having BL leprosy with the biopsy showing "BL, very

active lesion", (BI = 4.8, MI = 1%). Treatment with dapsone monotherapy 100 mg daily was started. She had an uneventful first pregnancy in the study (her third pregnancy, but the first by her second husband). The baby, who was small for gestational age, unfortunately developed acute respiratory distress syndrome after being born with an Apgar of 1/10, and died on the fourth day of life. Z.B. thus entered the study for a second time with her fourth pregnancy. During the third trimestre her biopsy was reported as "LL with some evidence of activity." She had persistent troublesome ENL postpartum with tender lymphadenopathy. A skin biopsy was reported as "LL regressing".

2. New cases, relapse of cured patients and deterioration in patients on treatment during pregnancy and lactation

One hundred and fourteen women with leprosy and 33 women without leprosy were studied during 120 and 36 pregnancies respectively. For simplicity, each pregnancy is considered as one woman. Two healthy controls developed leprosy during the study period; 12 of 25 women with cured tuberculoid leprosy relapsed with new lesions or nerve damage; 47 of 95 women with active tuberculoid and lepromatous leprosy showed increased activity of their leprosy either as a transient phenomenon (21 patients) or due to probable dapsone resistance (28 patients). The deterioration in leprosy status occurred chiefly during the third trimestre (Figure A2(i)).

Healthy Controls (HC)

Of 33 women observed during and after 36 pregnancies, 2 developed leprosy. Both had been BI negative and had had no sign of leprosy when first assessed. One asymptomatic woman developed a hypo-pigmented macule during the third trimestre of pregnancy which on biopsy showed indeterminate leprosy. Postpartum the lesion grew in size but the woman had no complications of leprosy. The second woman complained of severe "rheumatism" at 10 weeks postpartum when she was found to have enlarged nerves which on biopsy showed active BL leprosy. Hypopigmented skin lesions and skin infiltration were apparent by 6 months postpartum.

"Cured" tuberculoid and borderline tuberculoid leprosy (TT and BT/RFC)

Of these 25 patients, 9 relapsed with active leprosy (6 BT; 3 BL) within periods of 3 months to 3 years after stopping treatment (Figure A2(ii)). Eight of the 9 relapses were diagnosed on clinical grounds, 7 were confirmed on biopsy; 3 were BI positive. Five out of the 9 relapses occurred during the third trimestre of pregnancy.

In addition, 3 patients were considered to have incipient relapse on the evidence of new nerve enlargement or neuritis though skin biopsies and BI were negative.

Ten out of 12 patients relapsed in association with the first pregnancy and 2 during the second pregnancy after RFC. Details of clinical features and investigations are shown in Table A2(i).

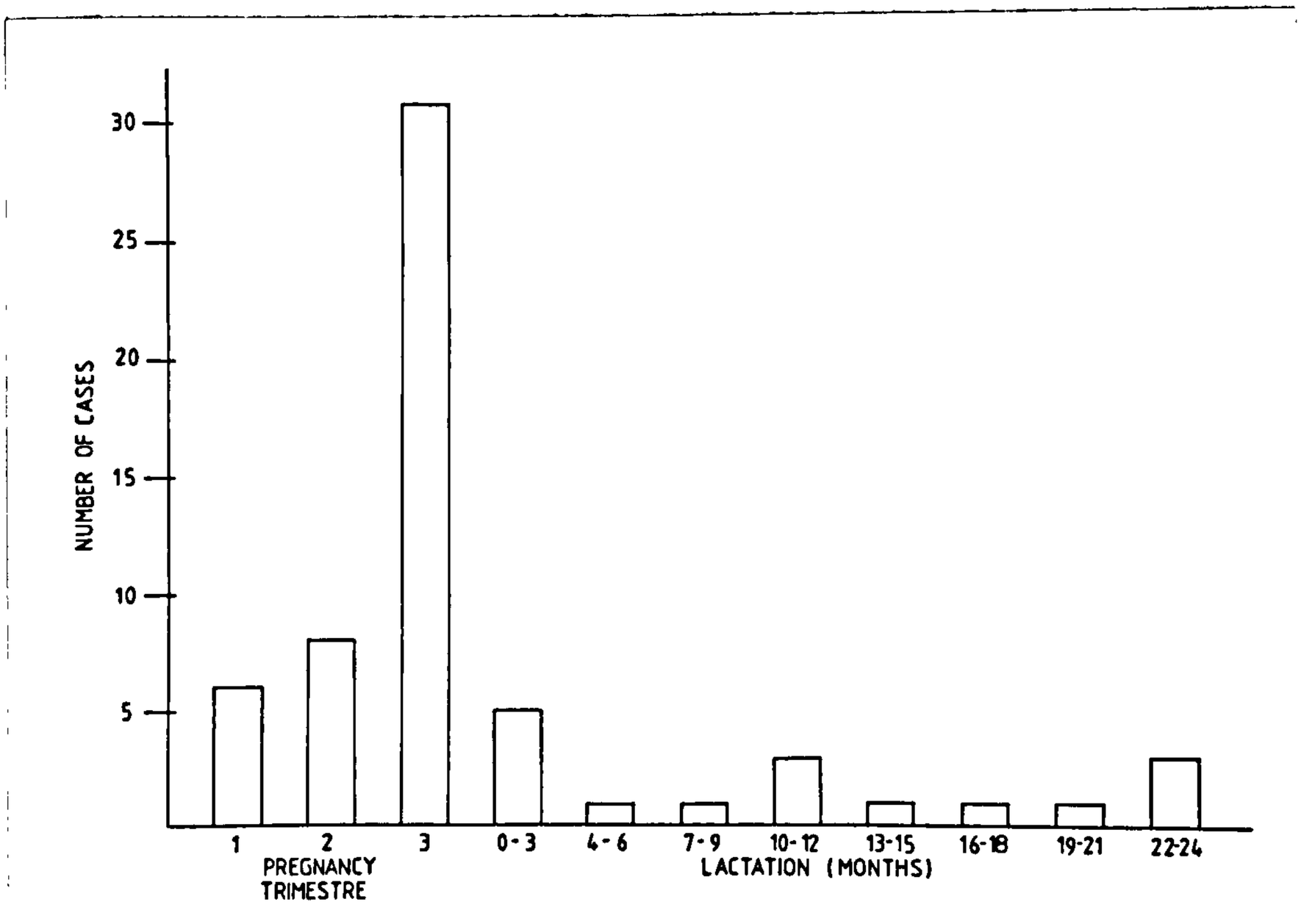


Figure A2(i). Timing of first evidence of deterioration of leprosy status (overt leprosy, relapse in "cured" TT and BT patients, increased activity of disease in TT, BT, BL and LL patients receiving treatment for leprosy) in relation to pregnancy and lactation.
(Note: The 4 patients relapsing between 19 and 24 months had not been assessed during the preceding 12 months: from the history and/or clinical findings, it is very likely that relapse had occurred by 12 months postpartum.)

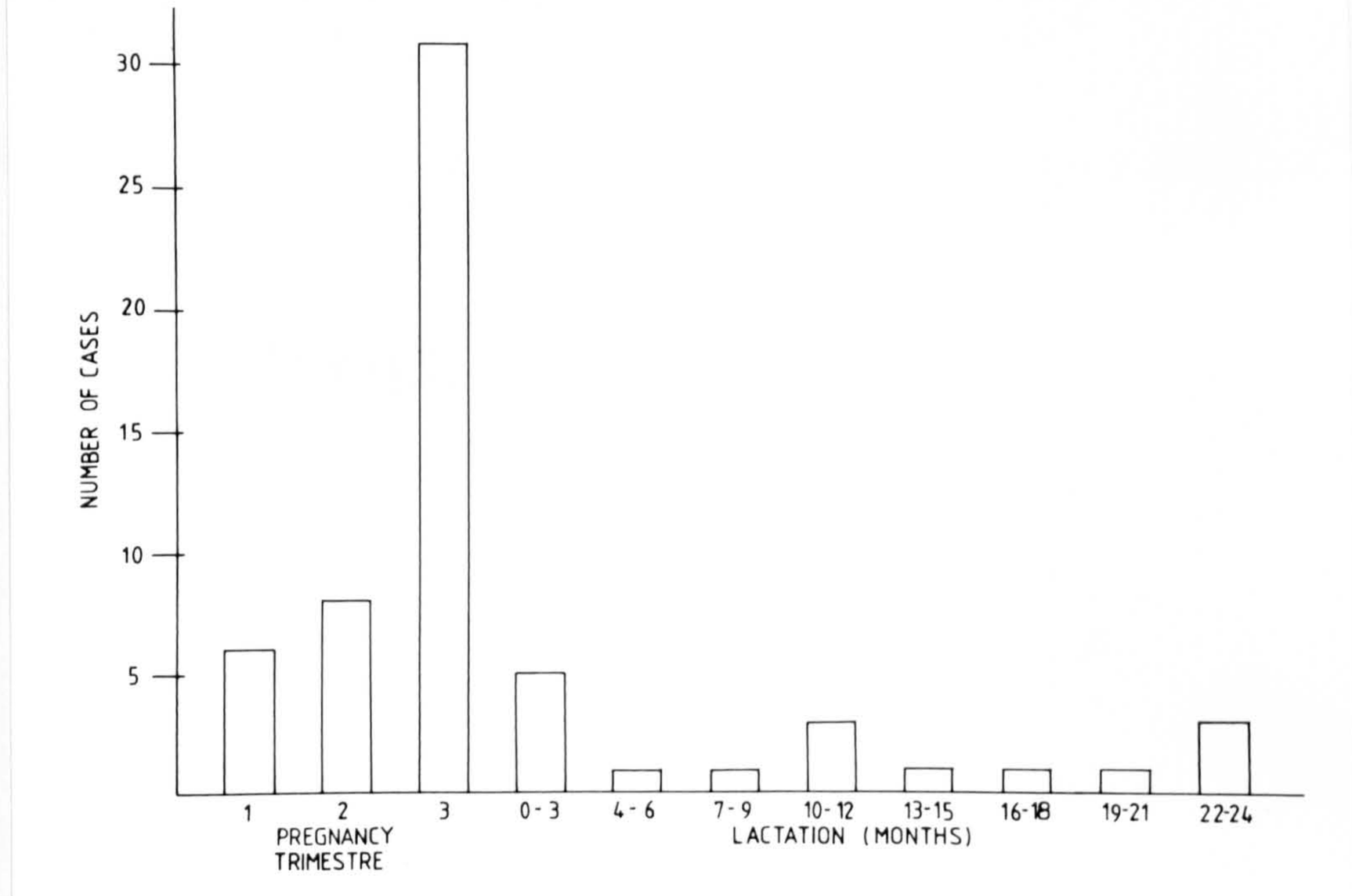


Figure A2(i). Timing of first evidence of deterioration of leprosy status (overt leprosy, relapse in "cured" TT and BT patients, increased activity of disease in TT, BT, BL and LL patients receiving treatment for leprosy) in relation to pregnancy and lactation.
(Note: The 4 patients relapsing between 19 and 24 months had not been assessed during the preceding 12 months: from the history and/or clinical findings, it is very likely that relapse had occurred by 12 months postpartum.)

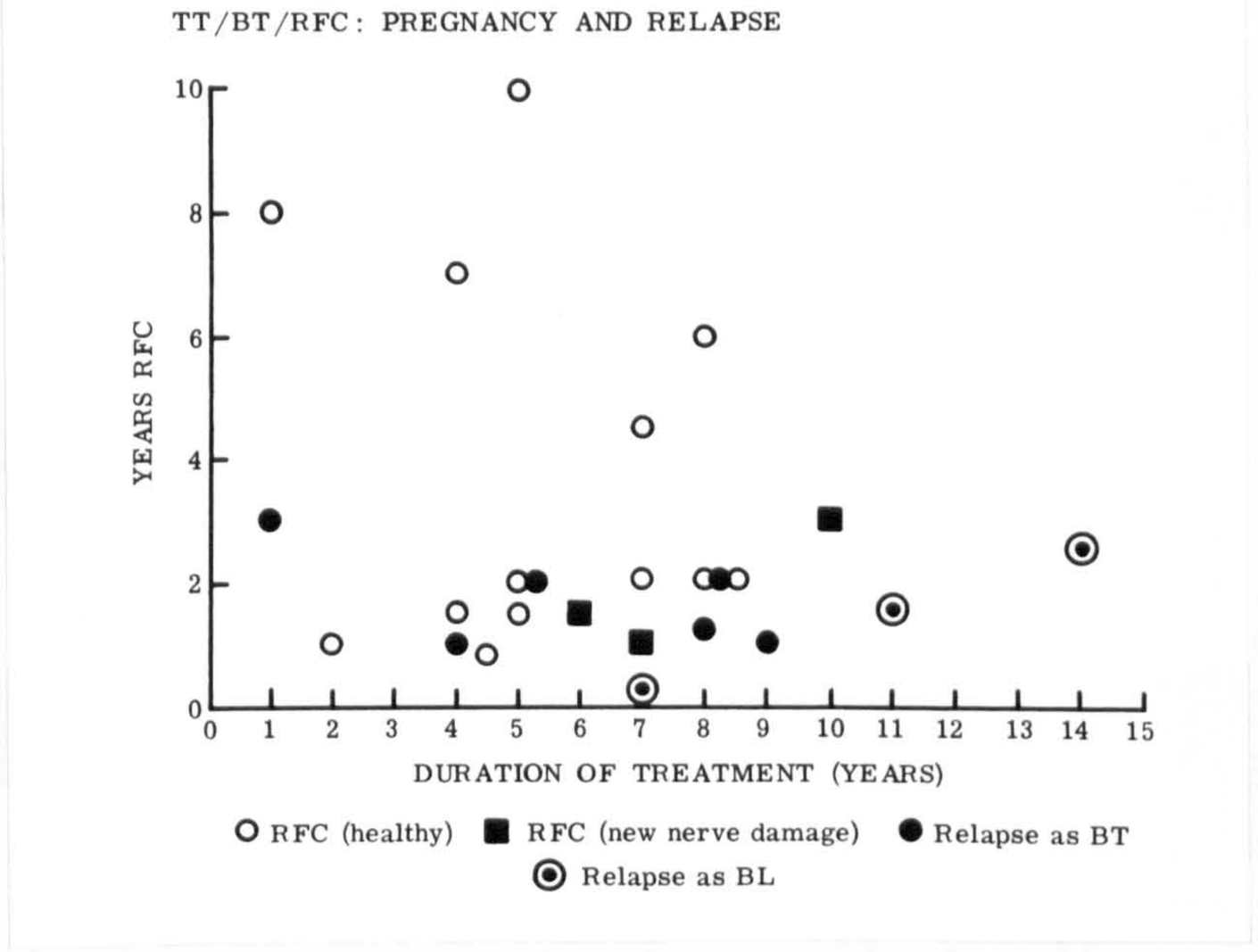


Figure A2(ii). Relapse of "cured" TT and BT patients in association with pregnancy and lactation, is shown in relation to the number of years of treatment prior to "cure" (RFC) on the abscissa and the years after stopping treatment before relapse occurred on the ordinate. The patients who relapsed whether as BL, BT or with new nerve damage (incipient relapse) all did so within 3 years of stopping treatment.

TABLE A2(i)

TT/BT/RFC CASES SHOWING RELAPSE IN ASSOCIATION WITH PREGNANCY

No.	PArity	Original Diagnosis	Duration of Treatment (Years)	Years RFC before present pregnancy	Symptoms	Clinical Features	Additional Tests	Clinical Diagnosis at Relapse	Histological Diagnosis	BI	Final Diagnosis	Timing of Relapse
'A' - Relapce Cases												
1	1 + 0	"T"	7	3/12	-	1 active new macule face; nerves normal		BT Active	Skin: BL, early, active AFB 3-4+ in granuloma	2.3	BL	3rd TM during 1st pregnancy after RFC
2	2 + 0	BT	11	1½	Rheumatism	New nodules ++ on legs; nerves normal		BL Active	Skin: Solid AFB in nerves and deep dermis	3 +	BL	3rd TM during 1st pregnancy after RFC
3	5 + 0	BT	14	2½	-	Inactive skin; nerves normal		Quiescent BT	Not done	0.8 (4+ at one site)	BL	3rd TM after 2nd pregnancy after RFC
4	1 + 0	BT	1	3	-	8 new macules (legs & arms); 6 enlarged nerves; New motor & sensory loss		BT Active	Skin: early BT. Nerve: early tuberculoid lepr.	0	BT	Lact. after 1st pregnancy after RFC
5	0 + 1	BT	4	1	"Burning" Paraesthesiae	New macule, face skin reaction neuritis	EMG active demyelination	BT Active	Skin: Normal. Nerve: BT active	0	BT	1st TM during 1st pregnancy after RFC
6	3 + 0	"T"	9	1	-	New macule face; nerves normal		BT Active	Not done	0	BT	Lact. after 1st pregnancy after RFC
7	1 + 0	BT	8	1¼	-	New nodules on face & ears; 3 enlarged nerves		BL Active	Skin: BT active. Solid AFB in nerve	0	BT	2nd TM during 1st pregnancy after RFC
8	1 + 0	BT	8	2	Rheumatism	2 new macules face; nerves normal		BT Active	Skin: BT active	0	BT	3rd TM during 2nd pregnancy after RFC
9	4 + 0	BT	5	2 *	"Burning" Paraesthesiae	Active erythematous edge of old macule, face; 3 enlarged nerves		BT Active	Skin: TT/BT active	0	BT	3rd TM during 1st pregnancy after RFC
'B' - Incipient Relapse Cases												
1	4 + 0	TT/BT	6	4/12	Rheumatism	Skin inactive; 2 enlarged nerves		Old BT	Skin: Almost normal	0	BT	Lact. after 1st pregnancy after RFC
2	3 + 0	BT	7	1½	Rheumatism	Skin inactive; 3 enlarged nerves	VMT & ST Motor & sensory loss	'Burnt Out BT'	Skin: Within normal limits	0	BT	Lact. after 1st pregnancy after RFC
3	2 + 0	BT	10	3 *	Rheumatism	Skin inactive; 1 enlarged nerve	VMT Motor loss	Inactive BT	Not Done	0	BT	Lact. after 1st pregnancy after RFC.

* RFC during previous pregnancy

Active tuberculoid leprosy (TT and BT)

Of 18 patients all on dapsone monotherapy, 8 had increased activity of the skin lesions, usually in the third trimestre, without any histological evidence of reaction. In 3 cases the lesions appeared more active with raised erythematous margins; in 4 cases there was conversion from BI negative to postive. In 2 cases there was increase in size and number of the skin lesions during lactation. In 5 cases this was a transient phenomenon.

Lepromatous leprosy (BL and LL)

Sixty-eight women (36 BL, 32 LL) were studied through 72 pregnancies and followed up after delivery. (Four others were assessed only during pregnancy.) Increased activity was found in 39 (54%) during pregnancy, puerperium or lactation (in 21 during the third trimestre or puerperium). At the time that increased activity was first observed, 18 out of the 39 patients had new nodules (a further 7 developed new skin lesions later); in 35 cases the increased activity was confirmed by a rise in BI, in 4 cases by biopsy only. In 16 cases the increased activity was a transient phenomenon, with fall in BI to less than the pre-pregnancy levels or reversion of the active biopsy to "LL regressing" during early lactation. However, 6 patients then went on, within 11-15 months, to show a subsequent rise in BI and clinical evidence of relapse during late lactation or the next pregnancy. The phenomenon of transient increase in activity was shown by patients receiving dapsone monotherapy, dual therapy with dapsone and rifampicin and clofazimine monotherapy.

Other clinical features associated with increased activity of leprosy (BL and LL)

The complaint of "rheumatism" either preceded or accompanied the increased activity of the disease in more than half of the patients. Erythema nodosum leprosum (ENL) preceded or accompanied the increased activity of the disease in one half of the patients, occurring for the first time during pregnancy in most cases. In contrast, reversal reaction was seen postpartum when recovery of CMI would be expected to occur. New nerve enlargement was observed in one-third of the patients, and neuritis (due to ENL or reversal reaction) was observed in more than half of the patients

who had increased activity of the disease, including those who had only a transient rise in BI during pregnancy. Details of patients with relapse due to dapsone resistance, with ENL, with neuritis and with "rheumatism" are given below.

Relapse in association with downgrading phenomena (BL and LL)

Six patients (all BL) downgraded from BL to LL, 5 during pregnancy and 1 during lactation. They were diagnosed on histology, but 3 also had clinical evidence of relapse due to dapsone resistance. In addition, 3 relapse patients showed, for the first time, the histological features of polar lepromatous leprosy (LL_p: Ridley and Waters, 1969) during the third trimestre or immediately after delivery. After delivery 3 of the patients who had downgraded to LL upgraded to BL or BT.

Illustrative Case Report

This case history illustrates the reactivation of skin lesions in a case of tuberculoid leprosy as a transient phenomenon during pregnancy, but with the suspicion of dapsone resistance emerging.

Case Report L.Z. A9/223 and A9/264

> L.Z. came from Gojam to Addis Ababa in 1969 on account of leprosy of 3 years' duration. She was diagnosed as having BT leprosy. Treatment with dapsone monotherapy was started, but because of frequent episodes of neuritis (at that time attributed to dapsone therapy), alternative therapy (thiambutosine) together with prednisolone was used for the next 3 years. Dapsone monotherapy was restarted in 1972. Four years later, during her first pregnancy in the study (1976) (she had had 3 pregnancies before), she was observed to have increased activity of her lesions, the margins of which were raised with an erythematous edge. Skin smears were negative but biopsy was reported as "BT slightly active". The baby died at the age of 7 days of a respiratory infection, thus L.Z. was included for a second time in the study with her fifth pregnancy. During early pregnancy her lesions had appeared inactive, but in the third trimestre she was again observed to have increased activity of the lesions with raised erythematous edges. Biopsy from one of these active lesions was reported as showing "Almost normal skin." One solid AFB was observed in a nerve bundle. Skin smears from standard sites showed the presence of 1^+ from the left ear, granular AFB. Seven months postpartum her skin lesions appeared quiescent, skin smears were negative, but she complained of rheumatism of recent onset and was found to have new nerve enlargement.

3. Pregnancy and dapsone resistant leprosy

Sixty-seven women (35 BL, 32 LL) were studied throughout 72 pregnancies. They were all receiving outpatient treatment for leprosy when first seen and taken into the study.

Assessment of leprosy was made during pregnancy and after delivery at 6 month intervals whenever possible. This included inspection of skin lesions, clinical drawings, palpation of nerves and regional lymphnodes, and slit skin smears and biopsies. When a patient was suspected of having developed dapsone resistant leprosy :

i) she was put onto chocolate coated dapsone 100 mg/day under trial conditions; or

ii) if her deterioration was extremely rapid during late pregnancy such as to cause a threat to her life or serious danger to the foetus, she was put immediately on to one of the dual/triple drug trial regimes for treatment of dapsone resistant leprosy.

In some cases, biopsy of an active skin lesion, with a positive MI, was taken and tested for dapsone resistance by the mouse foot-pad technique (Rees, 1967; Pearson, Rees and Waters, 1975). Resistance to dapsone was defined as multiplication of M. leprae in mouse foot-pads at a concentration of dapsone 0.0001% or more in the diet (Pearson et al, 1975).

For simplicity, each pregnancy is considered as one patient.

At the start of the study 6 patients (7 pregnancies) were already diagnosed as dapsone resistant, and were taking clofazimine or rifampicin plus thiacetazone and dapsone. An additional 5 patients were suspected of dapsone resistance, and were receiving dapsone 100 mg daily under trial conditions with the maximum possible supervision and frequent assessments. Thus the initial prevalence of proven and suspected dapsone resistant leprosy was 11/72, 15%; that is, much the same as the general prevalence among lepromatous patients at that time. The remaining patients were receiving dapsone 100 mg daily under routine outpatient clinic supervision.

During the course of the study an additional 24 patients showed clinical and/or bacteriological or histological deterioration despite apparently continuing to take dapsone, and were therefore considered to have prima facie evidence of dapsone resistant leprosy. In the majority of cases the diagnosis was clinical; the patients showed new active skin nodules, in which the BI and MI were raised. However, nearly half the patients (10/24) gave indications of relapse on routine skin smears and/or biopsies before new skin lesions became evident, and an additional 3 cases showed definite relapse on smears/biopsies when there was only minimal clinical evidence of relapse.

A striking feature of clinical relapse in these patients was the rapidity of development and increase in number of skin lesions after routine smears gave indications of relapse. The most rapid deterioration occurred toward the end of pregnancy when 7/24 women showed marked clinical deterioration during a 3 month period including part or all of the third trimestre. An additional 5 patients showed moderately rapid deterioration starting in the third trimestre and extending into the first 6 months of lactation.

It was not possible to perform mouse foot-pad tests for dapsone resistance in all cases. In some cases, because of shortage of mice, foot-pad tests were only done with DDS in low dietary concentrations. There were 4 "technical failures" on account of delays in the biopsied material reaching the U.K. laboratory; in these cases repeat biopsies were not carried out as alternative dual therapy had been instituted on account of rapid deterioration during pregnancy. Therefore results of the 7 patients tested successfully during the study are shown in Table A3(i). Of the patients whose detailed results are known, 11/11 were resistant at 0.0001% DDS in diet, 6/8 were resistant at 0.001% DDS in diet, and 6/6 were sensitive at 0.01% DDS in diet. None of the patients tested was proved dapsone sensitive.

The timing of relapse is shown in Table A3(ii). Half the patients (14/28; 15/29 pregnancies) relapsed clinically in the third trimestre (in some cases a rising BI was detected earlier in the pregnancy) and most of the remainder within 6 months after delivery.

TABLE A3(i)

Results of mouse foot-pad assessment of dapsone sensitivity

No. of Patients	Patient Number	Concentration of DDS in Mouse Diet: 0.01% (Human Equivalent Dose 100 mg)	0.001% (10 mg)	0.0001% (1 mg)	Conclusion
Tested during study	7	1	ND	+	+	R
		2	0	+	+	R
		3	0	+	+	R
		4	0	+	+	R
		5	0	ND	+	R
		6	0	0	+	R
		7	0	0	+	R

ND = not done

R = resistant

\+ = growth of bacilli

0 = no growth of bacilli.

TABLE A3(ii)

Timing of clinical relapse due to probable dapsone resistance

Classification of mother's leprosy	No. of pregnancies studied	No. of Relapses	Timing of clinical relapse										
			Pregnancy Trimestre			Lactation (by 3 month periods)							
			First	Second	Third	0–3	4–6	7–9	10–12	13–15	16–18	19–21	22–24
BL	36	15	2	1	8	2	1	–	1	–	–	–	–
LL	35	13 (14)*	–	2	6 (7)*	2	1	–	1	–	–	1	–
TOTAL	71	28 (29)*	2	3	14 (15)*	4	2	–	2	–	–	1	–

( )* Includes 2 pregnancies of 1 patient who relapsed during two consecutive pregnancies in the study.

In addition to these patients, all 5 patients already suspected of dapsone resistance and being treated under trial conditions, showed further deterioration during this study (4 in the third trimestre or lactation, 1 at the end of the first trimestre).

The complaint of "rheumatism" was commonly associated with relapse, it preceded relapse in about half the patients, and was almost always a complaint at the time of relapse. ENL and neuritis preceded relapse in about half the patients (but were not uncommon in smear positive cases who did not relapse). At the time when relapse became clinically evident, ENL was observed in half (14/28) of the patients. ENL was much more common than might be expected in BL patients, being recorded during pregnancy in 25% (9/36) of them: ENL was seen more frequently in BL patients who relapsed (33%: 5/15) than in BL patients who did not relapse (19%: 4/21).

During the course of the study it became evident that there was an unexpectedly high incidence of probable dapsone resistant leprosy associated with pregnancy. Therefore case records of women already diagnosed as suffering from dapsone resistant leprosy were reviewed, and the patients interviewed. An obstetrical history was obtained from 42 patients; 36 of them had had children after starting anti-leprosy treatment, of whom 31 first noticed new relapse nodules during pregnancy or soon after delivery or after a spontaneous abortion (Figure A3(i)). Only 5 relapsed independently of pregnancy. The patients themselves were well aware that pregnancy had made their leprosy worse.

Illustrative Case Reports

1. The following case history illustrates the stepwise appearance of dapsone resistance under trial conditions in two successive pregnancies. Because of rapid deterioration during pregnancy, foot-pad tests were not carried out.

Case Report E.A. A9/84 (a and b)

E.A. developed overt leprosy at the age of 15 in 1968 when she was living in Gojam. She was treated there for 2 years from 1971-73, for the next year she had no treatment because of moving to Addis Ababa. Treatment was re-started in 1974

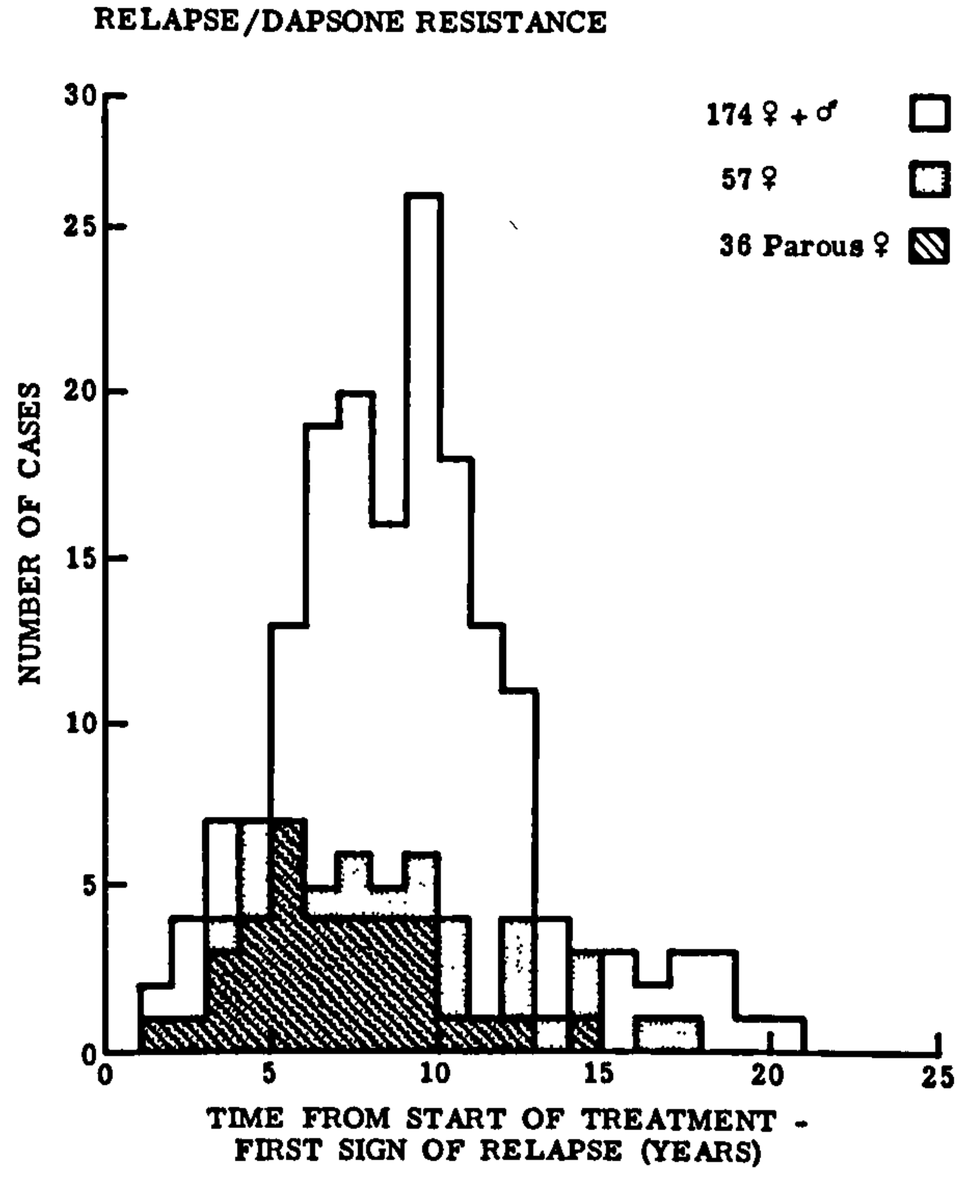


Figure A3(i). Duration of treatment (years) from start of treatment to first sign of relapse due to dapsone resistance, according to sex and parity of the patients.

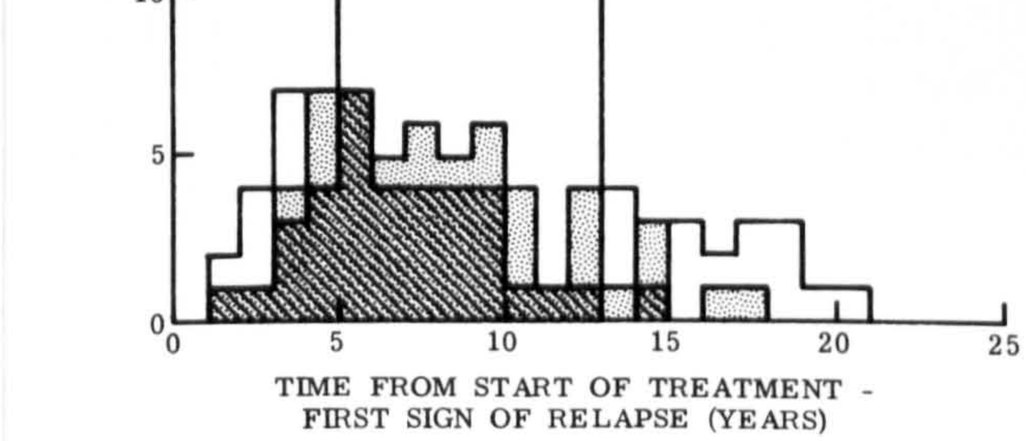


Figure A3(i). Duration of treatment (years) from start of treatment to first sign of relapse due to dapsone resistance, according to sex and parity of the patients.

when BI = 3.3. In December 1975 she showed clinical evidence of response to dapsone treatment 50 mg per day. In September 1975 she conceived her first pregnancy. During the third trimestre she developed crops of new nodules on arms and legs and in the ninth month of gestation BI = 4.3, MI = 4%. Biopsy of one of the active lesions was reported as "LL highly active". She delivered at term a male macerated anencephalic foetus weighing 2,380 g; the placenta was discarded. Post-partum her new nodules began to disappear; five months postpartum BI = 3.7, MI = 0.2%. However, in view of the clinical evidence of relapse with many active nodules and macules, she was considered to be developing dapsone resistance, and was started on chocolate coated dapsone 100 mg/day under trial conditions. The biopsy result reported later was: "LL, dense bacterial load with extremely active infiltration, AFB mostly granular". In April 1977, when she was 7 months pregnant, skin smears showed BI = 4.7, MI = 8%. At 9 months of gestation she was considered to have further clinical deterioration of her leprosy status during pregnancy; chocolate coated dapsone 100 mg/day under trial conditions was stopped, and she was started on clofazimine 100 mg twice daily with thiacetazone (given as thiazina, T.B. 450 1 tablet daily). She delivered at home a live male infant. At 6 months postpartum, despite showing clinical response to treatment in terms of flattening of the new nodules, she complained of having had "rheumatism" since delivery. This was found to be associated with new ulnar nerve enlargement and generalised lymphadenopathy.

2. This case history demonstrates the development of dapsone resistance in stepwise fashion under trial conditions, together with downgrading following and during two successive pregnancies.

Case Report N.T. A9/89

N.T. developed leprosy as a child at the age of 4. At her first hospital visit when she was 10 years old, she was recorded as having a history of nodules for 6 years. The nodules were situated on face, ears, buttocks, arms, thighs (both extensor and flexor surfaces). In addition, she had 3 scars on her buttocks from local cautery (treatment for leprosy). She was diagnosed as having BB leprosy and was treated with dapsone monotherapy from 1958 to 1976, with a break of treatment for 3 years from 1963-66. After her third pregnancy in 1973 the biopsy is reported: "BL active, looks as if it had downgraded from BB". She had at that time a rising BI (BI = 4.8, MI = 0). The possibility of dapsone resistance was considered and she was put on to dapsone by injection 1.5 cc/week. By January 1976 BI, which had fallen, had risen again and MI had become positive (BI = 4.2; MI = 4%). She was followed carefully through her pregnancy and in the third trimestre a biopsy was reported as showing "LL very active". Postpartum she had a further

rise in BI and MI and was put on to alternative dual therapy after mouse foot pads had been set up for assessment of dapsone resistance. She showed rapid response in clinical and bacteriological terms. Six months after her change of treatment, a biopsy was reported as "LL with no solid AFB seen, infection controlled by treatment, but not upgrading."

4. Erythema Nodosum Leprosum (ENL) in pregnancy and lactation

Seventy-six women with lepromatous leprosy were studied during 81 pregnancies and followed up during lactation. The classification according to Ridley and Jopling (1966) was :

Borderline lepromatous leprosy (BL)	- 44 women (45 pregnancies)
Lepromatous leprosy (LL_p and LL_s)	- 32 women (36 pregnancies)

Patients were admitted to the study over a 12 month period and followed up for varying lengths of time up to 24 months postpartum; the 3 months prior to pregnancy provided a baseline for the prevalence of complications of leprosy in women of child-bearing age. The incidence of ENL and increased activity of leprosy (relapse) are expressed as a percentage of the total number of women in the study in any 3 month period.

Incidence of ENL

Thirty out of 79 patients (37%) developed ENL during the course of the study; 10 out of 45 BL patients (22%) and 20 out of 36 LL patients (56%). Twenty-nine of these patients had clinically diagnoseable ENL in the skin on one or more occasions; the thirtieth, originally BT/RFC, developed iridocyclitis.

Occurrence of ENL in association with duration of treatment (Figure A4(i))

Fourteen patients (47%) had ENL within 4 years of the start of effective treatment for leprosy, either initial treatment with dapsone, or alternative therapy in the case of the 6 dapsone resistant patients (7 pregnancies). All but one of these were BI positive. The remaining 16 patients showed ENL after 6 to 14 years of treatment with dapsone. Only 3 were BI negative. Two patients who had been on treatment for 11 years and were BI positive, had had 2-3 years of additional treatment with clofazimine for persistent ENL. Twelve of the 30 patients were suspected of developing dapsone resistance during the study period.

Timing of first episodes of ENL during the study

The periods during which patients developed their first episodes of ENL during the study are shown in Figure A4(ii)b. The incidence

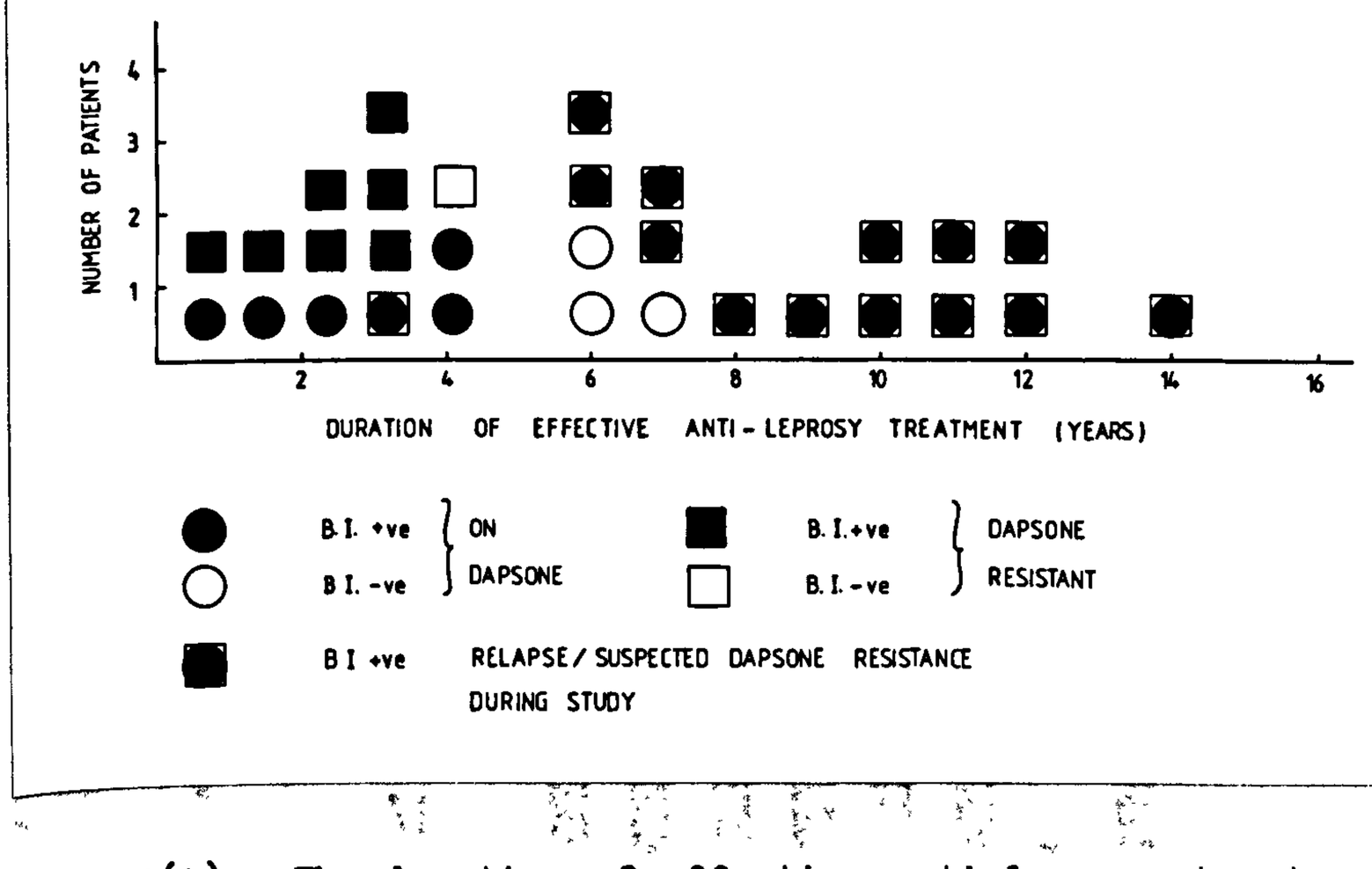


Figure A4(i). The duration of effective anti-leprosy treatment in women developing ENL in association with pregnancy and lactation.

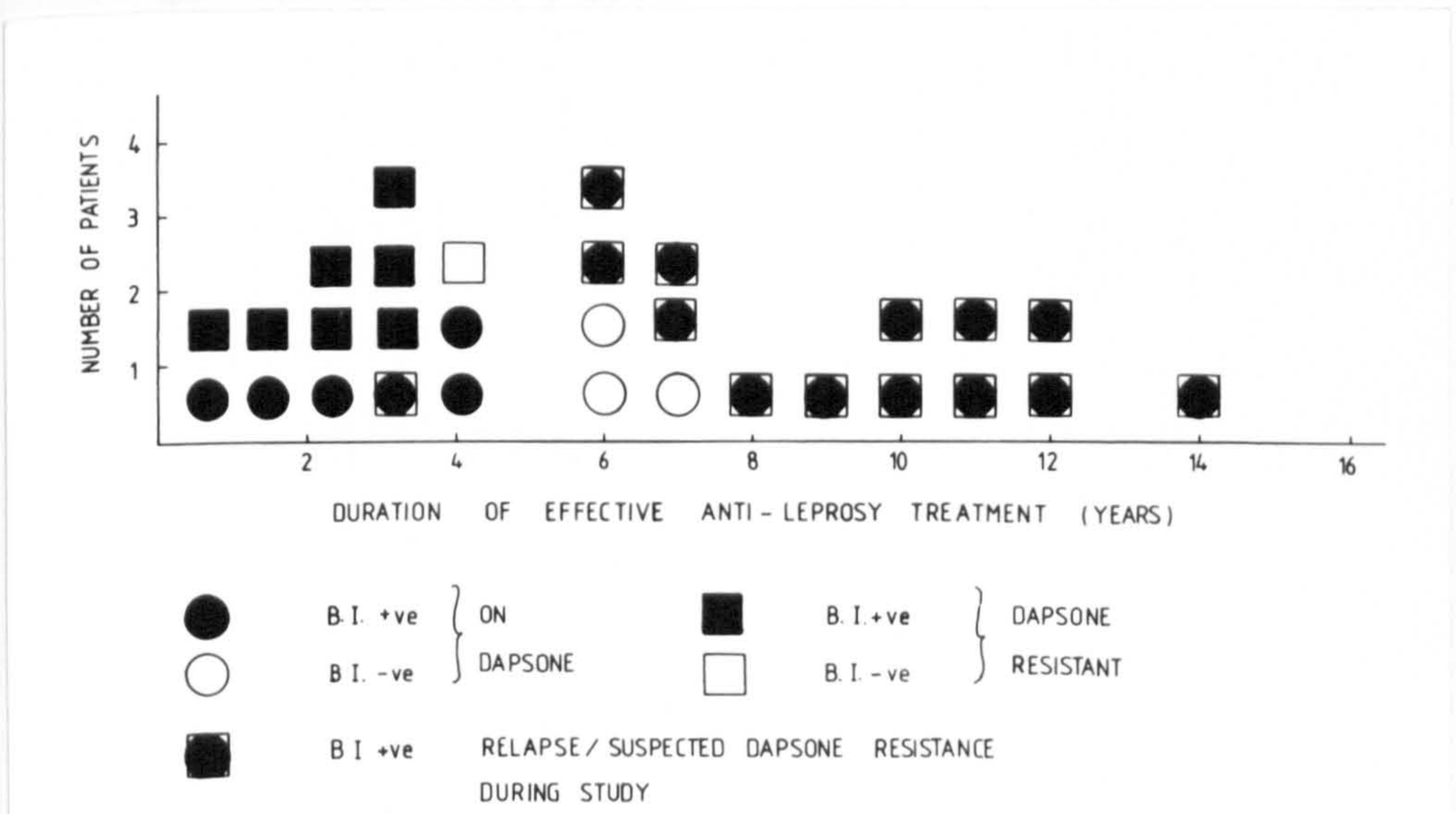


Figure A4(i). The duration of effective anti-leprosy treatment in women developing ENL in association with pregnancy and lactation.

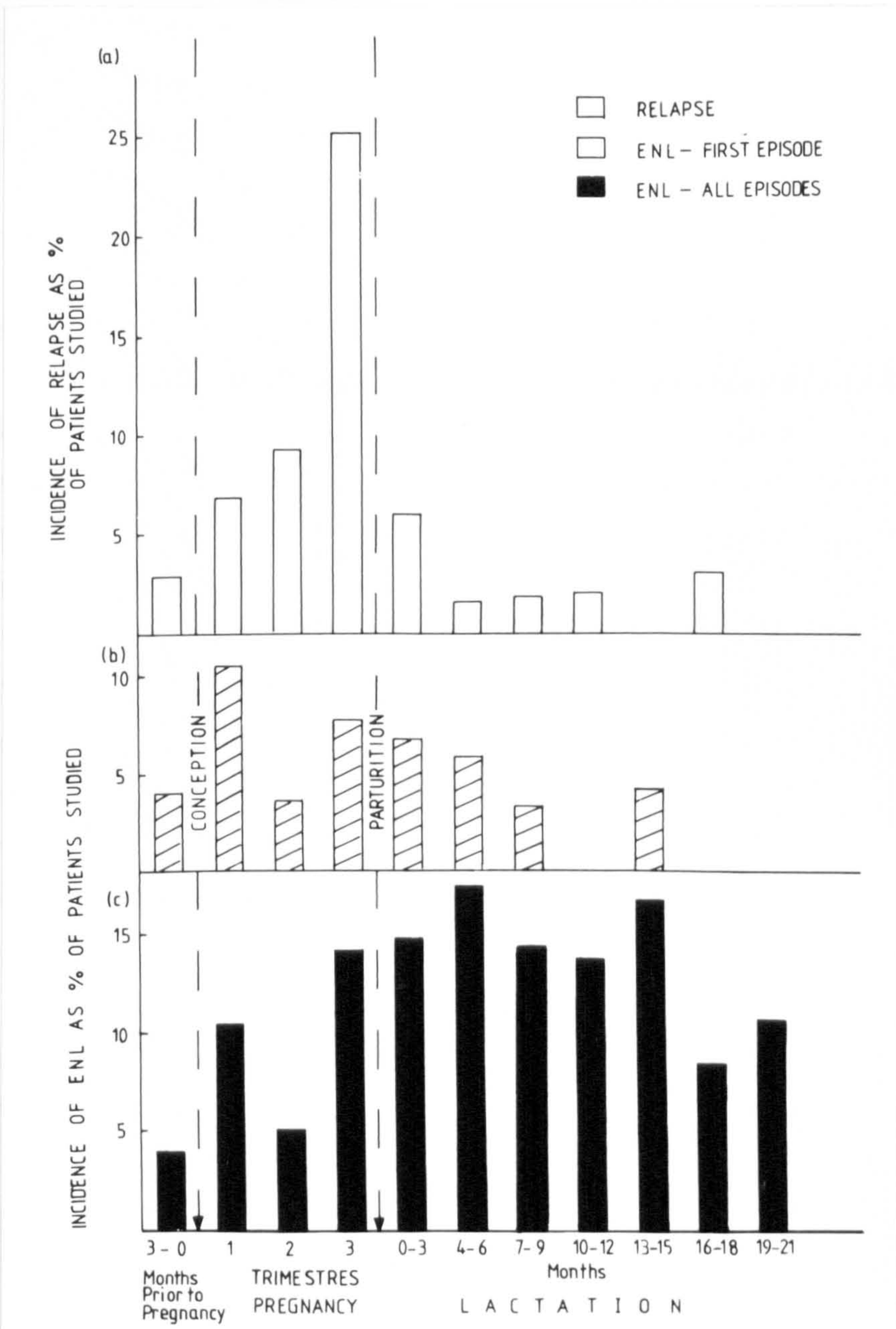


Figure A4(ii). The time of occurrence, prior to conception and during pregnancy and lactation of:
a) first evidence of relapse/development of dapsone resistance;
b) ENL - first episode;
c) ENL - all episodes.
The incidence is expressed as a percentage of the patients studied in each 3 month period.

was highest during the first trimestre with a fall in the second, then a rise in the third, coinciding with the peak of relapse. There was a steady decline during lactation, only 2 cases starting after 9 months. However, when all patients suffering from ENL during a period are recorded (Figure A4(ii)c), it can be seen that a high proportion of patients with ENL have it persistently for many months after delivery; indeed, 15% of the women suffered from ENL or neuritis for 18 months continuously from the third trimestre to 15 months postpartum.

ENL and tissues invdved, according to pregnancy and lactation

During the three months prior to pregnancy, ENL was recorded only in the skin. Throughout pregnancy ENL was seen more in the skin than in nerve or other tissue; however, after delivery, particularly in the recurrent or persistent episodes, ENL was seen more commonly in nerve than in skin (Figure A4(iii)). This was especially the case after the ninth month of lactation. Four patients (6 episodes) had evidence of ENL occurring in tissues other than skin or nerve; 2 episodes involved the eye, 2 episodes involved bone - both women had very tender tibiae and dactylitis, 2 had arthritis with concomittant nerve and/or skin ENL. All 4 women had ENL in association with relapse. Three other women had gross peripheral oedema in association with skin ENL. Six women had ENL in association with upgrading (5) or reversal reaction in skin (1) confirmed histologically.

ENL and clinical features of leprosy during pregnancy and lactation

Symptoms and clinical features of mothers who had ENL are shown in Table A4(i). Table A4(ii) shows the symptoms and clinical features of mothers who did not have ENL.

The total duration of treatment in the patients who had ENL was rather longer than in those who did not have ENL: the period of effective treatment, however, was shorter, due to the inclusion of patients who had already developed dapsone resistance. All clinical features, except reversal reaction in BL patients, were more commonly seen in patients who also had ENL than those who did not have ENL, especially in the LL patients. Thus there appears to be a correlation between ENL, relapse, new nerve enlargement,

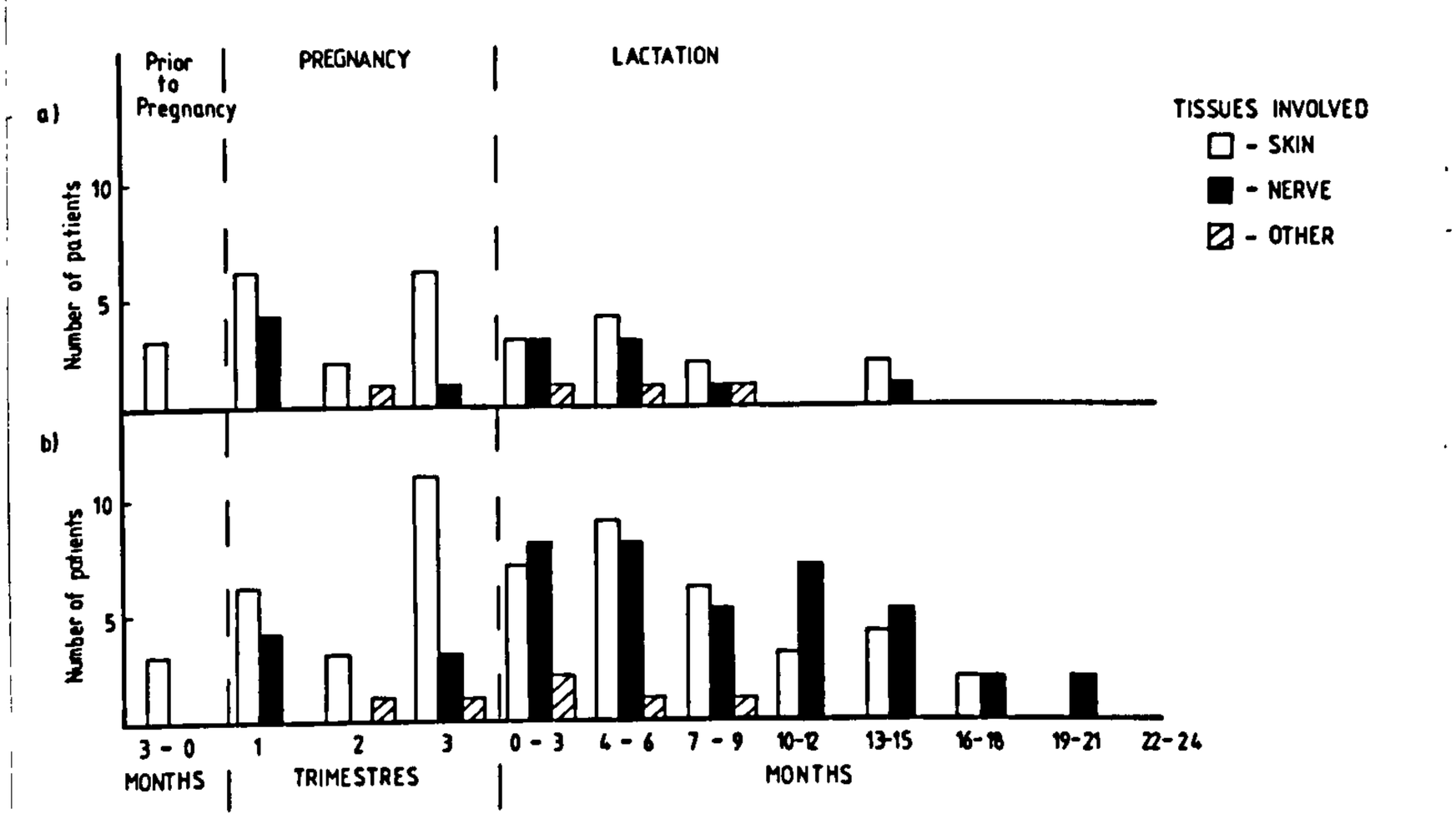


Figure A4(iii). The tissues involved in each episode of ENL in relation to the time of occurrence, prior to conception, during pregnancy, and during lactation:
a) during the first episode.
b) during the first and subsequent episodes, recurrent or continuing, of ENL.
In some cases more than one tissue was involved, i.e. skin and nerve or skin, nerve and other (e.g. bone).

TABLE A4(i)

Symptoms and clinical features in mothers who had erythema nodosum leprosum in pregnancy and lactation

Classif-ication	No. of Women	Total Duration of Treatment (Years) (Mean ± SEM)	Duration of Effective Treatment (Years) (Mean ± SEM)	No. BI +ve	Increased Activity of Leprosy	Reversal reaction in skin (including histological up and down-grading	Enlarged Nerves	Neuritis			Lymphad-enopathy	Complaint	
								Total No.	Overt	Silent		"Rheumatism"	Paraesthesiae
BL	10	7.93 ± 1.25	7.15 ± 1.47	9 (90%)	7 (70%) (1)ϕ	3 (3%)	8 (80%)	8* (80%)	6 (60%)	4 (40%)	6 (60%)	9 (90%)	2 (20%)
LL	20	7.85 ± 0.78	5.58 ± 0.7	17 (85%)	14 (70%) (6)ϕ	3 (15%)	10 (50%)	13** (65%)	8 (40%)	6 (30%)	13 (65%)	16 (80%)	3 (15%)
TOTAL	30	7.88 ± 0.66	6.1 ± 0.68	26 (87%)	21 (70%) (7)ϕ	6 (20%)	18 (60%)	21 (70%)	15 (50%)	12 (40%)	19 (63%)	25 (83%)	5 (17%)

ϕ Increased activity of leprosy as a transient phenomenon.
* One had silent neuritis initially, with overt neuritis later in lactation; one had overt neuritis initially, with silent neuritis later in lactation.
** One had overt neuritis during pregnancy and silent neuritis during lactation.

TABLE A4(ii)

Symptoms and clinical features in mothers who did not have erythema nodosum leprosum in pregnancy and lactation

Classification	No. of Women	Total Duration of Treatment (Years) (Mean ± SEM)	Duration of Effective Treatment (Years) (Mean ± SEM)	No. BI +ve	Increased Activity of Leprosy	Reversal reaction in skin (including histological up and down-grading	Enlarged Nerves	Neuritis			Lymphadenopathy	Complaint	
								Total No.	Overt	Silent		"Rheumatism"	Paraesthesiae
BL	35	6.53 ± 0.82	6.33 ± 0.83	30 (86%)	15 (43%) (2)ϕ	8 (23%)	13 (37%)	13* (37%)	7 (20%)	8 (23%)	13 (37%)	14 (40%)	4 (11%)
LL	16	6.97 ± 0.97	6.34 ± 0.77	10 (62.5%)	9 (56%) (4)ϕϕ	0	4 (25%)	2 (12.5%)	1 (6%)	1 (6%)	3 (19%)	3 (19%)	1 (6%)
TOTAL	51	6.67 ± 0.63	6.33 ± 0.61	40 (78%)	24 (47%) (6)ϕ	8 (16%)	17 (33%)	15 (29%)	8 (16%)	9 (18%)	16 (31%)	17 (33%)	5 (10%)

ϕ Transient increase in activity, in one case there was late relapse during lactation.
ϕϕ Transient increase in activity.
* One had overt neuritis with late silent neuritis, a second had silent neuritis with late overt neuritis.

neuritis and lymphadenopathy. The complaint of "rheumatism" was heard from 90% of BL patients with ENL and 80% of LL patients with ENL. Paraesthesiae, "burning sensation in the skin", was a complaint of pregnancy while "rheumatism" tended to be a complaint of lactation. In the BL group, enlarged nerves, neuritis and lymphadenopathy were also associated with reversal reaction during lactation and followed relapse in late pregnancy.

ENL and neuritis in pregnancy and lactation

The extent of nerve involvement in ENL in pregnancy and lactation has been shown in Figure A4(iii). The degree of nerve damage is shown in Table A4(iii). Six patients had 8 episodes of ENL nerve involvement with acutely tender peripheral nerves in which no motor or sensory loss was demonstrated. However, there were 30 episodes in which significant motor and/or sensory loss occurred. There was only one episode in which there was loss of function of a single nerve; however, the patient developed multi-nerve involvement during the next episode of neuritis.

It was observed that many nerves were involved in each episode of neuritis. Usually in overt neuritis twice the number of nerves were recorded as showing tender nerve enlargement, as showed loss of function. This may reflect the fact that the methods of sensory testing were designed to demonstrate loss or recovery of protective sensation rather than absolute loss of sensation. Severe motor nerve damage was recorded in 13 episodes of neuritis and severe sensory nerve damage was recorded in 9 episodes of neuritis.

A further measurement of the severity of the neuritis is seen in the number of patients who required treatment with prednisolone for nerve damage for periods of up to several months. Six out of 8 BL patients and 10 out of 13 LL patients received prednisolone; 5 out of 16 patients receiving prednisolone and 1 of the 5 without prednisolone showed improvement in nerve function at the time of the last assessment.

ENL and infections seen during pregnancy and lactation

Two patients, both LL, deserve special comment.

TABLE A4(iii)

Severity of nerve damage during each episode of neuritis

Type of neuritis		Degree of nerve damage				Total	No nerve damage detected (episodes)
		Motor *		Sensory **			
		Mild	Severe	Mild	Severe		
Overt	1 nerve only	1	1	0	0	2	8
	many nerves	7	5	5	4	21	
Silent	1 nerve only	1	1	2	1	5	
	many nerves	2	6	3	4	15	
Total		11	13	10	9	43	8

* Mild damage : loss of 1 or 2 VMT grades in 1 or more nerves, or EMG evidence only.
Severe damage: loss of 3 or more VMT grades in 1 or more nerves, or clinical assessment only.

** Mild damage : loss of 1 or 2 sensory test areas in 1 or more nerves.
Severe damage: loss of 3 or more sensory test areas in 1 or more nerves, or clinical assessment only.

i) S.M. A9/256, a secundigravida who had been treated with dapsone 100 mg daily for 3 years, was referred to the hospital, during the second trimestre, from an outlying clinic for severe ENL which had started during the first trimestre. During the first 6 months of pregnancy, she had severe ENL in the skin which required treatment with prednisolone 20–30 mg daily. In the third trimestre, she had a transient rise in BI, skin ENL persisted, and in addition she developed neuritis and arthritis with acutely tender swollen knees and hands. After delivery, the ENL stopped suddenly, but restarted as abruptly 21 days post-partum, with skin, nerve and bone involvement, acutely tender tibiae and dactylitis. Chest X-ray showed pulmonary tuberculosis. She improved on treatment with anti-tuberculous drugs, and clofazimine for ENL and was discharged home for further treatment by her home clinic.

ii) Y.T. A9/64, a gravida 4 who had received dapsone monotherapy for 9 years, had no problems during her pregnancy. At 6 months postpartum she was acutely ill with endemic typhus and required in-patient treatment for 3 weeks. During the first week of the febrile illness she developed skin ENL. This became very troublesome and after 2 months ENL involved nerves and bone as well. Like S.M., she had acutely tender tibiae and dactylitis. At this time biopsy showed ENL in the skin and short solid AFB in deep muscle, indicating incipient relapse. She developed new nerve damage with motor and sensory loss. Despite treatment with prednisolone 20–30 mg daily, she had persistent sensory loss at her last assessment.

5. Leprosy neuritis in pregnancy and lactation

In 116 women with leprosy (122 pregnancies) and 31 healthy controls (34 pregnancies) there was a total of 85 episodes of leprosy neuritis. In 11 episodes no nerve damage ensued. In the remaining 74 episodes, 29 showed pure motor loss, 12 pure sensory loss, and 33 mixed loss.

The number of patients studied, and the proportion developing neuritis during the study period, are shown in Table A5(i). There was considerable risk of neuritis in all types of leprosy. The timing of the episodes of neuritis is shown in Figure A5(i). BL cases were almost free of neuritis during pregnancy, but were greatly at risk in the first 6–9 months after delivery. In BT and TT cases neuritis was less common and showed little relationship to the events of pregnancy.

The general severity of the nerve damage, taking all types of leprosy together, is shown in Tables A5(ii) and A5(iii). The majority of patients with sensory loss were severely affected, whereas the motor deficits were in most cases mild. The apparently greater vulnerability of sensory nerves may indicate that damage occurred at both dermal nerve and nerve trunk levels; the danger that insidious silent neuritis will cause severe sensory loss is well shown. Neuritis affecting multiple nerves was usually more damaging than when one nerve only was affected.

Some indication of the severity of the neuritis may also be derived from the treatment that was employed. A total of 52 patients developed neuritis. However, in 14 cases the diagnosis (silent neuritis) was made only at the time of final assessment. Of the remaining 38 patients, 26 required treatment with corticosteroids during the study period.

Overt neuritis was usually associated with skin reaction, deterioration of the leprosy condition (due to relapse or dapsone resistance) or both. Early clinical deterioration not infrequently presented as apparent ENL (type 2) reaction (Figure A5(ii)c), and clinically puzzling mixtures of reversal (type 1) reaction and ENL (type 2) reaction were occasionally encountered (Figure A5(ii)b).

In general, overt neuritis was chiefly encountered just before delivery and during the first 9–12 months postpartum. Silent neuritis, on the other hand, occurred at all stages (Figure A5(ii)a), but became the predominant problem from about 6–9 months postpartum.

TABLE A5(i)

Classification of leprosy	No. of pregnancies studied	No. with neuritis	No. of episodes of neuritis	No. of episodes per patient
TT and BT	41	16 (39%)	24	0.6
TT and BT/RFC	22	6 (27%)	9	0.4
TT and BT/Active	19	10 (53%)	15	0.8
BL *	45	21 (47%	35	0.8
LL	36	15 (42%)	26	0.7
TOTAL	122	52 (43%)	85	0.7

* This group includes 3 patients (7 episodes of neuritis) originally classified as BT/RFC who relapsed with active BL leprosy during the study period.

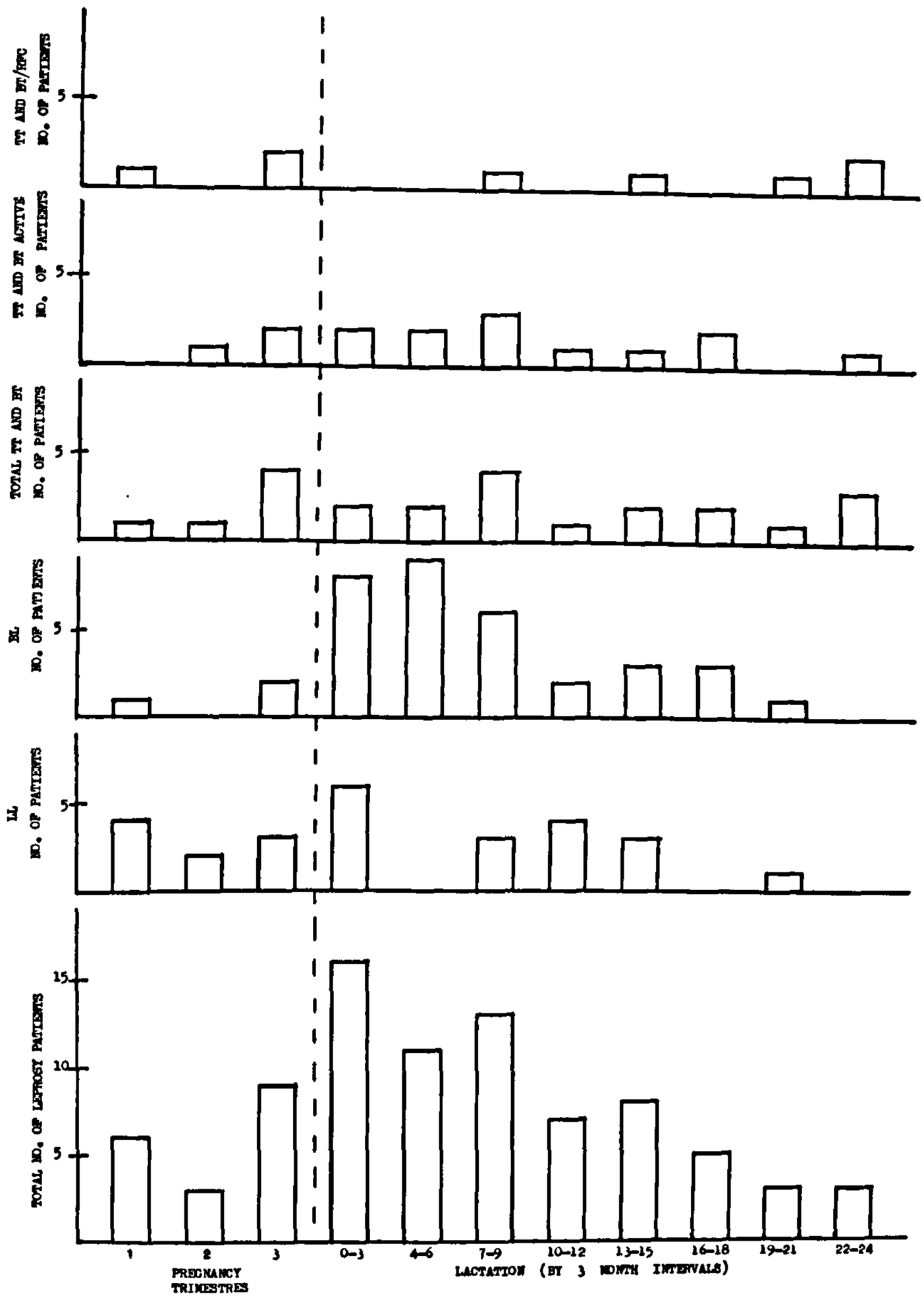


Figure A5(i). Timing of episodes of neuritis during pregnancy and lactation according to the clinical classification of the patients at the outset of the study.

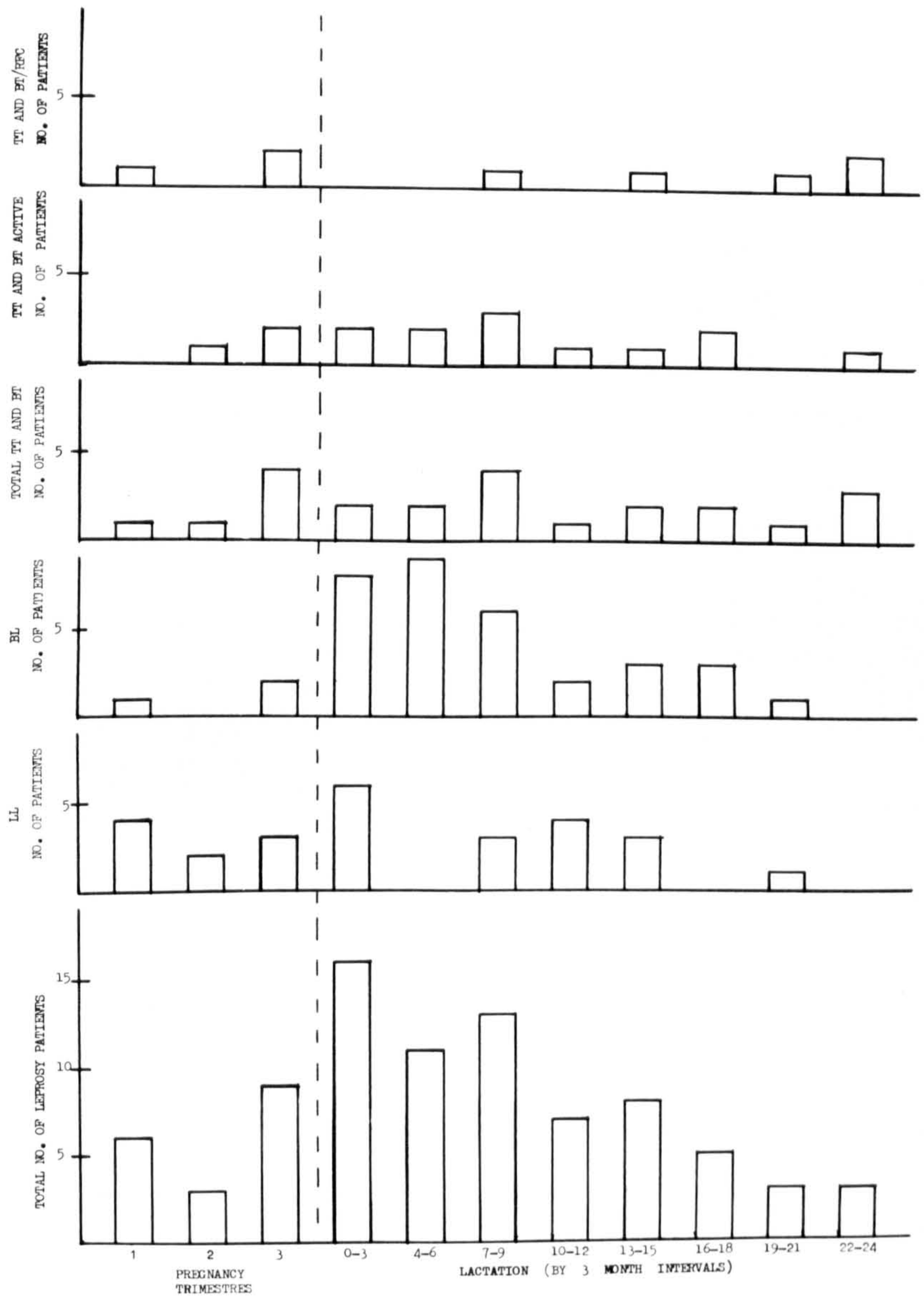


Figure A5(i). Timing of episodes of neuritis during pregnancy and lactation according to the clinical classification of the patients at the outset of the study.

TABLE A5(ii)

Severity of motor damage incurred during each episode of neuritis

Type of neuritis		Degree of nerve damage * mild	severe
Overt	1 nerve only	7	1
	many nerves	11	6
Silent	1 nerve only	4	1
	many nerves	17	15
Total		39	23

* Mild damage: loss of 1 or 2 VMT grades in 1 or more nerves, or NCV evidence only.

Severe damage: loss of 3 or more VMT grades in 1 or more nerves, or clinical assessment only.

TABLE A5(iii)

Severity of sensory damage incurred during each episode of neuritis

Type of neuritis		Degree of nerve damage* mild	severe
Overt	1 nerve only	1	2
	many nerves	3	6
Silent	1 nerve only	4	2
	many nerves	6	21
Total		14	31

* Mild damage : loss of 1 or 2 sensory test areas in 1 or more nerves.

Severe damage: loss of 3 or more sensory test areas in 1 or more nerves, or clinical assessment only.

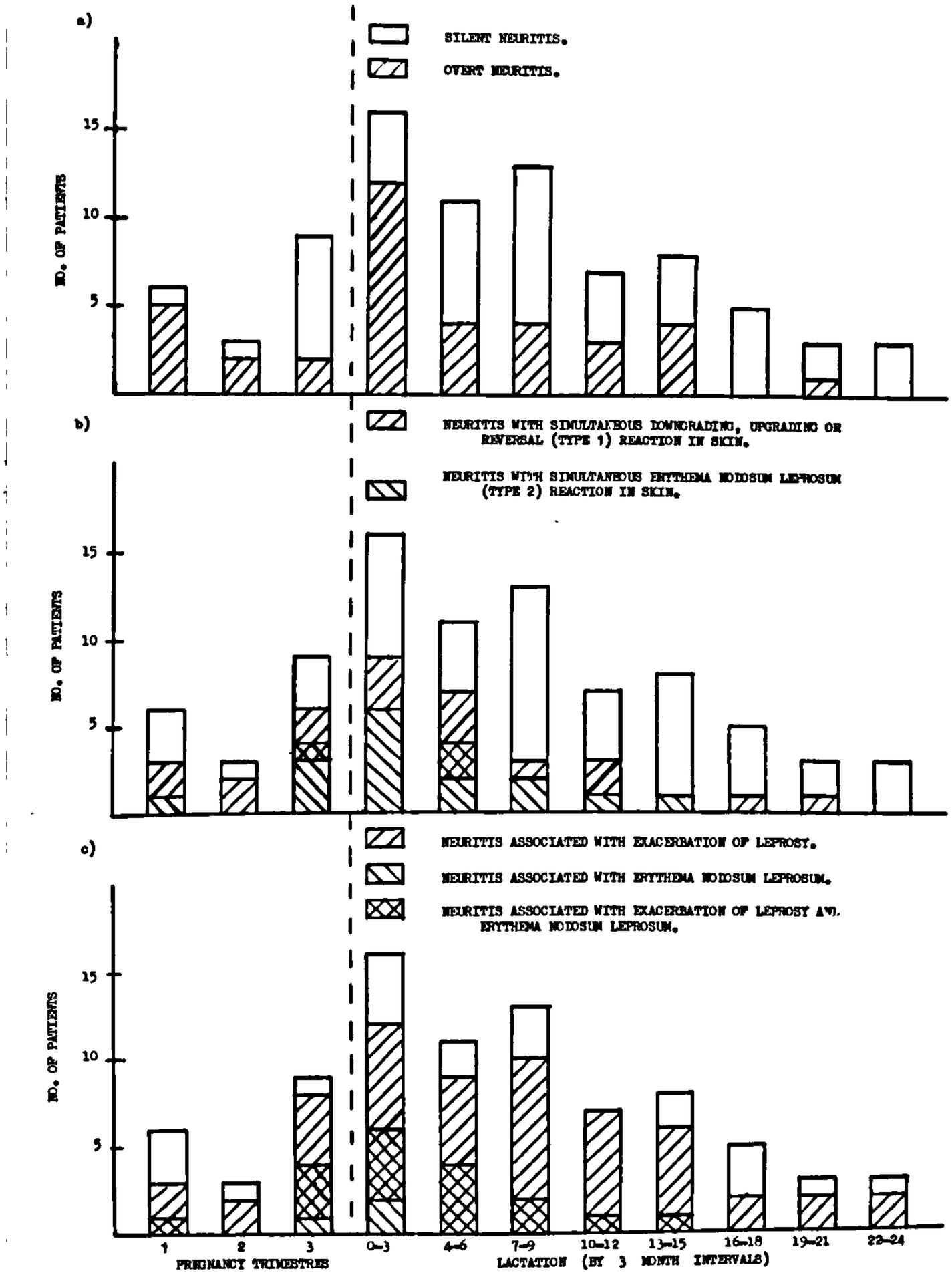


Figure A5(ii). Clinical aspects of neuritis in association with pregnancy and lactation.

a) Timing of silent and overt neuritis.
b) Timing of neuritis in association with simultaneous reversal (type 1) reaction and ENL (type 2 reaction).
c) Association of neuritis with exacerbation of leprosy and ENL (type 2 reaction).

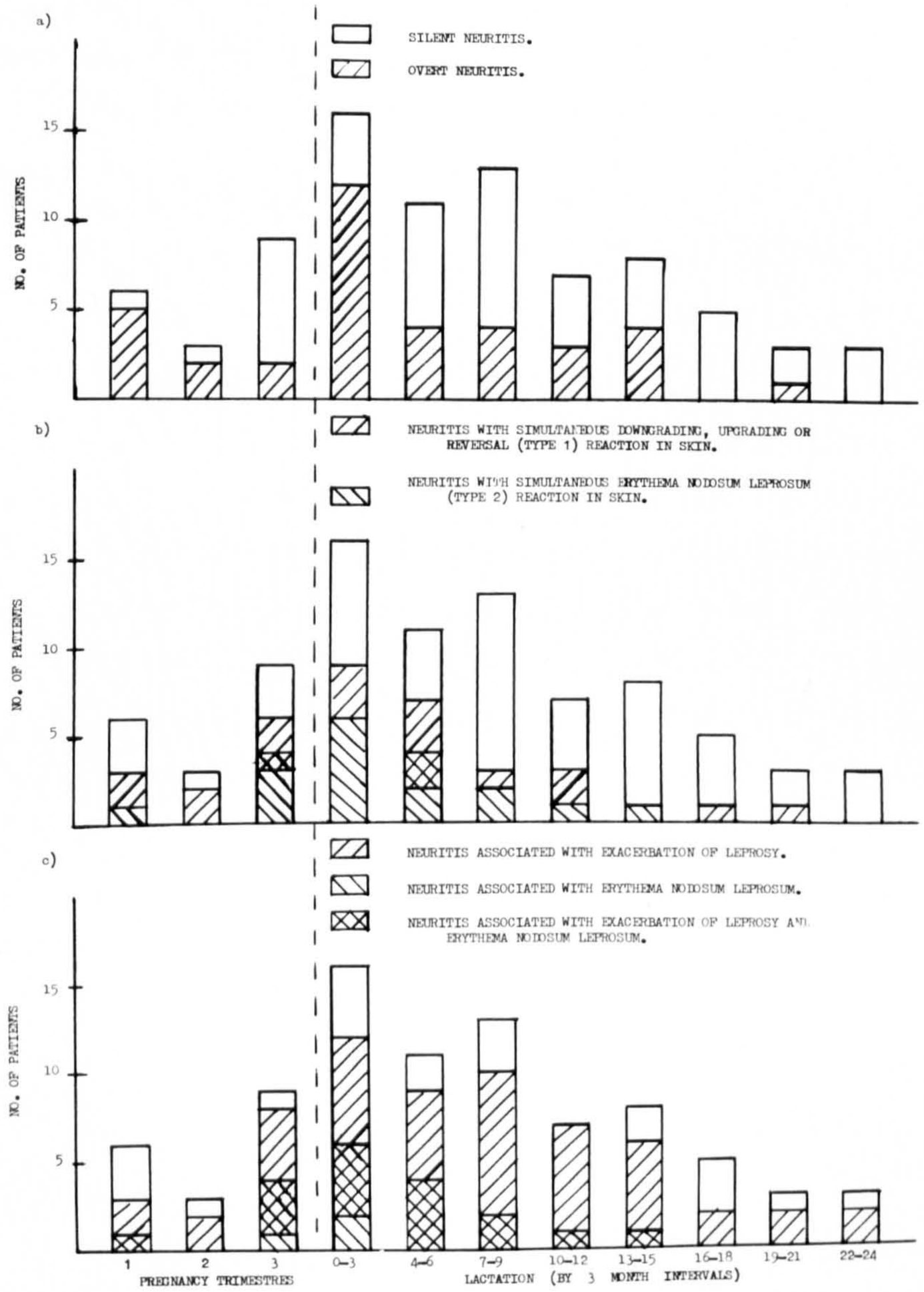


Figure A5(ii). Clinical aspects of neuritis in association with pregnancy and lactation.
a) Timing of silent and overt neuritis.
b) Timing of neuritis in association with simultaneous reversal (type 1) reaction and ENL (type 2 reaction).
c) Association of neuritis with exacerbation of leprosy and ENL (type 2 reaction).

Healthy Controls (HC)

One healthy control got overt neuritis. She complained of "rheumatism" and was found to have enlarged nerves at 10 weeks postpartum. At 6 months she had tender nerves with loss of motor and sensory function. Biopsy showed active BL leprosy. Thus she is here included in the BL group.

A second control mother developed BT leprosy during pregnancy - she is considered here in the TT and BT group.

Tuberculoid leprosy (TT and BT)

Of 25 patients initially classified as TT and BT/RFC (including the 3 who relapsed as BL during the study period), 8 patients had 12 episodes of neuritis in association with relapse.

Sixteen out of 41 patients had 24 episodes of neuritis in association with pregnancy or lactation. Five patients had neuritis (6 episodes) during pregnancy. These 5 patients, all of whom were either newly diagnosed or relapse cases, had silent neuritis which was diagnosed early, by clinical or physiological testing, after type 1 (reversal) reaction was observed in the skin. Six of 8 episodes of neuritis observed during pregnancy or the puerperium were in association with type 1 reaction of the skin lesions. One patient only, a newly diagnosed case, developed "classical" overt neuritis at 6 weeks postpartum. The remaining cases of neuritis occurring during lactation were all silent, the majority being preceded by the complaint of "rheumatism" (generalised aches and pains) and new, non-tender, nerve enlargement.

Lepromatous leprosy (LL)

Fifteen out of 36 patients had 26 episodes of neuritis. In 11 cases there was also mixed ENL and clinical deterioration: 2 patients showed deterioration without reaction and 2 had ENL only. In most cases ENL preceded the appearance of neuritis.

Fifteen episodes of neuritis were observed during pregnancy and the first 3 months of lactation; 12 were overt, 3 silent. Eleven episodes of neuritis occurred later in lactation, 6 of which were silent and usually preceded by the complaint of "rheumatism" and the finding of newly enlarged non-tender nerves. Nerve

conduction velocity was measured in 2 cases (at 12 months post-partum) and indicated active nerve damage rather than slow residual fibrosis.

Borderline lepromatous leprosy (BL)

Twenty-one out of 45 patients had 35 episodes of neuritis; only 3 episodes occurred during pregnancy. The neuritis was almost always associated with deterioration of the leprosy (15 cases) or reaction (ENL, 8 cases; reversal reaction, 7 cases), and many patients showed both reaction and deterioration.

Overt neuritis (13 patients) was associated with reaction (9 cases) and/or clinical deterioration (8 cases); 14 out of 19 episodes occurred during the first 12 months after delivery. Silent neuritis, on the other hand, was associated with reaction in only 4 cases and with clinical deterioration in 12 cases; it sometimes continued until 2 years after delivery, and was in 11 cases still present at the patient's final assessment. As in LL cases, silent neuritis was usually preceded by the complaint of "rheumatism" and the finding of nerve enlargement. Nerve conduction velocity studies performed on 3 patients 12-15 months after delivery showed evidence of active demyelination.

Treatment of neuritis

Twenty-six patients were treated with corticosteroids. Not all had completed their courses of treatment during the study period, but at the time of their final assessments 9/26 (35%) showed improvement (using the reverse of the criteria for deterioration) both clinically and on VMT and SST. Twelve patients with neuritis did not receive corticosteroids; only 2 of them (17%) improved.

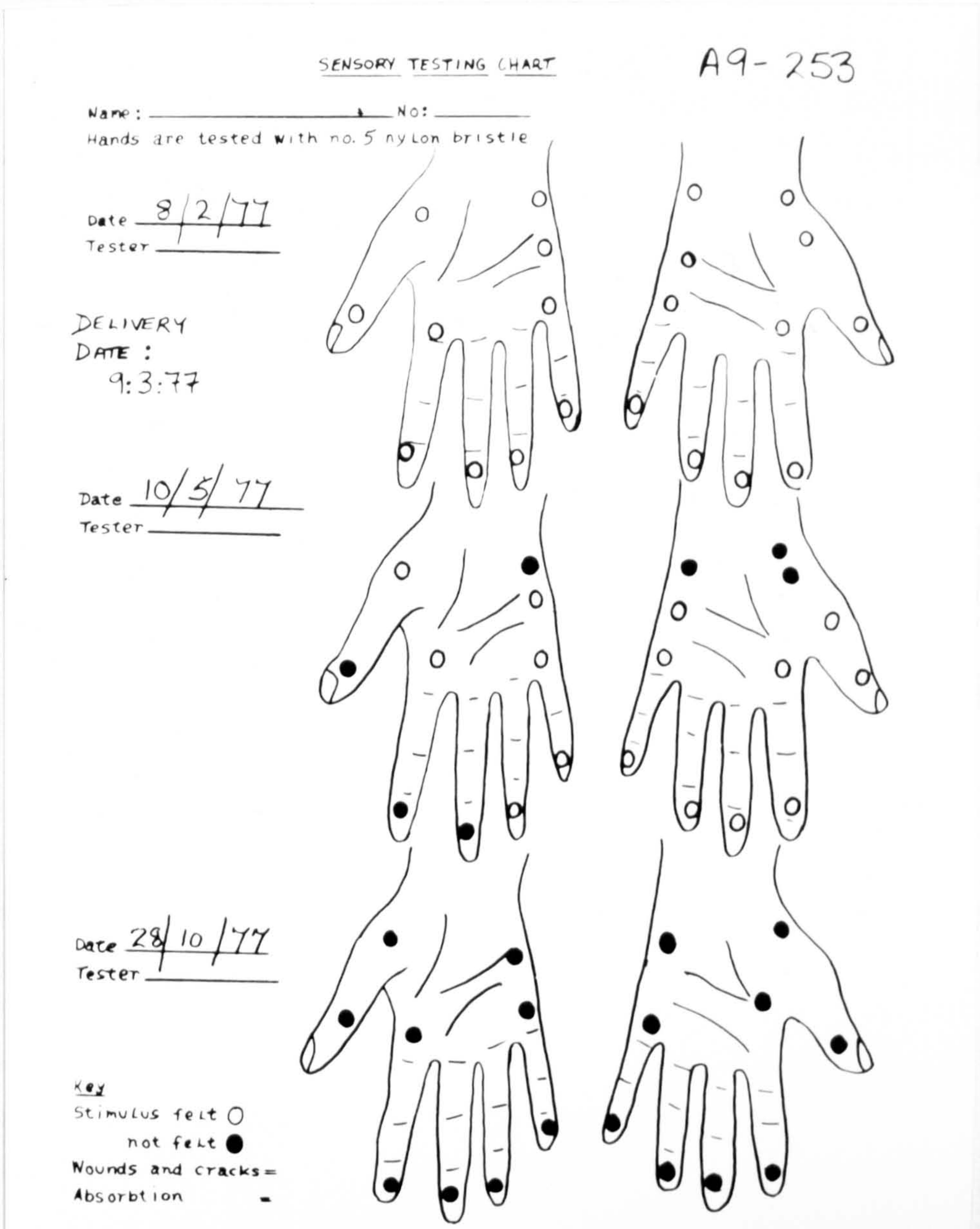
Illustrative Case Report

This case report illustrates the problem in diagnosing silent neuritis and treating it even in a patient who attended most regularly for treatment.

Case Report A.G. A9/253

A.G. had BL leprosy from the age of 16 for which she received regular treatment with dapsone monotherapy from the age of 17-24 when she entered this study. Prior to entering the study her BI had been negative for 2 years, clinically she

was considered to be BL quiescent. She continued taking dapsone 100 mg daily. In the third trimestre of her pregnancy she was noted to have a few new nodules on the lower part of her back, skin smears from standard sites were negative, but a biopsy of one of the new nodules showed "BL or LL early active lesion". At that time her hands and feet were fully sensitive to the normal test stimuli used. By two months after delivery she began to experience "rheumatism" which became progressively more severe. By 2 months postpartum she had evidence of motor and sensory nerve loss with new non-tender nerve enlargement. Biopsy was reported as "BL regressing with no sign of active leprosy, one acid fast bacillus (fragmented) was detected in one skin smear". By 8 months postpartum with persisting troublesome rheumatism, she was noted to have complete sensory loss of her hands and feet. There was no evidence of skin ENL and she had no tender nerves, although the ulnar and popliteal nerves, previously noted to be enlarged, remained enlarged. Treatment was started with prednisolone but despite several months of treatment, her feet remained totally insensitive, and there was only partial recovery of protective sensation in her hands. (Figure A5(iii) below.)



6. The message of "rheumatism", a forgotten symptom in leprosy

In 114 women with leprosy (120 pregnancies) and 32 healthy women (36 pregnancies), 93 episodes of qurtimat ("rheumatism") were recorded.

Most patients with tuberculoid leprosy are free of reactions and "clinically quiescent" after 2 years of effective treatment; tuberculoid patients were therefore divided into 2 groups: those treated for less than 2 years and those treated for more than 2 years.

Patients with lepromatous leprosy, which is sensitive to dapsone, are usually free of reaction and clinically quiescent after about 4 years of regular treatment. But in this study, a high proportion of women with lepromatous leprosy treated for more than 4 years showed reactivation due to the emergence of dapsone resistant leprosy (Duncan, Pearson and Rees, 1981). Thus, when the results were analysed, little difference was found between patients treated for less than 4 years and for more than 4 years. For this reason, the results of BL and LL cases are not divided into 0–4 years and more than 4 years' treatment groups.

Timing and duration of "rheumatism"

The numbers of patients who suffered from "rheumatism" during pregnancy, the puerperium (0–6 weeks after delivery) and lactation (6 weeks onwards) are shown in Table A6(i), together with the total number of episodes of "rheumatism" (many patients had it more than once) and the total number of patients who developed "rheumatism" (many suffered from it during more than one period).

About half the cases receiving treatment developed "rheumatism", which tended to start in lepromatous cases during late pregnancy and the puerperium, but in BT and TT patients was only common during lactation.

The duration of "rheumatism" varied between a few weeks to several months. Three patients had complained of "rheumatism" almost constantly throughout their pregnancies and for up to 12 months after delivery. Only 2 healthy controls developed "rheumatism"; one of them showed the first signs of leprosy during lactation.

TABLE A6(i)

Timing of episodes of rheumatism

Classification	No. of Pregnancies	No. of patients developing rheumatism			No. of Episodes of rheumatism	No. of patients who developed rheumatism
		Pregnancy	Puerperium	Lactation		
HC	36	0	1	2	6	2 (6%)
TT and BT/RFC	25	2	1	8	11	8 (32%)
TT and BT Active	18	2	1	7	8	8 (44%)
BL	41	5	5	20	30	20 (49%)
LL	36	11	9	18	38	20 (56%)
TOTAL	156	20	17	55	93	58 (37%)

Relapse and "rheumatism"

The numbers of patients who showed overt relapse or increased activity of leprosy (as shown by an increase in BI, MI, or histological activity) are shown in Table A6(ii). Relapse was twice as frequent in women with "rheumatism" as in women without it. There was little difference between the different types of leprosy.

Reaction and "rheumatism"

The numbers of patients who developed ENL and reversal reaction (RR) are shown in Table A6(ii). Both reactions were markedly more common among patients with "rheumatism".

Neuritis and nerve enlargement

The numbers of patients with nerve enlargement and silent and overt neuritis are shown in Table A6(iii). A number of patients developed both overt and silent neuritis one after the other (in either order). Enlarged nerves were 4 times more common, and neuritis 8–14 times more common in patients with "rheumatism".

Lymphadenopathy and paraesthesiae

The numbers of patients developing these complaints are shown in Table A6(iv). Both occurred 5–7 times more commonly when patients complained of "rheumatism".

Case Reports of patients complaining of gurtimat

1. B.D. A9/76

A primigravida, was well throughout her pregnancy and had no sign of leprosy when assessed during pregnancy. During her puerperium she first experienced gurtimat which became progressively more severe until at 10 weeks postpartum she presented herself at the diagnostic clinic. She was found to have an enlarged radial cutaneous nerve, but no skin lesions. Nerve biopsy showed globi with AFB 5+: skin biopsy showed the appearance of BL leprosy, but with few bacilli. Treatment with dapsone 100 mg daily was started. She developed acute overt neuritis with severe nerve damage, in a strong upgrading reaction confirmed by histology. Troublesome gurtimat persisted for 14 months. Additional treatment with prednisolone was required for 12 months for the neuritis. Full recovery was recorded 18 months postpartum.

TABLE A6(ii)

Number of patients showing increased activity of leprosy, ENL, and reversal reaction (RR)

Classification	No. of women with rheumatism				No. of women without rheumatism			
	Total	Increased Activity	ENL	RR	Total	Increased Activity	ENL	RR
HC	2	1	0	1	34	1	0	0
TT and BT/RFC	8	8 *	1	0	17	4 **	0	0
TT and BT Active	8	5 *** (3)	0	2	10	3 *** (2)	0	1
BL	20	11 *** (1)	8	6	21	6 *** (2)	1	4
LL	20	11 *** (4)	16	5	16	11 *** (6)	4	1
TOTAL	58	36 62%	25 43%	14 24%	98	25 26%	5 5%	6 6%

* 3 cases of incipient relapse with nerve damage, but no skin lesions;
5 cases overt relapse.

** All showed overt relapse.

*** ( ) = Number of cases in which increased activity was transient.

TABLE A6(iii)

Number of patients showing neuritis and nerve enlargement

Classification	Number of women with rheumatism					Number of women without rheumatism				
	Total	Enlarged Nerves	Neuritis Silent	Neuritis Overt	Total with Neuritis	Total	Enlarged Nerves	Neuritis Silent	Neuritis Overt	Total with Neuritis
HC	2	2	0	1	1	34	1	0	0	0
TT and BT/RFC	8	6	7	3	7	17	2	1	0	1
TT and BT Active	8	7	7	1	8	10	1	1	1	2
BL	20	12	8	11	15	21	5	3	0	3
LL	20	8	6	8	12	16	5	1	2	3
TOTAL	58	35 60%	28 48%	24 41%	43 74%	98	14 14%	6 6%	3 3%	9 9%

TABLE A6(iv)

Number of patients developing lymphadenopathy and paraesthesiae

Classification	Number of women with rheumatism			Number of women without rheumatism		
	Total	Lymphadenopathy	Paraesthesiae	Total	Lymphadenopathy	Paraesthesiae
HC	2	1	1	34	2 *	0
TT and BT/RFC	8	5	2	17	0	0
TT and BT Active	8	5	2	10	0	1
BL	20	9	4	21	6	2
LL	20	12	4	16	4	0
TOTAL	58	32 55%	13 22%	98	12 12%	3 3%

* 2 separate pregnancies in one woman who was a "healthy control" regarding leprosy but had a flare-up of cutaneous leishmaniasis during or after her 2 pregnancies in the study. The lymphadenopathy was in association with this "flare-up".

2. A.S. A9/261

A secundigravida was BT/RFC having stopped treatment 2 years prior to the study. During the third trimestre of her pregnancy she developed gurtimat, and was suspicious that her leprosy had become reactivated. She was found to have a hypopigmented lesion above her left eyebrow, with a small satellite lesion. There was no sensory loss. Biopsy showed "Active BT leprosy with no AFB seen". Treatment with dapsone 100 mg daily was started. She developed silent neuritis with enlarged nerves and lymphadenopathy during lactation.

3. A.G. A9/253

She was classified as BL quiescent after 7 years of dapsone therapy, BI = 0 for 3 years prior to the study. Although clinically quiescent during pregnancy, her fourth, her skin biopsy showed transient increase in activity during the third trimestre, being reported as "BL or LL early active lesion". She complained of paraesthesiae during pregnancy and gurtimat during lactation. "Rheumatism", persistent throughout lactation, was still present at her last assessment 13 months postpartum and accompanied by new nerve enlargement, lymphadenopathy and silent neuritis. She developed progressive sensory and motor loss from 2 months postpartum. At 8 months postpartum when NCV showed "fresh obvious ulnar nerve involvement", treatment with prednisolone was started. Five months later, she still complained of gurtimat although she had some recovery of nerve function.

4. E.A. A9/92

A secundigravida treated with dapsone for LL for 8 years prior to the study, showed small "atypical" new nodules during the third trimestre, BI = 0.8, MI = 0. A few solid staining AFB were present in the skin biopsy which was reported as"BL or LL active." She complained of gurtimat which started immediately after delivery. By 3 months postpartum, she had crops of new nodules on all 4 limbs, face and buttocks, BI = 4.7, MI = 4.6. Skin biopsy was reported as"LL with many plasma cells, somewhat histoid." She developed acute overt neuritis involving both median and ulnar nerves. Treatment with clofazimine and TB450 for dapsone resistance and prednisolone for neuritis was started at 3 months postpartum. Despite this she continued to have severe rheumatism, with paraesthesiae, skin ENL and tender nerves. NCV at 12 months postpartum showed active neuritis in the right ulnar and left median nerves. Treatment with prednisolone, clofazimine and TB450 was continued; but at her last assessment, at 15 months postpartum, she still complained of severe rheumatism, this time in her legs. Both legs were hot, with marked pretibial oedema, and there was new enlargement of both popliteal nerves with moderate tenderness of one.

B. THE EFFECT OF LEPROSY ON PREGNANCY

1. Oestrogen excretion in pregnant women with leprosy: Evidence of diminished foeto-placental function

Urinary oestrogen was determined by a continuous flow method using a fluorimetric end point (Oakey, 1977), on aliquots of 24h urine collections from 79 women with singleton pregnancies essentially free from obstetric complications.

Classification and treatment of patients

There were 64 patients with leprosy and 15 healthy controls. Patients with leprosy were classified, using the revised classification, as follows :

TT and BT	...	20 patients
BL	...	27 patients
LL	...	17 patients

Thirteen of the TT and BT patients and 2 BL patients were RFC and had stopped treatment. The remaining leprosy patients received dapsone monotherapy (50–100 mg daily); 4 of these (all LL) also received clofazimine (100 mg three times a week) or rifampicin and thiambutosine for dapsone resistant leprosy. No patients in these groups received corticosteroid therapy.

Four other patients were studied to examine the effects of corticosteroid therapy on oestrogen excretion in leprosy. These patients on dapsone therapy were treated with prednisolone 20–30 mg daily for leprosy reactions. One of them was also given immunosuppressive doses of clofazimine (300 mg daily).

Oestrogen excretion

There was no statistical difference between the groups of women in the means, or the range of oestriol assays before 32 weeks' gestation.

Oestrogen excretions, between 32–40 weeks of gestation, in patients and control subjects were assessed with reference to the lower limit of normal in European women for the corresponding gestation (Heys et al, 1968). For statistical analysis of differences between the groups, the student's 't' test was used after logarithmic conversion $\log (100 + x)$. The values shown

in Figure B1(i) represent urinary oestrogen values (μmol/24 h) greater or less than the corresponding lower limit of normal excretion for European women. It can be seen (Table B1(i))that the mean oestrogen excretion was lowest in LL women and highest among the HC group. Similarly, the incidence of subnormal values was lowest among the HC women and highest among the LL patients.

Birth weights, placental weights and placental coefficients

Birth weights and placental weights were known for 58 of the 79 pregnancies studied. Mean birth weights, placental weights and placental coefficients for each of the 4 groups of subjects are shown in Table B1(ii). There is a significant difference ($p < 0.001$, Mann Whitney U test) between mean birth weights of the HC and the LL groups. The difference between mean placental weights is even more striking ($p < 0.001$). Mean birth weights and placental weights for the TT and BT and BL groups were intermediate between these extremes.

Association between birth weights and frequency of subnormal oestrogen excretion

Birth weights of all infants were assessed relative to the data of Lubchenco et al (1963) which refers to subjects accustomed to comparable altitudes. Table B1(iii) shows that the proportion of women who gave birth to babies weighing below the tenth centile was much higher among the LL group than among the healthy controls. Similarly, the proportion of women with low oestrogen values was greatest among the women with light-weight babies.

Conditions of the babies at birth

Despite the relatively poor social and economic conditions of the women considered in this study, there were only 2 cases of perinatal death. The condition of the babies at birth in each group of women is described below.

a) HC group. There were no perinatal deaths nor operative deliveries for foetal hypoxia in this group. Apgar scores of 10 were recorded in all 8 babies on whom this measurement was made.

b) TT and BT group. Of the 20 babies delivered in this group, one required resuscitation after delivery by Caesarean section

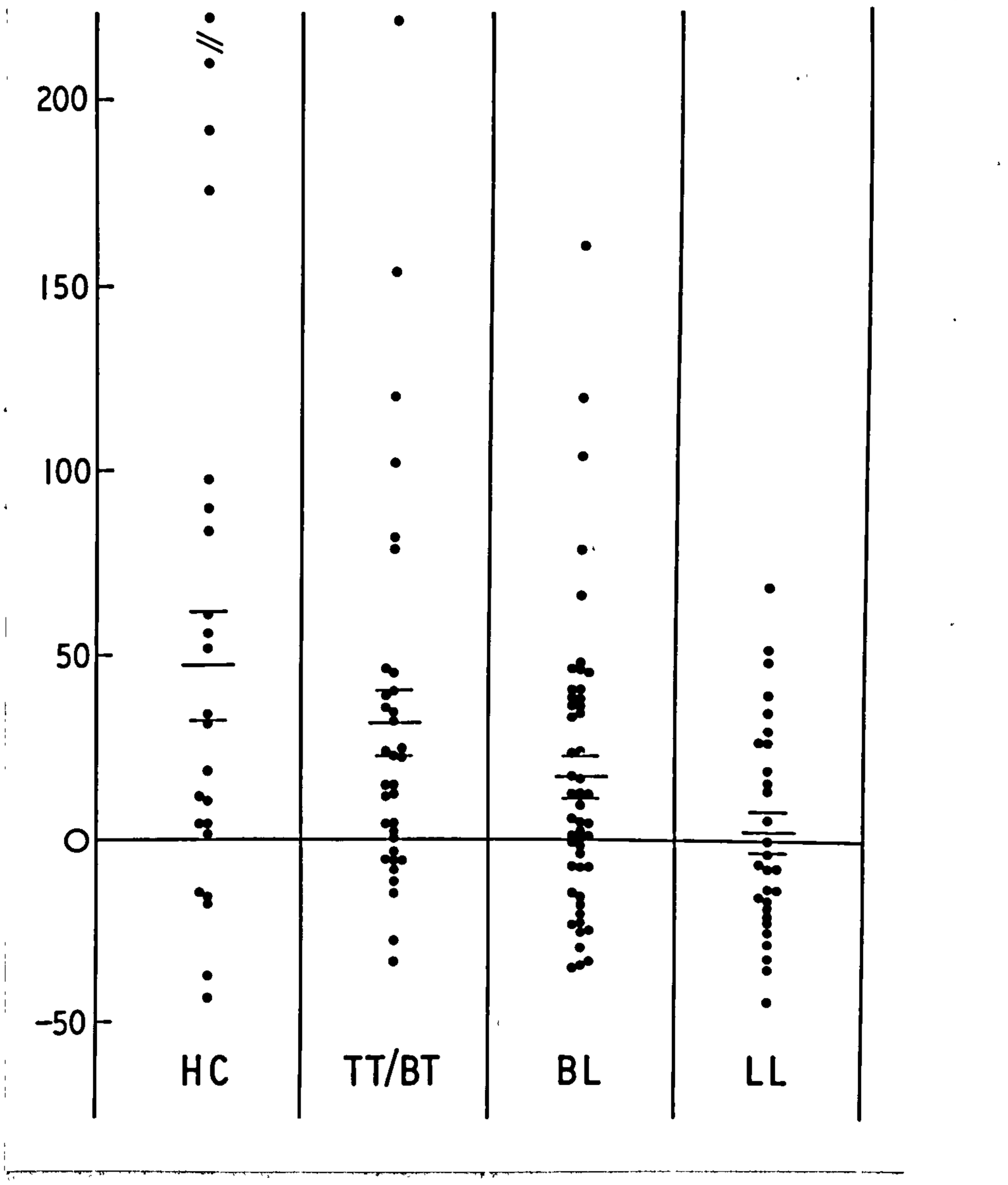


Figure B1(i). Oestrogen excretion according to the clinical classification of the mother. Values are plotted (in μmol/24 hr) relative to the lower limit of normal excretion in European women for the same period of gestation. (The means and standard error of the mean are indicated.)

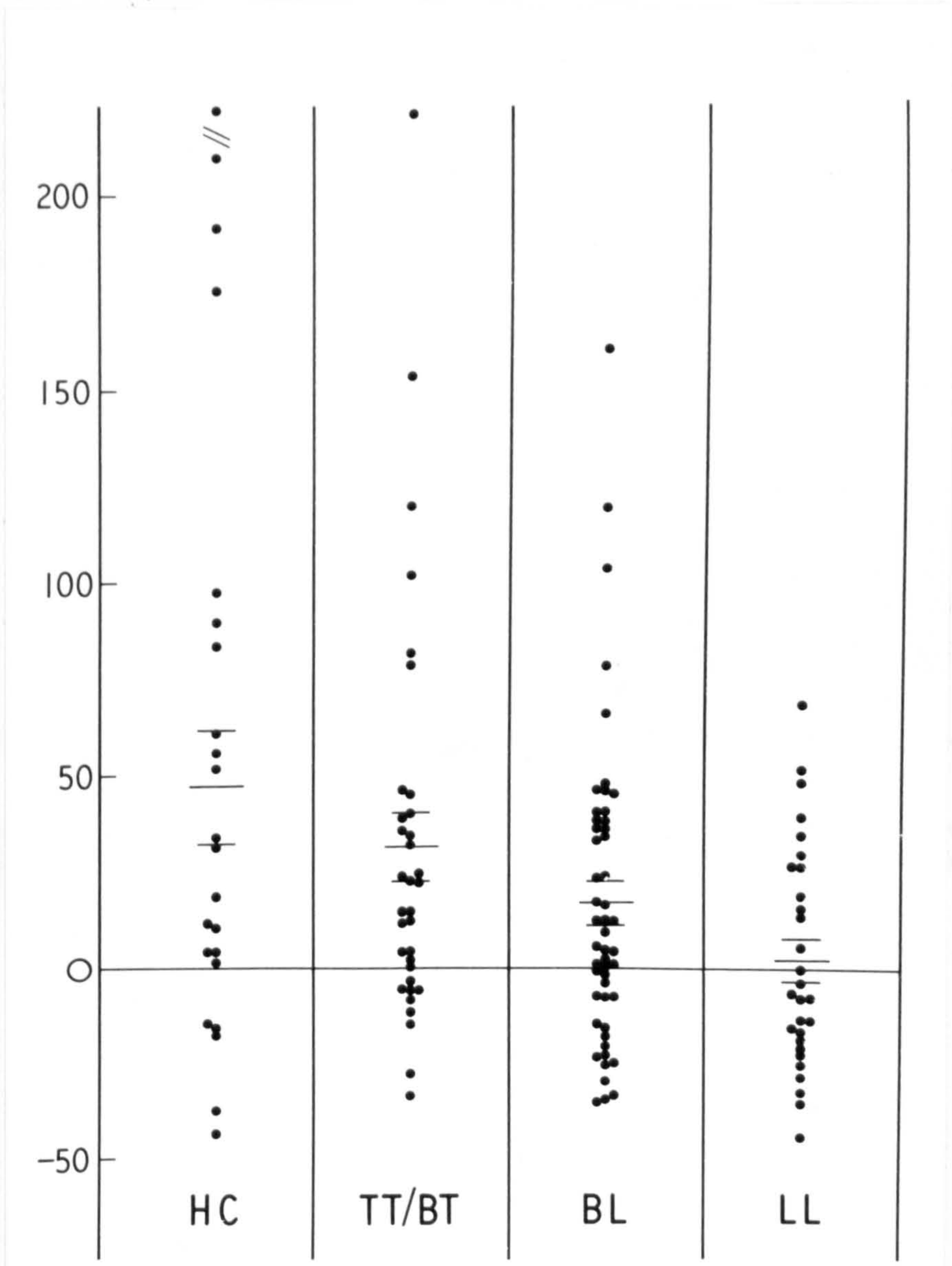


Figure B1(i). Oestrogen excretion according to the clinical classification of the mother. Values are plotted (in μmol/24 hr) relative to the lower limit of normal excretion in European women for the same period of gestation. (The means and standard error of the mean are indicated.)

TABLE B1(i)

Mean oestrogen excretion related to reference value for gestation and incidence of subnormal values in pregnant Ethiopian women in different leprosy classifications

Classification of the mother	No. of patients	No. of assays	Oestrogen excretion * Mean ± SEM	Proportion of subnormal values (as percentage of all assays)
HC	15	23	+ 47 (± 14.7)	22
TT and BT	20	34	+ 31 (± 9.1)	26
BL	27	50	+ 16 (± 5.7)	36
LL	17	29	+ 2 (± 5.4)	59

* Value is expressed in μmol/24 hours, oestriol, relative to the lower limit of excretion in normal European women for the same period of gestation (Heys et al, 1968).

TABLE B1(ii)

Infant birth weight, placental weight and coefficient of singleton term deliveries, according to the clinical classification of the mother

Values are mean ± SEM; number of observations in parenthesis

Classification of the mother	Birth Weight (g)	Placental Weight (g)	Placental Coefficient
HC	3252 ± 142.8 (9)	613 ± 50.1 (6)	0.19 ± 0.004 (6)
TT and BT	3100 ± 78.1 (18)	575 ± 52.0 (16)	0.18 ± 0.007 (16)
BL	2982 ± 92.6 (22)	540 ± 36.4 (17)	0.18 ± 0.009 (17)
LL	2522 ± 103.0 (9)	339 ± 18.2 (7)	0.14 ± 0.005 (7)

TABLE B1(iii)

Incidence of low birth weight (below Lubchenco's tenth centile) and subnormal oestrogen excretion in pregnant Ethiopian women with leprosy and in healthy subjects

	Patients where birth weight recorded	Birth Weight 10%	Low Oestrogen	Birth Weight 10%	Low Oestrogen
HC	9	9	1	0	0
TT and BT	18	17	4	1	1
BL	22	18	5	4	3
LL	9	5	2	4	3
TOTAL	58	49	12 (24%)	9	7 (78%)

for cephalo-pelvic disproportion and one died at 7 days from pneumonia. All other babies did well.

c) BL group. Three babies of the 27 delivered in this group showed signs of hypoxia in labour. There was one neonatal death in a baby delivered at home.

d) LL group. All 17 babies were in good condition on delivery except for one who died immediately after delivery at home, and one who suffered from acute hypoxia associated with prolonged labour.

Oestrogen excretion, infant birth weight and placental weight for women receiving corticosteroids

The oestrogen excretion of 3 patients receiving prednisolone for leprosy reactions throughout the study period was subnormal in 8 out of 10 assays, but the values for each patient showed a rising trend. The fourth patient who also received high dose clofazimine showed a continuous decline in oestrogen excretion (Figure B1(ii). All 4 babies were in good condition at birth; only one weighed less than Lubchenco's tenth centile, and the mother was not receiving clofazimine.

The suppressive effect of prednisolone on oestrogen excretion in women with tuberculoid leprosy is shown in Table B1(iv).

The effect of clofazimine on oestrogen excretion

The effect of immunosuppressive doses of clofazimine (300 mg daily) was studied in one patient who was already receiving prednisolone but whose initial oestrogen excretion was within the normal range. Introduction of clofazimine therapy was associated with diminished oestrogen excretion although a live, surviving infant (2.8 kg) was delivered at 39+ weeks of gestation. In 3 other women, who were already established on clofazimine (300 mg per week) for dapsone resistant leprosy before oestrogen assays were commenced, 5 out of 6 values (85%) were subnormal (relative to those of European women for the same period of gestation). The effect of dapsone and of clofazimine on oestrogen excretion is demonstrated in Figure B1(iii). where the oestriol assays relative to the lower limit of normal for European women for the same period of gestation are shown: in the HC group; patients with "cured" TT and BT/RFC who were not receiving dapsone; TT and BT patients receiving dapsone; LL

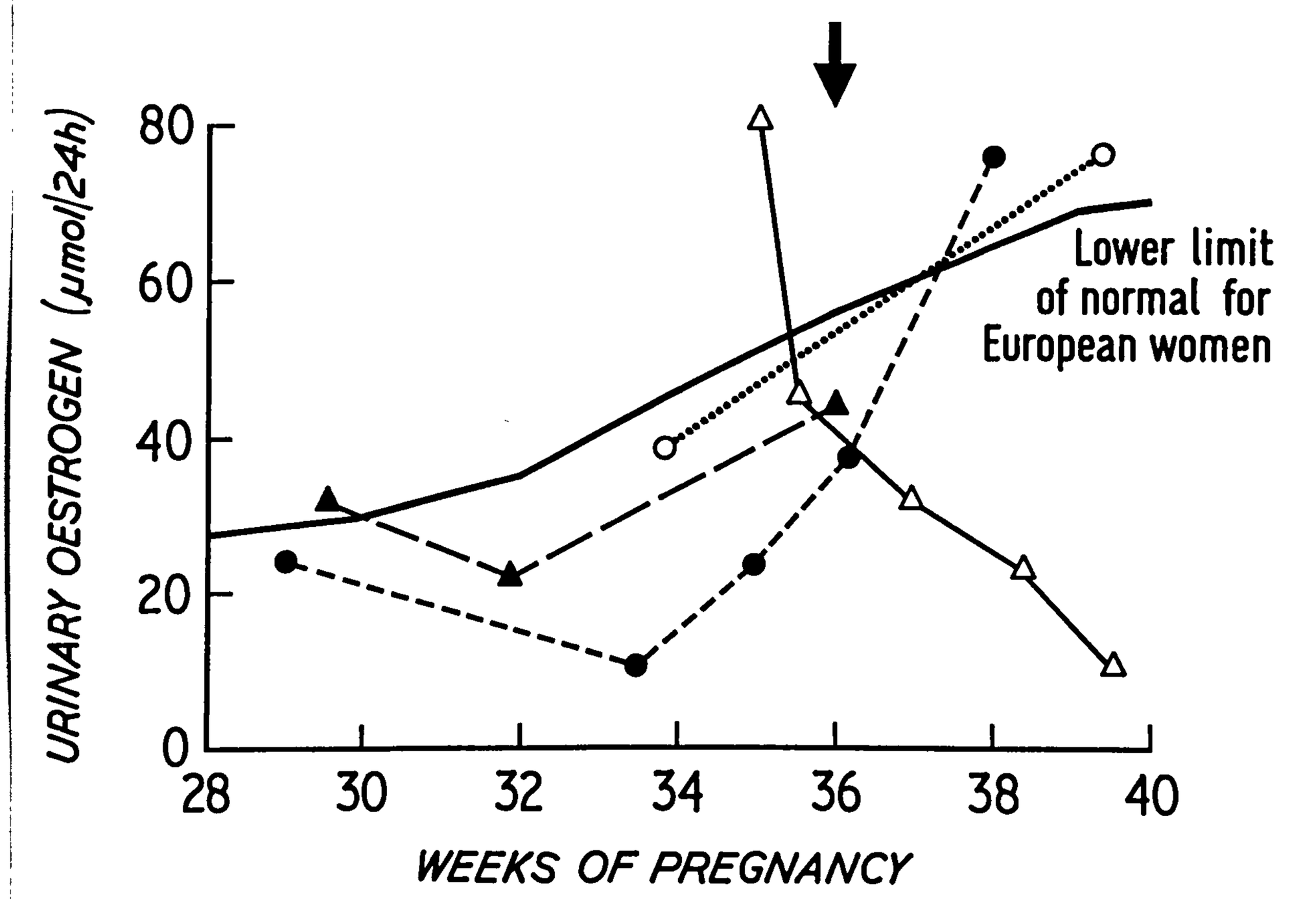


Figure B1(ii). Oestrogen excretion in four pregnant women receiving prednisolone (O....O, ● - - ● ; ▲— —▲), one of whom also received clofazimine (Δ——Δ) from 36 weeks' gestation, starting date indicated ↓

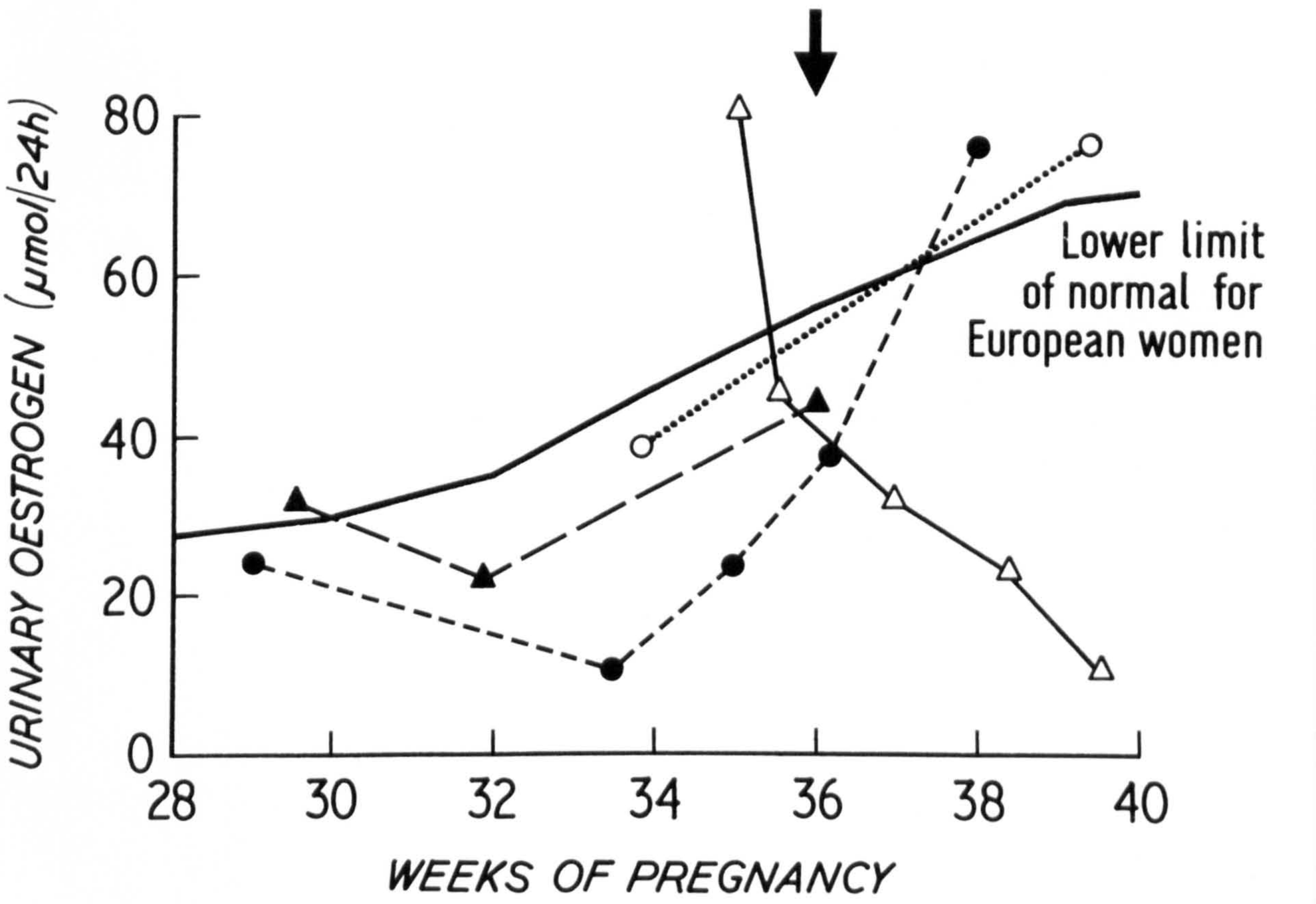


Figure B1(ii). Oestrogen excretion in four pregnant women receiving prednisolone (○....○, ●- - ●, ▲— —▲), one of whom also received clofazimine (△———△) from 36 weeks' gestation, starting date indicated ↓

TABLE B1(iv)

Oestrogen excretion in women with "cured" tuberculoid leprosy, and active tuberculoid leprosy receiving dapsone 50–100 mg daily or dapsone and prednisolone

Medication	Number of women	Number of assays	Number of subnormal assays*	Oestrogen excretion (Mean ± SEM) *
None	13	22	5 (23%)	29.1 (±9.4)
Dapsone 50–100 mg daily	7	13	4 (31%)	45.2 (±20.8)
Dapsone and prednisolone	2	5	4 (80%)	-16.4 (± 7.7)

* Value is expressed in μmol/24 hours, oestriol, relative to the lower limit of excretion in normal European women for the same period of gestation (Heys et al, 1968).

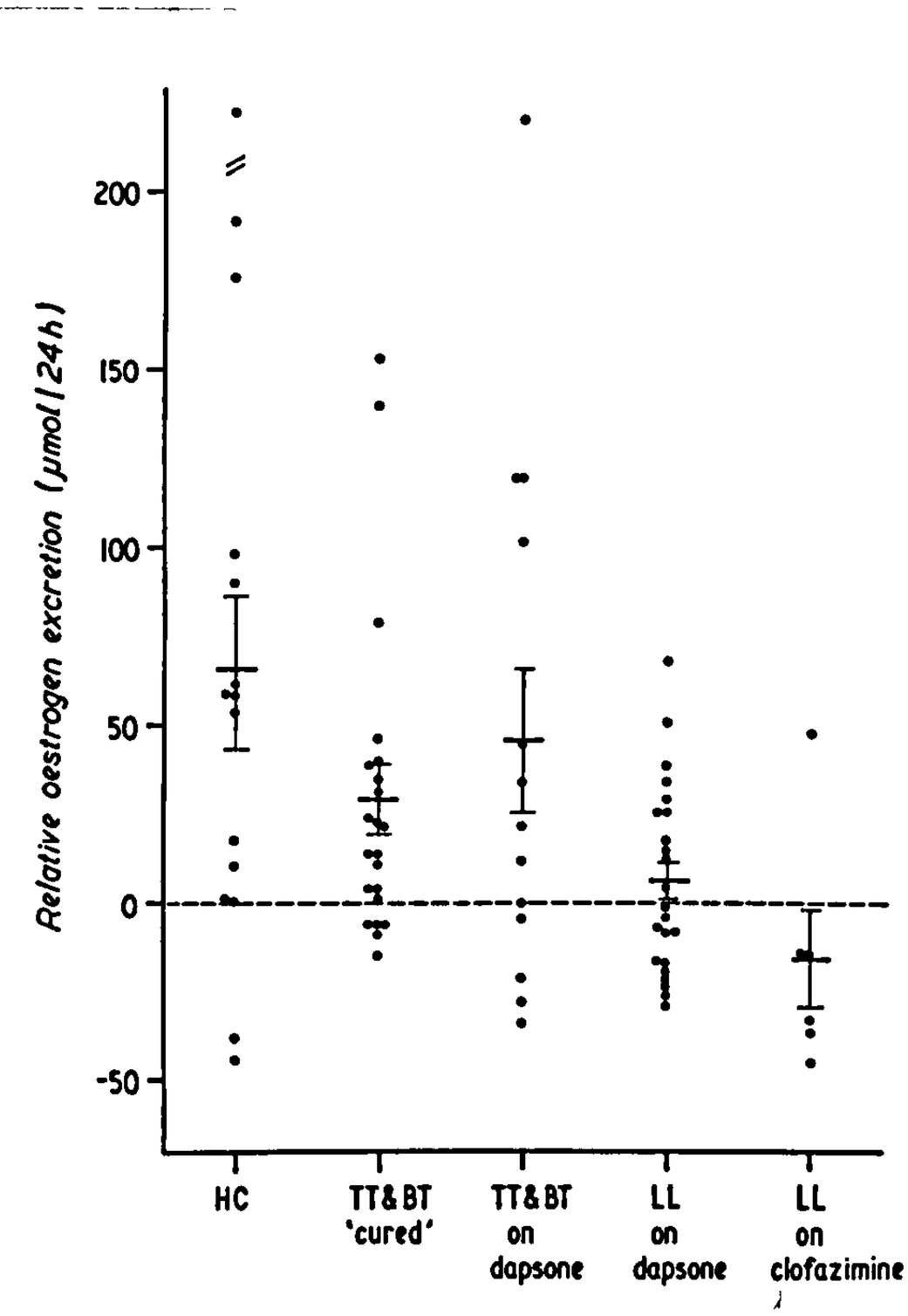


Figure B1(iii). Relative oestrogen excretion, according to the clinical classification of the mother, showing the effect of dapsone and clofazimine on oestrogen excretion.

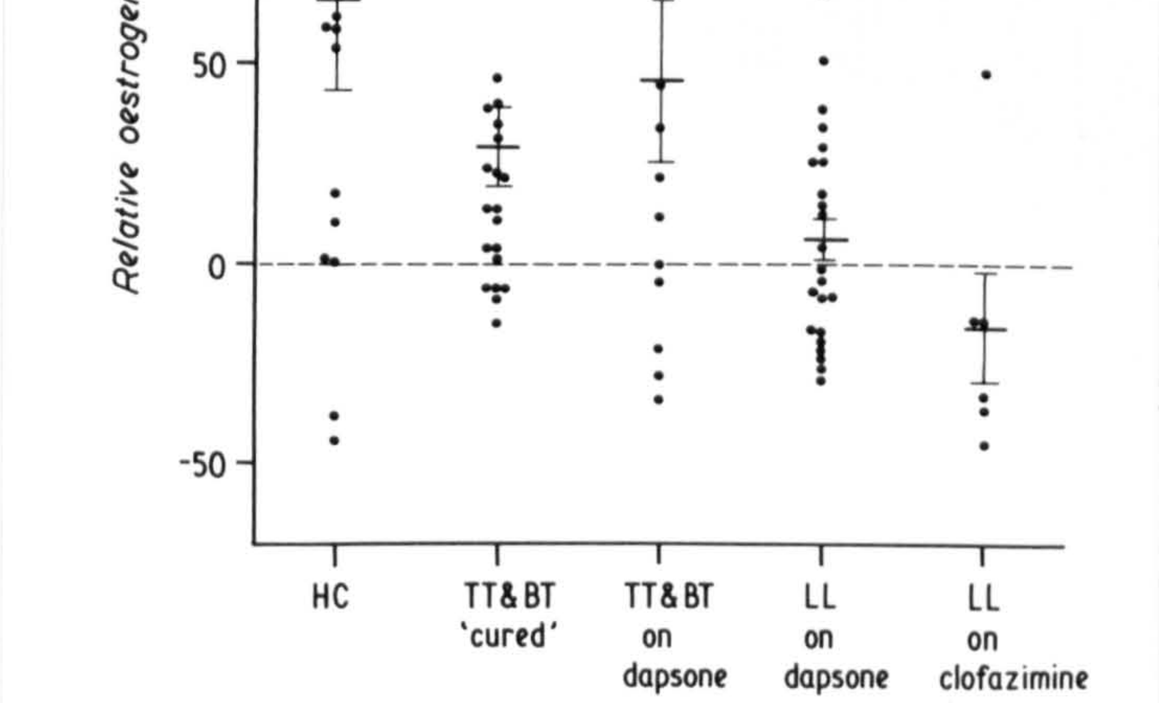


Figure B1(iii). Relative oestrogen excretion, according to the clinical classification of the mother, showing the effect of dapsone and clofazimine on oestrogen excretion.

patients receiving dapsone; and LL patients receiving clofazimine.

Oestrogen excretion is further suppressed by the combination of clofazimine and prednisolone (Table BI(v)).

TABLE B1(v)

Oestrogen excretion in women with lepromatous leprosy (LL) receiving dapsone, dapsone and prednisolone, clofazimine, clofazimine and prednisolone

Medication	Number of women	Number of assays	Number of subnormal assays *	Oestrogen excretion (Mean ± SEM) *
Dapsone	14	23	12 (52%)	+ 6.3 (± 5.5)
Dapsone + prednisolone	2	4	2 (50%)	+ 5.5 (± 9.2)
Clofazimine	3	6	5 (85%)	- 15.7 (± 13.7)
Clofazimine + prednisolone	1	3	3 (100%)	- 43.3 (± 8.7)

* Value is expressed in μmol/24 hours, oestriol, relative to the lower limit of excretion in normal European women for the same period of gestation (Heys et al, 1968).

2. Babies of mothers with leprosy have small placentae and low birth weights

Classification and treatment of mothers

One hundred and fifty-five pregnancies were studied; 121 were from mothers with leprosy, and 34 from 30 healthy mothers (healthy controls: HC).

The 116 mothers with leprosy were classified as follows using the scale of Ridley and Jopling (1966) :

Tuberculoid	(TT)	...	4 (4 pregnancies)
Borderline tuberculoid	(BT)	...	37 (38 pregnancies)
Borderline lepromatous	(BL)	..	43 (44 pregnancies)
Lepromatous	(LL)	...	32 (35 pregnancies

Most patients were receiving treatment (usually dapsone 50-100 mg daily) but 23 of them (1 BL, the rest BT or TT) were believed cured, had stopped treatment and been "released from control (RFC). Six patients (2 BL, 4 LL) had developed dapsone resistant leprosy, and were receiving clofazimine (4 patients all LL, 5 pregnancies) or rifampicin plus thiambutosine (2 patients both BL). Also 5 patients (2 BT, 3 LL) received prednisolone during most of the second and/or third trimestres for leprosy reactions.

Comparability of the groups of mothers

The mothers were all from the same low socio-economic group. There were no statistical differences within this group between the different clinical classifications of the mothers with regard to age, height, maximum weight recorded during pregnancy, skinfold thickness, haemoglobin levels or parity (Table B2(i)). Nor were there any significant differences in the blood pressure recordings with regard to either the peak systolic and diastolic measurements or the means of the systolic and diastolic blood pressures in each trimestre.

Expected date of delivery

The Ethiopian women, both Ethiopian orthodox and Moslem, followed traditional laws of purification after menstruation and were able to give the date of "cleansing" according to the Ethiopian (Church calendar. Thus their dates for last menstruation and hence

TABLE B2(i)

Status of the mothers according to their clinical classification

Clinical Classification		Age (years)	Height (cm)	Weight (kg) (maximum recorded)	Skinfold Thickness Right Sub-Scapular (mm)	Left Triceps (mm)	Haemoglobin (g %)	Gravidity	Primigravidae
HC (N = 34)	n	28	16	29	8	8	25	33	7
	$\bar{x}$	26.2	157.4	57.6	9.9	10.2	13.6	2.9	
	± SEM	0.94	1.21	0.96	0.89	1.01	0.22	0.30	(20.6%)
TT and BT (N = 42)	n	40	25	37	23	23	34	42	7
	$\bar{x}$	27.8	157.8	59.1	9.0	9.4	13.5	3.2	
	± SEM	0.96	1.71	1.32	0.54	0.86	0.18	0.28	(16.7%)
BL (N = 44)	n	39	24	39	24	24	29	41	8
	$\bar{x}$	27.0	156.4	55.3	9.1	9.1	12.9	3.2	
	± SEM	0.84	1.21	0.98	0.59	0.61	0.33	0.34	(18.0%)
LL (N = 35)	n	34	21	25	22	22	22	35	9
	$\bar{x}$	27.1	155.3	55.7	8.7	10.0	13.1	2.5	
	± SEM	1.03	1.28	1.26	0.78	0.99	0.27	0.23	(25.7%)

N = number of pregnancies.
n = number of mothers.
$\bar{x}$ = mean.
SEM = standard error of the mean.

expected date of delivery were remarkably accurate. Of 143 patients, 124 knew the date of the last menstrual period, 10 were exactly one calendar month wrong, 6 had lactated since the previous delivery, and only 3 were wildly out in their calculations. The mean period of gestation for the women studied was 40 weeks + 1.8 days ($\pm$ SEM 0.8 days).

Birth weight of babies

Babies born to LL mothers were the smallest (Figure B2(i)), weighing significantly less than the next smallest group, those of BL mothers ($p < 0.001$, Mann Whitney U test). Both LL ($p < 0.001$) and BL ($p < 0.01$) group babies were significantly smaller than those of TT and BT mothers, whether or not leprosy bacilli were found in the mother's skin smears (Tables B2(ii) and B2(iii)). Babies of healthy mothers weighed much the same as those of TT and BT mothers.

Placental weights

The placental weights followed the same trend as the baby weights (Figure B2(ii)), with the placentae of LL mothers weighing significantly less ($p < 0.001$, Mann Whitney U test) than those of healthy controls or other leprosy patients. The small size of the placenta in LL mothers is further demonstrated in Figure B2(iii) where the placental coefficients are shown. The placental coefficients (placental weight divided by the baby's weight) of the LL mothers are significantly less than those of the other leprosy patients ($p < 0.01$) or healthy controls ($p < 0.002$).

Effect of mother's parity on the weight of baby and placenta

The greater number of primigravidae in the LL group could have caused a lower mean baby birth weight in that group. However, when comparisons are made within the LL group, it is seen that there is no difference in the birth weight of babies of the primigravidae or multiparous mothers (Table B2(iv)). Furthermore, the trend of falling birth weight and decrease in placental weight seen in all the mothers from HC to LL is reflected in the primigravidae of each group (Table B2(v)).

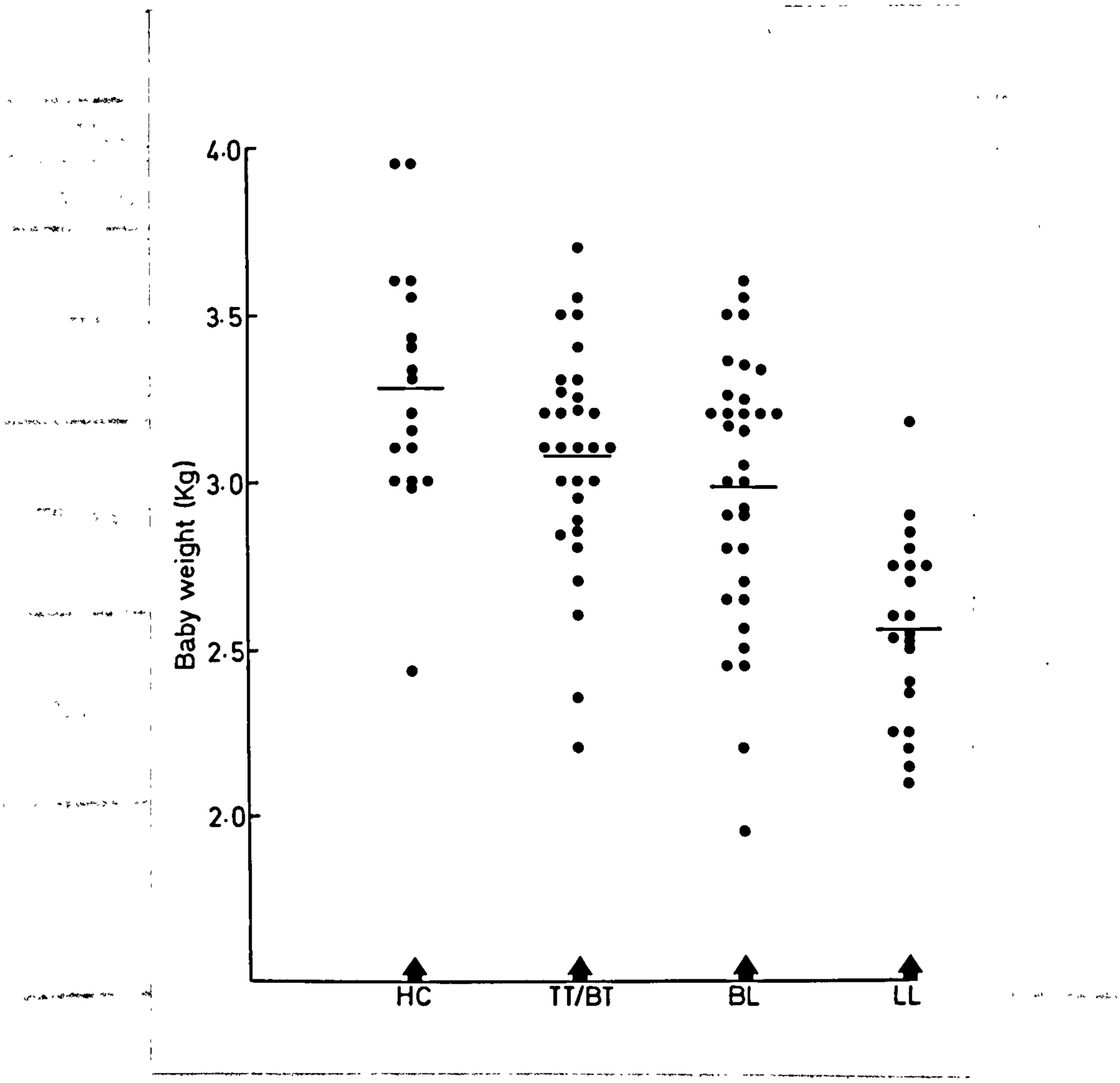


Figure B2(i). Birth weight (kg) of full term, singleton babies according to the clinical classification of the mother.

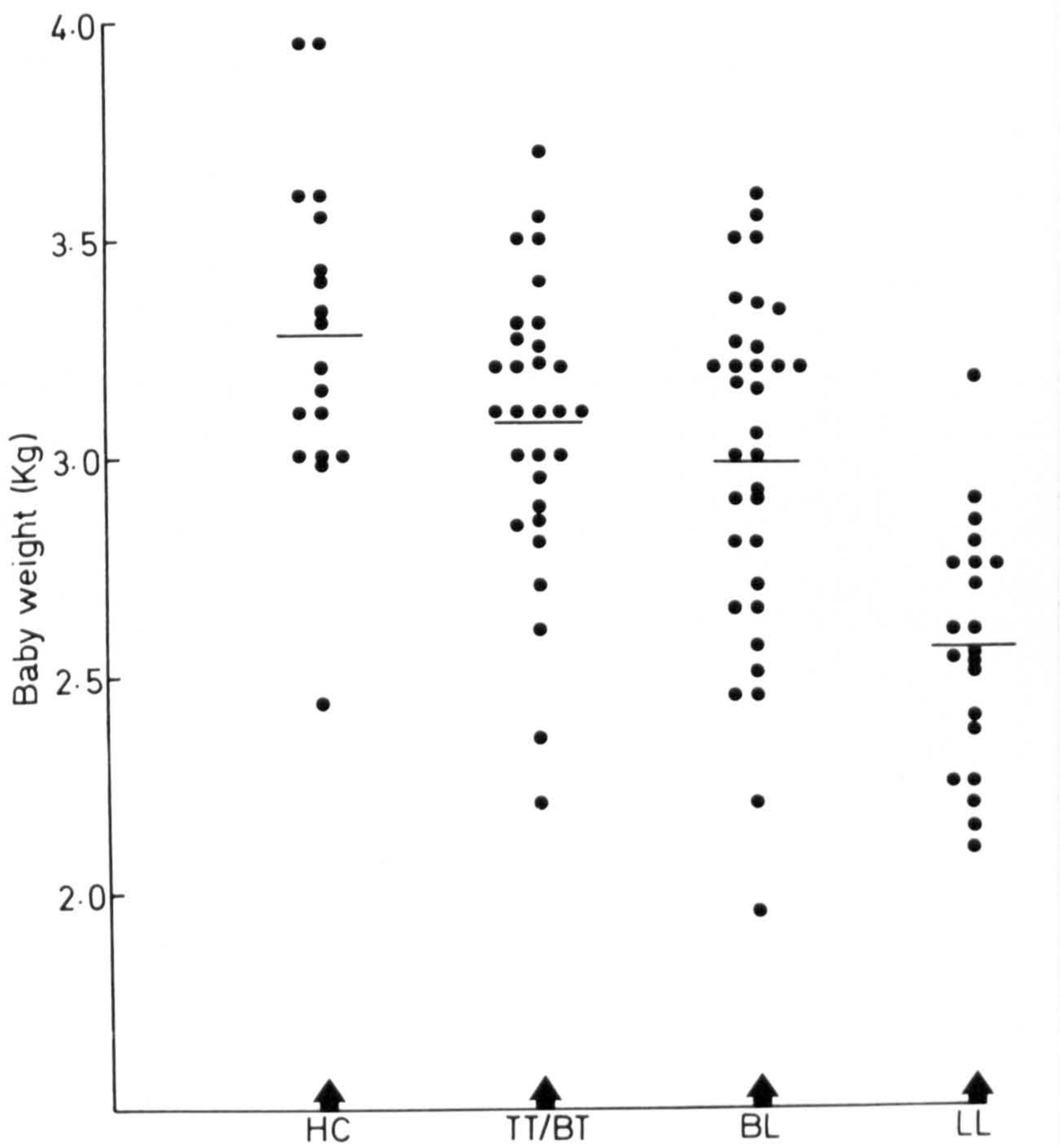


Figure B2(i). Birth weight (kg) of full term, singleton babies according to the clinical classification of the mother.

TABLE B2(ii)

Baby birth weight, placenta weight and placental coefficient according to the clinical classification of the mother

Clinical Classification of the mother		Baby Birth Weight (g)	Placenta Weight (g)	Placental Coefficient
HC	n	18	13	13
	$\bar{x}$	3280.6	595.0	0.184
	± SEM	87.6	35.4	0.01
TT and BT	n	30	25	25
	$\bar{x}$	3075.0	569.4	0.181
	± SEM	61.1	19.4	0.05
BL	n	33	26	26
	$\bar{x}$	2985.6	521.0	0.173
	± SEM	69.9	26.4	0.01
LL	n	21	15	15
	$\bar{x}$	2558.1	362.0	0.144
	± SEM	60.5	19.1	0.01

TABLE B2(iii)

Birth weight of babies and placenta weight of LL mothers according to the activity of the mother's leprosy during her pregnancy

BI +ve/MI +ve			BI +ve/MI -ve			BI -ve/ MI -ve		
Mother's Study No.	Birth Weight (g)	Placenta Weight (g)	Mother's Study No.	Birth Weight (g)	Placenta Weight (g)	Mother's Study No.	Birth Weight (g)	Placenta Weight (g)
A9/53	2800	425	A9/64	2250	300	A9/38	2700	375
A9/74	2750	400	A9/97(a)	2520	460	A9/46	2900	
A9/84	2380		A9/210	2600	430	A9/97(b)	2250	250
A9/91	2540		A9/256	2500	300	A9/208	2200	350
A9/95	3180		A9/260	2400	350	A9/215	2550	500
A9/96	2600	400	A9/267	2850		A9/226	2150	290
A9/257	2100	260				A9/231	2750	
						A9/254	2750	350
n	7	4	n	6	5	n	8	6
$\bar{x}$	2621.4	371.2	$\bar{x}$	2520.0	368.0	$\bar{x}$	2531.2	352.5
$\pm$ SEM	128.9	37.5	$\pm$ SEM	82.3	33.1	$\pm$ SEM	103.1	35.0

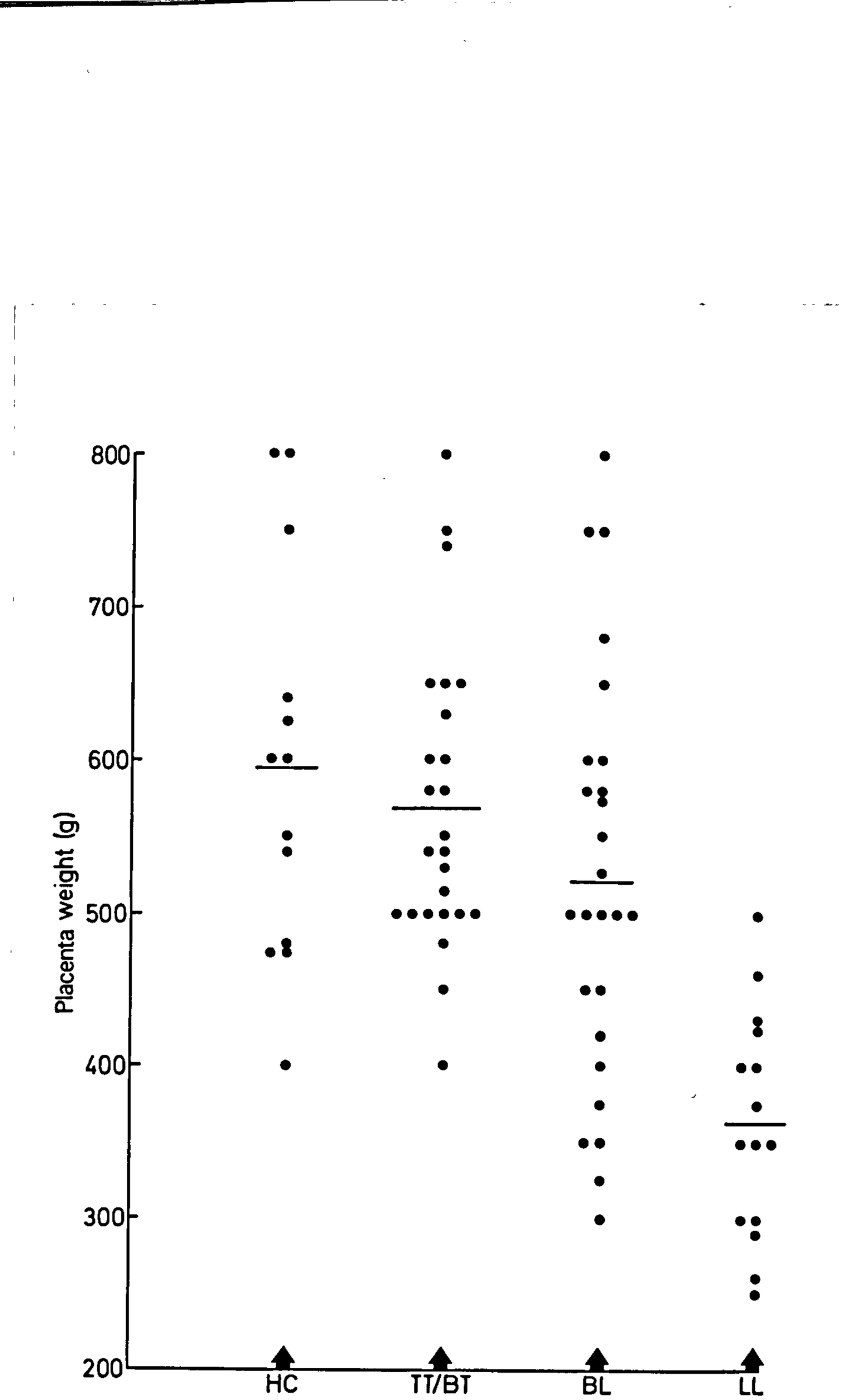


Figure B2(ii). Trimmed placental weight (g) of full term singleton pregnancies according to the clinical classification of the mother.

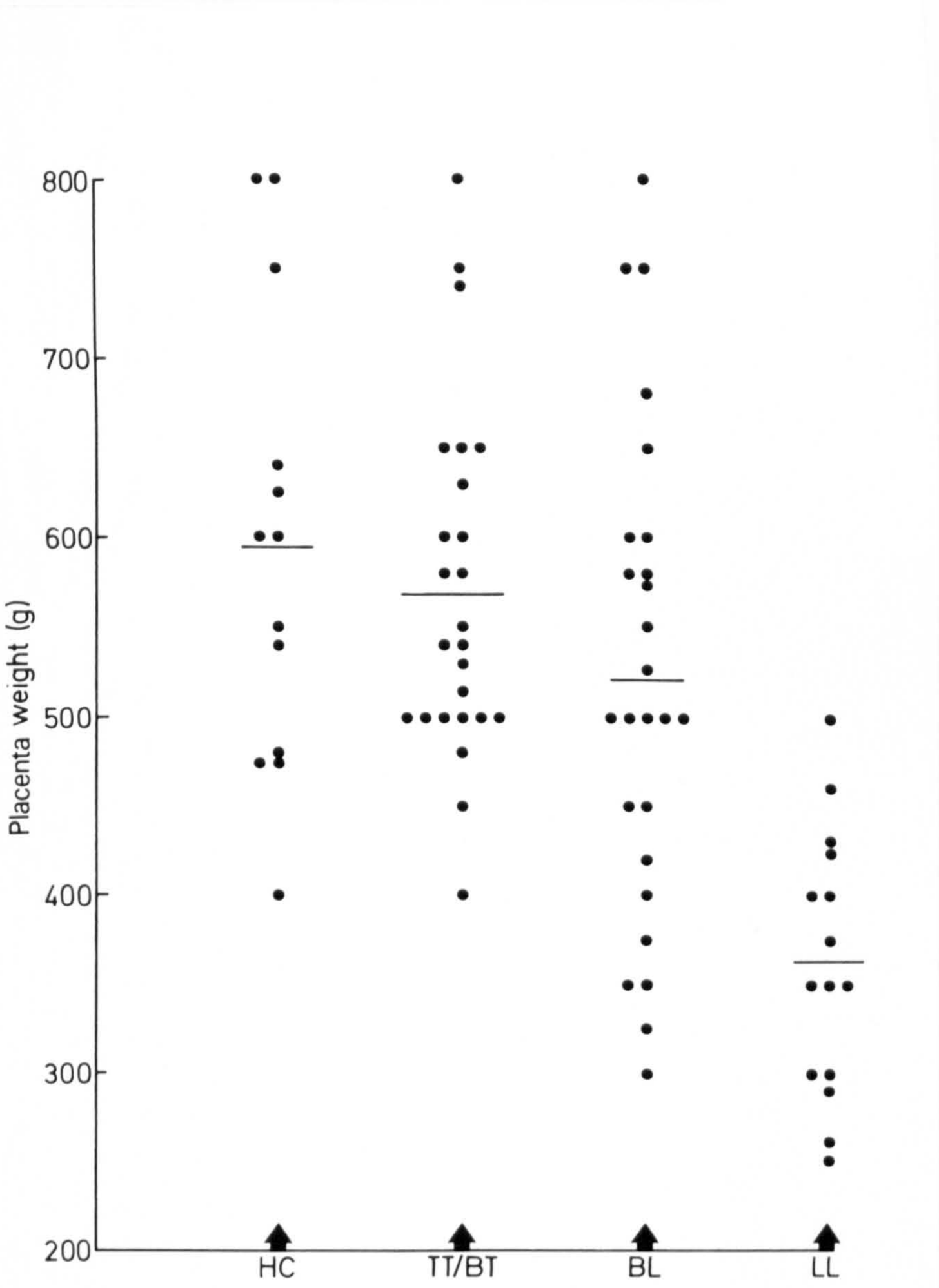


Figure B2(ii). Trimmed placental weight (g) of full term singleton pregnancies according to the clinical classification of the mother.

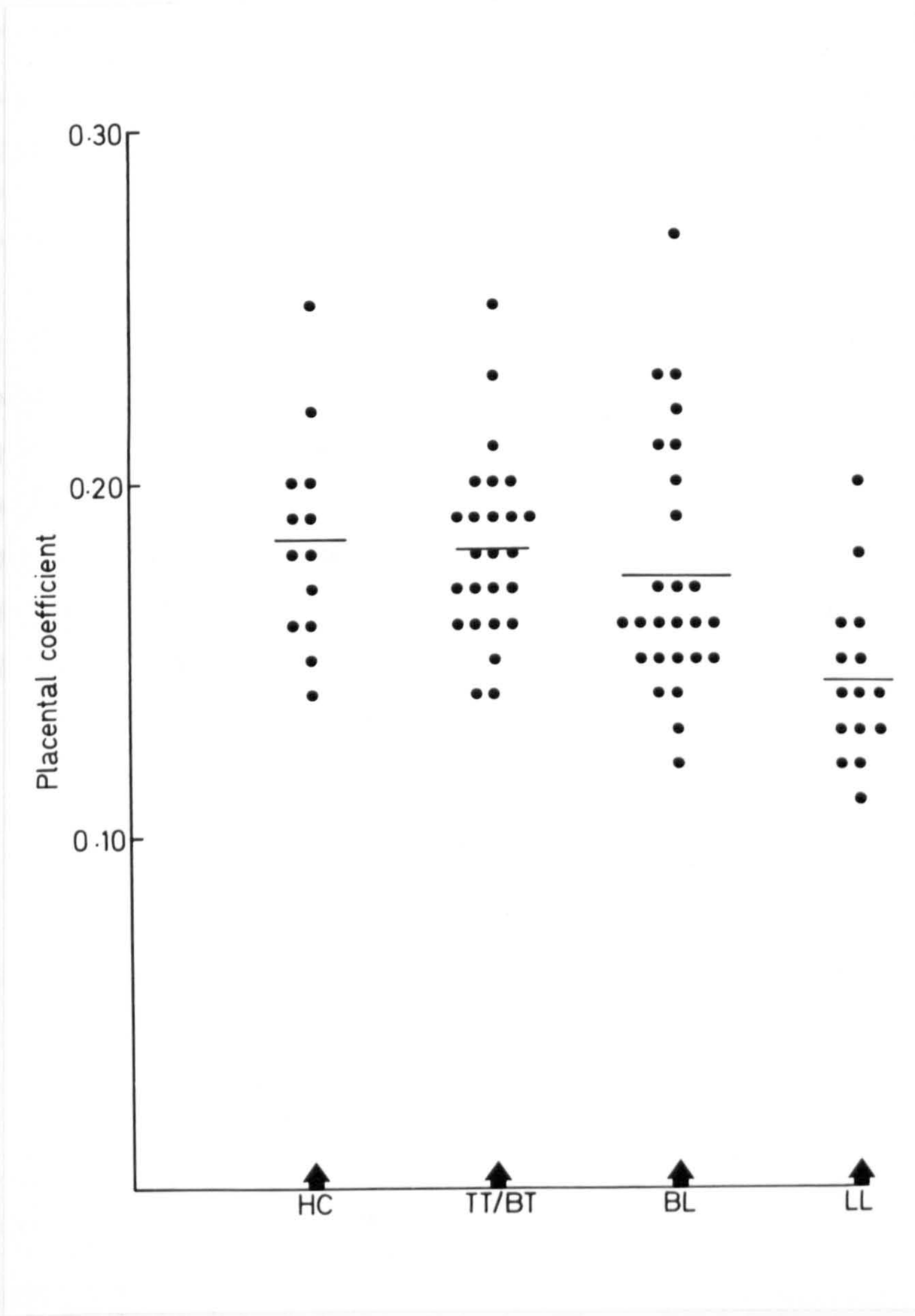


Figure B2(iii). Placental coefficient (ratio of placental weight : baby weight) according to the clinical classification of the mother.

TABLE B2(iv)

Birth weight of babies and placenta weight of LL mothers according to parity of the mother

Primiparous			Multiparous			
Mother's Study No.	Baby Weight (g)	Placenta Weight (g)	Mother's Study No.	Parity	Baby Weight (g)	Placenta Weight (g)
A9/46	2900		A9/38	1 + 0	2700	375
A9/53	2800	425	A9/64	3 + 0	2250	300
A9/84	2380		A9/74	5 + 1	2750	400
A9/91	2540		A9/95	1 + 0	3180	
A9/97(a)	2520	460	A9/96	2 + 0	2600	400
A9/210	2600	430	A9/97(b)	1 + 0	2250	250
A9/226	2150	290	A9/208	1 + 0	2200	350
A9/231	2750		A9/215	2 + 0	2550	500
			A9/254	2 + 0	2750	350
			A9/256	1 + 0	2500	300
			A9/257	2 + 0	2100	260
			A9/260	3 + 0	2400	350
			A9/267	2 + 0	2850	
n	8	4	n		13	11
$\bar{x}$	2580	401.2	$\bar{x}$		2544.6	348.6
$\pm$ SEM	85.6	37.8	$\pm$ SEM		84.8	21.6

Parity x + y

x ≡ No. of pregnancies > 28 weeks' gestation.

y ≡ No. of pregnancies < 28 weeks' gestation.

TABLE B2(iv)

Birth weight of babies and placenta weight of LL mothers according to parity of the mother

Primiparous			Multiparous			
Mother's Study No.	Baby Weight (g)	Placenta Weight (g)	Mother's Study No.	Parity	Baby Weight (g)	Placenta Weight (g)
A9/46	2900		A9/38	1 + 0	2700	375
A9/53	2800	425	A9/64	3 + 0	2250	300
A9/84	2380		A9/74	5 + 1	2750	400
A9/91	2540		A9/95	1 + 0	3180	
A9/97(a)	2520	460	A9/96	2 + 0	2600	400
A9/210	2600	430	A9/97(b)	1 + 0	2250	250
A9/226	2150	290	A9/208	1 + 0	2200	350
A9/231	2750		A9/215	2 + 0	2550	500
			A9/254	2 + 0	2750	350
			A9/256	1 + 0	2500	300
			A9/257	2 + 0	2100	260
			A9/260	3 + 0	2400	350
			A9/267	2 + 0	2850	
n	8	4	n		13	11
$\bar{x}$	2580	401.2	$\bar{x}$		2544.6	348.6
$\pm$ SEM	85.6	37.8	$\pm$ SEM		84.8	21.6

Parity x + y

x ≡ No. of pregnancies > 28 weeks' gestation.

y ≡ No. of pregnancies < 28 weeks' gestation.

TABLE B2(v)

Birth weight of babies and placenta weight of primiparous mothers according to the clinical classification of the mother

HC			TT and BT			BL			LL		
Mother's Study No.	Baby Birth Weight (g)	Placenta Weight (g)	Mother's Study No.	Baby Birth Weight (g)	Placenta Weight (g)	Mother's Study No.	Baby Birth Weight (g)	Placenta Weight (g)	Mother's Study No.	Baby Birth Weight (g)	Placenta Weight (g)
A9/13	3200	600	A9/3	3270	580	A9/7	2560		A9/46	2900	
A9/61	3600		A9/24	3700	740	A9/18	3200		A9/53	2800	425
A9/67	3400	640	A9/60	3550	650	A9/36	3250	500	A9/84	2380	
A9/252	2980	500	A9/66	3000	500	A9/76	3200	600	A9/91	2540	
			A9/245	3500	800	A9/80	2200	325	A9/97(a)	2520	460
			A9/246	2840	400	A9/216	2450		A9/210	2600	430
									A9/226	2150	290
									A9/231	2750	
n	4	3	n	6	6	n	6	3	n	8	4
$\bar{x}$	3295	580	$\bar{x}$	3310	611.7	$\bar{x}$	2810	475	$\bar{x}$	2580	401.2
$\pm$ SEM	133	41.6	$\pm$ SEM	137.2	61	$\pm$ SEM	188.1	80.4	$\pm$ SEM	85.6	37.8

Foetal distress and neonatal asphyxia

Foetal distress or Apgar scores of 4/10 or less at one minute after birth were recorded in 20% of the babies of BL or LL mothers (Table B2(vi)). Resuscitation was carried out immediately; babies with persistent respiratory difficulty were transferred to the paediatric intensive care unit.

TABLE B2(vi)

Apgar score of liveborn babies according to the clinical classification of the mother

Clinical Classification of the Mother	No. of babies with Apgar score recorded	No. of babies with Apgar score 10/10	No. of babies with Apgar score 4/10 or less, or with acute foetal distress in labour
HC	17	17 (100%)	0
TT and BT	17	14 (82%)	0
BL	24	16 (67%)	5 (21%)
LL	17	9 (53%)	3 (18%)

3. The placenta in leprosy

Placentae from 78 women with leprosy and 17 healthy controls were obtained at delivery. The trimmed placental weight was recorded. The placenta, membranes and cord were inspected and specimens obtained for light and electron microscopy, and immunohistology. Unfixed frozen specimens were used for i) searching for M. leprae by concentration methods, and ii) biochemical and enzyme studies including assays of DNA, total protein, alkaline phosphatase, β-glucuronidase and cytochrome oxidase.

Serum bilirubin was measured by spectrophotometry in 25 µl samples of undiluted cord sera from babies of mothers with lepromatous leprosy, together with samples from babies of mothers with tuberculoid leprosy and healthy mothers as controls.

Observations

i) Assessment of the women

Women who were found to have solid staining M. leprae (AFB) in skin smears or biopsies during pregnancy or early lactation were considered to be "potentially infectious" as far as haematogenous spread via the placenta is concerned. The numbers are shown in Table B3(i).

ii) Obstetrical complications

There were no cases of acute hypertension of pregnancy, nor of antepartum haemorrhage due to placenta praevia or abruptio placentae. There were two cases of twins, one binovular, one uni-ovular.

iii) Macroscopic examination

The placentae of women with lepromatous leprosy were much smaller than those with BL, BT and TT leprosy or the healthy controls. Placental weights and coefficients are shown in Table B3(ii). In general, these placentae were also much thinner, in other words the maternal surface area was not reduced. One placenta of a woman with lepromatous leprosy (BI = 0) was found to have an abscess measuring 1 x 1 cm diameter. Apart from this there were no gross macroscopic changes seen.

TABLE B3(i)

Classification of mother's leprosy	Number of Pregnancies Studied	Number of women "potentially infectious"
HC	18	0
TT and BT/RFC	11	0
TT and BT Active	15	1
BL	34 *	14
LL	21 *	10
TOTAL	99	25

* One case of twin pregnancy.

TABLE B3(ii)

Baby birth weight, placental weight, placental coefficient, foetal distress/Apgar < 4, foetal dysmaturity

Classification of mother's leprosy	Baby Weight ± SEM (grams)	Placental weight ± SEM (grams)	Placental coefficient ± SEM	No. of babies showing foetal distress/Apgar Score < 4	No. of clinically "dysmature" babies
HC	3252 ± 105 (16)*	581 ± 36 (14)	0.18 ± 0.008 (14)	0/12	0/16
TT and BT (total)	3088 ± 68 (25)	567 ± 21 (23)	0.18 ± 0.005 (23)	3/16	1/25
(TT and BT/RFC)	3091 ± 98 (11)	589 ± 34 (10)	0.19 ± 0.009 (10)	1/9	0/11
(TT and BT active)	3086 ± 98 (14)	550 ± 26 (13)	0.17 ± 0.006 (13)	2/7	1/14
BL	3000 ± 73 (31)	526 ± 26 (28)	0.175 ± 0.007 (27)	7/24	3/31
LL	2520 ± 68 (17)	363 ± 19 (15)	0.146 ± 0.006 (15)	3/15	4/17

* Number ( ) = number of observations.

iv) Search for M. leprae in the placenta

a) Routine light microscopy. No AFB nor AFB granules were seen in any of the sections examined.

b) Concentration methods. Of 7 patients all with extremely active BL or LL leprosy, only 2 were found to have AFB in the placenta; one patient had "? AFB debris" in the smears. The results are shown in Table B3(iii). Although a few AFB were seen in 2 of the homogenates, they represent an incredibly small infection, indicating that the placenta is not a site in which M. leprae are found in any significant quantities.

v) Placental morphology

Light and electron microscopy. No abnormality of placental morphology was observed, nor was any cellular infiltrate typical of M. leprae infection of the placenta seen.

vi) Immunohistology

Results are given for both test and control materials, as no reproducible differences were found in these two groups of tissues. Intervillous fibrin reacted positively with anti-sera to fibrin/fibrinogen, IgM and α-2-macroglobulin. The trophoblastic mantle was largely unreactive with any of the immunological reagents. Trophoblastic basement membranes (TBM) were positive for fibrin/fibrinogen and the C3d and C9 anti-complement sera, and the rare occurrence of IgG was noted. Not all villi contained such positive areas, and when TBM was reactive, it was only in intermittent portions. The mesenchymal stromata were unreactive per se, except for scattered patches of fibrinogen/fibrin, but Hofbauer cells in the stroma were noted to contain granules which reacted with antisera to C1q, C3d, C9 and sometimes with IgM and IgG. Foetal stem vessels were reactive with antibodies to Factor VIII and their apical aspects occasionally were positive with anti-α-2-macroglobulin and less commonly with antiserum to IgM.

These results must be considered as preliminary, as in

TABLE B3(iii)

Search for M. leprae by concentration methods
Results of smears from placentae

Patient No.	Skin smears		AFB in biopsy	No. of AFB
	BI	MI		
RR - A9/257	5.2	2.5%		10
RR - A9/ 70	2.3	0	Solid AFB 3-4+	4
RR - A9/235	5.0	6.7%		2 (probably not AFB, ? AFB debris)
RR - A9/89	4.4	2.0%		0
RR - A9/244	1.0	4.5%	Solid AFB ++	0
RR - A9/36	3.8	6.0%		0
RR - A9/225	3.3	5.5%		0

order to obtain maximal information, immunohistological studies should be carried out on freshly collected specimens. The specimens tested had of necessity been stored at -70° C for rather longer than the optimal acceptable period.

vii) Biochemical and enzyme studies

Results are shown in Table B3(iv). There is an elevation of DNA µg/mg protein comparing controls with placentae from women with lepromatous leprosy ($p = 0.01$ by F ratio). This suggests that there is a marked overall reduction in cytoplasmic protein relative to the size of the nucleus. By expressing the results of the enzyme activities per µg DNA, all enzyme activities were shown to fall. Total activities per cell probably fall whereas enzyme activities per mg protein showed a less marked rise, suggesting the loss of cytoplasm generally involves the relatively greater loss of proteins other than the enzyme measured. The enzymes show different patterns. Alkaline phosphatase, a membrane-bound enzyme, alters only in lepromatous leprosy, whereas β-glucuronidase, a lysosomal acid hydrolase, and cytochrome oxidase, a mitochondrial enzyme, change in both tuberculoid and lepromatous leprosy. Measurement of a cytosolic enzyme lactic dehydrogenase resulted in very low activities which we cannot comment on. These results are comparable with the simultaneously collected samples of controls, but not with results obtained from assays on fresh placentae.

viii) Serum bilirubin in cord sera

Results of the levels of bilirubin in cord sera are shown in Table B3(v). While no statistical significance can be drawn from the results, a trend is apparent: a measurable level of bilirubin in the cord serum was found in only 1/7 of the babies of healthy mothers compared with 5/8 babies of mothers with tuberculoid leprosy, and 11/18 babies of mothers with lepromatous leprosy. The highest bilirubin levels measured were in the babies of mothers with lepromatous leprosy. The significance of these results is not clear. There was no correlation with foetal distress, or clinical foetal dysmaturity, nor was there a correlation with placental coefficient.

TABLE B3(iv)

Placental enzyme studies
(Mean ± SD)

Classification of mother	DNA μg/mg wet wt.	DNA μg/mg protein	Alk. Phos. mU/mg protein	β-gluc. mU/mg protein	Cyt. Oxidase mU/mg protein
HC	2.16 ± 1.84	4.07 ± 3.32	35.4 ± 15.5	0.085 ± 0.016	0.098 ± 0.047
TT and BT	2.52 ± 1.58	4.89 ± 2.58	35.9 ± 19.5	0.103 ± 0.035	0.100 ± 0.042
BL	3.25 ± 2.16	6.94 ± 4.67	33.8 ± 10.4	0.136 ± 0.039	0.136 ± 0.053
LL	4.16 ± 1.51	9.47 ± 4.47 *	52.0 ± 27.7	0.124 ± 0.022	0.140 ± 0.066

* P = 0.01 by F ratio vs controls; TT and BT vs LL also shows significant difference; BL vs LL shows no significant difference.

TABLE B3(v)

Cord bilirubin levels (μmol/l) according to classification of the mother's leprosy

Classification of mother	Number of cord samples tested	Number with bilirubin <5 μmol/l (unrecordable)	Mean bilirubin (μmol/l) in those with measurable levels ± SEM
HC	7	6	6 (1)*
TT and BT (total)	8	3	7.4 ± 1.7 (5)
(TT and BT/ RFC)	4	1	5.7 ± 0.3 (3)
(TT and BT Active)	4	2	10.0 ± 4.0 (2)
BL	10	3	11.9 ± 2.4 (7)
LL	8	4	17.0 ± 2.9 (4)

* Number ( ) = number of observations.

There were no cases of ABO or Rhesus incompatibility, nor prematurity. None of the babies developed clinical jaundice during the first week of life.

4. Humoral defence factors in the breast milk of Ethiopian women with leprosy and healthy controls

Samples of breast secretions from day 1 of lactation to 2 years were collected and humoral defence factors measured. Secretory IgA, lactoferrin and albumin were measured in all samples by radial immunodiffusion, total protein was measured by the Folin Lowry technique; IgG, IgM, β1A and β1E proteins were measured in the samples collected within 24 hours following parturition. The samples from Ethiopian women were compared with the samples from normal Scottish nursing mothers grouped, where possible, over the same time periods.

Classification of mother's leprosy

The women were classified according to Ridley and Jopling (1966) as follows :-

	No. of mothers studied	No. of pregnancies studied	No. of milk samples
Healthy controls (HC)	15	15	23
Tuberculoid and borderline tuberculoid (total) (TT and BT)	22	22	47
"Cured" BT	7	7	12
Active TT and BT	15	15	35
Borderline lepromatous (BL)	29	29	80
Lepromatous (LL)	26	27	73
Total	92	93	223

Milk samples from mothers who were sero-positive for Hepatitis B were not examined. Milk samples from women who were well advanced into a second pregnancy while still lactating were excluded from the computed results.

Evaluation of the results

On the initial evaluation of the results, no significant differences were seen between the 5 groups of mothers: HC, "cured" BT, TT and BT active, BL and LL. As the numbers of samples in the first 3 groups were very small, and to make the results more meaningful,

the HC, TT and BT groups were grouped together on the grounds that their skin smears were negative for Mycobacterium leprae, although in 2 women with active BT leprosy, on one occasion each during late pregnancy, a single acid-fast bacillus had been observed in a skin biopsy. The results were computed with the samples grouped as follows :

1. Lepromatous leprosy - 72 samples
2. Borderline lepromatous leprosy - 77 samples
3. Non-lepromatous (NL) including women with tuberculoid leprosy and healthy controls - 66 samples

Further evaluation of the results in the women with lepromatous leprosy (BL and LL) was made by comparing those who were skin smear postive (BI and/or MI positive) with those who were skin smear (BI and MI) negative.

Observations

Tables B4(i) to (iv) illustrate respectively the levels of secretory IgA, lactoferrin, albumin and total protein which were found in the three Ethiopian groups (LL, BL and NL). In Table B4(i), statistical analyses of variance by the unpaired 't' test revealed only one difference between the three groups in that secretory IgA levels were significantly higher in the LL group relative to the BL group in the 8 day to 1 month sampling period ($p = < 0.02$). In Tables B4(ii), (iii) and (iv) there were no significant differences between the levels of lactoferrin, albumin or total protein between any group. Figure B4(i) illustrates the levels of secretory IgA, lactoferrin and albumin expressed as a percentage of the total protein relative to each group and includes the three groups of normal nursing mothers from Edinburgh. Although the sample numbers of the Ethiopian day 1 groups are relatively small, the concentration of secretory IgA is seen to fall from 40 to 60% of total protein at day 1 to between 10 and 20% thereafter. Lactoferrin shows a rise in percent of total protein on day 1 which remains between 15 and 25% until 6 months post-parturition when it subsides to between 5 and 10%. The albumin concentrations were relatively similar over the whole sampling period, between 1.8 and 4.3% of the total protein present. The percentage protein

Clinical Status	Parameter	POST PARTURITION SAMPLING TIME						
		Day 1	2 - 7 Days	Day 8 - 1 Month	1 - 2 Months	3 - 6 Months	7 - 12 Months	13 - 24 Months
L.L.	Mean	3202	325	*251	233	102	104	103
	Range	1167-5800	106-850	109-498	74-840	62-179	57-161	56-189
	S.D.	2367	225	124	246	32	41	41
	No.	3	14	7	9	15	12	12
B.L.	Mean	5355	239	*123	91	89	87	123
	Range	3688-9300	103-878	84-224	42-120	48-149	44-160	56-465
	S.D.	2010	242	42	32	31	28	111
	No.	6	8	9	9	11	20	13
N.L.	Mean	4702	391	221	138	84	136	153
	Range	2025-6320	164-903	94-348	74-302	47-132	39-429	48-591
	S.D.	2335	236	180	72	27	151	149
	No.	3	8	2	8	15	14	15

Table B4(i). Secretory IgA levels (mg/dl) in colostra and milk samples obtained from patients with leprosy.
LL = Lepromatous leprosy. BL = Borderline lepromatous leprosy. NL = Non lepromatous.
*' $p = <0.02$

Clinical Status	Parameter	POST PARTURITION SAMPLING TIME						
		Day 1	2 - 7 Days	Day 8 - 1 Month	1 - 2 Months	3 - 6 Months	7 - 12 Months	13 - 24 Months
L.L.	Mean	700	392	261	232	80	56	45
	Range	109-1663	137-795	67-810	51-668	26-165	27-156	20-78
	S.D.	841	178	260	227	48	35	20
	No.	3	14	7	9	15	12	12
B.L.	Mean	999	278	218	139	82	53	51
	Range	293-1830	74-555	69-405	52-269	39-189	15-207	23-142
	S.D.	500	164	95	76	53	43	30
	No.	6	8	9	9	11	21	13
N.L.	Mean	916	398	340	192	58	44	62
	Range	810-1097	161-1005	260-420	55-458	21-167	21-134	23-151
	S.D.	125	277	114	122	40	27	42
	No.	4	8	2	8	15	14	15

Table B4(ii). Lactoferrin levels (mg/dl) in colostra and milk samples obtained from patients with leprosy.
L.L. = lepromatous leprosy. B.L. = Borderline lepromatous leprosy. N.L. = Non lepromatous.

Clinical Status	Parameter	POST PARTURITION SAMPLING TIME						
		Day 1	2 - 7 Days	Day 8 - 1 Month	1 - 2 Months	3 - 6 Months	7 - 12 Months	13 - 24 Months
L.L.	Mean	216	32	34	44	34	33	31
	Range	36-525	11-145	19-49	30-55	23-65	25-45	24-44
	S.D.	269	34	10	9	10	7	7
	No.	3	14	7	9	15	12	12
B.L.	Mean	168	31	37	40	36	34	36
	Range	90-398	14-71	28-55	25-55	26-46	23-47	20-57
	S.D.	117	18	9	10	7	7	10
	No.	6	8	9	9	11	21	13
N.L.	Mean	184	20	44	39	33	41	34
	Range	147-219	13-32	43.5-44	13-58	15-46	17-232	18-53
	S.D.	38	7	0.35	15	8	53	11
	No.	4	8	2	8	15	14	15

Table B4(iii). Albumin levels (mg/dl) in colostra and milk samples obtained from patients with leprosy.

L.L. lepromatous leprosy. B.L. = Borderline lepromatous leprosy. N.L. = Non lepromatous.

Clinical Status	Parameter	POST PARTURITION SAMPLING TIME						
		Day 1	2 - 7 Days	Day 8 - 1 Month	1 - 2 Months	3 - 6 Months	7 - 12 Months	13 - 24 Months
L.L.	Mean	8366	1721	1581	1311	897	979	767
	Range	2475-17325	710-2540	1165-2670	910-2150	620-1165	630-2720	650-1280
	S.D.	7886	488	520	480	189	605	188
	No.	3	14	7	9	15	12	12
B.L.	Mean	9254	1901	1239	1013	1125	810	841
	Range	5810-21708	1400-2485	930-1675	860-1300	500-3720	445-1350	530-1280
	S.D.	5951	443	252	149	881	223	211
	No.	6	8	9	9	11	21	13
N.L.	Mean	7450	1896	1415	1101	847	870	915
	Range	6200-8700	1580-2375	1080-1750	785-1780	620-1040	540-2350	510-1290
	S.D.	1768	231	474	303	135	490	352
	No.	2	8	2	8	15	14	15

Table B4(iv). Total protein levels (mg/dl) in colostra and milk samples obtained from patients with leprosy.

L.L. = Lepromatous leprosy. B.L. = Borderline lepromatous leprosy. N.L. = Non-lepromatous.

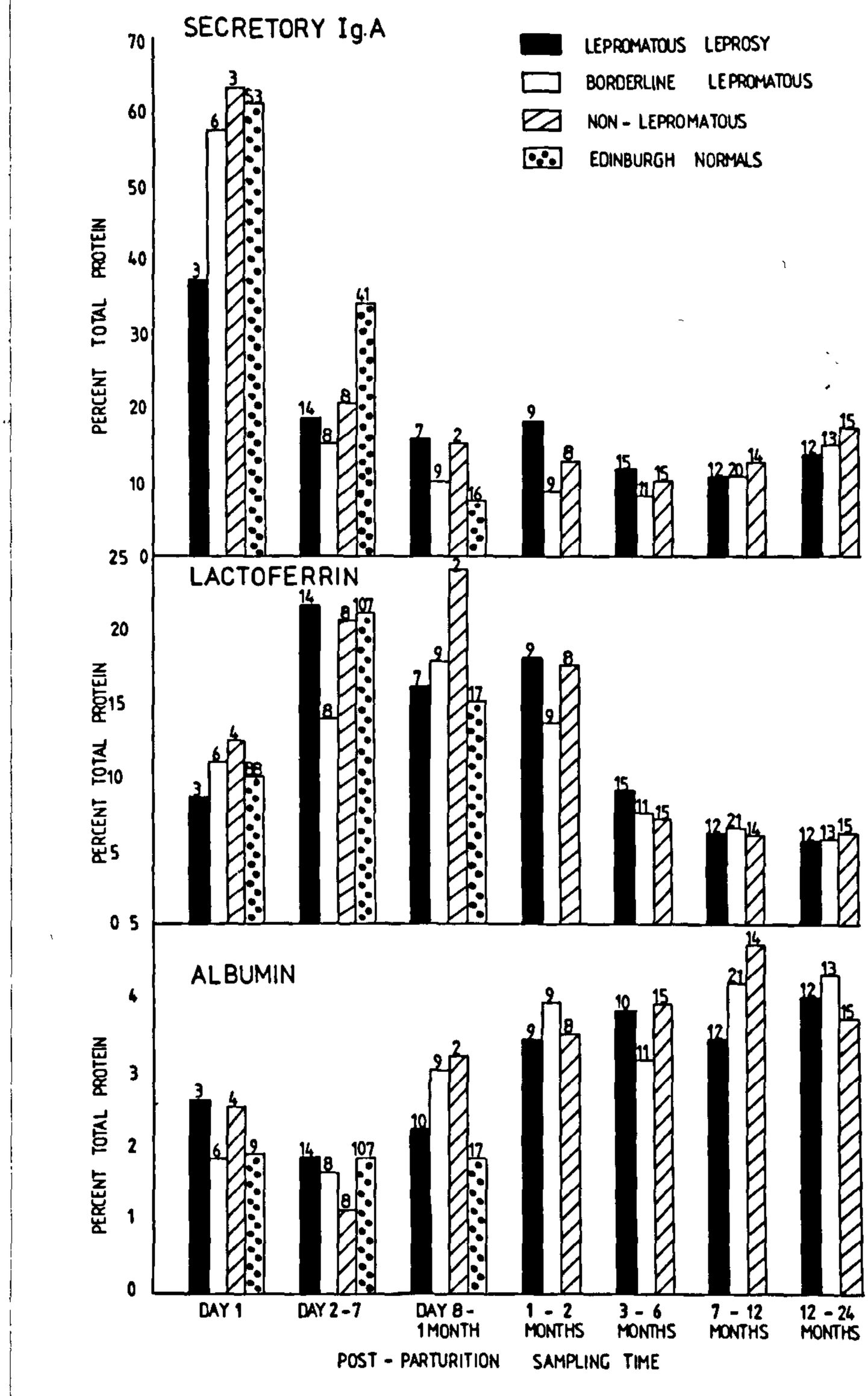


Figure B4(i). Levels of secretory IgA, lactoferrin and albumin (expressed as a percentage of the total protein) are shown for the three groups of Ethiopian women, lepromatous, borderline lepromatous and non-lepromatous (including tuberculoid and healthy controls) from day 1 to 24 months, and normal Edinburgh women from day 1 to 1 month.

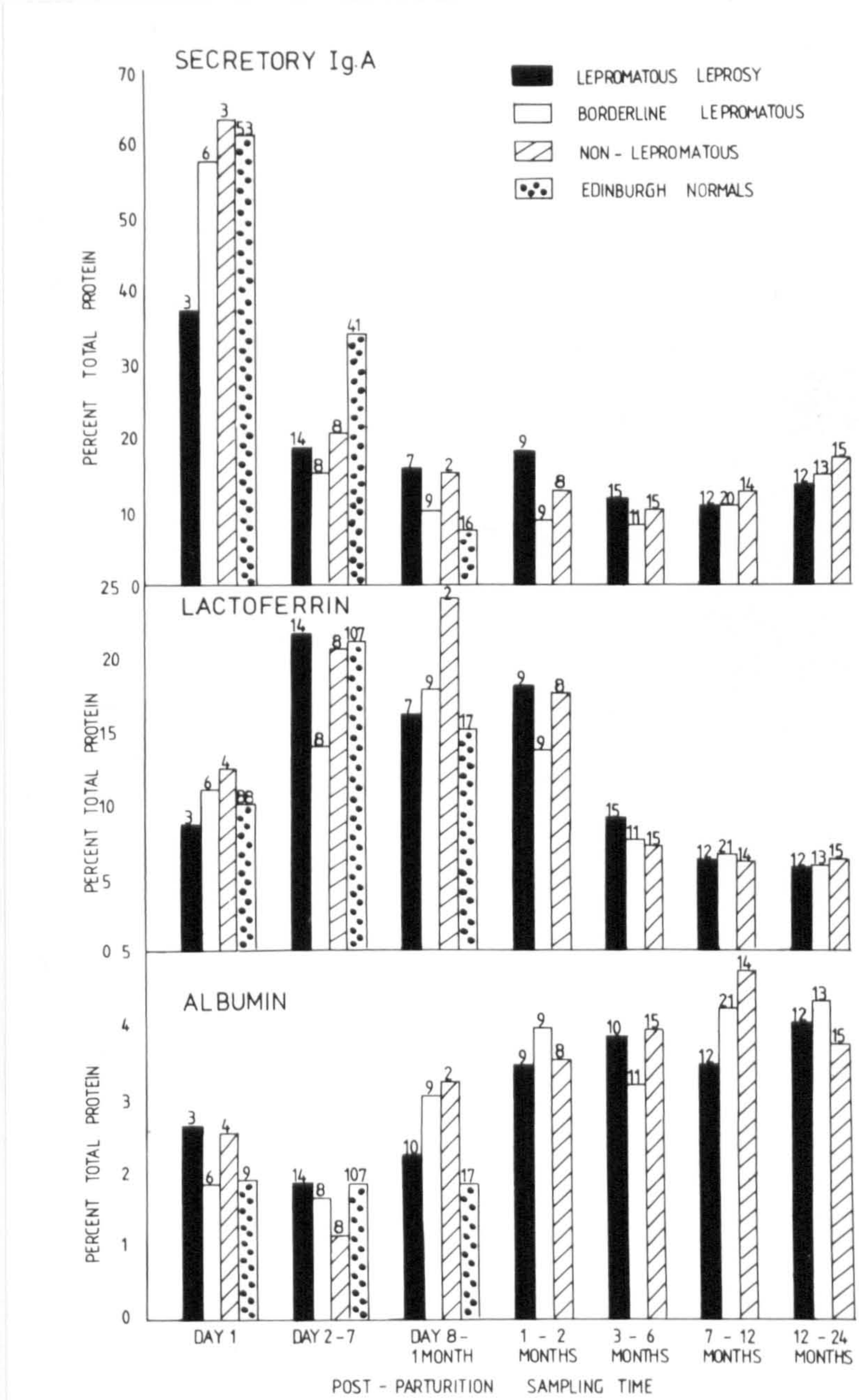


Figure B4(i). Levels of secretory IgA, lactoferrin and albumin (expressed as a percentage of the total protein) are shown for the three groups of Ethiopian women, lepromatous, borderline lepromatous and non-lepromatous (including tuberculoid and healthy controls) from day 1 to 24 months, and normal Edinburgh women from day 1 to 1 month.

concentrations of the three Ethiopian groups are not significantly different from the Edinburgh group, with the exception of the higher secretory IgA percentage at day 2 to 7.

Table B4(v) summarises the results from the Edinburgh study over the three sampling periods which could be compared with the Ethiopian groups. As no significant differences were seen in the three Ethiopian groups, the results of these groups were pooled in order to increase the sample numbers for comparison with the Edinburgh study.

Levels of secretory IgA were not significantly different between both groups. Lactoferrin levels revealed significantly higher means in the Edinburgh group at day 1 and at day 2 to 7. Albumin levels were significantly higher in the Edinburgh group at day 2 to 7 only. Total protein levels were significantly higher in the Edinburgh group at day 2 to 7 and at day 8 to 1 month (Table B4(vi)).

The results of the other proteins measured in the day 1 post-parturition samples of both groups are shown in Table B4(vi). No significant differences were found.

There was no statistical difference between the secretory IgA levels of the LL and BL, BI positive, and LL and BL, BI negative, groups.

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There was no statistical difference between the secretory IgA levels of the LL and BL, BI positive, and LL and BL, BI negative, groups.

		POST - PARTURITION TIME					
		Day 1		Day 2 - 7		Day 8 - 1 Month	
		Edinburgh Group	Ethiopian Group	Edinburgh Group	Ethiopian Group	Edinburgh Group	Ethiopian Group
Secretory IgA	Mean	8793	4654	1197	344	149	184
	Range	1911-17680	1167-9300	94-16320	103-903	108-198	84-498
	S.D.	4699	2165	2616	234	36	110
	No.	53	12	41	30	16	18
	p =	NS		NS		NS	
Lactoferrin	Mean	1426	905	748	363	345	248
	Range	57-3900	109-1830	216-2955	74-1005	156-720	67-810
	S.D.	38	491	575	205	144	174
	No.	88	13	107	30	17	18
	p =	<0.05		<0.001		NS	
Albumin	Mean	233	184	70	29	41	36
	Range	81-531	36-525	18-480	11-145	24-129	19-55
	S.D.	136	136	78	25	25	9
	No.	9	13	107	30	17	18
	p =	NS		<0.01		NS	
Total Protein	Mean	13989	9272	3523	1815	2422	1392
	Range	5138-28160	2475-21708	880-9500	710-2540	1700-4550	930-2670
	S.D.	6817	5541	2046	419	704	407
	No.	9	11	63	30	15	18
	p =	NS		<0.001		<0.001	

Table B4(v). Results of secretory IgA, lactoferrin, albumin and total protein levels in colostra and milk from normal nursing mothers in Edinburgh and patients and controls in Ethiopia (see text).

p = analysis of variance by unpaired t-test.

NS Not significant.

Table B4(vi) IgG, IgM, B1A and B1E globulin levels, (mg/dl), in colostra and milk from normal nursing mothers in Edinburgh and in patients and controls in Ethopia.

NS = not significant.

		Post-Parturition (Day 1)	
		Edinburgh Group	Ethiopian Group
IgG	Mean Range No. p =	48.9 31-100 76 NS	47.5 0-282 11 NS
IgM	Mean Range No. p =	177.4 26-458 76 NS	223.4 0-840 11 NS
B1A	Mean Range No. p =	67.4 0-136 76 NS	50.7 13-189 10 NS
B1E	Mean Range No p =	28.9 0-117 76 NS	28.1 0-109 11 NS

5. The outcome of pregnancies of women with leprosy and healthy controls: child survival, growth and development, response to immunisation and skin testing with purified protein derivative of M. tuberculosis and M. leprae

One hundred and fifty women were studied during 160 pregnancies, 17(including one woman who aborted, see below) dropped out during late pregnancy, 8 of these being healthy controls. In three cases when the woman delivered at home, the baby died within a few minutes of birth, and thus was not seen at all. The follow up of the children is shown in Table B5(i)(a) and (b), the first of which shows the number of children in the study during each 3 month period from birth to 24 months, and at the Phase II assessment. The second Table shows the number of children still in the study at the end of each 3 month period and in the Phase II assessment. There was a considerable drop out rate due to death or shift in population during the 2 year period of the study: 23 children died, 22 of them during the initial 2 year period, one of them prior to the Phase II follow up. Seventy-seven babies were followed up for 12 months or more, half of these were followed for a further 9-12 months. The follow up rate was best in the children of lepromatous mothers because of the mothers' natural concern that the children might contract leprosy.

Despite other reports (Zambaco, 1897; Montero, 1927) that abortion is common in women with leprosy, there was only one abortion recorded amongst the mothers of this study. The mother, a woman with BT leprosy, stated that her pregnancy "disappeared" at 5 months' gestation, and hotly denied the possibility of there having been interference. She was not seen again for follow up despite our strenuous efforts to trace her and was therefore taken out of the study. There were 3 premature deliveries :

i) A9/63, a healthy control primagravida delivered at 36 weeks with the baby's weight appropriate for gestational age, the baby progressed normally;

ii) A9/201, a quiescent BL mother,was noted to have severe intrauterine growth retardation and was admitted at 34 weeks for treatment of acute urethritis. Within 12 hours of admission, she went into premature labour and delivered a live female infant weighing 1300 grams. Despite intensive care in the neonatal paediatric unit,

TABLE B5(i)a

Follow up of children: Number of children studied

Classification	MOTHERS No. Studied	No. of Pregnancies/ Lactations	No. dropping out in late pregnancy	Home delivery (Baby died)) (unexamined)	No. of children in the study during each 3 month period At Birth	0–3	4–6	7–9	10–12	13–15	16–18	19–21	22–24	Phase II
HC	32	36	8	6 (0)	22	28	14	13	11	10	9	9	5	17
TT & BT/RFC	17	17	3	1 (1)	13	13	10	9	9	9	7	6	5	9 *
TT & BT on treatment	24ϕ	25ϕ	3	6 (0)	16	22	18	18	15	11	8	6	5	15
BL	45	46 **	3	6 (1)	38**	43**	30	29	28	24	19	13	8	28
LL	32	36 **	–	12 (1)	25**	36**	28	24	23	18	16	7	5	21
	150ϕ	160ϕ	17	31 (3)	114	142	100	93	86	72	59	41	28	90
No. of deaths of children during each study period				3	2	7	4	1	2	2	1			1***

* Two mothers of babies in this group relapsed with active leprosy before Phase II assessment.

ϕ Includes one woman who aborted in the second trimestre and was subsequently taken out of the study – see text.

** One set twins.

*** Child died at age 2 years & 10 months prior to Phase II assessment.

TABLE B5(i)b

Follow up of children: Number of children studied

Classification	MOTHERS: No. Studied	MOTHERS: No. of Pregnancies/ Lactations	MOTHERS: No. dropping out in late pregnancy	Home delivery (Baby died unexamined)	No. of children in the study at the end of each 3 month period: At Birth	0-3	4-6	7-9	10-12	13-15	16-18	19-21	22-24	Phase II
HC	32	36	8	6 (0)	22	16	14	11	11	10	9	5	4	17
TT & BT/RFC	17	17	3	1 (1)	13	10	9	9	9	7	6	5	5	9*
TT & BT/Active	24 ϕ	25 ϕ	3	6 (0)	16	19	18	17	11	9	6	5	3	15
BL	45	46**	3	6 (1)	38**	32	29	28	25	21	13	10	3	28
LL	32	36**	-	12 (1)	25**	28	25	24	21	16	10	7	4	21
	150 ϕ	160 ϕ	17	31 (3)	114	105	95	89	77	63	44	32	19	90

* Two mothers of babies in this group in fact relapsed with active leprosy.

** One set twins.

ϕ Includes one woman who aborted in the second trimestre and was subsequently taken out of the study - see text.

including exchange transfusion, the baby died, on the third day, of pneumonia and hyperbilirubinaemia;

iii) A9/235, a patient with active LL leprosy, was admitted to hospital with hepatitis at 36 weeks' gestation. She went into premature labour and delivered a live male infant weighing 1600 grams. The baby made satisfactory progress in the paediatric neonatal unit and both he and his mother were followed up for the next 15 months.

There were two sets of twins (incidence of 1 in 80 pregnancies, which is normal), one uni-ovular and one binovular.

The mortality rates of the children studied is shown in Table B5(ii). Twenty-two children died during the initial Phase I of the study and there was a further death prior to the Phase II assessment. There were no significant differences in the peri-natal mortality or neonatal mortality rates in the different groups of children studied, but there was a sharp increase in infant mortality in the children of mothers with lepromatous leprosy, especially in the LL group. Eight of the children of LL mothers became marasmic and required hospital treatment for this condition. Causes of death are shown in Table B5(iii).

Causes of death in children of mothers with leprosy and healthy controls

i) Stillbirth. Two stillbirths were unavoidable. One was due to intrauterine death of the foetus at 34 weeks: the mother had sero-positive syphilis (VDRL ++++). Treatment of the mother was too late to prevent foetal death. The second was due to anencephaly. This baby was born to a mother with lepromatous leprosy, who had received irregular treatment with dapsone monotherapy for 4 years prior to her pregnancy, and underwent an active relapse during the third trimestre. There was no history of anencephaly in the family.

ii) Neonatal deaths. All 7 neonatal deaths were due to respiratory difficulty, respiratory distress syndrome or respiratory tract infection. A further 3 children were admitted to the paediatric intensive care unit with respiratory difficulty at birth, but were treated successfully. The neonatal paediatricians not only remarked early on the serious significance of respiratory distress syndrome in babies of

TABLE B5(ii)

Mortality Rates of Children in A9 Study

Classification of Mother	No. of mothers	No. of pregnancies	No. of children born	No. of stillbirths (SB)	No. of Neonatal deaths	No. of deaths in 1st month excluding SB	No. of deaths in 1st year excluding SB	No. of later deaths 1 – 3 years	PMR*	NMR**	IMR***
HC	32	36	36	1	1	1	3	–	55.5	28.6	85.0
TT & BT	40	41	41	0	2	2	2	2	48.8	48.8	48.8
BL	45	46	47	0	3	3	6	–	63.8	63.8	127.7
LL	32	36	37	1	1	2	6	2	54.1	55.6	166.7
	149	159	161	2	7	8	17	4	55.9	50.3	106.9

* PMR = Perinatal mortality = Stillbirths (SB) + neonatal deaths in first 7 days/1000 live + stillbirths.

** NMR = Neonatal mortality rate = all deaths occurring in first month (28 days)/1000 live births.

*** IMR = Infant mortality rate = all deaths occurring in first year/1000 live births.

TABLE B5(iii)

Causes of death in children of mothers with leprosy and healthy controls

Classification of mother	Stillbirths	Neonatal Deaths	Deaths 8 – 28 days	Deaths 1 – 12 months	Deaths after 1 year of age
HC	I.U.D. at 34/52 (syphilis)	R.T.I., 3 days		7 months, unknown cause 12 months, unknown cause	
TT & BT/RFC		R.D., 1 day			34 months, measles bronchitis
TT & BT on treatment		R.D.S., 7 days			13½ months, R.T.I.
BL		R.D., 1 day R.D.S., 4 days R.D.S. + prematurity, 4 days		3 months, R.T.I. (twin) 4 months, feeding problem 10 months, R.T.I. (mongol)	
LL	Anencephaly	R.D., 1 day	28 days, D & V	2 months, R.T.I. 4 months, feeding problem (twin) 4½ months, feeding problem (twin) 6 months, unknown cause	12 months, heart failure (viral myocarditis) 18 months, unknown cause

I.U.D. = Intrauterine death.
R.D. = Respiratory difficulty.
R.D.S. = Respiratory Distress Syndrome.
R.T.I. = Respiratory tract infection.
D. & V.= Diarrhoea and vomiting.

mothers with lepromatous leprosy, a hitherto unrecorded association, but also requested advanced notice when any woman with lepromatous leprosy went into labour in order that an incubator could be prepared and ready to receive the baby.

iii) Deaths from 8–28 days of life. Only one death occurred during this period, and that was due to diarrhoea and vomiting in a fully breast-fed but severely underweight baby of a mother with lepromatous leprosy.

iv) Deaths from 1–12 months. Of 9 deaths, 3 were due to respiratory tract infection, one of these in a high grade mongol with a congenital heart defect; 3 were due to feeding problems in babies who were all fully breast-fed; and in 3 the cause of death was unknown.

v) Deaths occurring after 1 year of age. Two of 4 deaths were due to respiratory tract infection, one occurring after measles. One death was due to heart failure in a child with viral myocarditis. (Four children in all were admitted to hospital with the same signs and symptoms. Three children made a satisfactory recovery when treated with digitalisation and antibiotics;the fourth unfortunately went into heart failure at night and digitalisation was considered too late to be effective.) The fourth death was due to an unknown cause.

Assessment of baby growth by measurement of weight, crown-heel length and head circumference

i) Weights of babies during the first 2 years of life. The growth rate of the babies in the different groups, charting the mean of the babies' weights at 6 monthly intervals, is shown in Figure B5(i) and Table B5(iv). In all the groups, growth was most rapid during the first 6 months while the mothers had a plentiful supply of milk. The babies of LL mothers gained weight rapidly after birth, particularly during the first 3 months, which suggested that their low birth weight was due to intrauterine malnutrition, but after 6 months of reasonable

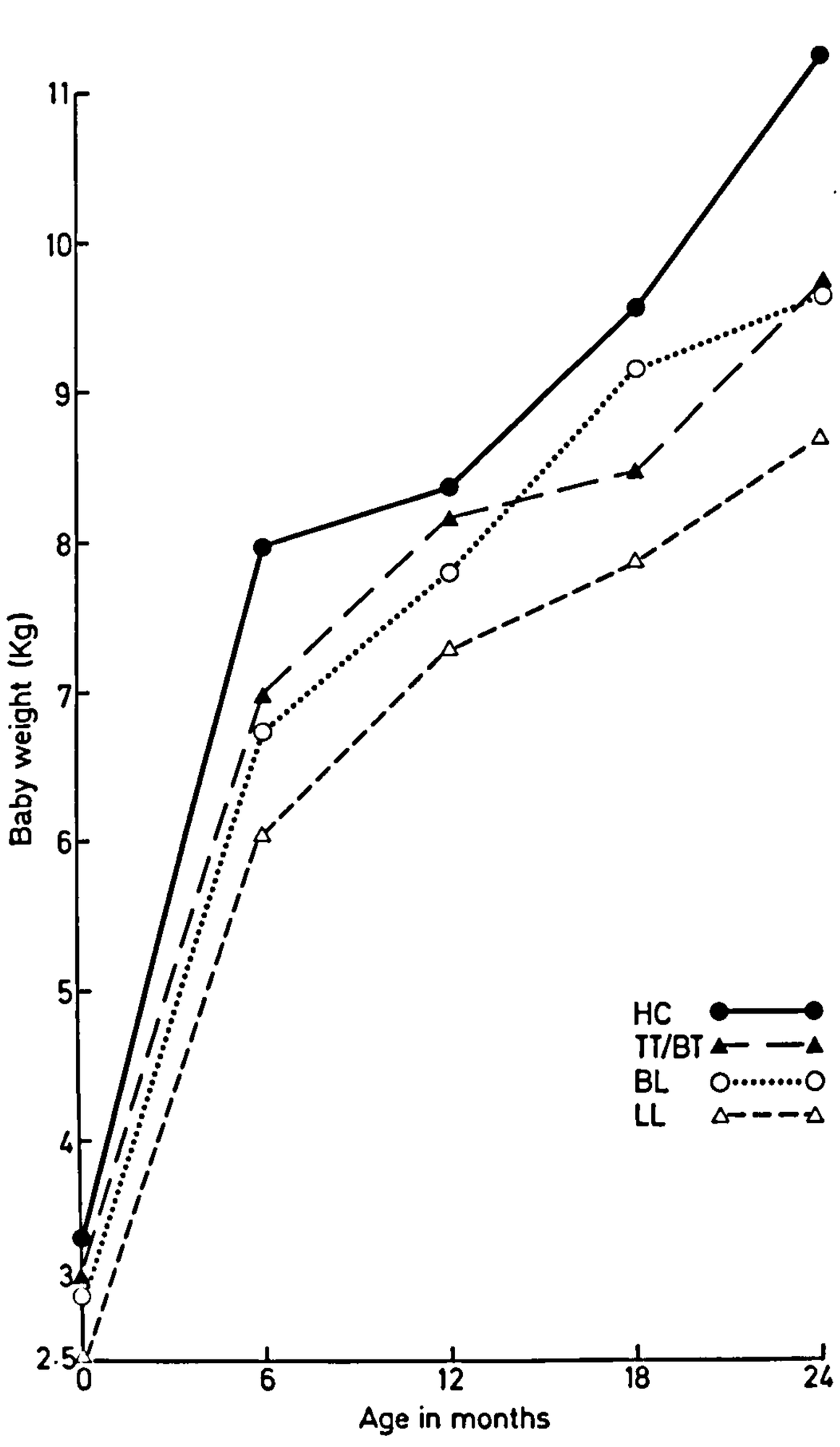


Figure B5(i). Mean infant weight (kg) for sexes combined at 6 monthly intervals from birth to 2 years of age, according to the clinical classification of the mother.

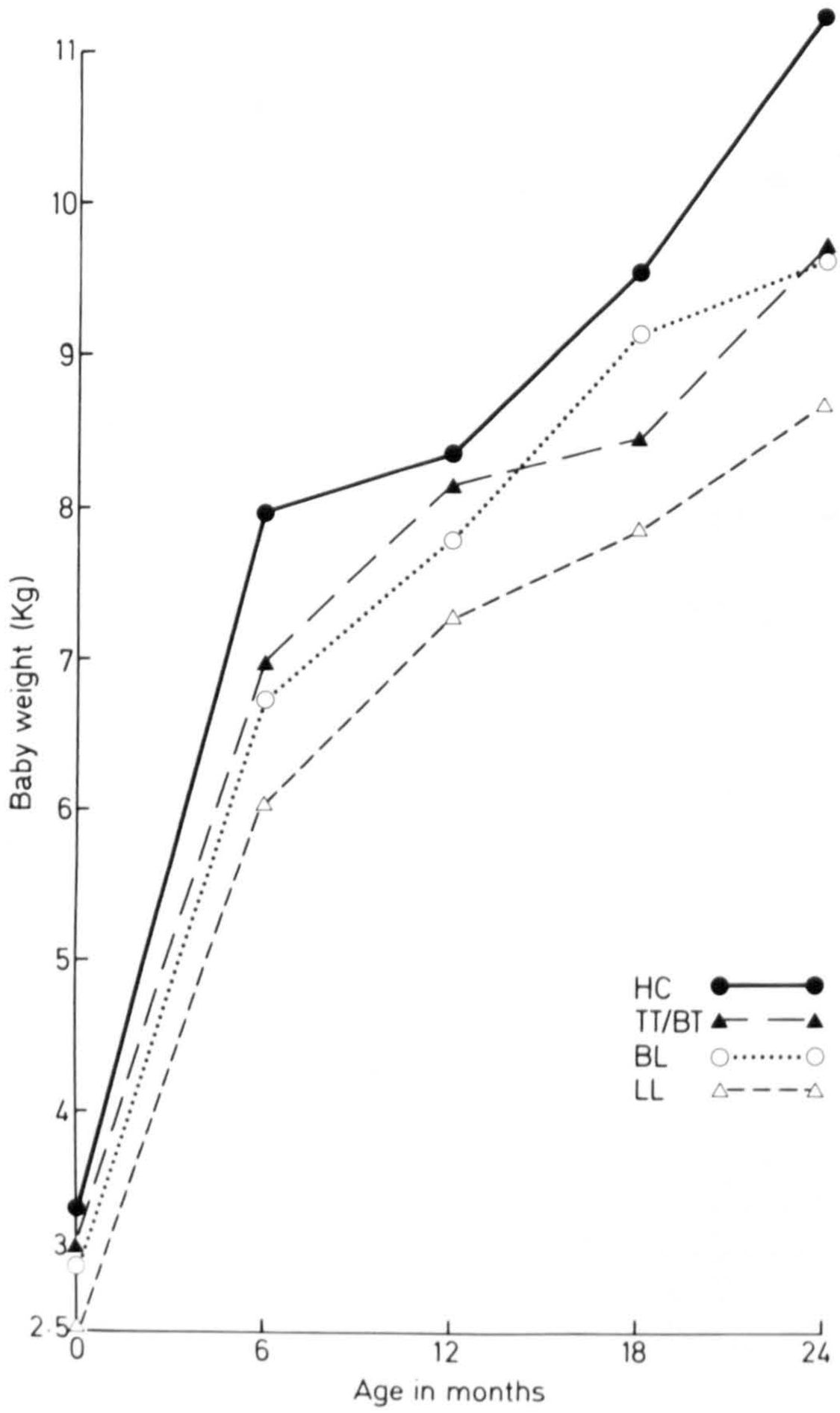


Figure B5(i). Mean infant weight (kg) for sexes combined at 6 monthly intervals from birth to 2 years of age, according to the clinical classification of the mother.

TABLE B5(iv)

Weights of babies, according to the clinical classification of the mother from birth to two years, at six monthly intervals

Clinical Classification of the mother		Weights of babies (g)				
		Birth	6 months	12 months	18 months	24 months
HC	n	18	4	3	5	4
	$\bar{x}$	3280.6	797.5	8366.7	9580.0	11037.5
	± SEM	87.6	415.1	145.3	387.8	520.9
TT & BT	n	30	8	9	4	9
	$\bar{x}$	3075.0	6912.5	8150.0	8462.5	9750.0
	± SEM	61.1	436.5	462.5	224.9	487.8
BL	n	33	19	20	12	6
	$\bar{x}$	2985.6	6694.7	7782.5	9129.2	9675.0
	± SEM	69.9	173.4	280.8	342.6	495.9
LL	n	21	14	15	9	3
	$\bar{x}$	2558.1	6007.0	7310.0	7877.8	8983.3
	± SEM	60.5	216.4	260.9	349.5	501.9

n = Number of babies.

$\bar{x}$ = Mean baby weight (g).

SEM = Standard error of the mean (g).

growth, they failed to gain weight at the normal rate, and were unusually susceptible to respiratory, gastrointestinal and skin infections. Several became marasmic and many required admission to hospital.

The growth rate of the babies of the leprosy patients can be further illustrated by classification of the baby weights according to the Boston standard (Jelliffe, 1966; Davidson et al, 1979) (Figure B5(ii)). In all the groups, the babies were slightly or moderately/severely underweight, but this is most striking in the LL group where at 12 months 80% of the babies were moderately/severely underweight, and at 24 months, all the babies were moderately/severely underweight.

ii) Length and head circumference during the first 2 years of life. The mean crown-heel length of babies at 6 monthly intervals is shown in Figure B5(iii). In all groups increase in length was most rapid during the first 6 months and less rapid during the next 18 months. A similar pattern is shown in the growth of head circumference using the occipito-frontal measurement in centimetres (Figure B5(iv)). The same trend observed in the baby weight increase in 2 years was observed in length and head circumference: at the end of 2 years there was a gradation of the three measurements from the highest in the babies of healthy mothers to lowest in the babies of LL mothers, as was observed at birth.

iii) Growth velocity. In order to assess the rate of growth of the babies in each group, the growth velocity of each baby was calculated using a decimal time scale. The growth periods studied were birth - 6 months, 7-12 months, 13-18 months and 19-24 months. A fifth calculation was added, namely, the growth velocity from birth - 3 months, as it seemed that the babies of LL mothers grew fastest during this 3 month period. The growth velocities for weight, length and head circumference were calculated for boys and girls separately. The sexes were subsequently combined in order to increase the numbers of babies in each group, particularly the babies of healthy mothers and those with tuberculoid leprosy, who after the first

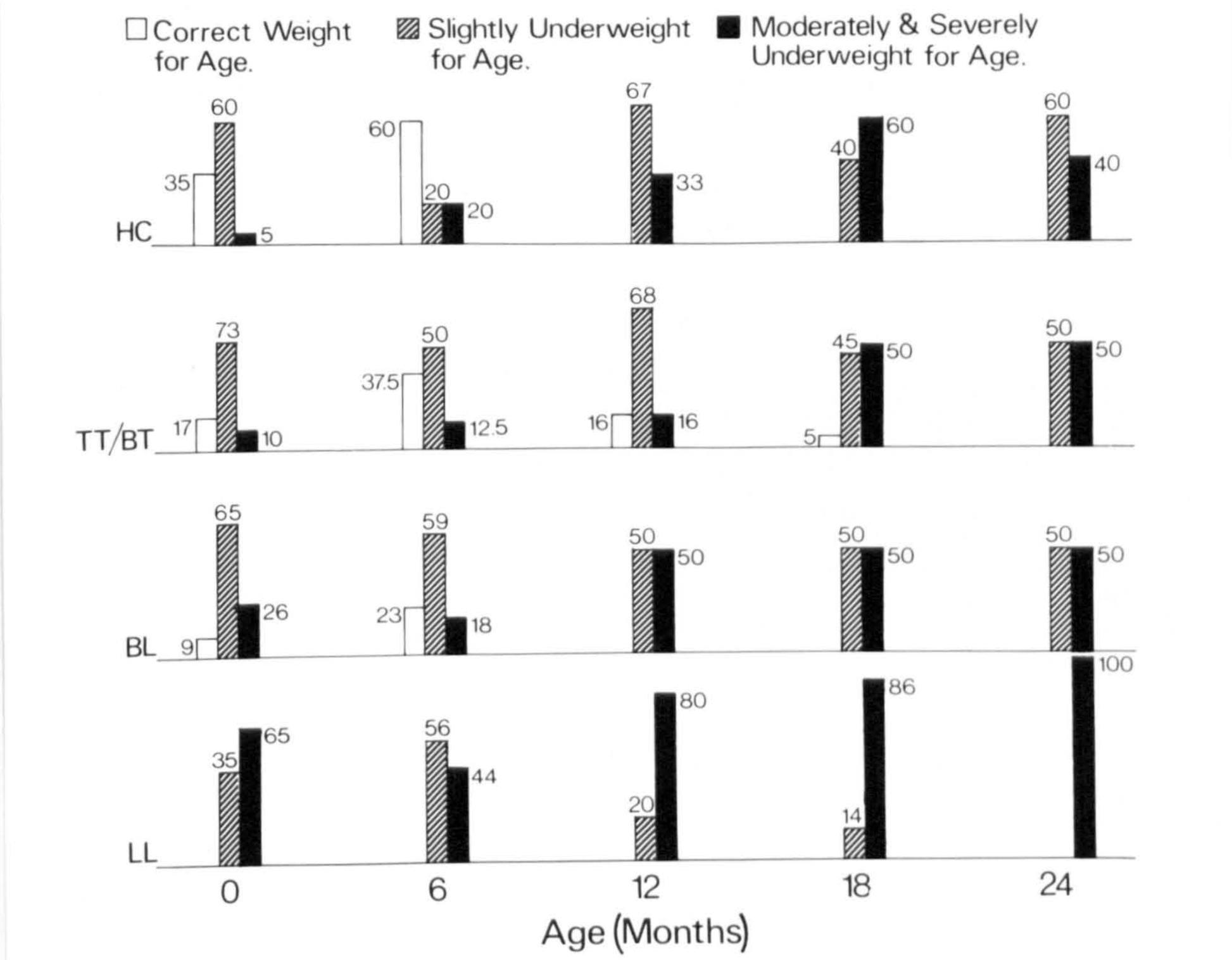


Figure B5(ii). Infant weights by the Boston Standard from birth to 2 years of age according to the clinical classification of the mother. Percentages of babies who were the correct weight for age (at or above 50th centile for both sexes) (☐), slightly underweight for age (80–99% of 50th centile) (▨), and severely/moderately underweight for age (below 80% of 50th centile) (■), are shown at 6 month intervals.

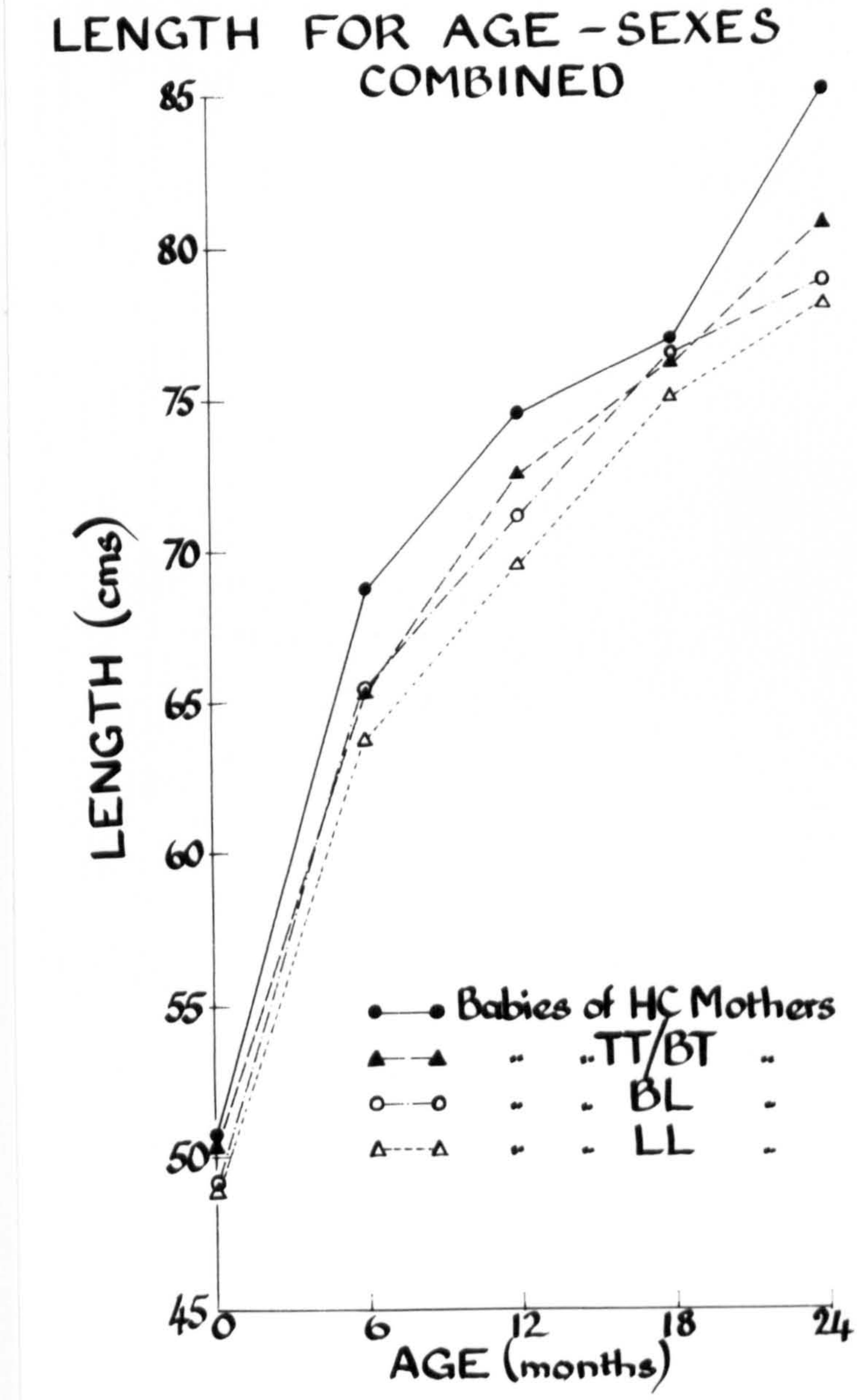


Figure B5(iii). Mean infant length (cms) for sexes combined at 6 monthly intervals from birth to 2 years of age according to the clinical classification of the mother.

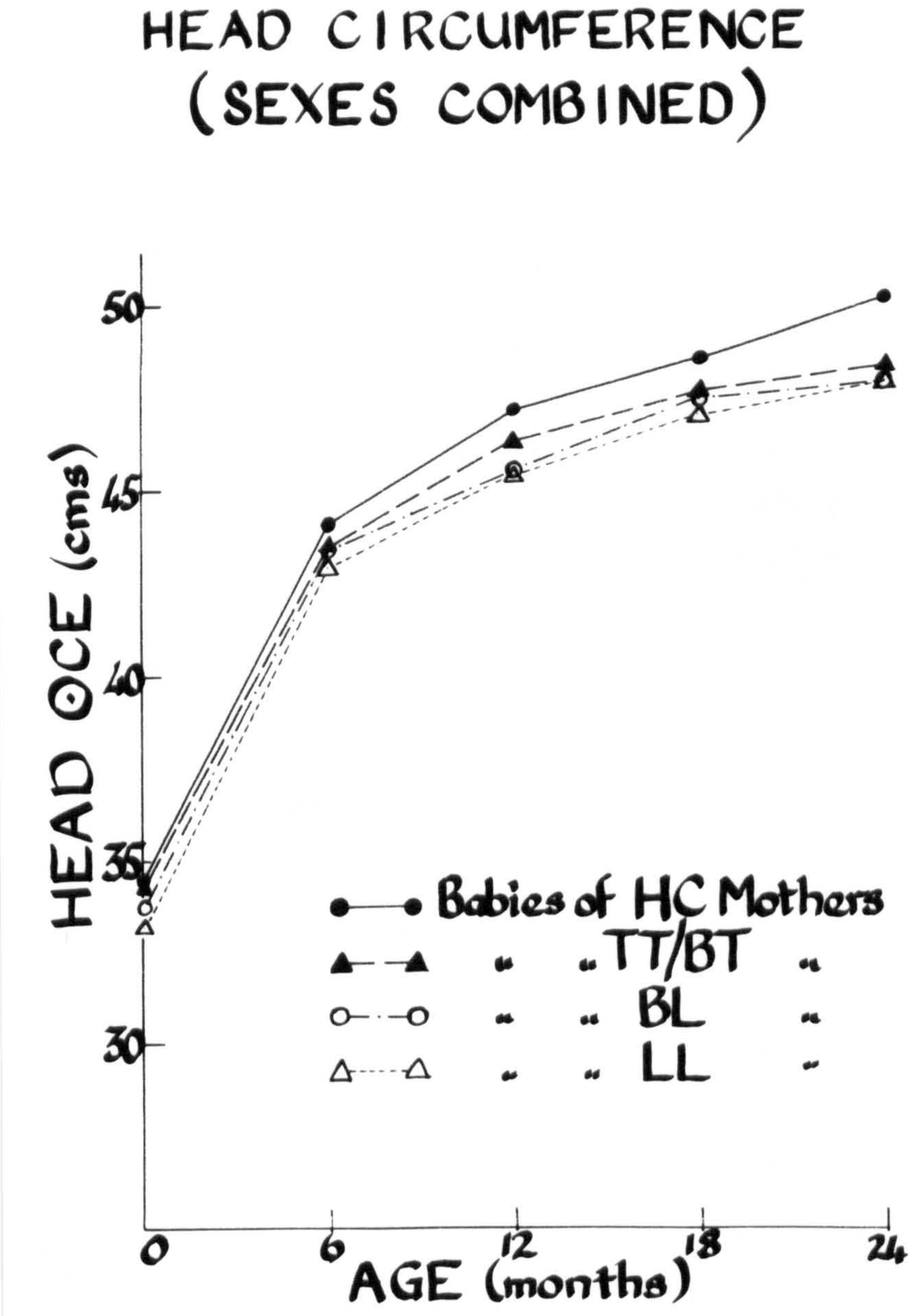


Figure B5(iv). Mean head circumference (cms) for sexes combined at 6 monthly intervals from birth to 2 years of age according to the clinical classification of the mother.

3-6 months were only seen if they were sick unless there was an additional incentive such as skin testing, BCG or a photograph!

Contrary to our clinical impression, the growth velocity for weight (Figure B5(v)) showed that the babies of LL mothers in fact was less at 3 months than the growth velocity of the other groups of babies. When studied according to sex of the baby, there was statistical significance between the growth velocity for weight of male babies of healthy mothers, babies of BL mothers and babies of LL mothers during the first 3 months of life (Figure B5(vi)). Furthermore, the growth velocity of male babies of healthy mothers was significantly higher than that of the male babies of all mothers with leprosy from birth to 6 months of age. The growth velocity for length for all babies (sexes combined) showed the babies of LL mothers grew more slowly than the others for the first 12 months of life (Figure B5(vii)). This, however, did not reach a statistical significance. There was no difference in growth velocity with regard to head circumference between the different groups of babies (Figure B5(viii)).

After the slowing down of growth velocity in weight and length in particular, during the 19-24 month period, a slight increase in growth velocity is observed, particularly in the babies of LL mothers with regard to weight. It is thus of interest to note that when the weights of the children recorded at the Phase II assessment are analysed according to the Boston Standard and the classification of the mother, there is a slight improvement in the weight of the children of LL and BL mothers when compared with the figures at 2 years of age (Table B5(v) and Table B5(vi)).

<u>ABO blood groups of mothers and children</u>

While ABO blood grouping was requested routinely for all antenatal patients and cord blood samples, carrying out the test was subject to availability of reagents. Table B5(vii) shows the ABO groups of mothers and children according to the classification of the mother's leprosy. The distribution of ABO blood groups amongst

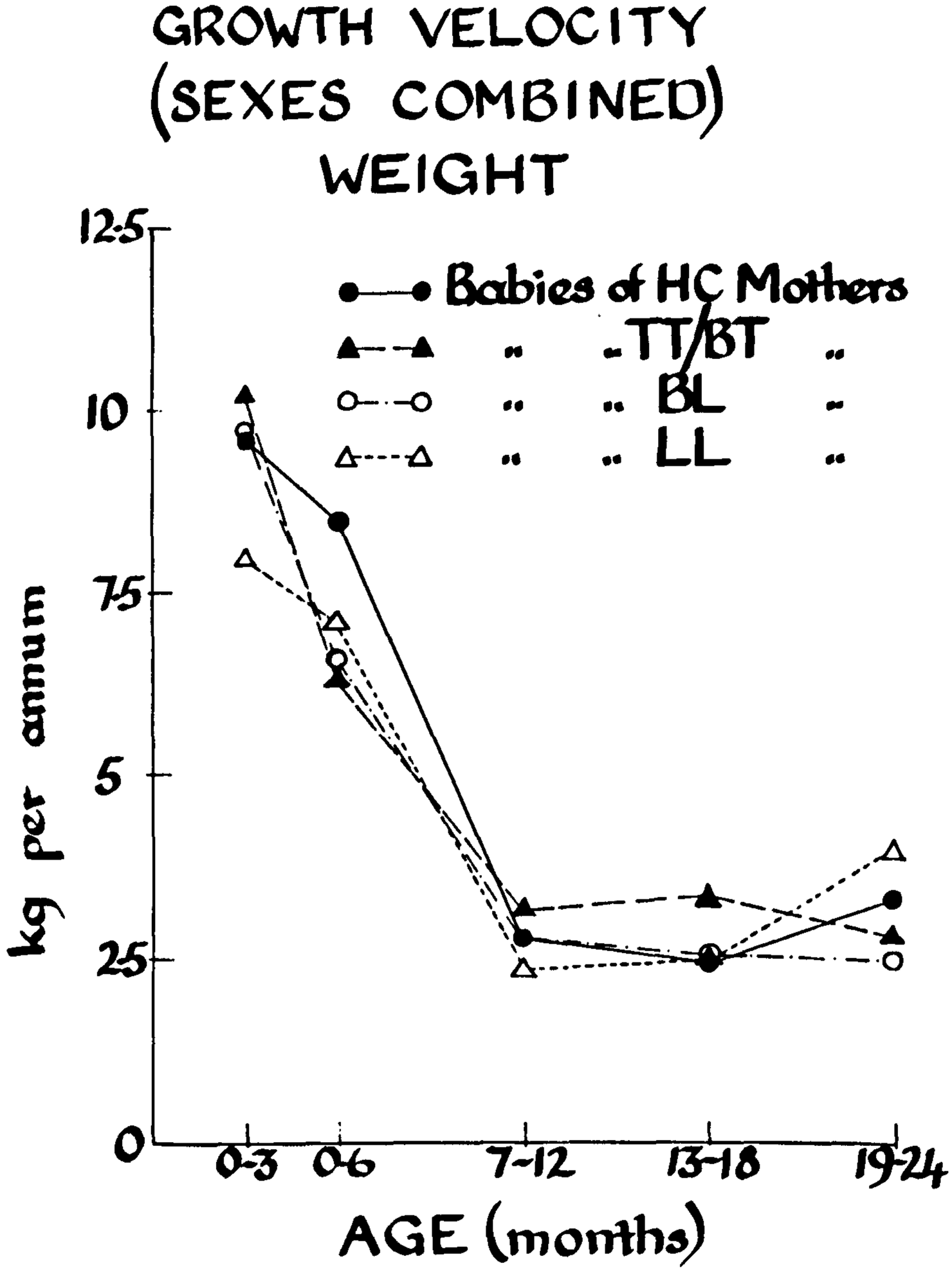


Figure B5(v). Mean growth velocity for weight (kg per annum) for sexes combined from birth - 3 months, birth - 6 months, 7 - 12 months, 13 - 18 months, and 19 - 24 months, according to the clinical classification of the mother.

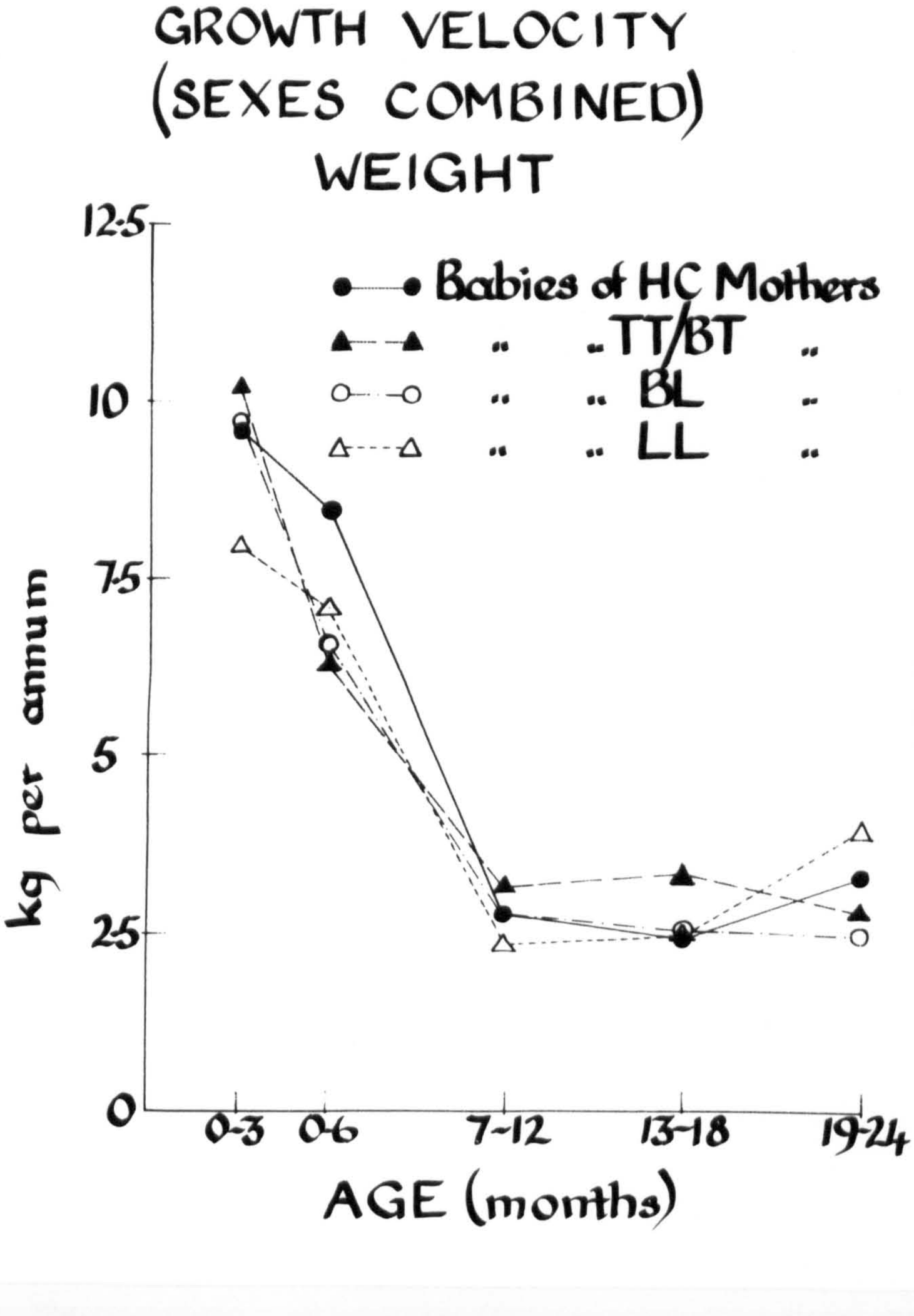


Figure B5(v). Mean growth velocity for weight (kg per annum) for sexes combined from birth - 3 months, birth - 6 months, 7 - 12 months, 13 - 18 months, and 19 - 24 months, according to the clinical classification of the mother.

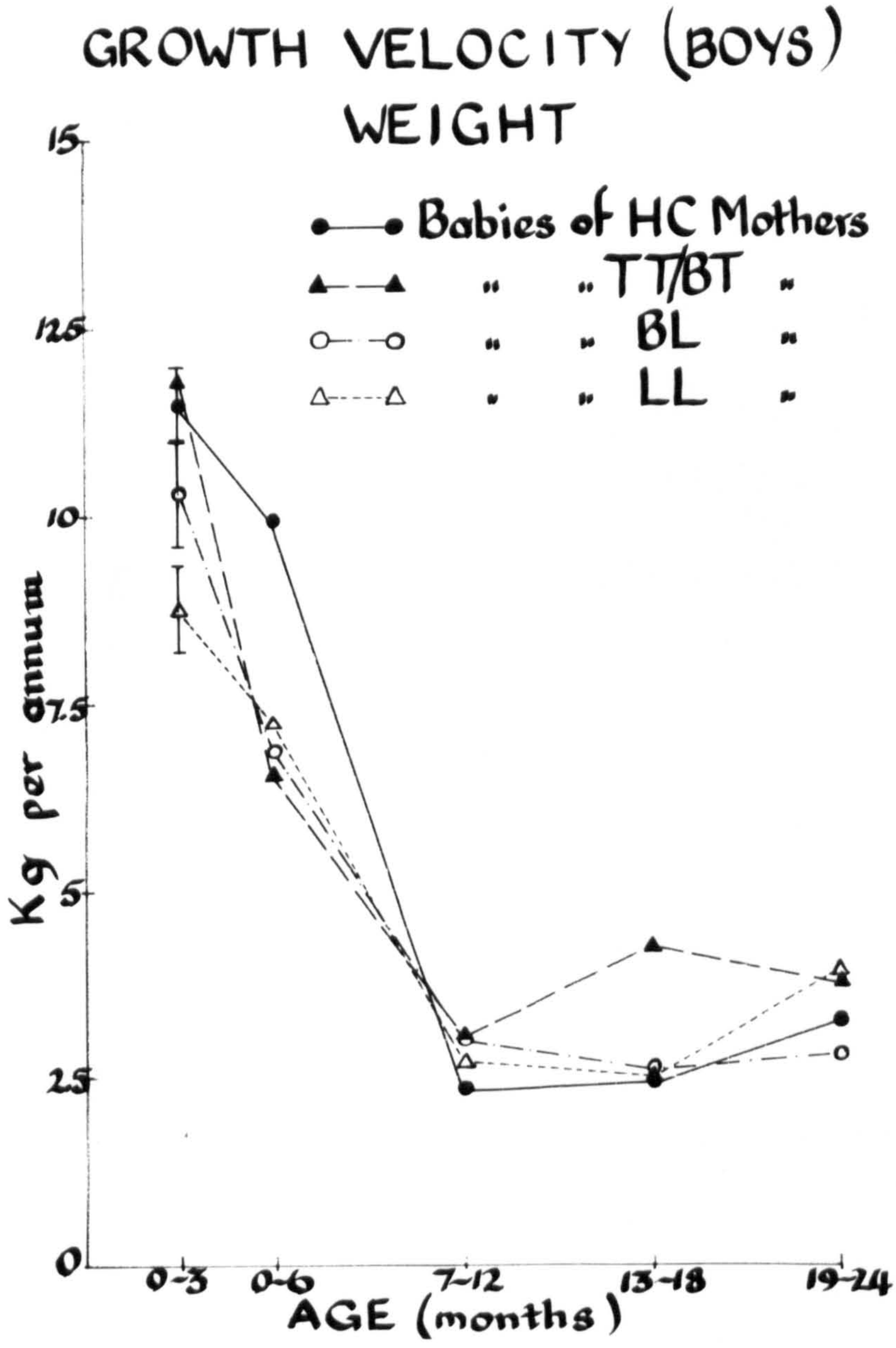


Figure B5(vi). Mean growth velocity for weight (kg per annum) for male infants from birth - 3 months, birth - 6 months, 7 - 12 months, 13 - 18 months, and 19 - 24 months, according to the clinical classification of the mother.

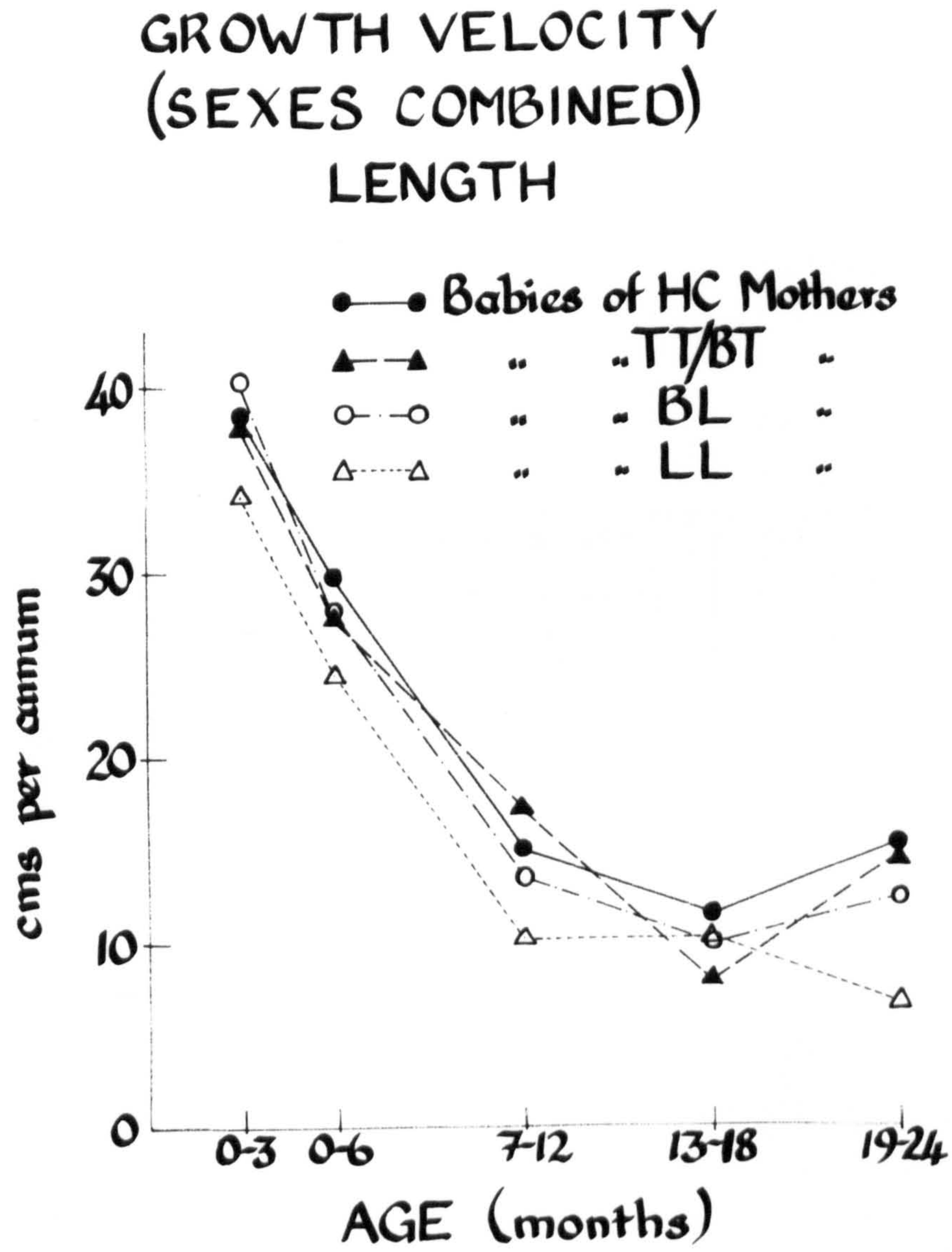


Figure B5(vii). Mean growth velocity for length (cms per annum) for sexes combined from birth - 3 months, birth - 6 months, 7 - 12 months, 13 - 18 months, and 19 - 24 months, according to the clinical classification of the mother.

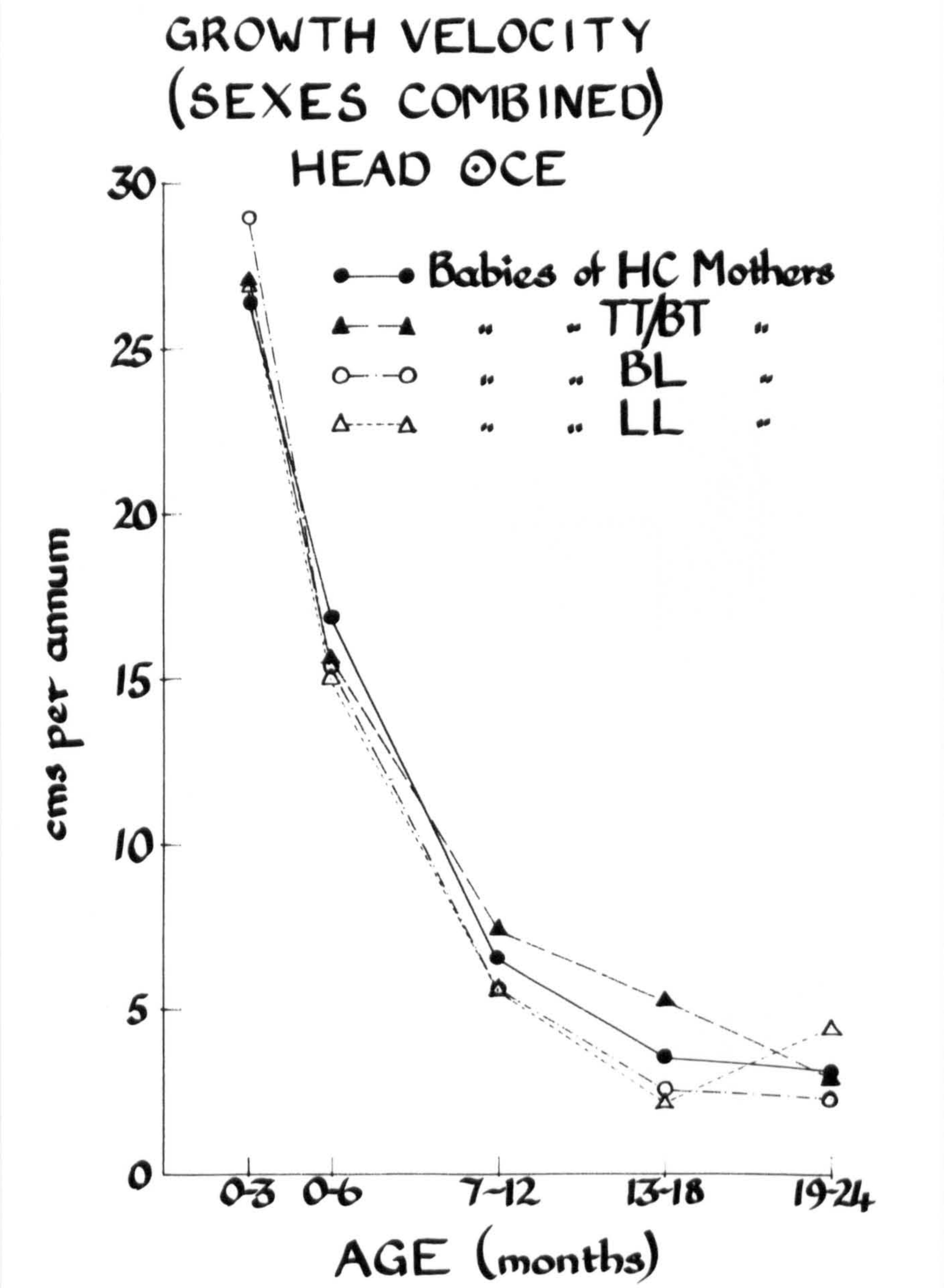


Figure B5(viii). Mean growth velocity for head circumference (cms per annum) for sexes combined from birth - 3 months, birth - 6 months, 7 - 12 months, 13 - 18 months, and 19 - 24 months, according to the clinical classification of the mother.

TABLE B5(v)

Weights of Children at Phase II assessment
according to Boston Standard

Classification of Mother	No. of Children	Number of children weighing 60–70%	70–80%	80–90%	90–100%	100%
HC	17	2	5	6	4	–
TT & BT	24	4	7	8	4	1
BL	28	3	8	11	6	–
LL	21	3	11	4	3	–
	90	12	31	29	17	1

TABLE B5(vi)

Weights of children at Phase II assessment, aged 3-4 years, according to the Boston Standard: Percentages of children who were correct weight for age, slightly underweight for age, moderately/severely under weight for age.
Number ( ) are the percentages at 24 months of age.

Classification of Mother	Correct weight for age (at or above 50th centile)	Slightly underweight for age (80-99% of 50th centile)	Moderately/severely underweight for age (< 80% of 50th centile)
HC	-	59% (60%)	41% (40%)
TT & BT	4%	50% (50%)	46% (50%)
BL	-	61% (50%	39% (50%)
LL	-	33%	67% (100%)
TOTAL	1%	51%	48%

TABLE B5(vii)

Blood groups of study patients according to the classification of the mother's leprosy

Leprosy Classification	MOTHERS No. Tested	Blood Group O	A	B	AB	B+AB	BABIES No. Tested	Blood Group O	A	B	AB	B+AB
HC	16	5 (31.0%)	6 (38.0%)	3 (19.0%)	2 (12.0%)	5 (31.0%)	14	7 (50.0%)	6 (43.0%)	1 (7.0%)	0	1 (7.0%)
TT & BT	26	10 (38.0%)	8 (31.0%)	7 (27.0%)	1 (4.0%)	8 (31.0%)	20	10 (50.0%)	5 (25.0%)	4 (20.0%)	1 (5.0%)	5 (25.0%)
BL	32	13 (41.0%)	10 (31.0%)	9 (28.0%)	0	9 (28.0%)	28	11 (39.0%)	5 (18.0%)	12 (43.0%)	0	12 (43.0%)
LL	27	11 (41.0%)	8 (30.0%)	6 (22.0%)	2 (7.0%)	8 (30.0%)	25	10 (40.0%)	8 (32.0%)	5 (20.0%)	2 (8.0%)	7 (28.0%)
TOTAL	101	39 (38.6%)	32 (31.7%)	25 (24.7%)	5 (4.9%)	30 (29.7%)	87	38 (43.7%)	24 (27.6%)	22 (25.3%)	3 (3.4%)	25 (28.7%)
BL & LL	59	24 (40.6%)	18 (30.5%)	15 (25.4%)	2 (3.4%)	17 (28.9%)	53	21 (39.6%)	13 (24.5%)	17 (32.1%)	2 (3.8%)	19 (35.8%)

all the mothers was in accordance with results already published for healthy communities of Ethiopians (Ghose, 1963; Huber, 1964): there were no significant differences between the different groups of mothers. In particular, the percentage of mothers with anti-B isohaemagglutinin (blood groups B and AB) was the same in each classification of leprosy.

The children as a whole showed a similar ABO group distribution, but there were differences between the classifications: the children of healthy mothers had a lower percentage of B and AB blood groups, compensated by a proportionate increase of O and A; while children of lepromatous mothers showed a higher percentage of B and AB groups at the expense of A.

Response to PPD skin testing and BCG immunisation in children of mothers with leprosy and healthy controls

The results are summarised in Table B5(viii). Eighty children were immunised by an intradermal injection of 0.1 ml BCG (Glaxo) between the ages of 6 months and 2 years, having had a negative skin test to PPD. (Three of the children had in fact received BCG at birth 9-18 months previously, but all were PPD negative on skin testing.) The local response to BCG was measured and recorded if the child was seen one week later; in many cases the local response was visible or palpable for several weeks/months after the injection. A negative response was recorded if there was neither visible nor palpable evidence of induration at the site of the BCG immunisation: this was observed in 3 children. A small 'take' (t) was recorded if the induration was less than 4 mm diameter at the height of the response: this was recorded in 24 of the children. A satisfactory 'take' (T) was recorded if the induration measured more than 5 mm at the height of the response, whether or not ulceration also occurred: this was observed in 39 of the children. In 14 children the response was neither measured nor recorded or the child failed to turn up when there was still evidence of local response. There was no significant difference between any of the groups of children with regard to local response to BCG.

TABLE B5(viii)

Response to BCG vaccination by children

Classification of mother	No. of Children	Local response to BCG				Result of PPD conversion 2 months after BCG			Result of PPD 2 years later		
		nil	t ⟨4 mm	T ⟩5 mm	DNA[a]	-ve	+ve	DNA[b]	-ve	+ve	DNA/ND[c]
HC	11 [8]	-	4	5	2	1	6	4	3(1*) [6]	4(1ϕ) [2]	4
TT & BT/RFC	7 [3]	-	1	3	3	-	1	6	2(1*) [2]	2 [1]	3
TT & BT/Active	14 [3]	1	4	7	2	5	6	3	7(2*) [2]	3 [1]	4
BL	24 [5]	1	7	14	2	8	7	9	11(3*) [3]	4 [2]	9
LL	24 [1]	1	8	10	5	10	5	9	6(3*) [1]	9(5ϕ)	9
TOTAL	80 [20]	3	24	39	14	24	25	31	29(10*) [14]	22(6ϕ) [6]	29

a Did not attend for measurement of local response to BCG.
b Did not attend for reading of skin tests 72 hours after they were done.
c No. of children previously tested who did not attend for skin tests or reading thereof because of death, removal from Addis Ababa, or untraceable; or skin test was not done because of shortage of supply.

[ ] Number of children given BCG by Kebele clinics, subsequently tested in Phase II A9 follow-up.

* PPD was +ve 2 years before but became -ve.

ϕ PPD was -ve 2 years before but became +ve.

Result of PPD conversion 2 months after BCG

Twenty-five out of 49 (51%) children showed a positive PPD conversion 2 months after BCG. Unfortunately, one-third of the children tested did not attend for reading of their skin tests largely due to economic reasons, as the mothers felt they could not afford a second day off work or away from begging within a week. While numbers are not large enough to allow statistical comparisons, a difference in response was observed between children in the different groups(according to the mother's classification), with positive PPD conversion being recorded in 6 out of 7 children of healthy mothers, 7 out of 12 children of tuberculoid mothers, 7 out of 15 children of mothers with BL leprosy, and only 5 out of 15 children of LL mothers.

Result of PPD 2 years later (Phase II)

When re-tested 2 years after BCG, 22 out of 51 children were positive (43%); of the 29 children recorded as giving a negative skin test, 10 had previously shown a positive test. Of 22 children showing a positive skin test, 6 had been negative following BCG 2 years previously. The late positive conversion was most marked in the children of LL mothers. Twenty-nine children of those who were previously tested did not attend for skin test or reading of the skin test, either because of death, removal from Addis Ababa, or because they were untraceable: in some cases skin testing was not done because of shortage of the reagent. Of an additional 20 children who had received BCG at the Kebele Clinics, 14 had a negative PPD and 6 a positive PPD. The conversion from positive PPD to negative could not be correlated with the child's weight or nutritional status.

While 5 of the mothers developed tuberculosis (4 pulmonary, 1 extra pulmonary) between the end of Phase I and the Phase II assessment, only in one case did the child become PPD positive:

> Baby A9/226, child of a mother with LL leprosy, had shown an adequate local response to BCG, but two months later had a negative PPD conversion,only 2 mm of induration being measured. The child herself developed pulmonary tuberculosis six months prior to the Phase II assessment.

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Effect of BCG on anti-M. leprae antibody activity

The results of IgA, IgM and IgG anti-M. leprae antibodies estimated by solid-phase radioimmunoassay (sRIA) in serum samples from birth to 2 years of age were analysed with regard to the effect of BCG vaccination on antibody activity. No effect, in the form of increase or decrease in IgA, IgM or IgG anti-M. leprae antibody activity, could be demonstrated in serum samples taken before and after BCG. Sampling immediately prior to BCG immunisation and 2 months afterwards was too infrequent to exclude the possibility of there having been a (transient) effect on the antibody activity by BCG immunisation, but there was no evidence to indicate that BCG immunisation affected the general results of the study of M. leprae antibody activity.

Response to skin test with purified protein of M. leprae prepared from armadillos

i) A6 skin test at 6-18 months of age. Of 81 children tested, 73 showed a negative skin test measured at 72 hours, 1 showed a weakly positive test and 7 a positive test. There was no difference between the different groups of children (Table B5(ix)).

ii) AB22 skin test at 3-4 years of age. Of 72 children tested, 37 were negative, 1 of these had been strongly positive at 12 months of age; 5 were weakly positive and 30 were positive with an induration equal to or more than 5 mm diameter at 72 hours. One child weakly positive 2 years before became strongly positive, a second child who was positive before remained positive. There were no statistical differences between the different groups of children (Table B5(ix)).

Response of children to immunisation with triple vaccine (DPT) and poliomyelitis oral vaccine - Diphtheria antitoxin antibodies

The response of 50 children (Table B5(x)) to DPT immunisation in terms of antibody production was assessed by measurement of diphtheria antitoxin antibodies before and after immunisation.

TABLE B5(ix)

Response to skin test with purified protein of M. leprae prepared from armadillos

Classification of Mothers	A6 skin test at 6–18 months of age				AB22 skin test at 3–4 years of age			
	No. of Children	– ve 0 mm	Weakly + ve 1–4 mm	+ ve >5 mm	No. of Children	– ve 0 mm	Weakly + ve 1–4 mm	+ ve >5 mm
HC	12	11	–	1 *	15	7	3	5
TT & BT/RFC	7	6	–	1 **	5	2	–	3
TT & BT/Active (on treatment)	14	12	–	2 ***	15 ϕ	7	–	8
BL	24	22	–	2 *	21	12	1	8
LL	24	22	1 ****	1 *	16	9	1	6
	81	73	1	7	72	37	5	30

* DNA for retesting 2 years later.
** Stayed +ve 2 years later.
*** 1 stayed +ve, 1 became –ve 2 years later.
**** Became strongly +ve 2 years later.
ϕ An additional 2 mothers previously TT & BT/RFC relapsed with TT & BT Active leprosy before Phase II follow up, are included here.

TABLE B5(x)

Diphtheria anti-toxin antibodies in children of mothers with leprosy and healthy controls after D.P.T. immunisation

Classification of mother's leprosy	No. of children immunised	No. of children tested for rise in antibody titre	No. of children with significant rise in antibody titre
HC	9	8	8
TT & BT	12	9	9
BL	14	14	12 *
LL	15	11	11
	50	42	40

* 2 children showing no significant rise in titre had received 1st dose only of D.P.T. The levels of antibody titre demonstrated were not considered abnormal in response to first dose.

Unlike the BCG immunisation which was assessed by means of pre- and post-immunisation PPD skin test, together with collection of blood samples for anti-mycobacterial antibodies, the DPT and polio vaccine which were given by the Kebele Clinics were not timed to coincide with the A9 assessments. Of the 50 children immunised, only 42 had blood samples collected at a suitable interval after immunisation for measurement of diphtheria antitoxin antibodies. Of these 42, 40 children had a significant rise in antibody titre, 2 children both of BL mothers showing no significant rise in titre, had received only the first dose of DPT. Levels of antibody titre demonstrated were not considered abnormal in response to a first dose. There was no significant difference in the response measured by any of the groups of children in response to DPT immunisation.

It is of interest, however, that within the year following DPT immunisation, 3 children (2 of LL mothers, 1 of a BT mother) had whooping cough sufficiently severe to require treatment from the out-patient hospital clinic. The 2 children of LL mothers were both under-weight, weighing between 70 and 80% of the 50th centile Boston Standard at the time of Phase II assessment; the child of the BT mother was not significantly under-weight, weighing 90-100% of the 50th centile Boston Standard.

One child of a healthy mother who had received oral poliomyelitis vaccine, developed paralytic poliomyelitis within a year of vaccination. Inadequate protection of children against poliomyelitis by oral poliomyelitis vaccine administered during 1977 and 1978 had already been noted and was causing considerable concern to paediatricians (Dr. N. Dekker, personal communication).

6. A clinical and immunological study of four babies of mothers with lepromatous leprosy, two of whom developed leprosy in infancy

Mothers were assessed as described earlier with slit skin smears, biopsies and "nose blows" to assess infectivity. Samples of milk were examined, by concentration methods, for presence of M. leprae, as were homogenates of placental tissue.

Babies were assessed at birth, monthly until 3 months, and 3 monthly thereafter. In addition to routine weighing and measuring, blood samples were obtained every 3-6 months from birth to 2 years of age. Suspicious skin lesions were subjected to sensory testing, and 3 were biopsied. Independent assessment was carried out by 2 senior leprologists. Vaccination with DPT, smallpox and BCG was carried out at local "kebele" clinics. Skin testing with A6 (purified protein of M. leprae grown in armadillos) and PPD was carried out by the author: babies who were negative to PPD were given BCG and retested with PPD two months later.

Follow up of the babies 2 years later, when the children were 3-4 years old, was carried out by Dr. S. Menzel. Skin tests with AB22 (similar to A6) and PPD were done together with full examination and slit skin smears.

Immunological methods

IgA, IgG and IgM were quantitated by single radial diffusion in gel. Antibodies against M. leprae antigen 7 were determined by RIA. IgA, IgG and IgM anti-M. leprae antibody activity was demonstrated and quantitated by sRIA.

Observations

Assessment of infectivity of mothers

1. Clinical, skin smear and biopsy results. Of the 36 mothers with BT or TT leprosy, 11 had active disease. However, only in 2 cases were solid staining bacilli seen in biopsies or skin smears (Table B6(i), and it seems very unlikely that these mothers could infect their babies.

Of the 76 mothers with lepromatous (LL or BL) leprosy, 40 showed some clinical deterioration during pregnancy or soon after delivery. In 28 cases the deterioration amounted

TABLE B6(i)

Number of patients in whose skin biopsies solid staining acid fast bacilli were demonstrated

Classification	Number studied	Number with solid AFB in skin biopsy
TT/BT	36	2
BL/LL	76	38

to frank relapse probably due to the emergence of sulphone-resistant leprosy. Solid staining bacilli were found in skin smears or biopsies in 38 cases; these patients must be considered as potentially highly infectious to their unborn children by haematogenous spread via the placenta.

2. Results of nose blows. Nasal mucus (nose blow) was examined in 43 patients with lepromatous leprosy, but only one was found to be positive; no solid staining bacilli were seen. The risk of droplet transmission of leprosy after delivery seems to be slight in this group of patients.

3. M. leprae in milk. Milk from 9 mothers with highly bacilliferous leprosy was examined for acid-fast bacilli by concentration methods; all 9 specimens were negative. It seems unlikely that the milk was, in this group of patients, a major source of M. leprae or risk to the babies.

4. The placenta as a site for M. leprae. Routine examination of placental sections failed to demonstrate the presence of acid-fast bacilli. Even the use of concentration methods to search for acid-fast bacilli in placental tissue from 7 of the most bacilliferous patients were negative in half the cases; the other half showed very scanty acid-fast bacilli or debris.

Clinical assessment of babies with suspected leprosy

Four babies developed hypopigmented lesions between 9 and 17 months of age which were suspected to be due to leprosy; in all cases the mothers had active lepromatous (BL) leprosy. The clinical data is summarised in Table B6(ii), and detailed accounts of the 4 babies are given at the end of this section. Babies 2 and 3 show strong evidence of leprosy, but Baby 1 did not have leprosy, and Baby 4 is doubtful.

None of the 4 infants received antileprosy treatment (other than via the breast milk prior to weaning); but all the lesions had almost completely disappeared when the children were finally assessed 2 to 3 years after the lesions appeared. At about this time a new macule appeared in Baby 4, but it was shown to be not due to leprosy. A new lesion in Baby 3 appeared at 27 months,

TABLE B6(ii)

Clinical data on 4 babies suspected of developing leprosy and their mothers

Case No.	Mother	Baby Age (Months)	Details of lesions (Baby)						Follow Up	Skin test with M. leprae antigen at 3 years of age
			No.	Size (cm)	Site	Margin	Sensory Loss	Biopsy		
1	LL* (BI = 3.5) (MI = 2.8)	9	2	1 x 1 1 x 1	L Leg L Leg	Well defined	No	Not leprosy	Clinical diagnosis ringworm. Treated with anti-fungal ointment and resolved within 2 weeks	Negative
2	BL ø (BI = 1.1) (MI = 0)	17	2	0.5 x 1 1.8 x 3	Neck R Leg	Well defined	Yes	Tuber-culoid leprosy	Gradual fading of lesions. No sign of leprosy at examination aged 4 and 4½ years	Positive
3	BL** (BI = 4.2) (MI = 4.0)	12	3	1 x 4 1 x 4 1 x 4	Back Back Back	Poorly defined	Yes	Consist-ent with indeter-minate leprosy	Lesions began to fade after 3 months. New macule 1.4 x 1.5 on buttock seen at 23 months, biopsy "not diagnostic of leprosy". All lesions almost disappeared at 3-4 years	Positive
4	BL* (BI = 3.0) (MI = 4.5)	14	1	1.5 x 1	Chest	Poorly defined	No	Not done	Lesion faded at 17 months, no abnormality seen at 39 months. New lesion 1 x 1 seen on thigh at 45 months, biopsy "nothing at all to indicate leprosy".	Negative

* Suspected dapsone resistance during pregnancy.
ø "Cured BT" relapsed "active BL" during pregnancy.
** Proven dapsone resistance during pregnancy.

but the biopsy was not diagnostic of leprosy and the lesion had resolved at the last assessment.

Skin testing with M. leprae antigen was performed at the end of the study; Babies 1 and 4 were negative, 2 and 3 positive (all 4 infants were negative when first tested at 9–15 months).

Immunological assessment of babies with suspected leprosy

1. Immunoglobulins. Immunoglobulin levels for cord sera of Babies 2, 3 and 4 are shown in Table B6(iii); (cord serum from Baby 1 was not available). The median figures shown in the Table are medians found in cord blood from the babies of all mothers with active lepromatous leprosy.

   The levels of IgA tended to be slightly lower than the median figures, while IgM was in the median range. The IgG levels of these 3 babies, however, were higher than the median, although the IgG levels of their mothers' sera (taken at delivery) were lower than average for mothers with active lepromatous leprosy.

2. Antibodies against M. leprae antigen 7. The concentrations of antibodies against M. leprae antigen 7 declined in all 3 babies from birth to 6–8 months of age (Figure B6(i)) as expected from the 28 day half life of maternal IgG in newborn babies (Melsom et al, 1980b). No rise was seen at the time the babies developed skin lesions suspicious of leprosy.

3. IgA, IgM and IgG anti-M. leprae antibody activity. The median IgA, IgM and IgG anti-M. leprae antibody activity of 26 babies of mothers with lepromatous leprosy in serial samples of serum from birth to 20 months is shown in Figure B6(ii). The antibody activity is expressed as percentage of the activity in a lepromatous serum pool (LSP). IgG antibody activity declined markedly from 25% in cord sera to 0.25% of LSP in sera taken 12 months after birth and thereafter showed a slight insignificant increase. IgM anti-M. leprae antibodies could be detected in 55% of the cord sera and the activity rose during this period to 10%. IgA antibodies could be detected in 30% of cord sera and the activity also rose steadily to 2.5% of LSP in sera taken at about 18 months of age.

TABLE B6(iii)

Immunoglobulin levels of cord sera of 3 babies and median figures of babies of all mothers with active lepromatous leprosy

	IgA	IgM	IgG
A9/70 (Case No. 2)	4-8 x 10^{-3} g/l	32 x 10^{-3} g/l	10.0 g/l
A9/89 (Case No. 3)	4-8 x 10^{-3} g/l	60 x 10^{-3} g/l	9.8 g/l
A9/244 (Case No. 4)	4-8 x 10^{-3} g/l	110 x 10^{-3} g/l	12.5 g/l
Median LL-Bl, BI+	9.5 x 10^{-3} g/l	54 x 10^{-3} g/l	9.5 g/l

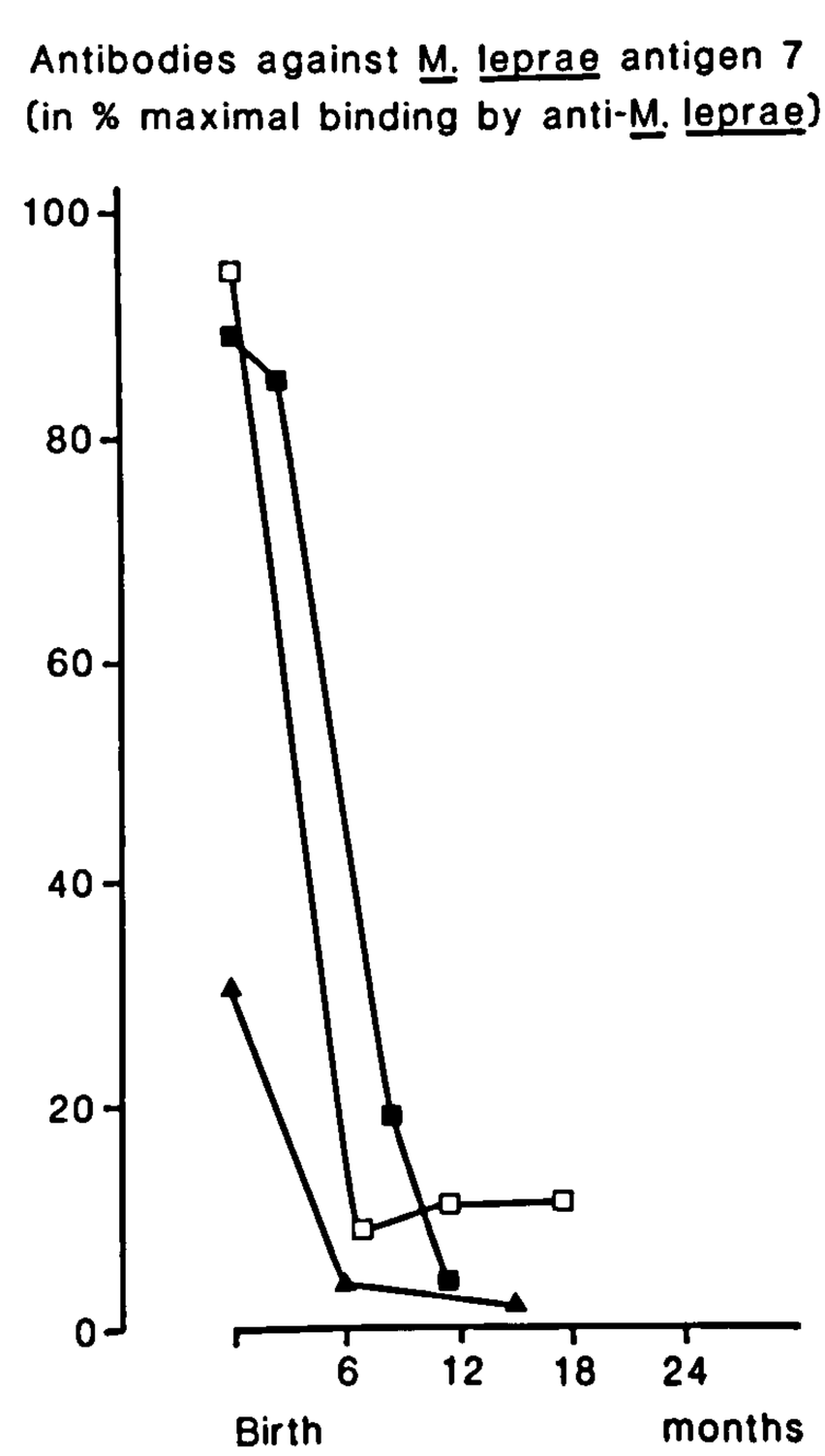


Figure B6(i). The activity of antibodies against M. leprae antigen 7 in cord serum and repeated serum samples taken from ▲ Case 2, □ Case 3 and ■ Case 4. The activity is expressed as percentage of the maximum binding using polyvalent rabbit anti-M. leprae antibody preparation.

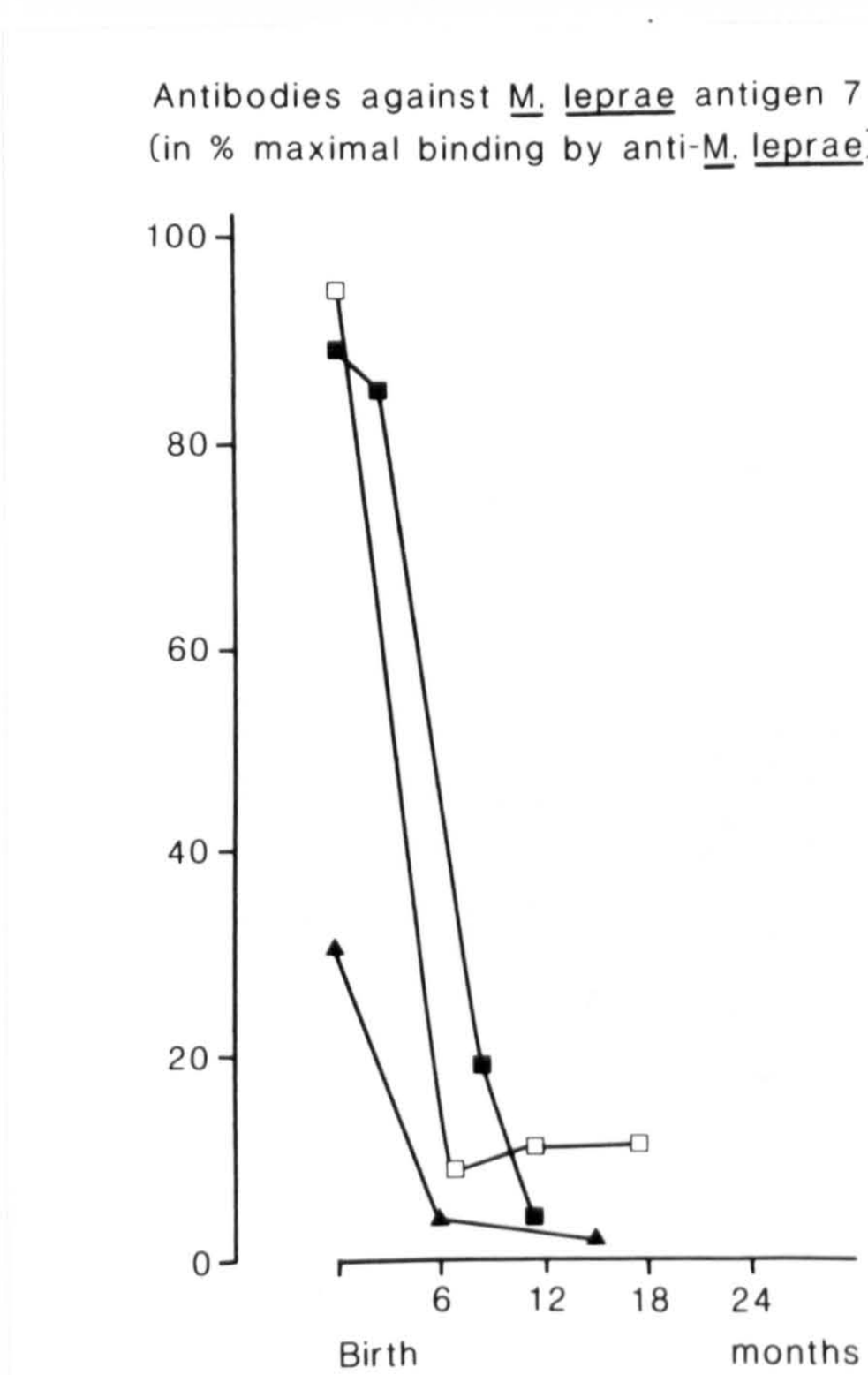


Figure B6(i). The activity of antibodies against M. leprae antigen 7 in cord serum and repeated serum samples taken from ▲ Case 2, □ Case 3 and ■ Case 4. The activity is expressed as percentage of the maximum binding using polyvalent rabbit anti-M. leprae antibody preparation.

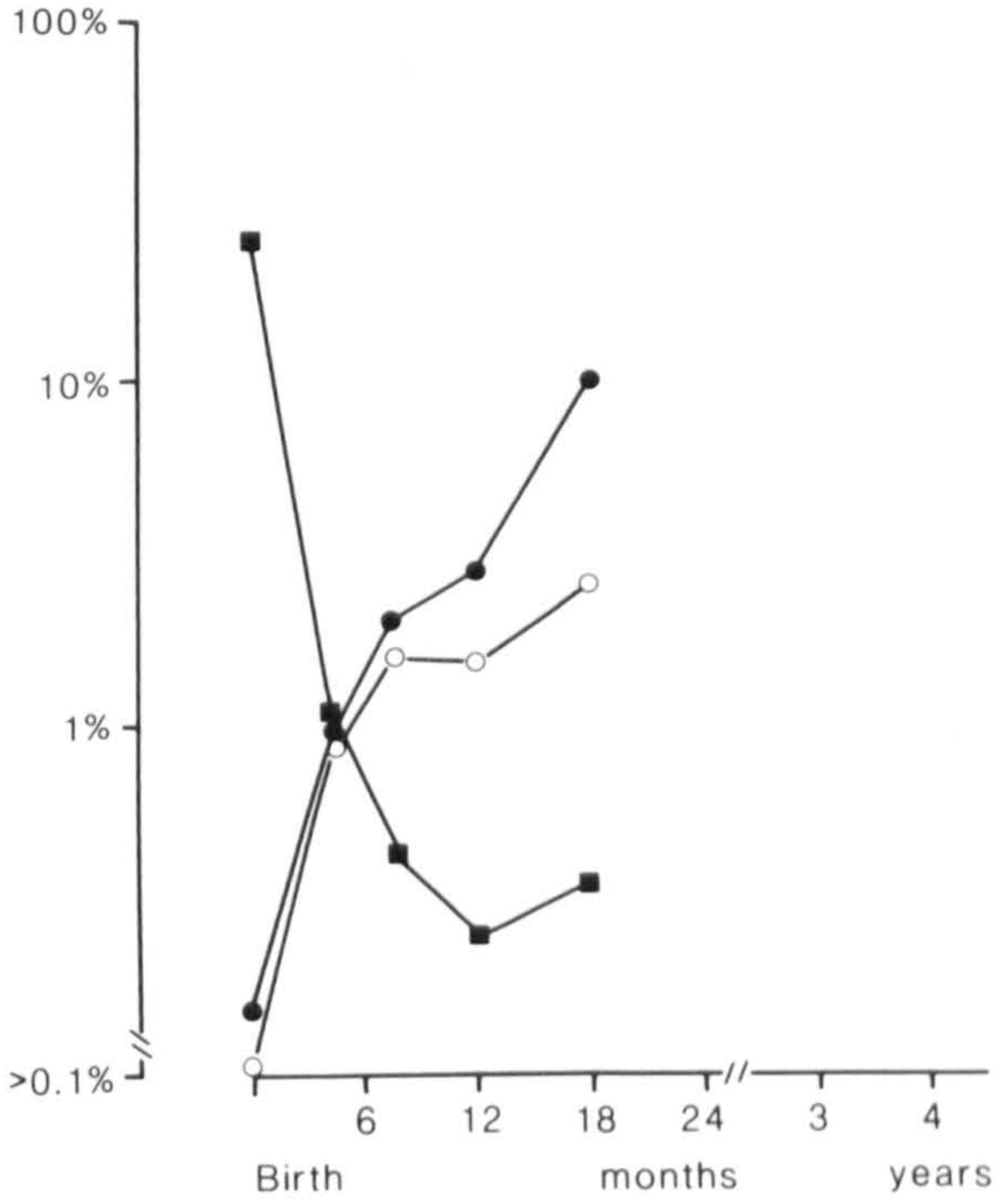


Figure B6(ii). Median IgA (O), IgM (●) and IgG (■) anti-M. leprae antibody activity in sera from 29 babies of mothers with lepromatous leprosy. The median results were calculated from all the individual results after the sera had been allocated to five groups, cord sera and sera taken around 3, 6, 12 and 18 months after birth. The results are expressed as percentage of IgA, IgM or IgG anti-M. leprae antibody activity in a lepromatous serum pool (LSP).

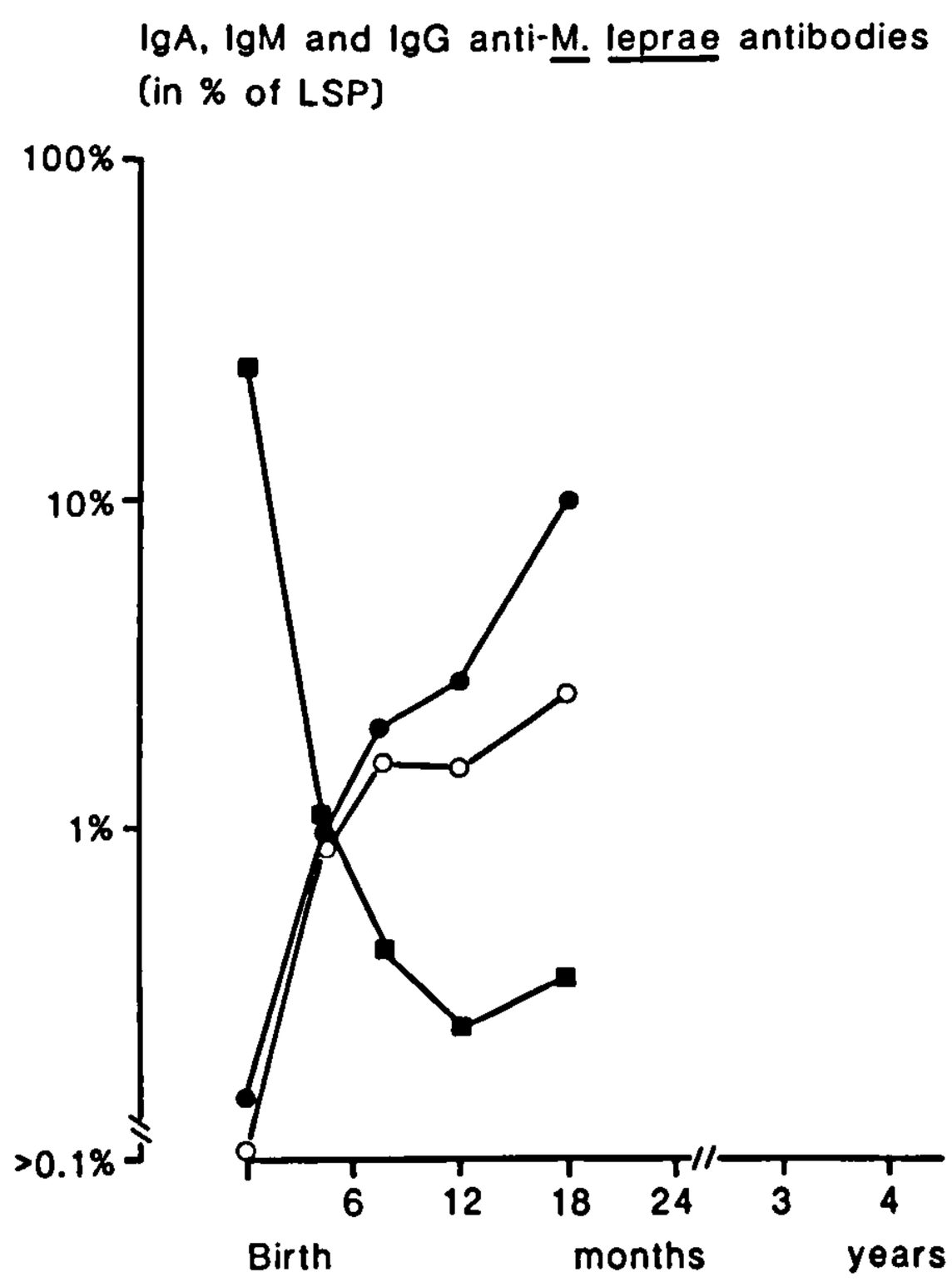


Figure B6(ii). Median IgA (O), IgM (●) and IgG (■) anti-M. leprae antibody activity in sera from 29 babies of mothers with lepromatous leprosy. The median results were calculated from all the individual results after the sera had been allocated to five groups, cord sera and sera taken around 3, 6, 12 and 18 months after birth. The results are expressed as percentage of IgA, IgM or IgG anti-M. leprae antibody activity in a lepromatous serum pool (LSP).

The IgA, IgM and IgG anti-M. leprae antibody activity of serial serum samples from 4 babies with suspected leprosy is shown in Figures B6(iii), (iv), (v) and (vi). Baby 1 shows a "normal" pattern (Figure B6(iii)) with lower IgA, IgM and IgG anti-M. leprae than illustrated in Figure B6(ii). Babies 2 and 3 show a different pattern. Both Babies 2 and 3 showed a marked rise in IgA and IgM anti-M. leprae antibody activity: in Baby 3 this rise was early, and in Baby 2 it was somewhat slower, the IgM activity reaching 30% of LSP activity (both babies) and the IgA activity reaching 35% and 25% of LSP activity respectively in sera taken 3 years after birth. Baby 4 with unproven leprosy showed a pattern of IgA, IgM and IgG anti-M. leprae activity between the pattern seen in Babies 2 and 3 and that made by the sera from all babies of mothers with lepromatous leprosy illustrated in Figure B6(ii).

Case Histories of the 4 babies suspected of developing leprosy

Case No. 1 (A9/36)

The first baby to show suspicious lesions was a female infant aged 9 months, born to a woman with very active lepromatous leprosy (BI = 3.5; MI = 2.8%) who had been treated irregularly for 14 years and had relapsed with active leprosy during her pregnancy. The two lesions which both measured 1.0 x 1.0 cm were hypopigmented, slightly raised lesions with well defined margins, situated on the lower left leg. There was no obvious sensory loss. The child was seen and assessed by the author and 2 senior hospital leprologists. Clinically, the skin lesions resembled those of early BT leprosy, but a biopsy was taken and reported as follows :

> "Non specific cellular infiltration in the upper dermis. Nerves appear normal. No AFB seen. Probably not leprosy." (JMHP)

On the basis that the most likely alternative diagnosis was ringworm, treatment was started with Whitfield's ointment and the lesions disappeared within 2 weeks, which would seem to confirm that this diagnosis was correct. Skin tests with A6 and PPD were both negative at 2 years of age. Because this baby did not have leprosy, although she did have ringworm, we have used her for comparison as a reasonable control.

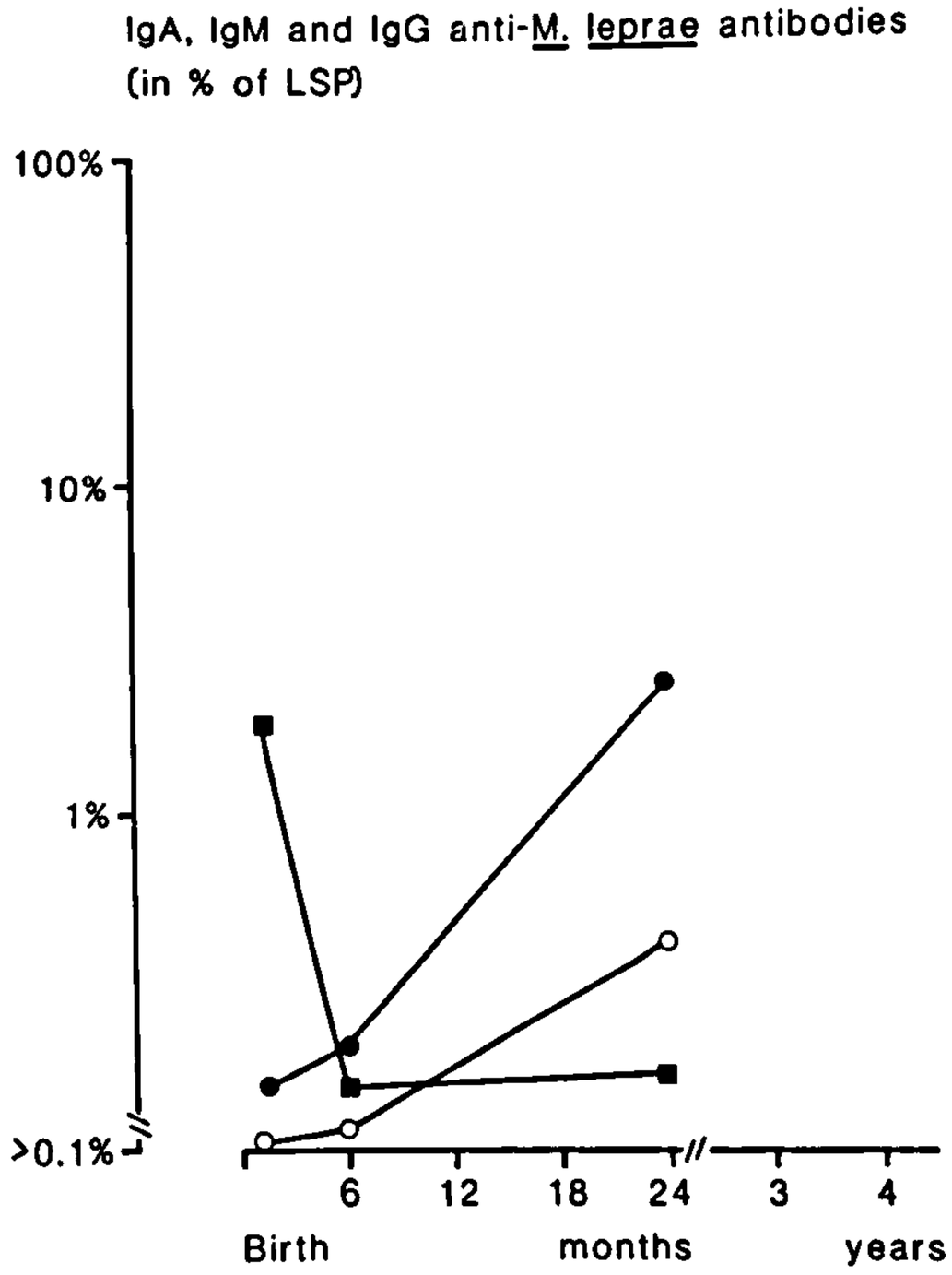


Figure B6(iii). IgA (O), IgM (●) and IgG (■) anti-M. leprae antibody activity in serum taken one month after birth and repeated sera taken up to 2 years after birth from A9/36 (Case No. 1). The results are expressed as percentage of IgA, IgM or IgG anti-M. leprae antibody activity in a lepromatous serum pool (LSP).

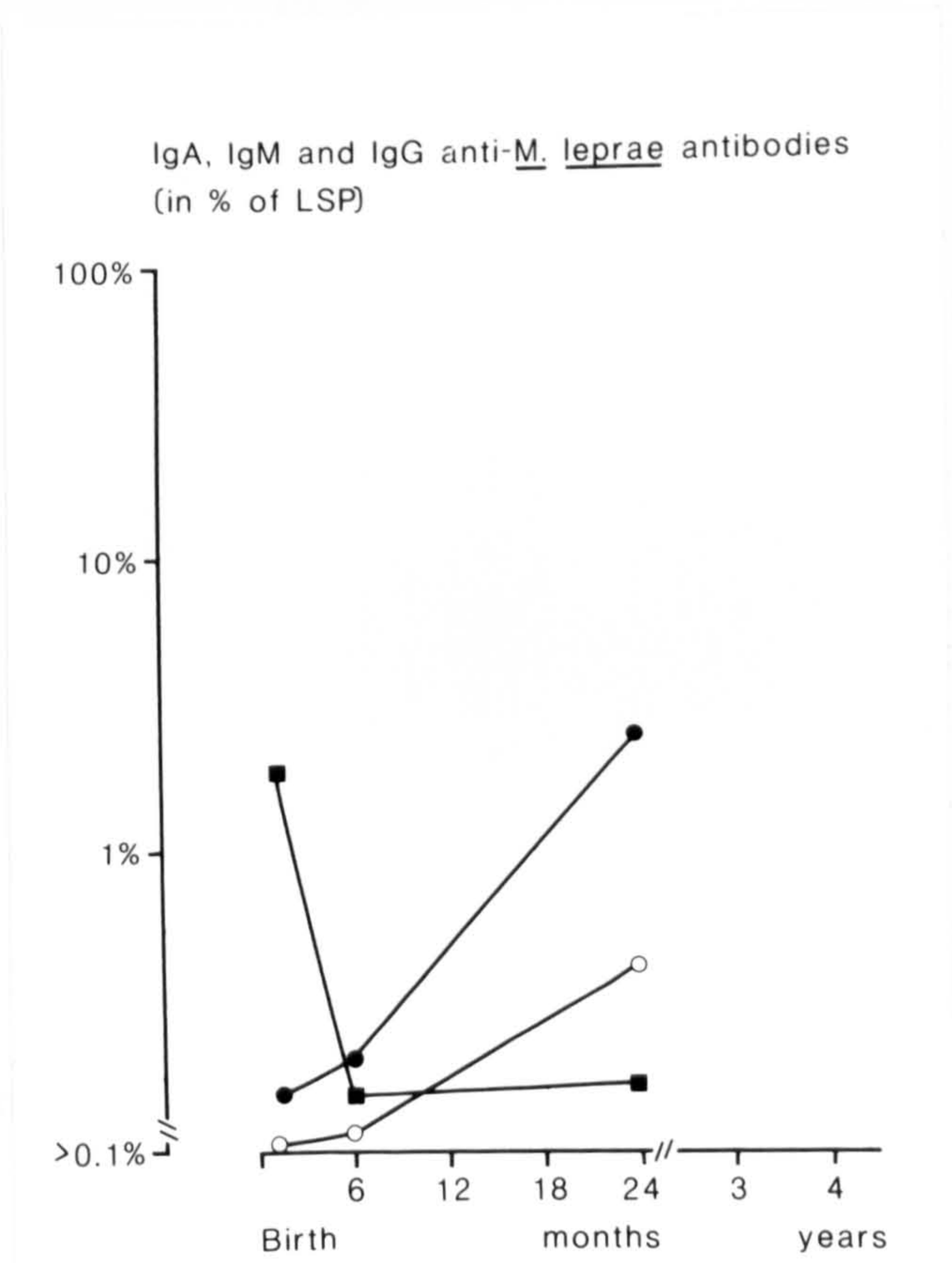


Figure B6(iii). IgA (O), IgM (●) and IgG (■) anti-M. leprae antibody activity in serum taken one month after birth and repeated sera taken up to 2 years after birth from A9/36 (Case No. 1). The results are expressed as percentage of IgA, IgM or IgG anti-M. leprae antibody activity in a lepromatous serum pool (LSP).

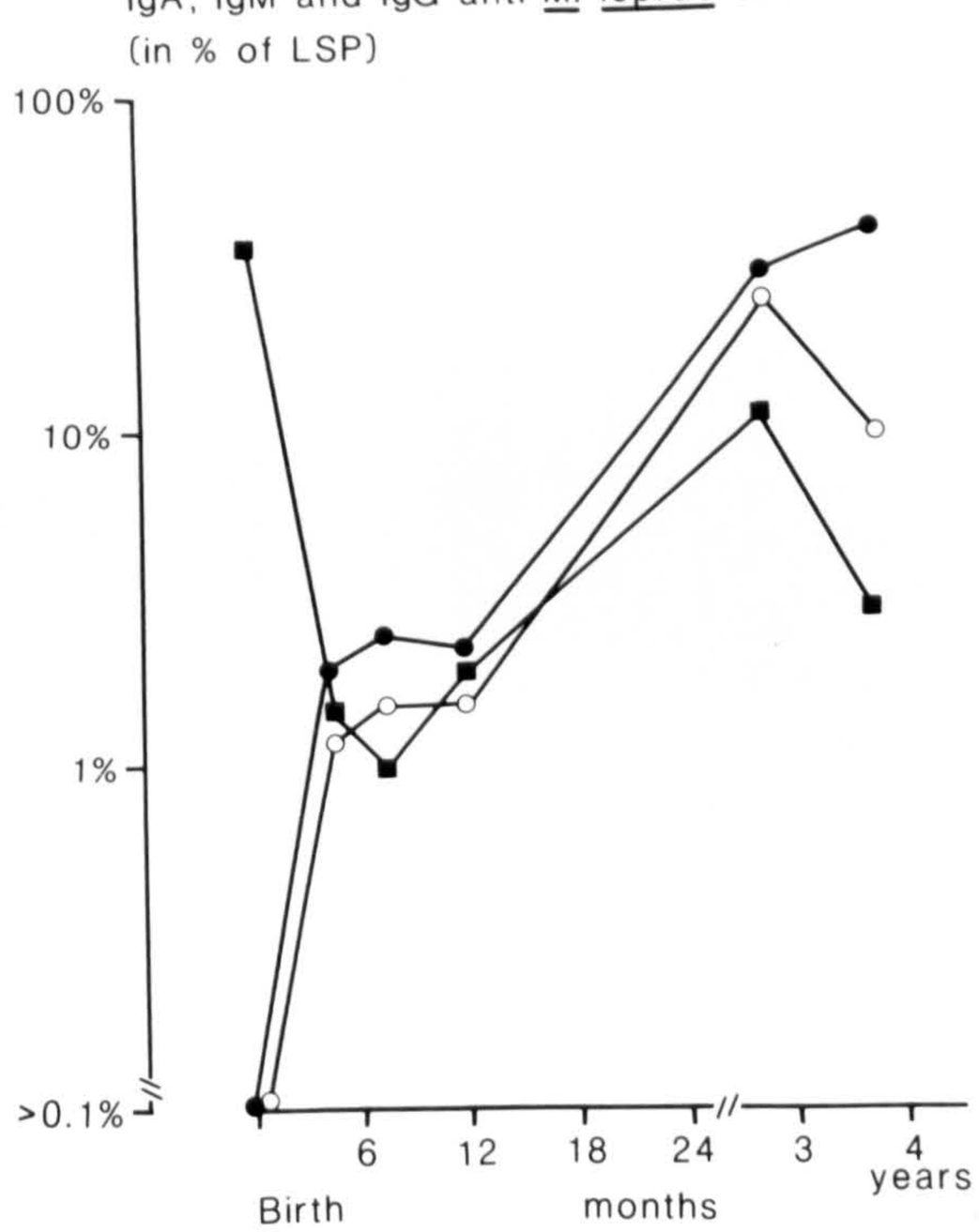


Figure B6(iv). IgA (O), IgM (●) and IgG (■) anti-M. leprae antibody activity in cord serum and repeated serum samples taken up to 4 years from birth from Baby A9/70 (Case No. 2). The results are expressed as percentage of IgA, IgM or IgG anti-M. leprae antibody activity in a lepromatous serum pool (LSP).

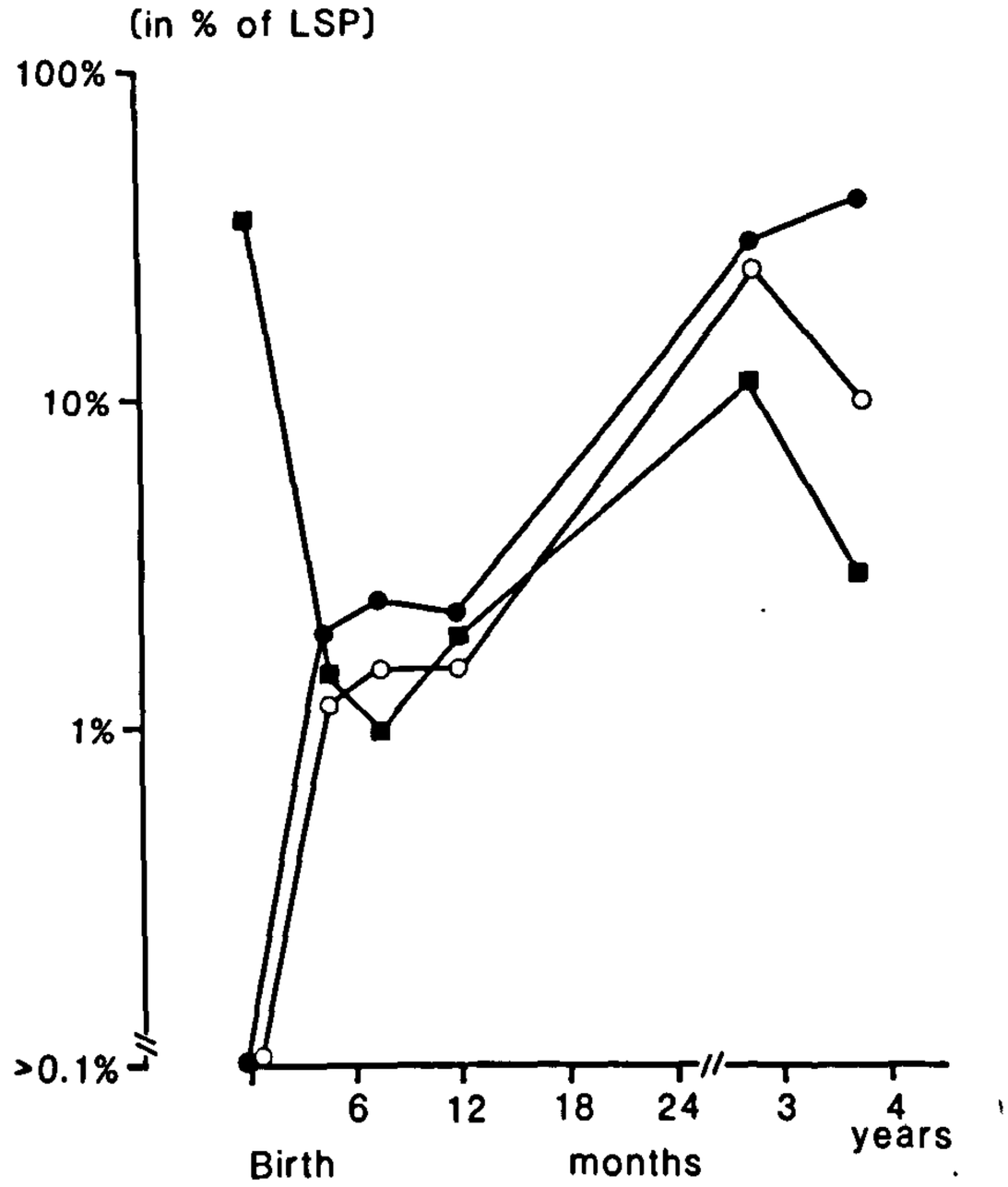


Figure B6(iv). IgA (O), IgM (●) and IgG (■) anti-M. leprae antibody activity in cord serum and repeated serum samples taken up to 4 years from birth from Baby A9/70 (Case No. 2). The results are expressed as percentage of IgA, IgM or IgG anti-M. leprae antibody activity in a lepromatous serum pool (LSP).

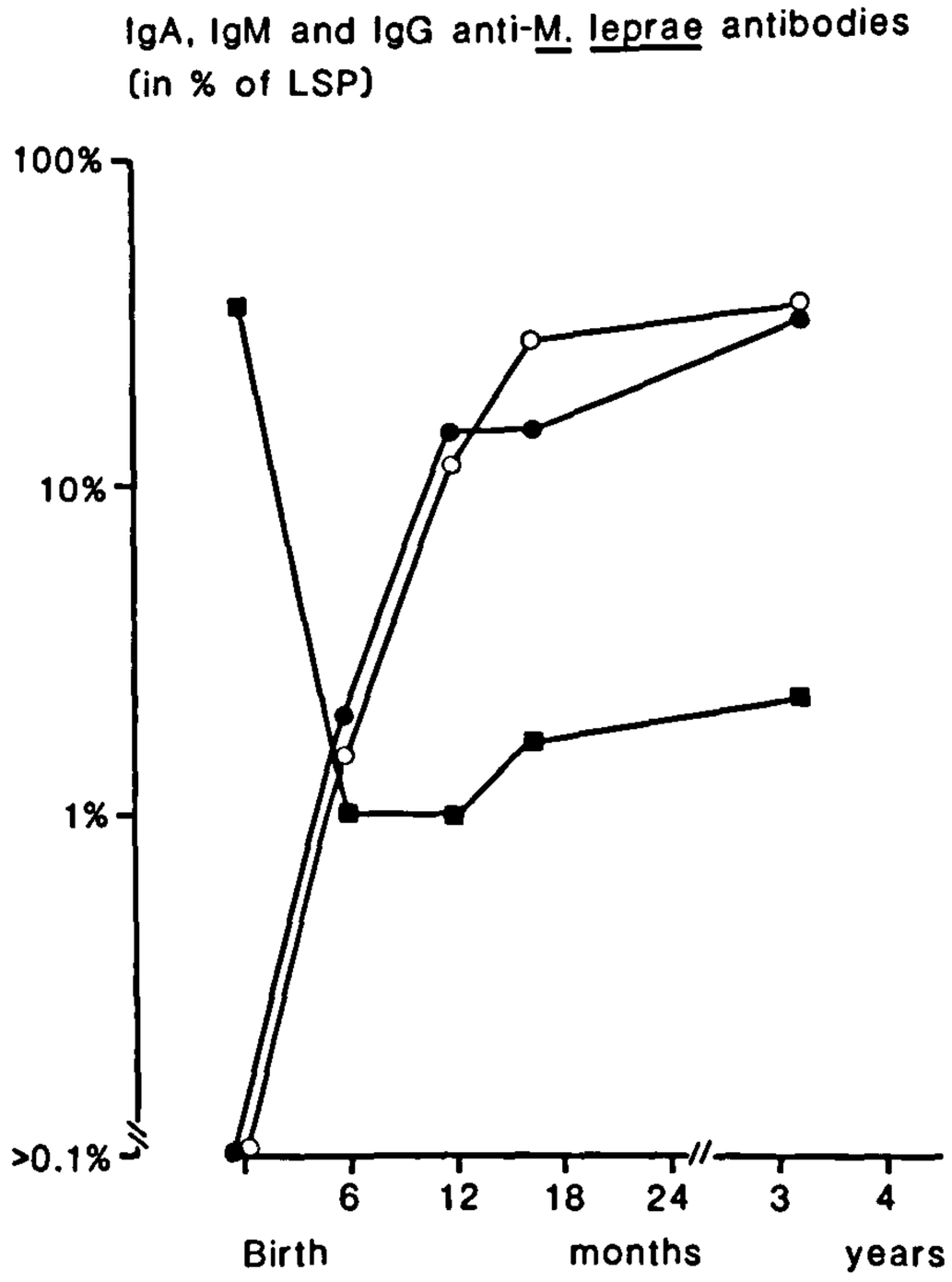


Figure B6(v). IgA (O), IgM (●) and IgG (■) anti-M. leprae antibody activity in cord serum and repeated sera taken up to $3\frac{3}{4}$ years after birth from Baby A9/89 (Case No. 3). The results are expressed as percentage of IgA, IgM or IgG anti-M. leprae antibody activity in a lepromatous serum pool (LSP).

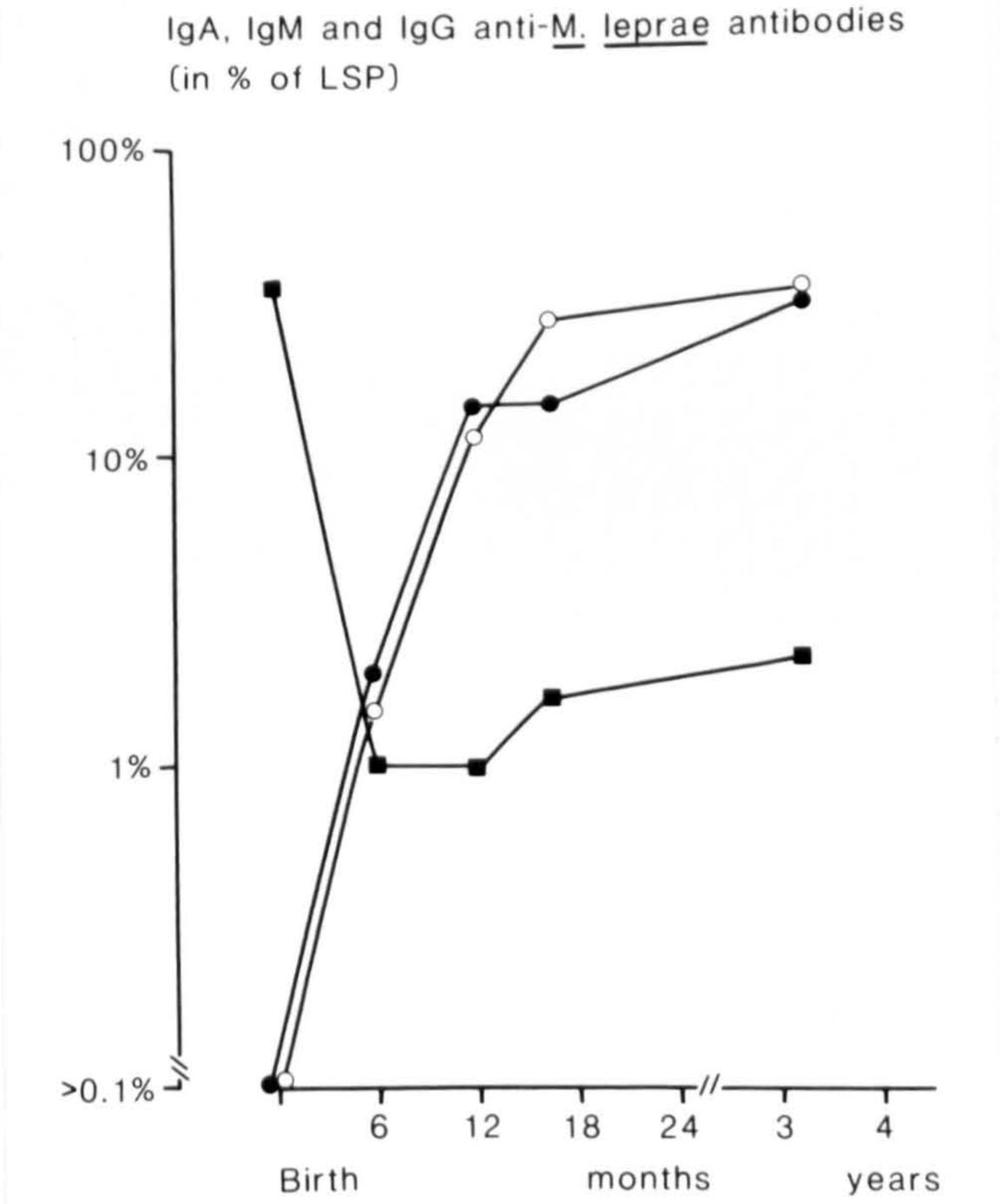


Figure B6(v). IgA (O), IgM (●) and IgG (■) anti-M. leprae antibody activity in cord serum and repeated sera taken up to $3\frac{3}{4}$ years after birth from Baby A9/89 (Case No. 3). The results are expressed as percentage of IgA, IgM or IgG anti-M. leprae antibody activity in a lepromatous serum pool (LSP).

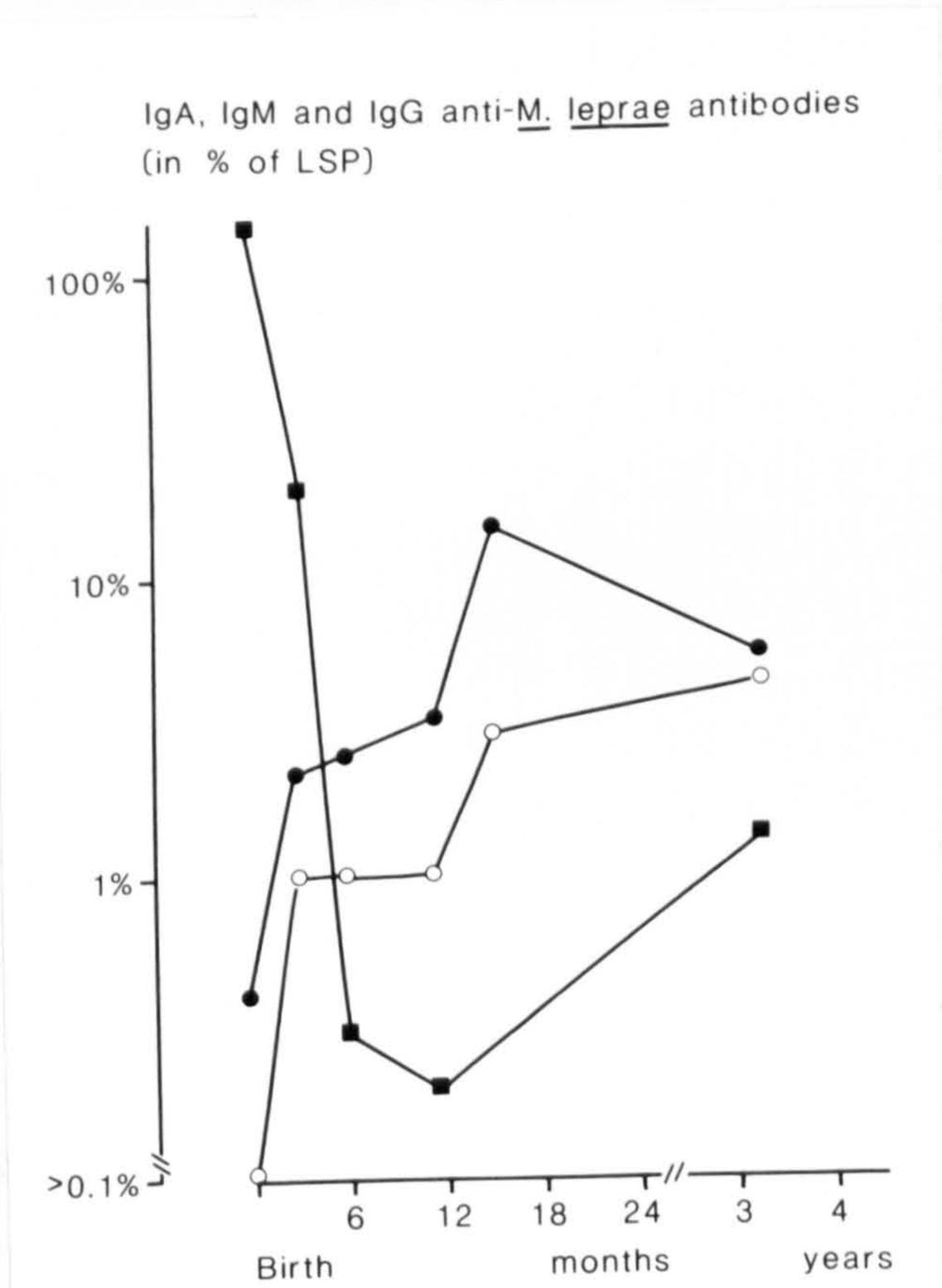


Figure B6(vi). IgA (O), IgM (●) and IgG (■) anti-M. leprae antibody activity in cord serum and repeated sera taken up to $3\frac{1}{4}$ years after birth from Baby A9/244 (Case No. 4). The results are expressed as percentage of IgA, IgM or IgG anti-M. leprae antibody activity in a lepromatous serum pool (LSP).

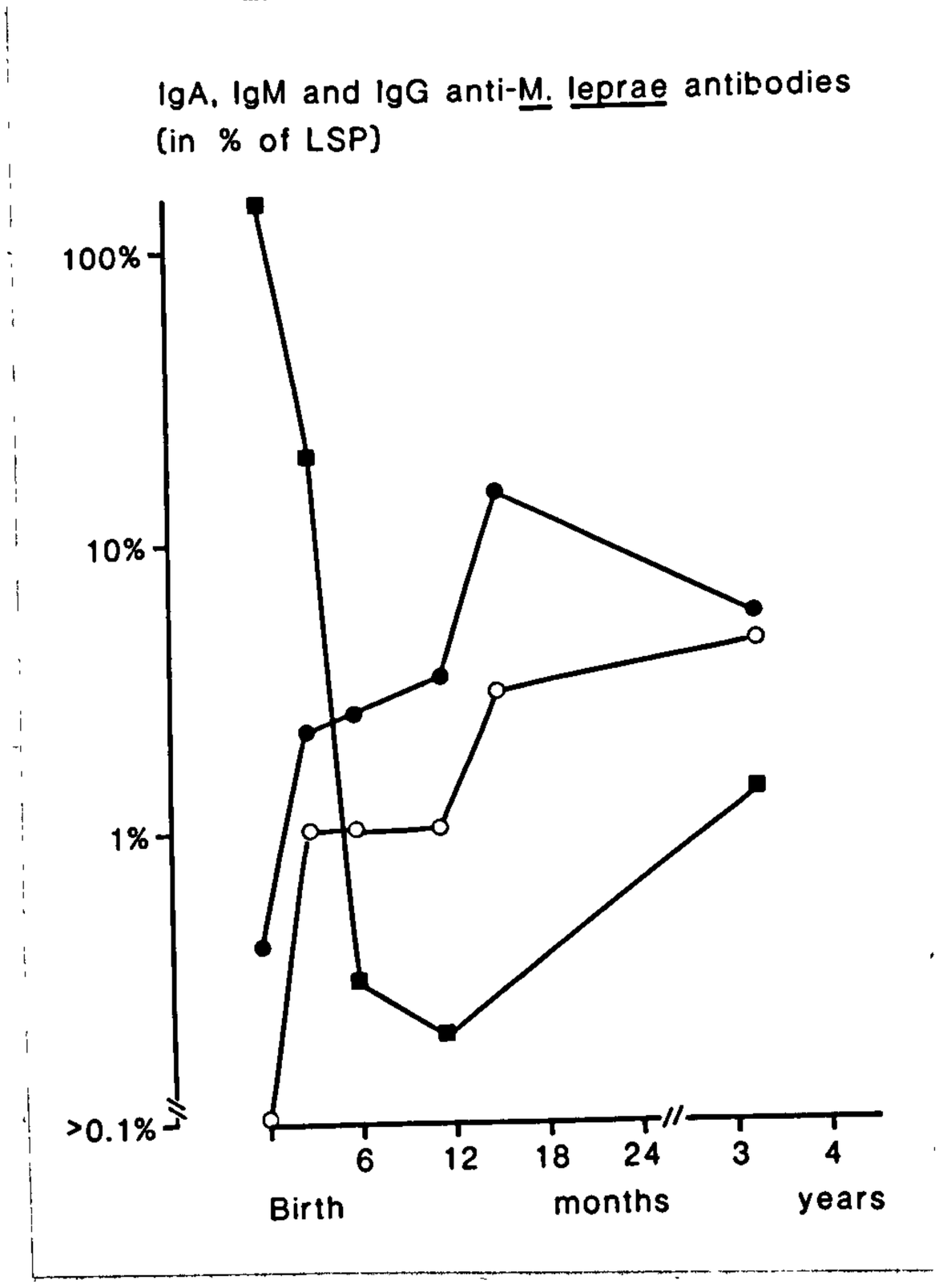


Figure B6(vi). IgA (O), IgM (●) and IgG (■) anti-M. leprae antibody activity in cord serum and repeated sera taken up to $3\frac{1}{4}$ years after birth from Baby A9/244 (Case No. 4). The results are expressed as percentage of IgA, IgM or IgG anti-M. leprae antibody activity in a lepromatous serum pool (LSP).

Case No. 2 (A9/70)

The mother, a 28 year old secundigravida, had been treated for tuberculoid leprosy (BI = 0) from April 1968 to August 1975, when she was considered to be cured and was released from control. During her second pregnancy she had iridocyclitis in the second trimestre; at 7 months' gestation she developed an anaesthetic erythematous macule in the centre of an old hypopigmented macule on the left cheek. Both ulnar nerves were enlarged but not tender. BI = 1.1 (from the lesion); BI = 0 (standard sites); biopsy showed :

"BL, early active lesion AFB 3-4^{+} in granuloma."

When the laboratory results were available, treatment with dapsone 100 mg per day was started during the third trimestre.

The baby, a healthy male weighing 3,200 grams, was born at 41 weeks' gestation. The placenta weighed 750 grams. The baby was seen regularly (on 24 occasions) until 21 months of age. He had recurrent infections: gastro enteritis (4x), bronchitis (5x), conjunctivitis (3x), otitis media (1x), scabies (4x) but, despite these, appeared well nourished (weight 11.5 kg at 21 months, Boston Standard 11.9 kg). He was fully breast fed until 11 months old when weaning was started, and was still being breast fed at 18 months.

At 17 months old, while suffering from his fourth generalised scabies infection, he developed 2 hypopigmented macules, one on the back of the neck (0.5 x 1.0 cm) and a second on the inner aspect of the right thigh (1.8 x 3.0 cm) (Plate 11). The second macule had 2 small satellite lesions. These macules, which became very obvious when the child was 19 months old, after the scabies infection had been treated, were hypopigmented with clearly defined, non-erythemous margins. There was some loss of sensation in the area of the macules, but no enlargement of peripheral nerves. Clinically, the lesions were those of early BT or indeterminate leprosy. The child was seen and assessed independently by the author and also by two senior hospital leprologists. Two punch biopsies were taken from the edge of the lesion on the thigh. They were reported as follows :

"The skin is almost normal except for a small focus of lymphocyte infiltration in the epidermis and subepidermal zone, and infiltration of a nearby neurovascular bundle



Plate 11. Case No. 2, Baby A9/70. A hypopigmented, slightly raised, macule, with a clearly defined margin, measuring 1.8 x 3.0 cm, is shown on the inner aspect of the thigh. Lesions of a generalised scabies infection are also visible.



Plate 12. Case No. 3, Baby A9/89. Three hypopigmented, flat macules all measuring 1.0 x 4.0 cm, with poorly defined margins are seen above the left sacro-iliac joint. Two (2 cm above the mother's index finger) have biopsy scars at the edge; the third (1 cm above the finger) was not biopsied. The lesions were not shown with B & W photography (Cochrane, 1938).

> with lymphocytes and mononuclear cells. In the latter the lymphocytes have penetrated the perineurium of the nerve twig, which shows also swelling and some proliferation of Schwann cell nuclei without disorganisation of structure. The nerve involvement makes a diagnosis of leprosy highly probable, even though acid-fast bacilli could not be demonstrated. Classification strictly would be indeterminate, but the involvement of epidermis and of nerve in the absence of bacilli point to tuberculoid leprosy." (DSR)

Two months later the macule on the back of the neck was fading while the macule on the thigh had extended, encircling the biopsy-sites, and measured 3.0 x 3.0 cm. The mother failed to attend for special follow up with the baby after the author left Addis Ababa in May 1978. Both mother and child were referred to the general hospital clinic for follow up.

Special follow up examinations were made when the child was 4 and 4½ years old by Dr. Menzel and a senior hospital leprologist. There was no evidence of leprosy on either occasion.

Case No. 3 (A9/89)

The mother, a 28 year old gravida 3, had had a history of "nodules" for 6 years prior to starting treatment in 1958 at the age of 10 years. Treatment was interrupted for 3 years, before her first pregnancy. The disease was reactivated after both her first two pregnancies and histologically she downgraded from BB→BL and the BL→LL, upgrading to BL before her third pregnancy. She developed new nodules (BI = 4.2, MI = 4.0%) during the third trimestre of her third pregnancy, and had a rising MI despite taking dapsone 100 mg daily. Dapsone resistance was suspected and treatment was changed to dapsone by weekly injections. However, further deterioration took place postpartum and improvement only occurred after treatment with oral clofazimine and TB 450 was initiated 6 months postpartum.

The baby, a healthy male infant weighing 3,350 grams, was born at full term. The placenta weighed 450 grams. Colostrum collected at birth and milk during the first week of lactation did not contain M. leprae. The baby initially did very well, gaining weight rapidly to 7.9 kg at 6 months (Boston Standard 7.4 kg); he was seen only once during the next 6 months, and weighed 9.0 kg at 12 months (Boston Standard 9.9 kg) and 9.5 kg at 15 months

(Boston Standard 10.6 kg). He had 3 minor upper respiratory infections during his first year, and 3 moderately severe chest infections and 1 episode of otitis media between the age of 12 and 15 months. He was seen 14 times in all.

At 12 months of age the child was found to have 3 hypopigmented lesions on the lower back just above the left sacro-iliac joint. His mother stated that the lesions had been present from 7 months of age. (They had not, however, been observed at the one check-up visit between 6 and 12 months.) The lesions which were flat, hypopigmented macules, all measuring 1.0 x 4.0 cm, had poorly defined margins. There was loss of sensation in the area of the macules, but no enlargement of peripheral nerves. The child was assessed independently by the author and also by two senior hospital leprologists and diagnosed, on clinical grounds, as having indeterminate leprosy, possibly with primary dapsone resistance. We decided to keep the child under observation initially. Two months later when the lesions were unchanged (Plate 12), two punch biopsies were taken and reported as follows :

> "The skin is normal except for a rather scanty infiltrate of lymphocytes and mononuclear cells around the sweat ducts and hair follicles, though there is no significant perivascular infiltrate. A small nerve bundle in the subcutis shows some slight Schwann cell proliferation. No acid-fast bacilli found. The histological evidence is not sufficient in itself to warrant a diagnosis of leprosy, though it is consistent with this. The classification would be indeterminate." (DSR)

The macules started to fade shortly after the biopsies were taken, but were still visible as vague macules 3 months later when the child was 17 months old. The child was fully breast fed throughout the study, despite attempts at weaning. After the mother started treatment with clofazimine her milk acquired a pinkish discolouration and the child assumed the pinkish skin hue typical of patients receiving clofazimine. Mother and child were referred to the regular hospital clinic for follow up.

In March 1979 when the child was 27 months old he was found to have a hypopigmented macule (1.4 x 1.5 cm) on the right buttock. Sensory status and condition of peripheral nerves was not recorded. Histology showed :

"Some tiny lymphocytic infiltration around sweat ducts, not diagnostic for leprosy." (JW)

Special follow up examinations were made when the child was $3\frac{1}{4}$ and $3\frac{3}{4}$ years old by Dr. Menzel and a senior hospital leprologist respectively. At the time of the second examination the macule on the buttock had faded, while there was residual vague hypopigmentation visible around the scars of the first biopsies. There was no clinical evidence of active leprosy.

Case No. 4 (A9/244)

The mother, a 37 year old gravida 4, started treatment in 1966 at the age of 26, having had erythematous macules and nodules for 9 years (BI = 3.5). Prior to her fourth pregnancy, slit skin smears from standard sites showed BI = 0. Clinically she was classified as quiescent BL. In the third trimestre she developed a few new nodules which were BI = 4; MI = 4%. Biopsy showed histoid features of early relapse with solid AFB in deep dermis. During lactation more new nodules appeared and smears from standard sites became positive (BI = 3; MI = 4.5%). During this time she was being treated with dual therapy with dapsone 100 mg daily and thiazina 1 tablet daily.

She delivered at 41 weeks' gestation a healthy male infant weighing 3,500 grams. The baby was seen regularly, was fully breast fed until 15 months, gained weight steadily weighing 9.7 kg at 15 months (Boston Standard 10.6 kg). He had two minor infections during the first year, recurrent otitis media from 12-15 months, and ringworm for the first time at 14 months. At 14 months he was found to have a hypopigmented macule with poorly defined edges over the right costal margin. The macule measured 1.5 x 1 cm in diameter. This lesion bore no resemblance to the patches of ringworm on the left cheek or right and left calves. Treatment with Whitfield's ointment cleared the ringworm, while the hypopigmented patch on the right costal margin grew to 1.2 x 2 cm and the edges of the macule became better defined. The baby was seen and assessed independently by the author and also by a senior leprologist. The lesion was not biopsied, but was kept under observation, and after 3 months it too had faded. There was no enlargement of nerves, nor was there definite loss of sensation.

The child was seen by Dr. Menzel when he was $3\frac{1}{4}$ years old. At that time there was no clinical sign of leprosy. Six months later, he was found to have a vague hypopigmented macule (1.0 x 1.0 cm) on the left thigh, lateral aspect; this was thought to have been present for about 6 months appearing shortly after the previous check-up. A senior hospital leprologist proposed reviewing the child after a 3 month interval. However, one month later, two more vague and ill defined new macules were seen. Biopsy was taken and reported as follows :

> "The biopsy shows fairly normal skin with only a slight non-specific dermatitis. There is nothing at all to indicate leprosy, and although it is always difficult to exclude the possibility, I think from the amount of pigment drop-out from the basal layer, for which there is no explanation, that it is a simple dermatitis rather than leprosy." (DSR)

The condition of peripheral nerves was not recorded, nor were sensory tests done.

At the time of the last check-up it was observed that while the mother was now skin smear negative (from standard sites) there were several other inmates of the home who were highly bacilliferous with positive skin smears.

C. SOME ASPECTS OF CELL MEDIATED IMMUNITY IN MOTHERS WITH LEPROSY AND THEIR CHILDREN

1. Hepatitis B infection in Ethiopian mothers with leprosy and their babies

Patients. One hundred and thirty-eight Ethiopian women were studied throughout 145 pregnancies and followed up with their babies after delivery. The number of mothers in each clinical group, according to the revised classification, is shown in Table C1(i).

Assays

Hepatitis B surface antigen (HBsAg). All samples were tested by a reverse passive haemagglutination assay (RHPA: Hepatest). Positive samples were confirmed by a radioimmunoprecipitation (RIP) test. Babies born to HBsAg positive mothers were also tested by RIP assay.

Anti-HBs. Samples from each of the mothers, and the follow up samples of the babies born to carrier mothers, were tested by a precipitation assay.

HBeAg and anti-HBe. Each of the HBsAg positive mothers was tested for HBeAg and anti-HBe by a solid-phase radioimmunoassay (sRIA).

Observations

Prevalence of HBsAg

Eleven (8.0%) of the 138 mothers were positive for HBsAg during pregnancy and at delivery. Ten were carriers as HBsAg was present throughout the follow up period. One patient who was HBsAg positive at delivery later became negative and anti-HBs was detected 15 months after delivery. The age distribution of the HBsAg carriers was similar to the overall population in that 8 mothers were 20-30 years old, one was 19 years and two did not know their ages. The distribution of the HBsAg positive patients in the different clinical groups is shown in Table C1(i).

Prevalence of anti-HBs

Seventy-four (53.6%) of the mothers had anti-HBs. The rates

TABLE C1(i)

Prevalence of HBsAg and anti-HBs according to classification of leprosy

Group	Total number in group (babies tested)	Number who were :		
		Anti-HBs +ve	HBsAg +ve	Anti-HBe +ve (of HBsAg +ve)
HC	28 (25)	14 (50%)	3 (11%)	1 *
TT and BT/RFC and TT and BT	40 (34)	25 (62.5%)	2 (5%)	2
BL and LL	70 (70)	35 (50%)	6 (8.6%)	5 *

* Other mothers were negative for both HBeAg and anti-HBe.

were 45% in those less than 20 years old, 48.3% in the 20–30 year old group, and 68.8% in those over 30 years. Thus, 61.6% of the patients (control and lepromatous) had evidence of present or past infection with hepatitis B virus.

Association of HBsAg and anti-HBs with leprosy status

The prevalence of HBsAg and anti-HBs according to the mother's leprosy status is shown in Table C1(i). Apart from the group of mothers with "cured" tuberculoid leprosy who were all HBsAg negative, there was no significant difference in the prevalence of HBsAg in the healthy controls and the different classifications of leprosy. The prevalence of anti-HBs was 50% in the control and lepromatous leprosy groups and 62.5% in the tuberculoid group. Although the numbers are small, variation was noted in the prevalence from 66.7% in cured TT and BT patients, 60% in active TT and BT, to 50% and 43.3% in BL and LL leprosy groups respectively.

Maternal HBeAg and anti-HBe status

Eight of the 11 HBsAg positive mothers had anti-HBe at delivery: one mother was HBsAg and anti-HBe positive during two pregnancies in the study. The other 3 carriers were negative for both HBeAg and anti-HBe. Only one of the 3 healthy mothers was anti-HBe positive, while 7 out of 8 mothers with leprosy were anti-HBe positive (Table C1(i)).

Evidence of transmission to babies

Nine babies were born to the 8 carrier mothers who were anti-HBe. There was no evidence of transplacental transmission of HBsAg.

2. Evidence for a soluble lymphocytic factor in the transplacental transmission of T-lymphocyte responses to Mycobacterium leprae

Lymphocyte transformation tests (LTT) were carried out by a micro-method on both maternal and neonatal (cord) blood; whole washed M. leprae were used as antigen, and in each case the responses to BCG and PPD were also determined.

Of the ten mothers tested, 5 showed a postive LTT response with M. leprae as antigen and 5 did not (Figure C2(i)). It was considered that a positive response existed when the LTT response with the antigen was more than twice the value of unstimulated control cultures. The values for these control cultures were much higher in the neonates (median: 13,171) than in the mothers (median: 532). In the 5 mothers with positive lymphocyte blastogenic responses, the response of the neonate reflected the maternal response, though the former were much greater. In the 5 unresponsive mothers, the neonatal response was much lower than in the neonates born of responders. The difference between the two neonatal groups is highly significant ($P < 0.005$), using the Mann-Whitney U test for statistical analysis. There was also a statistically significant difference in responses to PPD (but not BCG) between the maternal responders and non-responders to M. leprae (Table C2(i)), possibly due to some cross reactivity. There was, however, no significant difference of responses to PPD and BCG between the two neonatal groups (Barnetson, Bjune and Duncan, Nature (1976), 260, 150-151).

TABLE C2(i)

MEDIAN RESPONSES TO ANTIGENS

	M. leprae		BCG		PPD	
Mothers with positive responses to M. leprae	8,600	P<0.005	267	P>0.1	4,316	P<0.02
Mothers with negative responses to M. leprae	-11		21		-36	
Babies of mothers with positive responses to M. leprae	13,658	P<0.005	995	P>0.1	3,654	P>0.1
Babies of mothers with negative responses to M. leprae	602		-17		1,140	

Figures are c.p.m.

Median responses to antigens in the two maternal and the two neonatal groups. P, Probability of distribution between the two groups occurring by chance, calculated by Mann-Whitney U test.

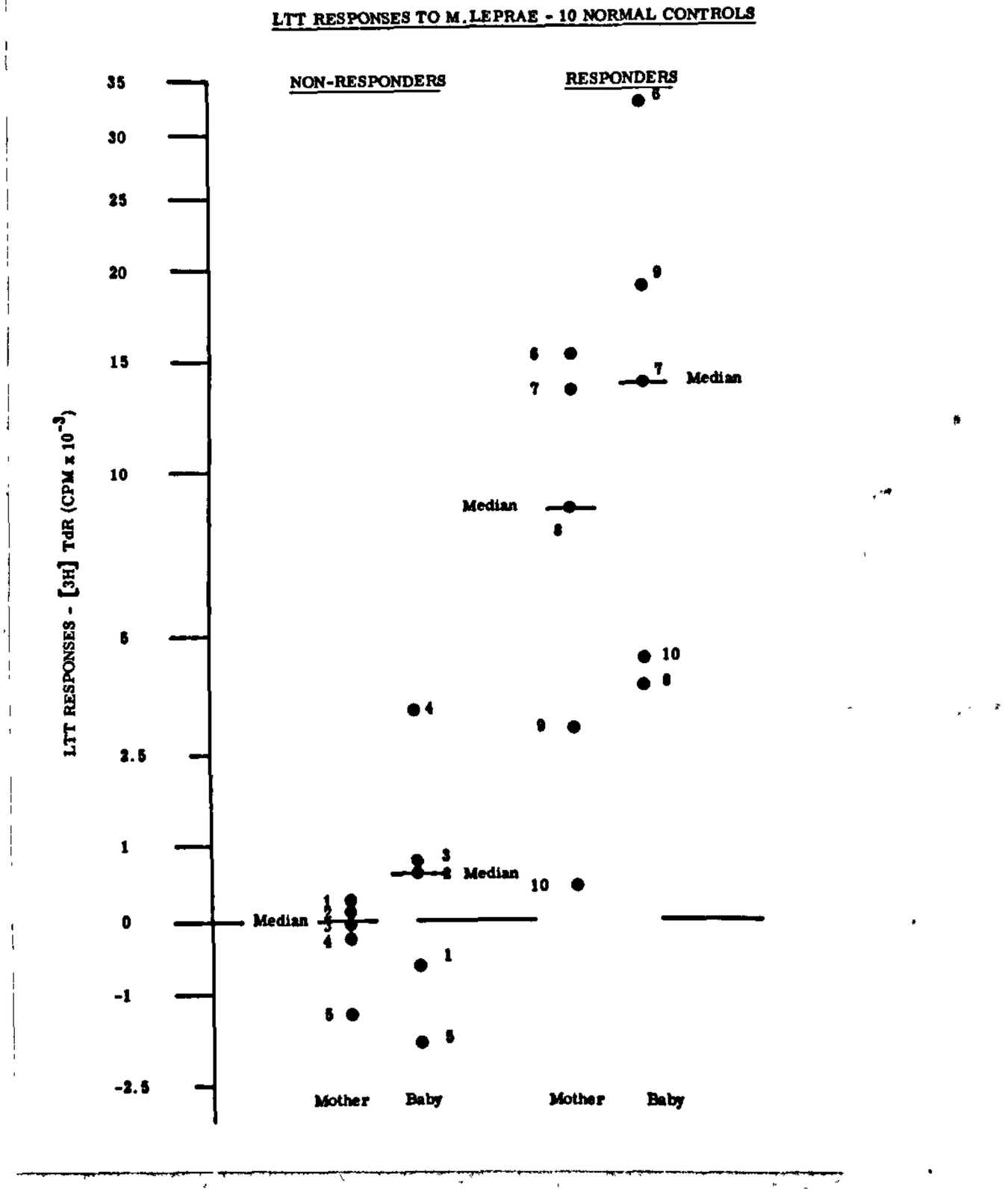


Figure C2(i). LTT responses to whole M. leprae in mothers and neonates, comparing the five mothers with positive responses and the five mothers with negative responses (unstimulated control values are subtracted). ^{3}H-TdR, Tritiated thymidine uptake.

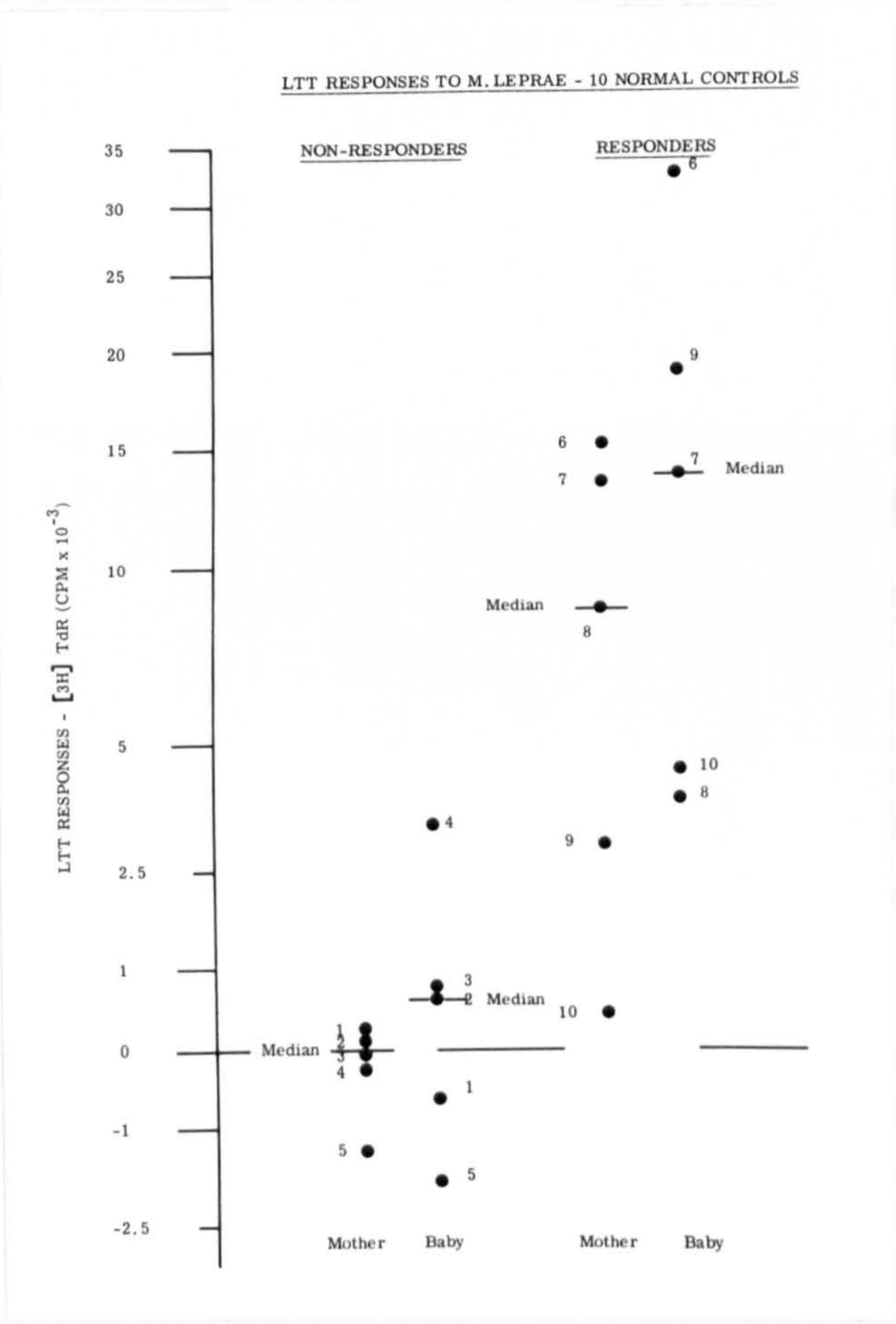


Figure C2(i). LTT responses to whole M. leprae in mothers and neonates, comparing the five mothers with positive responses and the five mothers with negative responses (unstimulated control values are subtracted). ^{3}H-TdR, Tritiated thymidine uptake.

3. In vitro modulation of lymphocyte responses to PHA by plasma in mother and baby at the time of birth

Peripheral blood lymphocytes from 19 healthy mothers, 16 mothers with BT leprosy, and 14 mothers with BL or LL leprosy, and their newborn babies were stimulated in vitro with PHA. The responses in medium supplemented by serum from a pool of healthy, non-pregnant individuals were compared with the responses in medium supplemented by plasma from the mothers or from their babies, to assay for the presence of non-specific effects on T-cell responses.

I. PHA responses of lymphocytes in mothers with leprosy were similar to those of healthy mothers (Figure C3(i)).

II. PHA responses of lymphocytes were significantly higher in babies of mothers with lepromatous leprosy than those of tuberculoid mothers or control mothers (Figure C3(ii)).

III. PHA responses of all mothers' lymphocytes were greatly inhibited by autologous plasma, but not by plasma from their babies (Figure C3(iii)).

IV. PHA responses of babies' lymphocytes were not significantly depressed by their mothers' plasma except in babies of mothers with leprosy (Figure C3(iv)).

(Bjune, Duncan, Barnetson and Melsom, Clinical and Experimental Immunology (1978), 32, 517-522.)

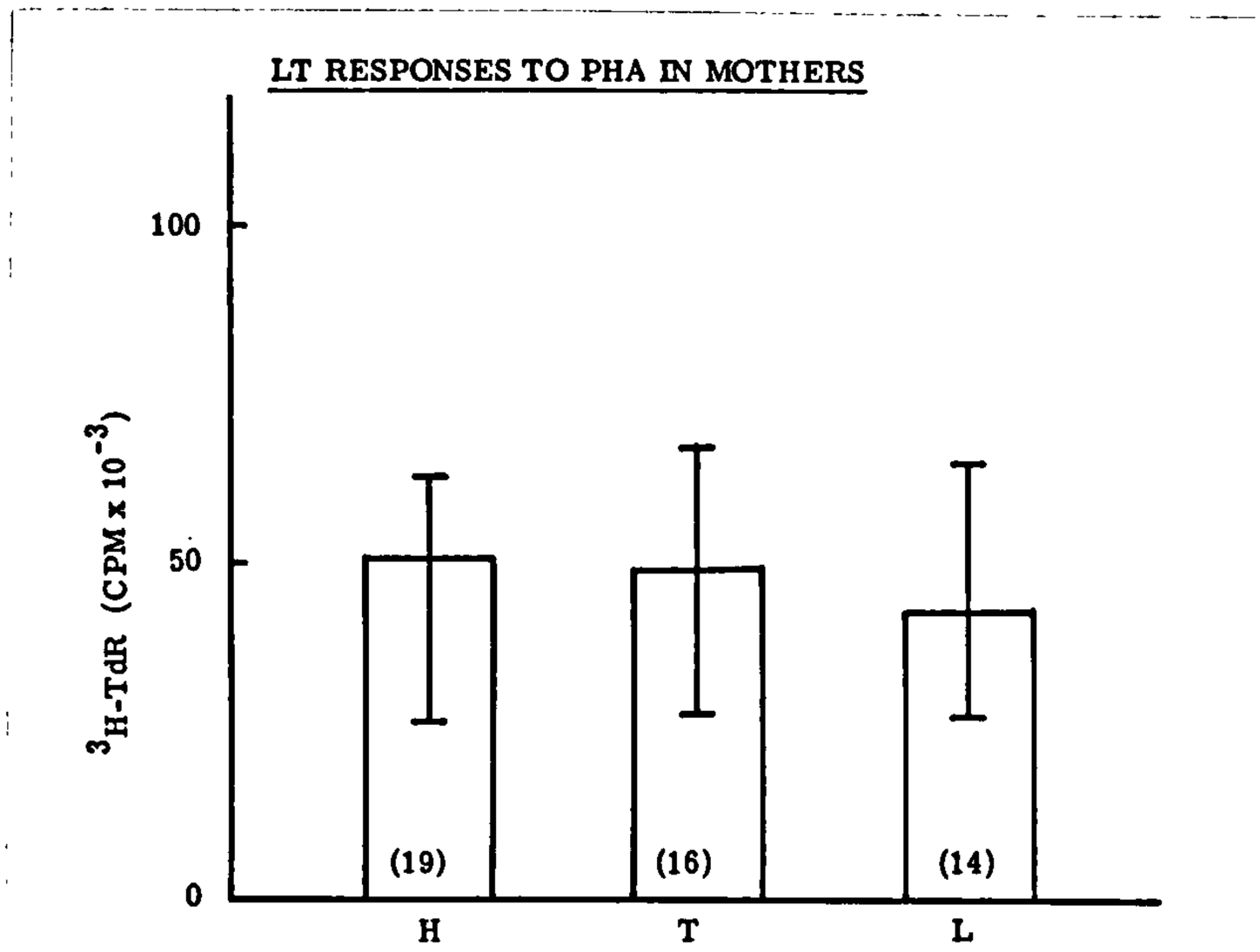


Figure C3(i). Median PHA responses of maternal lymphocytes in medium supplemented with 10% pooled serum from healthy non-pregnant individuals. The range is indicated as inter-quartile distance. (H) Healthy mothers; (T) mothers with borderline tuberculoid leprosy; (L) mothers with BL and LL leprosy. Figures in brackets give the number of individuals in the group.

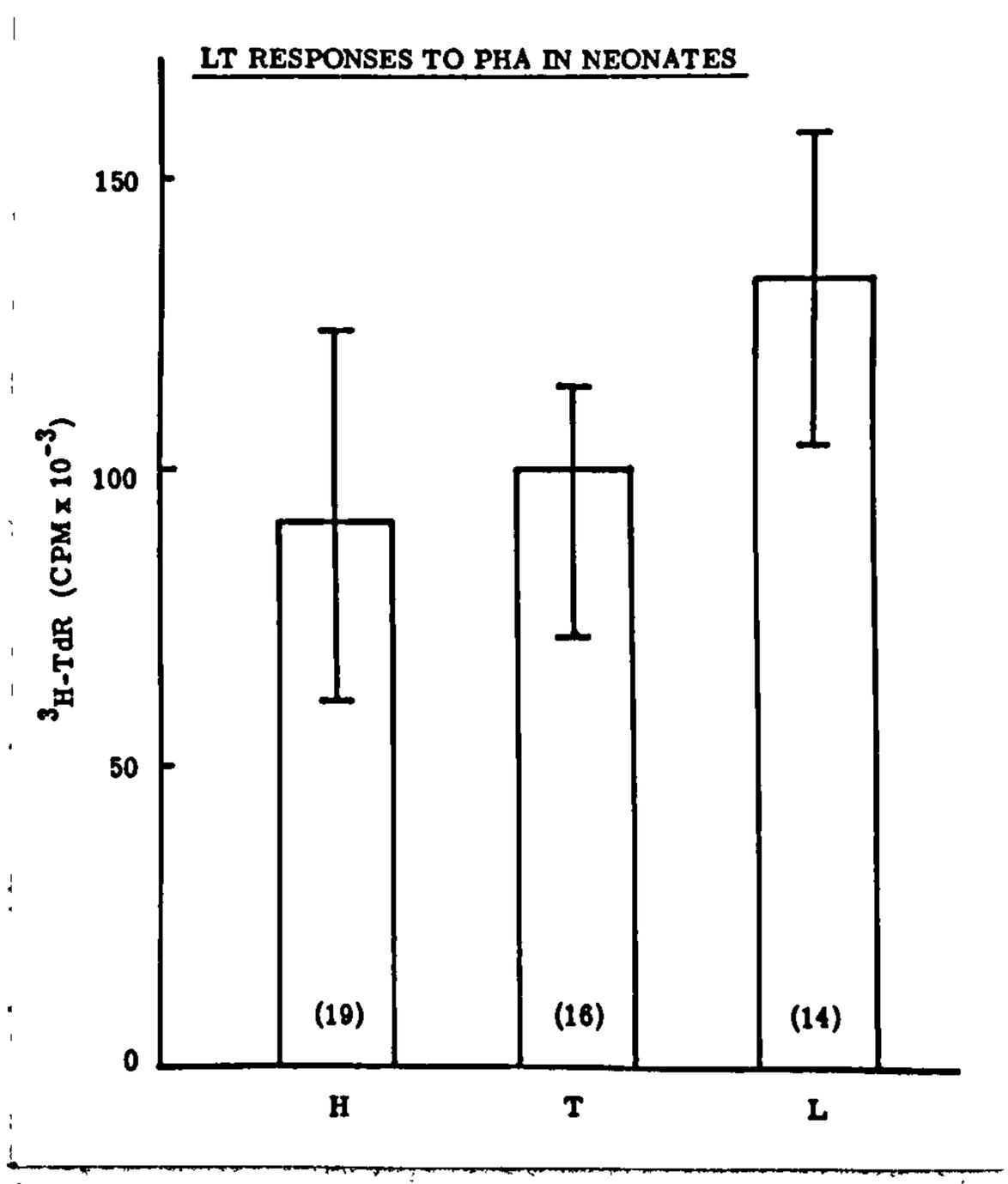


Figure C3(ii). Median PHA responses of cord blood lymphocytes in medium supplemented with 10% pooled serum from healthy, non-pregnant individuals. (H) Babies of healthy mothers; (T) babies of mothers with borderline tuberculoid leprosy; (L) babies of mothers with BL and LL leprosy. Figures in brackets show number in each group.

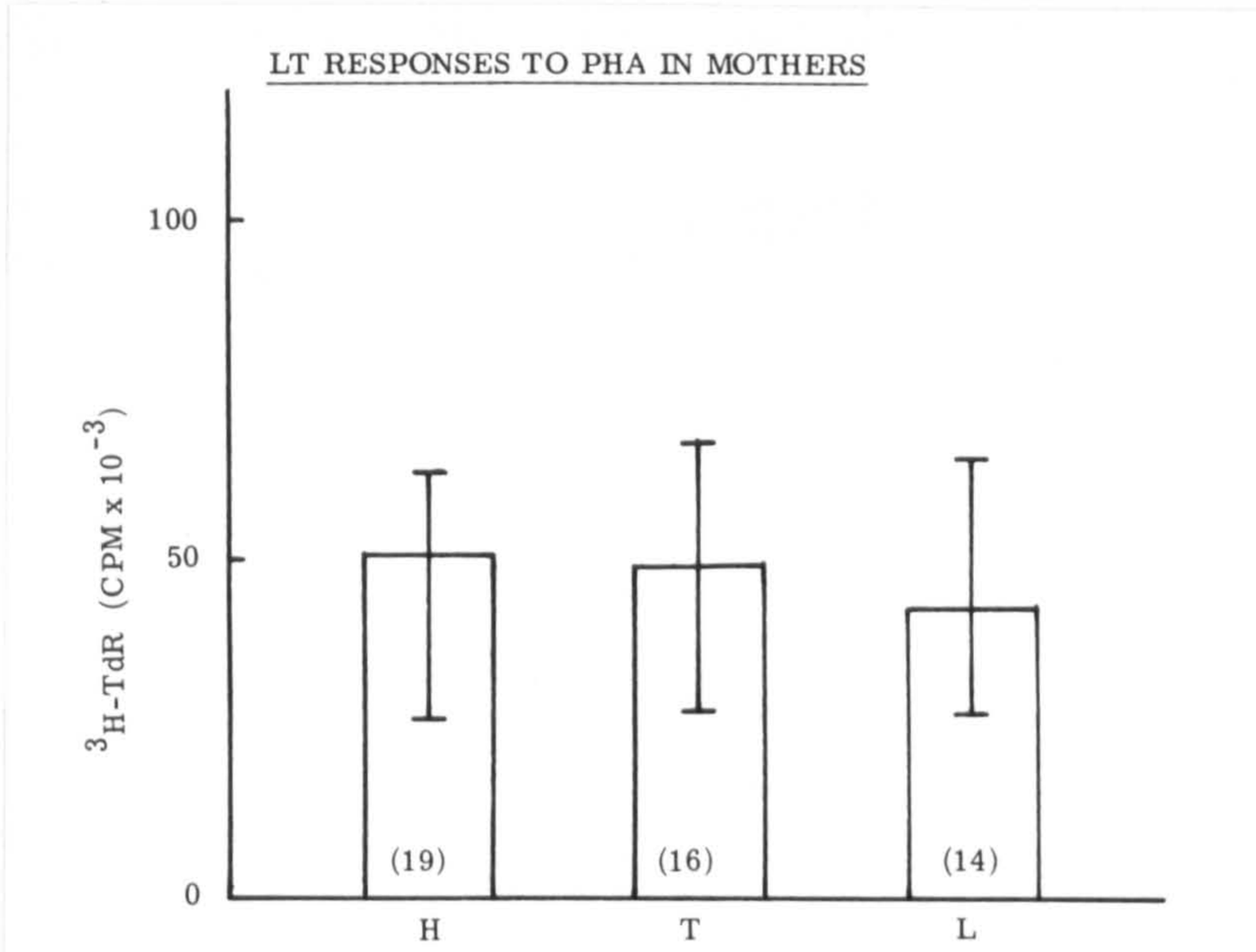


Figure C3(i). Median PHA responses of maternal lymphocytes in medium supplemented with 10% pooled serum from healthy non-pregnant individuals. The range is indicated as inter-quartile distance. (H) Healthy mothers; (T) mothers with borderline tuberculoid leprosy; (L) mothers with BL and LL leprosy. Figures in brackets give the number of individuals in the group.

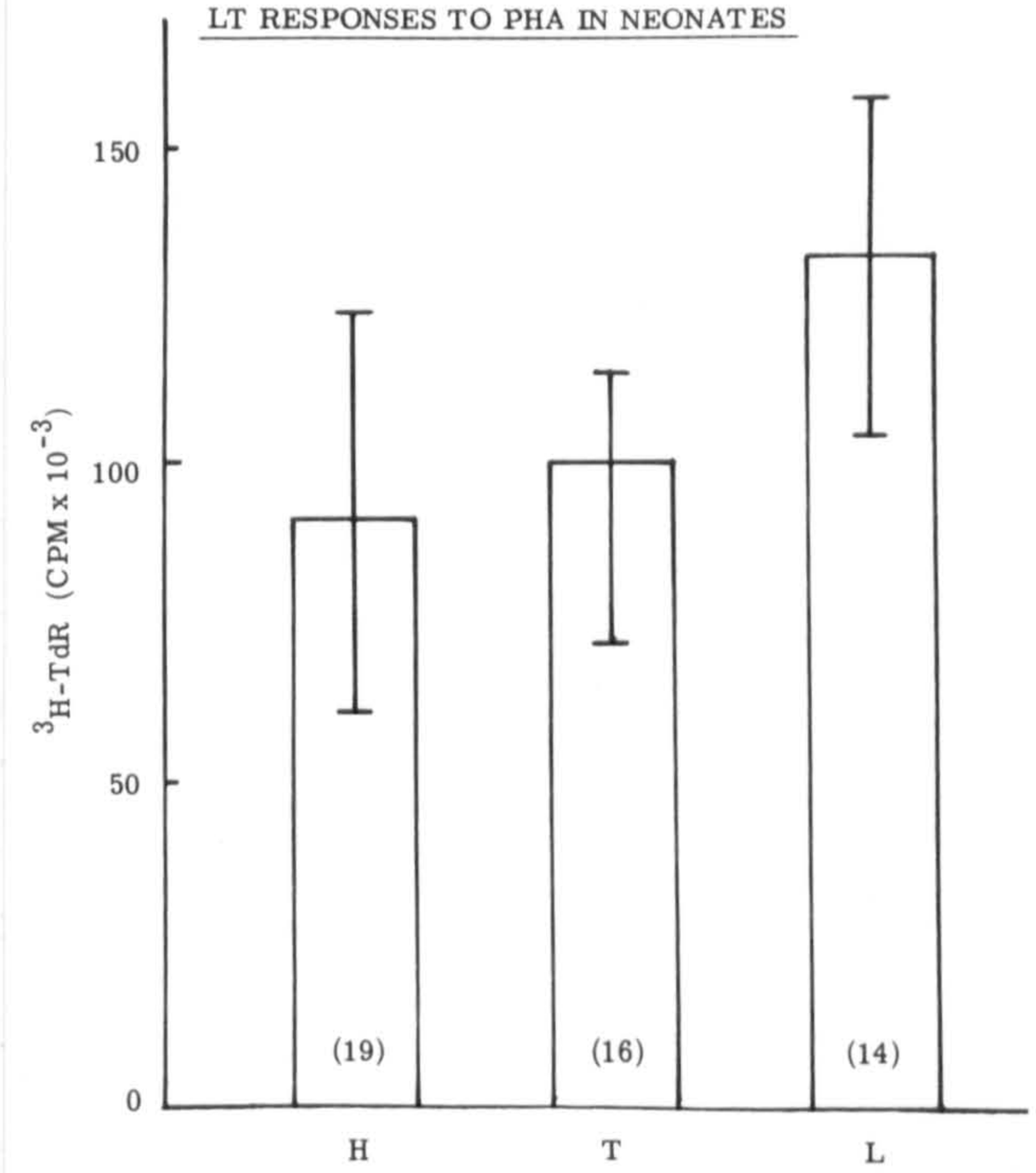


Figure C3(ii). Median PHA responses of cord blood lymphocytes in medium supplemented with 10% pooled serum from healthy, non-pregnant individuals. (H) Babies of healthy mothers; (T) babies of mothers with borderline tuberculoid leprosy; (L) babies of mothers with BL and LL leprosy. Figures in brackets show number in each group.

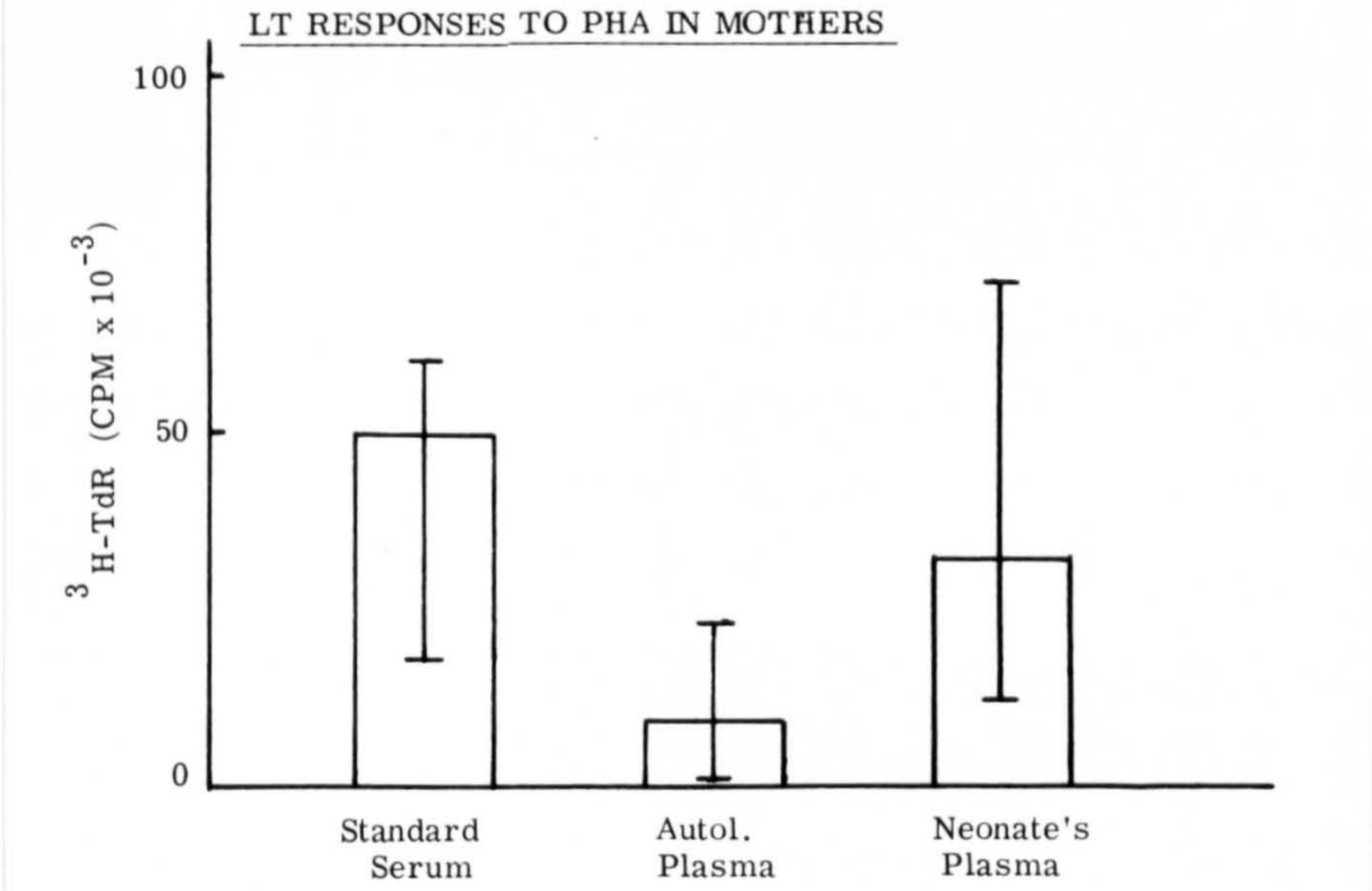


Figure C3(iii). Median PHA responses of healthy mothers' lymphocytes in medium supplemented with the standard pool of healthy non-pregnant serum, autologous plasma and in plasma of their babies (neonate's plasma). Nineteen pairs of mothers and babies were tested.

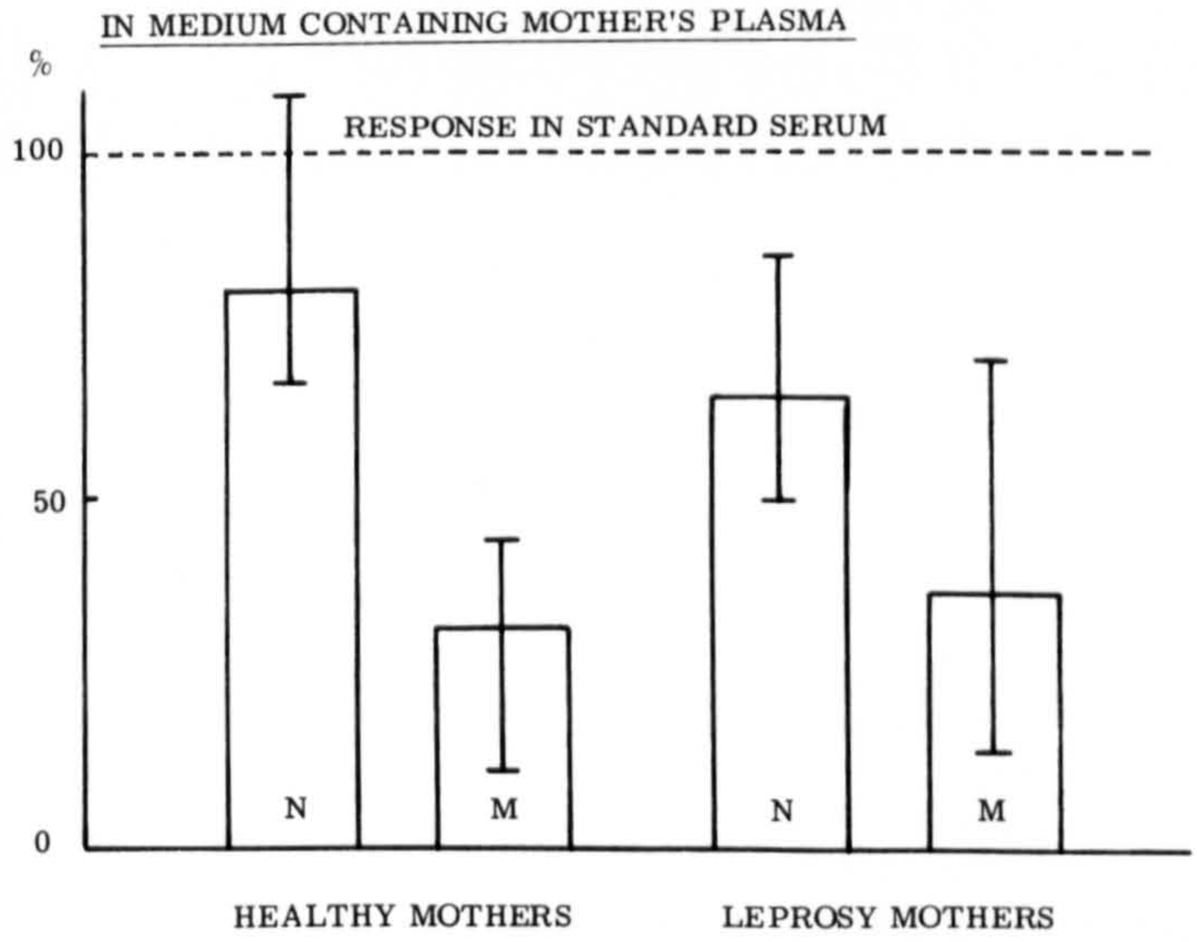


Figure C3(iv). Inhibition of neonate lymphocytes' responsiveness to PHA in medium with their mothers' plasma (N) compared to the PHA responsiveness of their mothers' lymphocytes in autologous plasma (M). Responses are recorded as a percentage of the simultaneous response to PHA in medium supplemented with pooled healthy, non-pregnant serum. The dotted line shows 100%, the response in standard serum.

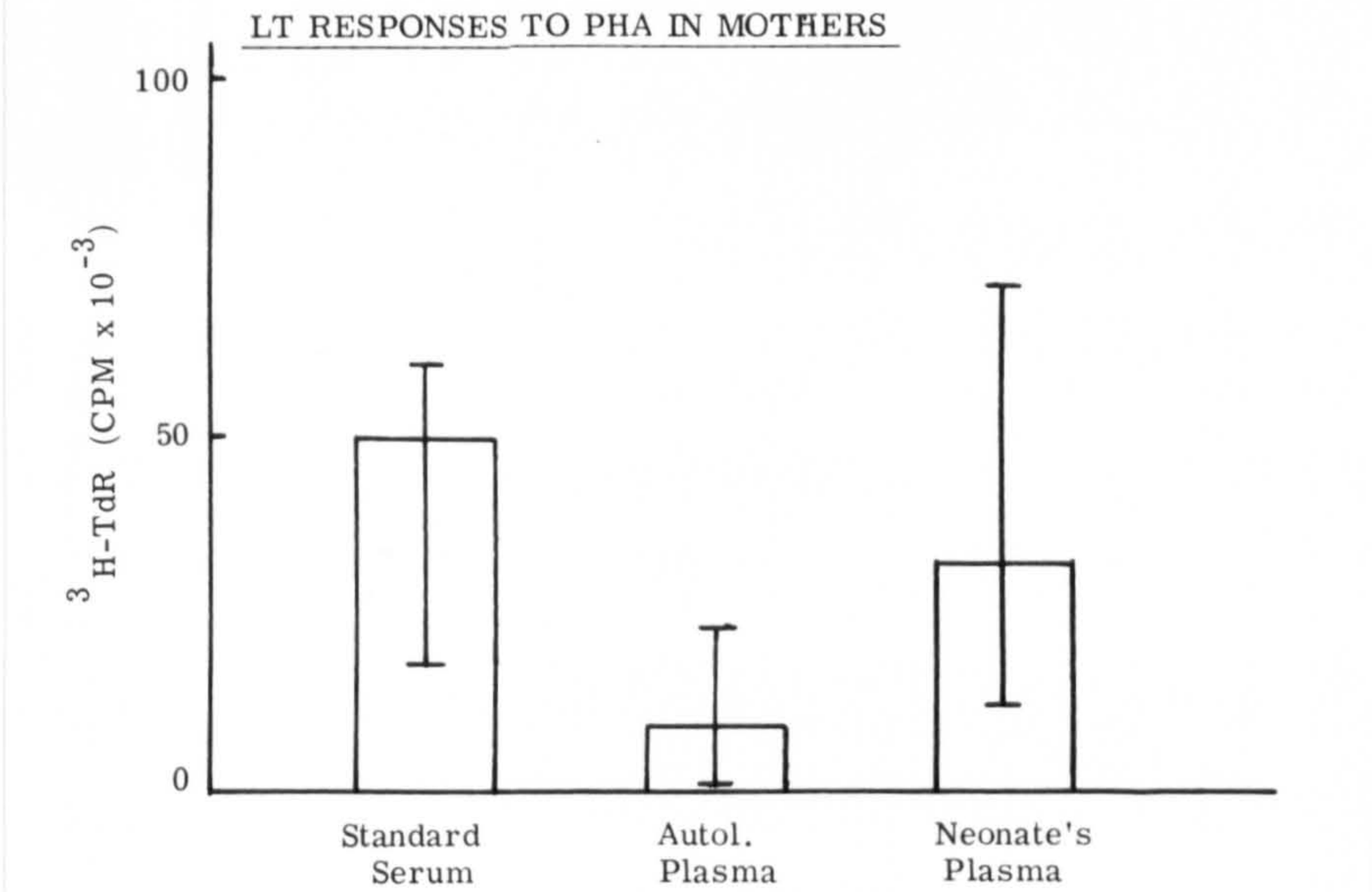


Figure C3(iii). Median PHA responses of healthy mothers' lymphocytes in medium supplemented with the standard pool of healthy non-pregnant serum, autologous plasma and in plasma of their babies (neonate's plasma). Nineteen pairs of mothers and babies were tested.

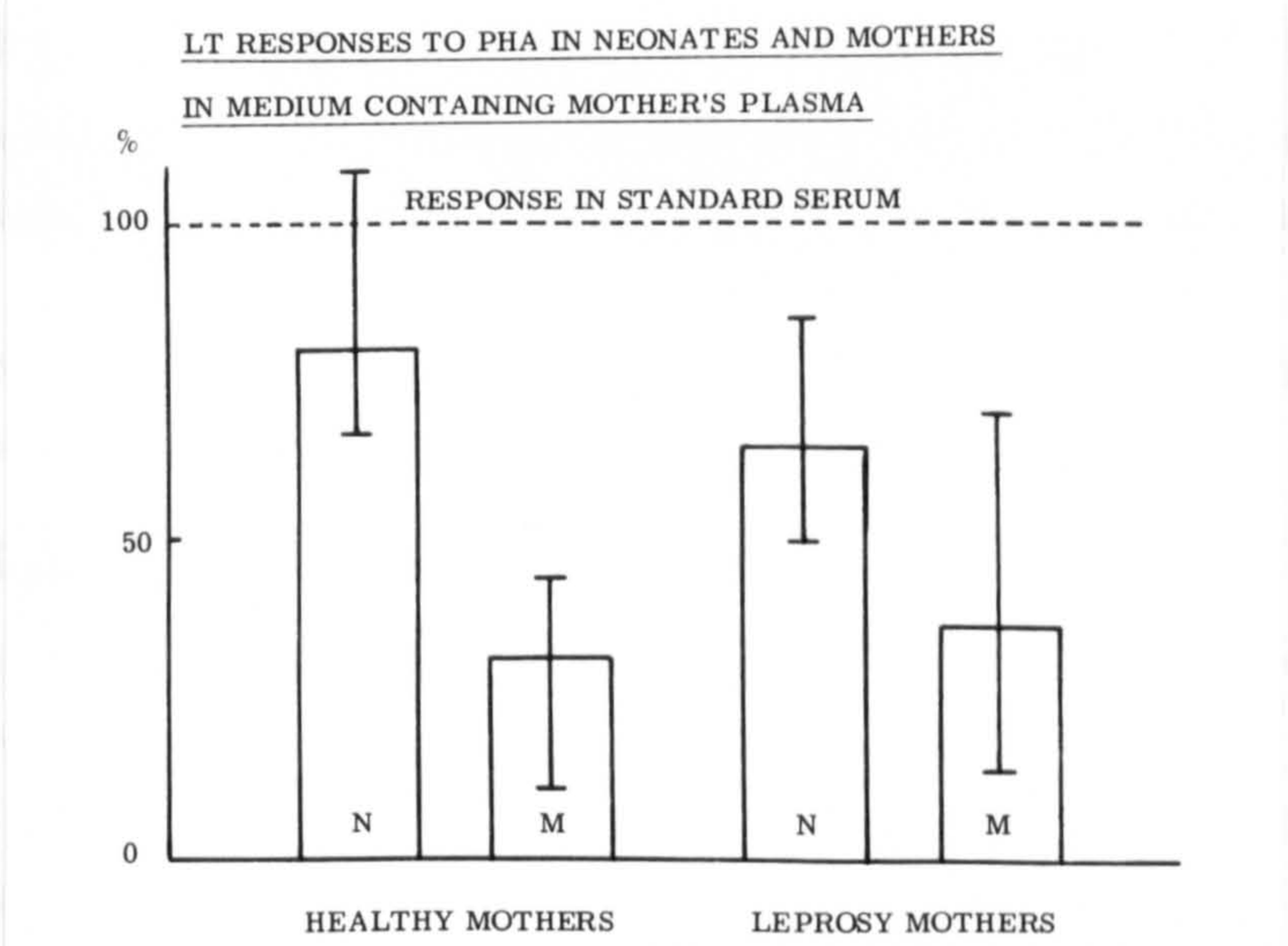


Figure C3(iv). Inhibition of neonate lymphocytes' responsiveness to PHA in medium with their mothers' plasma (N) compared to the PHA responsiveness of their mothers' lymphocytes in autologous plasma (M). Responses are recorded as a percentage of the simultaneous response to PHA in medium supplemented with pooled healthy, non-pregnant serum. The dotted line shows 100%, the response in standard serum.

D. STUDIES OF HUMORAL IMMUNITY IN MOTHERS WITH LEPROSY AND THEIR BABIES

1. Immunoglobulin concentration in mothers with leprosy and in healthy controls and their babies at the time of birth

Concentrations of immunoglobulin IgA, IgG and IgM were measured by single radial diffusion technique, with modifications for measuring the minute amounts of IgA and IgM in cord serum. Immunoglobulins were quantitated in the sera of 52 women and their newborn infants at the time of delivery. The mothers were grouped as follows :

Group 1. 17 mothers with lepromatous leprosy (LL and BL, BI +ve) with skin smears positive for M. leprae.

Group 2. 5 mothers with lepromatous leprosy (LL and BL, BI -ve) with skin smears negative for M. leprae.

Group 3. 16 mothers with tuberculoid leprosy (BT and TT), 6 of whom were on treatment for active disease and 10 of whom were considered cured.

Group 4. 14 mothers with no clinical or laboratory evidence of leprosy (NL) but with the same socio-economic background as the leprosy patients.

The babies were grouped according to the clinical classification of their mothers.

Immunoglobulin concentration in maternal sera at delivery

There was no significant difference (Figure D1(i)) in the median concentrations of IgG, IgA or IgM between the 4 groups of mothers studied. The IgG concentration in sera during the third trimestre of pregnancy was higher than at delivery, a feature observed in 24 out of 36 women.

Immunoglobulin concentration in cord blood

Figure D1(ii) shows the IgG concentration in the cord sera. The highest concentration was found in Group 1, but there was no significant difference between the 4 groups. There was a good correlation between the IgG concentration in cord blood and maternal sera taken at delivery in each mother and baby pair.

The IgA concentration could be measured at levels above

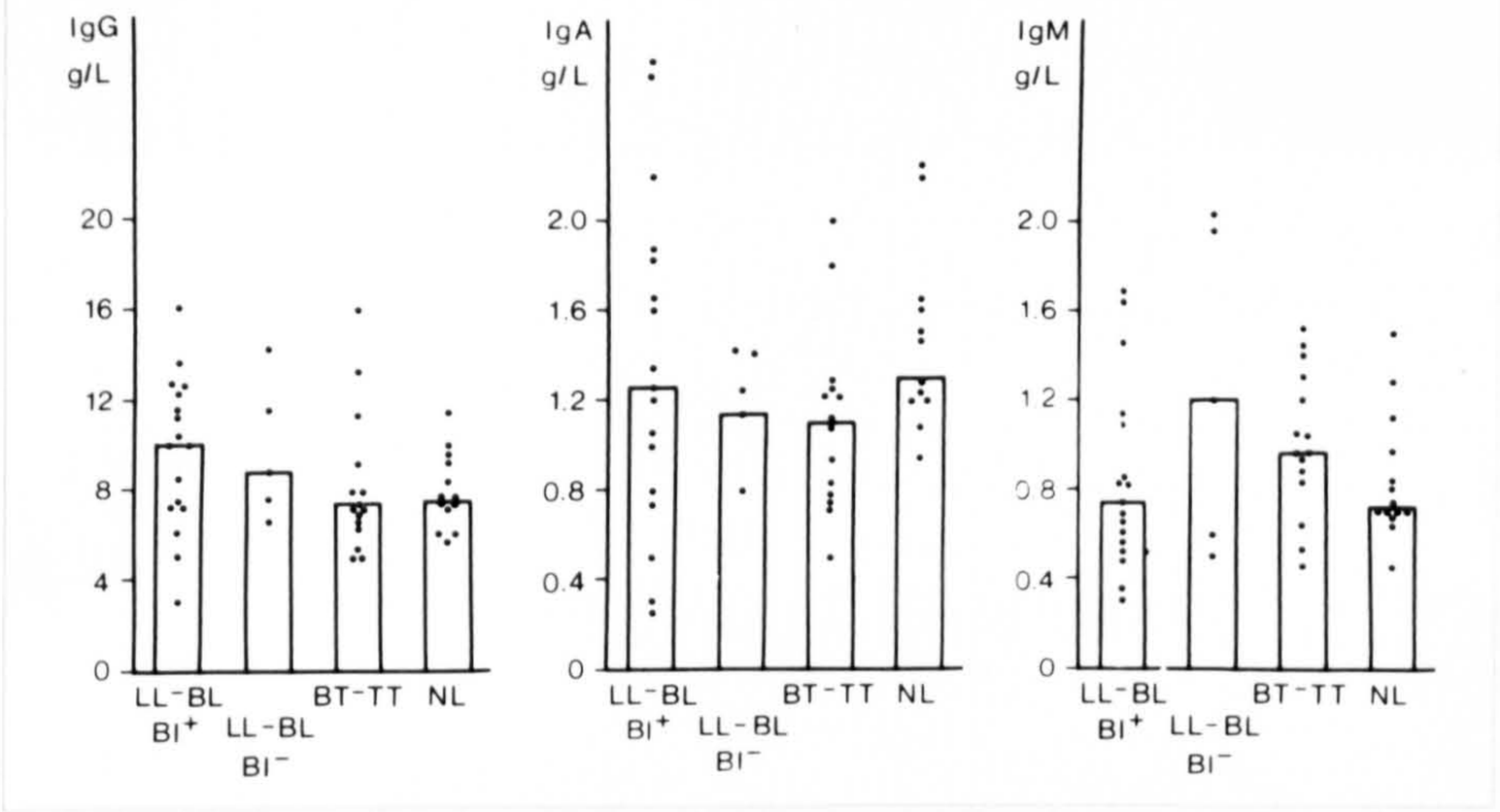


Figure D1(i). IgG, IgA and IgM concentration in maternal serum. Each point represents one individual and the top of the columns the median value.

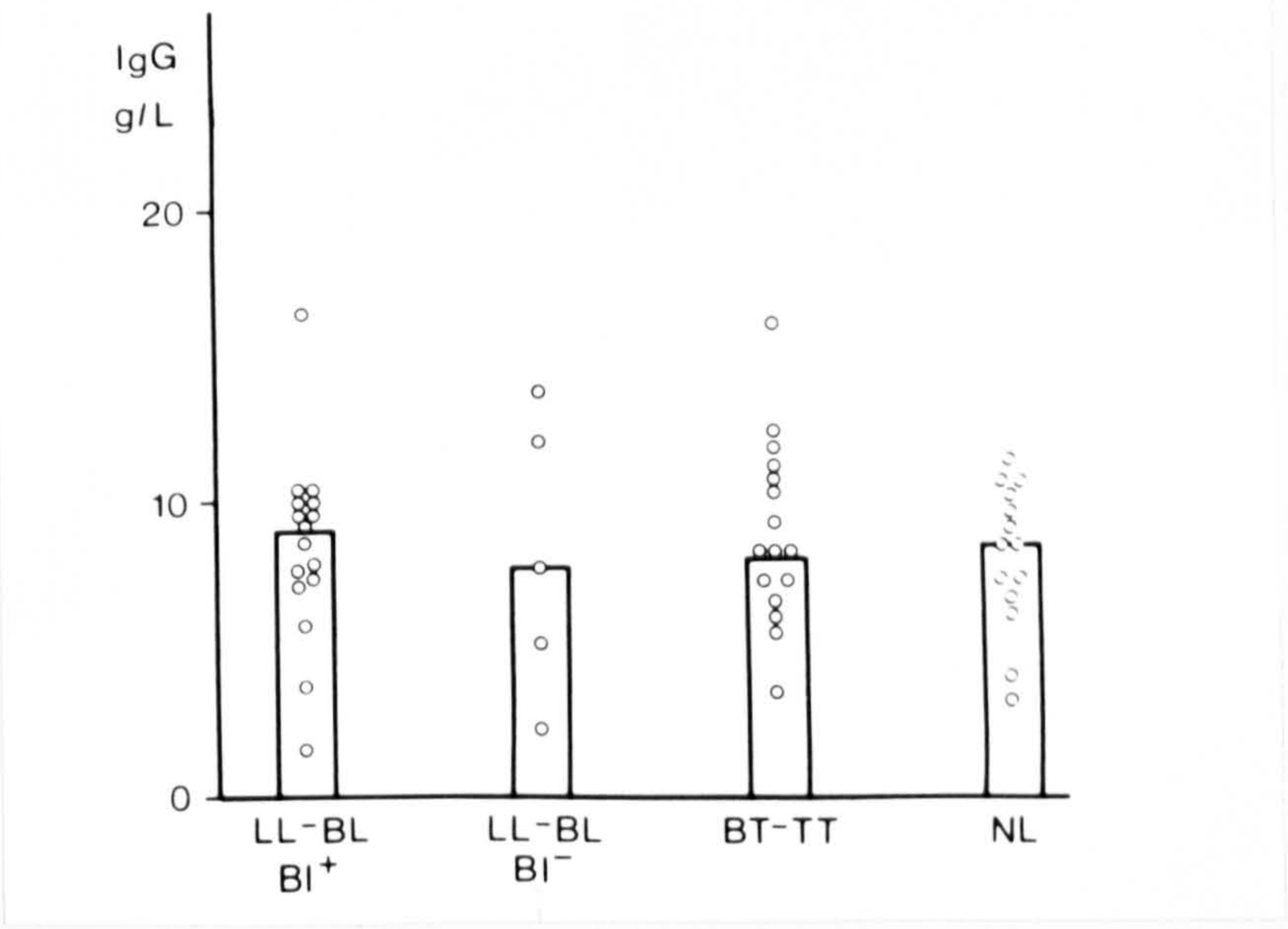


Figure D1(ii). IgG concentration in cord serum. Each open circle represents one baby, and the top of the columns the median value.

8×10^{-3} g/l. IgA could be detected but not quantitated if the concentration was above 4×10^{-3} g/l but below 8×10^{-3} g/l. These two limits are indicated on Figure D1(iii) with horizontal dotted lines. There was a significantly raised IgA in the babies of Group 1 compared with the other groups.

The IgM concentration in cord sera is shown in Figure D1(iv). While the highest concentration of IgM was seen in Group 4, the differences were not statistically significant (Melsom, Duncan and Bjune, Leprosy Review (1980), 51, 19-28).

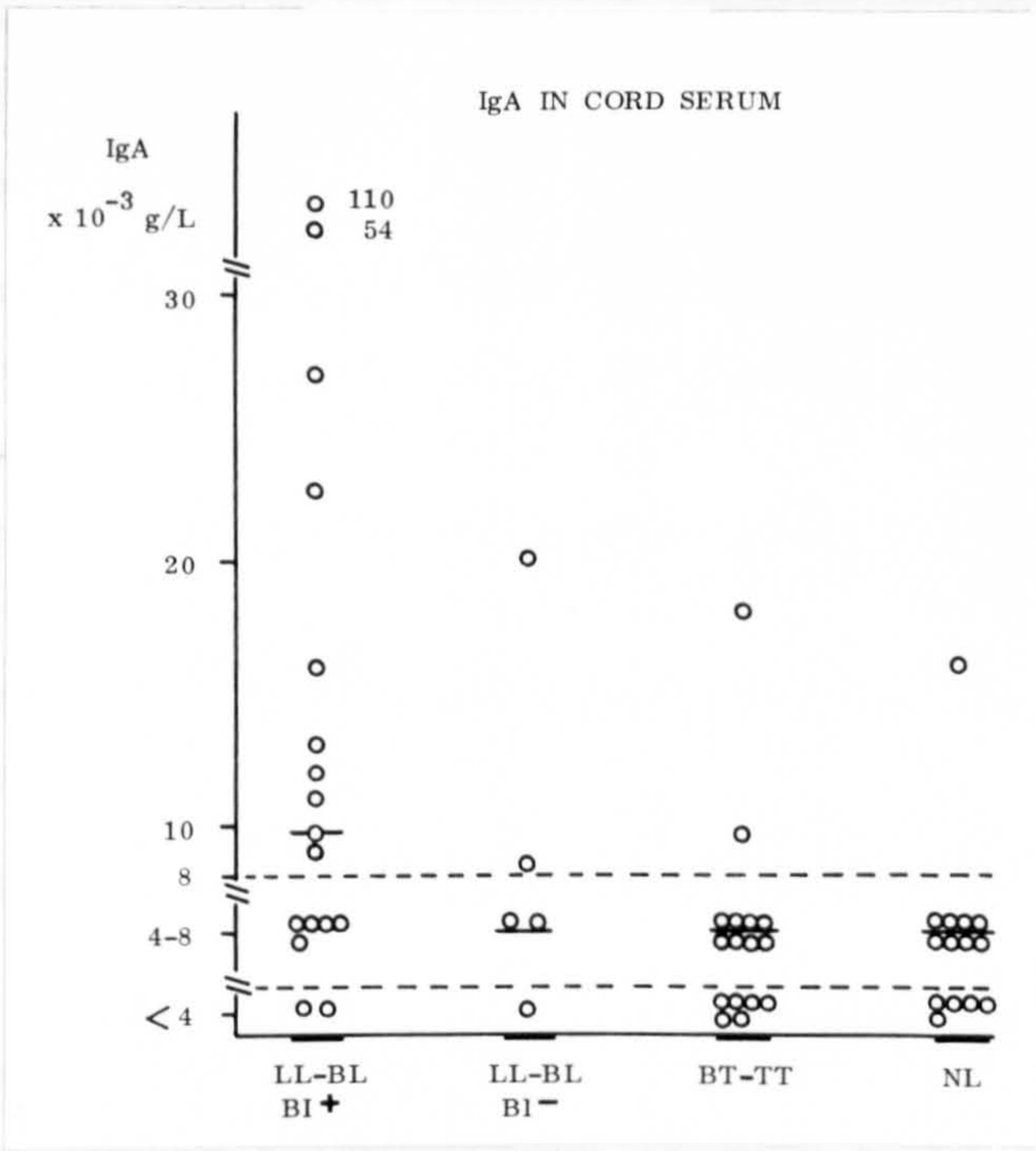


Figure D1(iii). IgA concentration in cord serum. The IgA concentration could be measured by single radial diffusion methods at levels above 8 x 10^{-3} g/l. IgA could be detected if the concentration was above 4 x 10^{-3} g/l, but it could not be quantitated at levels between 4 and 8 x 10^{-3} g/l. These two limits are indicated with horizontal dotted lines. Each open circle represents one baby, the horizontal bars show the median value for the four groups.

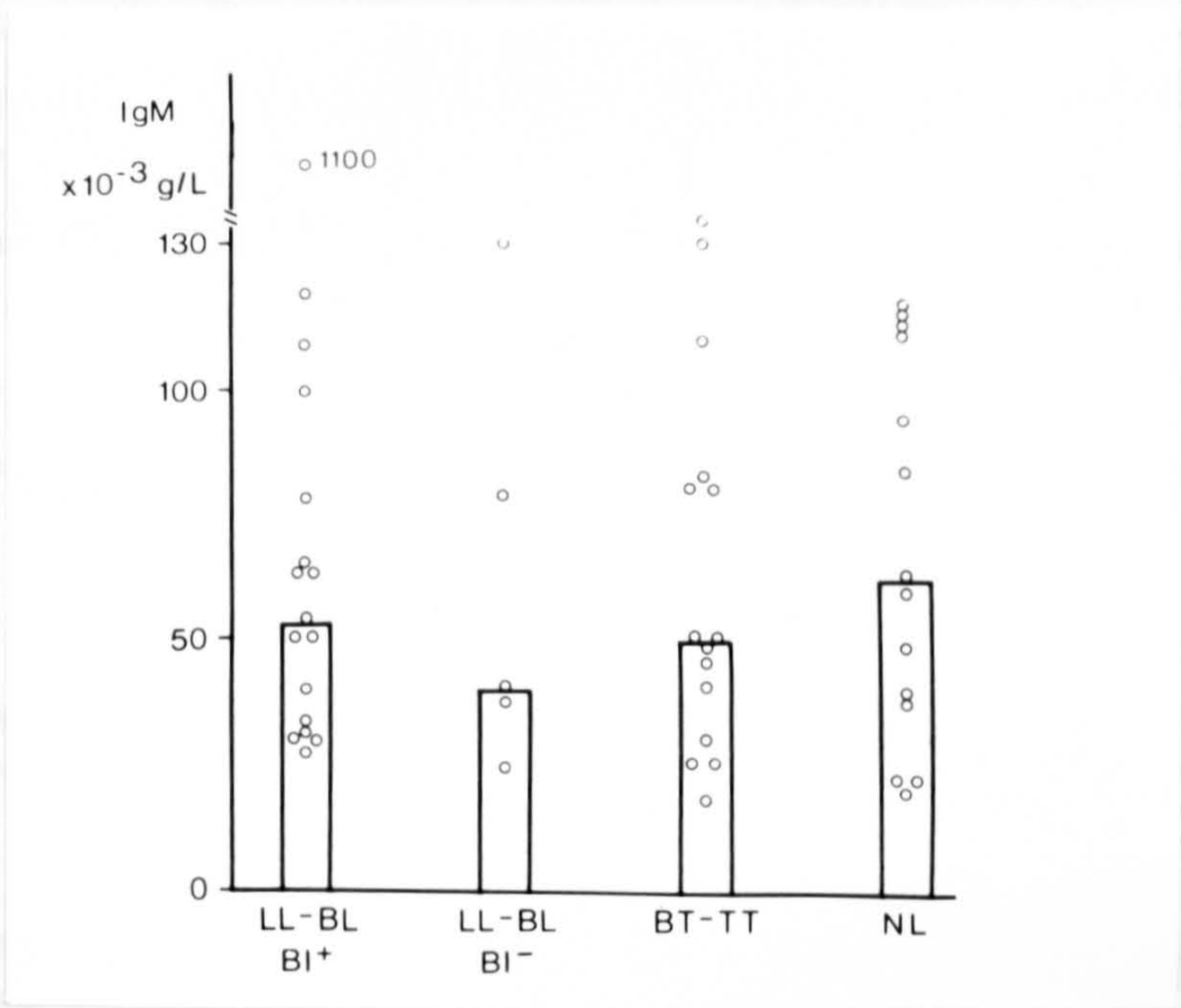


Figure D1(iv). IgM concentration in cord serum. Each open circle represents one baby and the top of the columns the median value.

2. Demonstration of antibodies against M. leprae antigen 7 both in IgG and IgM in sera from pregnant and non-pregnant lepromatous leprosy patients

Using a sensitive RIA technique, the following sera were tested for presence of antibodies against M. leprae antigen 7:

I. Pooled sera from 40 patients with active lepromatous leprosy recently diagnosed or on treatment for less than 6 months.

II. Four mothers with active lepromatous leprosy (LL and BL) and their babies.

III. One mother with tuberculoid leprosy and her baby.

IV. One healthy mother and her baby.

Samples were taken from mother and child (cord blood) at the time of delivery.

Antibodies against M. leprae antigen 7 were shown to consist of both IgG and IgM in a lepromatous leprosy serum pool (LSP) and in individual sera from patients with active lepromatous leprosy. The IgM fraction was separated from IgG by zonal ultracentrifugation. The IgM and IgG concentrations were quantitated by single radial diffusion methods. As seen in Figure D2(i), maximal IgM concentration corresponded to peak 1 and IgG concentration corresponded to the antibody activity in peak 2.

Cord sera, fractionated by density gradient ultracentrifugation, were tested by the RIA system. Figure D2(ii) shows a typical pattern of antibody activity against M. leprae antigen 7 in different fractions from both maternal and cord serum at delivery. While maternal serum showed antibody activity in fractions corresponding to IgM and IgG, the cord serum showed antibody activity only in the IgG fraction. IgM antibodies against M. leprae antigen 7 could not be detected in any cord sera of an additional 5 baby-mother pairs.

Three out of 4 mothers with active lepromatous leprosy contained antibodies of both the IgM and IgG class against M. leprae antigen 7 (Figure D2(iii)) while sera from the healthy control and patient with tuberculoid leprosy did not contain antibodies of IgM class against M. leprae antigen 7 detectable by the present technique (Figure D2(iv)) (Melson and Duncan, Leprosy Review (1980), 51, 125-135).

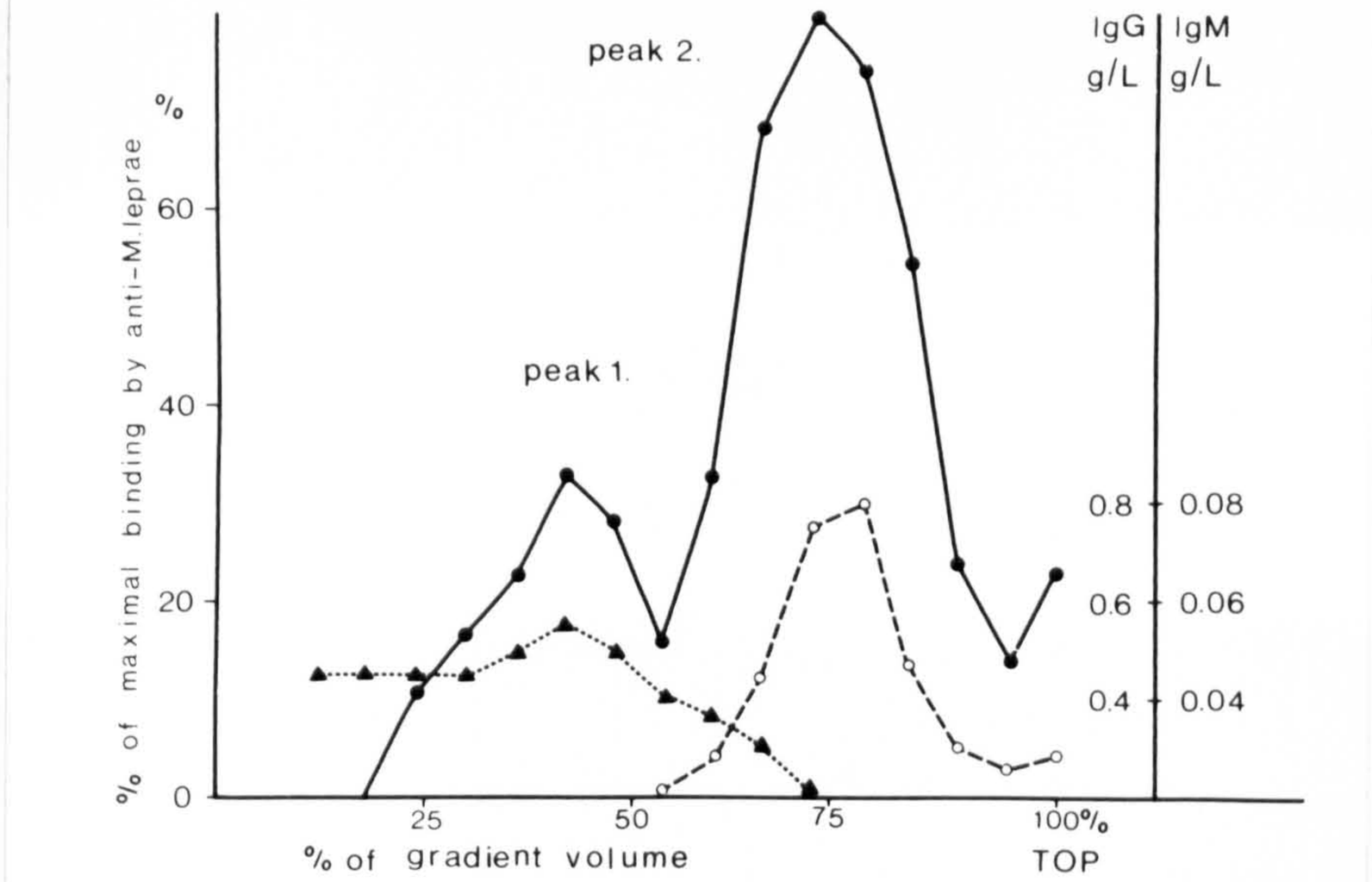


Figure D2(i). ●——● antibody activity against M. leprae antigen 7, ▲....▲ IgM concentration and o- - -o IgG concentration in different fractions after density gradient ultracentrifugation of LL serum pool.

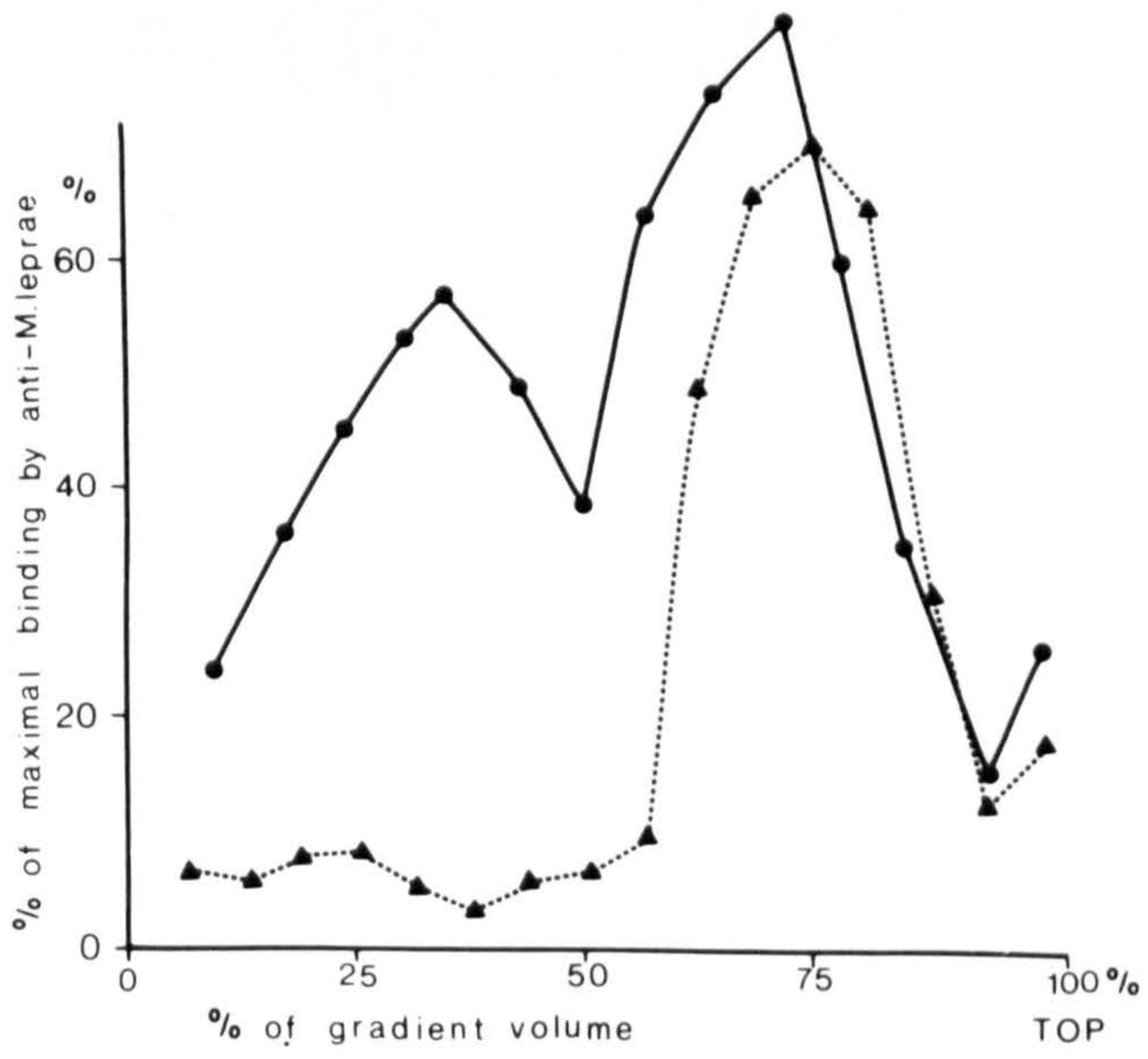


Figure D2(ii). Antibody activity against M. leprae antigen 7 in fractions after density gradient ultracentrifugation of ▲....▲ baby's cord serum and ●——● the corresponding maternal serum taken at delivery.

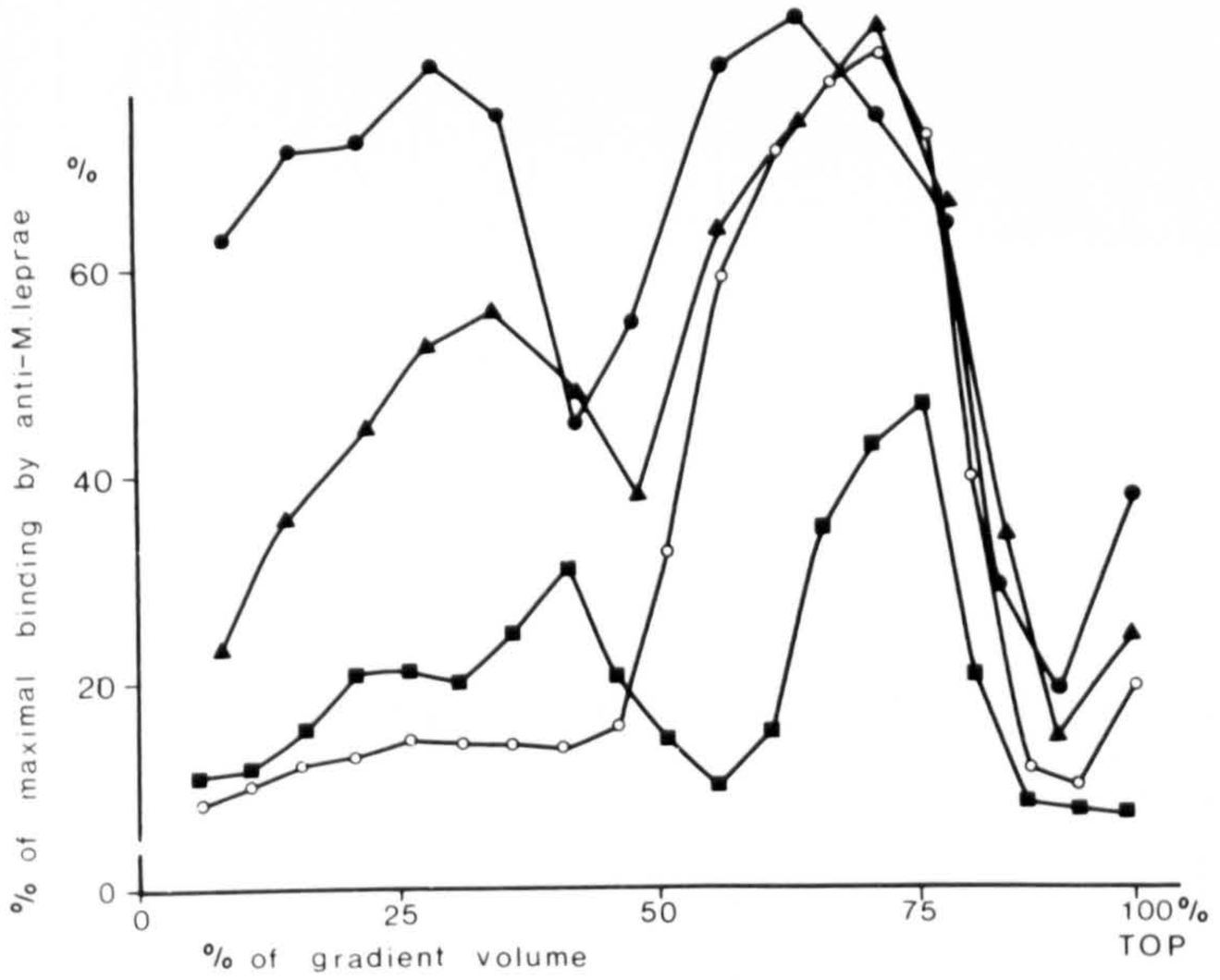


Figure D2(iii). Antibody activity against M. leprae antigen 7 in fractions after density gradient ultracentrifugation of 4 mothers with active lepromatous leprosy.

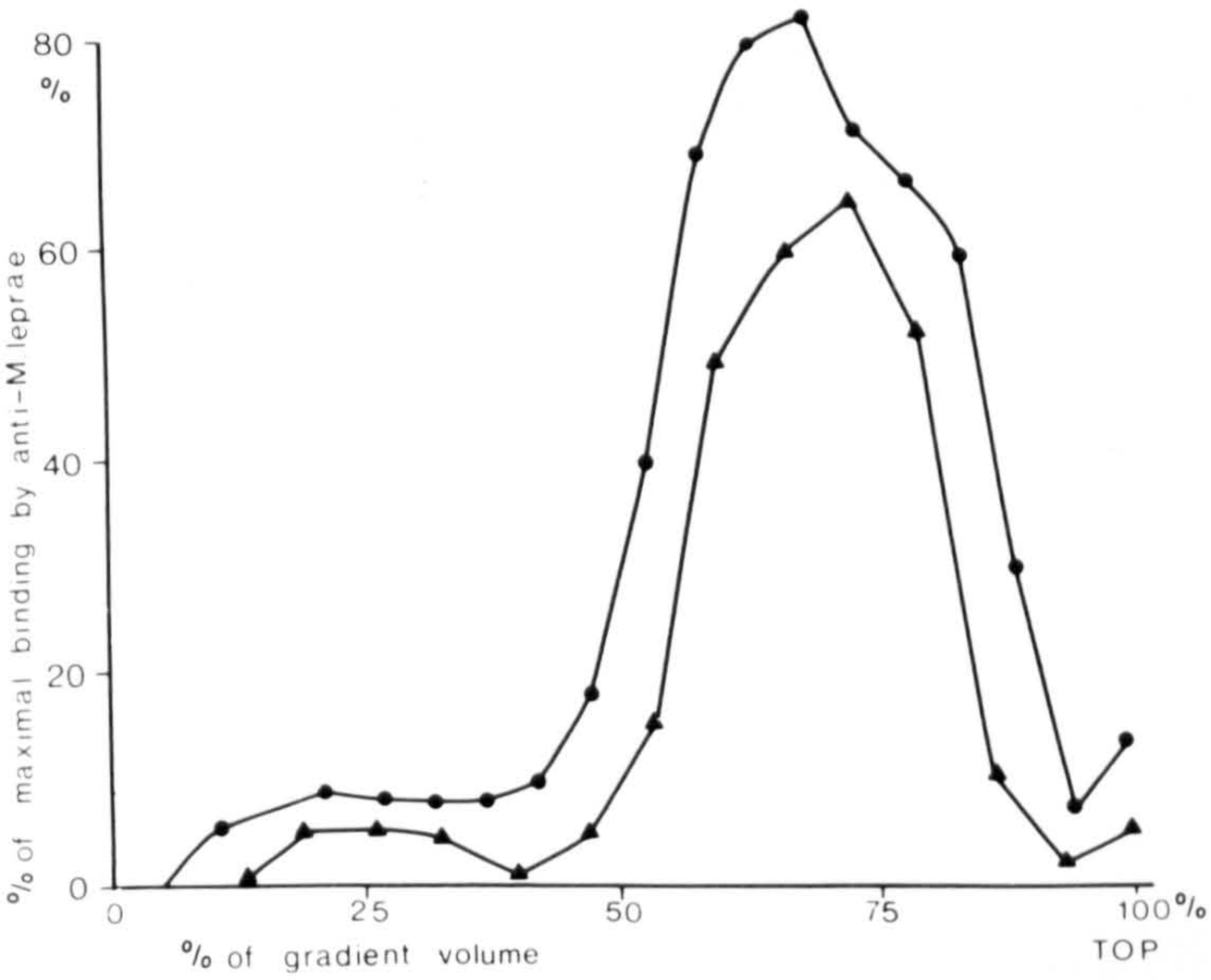


Figure D2(iv). Antibody activity against M. leprae antigen 7 in fractions after density gradient ultracentrifugation of ▲——▲ one healthy mother, ●——● one mother with tuberculoid leprosy.

3. Antibodies against M. leprae antigen 7 from birth to 18 months of age

Using an RIA technique, antibody activity against M. leprae antigen 7 was determined in babies of mothers with leprosy from birth to 4 months of age and compared with the expected decline in concentration of maternal IgG. The concentration of antibodies against M. leprae antigen 7 in sera from 3 babies of healthy mothers and 9 babies of tuberculoid mothers expressed as a percentage of antibody concentration in the corresponding maternal serum at birth, showed a decrease, as expected, corresponding to the half life of maternal IgG of 4 weeks (Figure D3(i)).

Four out of 5 babies of mothers with inactive lepromatous leprosy and 10 out of 20 babies of mothers with active lepromatous leprosy showed a similar fall in concentration of antibody activity against M. leprae antigen 7, while 1 out of 5 babies of mothers with inactive lepromatous leprosy and 10 out of 20 babies of mothers with active lepromatous leprosy did not show the expected decrease in antibody concentration (Figure D3(ii)).

In the serial study of antibody activity against M. leprae antigen 7 during the first 18 months of life, 3 patterns emerged :

I. Decline in antibody activity against M. leprae antigen 7 during the first 6 months of life, with the antibody activity remaining low thereafter (Figure D3(iii.a)). This was seen in the 3 children of healthy mothers, 4 out of the 5 babies of tuberculoid mothers, all 5 babies of mothers with inactive lepromatous leprosy, and 6 out of 17 babies of mothers with active lepromatous leprosy.

II. Partial decrease of antibody activity, less than expected decline (Figure D3(iii.b)). This was seen in 1 out of the 4 babies of tuberculoid mothers and 6 out of the 18 mothers with active lepromatous leprosy.

III. A partial decrease followed by an early increase in antibody activity against M. leprae antigen 7 (Figure D3(iii.b)). This was seen in 5 out of the 17 babies of mothers with active lepromatous leprosy.

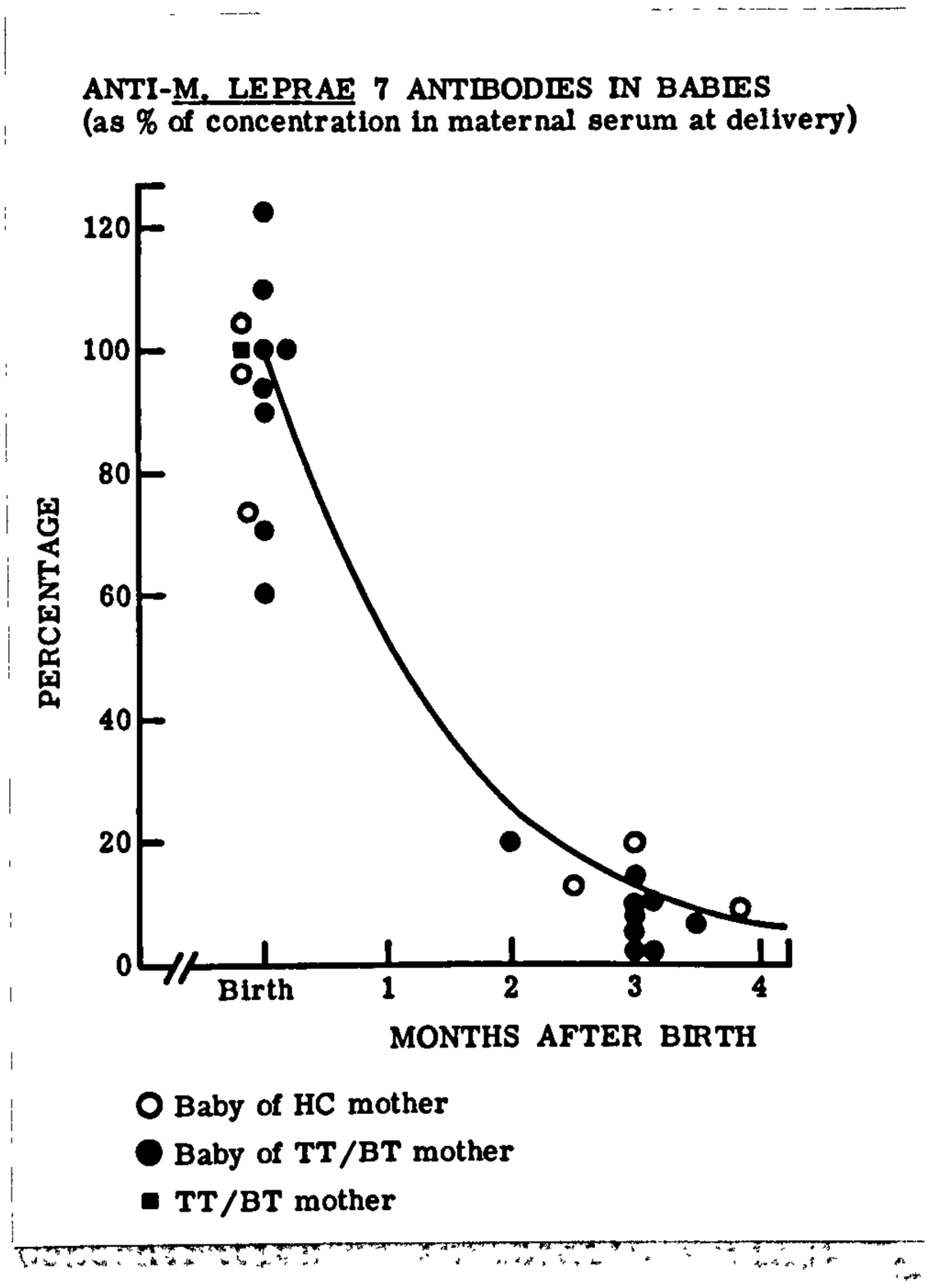


Figure D3(i). Concentration of antibodies against M. leprae antigen 7 during the first 4 months of life. Two values are shown for each baby, one at birth and one at 2-4 months of age. (○) Babies of non-leprosy (HC) mothers, (●) babies of tuberculoid leprosy mothers. The concentration is expressed as a percentage of the concentration in paired maternal sera at delivery. (■) Maternal concentration at delivery in one case where cord serum was not available.

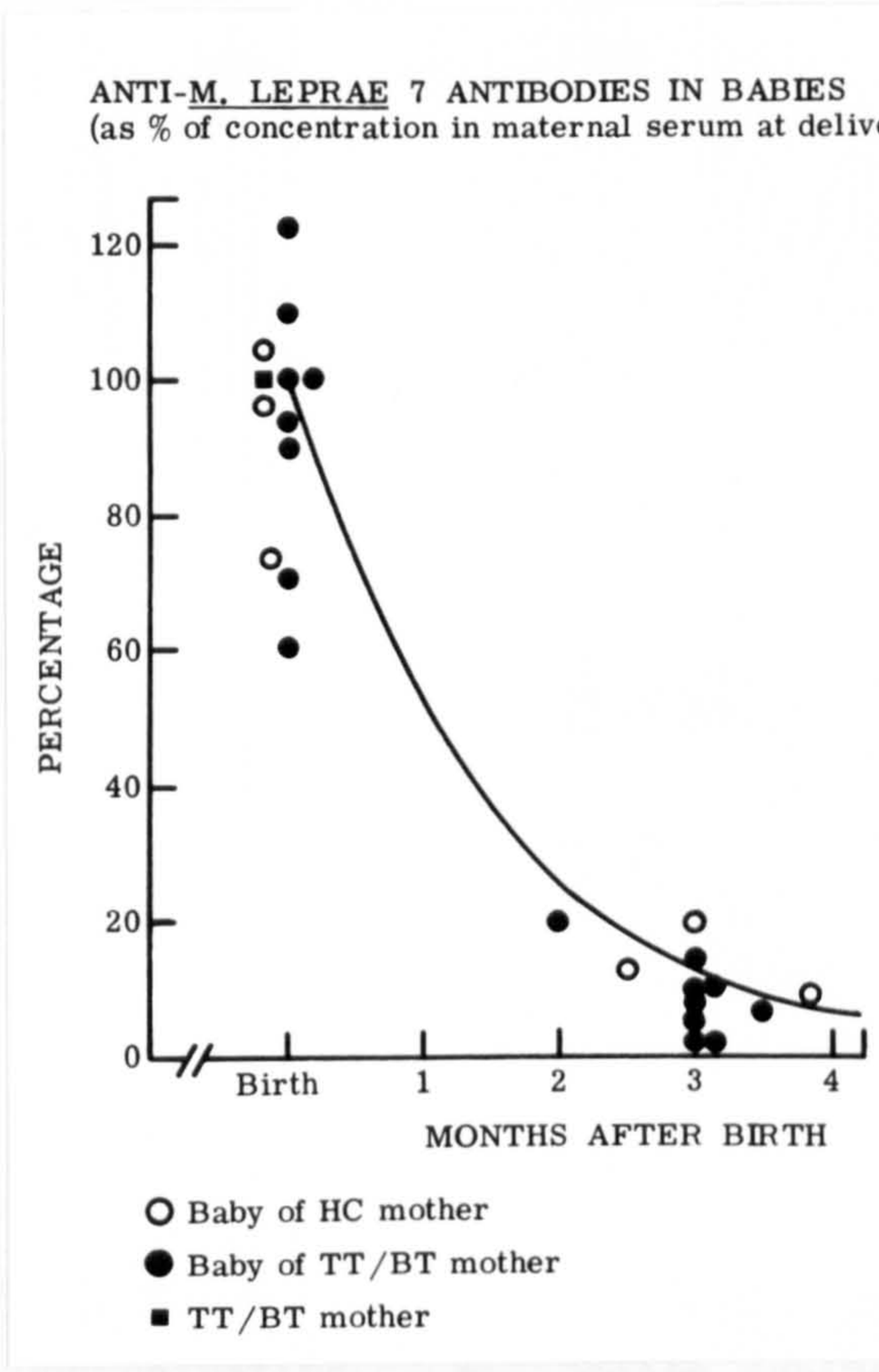


Figure D3(i). Concentration of antibodies against M. leprae antigen 7 during the first 4 months of life. Two values are shown for each baby, one at birth and one at 2–4 months of age. (○) Babies of non-leprosy (HC) mothers, (●) babies of tuberculoid leprosy mothers. The concentration is expressed as a percentage of the concentration in paired maternal sera at delivery. (■) Maternal concentration at delivery in one case where cord serum was not available.

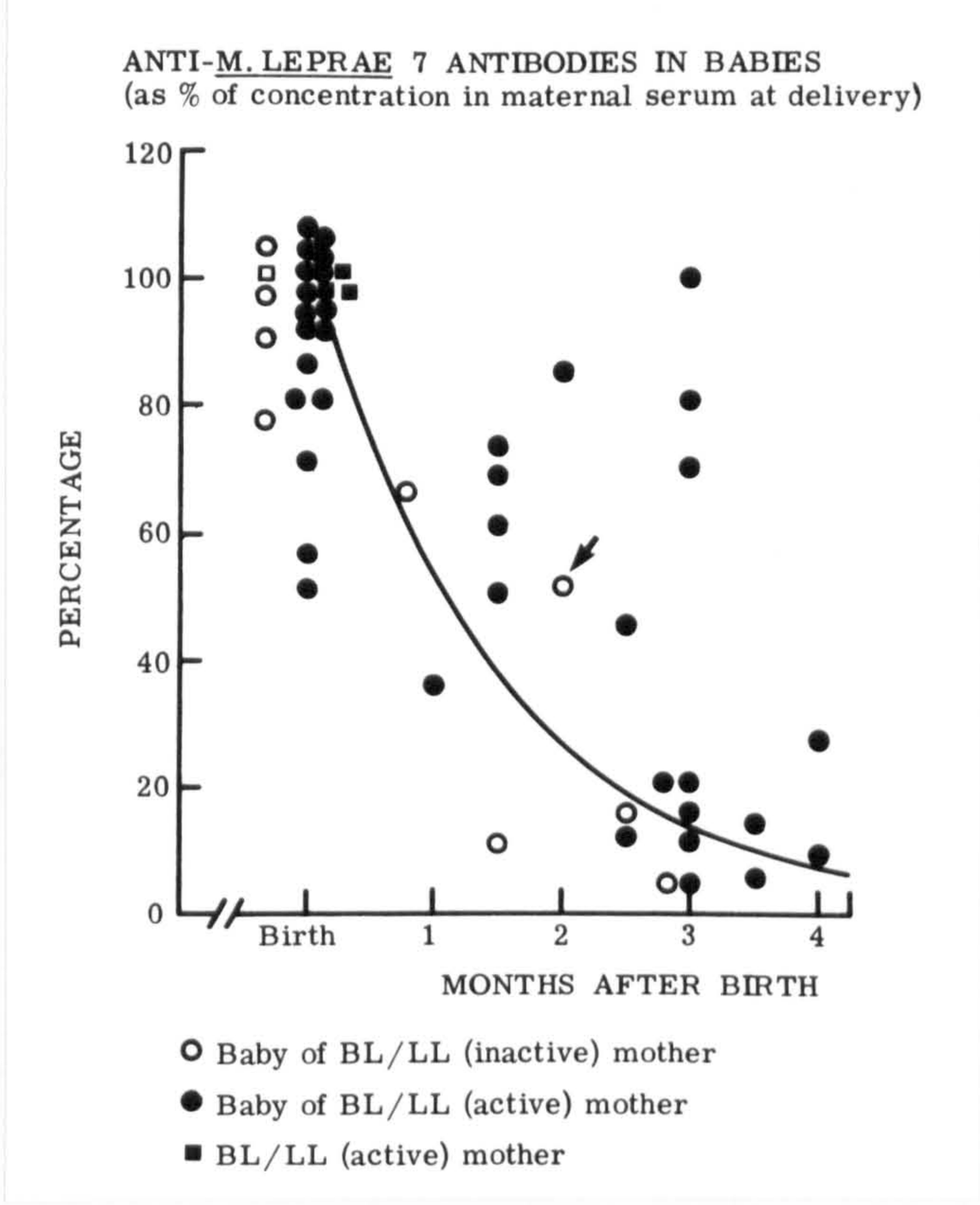


Figure D3(ii). Concentration of antibodies against M. leprae antigen 7 during the first 4 months of life. Two values are shown for each baby, one at birth and one at 2–4 months of age. (O) 4 babies of mothers with inactive lepromatous leprosy, (●) 20 babies of mothers with active lepromatous leprosy. The concentration is expressed as a percentage of the concentration in paired maternal sera at delivery. (■) maternal concentration at delivery in three cases where cord serum was not available, (□) maternal concentration at delivery in one case where cord serum was not available.

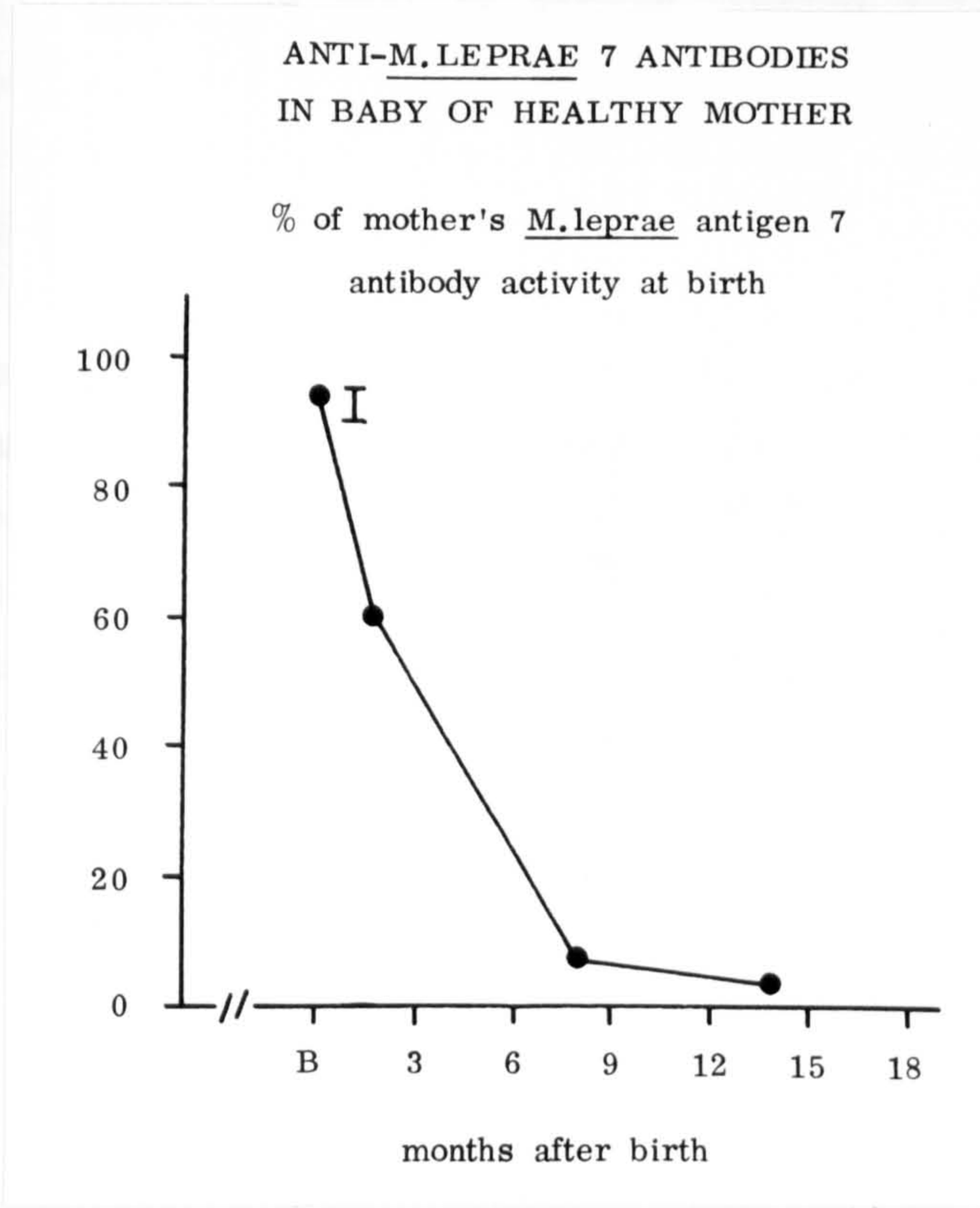


Figure D3(iii)a. The concentration of antibodies against M. leprae antigen 7 in a baby of a non-leprosy mother during the first 15 months of life (Pattern I).

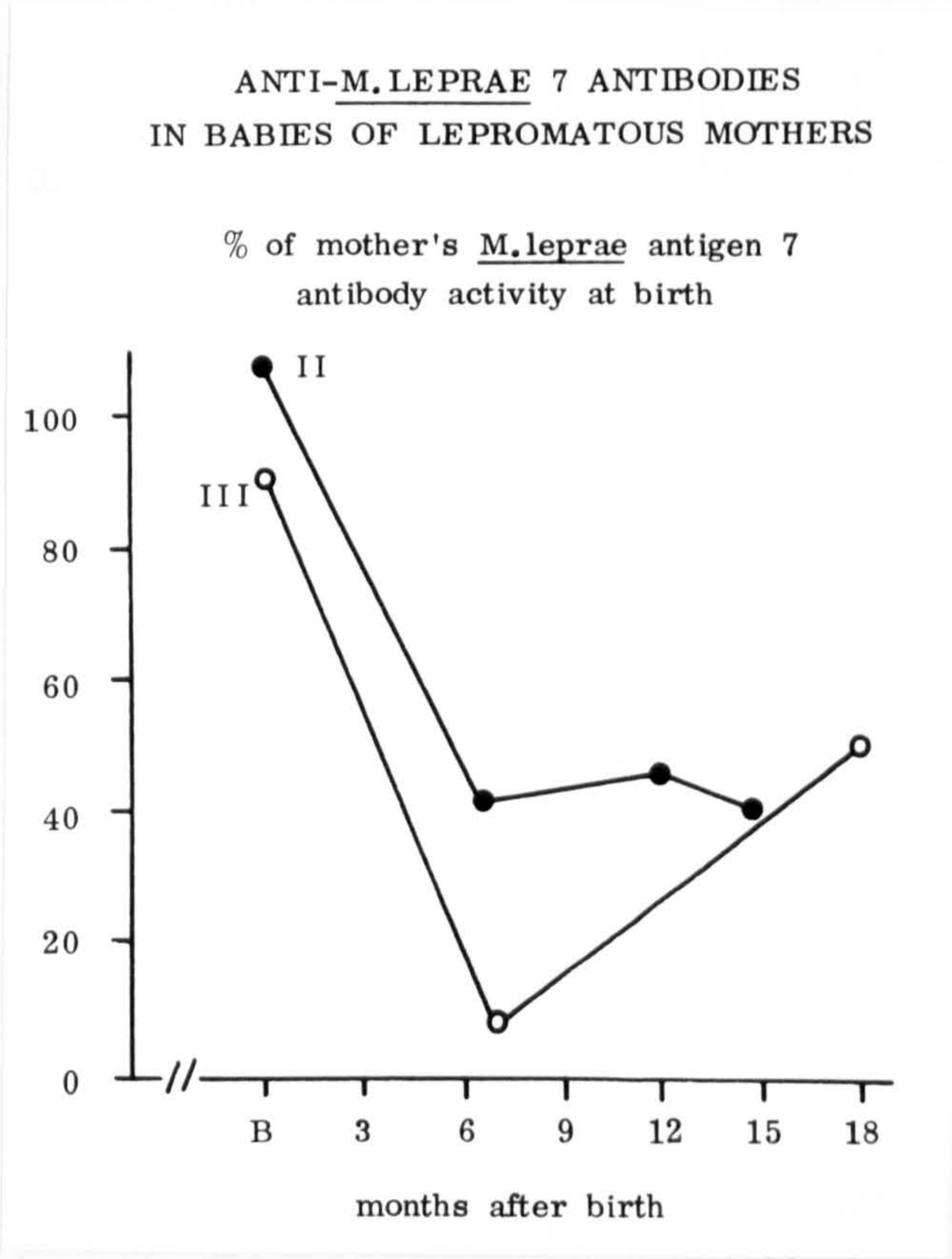


Figure 3(iii)b. Two different patterns of anti-M. leprae antibodies in two babies of mothers with active lepromatous leprosy during their first 18 months of life (Pattern II and III).

Table D3(i) shows the data on anti-M. leprae antibody levels in those babies, where serial blood samples were obtained, regarding the results between 6 and 18 months of age. In Table D3(ii), the data in two groups were pooled regarding the leprosy status of the mother and levels of antibody in the baby at 6 and 18 months after birth. The differences observed in babies of active lepromatous leprosy mothers versus the three other groups - inactive lepromatous leprosy, tuberculoid leprosy and non-leprosy, were statistically significant ($P < 0.002$).(Melsom, Duncan, Harboe and Bjune, Clinical and Experimental Immunology (1980) 42, 107-113).

TABLE D3(i)

Concentration of antibodies against *M. leprae* antigen 7 during the first 18 months in the four groups of babies

Leprosy status of the mother	Expected decline	Less than expected decline	Decline and later increase	Total
No sign of leprosy	3	0	0	3
BT and TT leprosy	4	1	0	5
BL and LL, BI -ve leprosy*	5	0	0	5
BL and LL, BI +ve leprosy**	6	6	5	17

* BL and LL, BI -ve = no acid-fast bacteria in skin smears, i.e. inactive lepromatous.

** BL and LL, BI +ve = acid-fast bacteria in skin smears, i.e. active lepromatous.

TABLE D3(ii)

Pooling of data in Table D3(i) on anti-*M. leprae* 7 antibodies

Leprosy status of the mother	Expected decline	Less than expected decline + increase	Total
No sign of leprosy			
BT and TT and BL and LL, BI -ve	12	1	13
BL and LL, BI +ve	6	11	17
Total	18	12	30

4. IgA and IgM antibodies against M. leprae in cord sera and in patients with leprosy

A solid-phase radioimmunoassay (sRIA) was developed for demonstration and quantitation of IgA and IgM anti-M. leprae antibodies. The sera tested by the sRIA technique included :

I. Lepromatous leprosy serum pool from 40 patients, either newly diagnosed or treated with dapsone for less than 6 months.

II. Thirty-five mothers with leprosy, together with 9 healthy mothers and their babies.

The mothers were grouped as follows :

Group 1. 22 mothers with active lepromatous leprosy (LL and BL, BI positive).

Group 2. 7 mothers with inactive lepromatous leprosy (LL and BL, BI negative).

Group 3. 6 mothers with tuberculoid leprosy (BT and TT), 3 had active leprosy and 3 were considered cured and had stopped treatment.

Group 4. 9 mothers with no clinical or laboratory evidence of leprosy.

The babies were grouped according to the classification of their mothers.

IgA and IgM anti-M. leprae antibodies were demonstrated in a lepromatous serum pool (LSP), in varying amounts in individual women with lepromatous leprosy, and in lower concentration in tuberculoid leprosy and non-leprous controls, in whom it was expressed as a percentage of anti-M. leprae antibody in the lepromatous serum pool (LSP).

IgA antibodies against M. leprae antigens were found in all of the maternal sera examined (Figure D4(i)). The concentration varied markedly from 30 to 500% of the concentration of LSP in LL and BL, BI positive mothers, with the median concentration 117%. The median value for mothers with inactive lepromatous leprosy was 140%, for tuberculoid leprosy was 40%, and for healthy controls 20%.

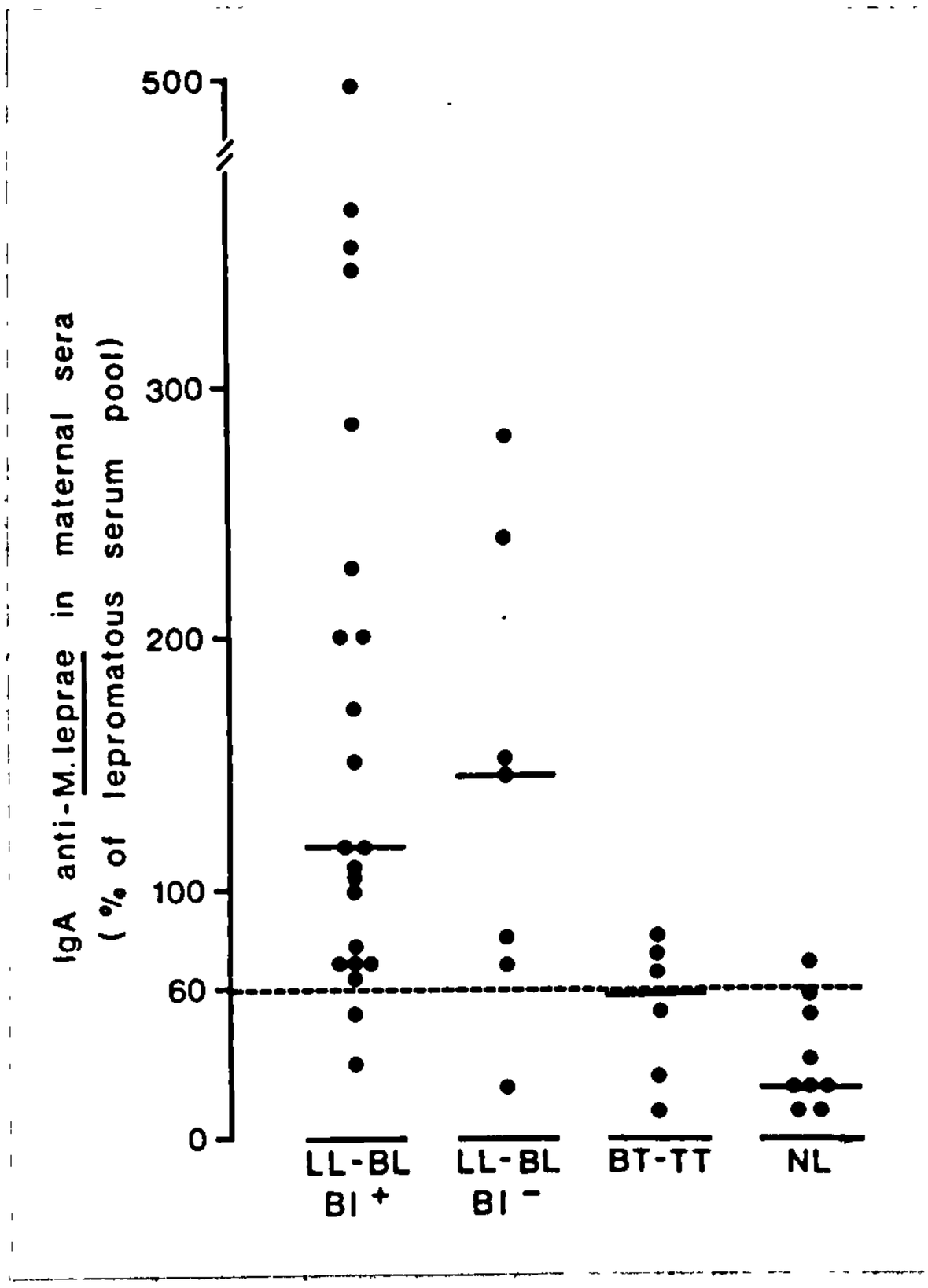


Figure D4(i). IgA anti-M. leprae antibodies in maternal sera at delivery. Each point represents one individual, and the amount is expressed as a percentage of the IgA antibody activity against M. leprae in lepromatous serum pool. The horizontal bars represent the median values.

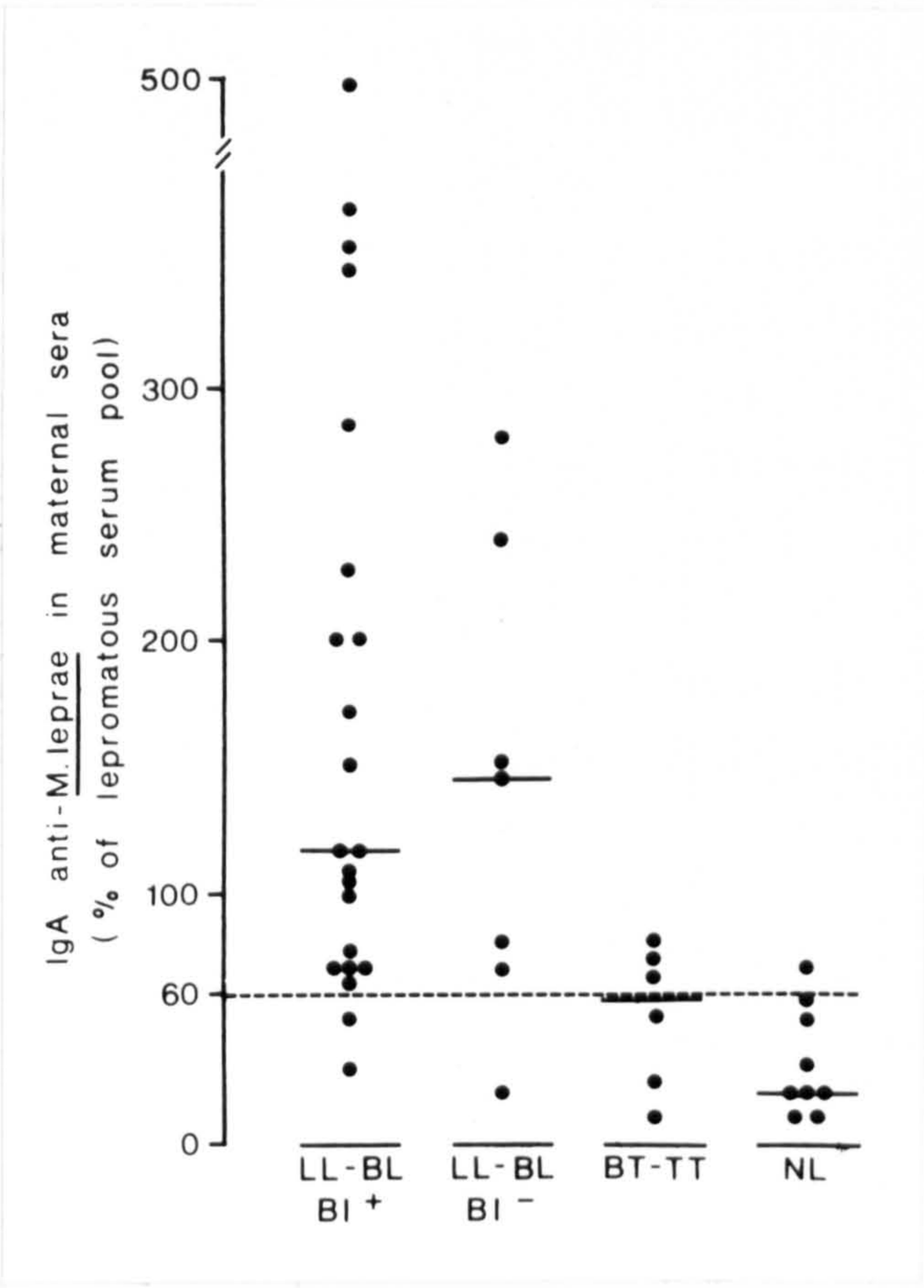


Figure D4(i). IgA anti-M. leprae antibodies in maternal sera at delivery. Each point represents one individual, and the amount is expressed as a percentage of the IgA antibody activity against M. leprae in lepromatous serum pool. The horizontal bars represent the median values.

IgM antibodies against M. leprae were present in all the sera examined in active lepromatous leprosy (Figure D4(ii)). The median value was 140% with a variation between 10 and 320%, the median value for inactive lepromatous leprosy was 100%, for tuberculoid leprosy 35%, and for non-leprous controls 40%.

IgA anti-M. leprae antibodies were demonstrated in the cord sera of 7 out of 22 babies of mothers with active lepromatous leprosy, but not in any of the 22 cord sera from the other groups (Figure D4(iii)). IgM anti-M. leprae antibodies were demonstrated in 12 out of 22 cord sera from babies of mothers with active lepromatous leprosy (Figure D4(iv)). The 7 cord sera with IgA anti-M. leprae antibodies also contained IgM anti-M. leprae antibodies. Two of the 7 babies of mothers with inactive lepromatous leprosy, and 1 out of 9 babies of healthy mothers also contained IgM anti-M. leprae antibodies (Melsom, Harboe, Duncan and Bergsvik, Scandinavian Journal of Immunology (1981), 14, 343-352).

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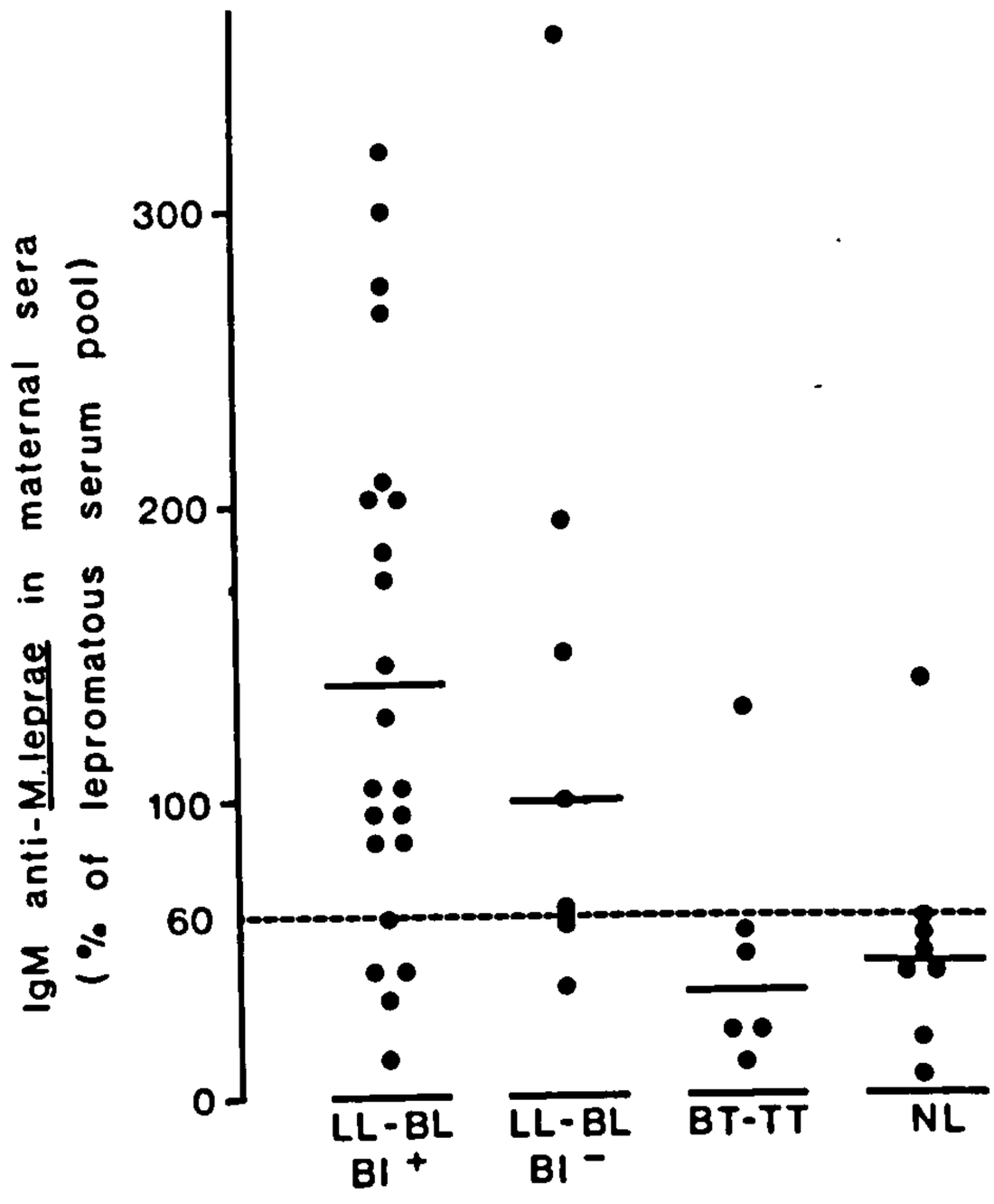


Figure D4(ii). IgM anti-M. leprae antibodies in maternal sera at delivery. Each point represents one individual and the amount is expressed as a percentage of the IgM antibody activity against M. leprae in lepromatous serum pool. The horizontal bars represent the median values.

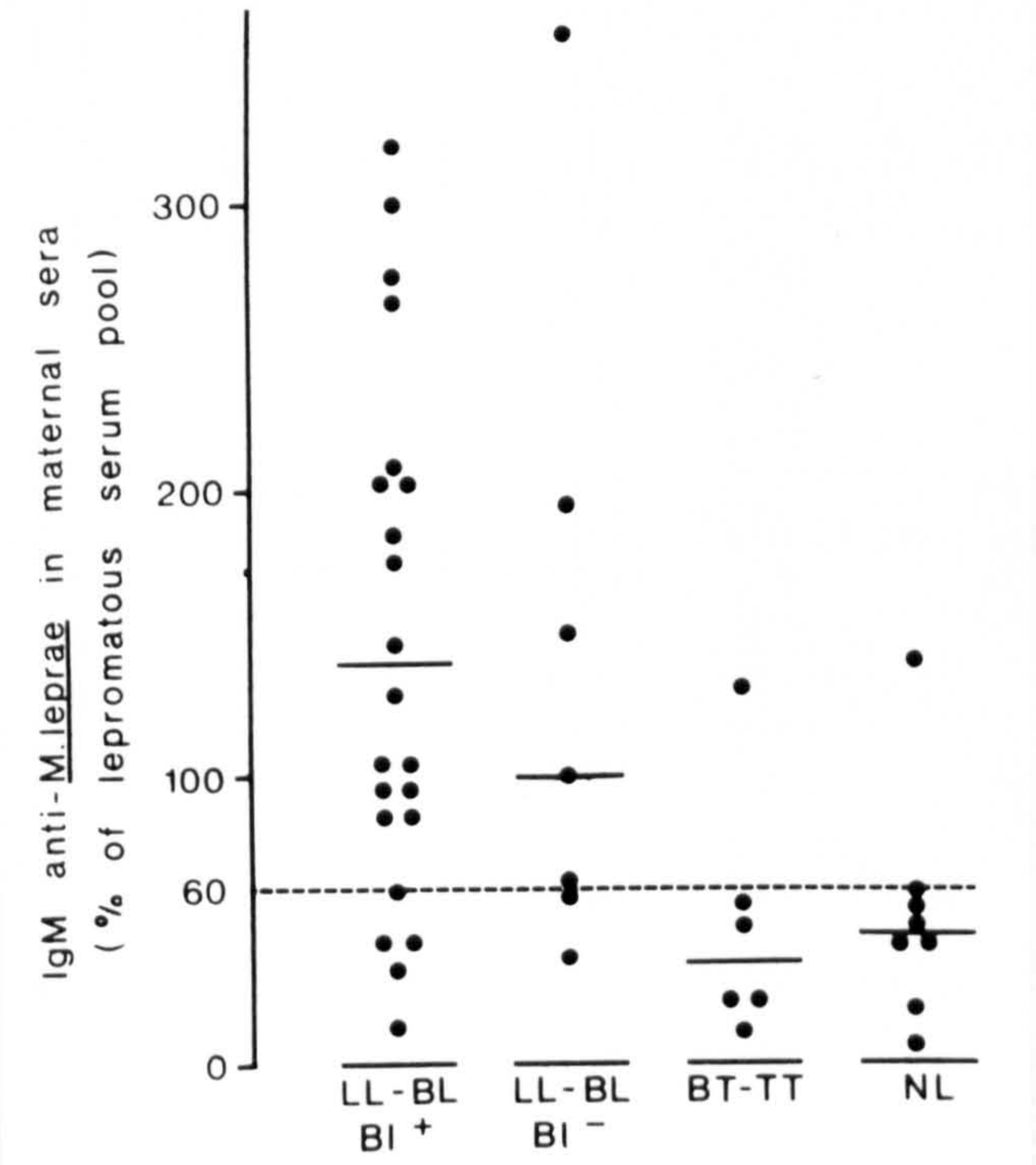


Figure D4(ii). IgM anti-M. leprae antibodies in maternal sera at delivery. Each point represents one individual and the amount is expressed as a percentage of the IgM antibody activity against M. leprae in lepromatous serum pool. The horizontal bars represent the median values.

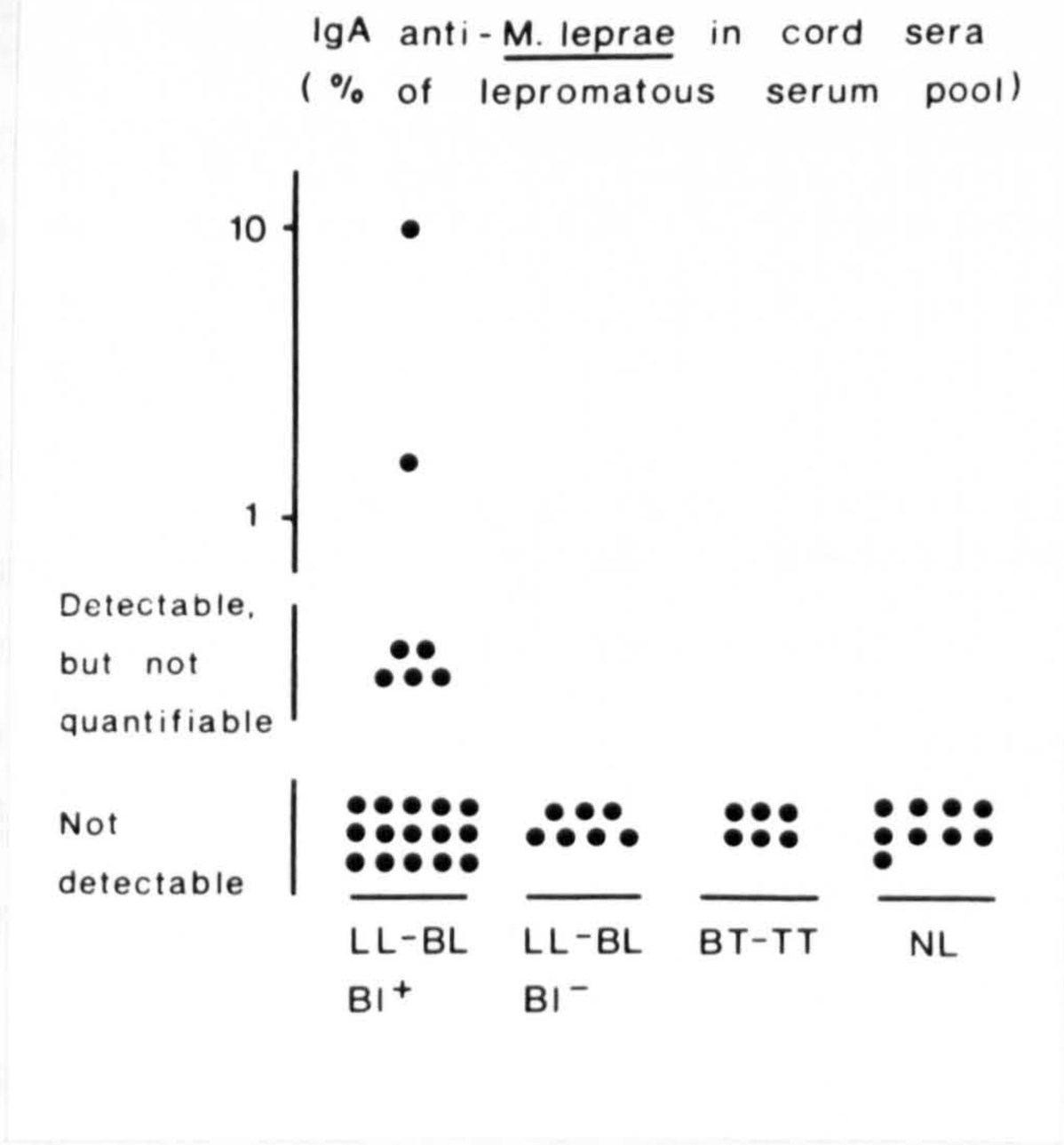


Figure D4(iii). IgA anti-M. leprae antibodies in cord sera diluted 10^{-1}. The amount is expressed as a percentage of IgA antibody activity in lepromatous serum pool.

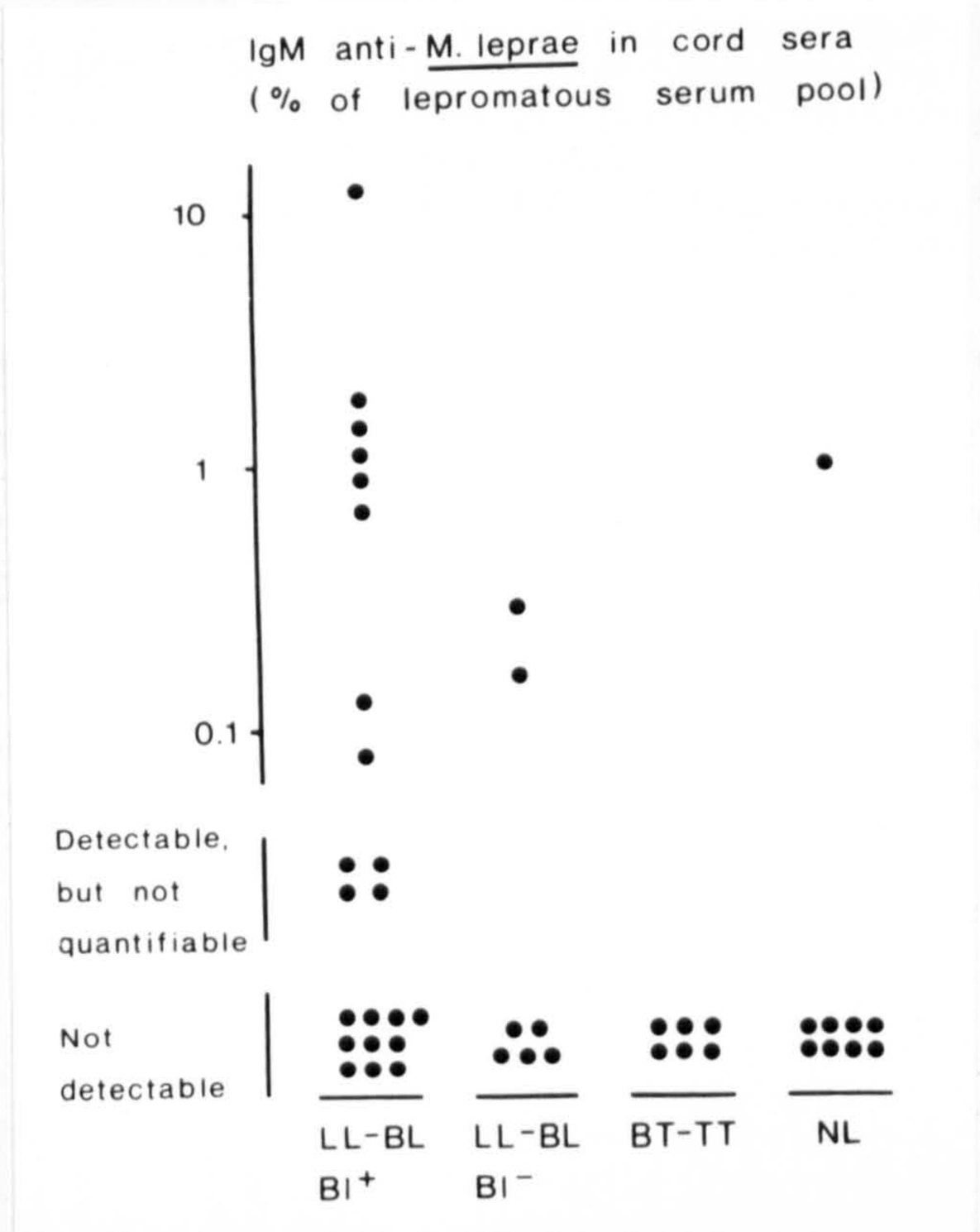


Figure D4(iv). IgM anti-M. leprae antibodies in cord sera diluted 10^{-1}. The amount is expressed as a percentage of IgM antibody activity in lepromatous serum pool.

5. IgA, IgM, IgG anti-M. leprae antibodies in babies of mothers with leprosy during the first 2 years of life

IgA, IgM and IgG anti-M. leprae antibody activity was estimated by solid-phase radioimmunoassay (sRIA) in serial serum samples from birth to 2 years of age, from 40 babies of mothers with leprosy and 5 babies of healthy controls. The babies were grouped as follows :

Group 1. Twenty-nine babies of mothers with lepromatous leprosy (active and inactive) (BL and LL). These babies may have been exposed to M. leprae in utero.

Group 2. Eleven babies of mothers with tuberculoid leprosy (BT and TT) and 5 babies of non-leprous (healthy control) mothers. These babies are very unlikely to have been exposed to M. leprae in utero.

IgA anti-M. leprae antibody activity could be detected in 30% and IgM anti-M. leprae antibody activity in 50% of cord sera from Group 1 babies, but not in any of the cord sera from Group 2. After birth, there was a significantly higher increase of both IgA and IgM anti-M. leprae antibody activity in sera taken at 3-6 months of age from babies of Group 1 compared with those of Group 2: after 6 months of age, the serum IgA and IgM activity showed similar increase in the two groups of babies (Figures D5(i) and D5(ii)).

Serum IgG anti-M. leprae antibody activity showed a marked decrease in both groups of babies at 3-6 and 6-9 months of age compared with the activity in the cord sera. No significant increase of IgG activity could be demonstrated even in sera taken 15-24 months after birth in either of the two groups of children (Figure D5(iii)) (Melsom, Harboe and Duncan, Clinical and Experimental Immunology (1982), 49, 532-542).

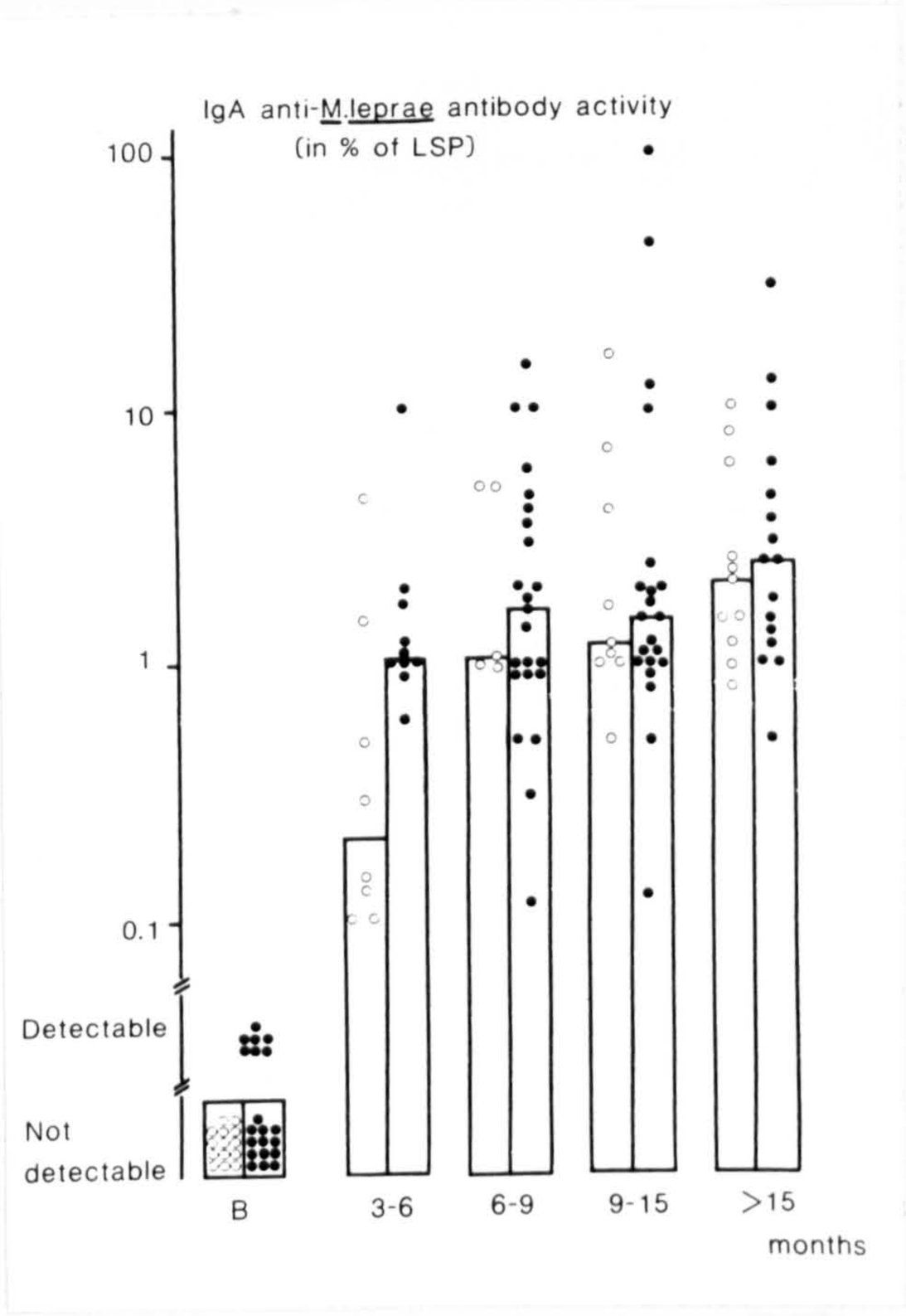


Figure D5(i). IgA anti-M. leprae antibody activity in individual sera from babies of mothers with lepromatous leprosy (•) and in sera from babies of tuberculoid leprosy mothers and healthy controls (o). The median results were calculated from all the individual results after the sera had been allocated to 5 groups, cord sera and sera taken at 3-6, 6-9, 9-15 and 15-24 months after birth. The results are expressed as a percentage of IgA anti-M. leprae antibody activity in a lepromatous serum pool (LSP).

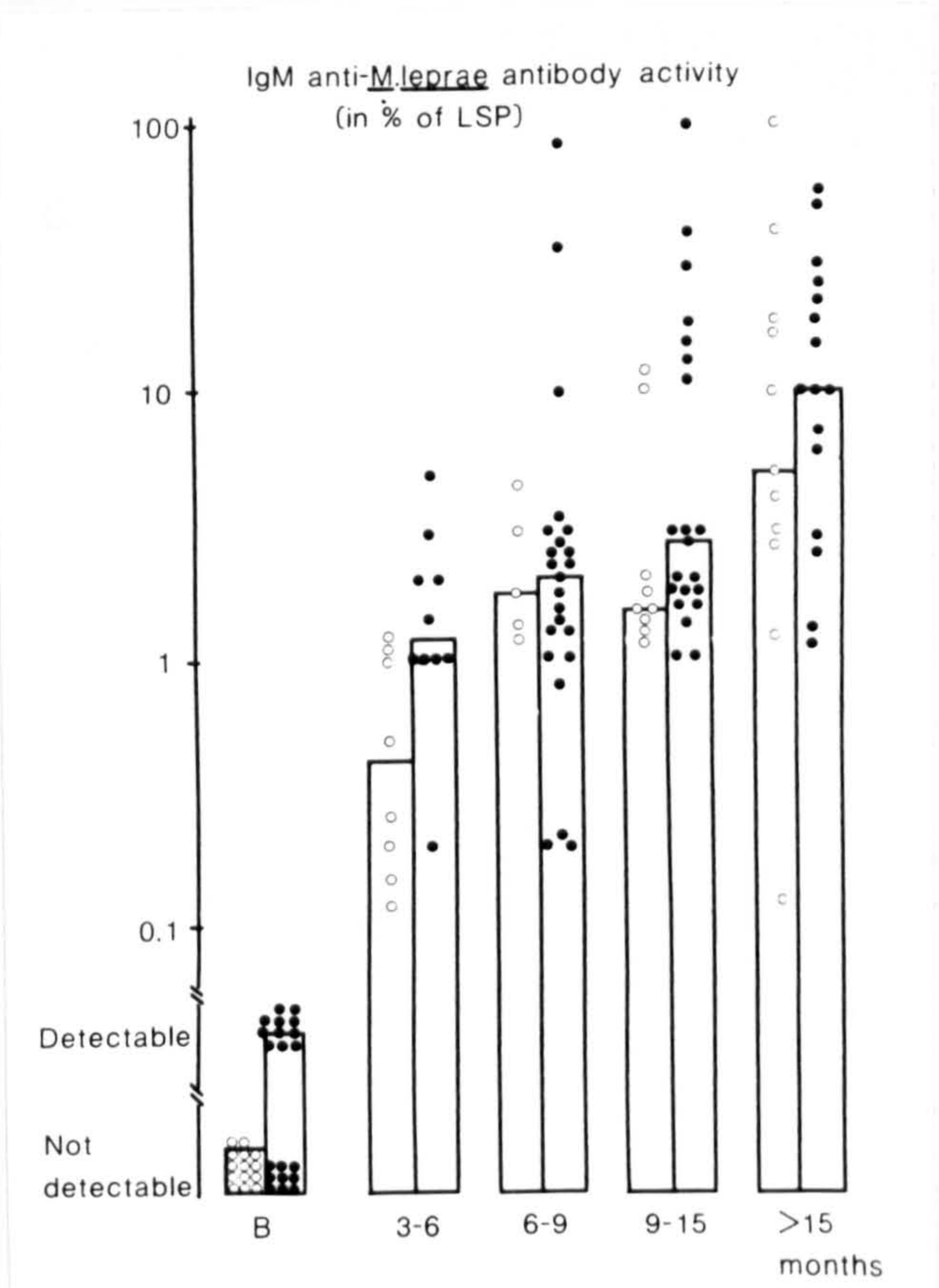


Figure D5(ii). IgM anti-M. leprae antibody activity in individual sera from babies of mothers with lepromatous leprosy (•) and in sera from babies of mothers with tuberculoid leprosy and healthy controls (o). The median results were calculated from all the individual results after the sera had been allocated to 5 groups, cord sera and sera taken 3-6, 6-9, 9-15 and 15-24 months after birth. The results are expressed as a percentage of IgM anti-M. leprae antibody activity in a lepromatous serum pool (LSP).

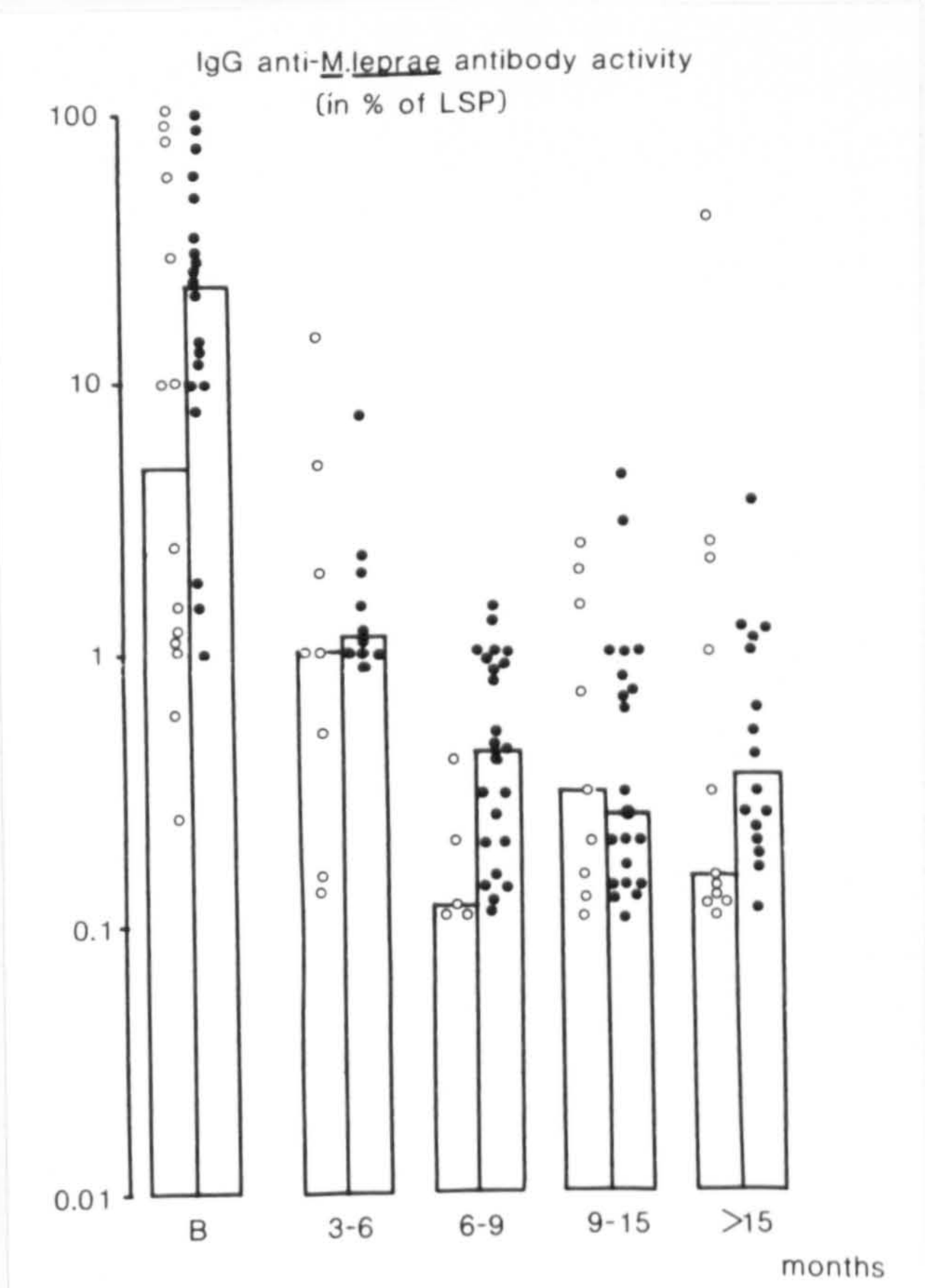


Figure D5(iii). IgG anti-M. leprae antibody activity in individual sera from babies of mothers with lepromatous leprosy (•) and in sera from babies of mothers with tuberculoid leprosy and healthy controls (o). The median results were calculated from the individual results after the sera had been allocated to 5 groups, cord sera and sera taken 3-6, 6-9, 9-15 and 15-24 months after birth. The results are expressed as a percentage of IgG anti-M. leprae antibody activity in a lepromatous serum pool (LSP).

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A. THE EFFECT OF PREGNANCY ON LEPROSY

1. Pregnancy and Leprosy: The consequences of alterations of cell mediated and humoral immunity during pregnancy and lactation

One hundred and fourteen Ethiopian women with leprosy and 33 healthy women without leprosy were studied prospectively throughout 120 and 36 pregnancies respectively and followed up during lactation. Fifty-five women showed worsening of their leprosy status; in 31 (56%) this was observed during the third trimestre of pregnancy. Forty women were diagnosed as having reversal (type 1) reaction; in 20 (50%) the first occurrence was during the first 6 months of lactation. Twenty-eight women had ENL (type 2) reaction, which in 19 (68%) first occurred during the third trimestre of pregnancy or the first 6 months of lactation.

The purpose of this section is to show the overall effect of pregnancy on leprosy in terms of worsening of the patient's leprosy status, reversal (type 1 lepra) reaction and ENL (type 2 lepra reaction) during pregnancy and the first 12 months of lactation. While many of the mothers were followed up for considerably longer than 12 months, this was the period during which most of them were seen; it was also the period during which the majority of them were lactating before the next pregnancy supervened.

More detailed accounts of relapse and the clinical manifestations of reaction, together with the effects of prolonged lactation on leprosy, are dealt with in the next 5 sections.

Most of the time impairment of cell mediated immunity (CMI) in pregnancy is of academic interest. Sometimes, it may be of practical importance in patient management - leprosy being an example of such a situation. Leprosy has at least 3 properties which make it of particular immunological interest :

1. It is an exceedingly chronic disease.
2. M. leprae is almost non-toxic.

3. The host parasite relationship in leprosy is often unstable, and variations in both CMI and humoral immune responses to M. leprae can cause reactions which are not directly related to the bacteriological progress or regression of the disease. Decrease in CMI or downgrading is frequently associated with worsening of the patient's leprosy and increase in the bacillary load (and secondarily with increase of humoral response), but only occasionally with type 1 reaction (Figure A1(iv)). Increase in DH will tend to cause up-grading or shift towards the tuberculoid end of the spectrum (reversal reaction), which may involve either skin or nerve lesions (Figure A1(v)). Although the trigger mechanisms of reaction are not as yet understood, conditions causing general diminution (or increase) of CMI may initiate reactions. Pregnancy is such a condition (Figure A1(iii)).

In certain diseases natural remission occurs with the advance of pregnancy followed by deterioration after delivery, while in other diseases, notably those caused by viruses and mycobacteria, pregnancy has an adverse effect, the disease becoming overt or progressing rapidly during late pregnancy and immediately post-partum. These observations have been attributed to :

1. Raised hormone levels during pregnancy, in particular increased levels of free cortisol and 17-hydroxy-corticosteroid.

2. Metabolic disturbances particularly in women who are already suffering from protein and calorie malnutrition.

3. Alterations in cell mediated immunity during pregnancy.

Recent publications have reported varying results regarding alterations in CMI during pregnancy. It is generally agreed that there is some suppression of CMI probably due to serum factor(s); cell mediated suppressor mechanisms may also be involved; inversion of T- and B-cell ratio and suppression of macrophage and poly-morphonuclear function during pregnancy may also play a role. This has been reviewed in the Introduction section.

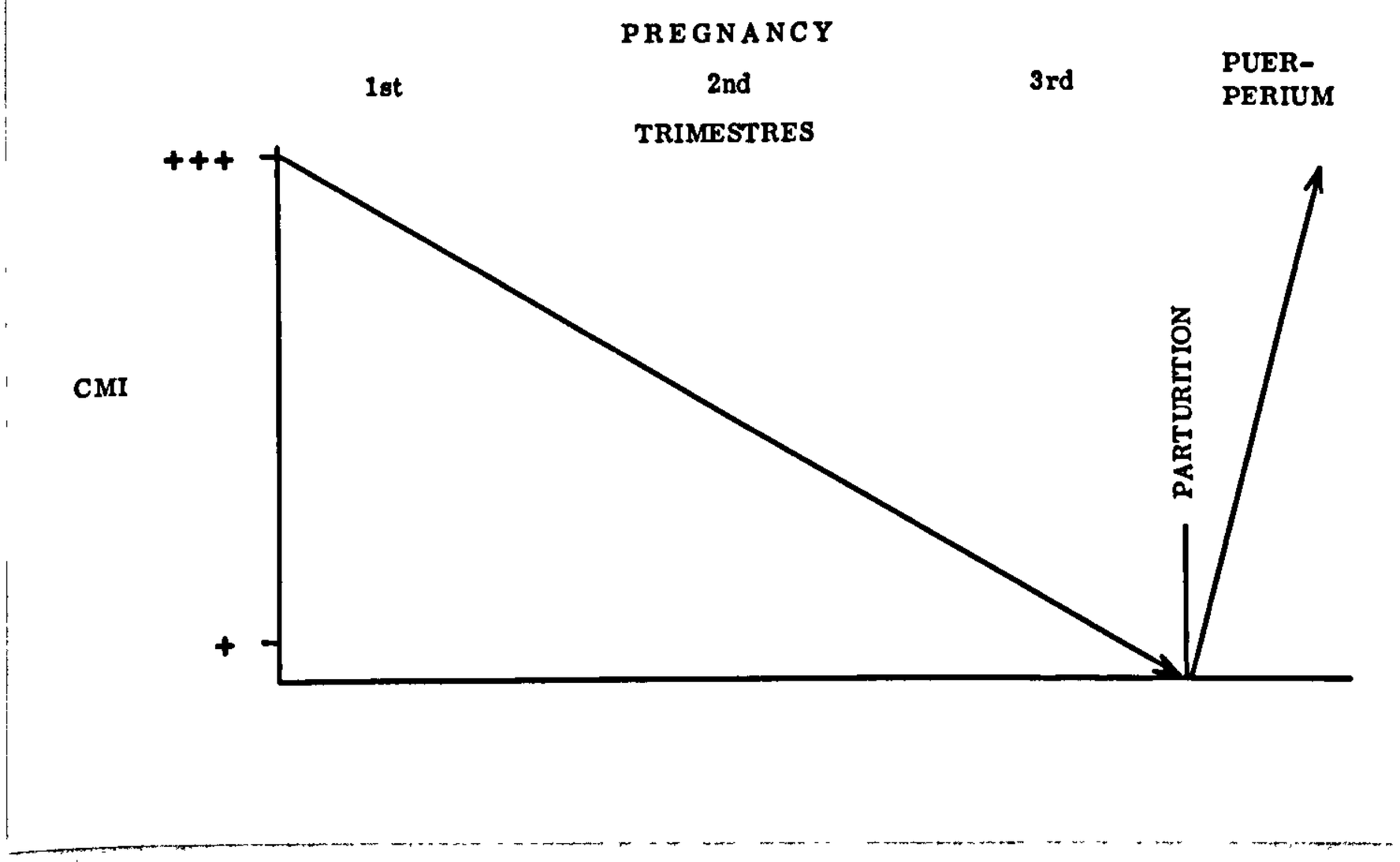


Figure A1(iii). During pregnancy there is some non-specific suppression of CMI, possibly due to serum factors, maximal during the third trimestre, which comes off soon after delivery.

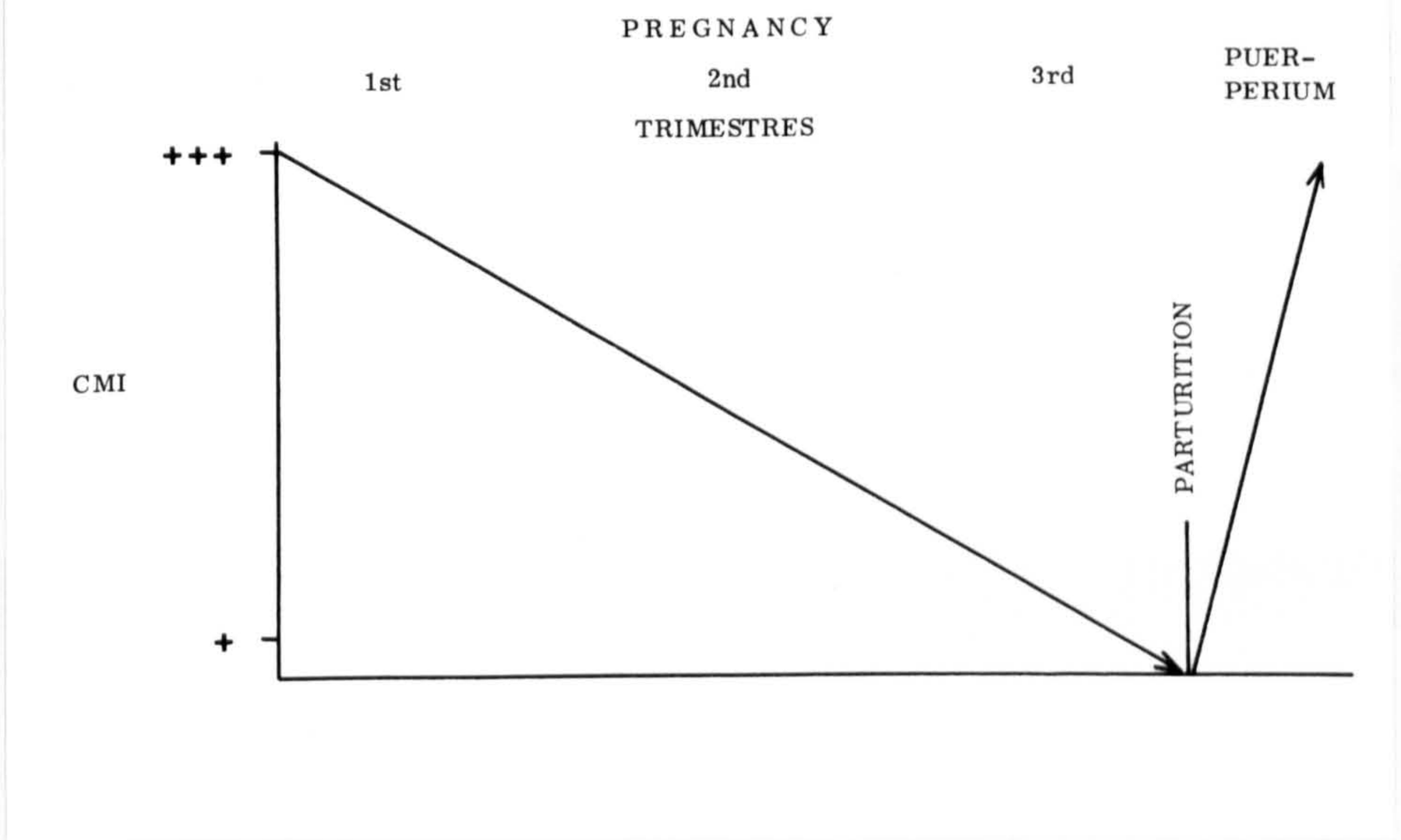


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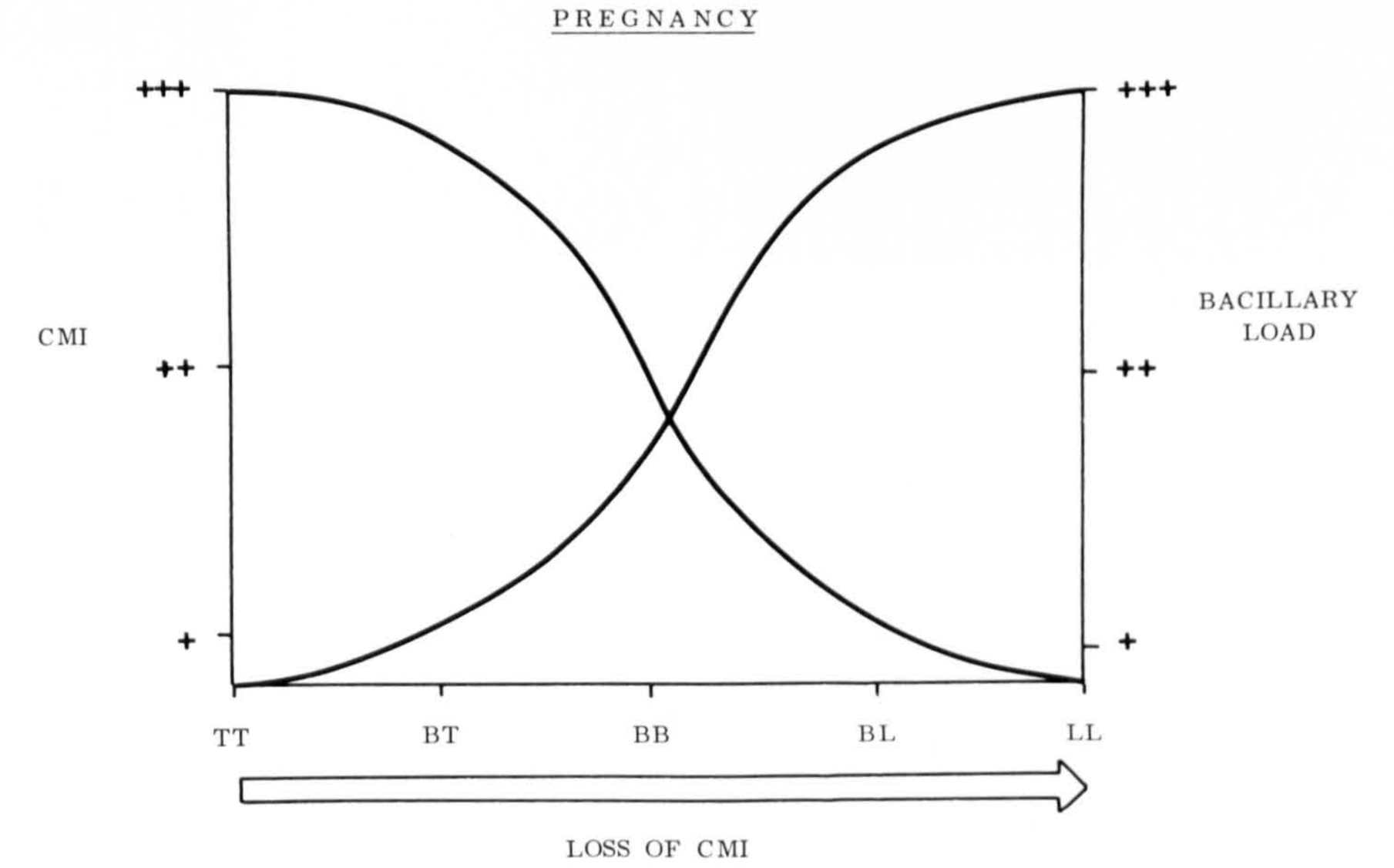


Figure A1(iv). During pregnancy, with suppression of CMI, there is a tendency for shift to the lepromatous end of the spectrum with increased bacillary multiplication (or decreased killing) and downgrading.

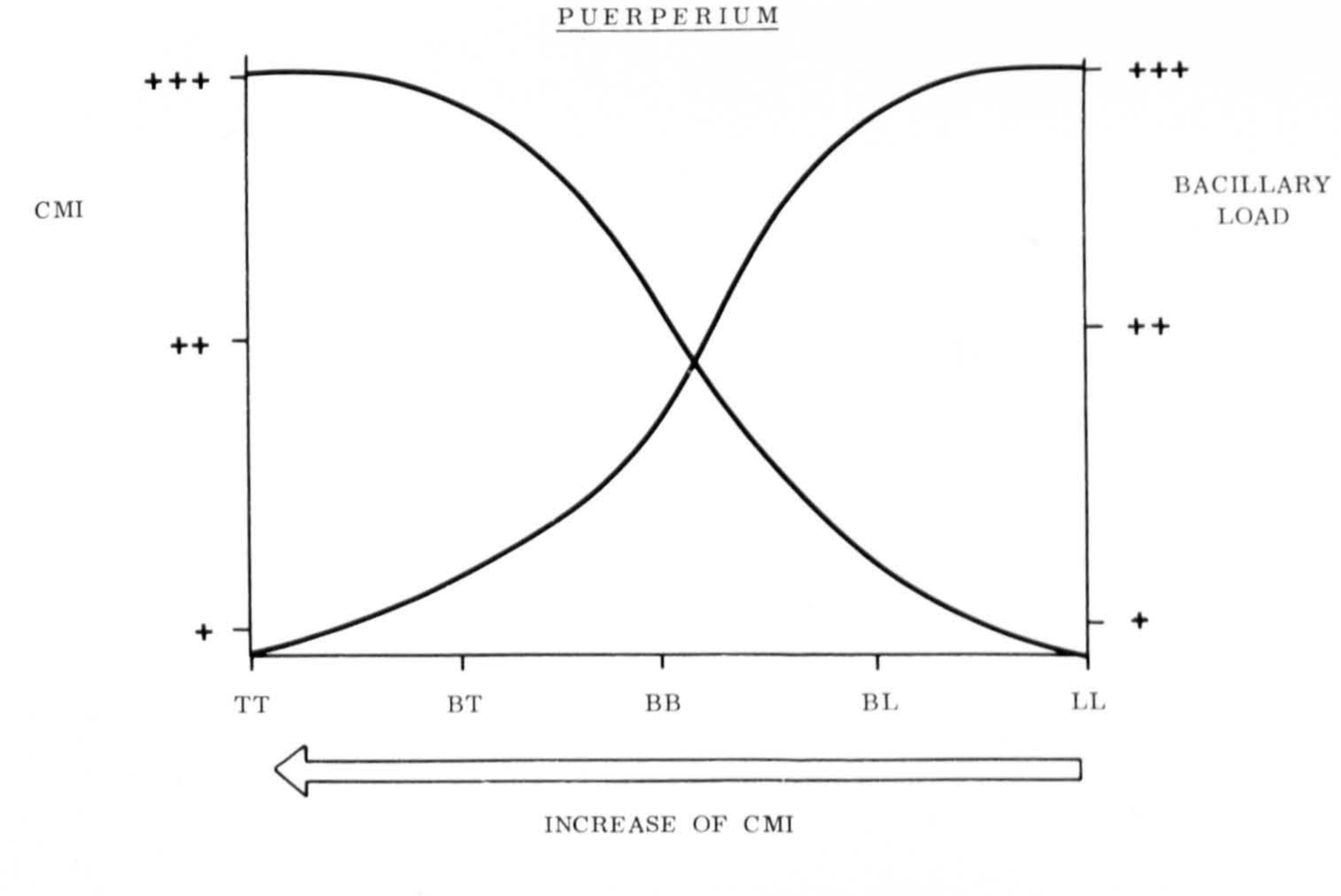


Figure A1(v). During the puerperium, with recovery of CMI, with a shift towards the tuberculoid end of the spectrum, upgrading and reversal reaction in skin and nerve are frequently seen.

Diseases such as rheumatoid arthritis, ulcerative colitis and sarcoidosis show a pattern of amelioration during pregnancy, with rebound deterioration postpartum. Leprosy, however, shows what may be called a biphasic adverse effect, with deterioration of the leprosy status during pregnancy and prolonged type 1 lepra reaction (RR) following delivery. While various factors as mentioned may influence the patient's leprosy status, the most likely explanation for this biphasic effect is suppression of the mother's CMI during pregnancy and recovery of CMI postpartum.

i) Deterioration of the patient's leprosy status

Previous reports indicate that pregnancy is apt to precipitate the appearance of overt leprosy (Tajiri, 1936; Lawson and Stewart, 1967; Hardas et al, 1972; Rose and McDougall, 1975; Coudert et al, 1978). Our results fully confirm this. Six per cent (2/33) of the "control" patients developed leprosy during the study, and 32% (8/25) of the apparently cured cases relapsed.

It has also been shown previously that pregnancy is associated with the exacerbation of existing leprosy (Tajiri, 1936; Ryrie, 1938; King and Marks, 1958; Lawson and Stewart, 1967; Hardas et al, 1972; Coudert et al, 1978; and Jopling, 1978a). The results of this study confirm this; 20% of the patients receiving treatment showed transient worsening of their leprosy during the study period despite apparently effective chemotherapy.

A novel and hitherto unrecorded finding was the high proportion of patients (38% of those receiving treatment) who showed significant and apparently progressive deterioration almost certainly caused by the emergence of dapsone resistant leprosy (proven in all 7 cases where mouse foot pad tests were undertaken). The high figure is related to the high proportion of new cases in Ethiopia (about 50%) showing primary low grade dapsone resistance (Pearson et al, 1977b). The immunosuppression of pregnancy may well afford opportunity for drug resistant bacilli to multiply and cause overt disease unusually rapidly. These findings are fully reported later.

The phenomenon of downgrading was observed particularly during pregnancy, and might be expected in any condition where CMI is suppressed. Within the LL group a number of patients showed a shift from subpolar lepromatous leprosy (LL_s) to polar lepromatous leprosy (LL_p) (Ridley, 1974) in association with exacerbation of the infection.

ii) Reversal (type 1) reaction

As this is usually due to an increase in CMI or DH it is not surprising that it occurs immediately after delivery, and our study confirmed findings of others (Chowduri and Ghosh, 1965; Rose and McDougall, 1975; Jopling, 1978b). However, the classical appearance of reversal reaction with erythema and oedema of skin lesions was not a prominent feature in our patients, except in those who also relapsed with active leprosy or who had very recently started treatment. The picture described by Rose and McDougall (1975) may reflect the natural evolution of the disease in untreated patients. We did, however, observe that reaction in skin or skin and nerve was a feature of pregnancy and early lactation, whereas reaction in nerve alone was a feature of the lactation period. This may reflect predominant exposure of the surface antigens of actively multiplying M. leprae during pregnancy and relatively increased uncovering of cytoplasmic antigens postpartum (Barnetson et al, 1975), if postpartum recovery of CMI leads to bacillary destruction. Reversal reaction was sometimes unusual in that it was seen coincidentally with ENL.

While the peak incidence of new cases of reversal reaction occurred immediately after delivery, new and recurrent episodes also occurred late in lactation, suggesting that residual Schwann cells in the nerve trunks contained small numbers of bacilli which previously went unrecognised (Weddell and Pearson, 1975), but with recovery of CMI were recognised and attacked. Alternatively, late reaction may be caused by the release of sensitised lymphocytes which can be trapped in the spleen for prolonged periods (Bullock, 1976).

iii) ENL (type 2 reaction)

Our observations confirmed other reports of an increase in ENL during pregnancy (Trần Đình Đề, Hoang Ngoc, Minh and Cao Minh Trung, 1964; Maurus, 1978; Jopling, 1978c) and during lactation (Lawson and Stewart, 1967; Jopling, 1978c). After a peak in the first trimestre, the reaction was associated with exacerbation of leprosy and occurred throughout pregnancy with a second peak in the third trimestre (Figure A1(ii), gradually decreasing during the first year of lactation. The incidence is higher than would be expected in non-pregnant patients on treatment, particularly in the BL group, and is probably associated with the suppression of CMI, increased multiplication of bacilli and hence increased antigen load, and a tendency to shift towards the lepromatous pole (Waters et al, 1971).

2. New cases, relapse of cured patients and deterioration in patients on treatment during pregnancy and lactation

One hundred and fourteen women with leprosy and 33 women without leprosy were studied during 120 and 36 pregnancies respectively. For simplicity, each pregnancy is considered as one woman. Two healthy controls developed leprosy during the study period; 12 of 25 women with cured tuberculoid leprosy relapsed with new lesions or nerve damage; 47 of 95 women with active tuberculoid and lepromatous leprosy showed increased activity of their leprosy either as a transient phenomenon (21 patients) or due to probable dapsone resistance (28 patients). The deterioration in leprosy status occurred chiefly during the third trimestre (Figure A2(i)).

Protection and survival of the foetus as an allograft is the result of adaptive maternal responses to pregnancy including transient suppression of CMI (Thong et al, 1973). Suggestive evidence for this is the increased survival time of adult skin homografts on pregnant hosts, especially during the third trimestre, compared with non-pregnant hosts (Andresen and Monroe, 1962); the depression of tuberculin sensitivity in the third trimestre of pregnancy (Lichtenstein, 1942); the increased severity of certain viral diseases during pregnancy (Greenberg et al, 1958; D'Cruz, Balani and Iyer, 1968; Pickard, 1968); and amelioration of disease such as rheumatoid arthritis (Hench, 1938 and 1949), ulcerative colitis (de Dombal et al, 1965) and sarcoidosis (Siltzbach, 1965) during pregnancy with deterioration postpartum. The pregnancy-associated alterations of these conditions pertain to cell-mediated immune reactions (Beer and Billingham, 1976d).

Host resistance to mycobacterial disease is dependent on CMI and can be measured in vitro by LTT. Results of such tests, using PHA and PPD, indicate suppression of CMI during pregnancy which ceases at delivery or shortly afterwards (Purtilo, Hallgreen and Yunis, 1972; Finn et al, 1972; St. Hill and Finn, 1973; Smith, Caspary and Field, 1972). It is possible that pregnancy-associated α-macroglobulin plays a part in this process (Stimson, 1976). In pregnant leprosy patients it is likely that plasma contains suppressive factors in addition to those normally associated with pregnancy, as plasma from mothers with leprosy had a greater

inhibitory effect on their babies' LTT than plasma from healthy mothers (Bjune et al, 1978).

Before the era of chemotherapy it was observed that there was a sex difference in the mortality from tuberculosis: according to the United States Census Bureau Statistics there was a consistently higher death rate in females aged 15 to 25 years of age from 1900 to 1942 (Rich, 1951). It was well recognised that pregnancy had an adverse effect on tuberculosis. In many cases the first sign of tuberculosis was observed soon after parturition and where tuberculosis was already established, mortality was increased during later pregnancy and the puerperium (Norris and Landis, 1918; Bridgman and Norwood, 1926; Robinson, 1931; Nicholson, 1932 and 1933), although with proper sanitorium care throughout pregnancy, the danger was greatly diminished if not avoided (Jennings, Mariette and Litzenberg, 1932). A similar adverse effect of pregnancy on tuberculosis was observed in cattle (Smith, 1917) and experimental animals (Hodes, 1939).

In leprosy the overall prevalence in men is greater than in women. However, women appear to develop the disease at an earlier age than men. For instance, among leprosy patients in India 50% of the women had developed leprosy by the age of 20 years, compared with 30% of men (Rogers and Muir, 1946). In Ethiopia as many as 75% of female patients in the studies of the Medical Research Council Leprosy Project had developed leprosy by the age of 20 (Duncan and Pearson, unpublished observations). It is tempting to link this early onset with an increased risk of infection and rate of evolution associated with increased endocrine activity during puberty and suppression of CMI in frequent pregnancies during the late teens.

In leprosy where the host resistance is dependent chiefly on CMI, one would expect pregnancy to be associated with i) the first appearance of leprosy; ii) relapse in cured patients; and iii) increased activity of the disease with a tendency to shift towards the lepromatous end of the spectrum and increase in bacillary load. These features were all seen in our study.

i) New Cases. In Addis Ababa the new case rate for the city is 1 per 3,000 population; in the villages surrounding the

Leprosy Hospital the rate is higher, 1 per 1,000 population (0.1%). It is therefore significant that of 33 women observed during 36 pregnancies, 2 (5.6%) showed the first sign of disease during the third trimestre or early lactation. Our observation confirmed earlier reports (Tajiri, 1936; Ryrie, 1938; Rogers and Muir, 1946; King and Marks, 1958; Lawson and Stewart, 1967; Hardas et al, 1972; Coudert et al, 1978). Women already infected with M. leprae and incubating the disease show overt leprosy in late pregnancy or early lactation as a result of decreased host resistance of pregnancy (Tajiri, 1936; Lawson and Stewart, 1967; Coudert et al, 1978).

ii) Relapse of "cured"patients. The relapse rate in patients with cured TT and BT leprosy in Ethiopia has been reported by Touw-Langendijk and Naafs (1979) as 5% per annum. A considerable number of patients relapsed because they had been misclassified as BT rather than BB or BL, and thus had received inadequate treatment prior to stopping therapy. Our observation that 9 "cured" TT and BT patients relapsed with active leprosy (3 as BL and 6 as BT) confirms the above findings. While the original clinical diagnosis had not been in doubt in any of our cases and all were BI negative, none had had histological confirmation. In pregnant women the skin lesions may not be typical of either BT or BL leprosy, thus causing difficulties in clinical classification as happened with two of the patients who relapsed in our study (Table A2(i)). Ideally, (to ensure adequate treatment) histological confirmation and classification is recommended in all patients, especially women presenting with overt leprosy in association with pregnancy or lactation. It is also possible that the initial "BT" classification in our patients was correct but that 3 of these women who were all parous had downgraded to BL during a previous pregnancy.

Nerve damage was a feature of relapse in BT/RFC patients, 4 out of 8 had nerve damage early. This is the same as is found in early active tuberculoid leprosy (J.M.H. Pearson, unpublished observation). The observation of Naafs (B. Naafs,

personal communication) that "rheumatism" was a symptom of relapse in these patients was confirmed in this study although we found it was more consistently a symptom of "late silent neuritis".

It has been suggested that pregnancy be regarded as a test of cure of leprosy (Lawson and Stewart, 1967). Seven of the 9 women who relapsed with active leprosy did so during the first pregnancy after stopping treatment and 2 relapsed in the second pregnancy after stopping treatment (Table A2(i)). Thus one pregnancy cannot be regarded as a test of cure and we recommend that all women with "cured leprosy" who have stopped therapy, be carefully assessed during and after all subsequent pregnancies if late nerve damage is to be avoided.

iii) <u>Increased activity of leprosy with a tendency to shift towards the lepromatous end of the spectrum and increase in bacillary load</u>

We observed increased activity of their leprosy in just under half of the patients with active TT or BT leprosy (8/18) and in rather more than half (39/72) of the BL and LL patients who were followed up. The very high rate of relapse or deterioration of leprosy status, half of which appeared to be a transient phenomenon, would undoubtedly have been overlooked had these patients not been assessed frequently with the use of routine skin smears and biopsies even in the absence of skin lesions. The importance of carrying out routine skin smears at regular intervals cannot be over-emphasised, as it is only by so doing that relapse can be detected early (Browne, 1977; Jopling, 1978d).

The increased activity of the patient's leprosy recorded in 17 of the 90 patients was diagnosed on the basis of a rise in BI and/or MI or on increased activity at the histological level in women who did not at any time during the study show new or active skin lesions. The timing and transient nature of this phenomenon was of interest in that it was related to the third trimestre of pregnancy when CMI would be maximally suppressed. A similar observation was made by Browne who refers to a transient non-significant rise in BI which he attributes to hormonal disturbances of pregnancy (Waters, 1969).

However, by having the opportunity to follow up these Ethiopian patients, we found that 6 out of 16 lepromatous women who had a transient rise in BI during pregnancy developed the clinical picture of dapsone resistance during the next 15 months (4 with new nerve damage) and 9 others developed new nerve damage during lactation. Thus, a transient rise in BI during pregnancy can no longer be considered as insignificant.

The conversion to BI positive with increase in size and number of new lesions in BT patients, the tendency to down-grade from BL to LL during pregnancy with upgrading following delivery, and the onset of leprosy with reversal reaction during early lactation (Rose and McDougall, 1975) are evidence of the increased instability of women with leprosy during pregnancy, especially those classified as borderline.

3. Pregnancy and dapsone resistant leprosy

Sixty-seven women with lepromatous leprosy were studied during 72 pregnancies and followed up during lactation; 6 patients (7 pregnancies) were already dapsone resistant and an additional 5 were receiving dapsone 100 mg daily under trial conditions for suspected dapsone resistance. During the study, 28 patients (29 pregnancies), including the 5 already suspected of having dapsone resistance, relapsed with probable dapsone resistance, 7 of them being confirmed by mouse foot-pad inoculation.

Emergence of dapsone resistant leprosy occurs more frequently when the dosage of dapsone is low or irregular; thus it will be associated with poor patient compliance in taking dapsone regularly. Studies in Ethiopia have indicated that outpatients swallow approximately half the dapsone issued to them (Low and Pearson, 1974). This is the usual finding in such studies, though figures as high as 89% have been reported in other parts of Africa (Huikeshoven and Bijleveld, 1978).

In this study, in response to questioning, less than 10% of women stated they had stopped taking dapsone for a few weeks during the first trimestre on account of emesis gravidarum. They all had resumed treatment during the second trimestre. The rest of the women said they never stopped taking dapsone. Furthermore, it appeared to be generally believed that dapsone (unlike some other drugs) would not harm the foetus. The degree of patient-doctor contact was high, and it was considered probable that the women took treatment regularly. However, from the results of the mouse foot-pad tests (Table A3(i)) there is evidence that patients 2-7 could not have been taking dapsone regularly or fairly regularly, otherwise the lower level of dapsone resistant mutants would have been killed. The failure of compliance demonstrated by these patients is disturbing. Nevertheless it follows the pattern of other diseases; attempts to improve compliance by educating diabetic, hypertensive, or tuberculosis patients about the importance of regular treatment have all failed (Haynes, 1979).

In Ethiopia where dapsone resistance has become a major problem, dapsone resistance at a concentration of dapsone 0.0001% in the diet is referred to as low grade resistance and has been

shown to respond, for a period of up to 4 years, to treatment with 100 mg dapsone daily (Pearson et al, 1977a), but as these patients harbour a number of more highly resistant dapsone mutants (Waters, 1977), in time resistance to higher dosage of dapsone emerges in a stepwise fashion (Pearson et al, 1977a; Waters, 1977). This is in contrast with the single step emergence of resistance to rifampicin (Jacobson and Hastings, 1976). Recurrent pregnancies by providing periods of physiological suppression of CMI could well be a factor in contributing to the progression of dapsone resistance among women.

The suppression of CMI during pregnancy is also probably responsible for the extremely rapid deterioration observed during the third trimestre of pregnancy - 3 to 6 months for half of our patients compared with 12 months for clinical relapse in a male patient under closely controlled conditions (Jopling et al, 1979). Downgrading and upgrading in association with relapse, occurring during pregnancy and lactation respectively, is further evidence of the increased immunological instability associated with pregnancy.

It may be argued, and indeed some eminent obstetricians with little knowledge of leprosy have done so, that the apparent deterioration of leprosy during pregnancy and early lactation is nothing more than the natural process of the disease. If this were the case, one would observe the appearance of dapsone resistance in male and female patients to occur after the same duration of dapsone monotherapy, assuming that therapy was equally available to patients of both sexes. In Ethiopia, the problems encountered in assessing and treating female patients, as encountered in India (Cochrane et al, 1938) and some Islamic communities, do not exist and we have no reason to assume that male or female Ethiopian patients are treated differently. Analysis of the results of the questionnaire and review of hospital records of women with dapsone resistance attending the MRC clinics in Addis Ababa has shown that dapsone resistance appears earlier in women than in the group of patients including men and women (Figure A3(i)) with an average duration of treatment of 7 years compared to 10 years. Of the women with dapsone resistance, dapsone resistance appeared earliest in the women who were known to have had one or more pregnancies even though in some cases the pregnancy only amounted to a late

first or early second trimestre abortion. Thus it appears that pregnancy does indeed have a detrimental effect on the patient's leprosy.

The association of pregnancy and the emergence of dapsone resistant leprosy is clear from the obstetrical histories of women already diagnosed as having developed dapsone resistant leprosy. It is fully confirmed by this prospective study. Indeed the difficulty is not to establish the relationship but to account for the excessively high incidence in the trial patients during the study period. Possible sources of error include :

i) Selection of patients. Although to the best of our knowledge no special selection of patients occurred, it is possible that patients who were already feeling that all was not well regarding their leprosy opted to be in the study, thus applying some degree of self selection.

ii) Overdiagnosis of relapse. This is not a serious possibility. The clinical and laboratory findings supported each other in most cases, as most of the patients showing (at first) only laboratory evidence of relapse, had relapsed clinically by the end of the study.

iii) Overdiagnosis of resistance. This again is unlikely. Four of the 6 patients already dapsone resistant (following relapse in a previous pregnancy) and 7 patients in the present study were tested by mouse foot-pad tests,and none showed dapsone sensitive bacilli.

iv) One possibility is that in the early stages of emergence of dapsone resistant leprosy the clinical signs are labile, and that relapse lesions might resolve between pregnancies, the condition progressing in a stepwise fashion. The relatively short period of this study prevents any definite conclusion,but when last seen 3 of the 24 patients suspected of dapsone resistant leprosy were still improving on dapsone monotherapy.

4. Erythema nodosum leprosum (ENL) in pregnancy and lactation

Seventy-six women with lepromatous leprosy were studied during 81 pregnancies and followed up during lactation for up to 24 months. Ten out of 45 BL patients (22%) and 20 out of 36 LL patients (56%) developed ENL during the course of the study. Only 4 out of 30 patients were BI negative, although the duration of effective treatment for leprosy ranged from 1 to 14 years. Twelve of the 30 ENL patients were suspected of developing dapsone resistance during the study period. The incidence of ENL was highest in the first trimestre with a second peak in the third trimestre, coinciding with the peak of relapse. Fifteen per cent of the women suffered from ENL almost continuously from the third trimestre to 15 months postpartum. In pregnancy ENL was seen more frequently in skin than in nerve or other tissue; however, after delivery, particularly in the recurrent or persistent episodes, ENL was seen more commonly in nerve than in skin. Significant sensory and/or motor loss occurred in 30 out of 38 episodes of ENL nerve involvement.

It has been generally accepted that lepromatous patients who develop ENL do so within the first 2 years of treatment. An overall rate of approximately 40% has been recorded, of which 90% of LL(LL_p), 62% of LI(LL_s) and 32% of BL patients suffer from the reaction within $2\frac{1}{2}$ years of starting treatment (Ridley and Waters, 1969). It has been suggested that late ENL may be the first indication of the development of dapsone resistance, but studies in Ethiopia failed to confirm this (Pearson et al, 1979). Release of antigen during initial therapy, together with immunoglobulin and complement has been thought to form the immune complexes which have been considered as causative of most cases of ENL (Wemambu et al, 1969; Waters et al, 1971; Gelber et al, 1974). Pregnancy has been reported as precipitating ENL and relapse without comment on mechanism (Jopling, 1964; Trần Diñh Dê et al, 1964; Lawson and Stewart, 1967; Jopling (1978c); Maurus, 1978).

During normal pregnancy there is an increase in the number of antibody-antigen complexes, particularly towards the end of pregnancy (Masson et al, 1977; Levinsky et al, 1978), but the

implications of this observation remain obscure. In studying patients with leprosy, it is tempting to link this general observation with the increased incidence of ENL observed in our study and assume a cause and effect association. However, this would not explain the initial peak of ENL in the first trimestre. During the first trimestre of pregnancy there is inversion of the T-cell/B-cell ratio due to physiological depletion of suppressor T-cells (Strelkauskas, Wilson and Dray, 1975).

The hypothesis that the initial phase of ENL is due to imbalance of T-cell subsets with decrease in the population of suppressor T-cells (Mshana, 1982) presents an interesting alternative which might well account for the upsurge of ENL seen in the first trimestre. Further evidence for the implication of disturbed T-cell function in the pathogenesis of ENL may be seen in the "clinically puzzling mixtures of type 1 and type 2 reaction" observed in some of our patients associated with relapse (Duncan and Pearson, 1982). Furthermore, pregnancy in leprous women is often associated with relapse in "cured" patients, and with a tendency to increased activity in patients under treatment, accompanied by a shift towards the lepromatous end of the leprosy spectrum (Duncan et al, 1981a), all of which are likely to be associated with an increase in levels of mycobacterial antigen.

In 11 out of 21 cases, ENL preceded or accompanied the first sign of relapse, which in these patients was associated with dapsone resistance (Duncan et al, 1981b). It may well be the case that in pregnancy where there is a state of immunological instability, ENL is a significant symptom in, or sign of, the development of dapsone resistance.

Probably the most important observation in this study is the association between ENL and severe nerve damage. It is sometimes stated that although ENL can occur in nerves, causing severe nerve tenderness, major nerve damage does not usually occur. For this reason, treatment with corticosteroids is frequently eschewed and most patients are treated symptomatically. While this may be true of non-pregnant patients, the same cannot be said of women during pregnancy or lactation. An increased prevalence of neuritis has been recorded in association with pregnancy and lactation

(Duncan and Pearson, 1982). It may be that with alterations of CMI during pregnancy and lactation, nerves previously considered as privileged sites for M. leprae become especially vulnerable.

The observation that ENL is more common in the skin during pregnancy and more common in nerve during lactation may be of interest in reappraising the pathogenesis of ENL. A similar observation has been made regarding the timing of reversal reaction in association with pregnancy and lactation (Duncan et al, 1982). It is tempting to suggest that as in reversal reaction where surface antigens of M. leprae play a part in skin reactions while cytoplasmic antigens are responsible for reactions in nerve (Barnetson et al, 1975), in ENL, skin reaction occurs during the period of relapse when surface antigens are predominant and nerve reaction is seen as cytoplasmic antigens are exposed. Or, if, as has been suggested, the CMI plays a part in the pathogenesis of ENL, a further explanation for the occurrence of ENL in the skin during pregnancy and in nerve during lactation could be as follows. During pregnancy, due to suppression of CMI, immune responses are weaker, hence antigen in nerves is less accessible and remains "hidden" until postpartum when reactions are observed in nerve. Reaction in skin, due to the antigen being more accessible, can occur during pregnancy even though the immune response is diminished (D.S. Ridley, personal communication).

Evaluation of lymphocyte transformation in pregnant women who were classified as cured tuberculoid (BT/RFC) increased our understanding of some of the hitherto "bizarre" results observed in some women with borderline leprosy in reaction (G. Bjune, personal communication). In similar fashion, a prospective study of a few well-controlled lepromatous patients (LL_p, LL_s and BL) with ENL in early pregnancy, including total white cell count, measurement of T and B-cells and LTTs, might throw light on the pathogenesis of ENL in terms of T and B-cell function and response to surface or cytoplasmic M. leprae antigen.

During the course of the study the coincidence of ENL and reversal reaction was observed in patients who relapsed during late pregnancy and the puerperium. It is possible that some of the cases of late silent neuritis seen in lepromatous patients with ENL

were actually due to reversal reaction rather than to ENL. A further study with serial nerve biopsies during pregnancy and lactation would be necessary to provide factual information on this point.

The role of infections other than leprosy in triggering off ENL is seen in 2 patients reported in detail. Persistent ENL has been associated with underlying tuberculosis (Pearson, unpublished observations), only remitting after treatment for tuberculosis was established. As both tuberculosis and endemic typhus are controlled to a variable extent by cell mediated immunity, it is possible that T-cell suppression associated with those infections causes ENL, thus providing further supportive evidence for Mshana's hypothesis.

The significance of ENL in association with pregnancy and lactation is :

i) first occurrence in early pregnancy when the woman may not even realise that she is pregnant, especially if she is still lactating after her previous pregnancy;

ii) significant morbidity during pregnancy and lactation, possibly necessitating hospital treatment as an in-patient;

iii) association with relapse/dapsone resistance;

iv) potential cause of permanent nerve damage; and

v) necessity for additional drug therapy.

In ideal circumstances unexplained ENL occurring in a woman treated with anti-leprosy drugs for more than 4 years should alert the leprologist to carry out a urinary pregnancy test, a urinary examination for levels of dapsone, initial assessment for early dapsone resistance and assessment of motor and sensory nerve function.

5. Leprosy neuritis in pregnancy and lactation

Of 116 women with leprosy and 31 healthy controls studied during 122 and 34 pregnancies respectively, and followed up during lactation, 51 leprosy patients and one healthy control developed leprosy neuritis. Neuritis was accompanied by type 1 and type 2 lepra skin reactions and/or deterioration of the patients' leprosy status; this was particularly the case when neuritis was associated with nerve pain or tenderness (overt neuritis). Neuritis without nerve pain or tenderness (silent neuritis), preceded by the complaint of "rheumatism" and the clinical finding of enlarged peripheral nerves, was seen more frequently than overt neuritis (48:37 episodes).

These findings are summarised simply. Nearly half of the Ethiopian women with leprosy who were studied suffered from deterioration of nerve function during a single pregnancy and/or during lactation. All patients, including those with tuberculoid leprosy treated for some years and apparently "cured" (RFC), were at risk. Insidious silent neuritis, leading to sensory and motor nerve damage during lactation, was a particularly dangerous and hitherto undescribed risk of pregnancy.

Overt neuritis is usually associated with reaction in leprosy, and is a manifestation of the reactional process as it occurs within nerves. Thus, in borderline and tuberculoid leprosy, there is initial oedema of the intraneural granuloma, and subsequently increased granuloma formation as the immune response to M. leprae progresses, so that Schwann cells are progressively replaced by epithelioid granuloma. The patients most at risk were those classified as BL, though newly diagnosed or relapsing BT or TT cases could also be affected. The neuritis usually started after delivery, thus coinciding with recovery of cell mediated immunity (CMI) after the immunosuppression of normal pregnancy. It has been shown that there is some increased bacillary multiplication during pregnancy, in some cases as a transient phenomenon (Duncan et al, 1981a), due to lowered host CMI and possibly also reluctance of women to take any medication, including anti-leprosy drugs, during pregnancy. Peripheral nerve is a partially immunologically privileged site (Weddell and Pearson, 1975) and the immuno-

suppression of normal pregnancy might therefore be particularly influential.

The description given by Rose and McDougall (1975) of adverse reactions following pregnancy in untreated patients with "dimorphous" leprosy gives us the picture of evolution of leprosy during late pregnancy or early lactation as a result of suppressed CMI and occurrence of skin and nerve reactions during lactation due to the recovery of CMI after delivery.

In lepromatous leprosy overt neuritis probably represents intraneural ENL. This reaction requires the presence of M. leprae within the nerve, and appears to be more common and severe when there is a high concentration of intraneural antigen. Thus bacillary multiplication unchecked by chemotherapy (as when a patient is developing dapsone resistant leprosy) or CMI (suppressed during pregnancy) is a likely precipitating factor that applies to many cases in this series. Neuritis in these patients was liable to occur at any stage of pregnancy or lactation.

While any form of neuritis is of concern to both patient and leprosy worker, overt neuritis is more readily diagnosed and therefore treated, and hence is the lesser evil for the lactating mother. Silent neuritis with its slow insidious progress means that permanent nerve damage can occur before the patient, or leprosy worker, is aware that anything is wrong. The diagnosis of silent neuritis was made possible in this study by regular sensory and motor function tests; however, among our patients persistent qurtimat ("rheumatism") was frequently associated with silent neuritis, and indicated that something was wrong. This symptom and its significance will be discussed later.

The aetiology of silent neuritis remains uncertain. One obvious possibility is that it is due to late fibrosis in nerves that have previously been damaged by intraneural granulomata. In this study, however, "new active demyelination" was reported in several patients with silent neuritis during lactation. Segmental demyelination at sites where bacilli were not present has been demonstrated in all types of leprosy (Antia, Shetty and Mehta, 1980). However, the apparent localisation of nerve damage to sites where they are particularly vulnerable to physical changes

in their environment makes autoimmune demyelination unlikely as a cause of silent neuritis.

Silent neuritis appears to cause more damage to sensory nerves than to motor nerves. This suggests that the process may cause damage at both dermal nerve and nerve trunk levels. The reversibility with corticosteroid treatment implies an immunological cause. This possibility is supported by the work of Bullock on trapping of sensitised lymphocytes (Bullock, 1976), but as little is known of immunological or endocrine function in women who prolong lactation for 2 or more years, it is difficult to discuss possible immune mechanisms.

Further studies, including nerve biopsies, will be required to elucidate the mechanisms of neuritis in lactating women.

6. The message of "Rheumatism", a forgotten symptom in leprosy

"Rheumatism" as a symptom in leprosy has received little attention in recent years, although the nineteenth century writers acknowledged its importance. In this prospective study, "rheumatism" as a symptom was recorded and correlated to relapse, reaction, neuritis and new nerve enlargement, lymphadenopathy and paraesthesiae. "Rheumatism" was experienced by 32% of "cured" TT and BT patients, and 50% of women with TT and BT, BL and LL leprosy receiving treatment; in contrast, only 2 healthy controls complained of "rheumatism" and one of those developed overt leprosy shortly afterwards. Relapse was seen more than twice as frequently in those who complained of "rheumatism" as in those who did not; reaction (ENL and reversal reaction) was 6 times more common in the "rheumatism" group; new nerve enlargement was seen 4 times as often and neuritis 8–14 times as often in those with "rheumatism" as those without; lymphadenopathy and paraesthesiae were found 5 times more often in the "rheumatism" group. A particularly sinister triad for the lactating mother was "rheumatism", new nerve enlargement and silent neuritis.

In the symptomatology of leprosy, loss of sensation rather than pain has been the feature receiving most attention (Danielssen and Boeck, 1848m; Hansen and Looft, 1895i). One of the earliest references to pain, in the symptomatology of leprosy, is to be found in the account of Rev. Andrew Fisken describing the case of the lepers in Papastour (1736) :

> "... and in the night time they are much troubled with deep-seated pains in their bodies, and have in the day-time frequent stitches and pains in all parts of their body ..." (Simpson, 1841a).

Douleurs rheumatismales were included in the prodromal symptoms of lepromatous leprosy while douleurs de jambes accompanied trophic ulceration with lymphadenitis (Danielssen and Boeck, 1848n). Neisser (1885e, Roose (1890g) and Leloir (1896g) all distinguished between douleurs rhumatoïdes (pains resembling rheumatism) and douleurs névralgiques (neuralgia or shooting pains along nerve trunks), as prodromal symptoms. Hansen and Looft (1895j) considered the rheumatoid "prodromal" pains of lepromatous leprosy to be a part of the disease, and rheumatoid pains of joints and

muscles to be prodromal symptoms of tuberculoid leprosy. Leloir observed that douleurs rhumatoïdes, especially in the legs could hamper mobility considerably and were frequently mistaken by doctors and sufferers as being due to a rheumatic disease (1896h); such a case has been reported recently (Fekete, Sarojini and Mock, 1982). Bowerbank (1867) described rheumatic pain about the extremities as a prodromal symptom of leprosy (Report R.C.P., 1867g),Thomson (1897b) recorded severe generalised body pain, especially in the joints, preceding the skin lesions of "mixed" (borderline) leprosy, while Impey (1895b) noted cessation of the pains with the self-cure of the disease.

Since the early classic descriptions, pain has been associated with reactions in leprosy and the painful lesions of ENL are well documented. In pregnant lepromatous women des arthralgies have been recorded as being a feature of fièvre de réaction (ENL) (Trần Diñh Dê et al, 1964). In Zaire anatisme, generalised vague pains, was a problem of pregnancy and accompanied exacerbation of pre-existing but apparently quiescent leprosy. It sometimes preceded by some months the appearance of skin lesions typical of BT leprosy; thus anti-rheumatic therapy was given, without effect, before leprosy became overt (E. Staples, unpublished observations). "Pseudo-rheumatism" has accompanied generalised lymphadenopathy in "mixed" leprosy prior to the appearance of lesions typical of reaction (Jeanselme, 1933e).

Reference to more generalised type of pain have on the whole been associated with descriptions of neuritis, namely nerve swelling accompanied by severe pain both in the main nerves and in their distribution (Rogers and Muir, 1946e), and with a neuritic type of pain which occurred before areas of anaesthesia or skin lesions were noticed (Cochrane, 1964; Bryceson and Pfaltzgraff, 1979b). Muscular discomfort and difficulty in walking has been recorded in a patient with leprous nodular interstitial neuritis (Jopling and Mehta, 1972). In 1956, Jopling described a BT patient who developed pains in the arms and legs just before the appearance of skin lesions of reversal reaction. Cochrane (1964), however, observes that the recording of subjective symptoms depends on careful questioning by the physician, implying that symptoms of unlocalised pain frequently go unrecorded.

In the present study, only 2 of the 33 healthy controls complained of "rheumatism", although they lived in the same environment as the leprosy patients; one of them was later shown to have developed leprosy. Among the 25 apparently cured TT and BT patients, there were 12 cases of relapse; all 8 of the women who complained of "rheumatism" relapsed, 7 with neuritis. The significance that the patients attached to "rheumatism" as a symptom was clear from a remark made by one of the "cured patients" - 'I think I have leprosy again because of the"rheumatism"'. However, 4 of the 15 BT and TT patients without rheumatism relapsed; the absence of rheumatism was not evidence that all was well with the patient.

Among the active BT and TT cases, there were 8 patients who showed deterioration of their disease, but in 5 of them, it was only transient; worsening of leprosy was almost as common among non-rheumatism cases as among those complaining of "rheumatism". Neuritis, however, was 4 times as common among the "rheumatism" group, affecting all 8 patients. In these patients "rheumatism" most commonly preceded, and was a warning of, the development 1 to 6 months later of nerve enlargement and silent neuritis. Such cases were easy to overlook, particularly if "rheumatism" was attributed to cold, wet weather and inadequate clothing or housing. "Rheumatism" was twice as common in the group of patients treated for less than 2 years compared with the group treated for more than 2 years. This confirms Barnetson's observation that "limb pains" were associated with activity of the disease in patients with borderline leprosy, and provided some evidence that those patients were taking their prescribed treatment.

Among patients with lepromatous (BL and LL) leprosy, overt relapse occurred about twice as frequently in patients with "rheumatism"(17/40 = 43%) as without (9/37 = 24%). "Rheumatism" clearly preceded relapse in 10 cases (38%), indicating that Naafs' observation in tuberculoid patients is applicable to lepromatous patients as well.

Neuritis in lepromatous patients was 4 times more common in patients with "rheumatism" (27/40 = 68%) as without (6/37 = 16%); the neuritis was more commonly overt, though the triad of

"rheumatism", nerve enlargement, and silent neuritis was seen occasionally.

Paraesthesiae in general occurred more commonly among patients with "rheumatism"; they tended to occur during pregnancy and be associated with skin reactions, both reversal reaction and ENL.

Lymphadenopathy occurred in over half of the patients with "rheumatism" (regardless of classification) but only in 12% of the 98 patients with no rheumatism (all were lepromatous apart from one non-leprous control who had lymphadenopathy in association with reactivation of cutaneous leishmaniasis). Its significance is uncertain.

In general, it is clear that pregnancy is a dangerous venture for leprosy patients. Among apparently cured patients who have stopped treatment, there is considerable risk of relapse; among lepromatous cases there is risk of reactivation due to the emergence of dapsone-resistant leprosy; and there is always a risk of the development of nerve damage, including particularly an insidious silent neuritis occurring during lactation. These problems are not always signalled by the complaint of "rheumatism". Nevertheless, it is rare for "rheumatism" to occur without some objective evidence of nerve damage or relapse. The message of "rheumatism" is clear: when a leprosy patient complains of "rheumatism"during or after her pregnancy, something is almost certainly wrong. The responsibility of the clinician is to elicit what is wrong and treat it.

APPLICATION OF OBSERVATIONS

1. Instruction of all leprosy workers

While further investigation is required to elucidate the mechanisms of the adverse effect of pregnancy on leprosy, the practical implications which should be made widely known to all leprosy workers are :

i) The pregnant woman, because of physiological suppression of CMI most marked during the third trimestre, is especially at risk. If she is a known leprosy contact incubating leprosy, she is most likely to show overt disease either in late pregnancy or during lactation when it may well be complicated by reaction. "Cured" BT patients run the risk of relapsing with active disease, and in patients receiving treatment for leprosy there is a 50% chance of the disease being aggravated with a shift towards the lepromatous end of the spectrum, increased bacillary load, subsequent risk of ENL and in the puerperium, reversal reaction possibly with severe nerve damage.

ii) In relapsing RFC patients and those who are developing dapsone resistant leprosy, with multiplication of viable bacilli during pregnancy, there is a real risk that the foetus may be infected in utero and go on to clinical leprosy in early childhood; furthermore, the woman herself will become a risk to her household and the community as she is likely to be infectious.

iii) Should any of the children of women with dapsone resistant leprosy develop leprosy at an early age, it is highly likely that they will have dapsone resistant leprosy - and hence would require alternative therapy.

iv) ENL may occur for the first time in early pregnancy when the woman herself may not even realise that she is pregnant. It may cause significant morbidity during pregnancy and lactation, be associated with relapse/dapsone resistance, be a potential cause of permanent nerve damage and necessitate additional drug therapy.

All drugs given during the first trimestre of pregnancy carry the risks of harming the foetus. Thalidomide, frequently used for treating ENL, is absolutely contra-indicated in all women of child-bearing age because of its teratotoxicity. Prednisolone given during the first trimestre carries a slight risk of causing cleft palate. It is suggested that the most suitable drug, currently available, for treating ENL in pregnancy is clofazimine.

v) Women with leprosy (even apparently cured) run a serious risk of deterioration of nerve function when they become pregnant. They may develop overt neuritis, or an insidious silent neuritis; in either case regular tests of nerve function are required to demonstrate nerve damage and follow the response to treatment.

vi) The complaint of "rheumatism" may be the first indication that something is wrong and merits immediate action.

In summary :

vii) BEWARE OF THE WOMAN WITH THE PROTRUBERANT ABDOMEN AND/OR THE BABY ON HER BACK!

Careful medical supervision during and after pregnancy should reduce the consequences (if not the incidence) of leprosy complications associated with pregnancy; awareness on the part of the community midwife/obstetrician of potential leprosy problems may play a critical part in their early recognition and correct management.

2. <u>Health education of women with leprosy</u>

The knowledge incorporated in 1. above should be incorporated into the health education of women in the reproductive age group. At the same time advice on family planning should be given so that as far as possible pregnancies can be postponed until after the leprosy is well under control.

3. <u>Increased surveillance during pregnancy</u>

i) For women with active leprosy: increased surveillance during pregnancy, a) to ensure a maximal patient compliance, if necessary substituting parenteral for oral dapsone therapy

during the first trimestre when emesis gravidarum is troublesome; b) routine assessments with skin smears and biopsies, as possible, during the second and third trimestre and at 3 and 6 months postpartum, by which time most relapses should have occurred.

ii) For women with cured leprosy (TT and BT/RFC): clinical assessment with particular attention to peripheral nerves during pregnancy and at 6, 12 and 18 months postpartum. Additional tests, namely skin biopsy,nerve biopsy (if possible), VMT, SST or NCV when relapse is suspected but clinical findings are not diagnostic.

iii) For healthy contacts, especially of infectious cases: assessment during the third trimestre of pregnancy and postpartum, ideally at 3 and 6 months.

iv) For the child born to a woman who has had an active relapse during pregnancy, there is risk of clinical leprosy in early childhood. This is likely to be of the indeterminate type and self-healing, particularly in the very young child,and probably occurs more frequently than realised hitherto. Regular inspection at child health care clinics when weighing and measuring the child, naked, provides diagnostic opportunities. A history of lactation should be obtained as anti-leprosy drugs are transmitted through the mother's milk.

4. Additional anti-leprosy drug therapy

The question of giving additional drugs in pregnancy requires careful consideration.

i) The possibility of giving supplementary chemotherapy in effective dosage during pregnancy and lactation.

This would aim both to prevent the emergence of dapsone resistant leprosy and also to lessen the risk of infecting the baby before and after delivery. Clofazimine (in the dosage of 100 mg at least 3 times a week) for one year starting at the beginning of the second trimestre would probably be the most suitable drug for the purpose, and would

have the additional advantage of reducing the amount of ENL occurring during pregnancy and lactation.

Clofazimine, however, has not been used extensively during pregnancy and all cases so treated should be carefully documented and reported. In rat pregnancy, clofazimine has been shown to cause abortion (Stenger et al, 1970) and in human pregnancy there is suggestive evidence that clofazimine suppresses the placental production of oestriol, although the significance of this is not clear (Duncan and Oakey, in press).

Alternatively, rifampicin 1200 mg could be given once monthly under supervision to all women with lepromatous and borderline leprosy after the end of the first trimestre. The once monthly dosage is better tolerated, much cheaper and as effective as daily rifampicin (Yawalkar et al, 1982) and could be administered when the patients attend for antenatal care. Such treatment would have the advantage of reducing numbers of any dormant bacilli present and preventing the relapse seen during late pregnancy, thus reducing the risk of exposure of the baby to M. leprae in utero and during lactation, and approaching the goal of making leprosy in childhood a preventable disease (Jayam et al, 1978).

ii) The possibility of giving prophylactic chemotherapy to patients at risk.

To prevent relapse in TT and BT/RFC patients, the recommendations made by Touw-Langendijk and Naafs (1979) must be practised. These are that very careful review of the patient's initial diagnosis, treatment, progress and date of leprosy "inactivity" be made before the patient is RFC. To this, two further recommendations are added: a) that no female patient be RFC either during pregnancy or within a year of delivery; b) that all pregnancies and dates of delivery should be noted in the patient's records. These two recommendations would reduce the number of patients being RFC prematurely. For those already RFC and becoming pregnant, increased surveillance during and after pregnancy and an awareness of the risk of relapse to allow early diagnosis is probably preferable to blind treatment of RFC patients for an empirical period of

time during and after pregnancy, which could mask clinical relapse.

5. Family planning advice

It would be reasonable to advise women with lepromatous leprosy to limit the size of their families by whatever means are locally acceptable. However, it should be remembered that the role of exogenous oestrogens (such as are found in the contraceptive "pill") in the causation of relapse is as yet unknown, and drugs such as rifampicin may render oral contraception less efficient; thus the use of oral contraceptives should be carefully monitored.

In communities where STD is prevalent, there is a very real risk that indiscriminate use of the intra-uterine contraceptive device (IUCD) will lead to endometritis, and acute and chronic salpingitis, causing troublesome (and culturally/socially unacceptable) bleeding, vaginal discharge, and pelvic and abdominal pain. The IUCD in such circumstances falls into disrepute. At the same time, it is well known that dapsone has a significant action against Neisseria gonorrhoea, to the extent that in Nigeria it was known as a fertility drug! Furthermore, there seemed to be very little symptomatic gonorrhoea amongst the women leprosy patients in Addis Ababa.

Personal interviews with patients who had suffered relapse or reaction in association with pregnancy not only revealed that the patients were aware of the adverse effect of pregnancy on leprosy, but in addition many patients volunteered the information that they wished for no more than one or two children at most.

To be effective, however, family planning advice must be linked with an efficient child welfare programme supplying curative and preventative health care, and health education regarding :

i) breast feeding, weaning and the best use of locally available foodstuffs;

ii) vaccination and immunisation;

iii) risks of local customs such as uvulectomy etc.

As most mothers with leprosy are concerned regarding the possible

transmission of leprosy to their children, this is probably the best time to give health education regarding the effect of pregnancy and lactation on leprosy, and of leprosy on childbearing.

With training, this sort of management could be provided in the context of a "vertical" leprosy control programme; it will be more difficult to ensure proper care in integrated primary health care programmes.

B. THE EFFECT OF LEPROSY ON PREGNANCY

1. Oestrogen excretion in pregnant women with leprosy: Evidence for diminished foeto-placental function

Oestrogen excretion was assayed in 64 women with leprosy and 15 healthy controls. The mean oestrogen excretion was lower in women with leprosy than in healthy controls, and the incidence of subnormal oestrogen values was higher in the leprosy patients than in the healthy controls. There was an association between the low infant birth weight and the frequency of subnormal oestrogen excretion. These features were most marked in women with lepromatous leprosy and are evidence of diminished foeto-placental function.

Oestrogen excretion in women with leprosy was further suppressed by prednisolone and clofazimine.

From the results obtained in this study, it appears that the difference in oestriol excretion in the different groups of mothers occurred after 32 weeks' gestation. The low levels of oestrogen in women with lepromatous leprosy is of great interest. In LL women 61.1% of the assays were below the lower limit of normal, compared with 36.0% of BL, 34.2% of TT and BT, and 21.7% of HC women. Furthermore, there was a statistical difference in the means in each group and a reduced range of oestriol levels towards the lepromatous end of the spectrum.

Drugs such as aspirin and ampicillin have been shown to reduce the excretion of oestriol without harm to the foetus (Castellanos et al, 1975; Willman and Pulkkinen, 1971). This effect may be due to drug interference with the method used for oestriol assays (Adlercreutz, 1975). Dapsone treatment had little effect on oestrogen excretion since there was no significant difference between the mean oestrogen excretion in 13 women with "cured" tuberculoid leprosy and in 7 women with active tuberculoid leprosy who received dapsone (50-100 mg daily). Nevertheless, in LL women receiving dapsone alone, 52% of oestrogen assays were subnormal.

In the BL and LL groups, there was an overall incidence of 20% of acute foetal distress or Apgar score of 4/10 or less.

While few of these women were amongst those who had oestriol assays, it is probably significant that BL and LL women had low oestriols and several of them delivered a baby that died shortly after birth.

The cause of low oestriols in LL women remains obscure. There seem to be three possible mechanisms:

i) the disproportionately small placenta as evidenced by the low placental coefficient;

ii) hypoplasia of the foetal organs responsible for the production of oestriol and its precursors;

iii) some vascular abnormality on the maternal side of the placenta in the decidua basalis.

i) <u>Disproportionately small placentae</u>

Using trimmed placental weight, as in this study, Younoszai and Hawarth (1969) showed that while the placental weight was decreased in intrauterine growth-retarded infants, the placental coefficient was normal. This is in contrast to the findings of this study that in addition to having small placentae, LL women had significantly lower placental coefficients, and hence disproportionately small placentae. Moreover, while Younoszai and Hawarth found the placentae of babies with intrauterine growth retardation to be as thick as those of normal babies, and hence to have a reduced decidual area, the placentae from LL, and to a lesser extent BL women, tended to be thin or very thin, and hence had less reduction of decidual area.

ii) <u>Hypoplasia of the foetal organs responsible for production of oestriol</u>

Naeye (1965) has shown marked adrenal hypoplasia in some small for gestational age babies born to diabetic mothers. While none of the women in this study were diabetic, 2 of the babies born to BL women and weighing less than the tenth centile were found at autopsy to have a small liver, 1 had small adrenals and both had very small thymus and spleen, using the standards of Schulz, Giordano and Schulz (1961)(Table B1(vi)). The role of the foetal adrenal in oestriol

TABLE B1(vi)

Weights of adrenals, liver, thymus and spleen (at autopsy) of two babies born to lepromatous mothers dying shortly after birth

	Baby 1		Baby 2	
	Weight of organ (g)	Percentage of normal*	Weight of organ (g)	Percentage of normal*
Adrenals	6.8	97.0	3.75	54.3
Liver	90.6	70.2	70.0	72.9
Thymus	3.3	38.0	3.5	56.5
Spleen	6.3	63.0	4.5	52.3

* Normal values for foetal organs according to gestational age (Schulz et al, 1962).

production is well known (Frandsen and Stakemann, 1961) and the foetal liver is also involved, particularly in the second trimestre. We were unable to carry out autopsies on any of the babies born to LL women, but it is likely that they would show similar hypoplasia of the same organs as those born to BL mothers.

iii) Vascular abnormality on the maternal side of the placenta

This is pure hypothesis based on the premise that women with lepromatous leprosy have small babies because of their depressed cell mediated immunity, and hence a poor immunological response to pregnancy.

Drug suppression of oestriol production

i) Prednisolone

Three women receiving prednisolone in excess of 75 mg cortisol equivalent per day had low oestriol levels but showed a rising trend, indicating that the foetus was not at risk, although one delivered a baby with birth weight less than the tenth centile.

ii) Clofazimine

As shown in Figure B1(iii), oestrogen levels in LL women receiving clofazimine were lower than those in LL women receiving dapsone. Clofazimine has generally been contra-indicated in pregnancy, because while its possible terato-toxicity in humans is unknown, it is known to cause abortion in rats (Stenger et al, 1970). With increasing dapsone resistance in leprosy and problems of ENL during pregnancy, clofazimine is being recommended as an alternative or in addition to dapsone therapy during pregnancy. There is clearly a need for further studies of the effect of clofazimine on oestrogen synthesis in the foeto-placental unit and on pregnancy outcome in humans.

2. Babies of mothers with leprosy have small placentae and low birth weights

One hundred and sixteen women with leprosy and 31 healthy controls were studied throughout 156 pregnancies, and their babies were observed for a period of up to 2 years. Babies of mothers with leprosy weighed less than those of healthy mothers; the placental weights and coefficients followed the same trend.

The normality of pregnancy and absence of obstetrical complications in leprosy patients has been well documented (King and Marks, 1958; Lawson and Stewart, 1967; Coudert et al, 1978), thus the cause of the low baby and placental weights observed in this study is not easy to define.

Zambaco (1897) observed that many of the children born to mothers with leprosy looked like "old men" or even "abortions at term" at the time of birth; these children were unduly susceptible to intercurrent disease. A recent study from Vietnam reported 19/64 (30%) "prematurity", more so in the women with lepromatous leprosy. From the report (Trần Diñh Dê et al, 1964) it is impossible to assess whether what is reported is true "prematurity" or "dysmaturity", namely infants born "small for gestational age" (SGA). Scattered reports throughout the older literature indicate that the observation of low birth weights in babies born to leprous mothers is not new, but this is the first study to correlate birth weight, placental weight, placental coefficient with the classification of the mother's leprosy.

In general, the causes of low birth weight include premature delivery; socio-economic factors; age, parity, and nutrition of the mother; obstetrical complications (multiple pregnancy, ante-partum haemorrhage and acute hypertension of pregnancy); inter-current infections in the mother; and immunological causes (Willcocks, 1971). But the babies whose birth weights are shown in Figure B2(i) were delivered at full term, and their mothers were comparable in regard to socio-economic status, age, parity and nutrition. Recent work has demonstrated that low birth weight is associated with low blood pressure in obstetrically normal women. Moreover, it now seems that many of the effects of under-nutrition are mediated through low maternal blood pressure and reduced utero-

placental perfusion (Naeye et al, 1980). No significant differences were found in either the peak systolic and diastolic or the means of the systolic and diastolic blood pressure in each trimestre. A haemodynamic cause of low birth weight in the BL and LL patients is therefore excluded. There were no cases of toxaemia or antepartum haemorrhage, and two sets of twins were excluded as were three babies born prematurely as a result of severe intercurrent infection in the mother. Therefore it seems certain that these babies were small because their mothers had leprosy.

Three factors associated with the disease itself can be excluded :

i) It could be postulated that the women with lepromatous leprosy were more ill than those with tuberculoid leprosy, and that the severity of their infection resulted in low birth weight of the baby. Such a finding was recorded by Coid and Wardman (1971) who reported that the severity of a maternal infection affected the foetus without the infecting organism being isolated from the conceptus. The degree of "sickness" of lepromatous patients can be assessed directly in terms of their bacillary load and indirectly in terms of their nutritional status. It was observed that their nutritional status was comparable and that there was no difference in the weight of the babies born to LL mothers with a high bacillary load (positive BI and MI) or a low bacillary load (negative BI and MI); and yet there was a significant difference between the babies of LL and BL mothers, both of whom had the same bacillary load. It would appear that the severity of the mothers' disease in terms both of nutrition and of bacillary load, can be ruled out as a cause of the low birth weights.

ii) Massive congenital infection (as occurs in syphilis, toxoplasmosis and malaria) involving the placenta and impairing its function; this was not observed histopathologically in these leprosy patients.

iii) "Toxic" damage to the placenta as occurs in animals injected with bacterial toxins (Coid, Lansdown and McFadyen, 1977).

This was not observed, nor is it likely that M. leprae can produce this type of toxin.

A further possibility that must be examined is that the anti-leprosy treatment was the cause of the low birth weight. This seems most unlikely. Mothers received the same dose of dapsone whether they were tuberculoid or lepromatous; but the TT and BT babies were not much smaller than normal. The four mothers on clofazimine had babies rather larger than the average in their group, as did those who received prednisolone.

It seems then that the low birth weights of these babies are due to immunological processes which are directly related to leprosy. Two main possibilities may be cited :

a) Deposition of immune complexes in the placenta. In leprosy, where circulating immune complexes are present, particularly in lepromatous patients (Bjorvatn et al, 1976), one might postulate that increased circulating immune complexes during pregnancy, if deposited in the placenta, could cause low birth weight. In this study, the placentae were all examined by light and electron microscopy and no evidence was found of any pathology which might be attributable to immune-complex deposition.

b) Depressed CMI in the mothers. All leprosy patients tend to have depression of their T-lymphocyte responses to both mitogens (Dierks and Shepard, 1968; Bullock and Fasal, 1971) and also to M. leprae (Godal et al, 1971), and this depression is certainly more marked in patients with lepromatous leprosy. The mechanisms by which depressed CMI causes low birth and placental weights, in humans, is obscure, but it has been shown in mouse experiments that mothers rendered tolerant at birth to paternal strain antigens produced smaller placentae than those from untreated mothers mated with allogenic males (James, 1965) and that both in experimental animals (Billington, 1964; Beer and Billingham, 1978) and in man (Jenkins and Good, 1972),histoincompatibility results in a larger placenta and larger babies. There is also suggestive evidence that women with drug induced immunosuppression produce babies of low birth weight (Scott, 1977).

Possible mechanisms by which female rats produced significantly smaller foetuses and placentae are suggested by the following experiments :

i) female Fischer rats pre-sensitized to DA tissue antigens and then mated with DA males produce (Fischer X DA)F_1 hybrid foeto-placental units that are larger and heavier than similar foetuses born by controlled unsentized mothers;

ii) the size and weight of (Fischer X DA) F_1 hybrid foeto-placental units of Fischer mothers rendered immunologically tolerant of DA tissue antigens were significantly smaller than those of untreated normal mothers mated with DA males. The weights of the foetuses and the placentae were assessed separately and both found to be significant.

The role of the draining para-aortic nodes and the spleen in maternal reactivity against foetal tissue histocompatibility antigens was assessed by experiments using Fischer female rates which were subjected to splenectomy or removal of para-aortic nodes or sham operation and a control group all mated with DA strain males. Both bilateral excision of para-aortic nodes and splenectomy reduced the mean weights of the placentae as well as the foetuses as compared with those in the control and sham operated series. As the spleen is the principal seat of the synthesis of antibodies when antigenic material is presented via the intravenous route, it seemed likely that humoral antibodies might be the principal mediators of the placental and foetal growth in utero. To test this premise, panels of Fischer females mated with DA males were passively immunised with Fischer anti-DA hyperimmune serum every third day starting after the implantation of the blastocysts. It was found that the foetuses and the placentae of these females were heavier than those of the allogeneic control group. Administration of serum from normal non-immune Fischer females to Fischer females bearing F_1 hybrid conceptuses had no influence on the weight of the latter (Beer, 1975).

In conclusion, it would appear that, due to depression of CMI, mothers with leprosy are incapable of producing an adequate cell-mediated immunological response to their pregnancy, and that this state of tolerance or partial tolerance tends to cause smaller placentae and smaller foetuses.

3. The placenta in leprosy

Twenty-five out of 78 women with leprosy and 17 healthy controls were considered to be potentially infectious on the grounds of having solid staining acid-fast bacilli in skin smears and/or biopsy.

The placentae of women with lepromatous leprosy were smaller than those with BL, BT and TT leprosy or the healthy controls. Of 7 women all with extremely active lepromatous leprosy, only 2 were found to have AFB and one had "? AFB debris" in smears from concentrated homogenates of the placenta. Placental morphology by light and electron microscopy showed no abnormality in any of the placentae, nor was there any difference between the placentae of women of different classifications of leprosy and healthy controls when placentae were examined by immunohistological methods. Placental enzyme studies showed a marked overall reduction in cytoplasmic protein relative to the size of the nucleus in the placentae from LL mothers. There was increased bilirubin in cord sera of babies of mothers with lepromatous leprosy.

Placental infection has been well established in pregnancies complicated by maternal tuberculosis in the pre-antibiotic era (Schmorl and Kockel, 1894; Schmorl and Geipl, 1904; Warthin and Cowie, 1904). The lesions vary from typical milliary tubercles to granulomata and large caseous foci. The position and type of lesions within the placenta suggests two likely modes of spread: direct spread,in cases of tuberculous endometritis, and haematogenous spread. It is this latter mode of spread which is of most interest to obstetricians.

It has been established that there is during pregnancy some non-specific suppression of maternal cell mediated immunity with impaired response of maternal lymphocytes to PPD and also skin testing with BCG. This would result in rapid progression of the disease in infected untreated women, hence the disease would become overt or deteriorate rapidly during pregnancy and immediately post-partum, as has been observed (see Discussion A2, pp.175-6). The resulting maternal bacteraemia could result in foetal infection via the placenta.

While congenital tuberculosis is not common, it is well documented (Corner and Brown, 1955). Unfortunately, there was little correlation of placental pathology with the degree and type of infection in the infant as most placentae were discarded after being recorded as macroscopically normal. Histological findings were recorded in only two cases where the placenta had "no gross pathologic change" (Hertzog, Chapman and Herring, 1949), or "the placenta was normal microscopically, but a direct smear of the placental material revealed the presence of acid-fast bacilli" (Harris and Trenchard, 1952).

A similar pattern of deterioration of the disease during pregnancy and early lactation has been observed in leprosy (Duncan et al, 1981a and b). Evidence for maternal bacteraemia and transplacental infection of the foetus was given by Rabinowitsch (1913) who observed acid-fast bacilli in the foetal heart blood at autopsy of an untreated lepromatous woman at 6 months' gestation. Other workers demonstrated the presence of acid-fast bacilli in cord blood of babies born to women with active lepromatous leprosy (Sugai and Monobe, 1913b; Montero, 1927; Trespalacios and Piñeyro, 1955). Further evidence for transplacental infection of the foetus has been :

a) raised IgA in cord blood of babies born to women with active lepromatous leprosy (Melsom et al, 1980a);

b) IgA, IgM and IgG anti-M. leprae antibodies in babies of lepromatous mothers at birth and during early extra-uterine life (Melsom et al, 1981 ; Melsom et al, 1982a); and

c) demonstration of raised IgA, IgM and IgG anti-M. leprae antibody activity in babies with clinical leprosy before the age of 18 months (Duncan et al, in press).

Specific study of the placenta has failed to demonstrate any morphological abnormality or lesions typical of infection by M. leprae except in one case, although acid-fast bacilli were demonstrated in placental tissue (Sugai and Monobe, 1913; Montero, 1927; Pineda, 1928; Cerruti and Bechelli, 1936; Inaba, 1938; Trespalacious and Piñeyro, 1955; Davison and Bernard, 1975). In some cases the M. leprae were clearly seen within macrophages

(Davison and Bernard, 1975). Our findings are in general agreement with those already reported although we were able to demonstrate acid-fast bacilli/debris only in three cases. It was postulated that although the criteria for maternal bacteraemia were present, due to the slow division time of M. leprae, there was insufficient time for the development of the typical inflammatory cell response to M. leprae.

The methods used until now for demonstrating the presence of M. leprae in the placenta, however, may not have been sufficiently sophisticated to show whether the M. leprae were on the foetal or maternal side. Particularly in the pre-dapsone era, women with lepromatous leprosy would have been liable to have a bacteraemia during pregnancy; thus one would anticipate that M. leprae in circulating macrophages could have been found in the placenta on the maternal side without any infection of the placenta having occurred. The use of fluorescent histological techniques and specific anti-sera in untreated lepromatous patients or in women relapsing with advanced degrees of dapsone resistant leprosy would be required to elucidate this point.

Other features of this study are, however, worthy of comment :

i) The placentae of women with leprosy were smaller than those of the healthy controls. This was most marked in the women with lepromatous leprosy. The smaller placentae seem to be due to the decreased cellular cytoplasm and hence smaller cell size, as evidenced by the increased levels of DNA protein in the placentae from lepromatous mothers. This is a very interesting observation and clearly demands further investigation.

ii) In contrast to the morphological changes seen in placentae from women with malaria (Galbraith et al, 1980), no morphological abnormality was detected when the placentae were examined by immuno-histological techniques.

iii) Electron miscroscopic examination failed to show significant morphological changes in placentae of women with leprosy. Furthermore, there was nothing to suggest the deposition of immune complexes on the trophoblastic basement membranes, possibly causing damage to the trophoblastic basement membrane or interfering with transfer of nutrients across it.

Possible significance of the placental findings to infant wellbeing

(1) Infection of the foetus in utero in the absence of placental infection. The placenta is a very large organ, and where lesions are small and scattered, the biopsied area may not contain evidence of tissue response to infection. Nonetheless, the results obtained by searching for bacilli by concentration techniques indicate that only very few M. leprae in fact lodge in the placenta even in women who have extremely active dapsone resistant leprosy who may have a considerable bacteraemia. In one of two placentae of "study babies" who were infected in utero we were able to find only scanty AFB in the placenta. Negative findings in the placenta do not rule out "transplacental transmission of leprosy". As the children of mothers with active lepromatous leprosy can be expected to develop leprosy by the age of 2 years, a combination of careful clinical surveillance and serological tests will be required for diagnosis of leprosy in children at risk ..... (Duncan et al, in press).

(2) Placental insufficiency in pregnancy and during labour. We have already shown that 59% of LL women excreted subnormal levels of oestriol and that this was related to a low placental coefficient (Duncan and Oakey, 1982). This was in contrast to the observation made by Younoszai and Haworth (1968) that in retarded foetal growth the placental coefficient is equal to that in pregnancies with well grown babies. Foetal distress or Apgar scores of 4/10 or less at one minute were recorded in 20% of the babies of BL and LL mothers (Duncan, 1980), and respiratory problems were a significant cause of neonatal mortality in babies of lepromatous mothers.

(3) Increased levels of bilirubin in cord sera. While the levels did not achieve statistical significance, a trend was observed. One out of 7 (14%) of babies of healthy mothers compared with 17/28 (61%) of babies of mothers with leprosy had raised levels of bilirubin in cord sera. The actual levels of raised bilirubin recorded were highest in the babies of lepromatous mothers (mean 15 mg % in the 6 babies with hyperbilirubinaemia). There were no cases of ABO or Rh incompatibility in any of the

groups of mothers. This observation may indicate placental inefficiency, foetal liver dysmaturity, or increased foetal haemolysis due to presence of immune complexes on red cells.

In conclusion, it seems most likely that the gross changes in the placenta: small size, low placental coefficient, small cell size, which were most marked in the placentae of women with lepromatous leprosy are secondary either to changes in the basal layer of the decidua or to the immune status of the women themselves. Clearly, further investigation in this field is required to establish at what level the defect in placentation occurs.

4. Humoral defence factors in the breast milk of Ethiopian women with leprosy and healthy controls

Secretory IgA (SIgA), lactoferrin, albumin and total protein were quantitated in 215 samples of colostrum or milk obtained from 85 Ethiopian women over a period ranging from 1 day to 2 years post-partum. IgG, IgM, β1A and β1E globulins were quantitated in 11 first day samples. The women were divided into three groups :

1. Lepromatous leprosy (LL).
2. Borderline lepromatous leprosy (BL).
3. Non-lepromatous (NL) (mothers with tuberculoid leprosy and healthy controls).

No major variation in levels of SIgA, lactoferrin, albumin or total protein was found between the three groups. Results from the Edinburgh group were significantly higher in the level of total protein in mature milk. When SIgA, lactoferrin and albumin were expressed as percentages of the total protein, there was no significant variation between the Ethiopian and Edinburgh mothers. When women with active lepromatous leprosy (BL and LL, BI positive) were compared with women with inactive or quiescent lepromatous leprosy (LL and BL, BI negative), no significant differences could be determined in levels of SIgA at any of the periods of lactation studied.

The milk study was initiated after the trends in low birth weights, slow growth rates and increased susceptibility to infection in the babies of LL mothers were observed. Hence there were relatively few milk samples collected within the first week after delivery. Furthermore, while test weighing was used for assessment of babies with early feeding problems, it was not possible to assess the daily intake of milk of any babies, by this method, once suckling was established.

No M. leprae were found in milk samples from 9 lepromatous mothers with active leprosy. These women were all on treatment with dapsone, and had relapsed with active leprosy due to the emergence of dapsone resistance during the third trimestre of pregnancy (Duncan et al, 1981b). The method of collection of the milk was such that contamination of the specimens was unlikely to have occurred.

M. leprae were found in 9 out of 14 milks from untreated LL patients, but only in one out of 3 treated LL mothers, a patient who had been on treatment for 8 years and who almost certainly had developed dapsone resistance (Girdhar et al, 1981). Most of their patients had bacillary counts in the order of 1.5×10^5/ml. Careful cleansing of the areola and nipple was carried out prior to collection of milk. Non-cultivatable acid-fast bacilli were isolated from the milk of 9 out of 12 LL patients, some bacilli being found intra-cellularly in macrophages (Saha et al, 1982). No bacillary counts were recorded, nor whether the mothers were receiving any treatment, although dapsone resistant leprosy had become a major problem (Saha, Mittal and Maheswari, 1982). Our negative results, which were in accordance with those of Girdhar et al (1981), may reflect the use of a less sensitive technique than that used by Saha et al (1982), or, alternatively, may be because most of our patients were relapsing with the rather localised lesions of early dapsone resistance: heavy breast milk infection may well require advanced disease involving the nipple and milk ducts.

Comparison of the levels of the various proteins within the three patient groups (Tables B4 i-iv) revealed no remarkable variations. In only one instance (Table B4(i), SIgA), was a statistically significant difference elicited between the LL and BL groups. All other six post-parturition periods were not significantly different. When the results were grouped for comparison with the Edinburgh nursing mothers (Table B4(v)), statistically significant differences were found for lactoferrin, albumin and total protein concentrations. However, the wide physiological variations in concentration found within the first week of lactation (McClelland et al, 1978) made it unlikely that differences found during this period were meaningful. Indeed, results from samples collected within the first day were extremely variable and the time of collection during this period was not standardised. In mature milk, a higher protein concentration within the Edinburgh group was evident. It was not possible to quantitate input to the infant due to lack of data concerning milk volume. When the results were expressed as percentage of protein (Figure B4(i)), similar trends were seen for all three parameters in the Ethiopian and Edinburgh samples. The increase in lactoferrin

concentration seen within the Ethiopian groups during the initial sampling period was similar to that of the Edinburgh group. Intake of milk volume was measured during the Edinburgh study and the increasing intake of lactoferrin demonstrated during the early period of lactation (McClelland et al, 1978) could well have been present also in the Ethiopian patients.

The values of total protein, which were shown to be significantly lower in the Ethiopian group, are comparable to those found in women in rural communities of the Ivory Coast during prolonged lactation, which were attributed to a protein deficient diet (Lauber and Reinhardt, 1979). A similar protein deficiency was seen in the daily dietary intake of Ethiopian women of the low income group with 1,539 calories containing only 41 g protein (55% of the FAO recommendations) (Ross, 1972).

Levels of other anti-bacterial proteins (Table B4(vi)) were similar in both groups. Concentrations in later milk samples from the Ethiopian group fell quickly, as did the Edinburgh samples, to levels which were undetectable by the radial-immunodiffusion method used.

In this study, no increase or decrease in the concentrations of the antibacterial proteins measured was found between the LL, BL and NL groups. This is in contrast to the decreased levels of SIgA which were observed in mature milk from women with lepromatous leprosy (Saha et al, 1982). The reason for this difference is obscure, but may be due to some technical difficulty in the standardisation of SIgA imported to India. While Saha et al (1978) recorded serum IgA, IgG and IgM which were in accordance with other studies, their assays for SIgA from saliva and the gastro-intestinal tract as well as from milk of LL patients all showed remarkably low levels.

While this study has shown no significant difference in concentrations of anti-bacterial proteins between the different groups and classifications of leprosy, there may well be differences in the specific anti-mycobacterial antibodies in IgG, IgM and the SIgA fraction. This is currently being investigated by Melsom (in Oslo) who is adapting the sRIA technique to measure the very low quantities of IgG and IgM antibodies in breast milk.

Regarding lactoferrin, higher levels have been found in the milk of Ethiopian privileged and non-privileged women than in a privileged Swedish group, between 2 and 6 weeks post-parturition (Lonnerdal et al, 1976). The sampling periods of this study are not strictly comparable but those authors found no major variation in earlier or later sampling periods. High levels of lactoferrin were attributed to the very high iron content of 'tef', a major component of the Ethiopian diet, although iron in this form may be poorly absorbed (Gebre-Medhin and Gobezie, 1975). There is evidence that the saturation of lactoferrin in human milk is not related to the intake of iron or haemoglobin concentration (Bowering, Sanchez and Irwin, 1976), and iron content has shown little variation as lactation progresses although there are daily and within-day variations (Picciano and Guthrie, 1976).

The present study indicates that there are no wide differences in the concentrations of parameters measured between Ethiopian women with different forms of leprosy and healthy controls, and the Edinburgh nursing mothers, although protein concentration in the Edinburgh subjects was significantly higher in mature milk.

5. The outcome of pregnancies of women with leprosy and healthy controls: child survival, growth and development, response to immunisation and skin testing with purified protein derivative of M. tuberculosis and M. leprae

In many third world tropical and sub-tropical countries, paediatric problems are well documented. For much of Africa, of every 10 live births, 3 children will be dead by the age of 5, 3 will be severely malnourished, and only 4 will be adequately nourished and healthy (King et al, 1972). The undernourished child has impaired growth in terms of weight and length and head circumference. The underweight child dies more easily of common infections. The clinically obvious cases of gross malnutrition represent the tip of the iceberg and milder degrees of malnutrition can only be detected by regular weighing and measuring and charting the measurements on a growth chart which is kept by the mother. This growth chart may also be used as a record of immunisations and treatment of minor ailments (King et al, 1972). Children weighing less than 70% of the standard weight for age are liable to suffer from immuno-deficiency problems (Kielmann, 1976). Serum antibody responses to most antigens is normal (World Health Organisation, 1978) including responses to measles, poliomyelitis, diphtheria and tetanus vaccines (Suskind, 1979), but antibody responses requiring T-cell help may be reduced. These underweight children have a reduction in total numbers of T-cells, but this reduction in cells is reversible with nutritional therapy (Chandra, 1974). Lymphocyte proliferation in vitro to antigens and mitogens is moderately depressed and cutaneous delayed hypersensitivity reactions are impaired. This is thought to be due to a combination of impaired cell mediated immunity and macrophage migration. Suppressive serum factors are also involved, as sera of malnourished infected children impair lymphocyte transformation in vitro (WHO, 1978).

In low birth weight infants with regarded intrauterine growth, various immunodeficiencies are observed transiently in the first month of life (Chandra, 1975) and impairment of cell mediated immunity may persist for several months after birth, particularly in those who fail to achieve normal growth (Chandra, 1977). This immunodeficiency associated with foetal malnutrition may have long-term effects.

While there has been considerable research carried out on the children of parents with leprosy, this has been largely of an epidemiological nature to determine the susceptibility of these children to leprosy and to investigate methods of preventing the infection, such as separation of children from their parents at birth. Such studies were carried out in the Philippines, in India and in South America (see Introduction pp. 17-33). More recently massive immunisation campaigns have been conducted to test the efficiency of BCG in preventing leprosy in children in leprosy endemic areas (Brown, Stone and Sutherland, 1968; Bechelli et al, 1974).

Relatively little has been recorded of the general state of health of children of mothers with leprosy, although Zambaco (1897) recorded that many children born to mothers with lepromatous leprosy were small "like an abortion at term" and that they died within a few months of birth of athrepsie. In another context he wrote :

> "D'autre fois les enfants des lépreux, nés comme des vieillots, ne se développent pas et succombent à l'athrepsie, sans présenter rien sur le corps, aucun indice de la lèpre. Cette cachexie foetale, qui amène la mort dans l'utérus ou peu après la naissance, sans lésions spéciales, est certes due à la lèpre, et peut être désignée sous le nom de para lépreuse ....." (Zambaco, 1897f)

More recently, Gomez et al (1922) noted that whereas minor ailments were similar to those seen in the healthy community, among the children of lepers, skin diseases were very common and the infant mortality was very high with 42% of the children dying of infections, debility, marasmus and athrepsie. A similar observation was made by Jeanselme (1933f) :

> ".... les enfants .... sont de vitalité faible et succombent souvent peu après la naissance."

He illustrated this by pointing out the high infant mortality reported from different leper colonies. Wallace (1944) recorded :

> ".... all children of leper parentage are very delicate and seem to have a predisposition to respiratory and gastric diseases which take a big toll of them."

The low birth weights, small placentae, and low placental coefficients

in pregnancies of mothers with leprosy, most marked in those with lepromatous leprosy, have already been recorded (Duncan, 1980). In pregnancies uncompromised by leprosy, the small group of intra-uterine growth retarded infants weighing less than 2500 g at birth accounts for the majority of perinatal deaths (Robinson, 1979).

Causes of death in children of mothers with leprosy and healthy controls

Twenty-three children of mothers with leprosy and healthy controls died during the first 3 years of the study. There were 2 still-births, 7 neonatal deaths, 1 additional death during the first month, 9 deaths from 1 to 12 months of life, and 4 deaths after the age of 1 year.

i) Stillbirth

Syphilis remains an important cause of perinatal mortality in Ethiopia. Latent (sero-positive) syphilis was diagnosed in 17.5% of 2,500 parturient women of low socio-economic class (M.E. Duncan and P.L. Perine, unpublished data). One study baby dying *in utero* at 34 weeks was thought to have died of syphilis. The cause of death was not confirmed by autopsy and it is of interest that neither the placenta nor cord of this pregnancy nor of any other woman reported as being sero-positive for syphilis during the study showed any histological evidence of infection.

Anencephaly is not observed commonly in Ethiopia. The incidence has been recorded as 1 in 2,500 to 5 in 3,000 hospital deliveries. This, of course, may present an abnormally low prevalence of the condition as many cases may be aborted or delivered prematurely at home because of hydramnios. There is no evidence to suggest that anencephaly is any more common in patients with leprosy or those receiving dapsone than in the normal population.

ii) Neonatal deaths

It is of interest that the neonatal deaths occurring in this study were all due to respiratory difficulties which were most marked in the babies of mothers with lepromatous (BL and LL) leprosy, whose birth weights were all significantly lower than

those of babies born to mothers with tuberculoid leprosy or healthy controls. While an investigation of the aetiology of perinatal mortality in 1,000 babies born in Addis Ababa showed that a significant cause of death was respiratory infection due to organisms of low pathogenicity causing amnionitis and intrauterine pneumonia (Naeye et al, 1977), there was nothing in our study to suggest a similar aetiology. In one case only was there evidence of amnionitis on histological examination of placenta, membranes and cord: in this case the child did not die.

iii) & iv) Deaths from 8–28 days and 1–12 months

While respiratory tract infections were an important cause of death, feeding problems were especially significant in the babies of mothers with lepromatous (BL and LL)leprosy. The cause of the failure to thrive in children under the age of 6 months who were fully breast fed is hard to evaluate, as all these mothers had a plentiful supply of milk and none of them had troublesome neuritis or ENL at the time.

Feeding problems in children over 6 months of age, however, presented a clear pattern. The babies were still being breast fed but they were all underweight, fractious, crying, horrible children. The Ethiopian mothers had a word to describe their children, "kifew lidj", and in many cases they despaired of being able to cope with their babies. At a slightly older age, 9–12 months, when these children were being weaned, they would turn away their heads to proffered food (all children were hand fed) and frequently knock the food out of the mother's hand on to the floor. Again, the mothers had a word to describe the children's behaviour, "imbiale" (coloquially, "I can't cope") and "imbialich" (she won't).

While not all children had routine stool analyses carried out, a considerable number of children in this group were observed to have giardia lamblia, and it was notable that the children's behaviour improved dramatically when the giardiasis was treated. Many of these children required hospital admission and careful supervised feeding for several weeks.

v) Deaths occurring after 1 year

Here there was no pattern suggesting that any group of children were particularly at risk. Measles bronchitis was still a killing condition in Ethiopia and measles vaccine was not then widely available. Viral myocarditis, which caused the death of one child of an LL mother, affected particularly the children of BL and LL mothers. The cause may have been an ECHO virus; such conditions have been reported elsewhere occurring in small epidemics and only diagnosed in the case of children actually admitted to hospital, otherwise they were likely to be recorded as deaths due to respiratory infection (International Paediatric Virology Conference, Jerusalem, Israel, 1963).

Assessment of baby growth by measurement of weight, length and head circumference

The mean weight, length and head circumference of babies at 6 monthly intervals from birth to 2 years showed most rapid growth during the first 6 months, with the babies of healthy mothers measuring more in terms of all 3 measurements than the babies of mothers with leprosy. Study of the growth velocity showed that the babies of lepromatous mothers grew more slowly than those of healthy controls during the first 6 months of life.

The observation that babies of LL mothers failed to grow satisfactorily after birth is of interest. The reason for this failure to thrive is obscure, although there are several possible explanations.

i) The milk of lepromatous mothers might have been of poor quality with regard to its content of protein or humoral defence factors, or in regard to quantity. The results of the analysis of the humoral defence factors of milk of mothers in this study indicate that there were no qualitative differences in the milk from the mothers of the different clinical classifications of leprosy. As test weighing on a large scale was impracticable in Addis Ababa, there might have been quantitative differences between the groups. From the observations on a few cases of BL and LL mothers, test weighing their babies during the first week of life, the

quantities of milk ingested were comparable to those of European babies.

ii) Babies of mothers with lepromatous leprosy might have failed to thrive because of circulating immune complexes. Failure to thrive in association with the presence of circulating immune complexes has been recorded in children suffering from cytomegalo virus infection (D.M. Weir, personal communication). Increased C1q binding activity was demonstrated in a number of children of lepromatous mothers (unpublished observations). Whether these immune complexes could have been interfered with, infant growth or not would need to be determined by a further prospective study.

iii) The period of reduced growth velocity of babies of LL mothers coincided with the time that these babies showed significantly increased antibody activity against M. leprae. It is suggested that a sub-clinical infection with M. leprae may directly, or indirectly, have been the cause of failure to thrive (R. Melsom, personal communication).

iv) The babies of lepromatous mothers were unusually prone to respiratory and gastro-intestinal infections which are not usually seen in breast fed children. It might have been that the babies "inherited" from their mothers a state of immune depression. A vicious circle would then be set up: infection → weight loss → impaired immune responsiveness → infection.

While most of the growth retardation of babies of lepromatous mothers appeared to have occurred in utero, it is possible that some factor(s) transmissible by breast milk might have contributed to their failure to grow as rapidly after birth as the babies of the other groups of mothers. It is of interest that by the time of the second follow up (Phase II) when the children were 3-4 years old, the children of lepromatous mothers were starting to catch up in terms of weight when compared with the other children. Because of the increased mortality of the children of LL and BL mothers, it may have been that most of the children already compromised in terms of poor weight gain and failure to thrive, had already died and thus the apparent catching up reflected the growth pattern of the less compromised children.

It is of interest that many of the children with impaired weight gain were found to have G. lamblia in specimens of stool when they presented with diarrhoea and vomiting. Treatment of the giardiasis not only cured the diarrhoea and vomiting, but was followed by a significant gain in weight. It is perhaps noteworthy that the mothers of these children, returning on a subsequent occasion when symptoms recurred, asked whether the child could have recurrent giardiasis. G. lamblia is known to have an increased prevalence in the lower socio-economic groups, especially in young children (Kidney and Holland, 1967) and in immunologically compromised individuals (Ament, Ochs and Davis, 1973; Webster, 1976). Chronic giardiasis may cause a reversible malabsorption syndrome which may include milk intolerance (Hoskins et al, 1967), disaccharide deficiency and protein losing enteropathy (Ament et al, 1973). Milk intolerance was observed in many of the study children admitted for treatment of failure to thrive whether or not G. lamblia was isolated from the stool. In fact, absence of G. lamblia from the stool does not exclude a diagnosis of giardiasis as trophozoites may be found in small intestinal biopsies or centrifugal jejunal aspirates when stool examinations are negative (Webster, 1976).

The probable importance of the local secretory immune system is demonstrated by high incidence of giardiasis (50-80% of individuals) with primary immunodeficiency syndromes affecting antibody-producing function (Ament et al, 1973; Webster, 1976). Infestation with G. lamblia, however, is not dependent on lack of secretory immunoglobulin, in particular IgA, as giardiasis is found in individuals with normal serum concentrations of IgG, IgA, IgM and IgE, and significantly raised levels of intestinal IgG (Jones and Brown, 1974).

Increased numbers of intra-epithelial lymphocytes in the biopsies of small children with diarrhoea due to giardiasis, a finding also seen in coeliac disease but not in other infective diarrhoeas, has been reported (Ferguson, McClure and Townley, 1976). While this is the only report suggesting an important role for CMI in human giardiasis, in animals G. muris causes a significant mortality in nude mice reduced by thymus transplantation (Boorman et al, 1973). Whether this susceptibility to infection is due to

failure of CMI to parasite antigens in local tissues or to failure of antibody synthesis (IgA antibody which is T-cell dependent in mice) remains to be determined.

While giardiasis has frequently not been considered a significant disease in children, it may well be that in immunologically compromised children, it presents a significant problem and major cause of failure to thrive.

ABO blood groups of mothers and children

The ABO blood groups of the mothers as a group and within the different classifications of leprosy were in accordance with the Ethiopian population in general (Ghose, 1963; Huber, 1964): there was no apparent association of any particular blood group with any classification of leprosy.

While the children as a whole showed a similar distribution of blood groups to their mothers, the apparent increase in the percentage of blood groups B and AB in the children of mothers with lepromatous leprosy is of interest. Although numbers are too small to show statistical significance, two points deserve comment :

i) The apparent increase in blood groups B and AB in the children compared with the mothers may suggest that there is some selection of foetal blood group/genetic determinant at the time of placentation.

ii) Such a strong correlation has been shown between increased susceptibility to infection and blood groups B and AB, that identification of blood groups may indicate those at risk (Kinane et al, 1982). The babies of mothers with lepromatous leprosy, who were unduly susceptible to infections not normally experienced by breast-fed infants, also had a higher infant mortality rate and a higher prevalence of blood groups B and AB. It is tempting to link the increased infection rate with the blood groups B and AB, assuming a cause and effect relationship. Clearly this requires further investigation.

Response to skin tests and BCG vaccination

The impaired response to BCG by children of mothers with lepromatous (BL and LL) leprosy is an indication of impaired CMI, to mycobacteria other than M. leprae, as the humoral response showed no significant differences between the different groups of children. The cause of this impairment of CMI is obscure, but may be related to the level of nutrition of children. Malnutrition is a well known cause of impaired T-lymphocyte responsiveness (Chandra, 1974) and the children of mothers with lepromatous leprosy have already been shown to be moderately or severely underweight for age at 24 months (Duncan, 1980). The results of PPD testing 2 years following BCG are of interest in two respects :

Firstly, that 10 out of 25 showing a strong positive 2 months after BCG became negative. Diminished responsiveness to PPD has been reported in children with protein calorie malnutrition. However, there was suggestive evidence that nutritional rehabilitation could lead to repair of a defective skin test response (Jayalaksmi and Gopalan, 1958). It is unlikely in this study that the negative response to PPD 2 years following BCG vaccination was due to the children's nutritional status, although it may be associated with it, as on the whole the nutritional status of all groups of children improved by the time of the Phase II assessment, when most children were aged 3-4 years. It is of practical importance, however, as it raises the question of re-vaccination of children at regular intervals. Vaccination with BCG has been and still is being assessed as a method of reducing the prevalence of leprosy in childhood, as well as providing protection against tuberculosis. At the time of this study there was already an increase in the prevalence of tuberculosis in Addis Ababa coinciding with a period of increased food shortage.

Secondly, it is of interest that of the 9 children of LL mothers who had a positive PPD 2 years after BCG, 5 had previously been negative. In most cases there was correlation between positive PPD and positive AB22 skin test. This raises the question of a positive PPD being due to some crossed reactivity with M. leprae or other mycobacteria in the environment.

The response to skin test with purified protein of M. leprae shows a marked increase from 10% to 49% after an interval of 2 years. During these 2 years in most cases the child would no longer be carried by the mother on her back, but would be mixing freely with other inmates of the house and neighbouring houses in the village. The positive response rate seen in children of 3-4 years of age is similar to that of the adult population of Addis Ababa.

The response of the children to triple vaccine (DPT) shows no impairment of B-cell function in any of the groups of children studied. The apparent failure of protection of 3 children against whooping cough and one child against poliomyelitis, may be an indication of impairment of the vaccine's efficiency by delays in importation, possibly without refrigeration, as well as a reflection of decreased/diminished immunity associated with malnutrition of children. For a fully effective immunisation campaign, vigorous efforts will be required to ensure better nutrition of the children at the same time as active immunisation.

The observations reported here underline the fact that children born to mothers with lepromatous leprosy are significantly compromised in terms of morbidity and mortality, probably due to some form of immunodeficiency. Further investigation of the nature of the defect is clearly required if the survival rate of these children is to be improved. In the meantime, however, it would probably be worthwhile attempting to improve their general standard of nutrition by teaching the mothers the use of supplementary feeding from the age of six months.

6. <u>A clinical and immunological study of four babies of mothers with lepromatous leprosy, two of whom developed leprosy in infancy</u>

One hundred and thirteen women with leprosy and 27 healthy controls were studied throughout pregnancy, at delivery, and followed up with their babies during lactation. Thirty-eight of the mothers with lepromatous leprosy were found to have solid staining bacilli in skin smears or biopsies, and hence are regarded as potentially highly infectious to their unborn children by haematogenous spread via the placenta. Two babies of mothers within this group were diagnosed as having leprosy on clinical and histological grounds: a third baby could well have had leprosy, but the case was not proven; the fourth baby did not have leprosy and, though it did have ringworm, was considered to be a reasonable control. The leprosy skin lesions were first observed at a special follow up clinic when the children were between the ages of 9 and 7 months.

The demonstration of IgA and IgM anti-<u>M. leprae</u> antibodies in cord sera was taken as an indication for intra-uterine stimulation of the foetus, and hence transplacental transmission of <u>M. leprae</u>. Two babies with proven leprosy showed an early and significant increase of serum IgA and in particular serum IgM anti-<u>M. leprae</u> antibody activity. A third baby, who was suspected of having leprosy, but in whom the diagnosis was not proven, showed a similar but lesser increase of serum IgA and IgM activity. The fourth baby showed no such rise in anti-M. leprae activity. Decrease of serum IgG anti-M. leprae antibody activity could be demonstrated in one of the babies with leprosy after healing of the leprosy lesions, but not in the second.

Of the four babies studied, Baby 1 had no evidence of leprosy and almost certainly had a ringworm infection. However, in contrast, the clinical evidence that Babies 2 and 3 were suffering from leprosy when examined at the ages of 17 and 12 months respectively is very strong. The lesions looked like leprosy, showed some sensory impairment, and histologically were considered to be tuberculoid and indeterminate leprosy respectively. The evidence that Baby 4 had leprosy is inconclusive.

The diagnosis of leprosy in Babies 2 and 3 was reinforced by the fact that they both developed positive skin tests to M. leprae: they also had a marked increase in IgA and IgM anti-M. leprae antibody activity on sequential studies of their sera during the first 3 years of life. Baby 4 had a negative skin test to M. leprae, but had a rise in IgA and IgM anti-M. leprae antibody activity on sequential testing of the sera, which gives evidence that the Baby might well have been infected in utero. No such rise in antibodies was seen in Baby 1.

To find two babies with proven leprosy was unexpected, and gave a prevalence of leprosy in children under 2 years of age whose mothers had active lepromatous leprosy of 5% (2/38). This figure is comparable with that reported by earlier workers (Rodriguez, 1926; Lara and Ignacio, 1956). It is likely that many such cases are missed. The lesions are transient, and unless they are carefully sought they can easily be overlooked, even if examinations are conducted in a well lit room (Lara, 1948).

Possible routes by which these children were infected include skin to skin contact, the breast milk, inhalation of droplets from mothers' nasal secretions, or transplacentally. Skin to skin contact seems unlikely (Pedley, 1967). The clothing habits in Ethiopia make such contact unusual except during breast feeding, and in any case few patients with lepromatous leprosy have bacilli on the skin surface except near the nose (Pedley, 1970).

Maternal milk may be a possible source of infection for babies. While high counts of M. leprae in breast milk have been recorded from a few patients with active untreated lepromatous leprosy (Sugai and Monobe, 1912; Pedley, 1967), a larger study failed to corroborate this (Rodriguez, 1926). In a recent study, M. leprae were isolated from the milk of 9 out of 14 untreated LL women, and from 1 out of 3 LL women on treatment - a patient treated for 8 years who was almost certainly developing dapsone resistance (Girdhar et al, 1981). Using a PEG precipitation technique, Saha et al (1982) demonstrated M. leprae in 9 out of 12 samples of milk from lepromatous mothers. However, the 9 milk samples tested in our study (from the mothers most likely to be positive) were negative for acid-fast bacilli. Our negative results may reflect the use of

a less sensitive technique (Saha *et al*, 1982) or, alternatively, may be because most of our patients with active lepromatous leprosy were relapsing with rather localised lesions; heavy breast milk infection may well require advanced disease involving the nipple and milk ducts. It seems unlikely that the babies in our study were infected in this way.

Droplet infection is now considered to be the usual route of spread of *M. leprae*, and although "casual nose blows" from these mothers were negative, it is possible that they would have been positive if taken in the early morning. However, one would expect this "normal" transmission of infection to cause leprosy with a normal incubation period. For babies aged 12 and 17 months to develop a disease whose incubation period is usually 4 years or so predicates very heavy exposure, an unusual route of infection, or both.

Placental transmission meets these conditions. Patients with active lepromatous leprosy have a bacteraemia of up to 10^5 *M. leprae* per ml (Drutz *et al*, 1972). The placenta is highly vascular, and even minor breaches of its integrity might lead to the passage of large numbers of *M. leprae* to the foetus. Increased concentration of IgA (Melsom *et al*, 1980a) and demonstration of IgA and IgM anti-*M. leprae* antibody activity in cord sera (Melsom *et al*, 1981) from babies of mothers with active lepromatous leprosy have already been reported from this study, indicating transfer of M. leprae antigens or bacilli across the placentae.

The placentae of babies in the study were examined. *M. leprae* were not observed histologically, and were present only in small numbers in homogenised and concentrated tissue. Previous studies, however, have shown *M. leprae* in both placenta and cord blood of babies born to mothers with active lepromatous leprosy (Sugai and Monobe, 1913a; Montero, 1927; Cerruti and Bechelli, 1936; Inaba, 1938). It may be noted in rare cases that congenital tuberculosis can affect the baby but leave no signs of placental infection (Hertzog *et al*, 1949).

The immunological tests of the cord sera, by the demonstration of IgM anti-*M. leprae* antibodies, suggested infection *in utero* had occurred in Baby 4. However, no evidence was found, either by

increased IgA or the presence of IgA or IgM anti-*M. leprae* antibodies in cord sera, for congenital infection in the two babies who later developed clinical signs of leprosy.

The most interesting results came from the solid phase radioimmunoassay for IgA, IgM and IgG anti-*M. leprae* antibodies. There was a marked increase of IgM anti-*M. leprae* antibody activity to about 30% of LSP in sera taken at 3 years of age from Babies 2 and 3. These values are above the highest IgM anti-*M. leprae* antibody activity found in sera from a group of healthy non-leprosy contacts to patients with active lepromatous leprosy. This IgM activity in the two babies was also about 3 times higher than the median activity found in sera from all the babies of mothers with lepromatous leprosy. The rise in IgA anti-*M. leprae* antibody activity was also significantly higher than normally seen in sera taken 6 to 12 months after birth from babies of both lepromatous and tuberculoid leprosy mothers (Melsom et al, 1982a). Therefore the demonstration of increased IgM anti-*M. leprae* antibody activity in the sera from the two babies who developed leprosy might be a specific indication of early *M. leprae* infection.

The results of assay for antibodies against *M. leprae* antigen 7 failed to demonstrate increased antibody formation in Babies 2 and 3 at the time their skin lesions appeared. This is due to the limited sensitivity of the method which detects IgG antibodies chiefly to one antigenic component of the *M. leprae* bacilli (Melsom and Duncan, 1980). It is felt this method is poorly suited for demonstration of IgA and IgM antibodies against *M. leprae*.

The clinical appearance of Babies 2 and 3 was that of indeterminate leprosy, though the recognition of impaired sensation in such young children suggests it was close to tuberculoid. This form of leprosy is often self-healing (Cochrane in Preventoria, 1945; Lara and Nolasco, 1956); and indeed in Babies 2 and 3 the lesions rapidly and spontaneously resolved, and skin tests to *M. leprae* antigen became positive. Nevertheless, it would be unwise to assume that these children are now cured. They have probably been infected by an unusual route and possibly exposed to an unusually heavy inoculum of *M. leprae.* Baby 2 showed a decrease of IgG anti-*M. leprae* antibody activity at 4 years compared to 2½

years: this coincides with the disappearance of the skin lesions and suggests healing of leprosy. A similar decrease of IgG anti-M. leprae antibody activity has been observed after dapsone treatment of patients with lepromatous leprosy (Melsom et al, 1982b; Melsom, Harboe and Naafs, in press). No such fall was seen in Baby 3. Furthermore, it would be unwise to asssume that no more cases of leprosy will occur in this group of children.

Evidence of infection of babies with M. leprae prior to birth has been shown in the study by presence of IgM anti-M. leprae antibodies in 50% and both IgA and IgM antibodies in 30% of cord serum of the children who were potentially at risk, namely the children born to mothers with positive skin smears for M. leprae (Melsom et al, 1981). Of these children only two presented indubitable clinical and histological evidence of infection after birth within the first 2 years of life; the third child was suspected of having leprosy, but this was not proven. These observations raise the question: why did only a small percentage of babies develop clinical leprosy? As has been said, these babies were probably infected by an unusual route and as their mothers all relapsed with active multi-bacillary leprosy during the third trimestre of pregnancy, the babies were possibly exposed to an unusually heavy inoculum of M. leprae. Did the babies have circulating immune complexes of maternal antibody and leprosy bacilli? Immune complexes have been shown in experimental models to suppress the immune response. This would lead to irability of the immune system to deal with the infection. The presence of granulomatous tissue in lymphnodes or spleen can affect lymphocyte circulation, and this could in theory affect the development of immunity (McKearn, 1974; Bullock, Carlson and Gershon, 1978). Were some of the babies exposed early to some other mycobacterium which could affect their immune response to M. leprae?

The presence of an unidentifiable non-cultivable acid-fast bacillus (NC AFB) has been demonstrated in human skin of adults, and also in the skin of 15 out of 82 foetuses, of 2 to 3 months' gestation, obtained at therapeutic abortion. None of the persons from whom specimens or foetuses were obtained had leprosy. This unidentifiable mycobacterium, which was also isolated from 1 out of

6 samples of amniotic fluid obtained at aspiration, was tested in parallel with lepromin and thought not to be M. leprae (Mori et al, 1969). The occurrence of NC AFB in the environment and their relationship to M. leprae is currently under investigation (Kazda, 1981).

Studies of perinatal infections and mortality in Ethiopia have demonstrated intrauterine infections due to organisms of low pathogenicity, namely, commensal bacteria of the lower genital tract. These infections have been attributed to dietary deficiencies, especially zinc, and associated with low household water utilisation, particularly in women of the low socio-economic class (Naeye et al, 1977). One might therefore postulate that in an environment where mycobacteria other than M. leprae, for example, M. smegmatis and others, abound, these organisms could gain access and be present from an early stage in the skin of foetuses in utero. The mycobacteria, while not causing any evidence of infection, could impair the ability of certain babies to deal effectively with an infecting dose of M. leprae received late in pregnancy. These babies would then be the ones to exhibit clinical evidence of the disease during the first year ot two of life.

Continued observation and repeated tests for some years to come is likely to bring to light more information which may greatly increase our understanding of the pathogenesis of leprosy.

C. SOME ASPECTS OF CELL MEDIATED IMMUNITY IN MOTHERS WITH LEPROSY AND THEIR CHILDREN

1. Hepatitis B infection in Ethiopian mothers with leprosy and their babies

Eleven (8%) of 138 mothers were positive for HBsAg during pregnancy and at delivery. Ten were carriers as HBsAg was present throughout the follow up period. Seventy-four (53.6%) of the mothers had anti-HBs, the percentage increasing with age. There was no significant difference in the prevalence of HBsAg in the healthy controls and the different classifications of leprosy. The prevalence of anti-HBsAg showed a falling trend across the spectrum of leprosy, from 66.7% in TT and BT/RFC to 43.3% in LL patients, thus confirming the findings of other workers which suggested that the prevalence of and response to infection by HBsAg in leprosy patients might be related to impaired immune responsiveness (Blumberg and Melartin, 1970; Serjeantson and Woodfield, 1978). There was no evidence in this study of transplacental transmission of HBsAg to the foetus.

2. Evidence for a soluble lymphocytic factor in the transplacental transmission of T-lymphocyte responses to M. leprae

This study provides evidence that when mothers are sensitized to M. leprae, this sensitization is transmitted to the foetus. The sensitization which seems to be specific could be transmitted by three methods, transplacental transmission of antigen or transplacental passage of significant numbers of maternal lymphocytes: in this study these two possibilities seem unlikely. The third and most likely method is that there could be transplacental passage of a soluble lymphocytic factor which was responsible for the foetal lymphocyte sensitization demonstrated (Barnetson, R.St.C., Bjune, G. and Duncan, M.E., Nature, (1976) 260, 150-151).

3. In vitro modulation of lymphocyte responses to PHA by plasma in mother and baby at the time of birth

The four conclusions of this study are as follows :

i) Plasma of mothers in late pregnancy has an inhibitory effect on lymphocyte responses to PHA. This finding is in agreement with reports by other workers and is thought to be part of the maternal immune response to pregnancy.

ii) The lymphocyte responses of healthy mothers were not significantly suppressed when cultivated in the presence of plasma from their babies, indicating that the suppressive factor(s) of normal pregnancy did not pass the placental barrier.

iii) Plasma from mothers with leprosy had a greater inhibitory effect on their babies' lymphocytes than plasma from healthy mothers. This raises the possibility that plasma from leprosy patients contains suppressive factors other than those associated with pregnancy.

iv) Babies of lepromatous leprosy mothers, who might have been exposed to mycobacterial antigens in utero, had higher PHA responses than the other babies, possibly due to a compensatory reaction to early stresses in the immune system (Bjune, G., Duncan, M.E., Barnetson, R.St.C and Melsom, R., Clinical and Experimental Immunology (1978), 32, 517-522).

D. STUDIES OF HUMURAL IMMUNITY IN MOTHERS WITH LEPROSY AND THEIR CHILDREN

Evidence for the transplacental transmission of leprosy is provided by the following studies :

1. Immunoglobulin concentration in mothers with leprosy and in healthy controls and their babies at the time of birth

The cord blood IgA concentration was significantly increased in babies of mothers with active lepromatous leprosy compared with those of healthy mothers and tuberculoid leprosy mothers. The cord IgM concentration was normal in all three groups of babies. Since IgA does not cross the placenta, this increase reflects an active increased production of IgA in the foetus of mothers suffering from active lepromatous leprosy. This could indicate transfer of M. leprae, or M. leprae antigens, across the placenta to the foetus (Melsom, R., Duncan, M.E. and Bjune, G., Leprosy Review, 1980, 51, 19-28).

2. Demonstration of antibodies against M. leprae both in IgG and IgM in sera from pregnant and non-pregnant lepromatous leprosy patients

IgG and IgM antibodies against M. leprae antigen 7 were demonstrated in patients with active lepromatous leprosy including pregnant women. IgM antibodies against M. leprae antigen 7 were not demonstrated in a tuberculoid leprosy or healthy mother, or in any of the babies of mothers with lepromatous leprosy, although IgG antibodies were present. The lack of IgM antibodies in the babies may be due to insensitivity of the technique, the IgG antibodies in the babies are expected to be mostly of maternal origin. This study, which describes a useful new serological technique utilised in D.3 (below), does not in itself provide evidence of transplacental transmission of M. leprae to the babies (Melsom, R. and Duncan, M.E. Duncan, Leprosy Review (1980), 51, 125-135).

3. Antibodies against M. leprae antigen 7 from birth to 18 months of age: an indicator of intrauterine infection in leprosy

The reduced rate of decline in concentration of anti-M. leprae antigen 7 antibodies in 10 out of 20 babies of mothers with active leprosy compared with a normal rate of decline of antibodies during the first 4 months of life of babies of healthy, tuberculoid or inactive lepromatous leprosy mothers indicates that the babies of mothers with active lepromatous leprosy have been stimulated, before birth, by M. leprae antigen 7, either as free antigen or by viable M. leprae. Studies of anti-M. leprae antibodies in serial serum samples during the first 18 months of life indicated that children of mothers with bacilliferous leprosy are frequently exposed to M. leprae to a sufficient extent to stimulate the immune system of the baby to production of anti-M. leprae antibodies during this period (Melsom, R., Duncan, M.E., Harboe, M. and Bjune, G., Clinical and Experimental Immunology,(1980), 42, 107-113).

4. IgA and IgM antibodies against M. leprae in cord sera and in patients with leprosy

The presence of IgA antibodies against M. leprae in 7 of 22 cord sera from babies of mothers with active lepromatous leprosy and IgM anti-M. leprae antibodies in 12 of 22 cord sera of the same babies (the 7 cord sera with IgA anti-M. leprae antibodies also contained IgM antibodies) is indicative of foetal antibody synthesis in utero. The stimulus could be either M. leprae antigens or live bacilli (Melsom, R., Harboe, M., Duncan, M.E. and Bergsvik, H., Scandinavian Journal of Immunology (1981), 14, 343-352).

5. IgA, IgM and IgG anti-*M. leprae* antibodies in babies of mothers with leprosy during the first two years of life

Two groups of babies were studied :

Group 1. The babies of mothers with multibacillary leprosy.

Group 2. The babies of mothers with paucibacillary leprosy or healthy controls.

After birth, there was a significantly higher increase of IgA and IgM anti-*M. leprae* antibody activity in sera taken 3–6 months after birth from babies of Group 1 compared to Group 2, but the IgA and IgM activity in sera taken after 6 months of age showed the same increase in the two groups of babies. IgG anti-*M. leprae* antibody activity showed a marked decrease in sera from both Groups 1 and 2 taken 3–6 and 6–9 months after birth compared to the activity in cord sera. No significant increase of the IgG activity could be demonstrated even in sera taken 15–24 months after birth in either of the two groups. The increased IgA and IgM activity after birth in babies of mothers with multibacillary leprosy is evidence for the stimulation of the immune system of some of the babies *in utero* and during the first few months of life, and is best explained by transmission of live *M. leprae* bacilli across the placenta with active multiplication in the children after birth (Melsom, R., Harboe, M. and Duncan, M.E., Clinical and Experimental Immunology (1982), 49, 532-542).



Plate 13.

"A study in which one or
two investigators .....
ponder one or two
specific questions ....

may develop ....



Plate 14. involving many experts in different fields ..."

POSTSCRIPT

As has been the case in many research projects in the past, a study in which one or two investigators have set out to unravel and ponder one or two specific questions (in the case the possibility of identifying transfer factor, and the cause of low birth weight of women with lepromatous leprosy), may develop into a highly complex multifaceted study involving many experts in different fields and eventually raising many more questions which in turn become complete research projects in themselves.

Further research avenues which are indicated by the results or trends observed in the present study include :

1. Further investigations of the effect of pregnancy on leprosy

   i) A prospective trial of additional chemotherapeutic agents to prevent relapse in pregnancy due to possible reactivation of dormant bacilli or development of dapsone resistance during a period of suppression of CMI. The trial should be carried out under very strict supervision, in pregnant women in an area where leprosy is endemic, the birth rate is high and dapsone resistance is a problem. Three (or four) drug regimes could be used.

   a) Rifampicin (given under supervision, at the antenatal clinic) 600 or 1200 mg orally every month after the first trimestre with dapsone 100 mg to be taken daily, self-administered by the patient.

   b) Regime as for a) with the addition of clofazimine 50 mg to be taken daily, self-administered by the patient.

   c) Clofazimine 50 mg and dapsone 100 mg to be taken daily, self-administered by the patient.

   d) Dapsone 100 mg daily, self-administered by the patient.

   In the light of the observations made in this study, it might not be considered ethical to run the fourth group on dapsone alone, although from the point of view of investigation of placental function, this would be most desirable.

   The trial period for each patient should run for at least

a year to cover the second and third trimestre of pregnancy and the first 6 months of lactation when relapse/drug resistance is most likely to occur. Clofazimine would have a secondary benefit, namely that of reducing the incidence/ severity of ENL in pregnancy and lactation.

Monitoring of the study, in addition to routine skin smears and biopsies, should include urine and serum sampling to test drug compliance, blood for anti-M. leprae antibodies as outlined in this study, and a questionnaire regarding local traditions and attitudes to drug ingestion during pregnancy.

ii) A prospective study of ENL in pregnancy and lactation

Ideally this should be linked to a prospective study of ENL in lepromatous patients including women of the reproductive age group, in order to obtain a pre-pregnancy baseline. (It has been observed that ENL may be cyclic and related to the menstrual cycle. The effect of endogenous and exogenous hormones could be studied at the same time by recording dates of menstruation and including a group of women on the "pill" but not on rifampicin(which is known to interfere with the efficacy of oral contraceptives).

Investigations should include: full assessment at 2-3 monthly intervals; skin smears and biopsies at every second visit, except during pregnancy when they should be done at each visit; collection of blood for serum and white blood cells - to be used subsequently for specific anti-M. leprae antibody tests, levels of immunoglobulins, complement factors and test of CMI: freezing techniques obviate discrepancies due to changing laboratory conditions, standards, etc. (Birkeland and Kristoffersen, 1977); biopsies of regional lymphnodes throughout pregnancy and lactation at 3 monthly intervals.

iii) A prospective study of neuritis in pregnancy and lactation

Because of the high incidence of neuritis in BL patients, and the clinically puzzling mixture of ENL and reversal reaction in this group, BL might be the best group to study. This might be combined with 1. ii) q.v. and should include in

addition regular assessment of nerve function using standard sensory tests and VMT; NCV; and nerve biopsies. It has already been shown that frequent nerve biopsy can be carried out without loss of nerve function (Pearson and Weddell, 1971). This, however, should only be done by one specially trained and experienced in the technique.

2. The effect of leprosy on pregnancy

i) Impaired placentation in women with lepromatous leprosy due to impaired CMI. This hypothesis clearly needs further investigation which will not only shed light on the problem for patients with leprosy, but could have far wider implications in the field of reproductive immunology. While investigation in rodents has suggested possible mechanisms (Beer, 1975), methods for investigating human pregnancy have to be worked out. Initial investigations could include mixed lymphocyte reactions using maternal, paternal and foetal cells (from cord blood), with/without addition of the mother's leprosy drugs; biopsy of the placental bed, e.g. at Caesarean section when biopsy can be made under direct vision (Brosens et al are currently investigating normal and abnormal placental bed morphology).

ii) Investigation of foeto-placental function in patients receiving dapsone, rifampicin and clofazimine. This study has indicated that the already reduced excretion of oestrogen in lepromatous women may be further suppressed by clofazimine. Further studies, involving the assay of serum and urinary levels of oestrogen and other steroid precursors, together with HPL (human placental lactogen) and HCG in patients on standard treatment regimens as outlined in 1. i) above should be carried out to investigate the effect of these drugs on placental function.

iii) Further studies of placental immunohistology. The study reported here has to be regarded as a pilot study in this respect, as for maximum information, specimens must be processed soon after collection. Use of specific antisera could be made to be incorporated into the study of placentae

from lepromatous women to see whether and where M. leprae/ M. leprae antigen lodge in relation to the maternal and foetal circulations. Maximum information would be obtained from placentae from lepromatous women reporting for the first time, untreated, late in pregnancy.

iv) The possible role of zinc deficiency in neonatal infections in babies born to mothers with lepromatous leprosy. Perinatal mortality in Addis Ababa has been shown to be due to a high incidence of respiratory infection, due in most cases to organisms with low pathogenicity, and associated with reduced bacteriocidal action of amniotic fluid due to zinc deficiency (Naeye et al, 1977). Zinc deficiency has been suggested as an aetiological factor in leprosy (B. Naafs, personal communication). Aspiration of amniotic fluid,preferably during early labour before membrane rupture occurred, would provide specimens for bacteriological and chemical investigation. The study would be simple to perform and the remedy, also simple,could have far-reaching effects.

3. The effects of the mother's leprosy on her child

i) Follow up of the cohort of children already studied. This should be done at regular intervals (2-3 years) for as long as possible, with clinical and serological assessments to investigate the long-term effect of in utero exposure to M. leprae.

In terms of extending our knowledge of the development of leprosy, this is probably the most important line of further research.

ii) A prospective study of nutritional factors in the milk of mothers with leprosy. This should be carried out initially in a few selected patients in each classification, with test weighing daily for the first week of lactation, and then once a week for a 24 h period until 1 month, then every 2 weeks until weaning has been completed. In this way the total nutritional content of the mother's milk could be measured, and correlated with the baby's growth velocity.

iii) An investigation of the possibility that failure to thrive in babies of LL mothers is due to transfer of cells across the placenta in utero and through the breast milk after birth.

iv) The possible association of babies' blood groups with infection during the first year of life. The present study has suggested that there may be a higher incidence of babies with blood group B born to mothers with lepromatous leprosy, and that the babies' increased susceptibility to infection may be associated with the B blood group. This requires investigation on a wider scale.

v) The possible role of immune complexes in pregnancies complicated by lepromatous leprosy. A pilot study showed suggestive evidence that immune complexes may be transferred from mother to child. Clearly, this requires further investigation in the light of the observation that there was nothing to suggest deposition of immune complexes in the placentae, and the hypothesis that impaired growth after birth may be due to the presence of circulating immune complexes.

In addition to the further lines of research outlined above, it is anticipated that additional serological methods will also become available, enabling further testing of sera already obtained from both mothers and babies, currently being stored at -70° C. Clearly the study reported here is not "an end" but "a beginning".

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Evidence for a soluble lymphocyte factor in the transplacental transmission of T-lymphocyte responses to *Mycobacterium leprae*

ANTIGENS introduced to the human foetus have been considered as "self" and thus not to provoke an immune response[1]. But, no sudden changes in the immune mechanisms seem to coincide with birth, and the development of adult immune responses is gradual. Moreover, in sheep, antigens can give rise to more or less mature immune responses in the foetus[2,3], dependent on the stage of gestation. The nature of transmission of these immune responses from mother to foetus remains unclear. Brody *et al.*[4] demonstrated in humans that T-lymphocyte sensitisation could be transmitted from mother to foetus and considered this was due to transplacental passage of antigen. Field and Caspary[5] showed that cell-mediated responses can be induced in the foetus, but thought that either maternal lymphocytes or some subcellular lymphocyte factor crossed the placenta to sensitise the foetal lymphocytes. We have tested ten randomly selected normal mothers and their babies at parturition for evidence of sensitisation to antigens of *Mycobacterium leprae*. We found that when the mothers showed sensitisation their babies did too: the children of unsensitised mothers were not sensitised. We consider the likeliest explanation for our findings is the transplacental passage of a soluble lymphocyte factor.

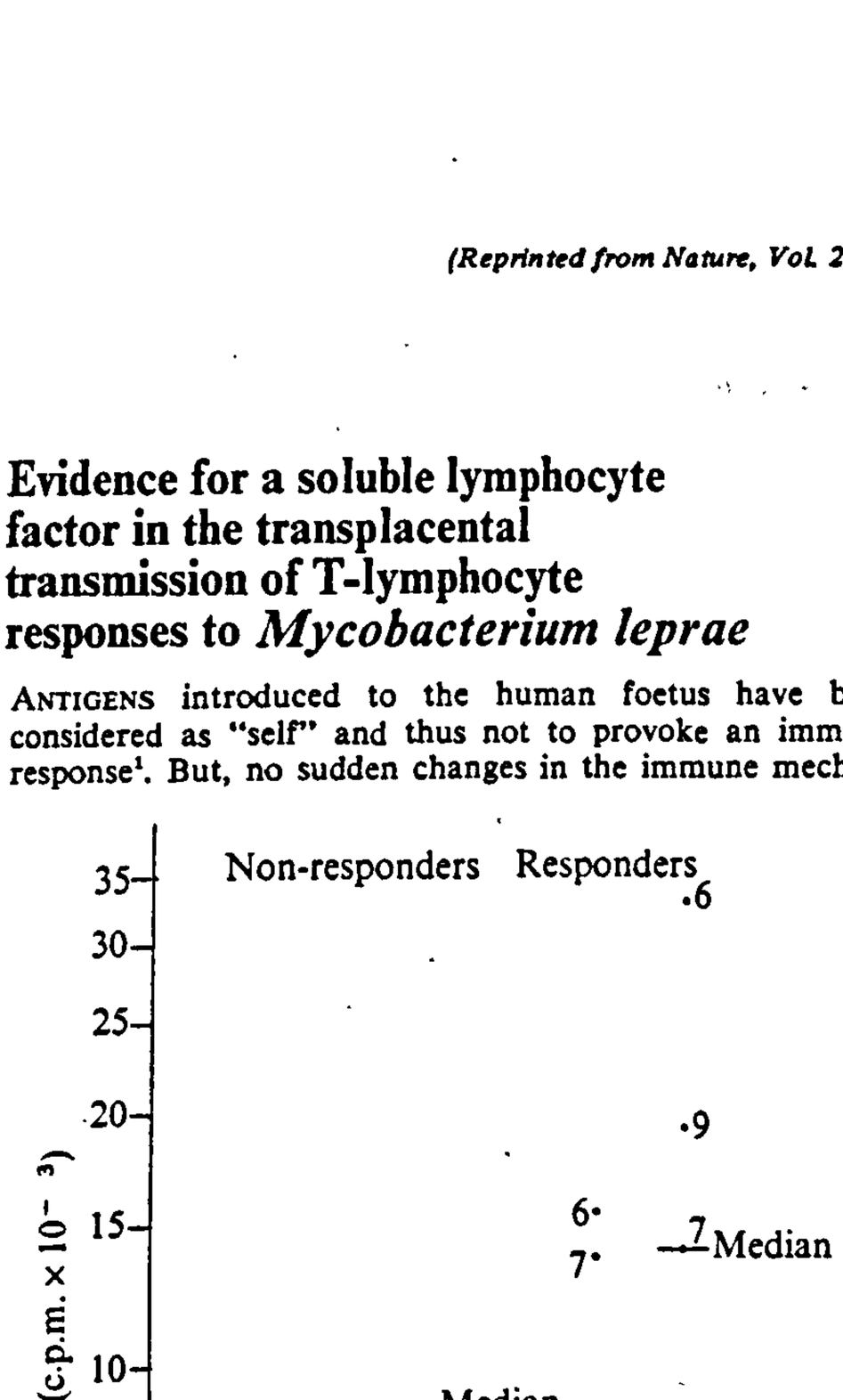


Fig. 1 LTT responses to whole *M. leprae* in mothers and neonates, comparing the five mothers with positive responses and the five mothers with negative responses (unstimulated control values are subtracted). ³H-TdR, Tritiated thymidine uptake.

Lymphocyte transformation tests (LTT) were carried out by a micromethod[6] on both maternal and neonatal cord blood; whole washed *M. leprae* were used as antigen, and in each case the responses to BCG and PPD were also determined. Of the ten mothers tested, five showed a positive LTT response with *M. leprae* as antigen and five did not (Fig. 1). It was considered that a positive response existed when the LTT response with the antigen was more than twice the value of unstimulated control cultures. The values for these control cultures were much higher in the neonates (median: 13,171) than in the mothers (median: 532). In the five mothers with positive lymphocyte blastogenic responses, the response of the neonate reflected the maternal response, though the former were much greater. In the five unresponsive mothers, the neonatal response was much lower than in the neonates born of responders. The difference between the two neonatal groups is highly significant ($P<0.005$), using the Mann–Whitney U test for statistical analysis. There was also a statistically significant difference in responses to PPD (but not BCG) between the maternal responders and non-responders to *M. leprae* (Table 1), possibly due to some cross reactivity. There was, however, no significant difference of responses to PPD and BCG between the two neonatal groups.

Table 1 Median responses to antigens

	M. leprae		BCG		PPD	
Mothers with positive responses to *M. leprae*	8,600	$P<0.005$	267	$P>0.1$	4,316	$P<0.02$
Mothers with negative responses to *M. leprae*	−11		21		−36	
Babies of mothers with positive responses to *M. leprae*	13,658	$P<0.005$	995	$P>0.1$	3,654	$P>0.1$
Babies of mothers with negative responses to *M. leprae*	602		−17		1,140	

Figures are c.p.m.
Median responses to antigens in the two maternal and the two neonatal groups. P, Probability of distribution between the two groups occurring by chance, calculated by Mann–Whitney U test.

Five out of ten may seem a high proportion of mothers to show evidence of sensitisation to *M. leprae;* but it is in accord with our experience that up to 90% of contacts with known cases of leprosy show evidence of previous subclinical infection[7]. Moreover, in Sao Paulo, Brazil, where the leprosy endemicity is similar to that of Addis Ababa, more than 50% of the normal population show positive lepromin (Fernandez) skin tests[8].

This study provides evidence that when mothers are sensitised to *M. leprae* this sensitisation is transmitted to the foetus. The sensitisation seems to be specific, as there was

no significant difference between the responses of the two neonatal groups when BCG or PPD were used as antigens.

For transmission of this sensitisation, there are three main possibilities. The first is transplacental passage of antigen. In our study, this seems unlikely as the mothers showed no signs of leprosy, or other mycobacterial disease. The second possibility is transplacental passage of maternal lymphocytes. It is still debatable whether maternal lymphocytes enter the foetal circulation[9–12], so if they do cross the placenta it must be in very small numbers. In view of the high neonatal LTT responses, recruitment of neonatal lymphocytes by maternal lymphocytes would again seem improbable. Third, there could be transplacental passage of a soluble lymphocyte factor. It seems most likely that such a factor was responsible for the foetal lymphocyte sensitisation we have demonstrated.

The nature of this lymphocyte factor remains unclear. Some immunoglobulins are known to cross the placenta, but none has been shown to raise antigen-specific T-lymphocyte responses in unsensitised individuals. Several soluble factors for specific T-cell stimulation however, have now been described. One possibility in this context is transfer factor[13] which can transfer delayed type hypersensitivity and, having a molecular weight of less than 10,000, could cross the placenta passively. The nature of the factor suggested by our study clearly needs further investigation.

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In vitro modulation of lymphocyte responses to phytohaemagglutinin by plasma in mother and baby at the time of birth

INCREASED LYMPHOCYTE RESPONSES IN BABIES OF MOTHERS WITH LEPROMATOUS LEPROSY

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(*Received* 18 *January* 1978)

SUMMARY

Peripheral blood lymphocytes from nineteen healthy mothers, sixteen mothers with borderline tuberculoid leprosy and fourteen mothers with borderline or polar lepromatous leprosy, and their newborn babies, were stimulated *in vitro* with phytohaemagglutinin (PHA). The responses in medium supplemented by serum from a pool of healthy non-pregnant individuals were compared with responses in medium supplemented by plasma from the mothers or from their babies, to assay for the presence of non-specific effects on T-cell responses. It was found that plasma from the mothers at the time of labour profoundly suppressed their own lymphocyte responses to PHA. However, the lymphocyte responses of healthy mothers were not significantly suppressed when cultivated in the presence of plasma from the babies, indicating that the suppressive factor(s) of normal pregnancy did not pass the placental barrier. Plasma from mothers with leprosy had a greater inhibitory effect on their babies' lymphocytes than plasma from healthy mothers. This raises the possibility that plasma from leprosy patients contains suppressive factors other than those associated with pregnancy. Babies of lepromatous leprosy mothers, who might have been exposed to mycobacterial antigens *in utero*, had higher PHA responses than the other babies, possibly due to a compensatory reaction to early stresses in the immune system.

INTRODUCTION

Pregnancy is a major challenge to control systems of the immune response. The foetus, which may be considered as an allograft, must not be rejected: at the same time, resistance to infections should remain intact, and the foetus should be able to eliminate foreign lymphocytes which may invade its circulation from the mother.

An adjustment of immune regulation in the pregnant state must necessarily influence the response to a chronic infection like leprosy. It has been shown that the bacteriological indices of leprosy patients tend to increase during pregnancy (King & Marks, 1958), and also that complications due to T-cell reactions to *M. leprae* antigens, namely reversal reactions, are less frequent (Rose & McDougall, 1975).

Many intracellular infections are transmitted to the foetus *in utero*, e.g. syphilis, toxoplasmosis and viral diseases. This is probably not the case in leprosy (King & Marks, 1958). The explanation for the lack of congenital and infantile leprosy could be (a) the extraordinarily long incubation period for the disease, (b) the foetus (and neonate) is protected by some non-immunological mechanism, (c) leprosy

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bacilli are unable to pass the placental barrier and (d) the foetus can mount such a vigorous cell-mediated immune response to the parasite that it is fully protected, even inside the immunologically unresponsive mother.

Several hormones and pregnancy-associated peptides are known to influence the immune response, some of which could easily cross the placenta. However, some might exert their effect exclusively in the mother, or in the placenta, which is the site of production for many of them.

In this study we have measured peripheral blood lymphocyte responses to phytohaemagglutinin (PHA) as an indicator of T-cell responsiveness. Responses in medium containing plasma from babies or mothers were compared with the responses in medium supplemented with a serum pool from healthy non-pregnant individuals in order to assay the presence of non-specific modulators of cell-mediated immunity at the time of birth.

PATIENTS AND METHODS

Blood samples were withdrawn from the umbilical cord between delivery of the baby and placenta, and from the mother immediately after delivery of the placenta. All the mothers were given 0·5 mg of ergometrine tartrate i.m. after delivery of the placenta, but no other medication. The control group consisted of nineteen mothers who were healthy individuals of the same age group and social status as the leprosy patients: none of them had had leprosy previously nor showed any clinical signs of the disease at the time of testing. Of the mothers with leprosy, sixteen were classified clinically as having borderline tuberculoid leprosy (acid-fast bacilli were not detected in skin smears taken from these patients) and fourteen were classified as having borderline lepromatous (BL) or polar lepromatous (LL) leprosy (acid-fast bacilli were detected in skin smears of this group at the time of diagnosis). All patients, except five borderline tuberculoid patients released after 4 years of treatment, were on anti-leprosy treatment with dapsone. Four of the lepromatous patients (BL or LL) had received less than 6 months treatment with dapsone, six had received treatment from 6 months to 5 years and four had been treated for more than 5 years.

Details of the lymphocyte transformation assay are reported elsewhere (Bjune *et al.*, 1976). In brief, peripheral blood was anti-coagulated with heparin and centrifuged at 400 *g* for 30 min after addition to Ficoll–Isopaque (sp. gr. 1·077). The cells obtained from the interface were washed three times in ice cold Hanks's balanced salt solution and adjusted to a concentration of $0{\cdot}5 \times 10^6$ mononuclear cells per ml in medium TC 199 supplemented with sodium bicarbonate, penicillin–streptomycin, fresh glutamine and either 10% serum (standard serum) from a pool of twenty healthy non-pregnant individual sera, or 10% of test plasma from either mother or baby. The cells were cultured in 0·25 ml with PHA (Reagent Grade, Wellcome, Beckenham) in flat-bottomed wells of microtitre trays (Linbro Chemical Company, Newhaven, Connecticut) for 96 hr at 37°C, 100% humidity and 5% CO_2 in air. 16 hr before harvesting 0·5 μCi of [^{3}H]thymidine (sp. act. 2·0 Ci/mmol) was added to the cultures. In control experiments with lymphocytes from normal individuals, the responses to PHA reached a peak on day 4 of culture. The cells were harvested on glass fibre filters in a commercial harvester (MASH II) and rinsed with distilled water. The filters were dried, placed in separate vials with scintillation fluid and counted in a liquid scintillation counter (Intertechnique SL-31, Plaisire, France).

Results are expressed as the mean net counts per minute (ct/min) of triplicate cultures (mean counts of wells containing PHA minus mean counts of wells without PHA). Statistical significance of differences between groups was estimated with the Wilcoxon matched pairs signed ranks test when observations were paired, and by the Kolmogorov–Smirnov two sample test when observations were not paired. In each figure the median and interquartile distance are indicated.

RESULTS

The PHA stimulation in medium supplemented by 10% standard serum of the lymphocytes from the nineteen control mothers (median: 56,500 ct/min) was not significantly different from that of the sixteen mothers with borderline tuberculoid leprosy (median: 51,200 ct/min), nor from that of the fourteen mothers with lepromatous leprosy (median: 46,300 ct/min) (Fig. 1).

The PHA responses of cord blood lymphocytes in 10% standard serum are depicted in Fig. 2. The responses were significantly ($P<0{\cdot}05$) higher in babies of lepromatous leprosy mothers (median: 144,000 ct/min) than those in babies of tuberculoid leprosy mothers (median: 95,000 ct/min) or those in healthy mothers (median: 92,500 ct/min). Although there was no significant difference in the background thymidine incorporation (cultures without PHA) between the lymphocytes of healthy mothers, tuberculoid leprosy mothers or lepromatous leprosy mothers, or between those of their babies, the cord blood lymphocytes demonstrated significantly higher responses (median:1840 ct/min) than did the mothers' lymphocytes (median: 230 ct/min) ($P<0{\cdot}001$).

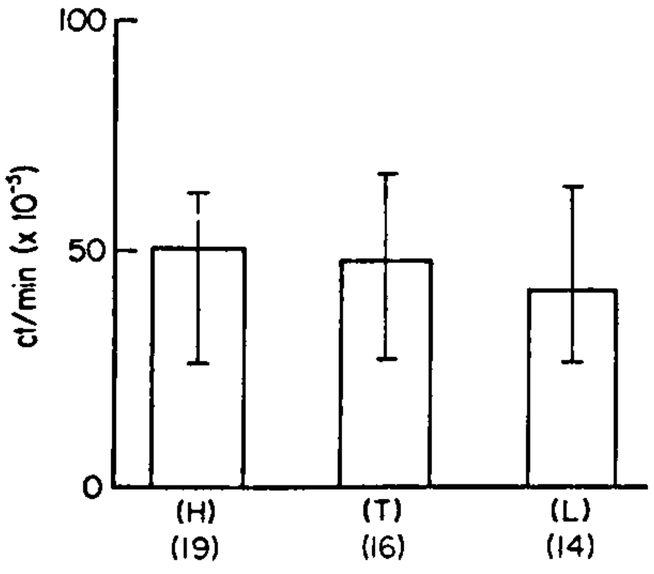


FIG. 1.

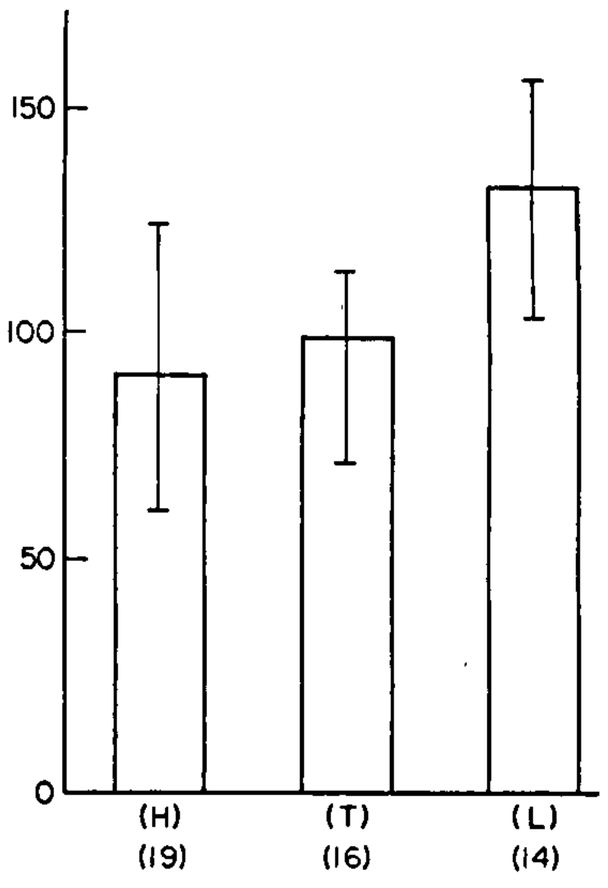


FIG. 2.

FIG. 1. Median PHA responses of maternal lymphocytes in medium supplemented with 10% pooled serum from healthy non-pregnant individuals. The range is indicated as interquartile distance. (H) Healthy mothers; (T) mothers with borderline tuberculoid leprosy; (L) mothers with BL and LL leprosy. Figures in brackets give the number of individuals in the group.

FIG. 2. Median PHA responses of cord blood lymphocytes in medium supplemented with 10% pooled serum from healthy non-pregnant individuals. (H) Babies of healthy mothers; (T) babies of mothers with borderline tuberculoid leprosy; (L) babies of mothers with BL and LL leprosy. Figures in brackets show number in each group.

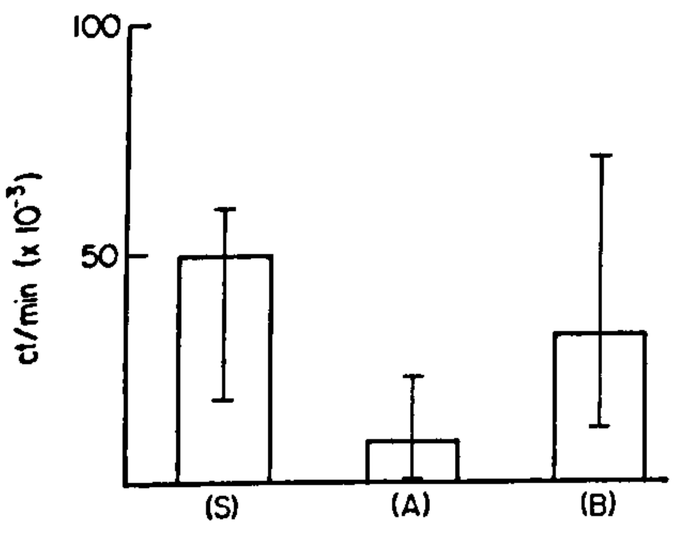


FIG. 3.

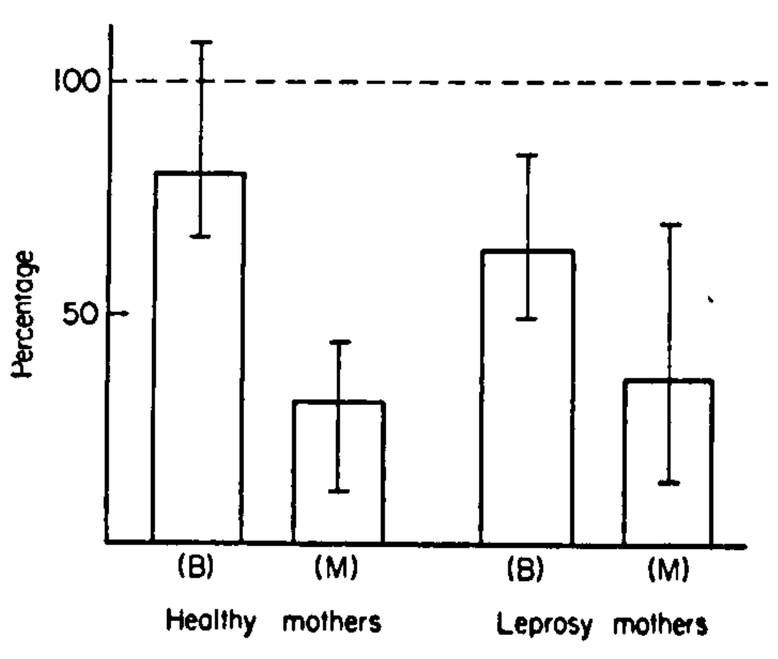


FIG. 4.

FIG. 3. Median PHA responses of healthy mothers' lymphocytes in medium supplemented with the standard pool of healthy non-pregnant serum (S), autologous plasma (A) and in plasma of their babies (B). Nineteen pairs of mothers and babies were tested.

FIG. 4. Inhibition of baby lymphocytes' responsiveness to PHA in medium with their mothers plasma (B) compared to the PHA responsiveness of their mothers' lymphocytes in autologous plasma (M). Responses are recorded as a percentage of the simultaneous response to PHA in medium supplemented with pooled healthy non-pregnant serum. The dotted line shows 100%, the response in standard serum.

The PHA responses of mothers' lymphocytes (all groups) were greatly inhibited when 10% fresh autologous plasma was included in the wells instead of standard serum: the inhibition was highly significant ($P<0{\cdot}001$) for all three groups of mothers. Responses were not, however, significantly inhibited when cultured in 10% fresh plasma from their respective babies. The median PHA responses of the control group of mothers in the standard serum, the autologous plasma and babies' plasma are shown in Fig. 3. Control experiments with four healthy non-pregnant individuals revealed no significant difference in the ability of serum and heparinized plasma to support stimulation (results not shown).

The responsiveness of the babies' lymphocytes to PHA in their mothers' plasma was compared with that of their mothers' lymphocytes in autologous plasma and the results are shown in Fig. 4 for both healthy mothers and their babies, and leprosy mothers and their babies. The mothers' plasma, which profoundly suppressed their lymphocytes' responsiveness to PHA, had only a weak inhibitory effect on their babies' lymphocytes. The inhibition only reached significance for babies of the mothers with leprosy. A correlation plot for percentage inhibition of the babies' lymphocyte responses to PHA in autologous plasma *vs* the responses in mothers' plasma revealed a very low degree of explanation (20%), with a correlation coefficient, $r = 0{\cdot}61$. Both results indicated that more than one suppressive factor was operating in the experiments.

DISCUSSION

This study has produced a number of interesting observations, listed below.

(a) *Plasma of mothers in late pregnancy has an inhibitory effect on lymphocyte responses to PHA.* This finding is in agreement with previous reports (Gatti, 1971; Purtilo, Hallgren & Yunis, 1972). Evidence of a diminished T-cell responsiveness during pregnancy has also been produced from *in vivo* experiments as well as from experiments with cells cultivated in serum from non-pregnant individuals, e.g. diminished responses in mixed lymphocyte culture (Kasakura, 1971), decreased leucocyte migration inhibition responses in the presence of antigen (Stimson, Bridge & Blackstock, 1974) and prolonged survival of skin allografts (Heslop, Krohn & Sparrow, 1954). Antibody levels, on the other hand, have been found to be normal or even enhanced during pregnancy (Fabris, 1973).

Plasma factors which inhibit *in vitro* responses to PHA have been described in a large number of diseases. In Hodgkin's disease the apparent suppression was later found to be due to binding of PHA by a factor in the patients' serum, made evident by a shift in the dose–response curve (Amlot & Unger, 1976). In experiments with a wide spectrum of PHA concentrations we were, however, unable to find any indication of PHA binding to a plasma factor in the plasma from pregnant mothers. The optimal dose of PHA stimulation was the same in pregnant plasma as in standard non-pregnant serum.

(b) *The suppressive factor(s) is(are) not operative in the cord blood.* Lymphocytes from mothers which had proven sensitive to the suppressive effect of autologous plasma were not significantly suppressed in their responses to PHA by the plasma of the babies.

Nor were the babies' own lymphocytes significantly suppressed when cultured in 10% autologous plasma. Others (St. Hill, Finn & Denye, 1973; Yu *et al.*, 1975) have described some suppressive effects of high concentrations of baby plasma at delivery. Large differences in the techniques make direct comparisons with their data impossible, but their findings could indicate that some suppressive factor(s) does (do) pass. However, sufficient levels to give suppression of the PHA responses in cultures supplemented with 10% plasma were not achieved. Alpha-foetoprotein is very unlikely to be the cause of pregnancy-associated T-cell suppression (Reggio *et al.*, 1971), as it is mainly found on the foetal side of the placenta.

It has been proposed that 17-hydroxycorticosteroids are the cause of CMI depression in pregnancy, because serum concentrations increase parallel with the inhibitory effect of pregnancy plasma on the mixed lymphocyte reactions (Kasakura, 1973). However, carrier globulin for corticosteroids also increases with the gestational age, leaving the concentration of free active steroids virtually constant. Also, there was no correlation between steroid levels and the inhibitory effect of plasma on an individual basis when correction for gestational age was made (Kasakura, 1973).

Blood concentrations of oestrogen and progesterone are generally low in the foetus when compared

with those in the mothers. Both hormones can inhibit lymphocyte responses to PHA as well as other T-cell functions (Waltman, Burde & Berries, 1971; Munroe, 1971), but the concentration needed to give a measurable effect is not achieved, except possibly in the placenta. Also, chorionic gonadotrophin and chorionic somatotrophin have been reported to affect the immune response (Contractor & Davies, 1973), but doubts have been raised as to the purity of the preparations used (Caldwell, Stites & Fudenberg, 1975); in addition the two hormones are able to pass through the placenta freely.

Pregnancy-associated alpha2 macroglobulin (Stimson & Eubank-Scott, 1972) and pregnancy zone protein (von Schoultz, Stigbrand & Tärnvik, 1973) are large molecules with a suppressive effect on T cells. Both are produced in the mother but do not cross the placenta. The presence of either protein could explain our observations, although it is somewhat doubtful in this case if such a profound inhibition of the lymphocyte responses to PHA could result from the addition of only 10% plasma to the cultures.

Blocking antibodies (Hellström, Hellström & Brawn, 1969), placental lactogen (Contractor & Davies, 1973), thyroxine (Fabris, 1973) and as yet undefined peptides (St. Hill *et al.*, 1973; Gatti, 1971) have been shown to inhibit T-cell activation *in vitro*. The incomplete knowledge of their distribution in the mother and baby provides an obstacle to any discussion of their possible role in relation to the present data.

(c) *There is increased PHA responsiveness of lymphocytes from babies of lepromatous leprosy mothers.* Babies of mothers with lepromatous leprosy who might have been exposed to leprosy bacilli or mycobacterial antigens *in utero* showed higher lymphocyte responses to PHA than did babies of mothers without the disease, or babies of mothers with borderline tuberculoid leprosy (in whom there is very limited amounts of antigen). This increase in the T-cell responses could possibly indicate compensatory reactions of the foetal immune system to early stresses.

(d) *Suppressive plasma factor(s) associated with leprosy affect(s) the foetus.* Plasma from non-pregnant leprosy patients tends to inhibit T-cell reactivity *in vitro* (Nelson *et al.*, 1971; Bjune & Barnetson, 1976), but this less pronounced suppression seemed to be completely surpassed by the pregnancy-associated inhibitory factors, giving no significant difference in the degree of inhibition between plasma from healthy mothers and mothers with leprosy when the effect was assayed on the mothers' own lymphocyte responses. However, the babies' lymphocytes, which were less sensitive to the suppressor factor(s) of pregnancy, showed evidence of an additional inhibitor in the plasma of mothers with leprosy.

The nature of this(these) substance(s), whether bacterial product(s), immunoregulatory factor(s) produced in the mother or soluble immune complexes, remain(s) to be elucidated.

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BABIES OF MOTHERS WITH LEPROSY HAVE SMALL PLACENTAE, LOW BIRTH WEIGHTS AND GROW SLOWLY

BY

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Summary

One hundred and sixteen women with leprosy and 31 healthy controls were studied throughout 155 pregnancies, and their babies were observed for a period of up to two years. Babies of mothers with leprosy weighed less than those of healthy mothers; the placental weights and coefficients followed the same trend. The babies of the mothers with leprosy grew more slowly than those of the healthy mothers and these findings were most marked in the babies of mothers with lepromatous leprosy. The cause of the reduced feto-placental weight is thought to be related to the immune status of the mother.

THE causative organism of leprosy (*Mycobacterium leprae*) has a multiplication time of about 12 days (Shepard and McRae, 1965) and is virtually non-toxic. The disease is therefore chronic, and its clinical manifestations depend not on the virulence of the bacterium but on the host's immunological response to its presence. Thus a spectrum of manifestations can be defined clinically, histopathologically and immunologically (Myrvang *et al*, 1973). When there is a powerful cell mediated immune response, the lesions are localised and bacilli scanty; this is tuberculoid leprosy. When cell mediated immunity is low (probably because of a specific defect of T lymphocyte reactivity to *M. leprae*) the disease is diffuse and generalised; this is lepromatous leprosy and such patients may harbour as many as 10^{13} bacilli. Between these polar forms of leprosy lies the intermediate borderline group.

Hitherto there has been no recorded association of chronic maternal infection with low placental weight and low birth weight. This paper documents, for the first time, this effect in children of mothers with leprosy, and in particular lepromatous leprosy. In addition, the babies of lepromatous mothers were found to grow slowly after the first few months of life.

PATIENTS AND METHODS

All mothers included in the study were from the low socio-economic group in Ethiopia, and lived in villages adjacent to the Addis Ababa Leprosy Hospital; they included 116 leprosy patients and 31 apparently healthy women.

On reporting their pregnancy at the antenatal clinic, patients had a complete physical examination including measurement of height, weight, skinfold thickness and blood pressure. The urine was tested for protein and sugar, and blood was sent to the laboratory for estimation of haemoglobin, determination of blood group and serological test for syphilis. Most patients had a chest X-ray taken during the puerperium.

For patients with leprosy, clinical drawings of skin lesions were made, slit skin smears were taken for bacteriological assessment, and a biospy of skin was taken for classification of leprosy.

During pregnancy, patients were seen every two weeks until the 34th week and every week

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TABLE I

Status of mothers according to clinical classification

	Age (years)	Height (cm)	Maximum weight recorded (kg)	Right sub-scapular skinfold thickness (mm)	Left triceps skinfold thickness (mm)	Haemoglobin (g/dl)	Gravidity	Primigravidae	
								No.	Per cent
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM		
Healthy controls (n = 34)	26·2 ± 0·94 (28)	157·4 ± 1·21 (16)	57·6 ± 0·96 (29)	9·9 ± 0·89 (8)	10·2 ± 1·01 (8)	13·6 ± 0·22 (25)	2·9 ± 0·30 (33)	7	20·6
Tuberculoid or borderline tuberculoid leprosy (n = 42)	27·8 ± 0·96 (40)	157·8 ± 1·71 (25)	59·1 ± 1·32 (37)	9·0 ± 0·54 (23)	9·4 ± 0·86 (23)	13·5 ± 0·18 (34)	3·2 ± 0·28 (42)	7	16·7
Borderline lepromatous leprosy (n = 44)	27·0 ± 0·84 (39)	156·4 ± 1·21 (24)	55·3 ± 0·98 (39)	9·1 ± 0·59 (24)	9·1 ± 0·61 (24)	12·9 ± 0·33 (29)	3·2 ± 0·34 (41)	8	18·0
Lepromatous leprosy (n = 35)	27·1 ± 1·03 (34)	155·3 ± 1·28 (21)	55·7 ± 1·26 (25)	8·7 ± 0·78 (22)	10·0 ± 0·99 (22)	13·1 ± 0·27 (22)	2·5 ± 0·23 (35)	9	25·7

Number of observations in parenthesis.

thereafter. The mothers were admitted to the Addis Ababa Leprosy Hospital for one or two days for collection of urine for oestriol assay, for complications of leprosy or intercurrent illness and when possible they were routinely admitted for rest one week before the estimated date of delivery. Most vaginal deliveries took place in a nearby delivery centre, Caesarean sections were done in the University obstetric units. Surveillance of labour followed standard practice; duration of contractions and fetal heart rate were recorded at 30-minute intervals during the first stage of labour, at 10 to 15-minute intervals during the latter part of the first stage of labour, and after each contraction during the second stage of labour. Fetal distress was said to be present if the fetal heart rate was faster than 160/minute or slower than 120/minute and if there was fresh meconium staining of the amniotic fluid with a cephalic presentation. Analgesic agents, including 100 mg of pethidine, were given as required during labour. Ergometrine maleate, 0·5 mg, was given intramuscularly with the delivery of the anterior shoulder, or intravenously with delivery of the head. The baby's Apgar score was recorded, and cord blood was collected after the cord was clamped and before delivery of the placenta. The baby was weighed immediately after birth, and its crown–heel length and head circumference measured. The placenta was weighed after membranes and cord had been trimmed, and samples were then taken for light and electron microscopy. Whenever possible, mother and baby were kept in the Addis Ababa Leprosy Hospital for four to seven days post partum but in accordance with local custom some mothers went home within 6 to 12 hours of delivery.

The mothers with leprosy and their babies were reviewed at monthly intervals. Each time the baby was weighed and its length and head circumference were measured. The babies of healthy mothers were usually only seen for follow up if they were brought to a sick baby clinic because of an acute infection. The custom of the mothers living in the vicinity of the leprosy hospital is to breast feed babies until 12 months, after which weaning is begun; the majority of mothers lactate for two years post partum. All mothers had similar diets and nutritional status.

Results

Classification of patients and treatment

Using the classification of Ridley and Jopling (1966), there were 4 mothers with tuberculoid leprosy studied in 4 pregnancies, 37 mothers with borderline tuberculoid leprosy studied in 38 pregnancies, 43 mothers with borderline lepromatous leprosy studied in 44 pregnancies, and 32 mothers with lepromatous leprosy studied in 35 pregnancies. We also studied 31 healthy mothers during 34 pregnancies; thus the total number of pregnancies studied was 155.

Most patients were receiving treatment (usually dapsone 50 to 100 mg daily) but 23 of them (one with borderline lepromatous leprosy, the rest with tuberculoid or borderline tuberculoid leprosy) were believed cured and had stopped treatment. Six patients (two with borderline lepromatous leprosy and four with lepromatous leprosy) were considered to have dapsone-resistant leprosy, and were receiving clofazimine (four patients with lepromatous

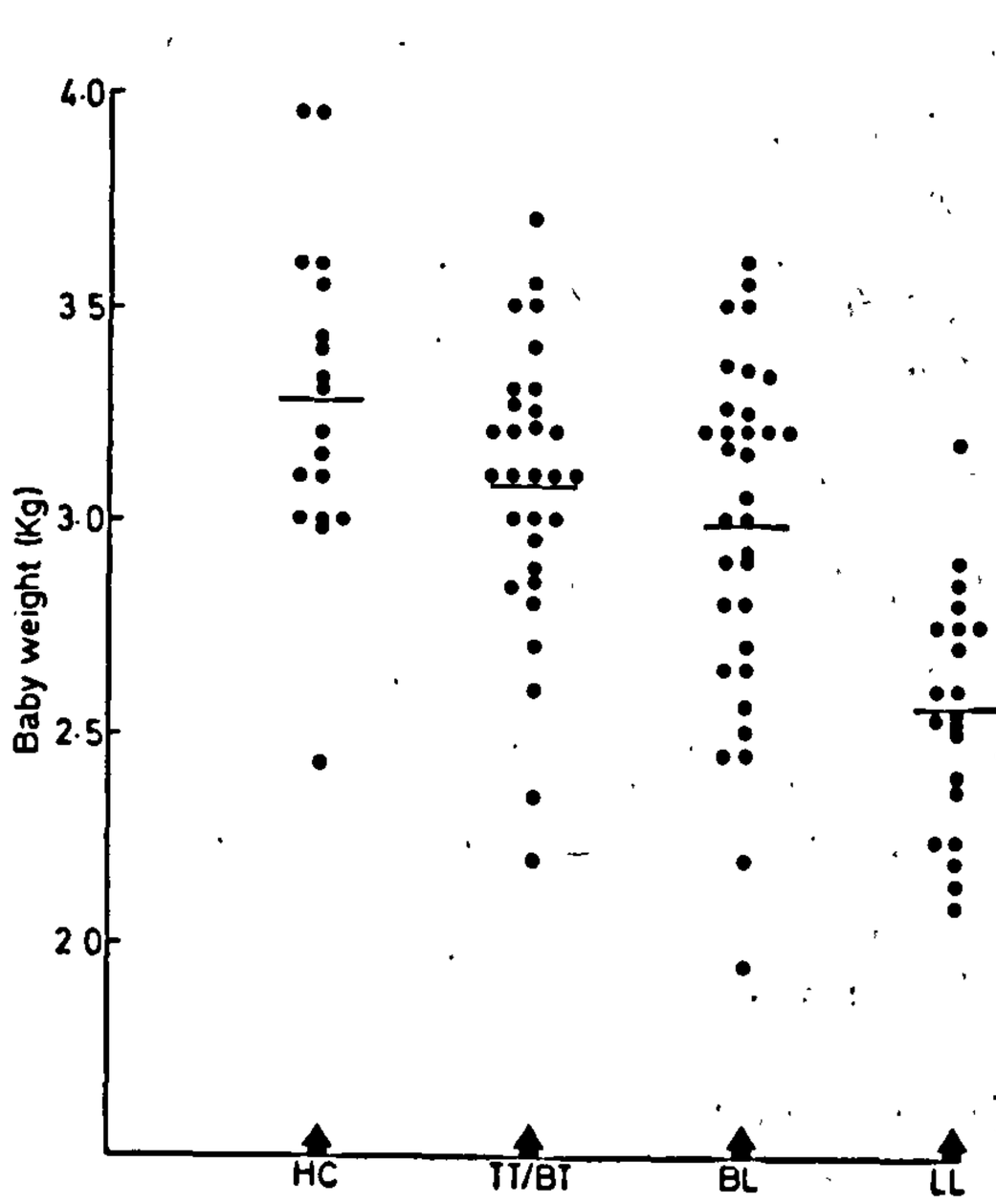


FIG. 1

Birth weight (kg) of full term, singleton babies according to the clinical classification of the mother. HC = healthy controls; TT/BT = tuberculoid/borderline tuberculoid leprosy; BL = borderline lepromatous leprosy; LL = lepromatous leprosy.

TABLE II

Infant birth weight, placental weight and placental coefficient according to the clinical classification of the mother

	Birth weight (g) Mean ± SEM	Placental weight (g) Mean ± SEM	Placental coefficient Mean ± SEM
Healthy controls	3280·6 ± 87·6 (18)	595·0 ± 35·4 (13)	0·184 ± 0·01 (13)
Tuberculoid or borderline tuberculoid leprosy	3075·0 ± 61·1 (30)	569·4 ± 19·4 (25)	0·181 ± 0·05 (25)
Borderline lepromatous leprosy	2985·6 ± 69·9 (33)	521·0 ± 26·4 (26)	0·173 ± 0·01 (26)
Lepromatous leprosy	2558·1 ± 60·5 (21)	362·0 ± 19·1 (15)	0·144 ± 0·01 (15)

Number of observations in parenthesis.

leprosy seen in five pregnancies) or rifampicin and thiambutosine (two patients with borderline lepromatous leprosy). Two patients with borderline tuberculoid leprosy and three with lepromatous leprosy received prednisolone for leprosy reactions during most of the second and/or third trimesters.

The clinical groups of patients are shown in Table I. There was no statistical difference between the groups with regard to the mothers' age, height, weight, skinfold thickness, haemoglobin level or gravidity.

Birth weight

Babies born to mothers with lepromatous leprosy were the smallest (Fig. 1, Table II), weighing significantly less than those born to mothers with borderline lepromatous leprosy ($p < 0·001$, Mann Whitney U test). Babies of healthy mothers and those with tuberculoid or borderline tuberculoid leprosy were heavier than the babies of mothers with lepromatous or borderline lepromatous leprosy ($p < 0·001$ and $p < 0·01$, Mann Whitney U test).

Placental weight

The placental weight (Fig. 2 and Table II) of mothers with lepromatous leprosy were significantly less ($p < 0·001$, Mann Whitney U test) than those of healthy controls or other leprosy patients. The placental coefficients (placental weight divided by baby's weight) of mothers with lepromatous leprosy were also significantly less than those of the other leprosy patients ($p < 0·01$) or the healthy controls ($p < 0·002$; Fig. 3 and Table II).

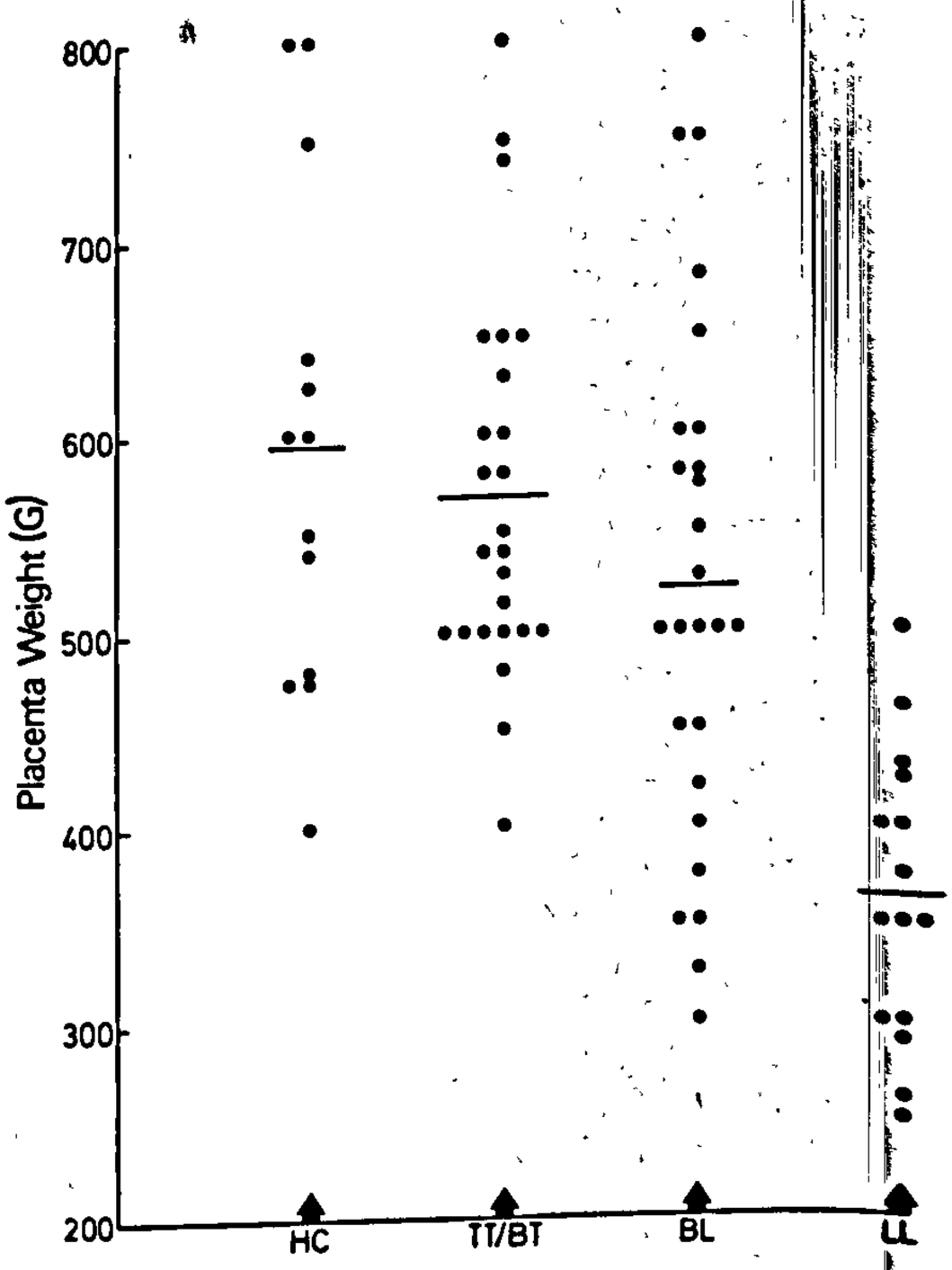


FIG. 2

Trimmed placental weight (g) of full term singleton pregnancies according to the clinical classification of the mother (see Fig. 1).

TABLE III

Infant weights at birth and six-monthly intervals according to the clinical classification of the mother

	Weight of babies (g) Mean ± SEM				
	Birth	6 months	12 months	18 months	24 months
Healthy controls	3280·6 ± 87·6 (18)	7975·0 ± 415·1 (4)	8366·7 ± 145·3 (3)	9580·0 ± 387·8 (5)	11037·5 ± 520·9 (4)
Tuberculoid or borderline tuberculoid leprosy	3075·0 ± 61·1 (30)	6912·5 ± 436·5 (8)	8150·0 ± 462·5 (9)	8462·5 ± 224·9 (4)	9750·0 ± 487·8 (9)
Borderline lepromatous leprosy	2985·6 ± 69·9 (33)	6694·7 ± 173·4 (19)	7782·5 ± 280·8 (20)	9129·2 ± 342·6 (12)	9675·0 ± 495·9 (6)
Lepromatous leprosy	2558·1 ± 60·5 (21)	6007·0 ± 216·4 (14)	7310·0 ± 260·9 (15)	7877·8 ± 349·5 (9)	8983·3 ± 501·9 (3)

Number of infants in parenthesis.

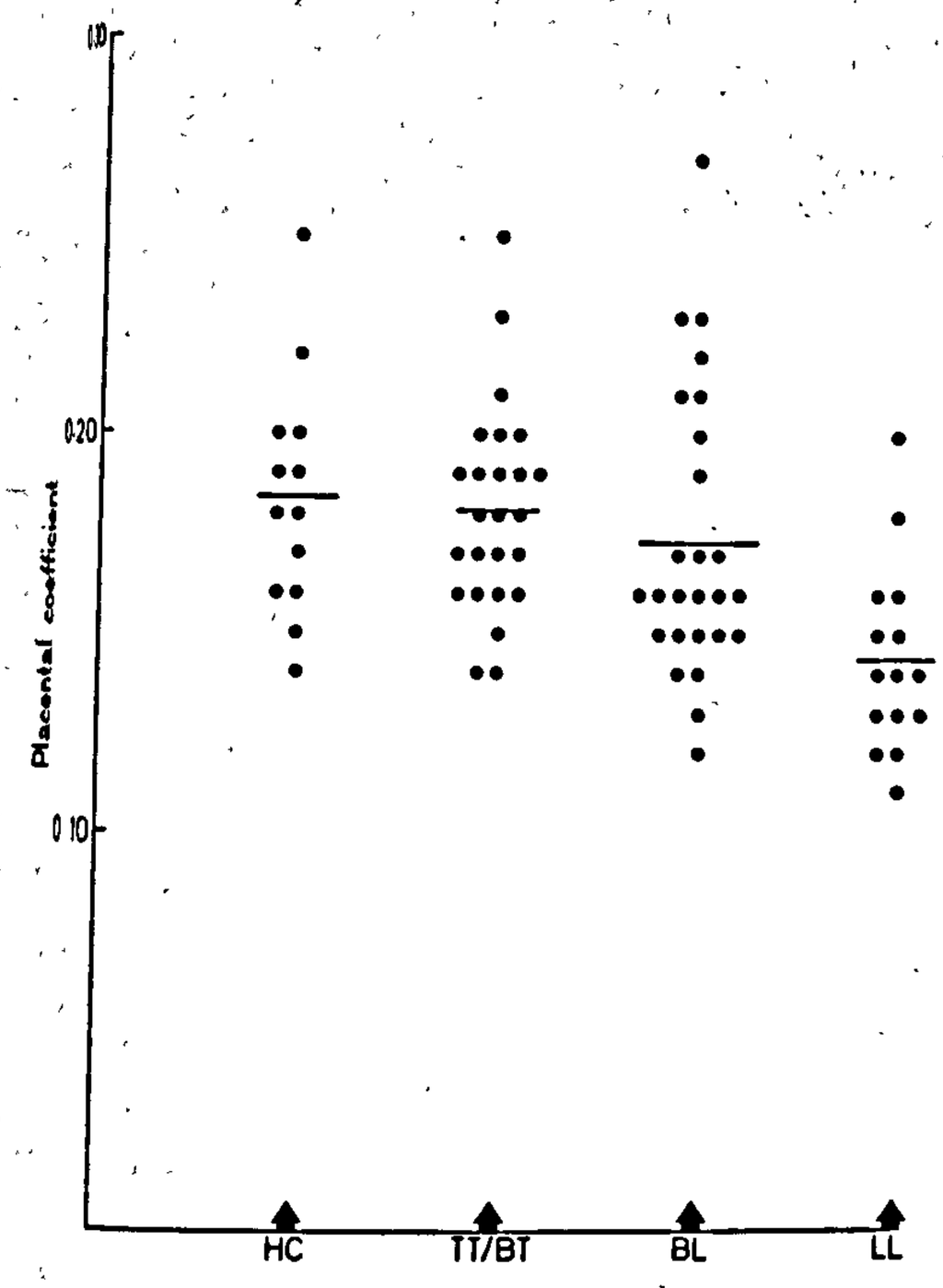


FIG. 3

Placental coefficient (ratio of placental weight:baby weight) according to the clinical classification of the mother (see Fig. 1).

Fetal distress and neonatal asphyxia

Fetal distress or Apgar scores of less than 3 at one minute after birth were recorded in 20 per cent of the babies of mothers with borderline lepromatous or lepromatous leprosy. Resuscitation was carried out immediately; babies with persistent respiratory difficulty were referred to the pediatric intensive care unit.

Infant weight during the first two years

The mean weight of babies at six-monthly intervals is shown in Figure 4 and Table III. In all groups, weight increase was most rapid during the first six months while the mothers had a plentiful supply of milk. The babies of mothers with lepromatous leprosy gained weight particularly rapidly during the first three months, suggesting that the low birth weight was due to intrauterine malnutrition. After six months they gained weight slowly and were unusually susceptible to infections; several became mar-

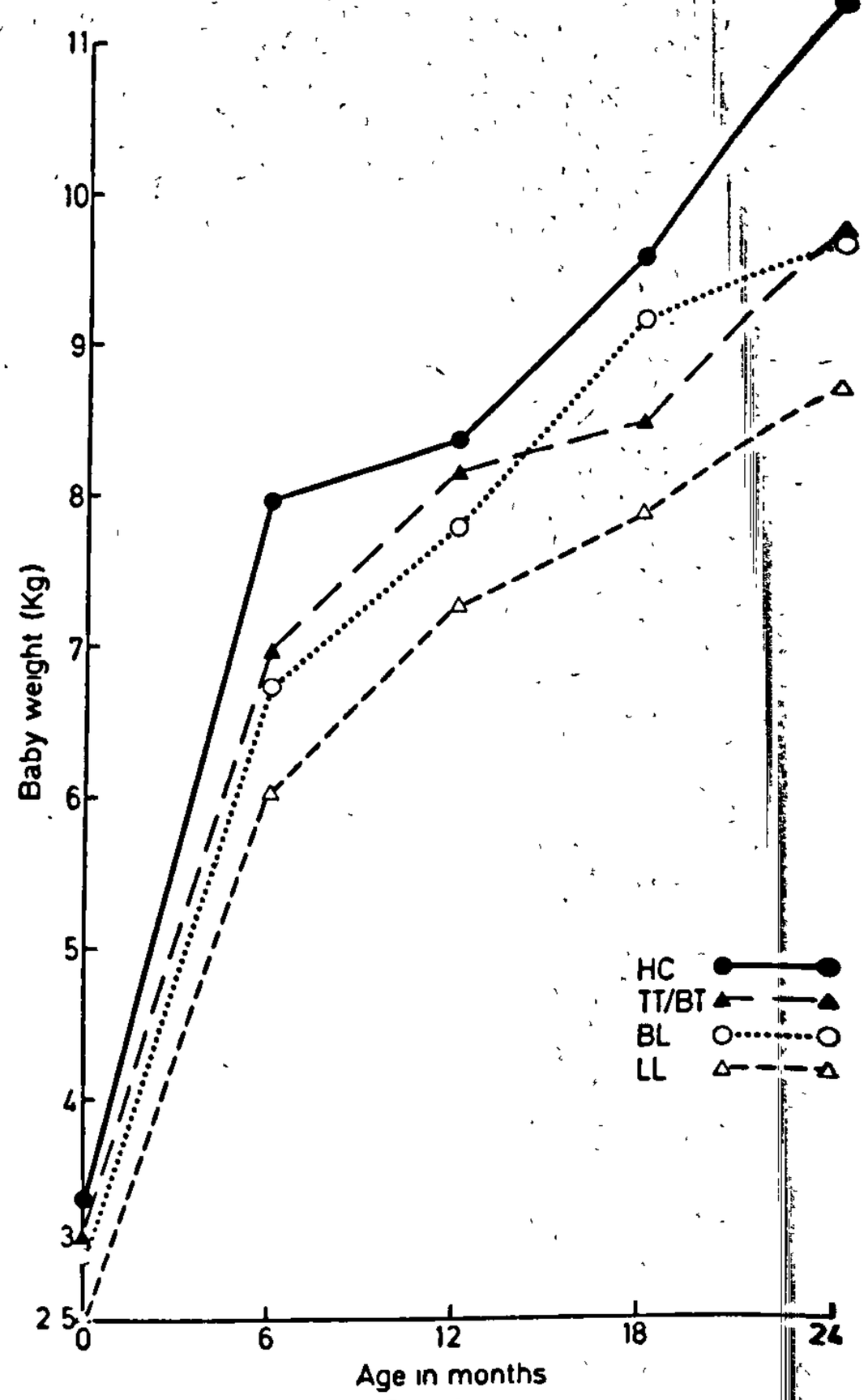


FIG. 4

Mean infant weight in the first two years according to the clinical classification of the mother (see Fig. 1).

asmic and many required admission to hospital. When infant weights were classified according to the Boston standard (Jelliffe, 1966; Davidson *et al*, 1979; Fig. 5), the babies in all groups were underweight, but this was most striking in the babies of mothers with lepromatous leprosy; at 12 months, 80 per cent of the babies were moderately or severely underweight and at 24 months, all the babies were moderately or severely underweight.

Perinatal and infant mortality

Perinatal and infant mortality was increased in babies of mothers with leprosy when compared with healthy controls but the difference did not reach significance.

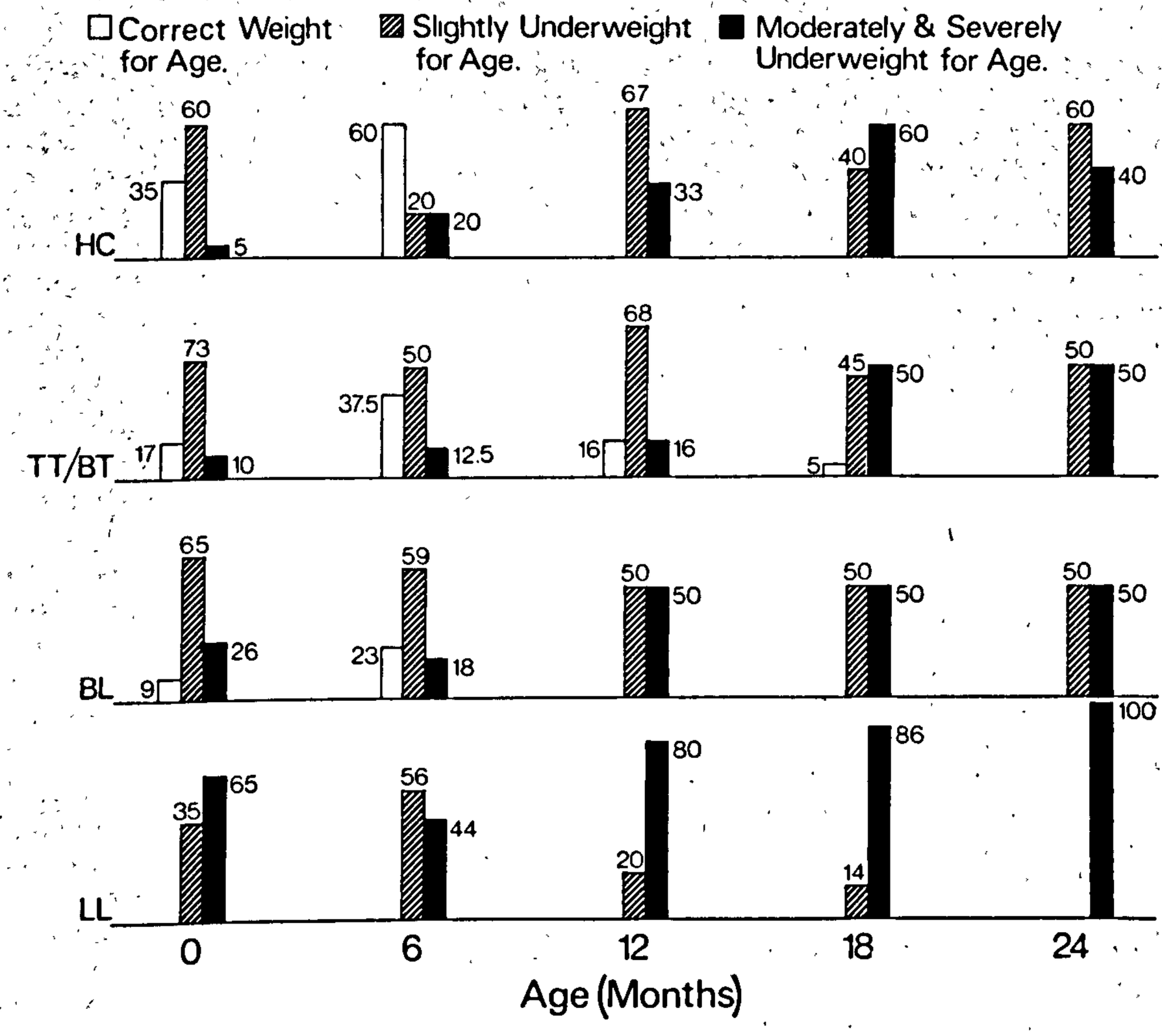


FIG. 5

Infant weights by the Boston Standard according to clinical classification of the mother. Percentages of babies who were the correct weight for age (at or above 50th centile for both sexes) (□), slightly underweight for age (80 to 99 per cent of 50th centile) (▨) and severely/moderately underweight for age (below 80 per cent of 50th centile) (■) are shown at six month intervals.

DISCUSSION

The absence of obstetric complications in leprosy patients has been well documented (King and Marks, 1958; Lawson and Stewart, 1967; Coudert *et al*, 1978) and was confirmed in this study; thus the cause of the low baby and placental weights observed in this study is not easy to define.

It could be that the women with lepromatous leprosy were more ill than those with tuberculoid leprosy, and that the severity of their infection resulted in low birth weight of the baby. Such a finding was recorded by Coid and Wardman (1971), who reported that the severity of a maternal infection with para-influenza affected the fetus without the infecting virus being isolated from the conceptus. The sickness of lepromatous patients can be assessed directly in terms of bacillary load and indirectly in terms of nutritional status. But no difference was found in the nutrition of the mother nor in the weight of babies born to mothers with lepromatous leprosy and a high bacillary load (positive bacteriological index and morphological index) when compared to mothers with lepromatous leprosy and a low bacillary load (negative bacteriological index and morphological index); and yet there was a significant difference between the birth weight of babies of mothers with lepromatous leprosy when compared with those

of borderline lepromatous leprosy although both groups had the same bacillary load. Thus the severity of the mother's disease in terms of both nutritional status and bacillary load is unlikely to be the cause of the low birth weight.

Massive congenital infection (as occurs in syphilis, toxoplasmosis and malaria) involving the placenta and impairing its function can also be excluded as a cause of low birth weight as we found no histological evidence of such placental involvement in these leprosy patients. For the same reason, damage to the placenta due to toxins, such as occurs in animals (Coid *et al*, 1977), can be ruled out, and it is unlikely anyway that *M. leprae* can produce this type of toxin.

Mothers with tuberculoid and lepromatous leprosy received the same dose of dapsone and yet it was only the babies of mothers with lepromatous leprosy that were of low birth weight. Thus anti-leprosy treatment was a further unlikely cause of the low birth weight. To us it seems likely that the low birth weight of babies of mothers with leprosy is due to immunological factors related to leprosy. Though immune complexes are known to occur in leprosy, particularly in lepromatous leprosy (Bjorvatn *et al*, 1976) it seems unlikely that such complexes in the placenta were responsible for low birth weight since our examination of the placenta by light and electron microscopy showed no evidence of immune complexes. However, patients with leprosy tend to have depression of their T lymphocyte responses to both mitogens (Dierks and Shepard, 1968; Bullock and Fasal, 1971) and to *M. leprae* (G. Bjune, 1976, personal communication) and this depression is certainly more marked in patients with lepromatous leprosy. How depressed cell-mediated immunity leads to low birth and placental weights is obscure, but mice rendered tolerant at birth to paternal strain antigens have smaller placentae than untreated mothers mated with allogenic males (James, 1965) and in both experimental animals (Billington, 1964; Beer and Billingham, 1978) and man (Jenkins and Good, 1972), histo-incompatibility results in larger placentae and larger babies. Scott (1977) also found that women on immunosuppressive drugs tend to have low birth weight babies.

It may be that mothers with lepromatous leprosy have an inadequate cell-mediated immune response to pregnancy, and this state of tolerance or partial tolerance might be associated with smaller placentae and smaller babies. The observation that babies of mothers with lepromatous leprosy failed to thrive is also of interest. Seven of the babies born to mothers with lepromatous or borderline lepromatous leprosy died during the first year, and the remainder failed to gain weight normally. This may be because the milk of lepromatous mothers is of poor nutritional content or is defective regarding humoral defence factors; this is being studied. Alternatively, babies of lepromatous mothers may inherit depressed immunological responses and thus be particularly prone to infection. The results of lymphocyte transformation and other immunological tests in the babies will be reported elsewhere.

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Immunoglobulin concentration in mothers with leprosy and in healthy controls and their babies at the time of birth

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Summary Immunoglobulins were quantitated in sera from 52 matched mothers at delivery and in the corresponding cord blood samples. The cord IgA concentration was significantly increased in babies from mothers with active lepromatous leprosy compared to a control group, and a group where the mothers suffered from tuberculoid leprosy. The cord IgM concentration was normal both in babies from mothers with active lepromatous leprosy, the control group and the group of mothers suffering from tuberculoid leprosy. Since IgA does not cross the placenta, this increase reflects an active increased production of IgA in the foetus of mothers suffering from active lepromatous leprosy. This could indicate transfer of *M. leprae* or *M. leprae* antigens across the placenta into the foetus.

Introduction

In some maternal infections, namely rubella, cytomegalovirus infection, toxoplasmosis and syphilis, transplacental transfer of pathogens occurs (Alford, 1962;[1] Scotti and Logan, 1968;[2] McCracken and Shinefield, 1965)[3] causing a severe generalized infection in the foetus. Under these circumstances, the foetus can start to produce antibodies in utero. This antibody production is sometimes indicated by increased IgM and/or IgA concentration in cord blood (Stiehm *et al.*, 1966),[4] and may be proven by specific antibodies of the IgM or IgA class against the infectious agent (Scotti and Logan, 1968;[2] Reimer *et al.*, 1975).[5]

Up to the present time leprosy has not been demonstrated to cause intrauterine infection. The first clinical signs of manifest leprosy have been

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demonstrated at 1.5 to 2 years of age (Soul, 1958;[6] Worth, 1960).[7] Based upon these observations, it was generally thought that leprosy bacilli do not infect the foetus. The long incubation period before the appearance of definite clinical signs and symptoms has made the subject difficult to study.

A prospective study on the effect of leprosy on pregnancy, parturition and of the baby was carried out in Addis Ababa, Ethiopia, from 1975 to 1978. As part of this study, IgA, IgM and IgG were quantitated during pregnancy and at birth. We were especially interested in the increase of IgM and/or IgA concentration in cord blood as an indication of foetal exposure to *M. leprae* antigen(s).

Materials and methods

PATIENTS

Fifty-two pregnant women were selected for this study. All of them attended the outpatient clinic at the Addis Ababa Leprosy Hospital. The patients were clinically and histologically classified according to the Ridley-Jopling scale (Ridley and Jopling, 1966)[8] and were divided into four groups.

Group 1

Seventeen mothers who suffered from lepromatous leprosy (LL-BL) with a positive bacterial index (BI), i.e. with acid fast bacilli in one or several skin smears taken from 6 different sites. All of them were treated with 100 mg DDS daily.

Group 2

Five mothers who suffered from lepromatous leprosy with a negative BI (LL-BL). Four of these patients received DDS, 50 to 100 mg daily, and had been on continuous treatment for at least 5 years. One patient had stopped treatment prior to the study.

Group 3

Sixteen mothers who suffered from tuberculoid leprosy (BT). Six patients still received DDS treatment (50–100 mg daily) and 10 patients were released from control before the trial started and did not receive any treatment.

Group 4

Fourteen mothers without any clinical sign of leprosy (NL), but with the same socio-economic background as the leprosy patients.

The patients were divided into these 4 groups to separate the mothers with highly bacilliferous disease from the others, since the foetus in the first group would have the greatest chance of being exposed to *M. leprae* or their antigens. All the patients were Ethiopians living in the villages around the leprosy hospital under poor socio-economic conditions. Serum samples were obtained during the last trimester of pregnancy, and from the mother at delivery and from cord blood. The serum samples were stored at −20°C and freeze dried before transportation to Norway. Prior to estimation of the immunoglobulin concentration, the sera were reconstituted with distilled water, and 0.01% NaN_3 was added as a preservative. They were subsequently stored at +4°C.

IMMUNOLOGICAL METHODS

Immunoglobulin concentrations were determined by the single radial diffusion technique (Mancini *et al.*, 1964;[9] Mancini *et al.*, 1965;[10] Fahey and McKelvey, 1965[11]) with specific anti-IgG, anti-IgA and anti-IgM (Dakopatts a/s, Copenhagen, Denmark) in 1% Litex agarose gel (Litex a/s, Glostrup, Denmark) containing 0.05 M barbiturate buffer of pH 8.6. The antisera were tested for specificity by immunoelectrophoresis and single radial diffusion using sera and isolated immunoglobulins from patients with myeloma and macroglobulinaemia, and sera from individuals with isolated lack of IgA. The sera were found to be monospecific by these methods.

Immunoglobulins in the maternal sera were quantitated by the single radial diffusion method routinely used in our laboratory. A 1.5 mm thick agarose gel was made on a glass plate of 11 x 20 cm. The total volume of agarose gel on the plate was 44 ml and the amount of anti-IgG 3.7 ml, i.e. 0.17 ml anti-IgG/cm^2. In this plate, 66 wells were punched out with a diameter of 2 mm. A volume of 5 μl of either standard or sera to be tested was filled in the wells. Locally prepared IgG standard solutions were used and controlled at regular intervals against the Behringwerke's IgG standard (Behringwerke AG, Marburg, Frankfurt/M, Germany). The plates were left in a moist chamber at room temperature for 24 hrs. The precipitin rings had a sharply defined edge and were measured directly on the unstained plates.

The IgA and IgM concentrations in maternal sera were determined in the same way using 0.04 ml anti-IgA or anti-IgM per cm^2 gel. The Behringwerke IgA and IgM standards were used to prepare the standard curves.

The amount of IgA and IgM in cord blood is so low that it is difficult to determine the concentration by single radial diffusion methods (Papadatous *et al.*, 1969;[12] Evans *et al.*, 1971[13]). The technique was modified to ensure that minute amounts of IgA and IgM could be detected. The concentration of anti-IgA was lowered to 0.35 μl anti-IgA/cm^2 in the agarose gel. At this point, weak but definite precipitin rings with sharply defined edges could be seen after staining with Coomassie brilliant blue when minute amounts of IgA were put in the wells. This concentration of anti-IgA in the gel was therefore chosen

to detect IgA in the cord sera. Wells with a diameter of 2 mm were made and filled with 5 μl either standard or test sera. The plates were left in a moist chamber for 48 h, washed and pressed 4 times, left for a final wash overnight, dried and stained with Coomassie brilliant blue. In this way distinct precipitin rings were obtained demonstrating IgA in concentrations down to 4×10^{-3} g/l, and the IgA concentration could be determined if above 8×10^{-3} g/l.

The concentration of IgM in the cord blood is 5 to 10 times higher than IgA (Faulkner and Borella, 1970;[14] Hardy *et al.*, 1969[15]). It was therefore easier to determine the IgM concentration in cord blood. The agarose gel contained 0.7 μl anti-IgM/cm^2. The plates were left at room temperature for 48 h, washed and stained as for the IgA plates. The standard used was diluted Behringwerke IgM standard.

For calculation of the statistical significance of difference between groups, Wilcoxon's modified ranking test was used (Documenta Geigy, 1962).[16]

Results

IMMUNOGLOBULIN CONCENTRATION IN MATERNAL SERA AT DELIVERY

The median IgG concentration in maternal sera at delivery was 8 g/l with a range of 3 g/l to 16 g/l. Figure 1 shows that there was no significant difference between the four groups of patients.

The median IgA concentration was 1.24 g/l with a range of 0.25 to 2.6 g/l.

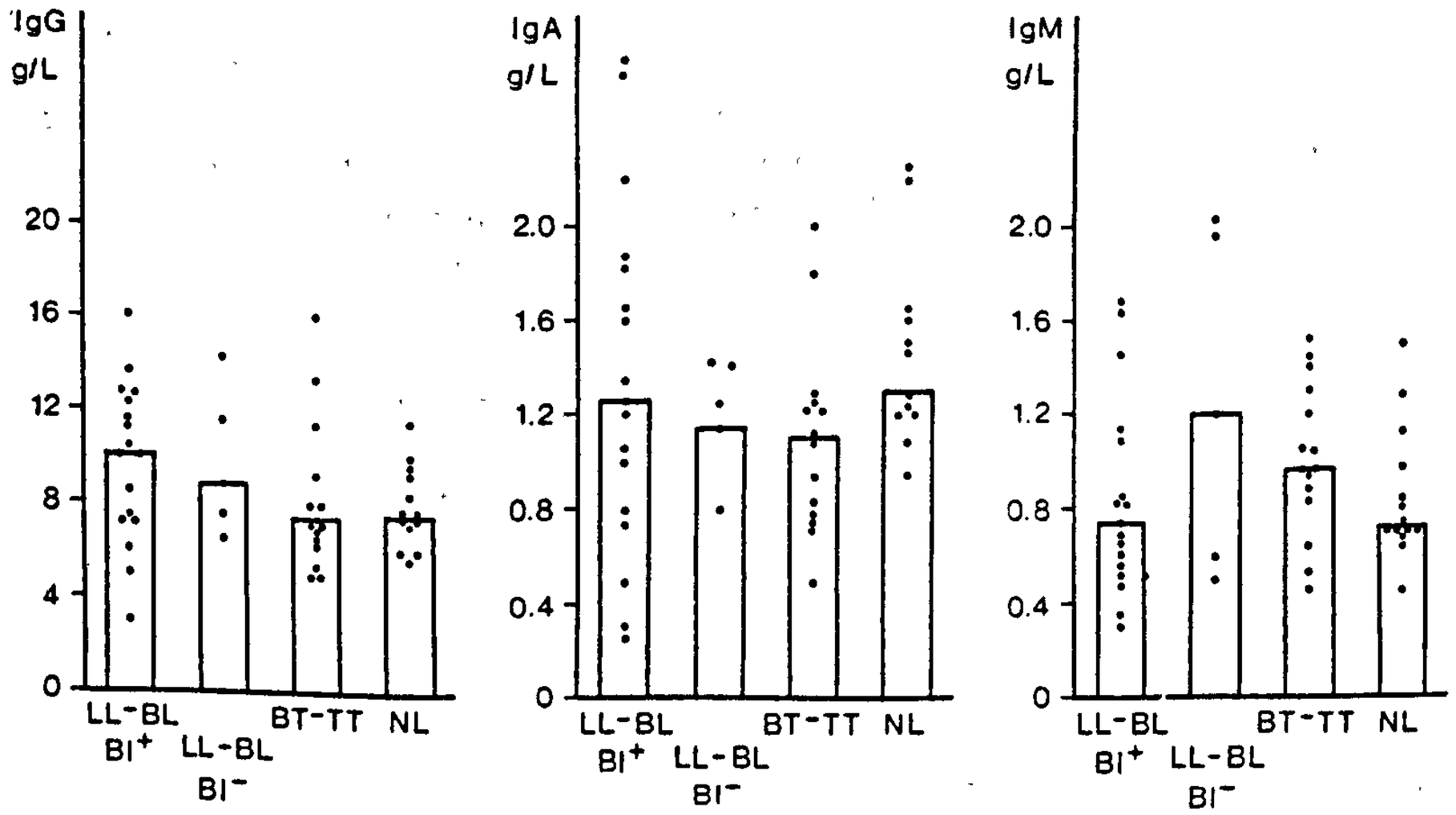


Figure 1. IgG, IgA and IgM concentration in maternal serum. Each point represents one individual and the top of the columns the median value.

The median IgM concentration was 0.82 g/l with a variation between 0.3 and 2.0 g/l. Neither IgA nor IgM concentration showed any significant difference between the four groups of patients, as shown in Fig. 1.

The IgG concentration in sera obtained from the mothers during the last three months of pregnancy was higher than at delivery. This fall of IgG concentration from pregnancy to delivery was observed in 24 out of 36 women. The 3 women with IgG concentration below 6 g/l at delivery had IgG concentration below 6 g/l when tested in the 3rd trimester.

IMMUNOGLOBULIN CONCENTRATION IN CORD BLOOD

Figure 2 shows the IgG concentration in the cord sera. The highest concentration was found in group 1 with a median value of 9.5 g/l, but there was no significant difference between the four groups. There was a good correlation between the IgG concentration in cord blood and maternal sera taken at delivery in each mother–baby pair. Out of 52 pairs, only 12 pairs showed a difference greater than 25% between the IgG concentration in cord blood and maternal blood taken at delivery.

The IgA concentration could be measured by single radial diffusion methods at levels above 8×10^{-3} g/l. IgA could be detected if the concentration was above 4×10^{-3} g/l, but it could not be quantitated at levels between 4 and 8×10^{-3} g/l. These two limits are indicated on Fig. 3 with horizontal dotted lines. Out of 17 cord sera in group 1, two fell below the detection limit of 4×10^{-3} g/l, while 12 out of 35 in group 2, 3 and 4 fell below this limit. The median value of each group is indicated on Fig. 3 with a horizontal bar. Group 1 had a median value of 9.5×10^{-3} g/l while the median value of group 2, 3

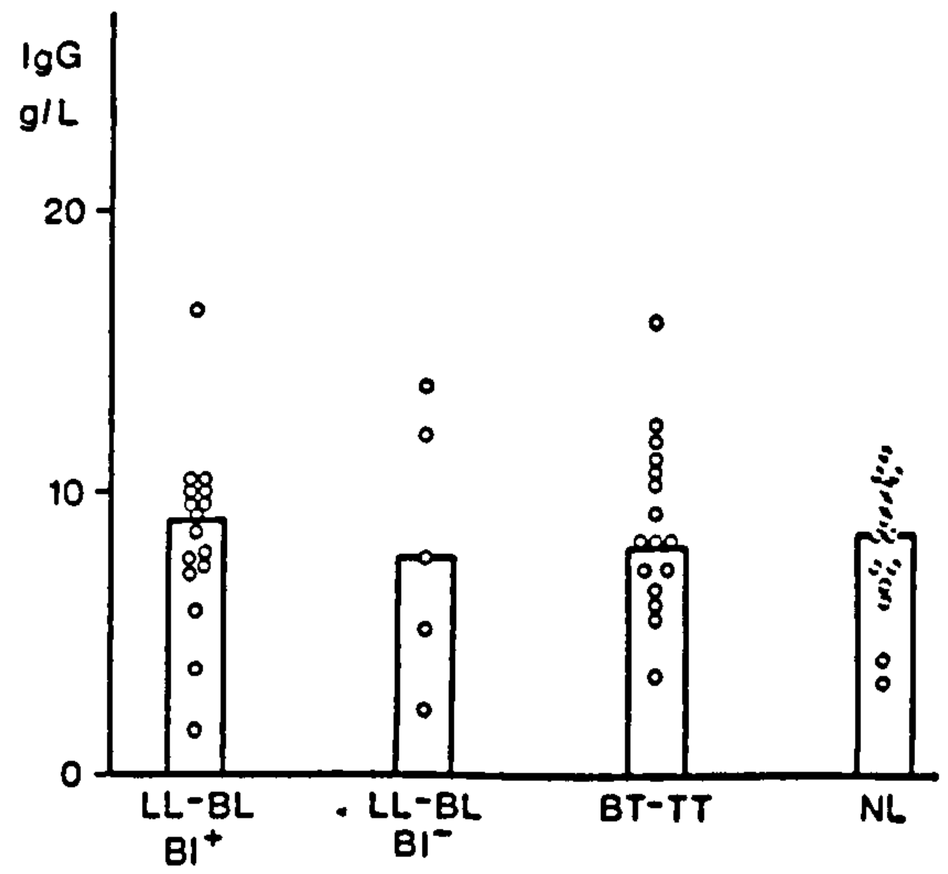


Figure 2. IgG concentration in cord serum, otherwise as for Figure 1.

and 4 fell below 8×10^{-3} g/l. This difference is significant using Wilcoxon's ranking test. The cord serum labelled 110 on Fig. 3 is excluded from the series because of possible leakage of maternal blood into this cord blood sample. The IgM concentration of this cord blood sample was 110×10^{-3} g/l, and both the IgA and the IgM concentration was lower in a sample taken 6 weeks after birth from the same baby. In the other cord sera, there was no correlation between high concentration of IgA and IgM, nor between IgA and IgM concentration in matched maternal and cord blood samples.

The IgM concentration in cord sera is shown in Fig. 4. The control group had the highest concentration with a median value of 74×10^{-3} g/l, while the median value of the three other groups varied from 40 to 54×10^{-3} g/l. These differences were not statistically significant ($p > 0.1$).

Discussion

Leprosy has not yet been described in patients below 1.5 yr (Noussitou *et al.*, 1976),[17] in children it is still uncommon below the age of four, thus it is generally thought that leprosy is not transferred to the foetus.

Leprosy has an incubation time of 2 to 5 yrs (Newell, 1966)[18] which can be partially explained by the slow multiplication rate of *M. leprae* (Shepard and McRae, 1965).[19] After experimental inoculation of armadillo, more than 9

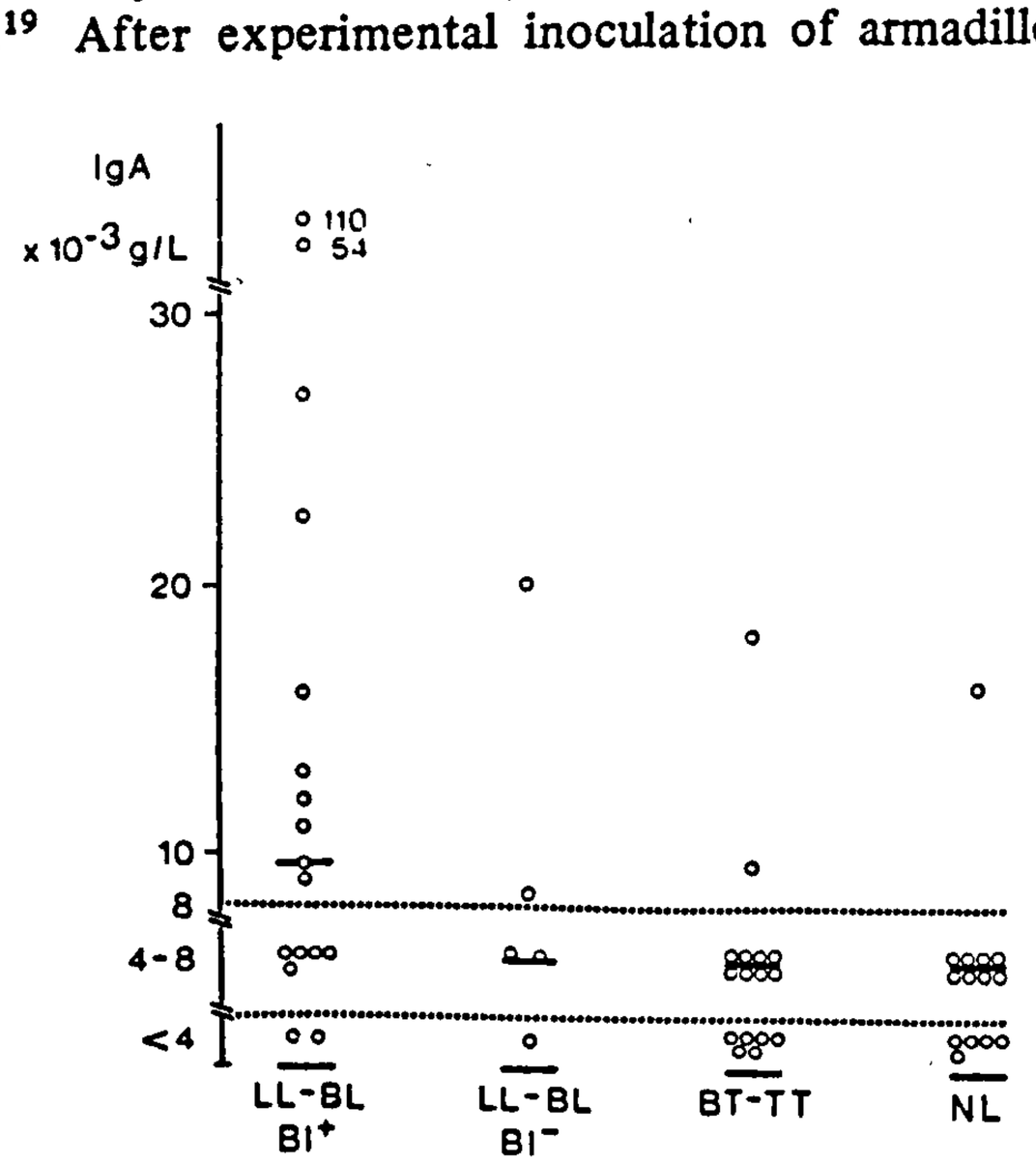


Figure 3. IgA concentration in cord serum. See text for explanation of the two horizontal dotted lines. The horizontal bars show the median value for the 4 groups.

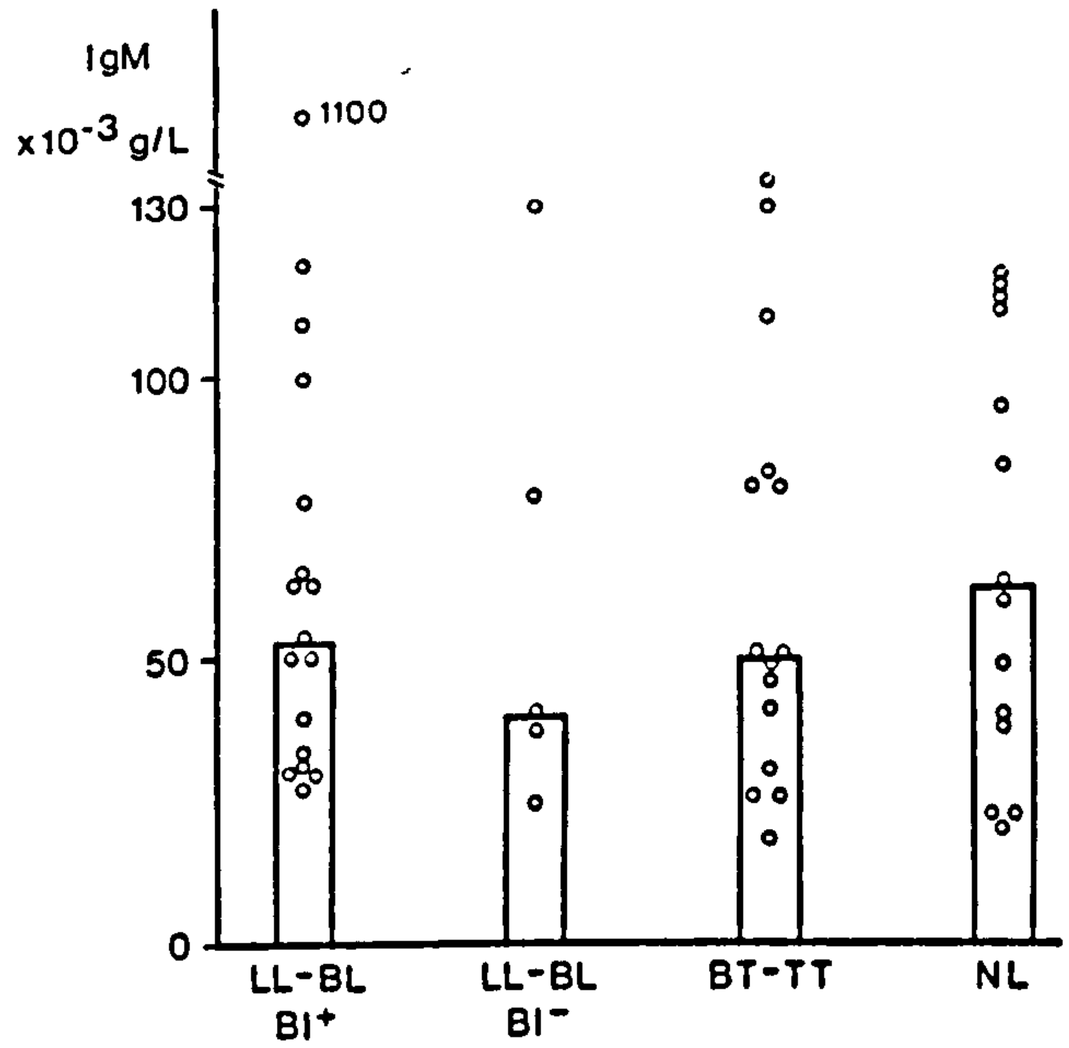


Figure 4. IgM concentration in cord serum, otherwise as for Fig. 1.

months elapsed before the leprosy bacilli had multiplied sufficiently to cause any clinical sign of leprosy (Kirchheimer and Storrs, 1971;[20] Storrs, 1973[21]). The absence of published reports of leprosy in humans occurring within the first 1.5 years of life could be explained by the long incubation time even though the infection was acquired in utero.

Patients with active lepromatous leprosy, can have up to 10^5 leprosy bacilli per ml of blood (Drutz *et al.*, 1972).[22] In pregnant women with active lepromatous leprosy, the exposure of the placenta to *M. Leprae* bacilli is intense and leprosy bacilli could cross the placenta and infect the foetus. However, soon after birth the newborn baby will be exposed to a heavy dose of *M. leprae.* The lepromatous leprosy mother can shed up to 2.4×10^8 leprosy bacilli from her nose in 24 h (Davey and Rees, 1974)[23] and leprosy bacilli can be present in breast milk (Pedley, 1967)[24] The long incubation period of leprosy makes it impossible to decide if the baby was infected with leprosy before or after birth.

IgG crosses the placenta, therefore most of the IgG present in the cord blood has been made by the mother. The lower IgG concentration at parturation compared with that of the last trimester of pregnancy has been documented previously (Wagner and Knobloch, 1973;[25] Maroulis *et al.*, 1971[26]). It is probably caused partially by active transport of IgG across the placenta and by increased catabolism in the mother around delivery.

IgM and IgA do not cross the placenta. Increased concentration of these

immunoglobulins in cord blood could be taken as an indication of stimulation of the immune system of the foetus by transfer of antigen(s).

Increased IgM concentration in cord blood may be found in congenital infections such as syphilis, toxoplasmosis and cytomegalovirus infections but even in these diseases heavy congenital infections are not always associated with increased IgM concentration in cord blood (Reimer *et al.*, 1975;[5] McCraken *et al.*, 1965;[3] Hardy *et al.*, 1969[15]). Several workers have determined the IgM concentration in large numbers of cord sera and babies with abnormal IgM concentration were followed for clinical and serological signs of congenital infections. In many instances (from 60 to 80%), babies with increased IgM concentration in cord blood could not be associated with any sign of congenital infection or disease during the first year of life (Hardy *et al.*, 1969;[15] Miller *et al.*, 1969[27]). Furthermore, intra-uterine infections such as syphilis and rubella do not always cause an increase in foetal IgM, therefore increased foetal IgM must be regarded as a non-specific and poor indicator of intra-uterine infection.

Little information is available regarding the IgA concentration in cord blood. In many instances, IgA has only been demonstrated in 5 to 10% of the cord sera examined. This has been due to insufficient sensitivity of the assay, the detection limit being $50–200 \times 10^{-3}$ g/l (Stiehm *et al.*, 1966;[4] Evans *et al.*, 1971;[13] Seth *et al.*, 1971[28]). Faulkner and Borella 1970[14] developed a radioimmunoassay for quantitation of IgA in cord blood samples. They found that IgA was present in all cord sera tested in concentrations ranging from 1.5 to 25.5×10^{-3} g/l with a mean value of 8×10^{-3} g/l. In our sera, we could determine IgA concentration down to 8×10^{-3} g/l, and detect but not accurately quantitate down to 4×10^{-3} g/l. IgA could be detected in 43 out of 52 cord sera we examined, and the concentration could be determined in 20 out of 52 sera. The median IgA cord concentration in our series is between 4 & 8×10^{-3} g/l. Our IgA cord blood concentrations are in agreement with the concentrations found by Faulkner and Borelli.

Increased IgA concentrations were demonstrated in cord blood from babies of mothers with active lepromatous leprosy (group 1). It is significant that at least 9 out of the 16 women in group 1 had an active relapse or were diagnosed as having lepromatous leprosy during this pregnancy. These women would have a large quantity of *M. leprae* bacilli in their blood stream throughout the pregnancy thus exposing the placenta, and possibly the foetus, to massive antigenic stimulation. The median cord IgA concentration in group 1 was 9.6×10^{-3} g/l, while the median cord IgA concentrations in the three other groups (2, 3 and 4) was below 8×10^{-3} g/l. Cord IgA concentration was above 8×10^{-3} g/l in 9 samples out of 16 in group 1, while the cord IgA concentration was above 8×10^{-3} g/l in only 5 samples out of 35 from groups 2,3 and 4. These differences are significant using Wilcoxon's ranking test. These results indicate that the immune system of the foetus was often stimulated when the mother suffered from active lepromatous leprosy.

Other possibilities should also be considered. Leakage through the placenta could occur due to damage of the placenta in mothers with active lepromatous leprosy. The difference in IgA concentration in maternal and cord serum is great, about 500 times higher in the mother. A small placental leakage would lead to a marked increase in cord IgA concentration. Placental leakage ought to lead to simultaneous leakage of IgM and IgA. IgM concentrations in cord sera were normal, also in group 1. Except for one cord serum, marked 110 on Fig. 3, we have found no indication that the increased IgA in cord blood from group 1 could be caused by leakage. The cord serum marked 110 has been excluded from the calculation due to possibility of placental leakage. The increased IgA concentrations in the other cord sera in group 1 must have been produced by the foetus before birth. This may have been caused by transfer of *M. leprae* or *M. leprae* antigen(s) across the placenta. Studies of the antigenic specificities of these babies' IgA will be studied later.

Acknowledgements

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Demonstration of antibodies against *Mycobacterium leprae* both in immunoglobulin G and M in sera from pregnant and non-pregnant lepromatous leprosy patients

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Summary Antibodies against *M. leprae* antigen 7 have been shown to consist of both immunoglobulin G and M in a lepromatous leprosy serum pool and in individual sera from patients with active lepromatous leprosy. Various implications of the occurrence of anti-*M. leprae* antibodies in several immunoglobulin classes are discussed, particularly their use as an indicator of transfer of *M. leprae* antigens or of live leprosy bacilli to the foetus during pregnancy. With the present techniques, no IgM antibodies against *M. leprae* antigen 7 could be detected in several cord sera from babies born of mothers with active lepromatous leprosy.

Introduction

About 20 distinct antigenic components have so far been detected in *Mycobacterium leprae* by crossed immunoelectrophoresis (CIE) using rabbit antisera against purified armadillo-grown leprosy bacilli (Harbo *et al.*, 1977a;[1] 1977b;[2] Closs *et al.*, 1978).[3] One of these components, *M. leprae* antigen 7, has been purified and labelled with ^{125}I, and this preparation has been used to develop a radio-immuno-assay (RIA) for detection and quantitation of anti-*M. leprae* 7 antibodies (Melsom *et al.*, 1978;[4] Yoder *et al.*, 1979).[5]

Patients with lepromatous leprosy have on average the highest concentration of antibodies towards *M. leprae* antigen 7 in their sera with a steady decline towards the tuberculoid end of the spectrum. Within each group there

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is a large variation in concentration of anti-*M. leprae* antigen 7 in sera from individual patients (Yoder *et al.*, 1979).[5]

In lepromatous leprosy patients there is only a small decline in anti-*M. leprae* antigen 7 activity during one and a half years of treatment with DDS (Melsom *et al.*, 1978),[3] while in tuberculoid leprosy the decline appears to occur faster (Yoder *et al.*, 1979).[5]

The specificity of anti-mycobacterial antibodies in leprosy has been characterized by various gel precipitation techniques, particularly CIE with patient serum in the intermediate gel. The number of antibody specificities is lower in tuberculoid than lepromatous leprosy. However, even in most cases of lepromatous leprosy the antibodies are directed against only a limited number of antigenic components of bacillus (Axelsen *et al.*, 1974;[6] Myrvang *et al.*, 1974;[7] Kronvall *et al.*, 1975).[8]

Patients with active lepromatous leprosy have an increased concentration of IgG and possibly also of IgM and IgA in their sera (Gupta *et al.* 1978).[9] Antibodies towards *M. leprae* has been demonstrated but not quantitated in both IgG and IgM class (Abe *et al.*, 1972)[10] but antibodies of IgA class have not yet been demonstrated.

The present RIA is based upon using protein A on the surface of Cowan I strain of staphylococci (NCTC 85308) as solid phase to separate free antigen from antibody-bound antigen. It was previously believed that protein A could only bind IgG immunoglobulins of subclass IgG1, IgG2 and IgG4 (Kronvall and Williams, 1969).[11] It has later been shown that protein A also can bind IgA of subclass IgA2 and IgM of subclass IgM2 (Harboe and Følling, 1974;[12] Saltvedt and Harboe, 1976).[13] The assay is therefore now also expected to detect IgA2 and IgM2 antibodies.

The purpose of the present work was to use this sensitive RIA to establish if antibodies against *M. leprae* antigen 7 occurred both in IgG and IgM in leprosy.

Materials and methods

PATIENT SERA

A serum pool was made from 40 sera obtained from patients suffering from lepromatous leprosy (LL, LI and BL) who had been either newly diagnosed or had been treated with DDS for less than 6 months. Sera were also collected from 4 mothers suffering from active lepromatous leprosy (LL, LI and BL) (Ridley and Jopling, 1966;[14] Ridley and Waters, 1969;[15] Myrvang *et al.*, 1973)[16] and their babies, from one mother suffering from tuberculoid leprosy and her baby, and from one healthy mother and her baby. The samples were taken at delivery from the mother and from the cord of the newborn baby.

PREPARATION AND LABELLING OF *M. LEPRAE* ANTIGEN 7

M. leprae purified from infected armadillo liver by Draper's technique (Draper, 1976)[17] were provided by R.J.W. Rees and P. Draper through the IMMLEP programme. ^{125}I-labelled *M. leprae* antigen 7 was made by electrolytic iodination and tested for purity by crossed immunoelectrophoresis and autoradiography. The properties of the presently used preparation corresponded closely to those of the preparation described in detail previously (Melsom *et al.*, 1978).[4]

RADIO-IMMUNO ASSAY (RIA)

The procedure described previously for assay of antibodies against *M. leprae* antigen 7 was used. In brief, the fractions after density gradient ultracentrifugation were diluted 1:50 in phosphate buffered saline (PBS) containing 0.2% bovine serum albumin (BSA). 100 µl of these dilutions were added to 100 µl of labelled *M. leprae* antigen 7, and protein A containing staphylococci were used as solid phase to separate labelled antigen 7 bound to antibody from free labelled antigen 7 (Melsom *et al.*, 1978).[4]

DENSITY GRADIENT ULTRACENTRIFUGATION

The IgM fraction was separated from IgG in human serum by zonal ultracentrifugation on a 10–40% sucrose density gradient in Tris-NaCl buffer of pH 8.0. When the effect of the acid pH was tested, a similar sucrose gradient in 0.1 M/glycine-HCl buffer of pH 3.0 was used (Hannestad, 1967).[18] Ultracentrifugation was carried out in a Spinco L-50 preparative ultracentrifuge equipped with a SW 65 K rotor 18 hours at 4°C at 36,000 rev./min. Fractions of 4–5 drops each were collected from a pinhole in the bottom of each tube.

DETERMINATION OF IGM AND IGG CONCENTRATIONS

The IgM and IgG concentrations were determined in the fractions after density gradient ultracentrifugation by single radial diffusion method as previously described (Melsom *et al.*, 1979).[19]

REDUCTION WITH 2-MERCAPTOETHANOL

To abolish antibody activity in the IgM fraction, reduction with 2-mercaptoethanol was performed as follows: To 100 µl of serum, 100 µl of 0.9% NaCl and 7.5 µl of 2-mercaptoethanol were added. The mixture was incubated at 20°C for 2 minutes and layered over a sucrose gradient followed by immediate start of the centrifuge. This provided a concentration of 0.5 M.

2-mercaptoethanol in the sample for reduction and did not lead to solidification of serum which occurred when higher concentrations of 2-mercaptoethanol or undiluted serum were used (Solheim and Harboe 1972).[20]

Results

When the lepromatous leprosy serum pool was subjected to density gradient ultra-centrifugation and the fractions tested by RIA, antibody activity against *M. leprae* antigen 7 was found in two distinct peaks; denoted peak 1 and peak 2 in Fig. 1. The concentration of IgM and IgG as determined by single radial diffusion in the different fractions, is also indicated in the figure. IgM was clearly separated from IgG. As seen in Fig. 1, maximal IgM concentration corresponded to maximal antibody activity in peak 1 and the distribution of IgG concentration corresponded to antibody activity in peak 2.

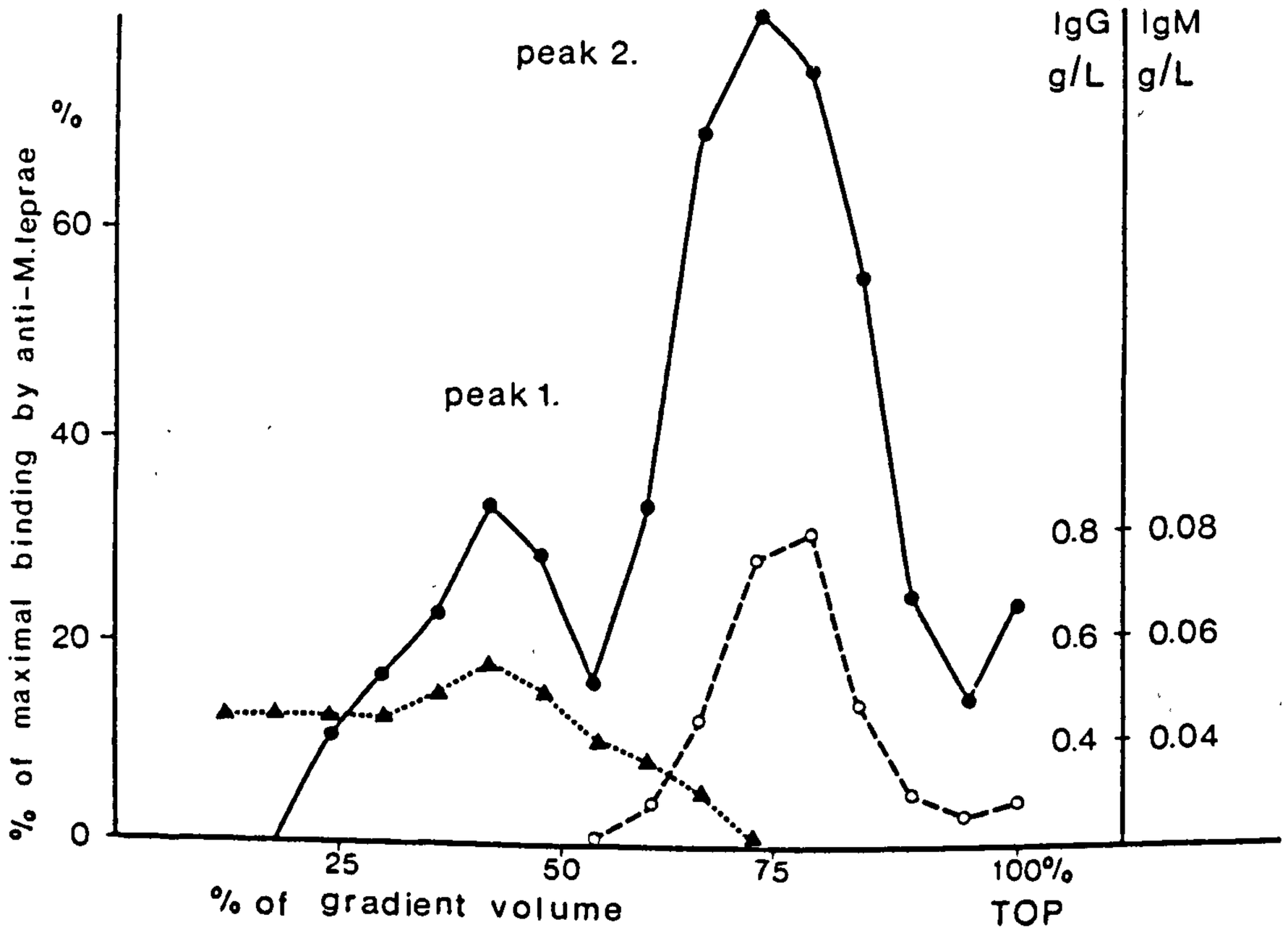


Figure 1. ●——● antibody activity against *M. leprae* antigen 7, ▲---▲ IgM concentration and o---o IgG concentration in different fractions after density gradient ultracentrifugation of LL serum pool.

The lepromatous leprosy serum pool was then tested in the same way after reduction of the serum with 2-mercaptoethanol. RIA with labelled *M. leprae* antigen 7 showed virtually no antibody activity corresponding to peak 1 after reduction of the serum with 2-mercaptoethanol. Nor could IgM be detected in the rapidly sedimenting fractions, while both peak 2 antibody activity and the IgG peak were found in the usual location (Fig. 2). The same results are seen in Fig. 3 where one individual serum has been treated with 2-mercaptoethanol. No antibody activity against *M. leprae* antigen 7 nor any IgM could be detected in the rapidly sedimenting fractions.

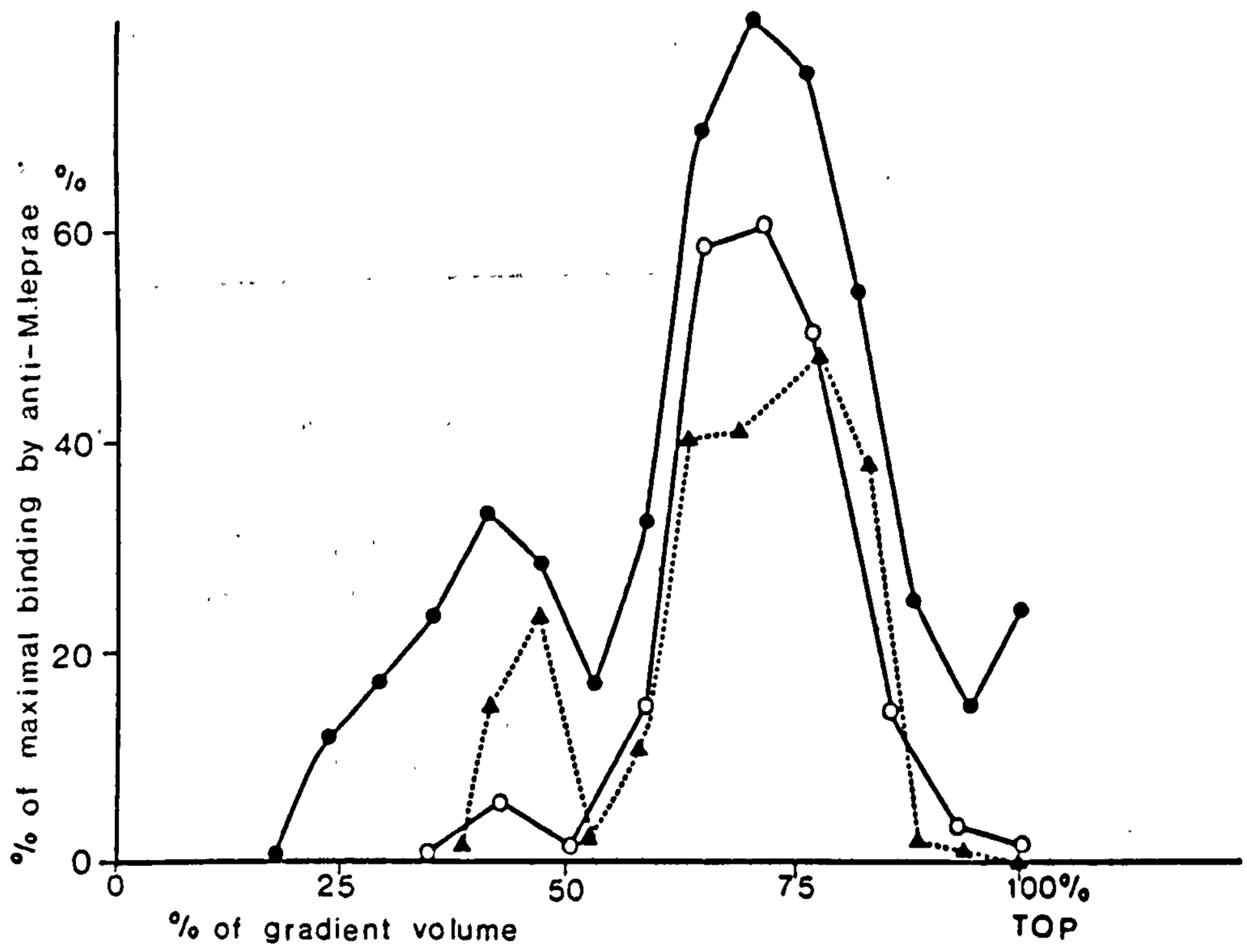


Figure 2. Antibody activity against *M. leprae* antigen 7 in fractions after density gradient ultracentrifugation of ●—● LL serum pool at pH 8.0, ○—○ LL serum pool at pH 3.0 and ▲---▲ LL serum pool after reduction with 2-mercaptoethanol.

Pretreatment with low pH would dissolve immune complexes consisting of labelled *M. leprae* antigen 7 and corresponding antibodies and other types of IgG complexes. At pH 8.0 such complexes would sediment faster than the bulk of monomeric IgG and together with other faster sedimenting components such as IgM. To exclude that the antibody activity in peak 1 consisted of IgG complexes, one individual serum from a mother suffering from lepromatous leprosy

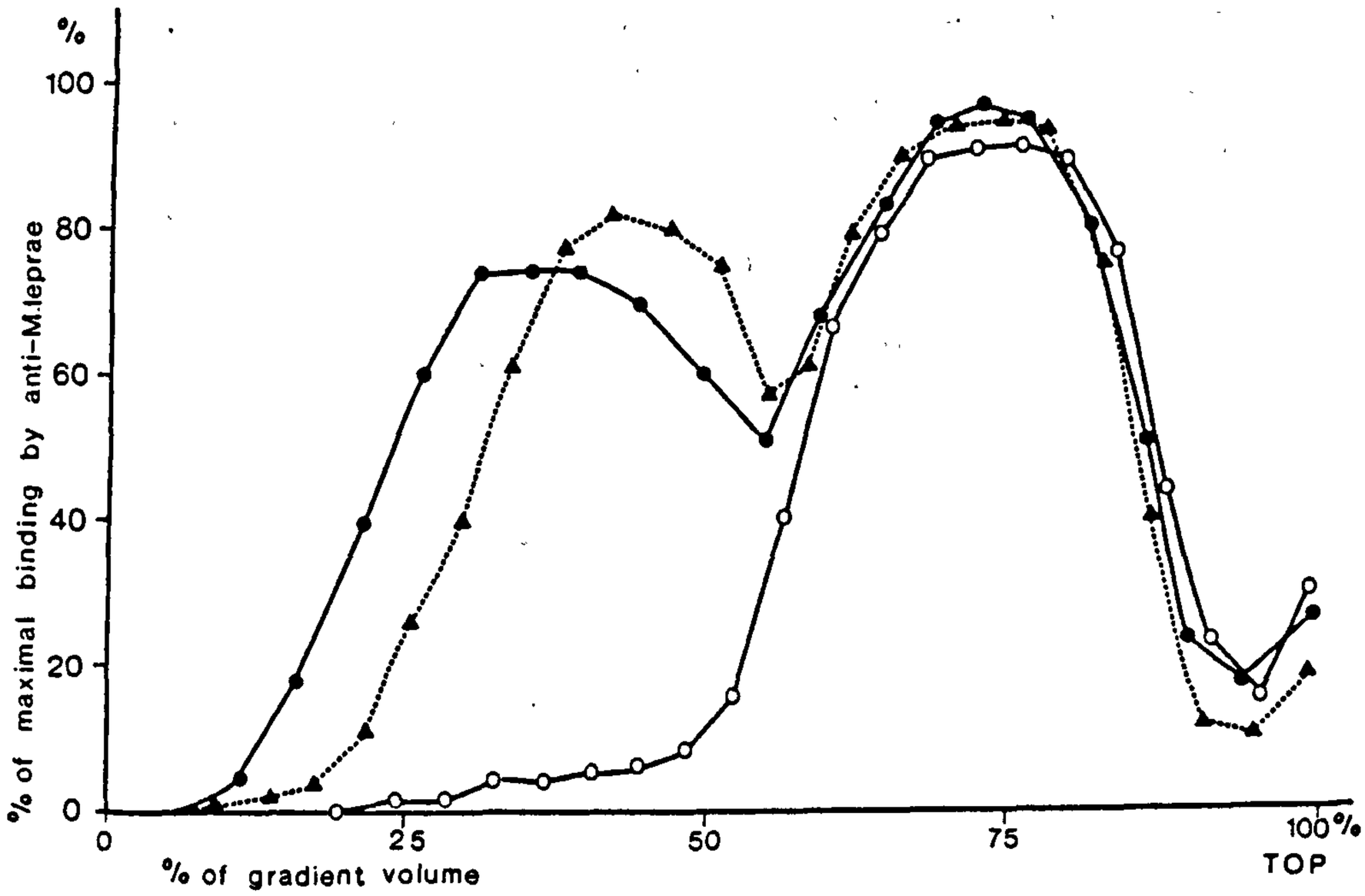


Fig 3. As for Figure 2 but with serum 242/76 from a patient with active lepromatous leprosy.

was fractionated by density gradient ultracentrifugation at pH 3.0. IgM and IgG were still demonstrated in fast and slow sedimenting fractions respectively. The *M. leprae* antigen 7 antibody activity was still present in the fractions containing IgM and IgG, again forming two distinct peaks, peak 1 and peak 2, as seen in Fig. 3. The position and the amount of *M. leprae* antigen 7 antibody activity was almost identical with the pattern seen after density gradient ultracentrifugation at pH 8.0. Similar results were obtained after density gradient ultracentrifugation of the LL serum pool. These findings indicated that the *M. leprae* antigen 7 antibody activity demonstrated in the fast sedimenting fractions after density gradient ultracentrifugation (denoted peak 1 in Fig. 1) did not consist of immune complexes.

Cord sera were fractionated by density gradient ultracentrifugation and the fast sedimenting fractions were tested in the RIA for possible *M. leprae* antigen 7 antibodies of IgM class. Fig. 4 shows a typical pattern of antibody activity against *M. leprae* antigen 7 in different fractions from both maternal and cord serum at delivery. After separation by density gradient ultracentrifugation, no antibody activity against *M. leprae* antigen 7 could be demonstrated in the cord serum sample in the rapidly sedimenting fractions, while the maternal serum had strong antibody activity in the fractions corresponding to IgM. Five additional cord-mother pairs were examined, and IgM antibodies against

M. leprae antigen 7 could not be dected in any of these cord sera. IgM does not cross the placenta, while IgG does. Therefore, the demonstration of *no* antibodies in rapidly sedimenting fractions of cord serum support the conclusion that the activity in these fractions of maternal sera was due to IgM antibodies towards *M. leprae* antigen 7.

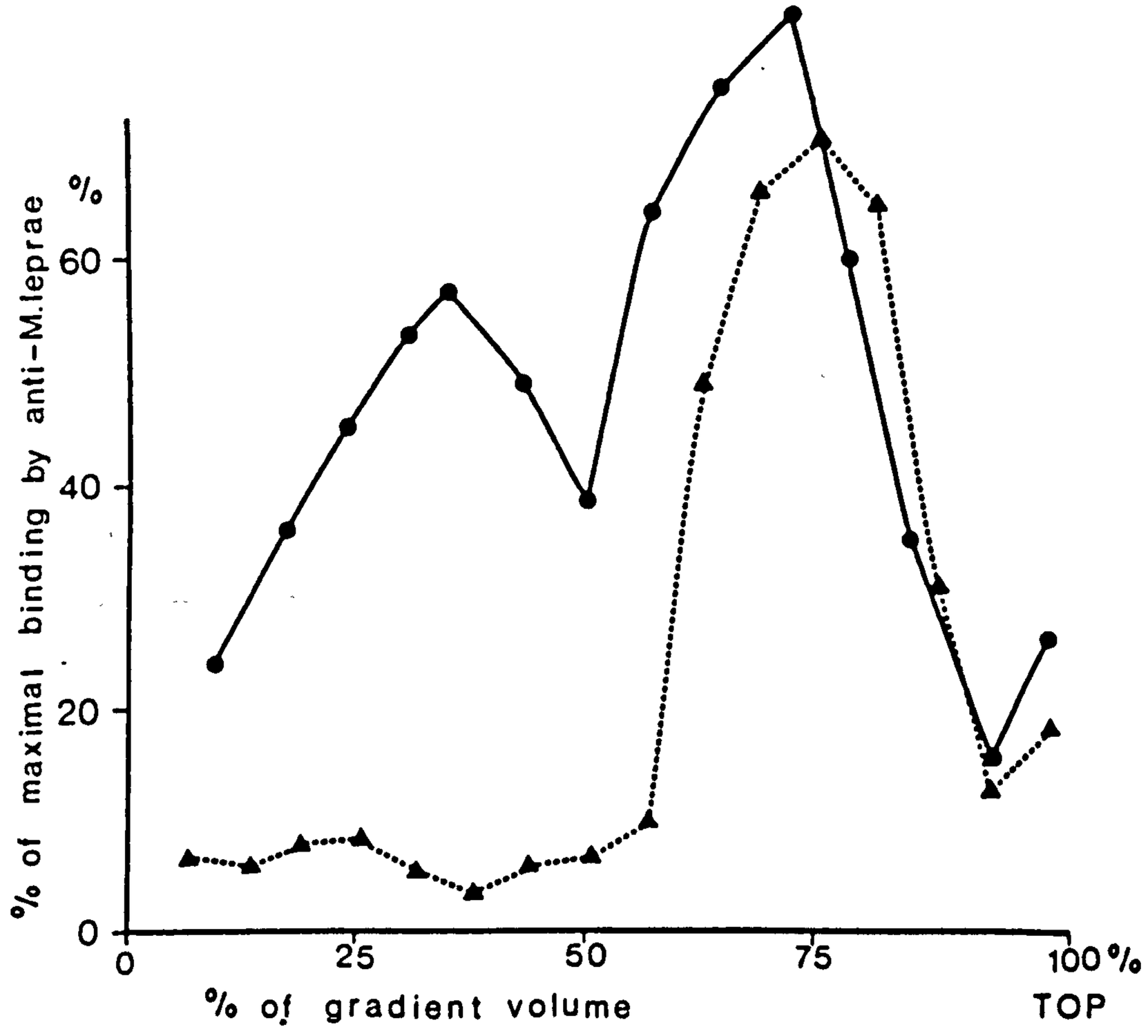


Figure 4. Antibody activity against *M. leprae* antigen 7 in fractions after density gradient ultracentrifugation of ▲---▲ baby's cord serum and ●—● the corresponding maternal serum taken at delivery.

Fig. 5a and 5b illustrate that different amounts of antibodies against *M. leprae* antigen 7 occurred in the IgM and the IgG class in four mothers with active lepromatous leprosy (Fig. 5a), in one mother with tuberculoid leprosy and in one mother who was a normal, non-leprosy control (Fig. 5b). These two figures showed that the sera from three out of four pateints with active leprosy contained antibodies of both the IgM and the IgG class against *M. leprae*

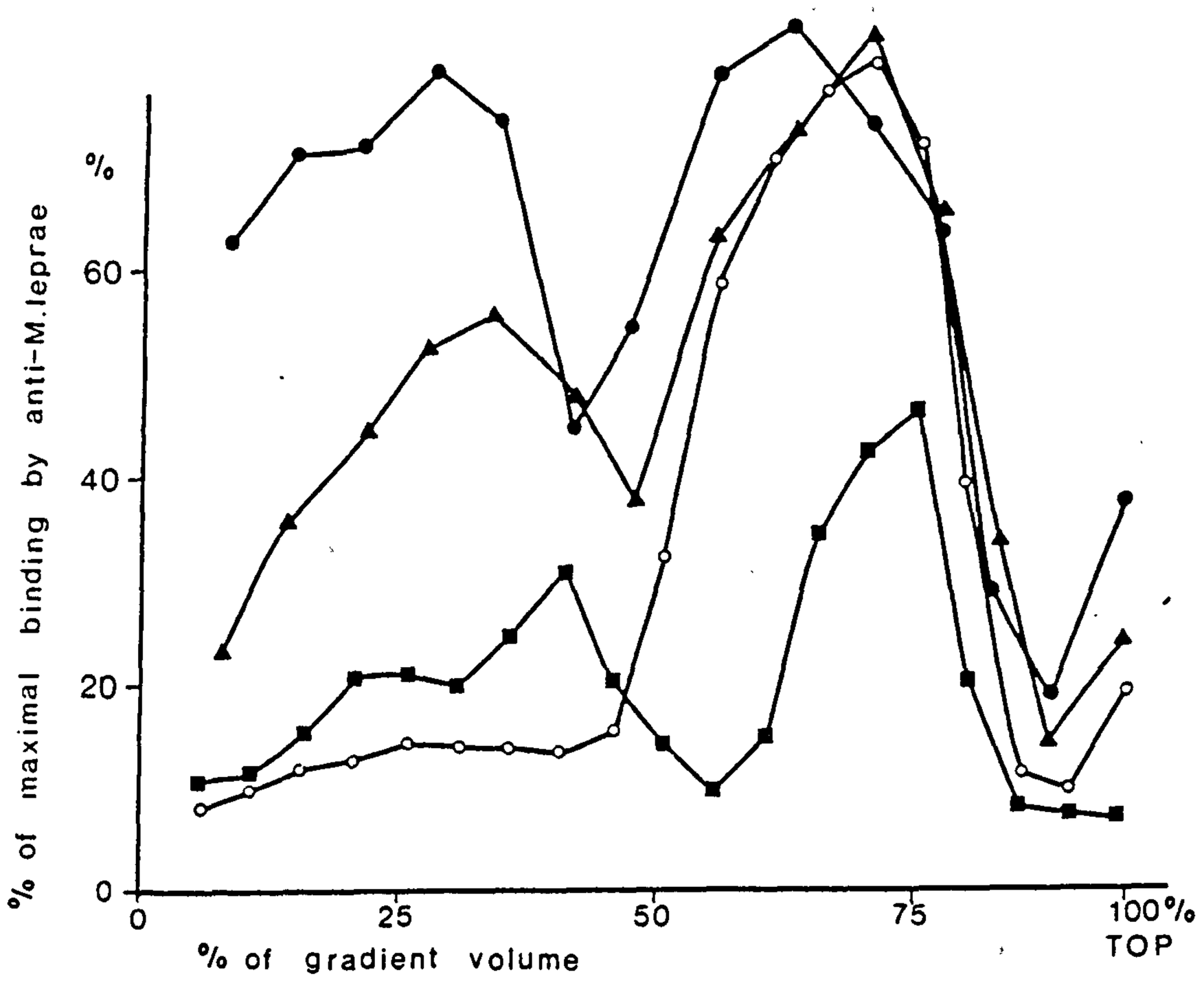


Figure 5a and b. Antibody activity against *M. leprae* antigen 7 in fractions after density gradient ultracentrifugation of: (a) 4 mothers suffering from active lepromatous leprosy, (b) 1 healthy mother ▲—▲, and 1 mother with tuberculoid leprosy ●—●.

antigen 7, while sera from the normal control and the patient with tuberculoid leprosy did not contain antibodies of this class against *M. leprae* antigen 7 detectable by the present technique.

Discussion

Patients with active lepromatous leprosy have more antibodies (both in concentration and specificity) than patients with tuberculoid leprosy or non-leprosy normal controls (Axelsen *et al.*, 1974;[6] Yoder *et al.*, 1979).[5] These antibodies have been considered mostly to be of the IgG class. Besides this no information is available regarding specific antibodies towards *M. leprae* in the different immunoglobulin classes.

IgM can be separated from IgG based on its faster sedimentation rate during density gradient ultracentrifugation of LL serum pool. Quantitation of IgM and

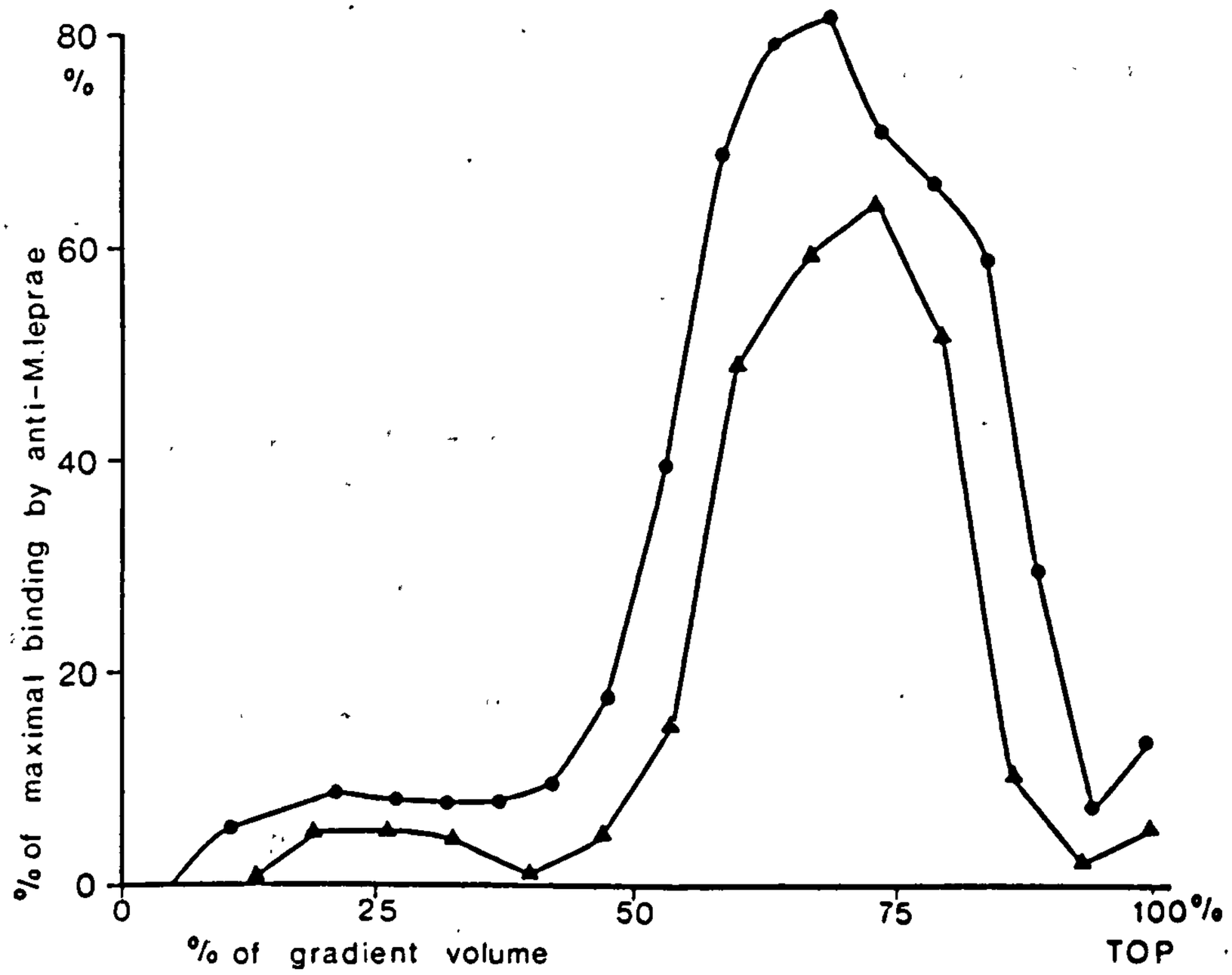


Figure 5a and b. Antibody activity against *M. leprae* antigen 7 in fractions after density gradient ultracentrifugation of: (a) 4 mothers suffering from active lepromatous leprosy, (b) 1 healthy mother ▲—▲, and 1 mother with tuberculoid leprosy ●—●.

IgG in the fractions showed peak of IgM concentration corresponding to the fractions denoted peak 1 in Fig. 1, while the peak of IgG concentration occurred in the slower sedimenting fractions corresponding to peak 2. The concentration of IgA was also quantitated in the fractions, and the distribution of IgA corresponded closely to that of IgG with maximum concentration in the same fraction as for IgG (data not shown).

M. leprae antigen 7 is one antigenic component of about 20 distinct antigenic components so far detected in *Mycobacterium leprae* by crossed immunoelectrophoresis. This preparation was labelled with ^{125}I, and was tested by crossed immunoelectrophoresis, and autoradiography and gel filtration. By these three techniques we could demonstrate that more than 95% of the radioactivity was localized to *M. leprae* antigen 7 (Melsom *et al.*, 1978).[4] We therefore used an isolated, defined labelled cell wall antigen which cross-reacts with several other mycobacteria.

RIA was carried out with labelled *M. leprae* antigen 7 on fractions obtained after density gradient ultracentrifugation of LL serum pool. Antibody activity

was found in the fractions with the highest concentration of IgM, peak 1, and in the fractions with IgG, peak 2. If any antibodies of IgA2 subclass against *M. leprae* antigen 7 are present in LL serum pool, these would be present in the same fractions as IgG, i.e. as part of peak 2.

After reduction and inactivation of IgM with 2-mercaptoethanol, almost no antibody activity towards *M. leprae* antigen 7 was found in the fraction corresponding to peak 1, while antibody activity towards *M. leprae* antigen 7 could be demonstrated in the fast sedimenting fractions after density gradient ultracentrifugation at pH 3.0, a procedure used to split IgG complexes whereas antibody activity due to IgM is not abolished.

We have demonstrated here antibodies towards *M. leprae* antigen 7 in both the IgM and the IgG classs. Thus several criteria used to distinguish IgM from IgG antibodies, i.e. presence in fast sedimenting fractions after density gradient ultracentrifugation, loss of activity after reduction, resistance to exposure to low pH, and lack of activity in cord blood have all been met in the present investigation.

It is still an open question if *M. leprae* bacilli or *M. leprae* antigens can cross the placenta. If the antibodies of the IgM class against *M. leprae* antigen(s) could be demonstrated in cord blood, this would strongly indicate that leprosy bacilli or antigen(s) from the bacilli cross the placenta. The present system used to demonstrate antibodies towards *M. leprae* antigen 7 in the IgM class, was therefore used to test if small amounts of IgM antibodies against this antigen are present in cord sera from babies of mothers with active lepromatous leprosy. But so far we have not been able to demonstrate IgM antibodies towards *M. leprae* antigen 7 in any of the cord sera we have tested. This can be due to our system being too insensitive. The protein A used as solid phase in this RIA system will only bind about 30% of the IgM present in adult sera (Harboe and Følling, 1974).[12] But these results could also indicate that *M. leprae* or *M. leprae* antigen 7 do not cross the placentae. A third possibility is that the foetus is unable to produce antibodies towards *M. leprae* antigen 7. To obtain more information on whether *M. leprae* or *M. leprae* antigens cross the placenta and affect the immune system of the foetus, we are presently developing other sensitive solid phase radio-immuno-assays for specific demonstration of anti-*M. leprae* antibodies in the IgG, IgA and IgM classes in cord serum.

Acknowledgements

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Antibodies against *Mycobacterium leprae* antigen 7 from birth to 18 months of age: an indicator of intra-uterine infection in leprosy

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SUMMARY

All babies of three non-leprosy mothers and ten tuberculoid leprosy mothers and four of five babies of mothers with inactive lepromatous leprosy showed a decline in serum concentration of antibodies against *M. leprae* antigen 7 during the first 4 months of life, as expected from catabolism of maternal IgG. By contrast, ten of twenty babies of mothers with active lepromatous leprosy showed a decline in concentration of anti-*M. leprae* 7 antibodies considerably less than expected. This indicates that these babies have been stimulated by *M. leprae* antigen 7, either as free antigen or by viable *M. leprae* before birth, and thus that leprosy may occur as a congenital infection. Studies of anti-*M. leprae* antibodies in repeated serum samples obtained during the first 18 months of life indicated that children of mothers with bacilliferous leprosy are frequently exposed to *M. leprae* to a sufficient extent to stimulate the immune system of the baby to production of anti-*M. leprae* antibodies during this period. The consequences of this exposure to *M. leprae* should be ascertained by careful clinical studies.

INTRODUCTION

One of the antigenic components of *Mycobacterium leprae*, antigen 7 (Harboe *et al.*, 1977a), has been labelled with ^{125}I and this preparation has been used to develop a radioimmunoassay (RIA) for demonstration and quantification of antibodies against this component (Harboe *et al.*, 1977b; Melsom *et al.*, 1978). Lepromatous leprosy patients have on average the highest concentration of antibodies against *M. leprae* antigen 7 with a steady decline throughout the spectrum towards the tuberculoid end (Yoder *et al.*, 1979). Most of the armadillos which develop a systemic mycobacterial infection after inoculation with *M. leprae* have an increased concentration of antibodies against *M. leprae* antigen 7 in their serum (Harboe *et al.*, 1978).

Antibodies against *M. leprae* antigen 7 have been demonstrated both in the IgM and the IgG classes (Melsom & Duncan, 1980), but no antibodies against *M. leprae* antigen 7 have so far been demonstrated in the IgM fraction in cord sera.

IgG crosses the placenta due to active transport of maternal IgG to the foetus during the last trimester of pregnancy. Therefore cord serum contains about the same amount of IgG and specific

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IgG antibodies as maternal serum at delivery. The concentration of IgG antibodies against *M. leprae* antigen 7 is thus expected to be similar in cord serum and maternal serum at delivery. The maternal IgG is catabolized in the baby at about the same rate as in adults with a half-life of about 4 weeks (Gitlin & Gitlin, 1975). After 3 months, less than 5% of the maternal IgG is still present in the baby. If the newborn baby does not produce any antibodies towards *M. leprae* antigen 7 itself, the decrease in antibody concentration should follow the decrease in concentration of maternal IgG.

In the present investigation antibody activity against *M. leprae* antigen 7 was determined by RIA in babies of mothers with leprosy from birth to 5 months of age and compared with the expected decline in concentration of maternal IgG. The concentration of anti-*M. leprae* 7 antibodies was assayed in serial serum samples during the first 18 months of age in a smaller group of children to study the formation of the anti-*M. leprae* 7 antibodies in babies of mothers with various forms of leprosy during this period.

MATERIALS AND METHODS

Sera

Forty-eight pregnant women were selected for this study. They were to a great extent the same women reported on in earlier publications (Melsom & Duncan, 1980; Melsom, Duncan & Bjune, 1980) living in Addis Ababa, Ethiopia. All lived under similar socio-economic conditions, and they were divided into four groups according to the type of leprosy (Ridley & Jopling, 1966), and one group was selected to contain women with active lepromatous (LL-BL) leprosy. These patients had a positive bacterial index (BI) during pregnancy. Patients of this kind are known often to have a pronounced bacteraemia with up to 1×10^5 *M. leprae*/ml blood (Drutz, Chen & Lu, 1972). In this group it is thus expected that the placenta is exposed to *M. leprae*, and there is a definite risk that these bacilli can cross the placenta and infect the foetus.

Group 1 (denoted LL-BL, BI^+) consisted of twenty-six patients with active lepromatous leprosy (LL-BL), where acid-fast *M. leprae* bacilli were demonstrated during this pregnancy in one or several skin smears taken from six different sites on the patients (BI^+). All the patients received 100 mg DDS daily.

Group 2 (LL-BL, BI^-) consisted of six patients with inactive lepromatous leprosy. No *M. leprae* bacilli were present in any of the skin smears. The patients received 50 to 100 mg DDS daily for at least 5 years prior to the start of this study.

Group 3 (BT-TT) consisted of ten patients with tuberculoid leprosy. Six of these patients had stopped anti-leprosy treatment prior to the pregnancy and the start of the study. In two of these six patients the disease was reactivated and new patches appeared after delivery of the baby. The other four patients received 50 to 100 mg DDS daily during the pregnancy.

Group 4 (NL) consisted of four healthy, pregnant women with no clinical sign of leprosy. They came from the same area and the same socio-economic class as the leprosy patients.

From most of the babies (37 out of 48) two serum samples were available and tested by RIA; one sample was from cord blood and the other was obtained somewhere between 1 and 4 months of age. Serial samples were available in thirty babies. This group comprised nineteen of the thirty-seven babies mentioned above, and eleven additional babies; they were tested at birth and at intervals until about 18 months of age. It was planned to test these babies at regular intervals. Difficulties in attendance, mostly due to local factors outside our control and occurrence of minor illnesses in the babies or their families led to serum sampling at irregular intervals.

Radioimmunoassay (RIA)

M. leprae was obtained from R. J. W. Rees and P. Draper as freeze-dried bacilli purified from armadillo liver tissue. *M. leprae* antigen 7 was prepared, labelled with ^{125}I and tested by crossed immunoelectrophoresis and autoradiography. The properties of the preparation corresponded closely to those of the preparation described previously (Melsom *et al.*, 1978). Protein A on the surface of the Cowan I strain of staphylococci (NCTC 83508) was used as solid phase to separate antibody-bound labelled antigen from free labelled antigen. In brief, the patient sera were diluted 1:100 and 1:1,000 in RIA buffer (phosphate-buffered saline containing 0·2% bovine serum albumin

and 0·02% NaN_3); 100 μl of these dilutions was added to 100 μl of labelled *M. leprae* antigen 7 and incubated for half an hour at 20°C before addition of 2 ml 1% suspension of staphylococci. The tubes were centrifuged for 15 min at 3,000 r.p.m. and the supernatant was sucked off. The radioactivity in the pellet was counted in an electronic gamma counter for 400 sec. All tests were made in duplicate.

The amount of antibodies against *M. leprae* antigen 7 in the baby sera is expressed as a percentage of antibody concentration in the corresponding maternal sera taken at delivery. A figure of 100% represents the same number of counts recovered in the pellet in the tube with baby serum diluted 1:100 as in the tube with the paired maternal serum diluted 1:100, while a figure of 10% means that the same number of counts was found with baby serum diluted 1:100 as with the paired maternal serum diluted 1:1,000.

Statistical analysis

The statistical analysis was carried out according to the 2 × J table described by Mather (1951).

RESULTS

Fig. 1 shows the concentration of antibodies against *M. leprae* antigen 7 in sera from three babies of healthy mothers and nine babies of mothers with tuberculoid leprosy. In each baby two serum samples were tested, one obtained from cord blood and the other from 2 weeks to 4 months after birth. The solid line in Fig. 1 represents the expected decrease in concentration of maternal IgG and antibodies towards *M. leprae* antigen 7, assuming that maternal IgG is catabolized in the baby with a half-life of 4 weeks (Gitlin & Gitlin, 1975). The open circles show the antibody concentration in three babies of non-leprosy mothers expressed as a percentage of antibody concentration in the corresponding maternal serum at birth. The closed circles show the antibody activity against *M. leprae* antigen 7 in sera from nine babies of mothers with tuberculoid leprosy. The decrease in antibody activity against *M. leprae* antigen 7 is as expected from a half-life of maternal IgG of 4 weeks.

Fig. 2 shows the antibody activity against *M. leprae* antigen 7 in sera from five babies from

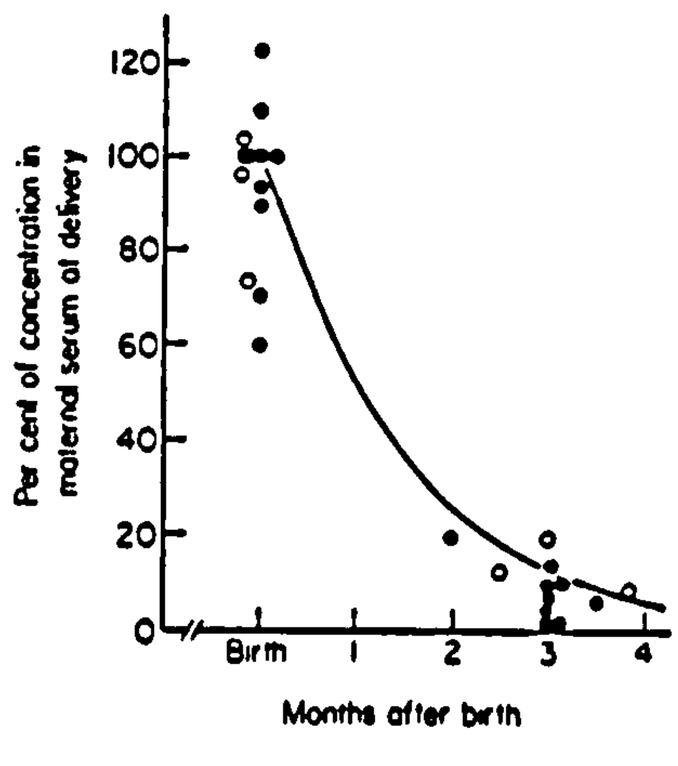


Fig.1

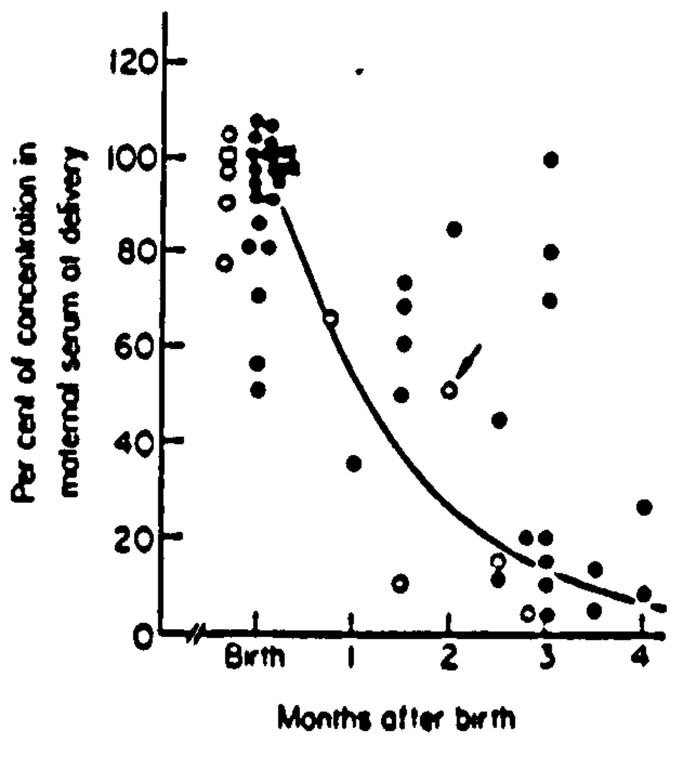


Fig.2

Fig. 1. Concentration of antibodies against *M. leprae* antigen 7 during the first 4 months of life. Two values are shown for each baby, one at birth and one at 2–4 months of age. (o) Babies of non-leprosy mothers. (•) babies of tuberculoid leprosy mothers. The concentration is expressed as a percentage of the concentration in paired maternal sera at delivery. (▪) Maternal concentration at delivery in one case where cord serum was not available.

Fig. 2. Concentration of anti-*M. leprae* 7 antibodies similarly recorded as in Fig. 1 in four babies of inactive lepromatous leprosy mothers (o) and in twenty babies of mothers with active lepromatous leprosy (•).

mothers with inactive lepromatous leprosy as open circles and from twenty babies of mothers with active lepromatous leprosy as closed circles. Four of the sera from babies of mothers with inactive lepromatous leprosy contained an 'expected' amount of antibodies against *M. leprae* antigen 7. Serum from one baby (marked with an arrow) taken at 2 months of age, contained more anti-*M. leprae* 7 antibodies than expected if all the antibodies had been of maternal origin. Ten of the twenty babies born of mothers with active lepromatous leprosy did not show the expected decrease in antibody concentration, while ten showed the expected fall.

Fig. 3a, and b shows examples of antibody activity against *M. leprae* antigen 7 during the first 18 months of life in babies of leprosy mothers. Fig. 3a shows a typical pattern (pattern I) of decline in antibody activity against *M. leprae* antigen 7 during the first 6 months of life, and the antibody activity remained low thereafter. Table 1 presents the data of the anti-*M. leprae* antigen 7 concentration in baby sera taken between 6 and 18 months of age. This table shows that all three babies of non-leprosy mothers, four of five babies born of tuberculoid leprosy mothers, all five babies of inactive lepromatous leprosy mothers, and six of seventeen babies of mothers with active lepromatous leprosy followed pattern I in Fig. 3a.

Fig. 3b shows a partial decrease of antibody activity against *M. leprae* antigen 7 in pattern II, where anti-*M. leprae* antigen 7 antibody levels in baby sera 10 months after delivery did not decrease to $<10\%$ of the levels in their respective sera at birth. A similar pattern was found in one of five babies of tuberculoid leprosy mothers and in six of seventeen babies of mothers suffering from active lepromatous leprosy.

Pattern III in Fig. 3b indicates a partial decrease, followed by an early increase, in antibody

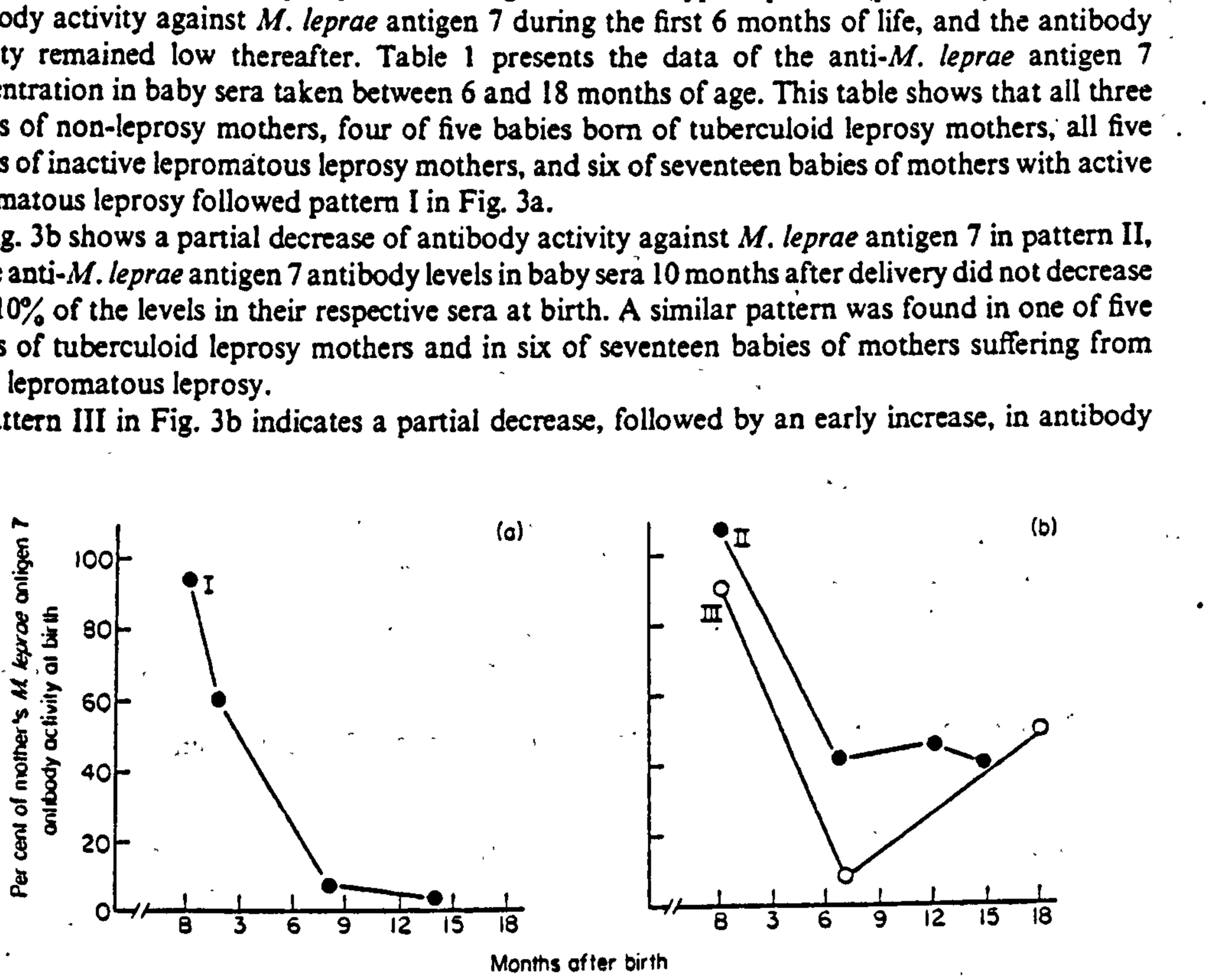


Fig. 3. (a) The concentration of antibodies against *M. leprae* antigen 7 in a baby of a non-leprosy mother during the first 15 months of life. (b) Two different patterns of anti-*M. leprae* 7 antibodies in two babies of mothers with active lepromatous leprosy during their first 18 months of life.

Table 1. Concentration of antibodies against *M. leprae* antigen 7 during the first 18 months in the four groups of babies

Leprosy status of the mother	Expected decline	Less than expected decline	Decline and later increase	Sum
No sign of leprosy	3	0	0	3
BT-TT leprosy	4	1	0	5
BL-LL$^-$ leprosy*	5	0	0	5
BL-LL$^+$ leprosy†	6	6	5	17

* BL-LL$^-$ = no acid-fast bacteria in skin smears.
† BL-LL$^+$ = acid-fast bacteria in skin smears.

Table 2. Pooling of data in Table 1 on anti-*M. leprae* 7 antibodies

Leprosy status of the mother	Expected decline	Less than expected decline + increase	Total
No sign of leprosy BT-TT and BL-LL, BI$^-$	12	1	13
LL-BL, BI$^+$	6	11	17
Total	18	12	30

activity against *M. leprae* antigen 7 in the baby of a mother with active lepromatous leprosy. This pattern was seen in five of seventeen babies of mothers with active lepromatous leprosy.

Table 1 shows the data on anti-*M. leprae* 7 antibody levels in those babies where serial blood samples were obtained up to 18 months of age, regarding the results between 6 and 18 months of age. In Table 2, the data in two groups were pooled regarding the leprosy status of the mother and levels of antibody in the baby at 6 and 18 months after birth. The differences observed in babies of active lepromatous leprosy mothers versus the three other groups—inactive lepromatous leprosy, tuberculoid leprosy and non-leprosy—were statistically significant ($P < 0{\cdot}002$).

DISCUSSION

In an earlier study (Melsom *et al.*, 1980) we found that a group of babies of mothers with active lepromatous leprosy had a statistically significant increased concentration of IgA in cord serum. This led to the suspicion that *M. leprae* bacilli or antigens of *M. leprae* cross the placenta and stimulate the foetus to produce antibodies towards *M. leprae* antigens.

The purpose of the present work was to investigate if the decrease in antibody activity against *M. leprae* antigen 7 after birth followed a course as expected from catabolism of maternal IgG with a half-life of about 28 days in the newborn baby (Gitlin & Gitlin, 1975). The solid line in Figs 1 and 2 shows the calculated decline in concentration of maternal IgG in the baby during the first 4 months of life based on this assumption concerning IgG catabolism.

The decline in concentration of antibodies against *M. leprae* antigen 7 followed roughly this line in the babies of non-leprosy and tuberculoid leprosy mothers, as shown in Fig. 1. In these babies, anti-*M. leprae* 7 gradually disappeared as expected from catabolism of maternal IgG, and there was no early or significant production of new antibodies against *M. leprae* antigen 7 in these babies detectable by RIA.

It would be expected that the first antibodies against *M. leprae* antigen 7 produced in exposed newborn babies would be of the IgM class. In an earlier study (Melsom & Duncan, 1980), we have shown that the present RIA can be used to demonstrate both IgM and IgG antibodies against *M. leprae* antigen 7. We can thus assume that the RIA would have detected a significant production in the newborn baby of IgM antibodies against *M. leprae* antigen 7.

Fig. 2 shows that the decline in concentration of antibodies against *M. leprae* antigen 7 was considerably less than expected during the first 4 months of life in ten out of twenty babies of mothers with active lepromatous leprosy. The catabolism of maternal IgG might be reduced in babies of mothers with active lepromatous leprosy thereby causing less decrease in concentration of anti-*M. leprae* 7 antibodies. At 3 months of age, the IgG concentration had fallen to about 35–40% of the concentration in cord sera in all four groups (unpublished observations). This is as in healthy infants (Gitlin & Gitlin, 1975). The IgG in baby serum at 3 months of age consists of a smaller part of maternal IgG and a larger part of IgG produced by the baby itself. Therefore, this reduced decline in concentration of antibodies against *M. leprae* antigen 7 indicates strongly that these antibodies have been produced by the babies themselves.

Stimulation of the immune system of the offspring to production of antibodies against *M. leprae* antigen 7 could be by transfer of *M. leprae* antigens or live leprosy bacilli *in utero* or by infection with *M. leprae* shortly after birth. The intimate contact with a mother having active lepromatous leprosy probably implies a great risk of infection shortly after birth by *M. leprae* often present in nasal discharge (Davey & Rees, 1974) and in breast milk (Pedley, 1967). *M. leprae* has a very slow growth rate with a doubling time of not less than 12 days (Shepard & McRae, 1965). The progress of infection after initial exposure is usually slow (Newell, 1966). The incubation period for leprosy is in most cases several years, but it may take up to 20 years after exposure until clinical signs of leprosy appear. Children who have been heavily exposed to leprosy since birth usually do not develop clinical signs of leprosy before 2 years of age (Worth, 1960). It is therefore unlikely that infection with *M. leprae* a few days after birth would cause an increase in the load of *M. leprae* antigen 7 sufficient to induce antibody production against *M. leprae* antigen 7 as seen in some of the babies before 2 months of age.

Increased amounts of anti-*M. leprae* 7 antibodies have been demonstrated in most armadillos which develop a systemic mycobacterial infection after inoculation with *M. leprae* (Harboe *et al.*, 1978). When individual animals were tested repeatedly after inoculation, significant increase in the antibody concentration usually did not occur until about 5 months (Harboe *et al.*, unpublished observations).

M. leprae antigen 7 is a strong cross-reaction antigen. It corresponds closely to BCG antigen 60 (Harboe *et al.*, 1977a), and it also cross-reacts extensively with other mycobacteria (Harboe *et al.*, 1979). Exposure to mycobacteria other than *M. leprae* can therefore induce production of antibodies which cross-react with *M. leprae* antigen 7. However, it is highly unlikely that during pregnancy women would be so severely infected with these other mycobacteria to have an associated bacteraemia. Moreover, after birth, the babies in the four groups would be exposed equally to these other mycobacteria. Therefore, these other mycobacteria cannot explain the differences in the antibody response against *M. leprae* antigen 7 seen between group 1 and the three other groups.

The findings thus show that synthesis of antibodies against *M. leprae* antigen 7 occurs frequently in babies of mothers with active lepromatous leprosy during the first 4 months of life. Production of the antibodies very soon after birth indicates that the immune system is stimulated *in utero*, and the findings are best explained by transfer of live leprosy bacilli or their antigens across the placenta.

One of the babies of mothers with inactive lepromatous leprosy (marked with an arrow in Fig. 2) showed a smaller decrease in concentration of antibodies against *M. leprae* antigen 7 than expected from catabolism of transferred maternal IgG. In a study of about 150 pregnant Ethiopian women including those described here, eleven out of thirty patients initially diagnosed as inactive lepromatous leprosy showed signs of reactivation of the disease with new nodules and positive BIs appearing during pregnancy or within the first few months after delivery (Duncan, Pearson & Melsoy, unpublished observations). Accordingly, they had to be reclassified as active lepromatous leprosy. The real frequency of reactivation of the disease among women with inactive lepromatous leprosy during pregnancy is unknown. Furthermore, a number of these women may have a partial reactivation without overt clinical signs during pregnancy. They may even have a bacteriaemia resulting in exposure of the placenta to *M. leprae* or their antigens, and subsequent transfer to the foetus.

Fig. 3a, pattern I, represents a typical pattern of concentration of antibodies against *M. leprae* antigen 7 in a newborn baby, with a decline in antibody activity to $< 10\%$ of the concentration in maternal serum at delivery within the first 6 months after birth and no significant increase in antibody concentration during the next 12 months. Table 1 shows that all the babies of non-leprosy mothers, four of five babies of tuberculoid leprosy mothers, and all the babies of inactive lepromatous leprosy mothers followed this pattern. On the other hand, only six of seventeen babies of mothers with active lepromatous leprosy had a decline to $< 10\%$ without a subsequent rise within the following year.

In Table 2, data from babies of non-leprosy, tuberculoid leprosy and inactive lepromatous leprosy mothers are pooled. Most of these babies were presumably exposed to none or low numbers of *M. leprae* during the first 18 months of life. On the other hand, most of the babies of active

lepromatous leprosy mothers are likely to have been exposed to large numbers of *M. leprae* in nasal discharge (Davey & Rees, 1974) and breast milk (Pedley, 1967). The difference in antibody concentration between the two groups is statistically significant ($P < 0{\cdot}005$). The data thus indicate that the production of antibodies against *M. leprae* antigen 7 during the first 18 months of life by babies of mothers with bacilliferous leprosy results from the exposure of the babies to *M. leprae*. Careful clinical studies of such babies are strongly indicated to determine the clinical consequence(s) of their early exposure to *M. leprae*.

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The association of pregnancy and leprosy

I. New cases, relapse of cured patients and deterioration in patients on treatment during pregnancy and lactation – results of a prospective study of 154 pregnancies in 147 Ethiopian women

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Summary One hundred and fourteen women with leprosy and 33 women without leprosy were studied during 118 and 36 pregnancies respectively. Two healthy controls developed leprosy during the study period: 12 of 25 women with 'cured' tuberculoid leprosy relapsed with new lesions or nerve damage; 46 of 93 women with active tuberculoid or lepromatous leprosy showed increased activity of their leprosy either as a transient phenomenon (21 patients) or due to probable dapsone resistance (28 patients). These occurred chiefly during the third trimestre and are thought to be due to decreased host resistance and increased immunological instability during pregnancy.

Introduction

Pregnancy has long been associated with the first appearance of leprosy or aggravation of the disease.[1–3] One study shows 75% of women studied to have developed the first sign of leprosy in association with child bearing, of whom two-thirds had the first signs of leprosy during the puerperium (the first 6 weeks after delivery).[4] Suggested reasons for this are hormonal,[5] metabolic[6] or some suppression of host resistance.[4, 7] Suppression of cell-mediated

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immunity (CMI) during pregnancy may also be associated with downgrading and a shift toward the lepromatous end of the spectrum. Hence one might expect recovery of CMI following delivery to be associated with upgrading phenomena and reversal reaction. Some patients present with the onset of leprosy and in reaction during the puerperium or first few weeks of lactation.[8] Many of the observations quoted have been based on retrospective studies. This paper presents the results of a prospective study on the effect of pregnancy on leprosy carried out at the Addis Ababa Leprosy Hospital between 1975 and 1978.

Patients and methods

One hundred and forty-seven Ethiopian women were studied during 154 pregnancies. There were 114 women with leprosy (118 pregnancies) and 33 women without leprosy (healthy contacts: HC, with 36 pregnancies). The women who were all from the low socio-economic class lived, for the most part, in the villages surrounding the Addis Leprosy Hospital. They were first seen, for this study, when they presented themselves at the Hospital ante-natal clinic which supplied ante-natal care for registered leprosy patients, wives of leprosy patients and members of staff. Initially the patients studied were those with active tuberculoid leprosy, active lepromatous leprosy with positive skin smears and healthy contacts; later the study group was broadened to include women with 'cured' tuberculoid leprosy who had stopped treatment and women with chronic, quiescent lepromatous leprosy with negative skin smears. Selection of the patients within the above general classification was based on their willingness to participate in the study, to deliver their babies in hospital rather than at home and to be seen with their babies for regular assessment, including blood tests, for a period of up to 2 years during lactation.

CLASSIFICATION AND TREATMENT OF MOTHERS

The 114 women with leprosy were classified initially as follows using the scale of Ridley & Jopling:[9]

Cured tuberculoid and borderline tuberculoid leprosy (released from control)	(TT and BT/RFC)	25 (25 pregnancies).
Active tuberculoid and borderline tuberculoid leprosy	(TT and BT)	17 (18 pregnancies).
Borderline lepromatous leprosy	(BL)	40 (41 pregnancies).
Lepromatous leprosy	(LL)	32 (34 pregnancies).

Eighty-two patients were receiving dapsone monotherapy (50–100 mg daily)

but 26 patients (1 BL, the rest BT or TT) were believed cured, had stopped treatment and had been 'released from control' (RFC, see below). Six patients (2 BL, 4 LL) had already developed dapsone-resistant leprosy, as defined[10] and were receiving clofazimine (4 patients, all LL, 5 pregnancies) or rifampicin plus thiambutosine and dapsone (2 patients both BL). Treatment and supervision of these patients was carried out through the hospital outpatient clinics: 81 patients receiving dapsone monotherapy (18 TT and BT, 36 BL, 27 LL) were supplied with dapsone tablets on a weekly or fortnightly basis by paramedical leprosy workers at hospital or municipality clinics, were referred to hospital clinics for treatment of reactions or other complications of leprosy, and were assessed by a doctor at the hospital 'Review Clinic' every 6 months when routine slit-skin smears were examined, 4 patients (2 BL, 2LL) receiving dapsone monotherapy 100 mg daily in a chocolate-coated tablet for suspected dapsone resistance[10] and 7 patients (2 BL, 5 LL) with proven dapsone resistance were seen every 6 months at a special clinic for the treatment of drug-resistant leprosy, routine slit-skin smears were done every 6 months and biopsies were taken annually.

PATIENTS RELEASED FROM CONTROL (RFC)

At the start of the study the practice in the hospital for stopping treatment of leprosy was as follows: TT patients were RFC after 2–3 years of treatment with dapsone 50–100 mg daily; BT patients were RFC after 4 or more years of treatment with dapsone 50 mg daily (300 mg weekly) when there had been no clinical evidence of active leprosy for at least 2 years; BL patients were RFC when they had received treatment for 15–20 years and had been skin-smear (bacteriological index: BI) negative with no clinical evidence of active leprosy for 10 years; LL patients continued on treatment for life and hence were not RFC.

The 25 patients classified as TT and BT/RFC were originally classified as TT or BT at the hospital new case clinic. Diagnosis had been made on clinical grounds supported by negative BI but without histological confirmation. Seventeen patients had been diagnosed at the Addis Ababa Leprosy Hospital and 8 at rural clinics or hospitals where they received their initial treatment before being transferred to Addis Ababa. One patient seen first in Addis Ababa and 1 patient coming from a rural clinic had had doubts raised regarding classification of leprosy and had been recorded as being 'LI' and 'BB' respectively on one occasion; on subsequent assessment by a senior hospital leprologist both were recorded as 'BT' on clinical grounds. The duration of stopping treatment ranged from 3 months to 10 years (mean 2.6 years).

ENTRY OF WOMEN TO THE STUDY: TIMING AND ASSESSMENT

At the time of entry to the study (Table 1) in addition to full obstetrical assessment, a general physical examination was made and the patient's leprosy

Table 1. Time of entry to study

Histological classification of leprosy	Number of women entering the study			Total pregnancies
	1st trimestre	2nd trimestre	3rd trimestre	
HC	2	11	23 (7)*	36
TT and BT/RFC	–	13	12 (2)	25
TT and BT/Active	1	6	11 (3)	18
BL	4	19	18 (4)	41
LL	4	13	17 (4)	34

*The number ( ) indicates the number of women entering the study at or after 36 weeks gestation.

HC = healthy contacts; RFC = released from control.

status assessed clinically; skin smears were taken from leprosy patients and a biopsy for histological classification, if it had not already been taken. Subsequent detailed leprosy assessments were made as indicated by the patient's symptoms and clinical state.

The first group of women admitted to the study were taken in during the third trimestre, several of them at or after 36 weeks gestation. After the first 3 months of the study it was apparent that leprosy deteriorated during pregnancy, thereafter whenever possible, patients were admitted to the study during the first or second trimestre with detailed leprosy assessment at the time of entry and during each following trimestre and at 6-month intervals during lactation, more frequently if indicated.

ROUTINE ASSESSMENT OF STUDY PATIENTS

Women in this study were seen for routine ante-natal care at monthly intervals until 28 weeks gestation, every 2 weeks until 34 weeks and weekly thereafter. In addition to receiving the routine ante-natal care their leprosy status was assessed clinically, complications were recorded, and additional investigations arranged as indicated. They were admitted to hospital for 24-hour collections of urine for oestriol analysis and also for medical, obstetrical or social reasons as necessary. LL patients in particular were admitted to hospital for several weeks prior to delivery to prevent foetal wastage by precipitate delivery at home. As inpatients they received routine ante-natal surveillance but stopped attending outpatient ante-natal clinics (ANC). This factor accounts largely for the reduced attendance at ANC by LL patients (Table 2).

At detailed leprosy assessment the patient's complaints, state of health and drug treatment were recorded. The patient was then examined in a well-lit room, with inspection and palpation of the skin, peripheral nerves and regional lymph nodes. Clinical drawings were made of the skin lesions, slit-skin smears were taken from 6 sites (both ears and 4 smears from active lesions, or from both ears, elbows and knees when no active lesions were seen; smears were

Table 2. Frequency and timing of assessment during pregnancy

Classification of leprosy	No. of women	No. of pregnancies	No. of attendances at ANC (Mean ± SEM)*	Frequency of leprosy assessments†						Frequency of laboratory investigations‡									No. of admissions for obstetrical/ medical/social reasons
				X1	X2	X3	X4	X5	X > 5	X1	X2	X3	X4	X5	X > 5	Puerperal[1]	Lactation[2]	Not done	
HC	33	36	5.9 ± 0.5	32	4	–	–	–	–	14	–	–	–	–	–	1	8	13	4
				(31)	(5)					(14)						(1)	(8)	(13)	
TT and BT/RFC	25	25	6.2 ± 0.5	19	5	1	–	–	–	11	5	–	–	–	–	3	6	–	3
				(17)	(6)	(1)			(1)	(11)	(4)				(1)	(3)	(6)		
TT and BT/Active	17	18	5.1 ± 0.6	15	3	–	–	–	–	13	3	–	–	–	–	–	2	–	4
				(3)	(5)	(8)			(2)	(12)	(3)			(2)			(1)		
BL	40	41	5.9 ± 0.5	32	9	–	–	–	–	19	15	2	–	–	–	3	–	2	4
				(7)	(16)	(13)	(3)	(1)	(1)	(17)	(14)	(3)	(2)			(3)		(2)	
LL	32	34	4.5 ± 0.6	16	18	–	–	–	–	16	13	3	–	–	–	1	1	–	9
				(4)	(15)	(9)	(5)	(1)		(13)	(12)	(3)	(1)	(1)	(2)	(1)	(1)		

*Assessment by doctor (MED).

†First number equals the number of patient-assessments during pregnancy for this special study only; number within ( ) equals the number of patient-assessments for the special study together with the routine 'full clinical assessments' at hospital review clinics and clinics monitoring suspected dapsone resistance.

‡First number equals frequency of laboratory investigations (skin smear for BI and MI/biopsy for histology/biopsy for mouse foot pad tests), singly or in combination; number ( ) equals frequency of laboratory investigations and additional tests (VMT with ST/EMG/skin test with A6), singly or in combination.

HC = healthy contacts (without leprosy); TT and BT/RFC = tuberculoid and borderline leprosy 'released from control', i.e. 'cured'; TT and BT/Active = tuberculoid and borderline tuberculoid leprosy, active; BL = borderline lepromatous leprosy; LL = lepromatous leprosy; ANC = ante-natal clinic.

BI = bacteriological index, MI = morphological index.

[1]When it was not possible to perform skin smears for BI or biopsy during pregnancy some were done immediately after delivery, during the puerperium.

[2]When skin smears for BI or biopsy had not been done before, some were done for the first time in the study during lactation at follow-up assessments.

taken from the same sites on subsequent occasions unless new lesions had appeared, in which case smears were taken from them) for bacteriological and morphological index (BI and MI).

Biopsies were taken for diagnosis and classification from active lesions or when the disease was quiescent from the buttocks. The biopsies were divided in two and read by two independent leprologists. Patients who were deemed healthy contacts were assessed in the same way as leprosy patients with the exception of the skin biopsy, which was only done if there was a suspicious lesion or nerve enlargement. Clinical classification was undertaken by two independent observers. When a patient was suspected of having developed dapsone-resistant leprosy a biopsy of an active nodule with a positive morphological index was taken and tested in mouse foot pads for dapsone resistance.[11]

Sensory skin testing (ST)[12] and voluntary muscle testing (VMT)[13] was done by the physiotherapy department. Nerve conduction velocity (EMG) was estimated in a few patients where it was difficult to decide whether nerve damage was of recent onset.

Skin testing using a standardized purified protein of *Mycobacterium leprae* grown in armadillos (A6) was done during lactation instead of lepromin testing.

The patients' hospital records were reviewed periodically and additional data regarding the initial diagnosis and treatment of leprosy, routine leprosy assessments, complications of leprosy and special investigations not obtained at the study assessments was abstracted and used in the final analysis of results. The frequency of assessment and of laboratory tests and other investigations carried out during pregnancy and lactation is shown in Tables 2 and 3. The total number of special investigations is not shown as tests carried out at the same time, regardless of number, are recorded as one time of testing.

DIAGNOSIS OF DETERIORATION OF LEPROSY STATUS

(i) New 'overt' cases of leprosy were diagnosed clinically and confirmed by skin smears and biopsy.

(ii) Relapse in RFC patients was diagnosed clinically, by the appearance of new lesions and/or new nerve damage or by positive BI or biopsy showing active leprosy.

(iii) Deterioration in leprosy status of patients receiving treatment was defined as the occurrence of one or more of the following: conversion from negative to positive or rise in the patient's BI or MI, appearance of new lesions, extension of existing lesions, erythema and oedema of margins or tuberculoid lesions (in the absence of reaction) or increased activity of the lesion as diagnosed by histology.

Table 3. Frequency of leprosy assessments and investigations during lactation

Classification of leprosy	No. of women	Frequency of leprosy assessments*								Frequency of investigations†						
		X1	X2	X3	X4	X5	X > 5	Seen with baby, asymptomatic, no leprosy assessment	Not seen	X1	X2	X3	X4	X5	X > 5	Not done
HC	36	11	3	–	2	–	–	7	13	12	2	–	–	1	–	21
		(11)	(3)		(1)	(1)		(7)	(13)	(11)	(4)			–	(1)	(20)
TT and BT/RFC	25	9	5	3	–	–	–	2	6	9	8	1	–	–	–	7
		(5)	(11)	(2)			(1)	(1)	(5)	(6)	(8)	(3)	(1)			(7)
TT and BT/Active	18	9	3	1	1	–	–	–	4	8	3	2	–	–	–	5
		(4)	(5)	(1)	(2)	(1)	(3)		(2)	(3)	(5)	(2)	(3)		(1)	(4)
BL	41	8	6	6	6	2	1	6	6	10	14	4	1	2	2	8
		(7)	(3)	(7)	(3)	(5)	(11)	(1)	(4)	(9)	(8)	(4)	(5)	(4)	(3)	(8)
LL	34	10	9	5	2	1	1	4	2	11	15	2	2	1	–	3
		(3)	(7)	(10)	(5)	(3)	(5)	(1)	–	(7)	(12)	(6)	(2)	(1)	(3)	(3)

*First number equals the number of patient-assessments during lactation for this special study only; number within ( ) equals the number of patient-assessments for this special study together with the routine 'full clinical assessments' at hospital review clinics and special clinics monitoring suspected dapsone resistance.

†First number equals frequency of laboratory investigations (skin smears for BI and MI/biopsy for histology/biopsy for mouse foot pad test) singly or in combination; number within ( ) equals frequency of laboratory investigations and additional tests (VMT with ST/EMG/skin test with 'A6') singly or in combination.

Results

HEALTHY CONTROLS (HC)

Of 33 women observed during and after 36 pregnancies, 2 developed leprosy. One asymptomatic woman developed a hypopigmented macule during the third trimestre of pregnancy which on biopsy showed indeterminate leprosy. Postpartum the lesion grew in size but the woman had no complications of leprosy. The second woman complained of severe 'rheumatism' at 10 weeks postpartum when she was found to have enlarged nerves which on biopsy showed active BL leprosy. Hypopigmented skin lesions and skin infiltration were apparent by 6 months postpartum.

'CURED' TUBERCULOID AND BORDERLINE TUBERCULOID LEPROSY (TT AND BT/RFC)

Of these 25 patients, 9 relapsed with active leprosy (6 BT, 3 BL) within periods of from 3 months to 3 years after stopping treatment. Eight of the 9 relapses were diagnosed on clinical grounds, 7 were confirmed on biopsy; 3 were BI positive. Five out of the 9 relapses occurred during the third trimestre of pregnancy.

In addition 3 patients were considered to have incipient relapse on the evidence of new nerve enlargement or neuritis though skin biopsies and BI were negative.

Ten out of 12 patients relapsed in association with the first pregnancy and 2 during the second pregnancy after RFC. Details of clinical features and investigations are shown in Table 4.

ACTIVE TUBERCULOID LEPROSY (TT AND BT)

Of 18 patients all on dapsone monotherapy, 8 had a transient increase in activity of the skin lesions, usually in the third trimestre, without any histological evidence of reaction. In 3 cases the lesions appeared more active with raised erythematous margins, in 4 cases there was conversion from BI negative to positive. In 2 cases there was increase in size and number of the skin lesions during lactation.

LEPROMATOUS LEPROSY (BL AND LL)

Sixty-eight women (36 BL, 32 LL) were studied through 71 pregnancies and followed up after delivery. (Four others were assessed only during pregnancy). Increased activity was found in 38 (54%) during pregnancy, puerperium or lactation (in 20 during the third trimestre or puerperium). At the time that

Table 4. TT and BT/RFC cases showing relapse in association with pregnancy

No.	Parity	Original diagnosis	Duration of treatment (years)	Years RFC before present pregnancy	Symptoms	Clinical features	Additional tests	Clinical diagnosis at relapse	Histological diagnosis	BI	Final diagnosis	Timing of relapse
A – relapse cases												
1	1+0	'T'	7	3/12	–	1 active new macule face; nerves normal		BT Active	Skin: BL early active AFB 3–4^{+} in granuloma	2.3	BL	3rd TM during 1st pregnancy after RFC
2	2+0	BT	11	1½	Rheumatism	New nodules ++ on on legs; nerves normal		BL Active	Skin: solid AFB in nerves and deep dermis	3+	BL	3rd TM during 1st pregnancy after RFC
3	5+0	BT	14	2½	–	Inactive skin; nerves normal		Quiescent BT	Not done	0.8 (4^{+} at one site)	BL	3rd TM during 2nd pregnancy after RFC
4	1+0	BT	1	3	–	8 new macules (legs and arms); 6 enlarged nerves; new motor and sensory loss		BT Active	Skin: early BT. Nerve: early tuberculoid lepr.	0	BT	Lact. after 1st pregnancy after RFC
5	0+1	BT	4	1	'Burning' paraesthesiae	New macule, face skin reaction neuritis	EMG active demyelination	BT Active	Skin: normal. Nerve: BT Active	0	BT	1st TM during 1st pregnancy after RFC
6	3+0	'T'	9	1	–	New macule face; nerves normal		BT Active	Not done	0	BT	Lact. after 1st pregnancy after RFC
7	1+0	BT	8	1¼	–	New nodules on face and ears; 3 enlarged nerves		BL Active	Skin: BT Active. Solid AFB in nerve	0	BT	2nd TM during 1st pregnancy after RFC
8	1+0	BT	8	2	Rheumatism	2 new macules face; nerves normal		BT Active	Skin: BT Active	0	BT	3rd TM during 2nd pregnancy after RFC
9	4+0	BT	5	2*	'Burning' paraesthesiae	Active erythematous edge of old macule, face; 3 enlarged nerves		BT Active	Skin: TT/BT Active	0	BT	3rd TM during 1st pregnancy after RFC
B – incipient relapse cases												
1	4+0	TT/BT	6	4/12	Rheumatism	Skin inactive; 2 enlarged nerves		Old BT	Skin: almost normal	0	BT	Lact. after 1st pregnancy after RFC
2	3+0	BT	7	1½	Rheumatism	Skin inactive; 3 enlarged nerves	VMT and ST motor and sensory loss	'Burnt out BT'	Skin: within normal limits	0	BT	Lact. after 1st pregnancy after RFC
3	2+0	BT	10	3*	Rheumatism	Skin inactive; 1 enlarged nerve	VMT motor loss	Inactive BT	Not done	0	BT	Lact. after 1st pregnancy after RFC

*RFC during previous pregnancy. TM = Trimestre; lact = lactation.

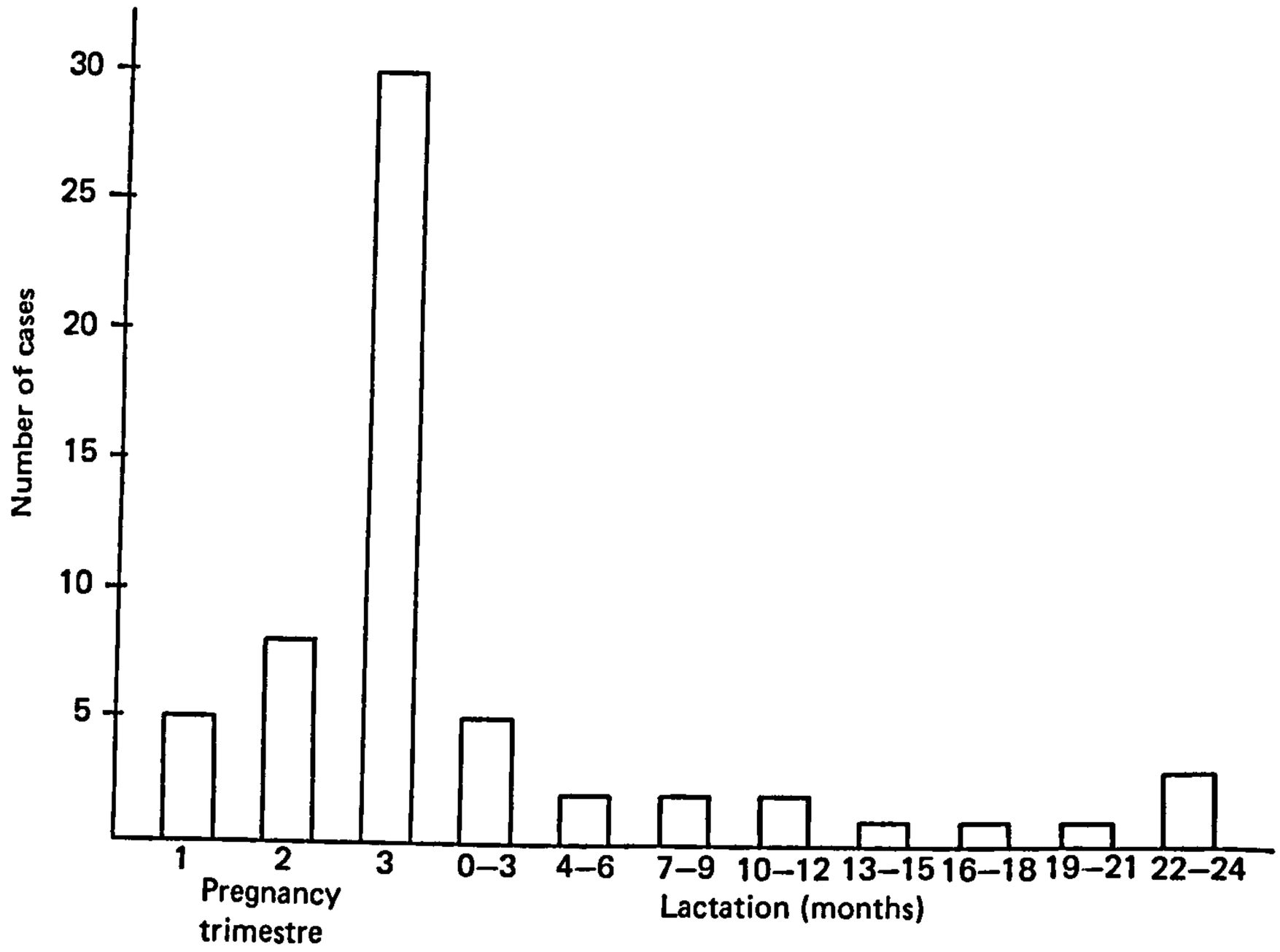


Figure 1. Timing of first evidence of deterioration of leprosy status (overt leprosy, relapse in 'cured' TT and BT patients, increased activity of disease in TT, BT, BL and LL patients receiving treatment for leprosy) in relation to pregnancy and lactation. (Note. The 4 patients relapsing between 19 and 24 months had not been assessed during the preceding 12 months: from the history and/or clinical findings, it is very likely that relapse occurred by 12 months postpartum.)

increased activity was first observed 17 out of the 38 patients had new nodules (a further 7 developed new skin lesions later); in 34 cases the increased activity was confirmed by a rise in BI, in 4 cases by biopsy only. In 16 cases the increased activity was a transient phenomenon, with fall in BI to less than the pre-pregnancy levels or reversion of the active biopsy to 'LL regressing' during early lactation. However, 6 patients then went on, within 11–15 months, to show a subsequent rise in BI and clinical evidence on relapse during late lactation or the next pregnancy. The phenomenon of transient increase in activity was shown by patients prescribed dapsone monotherapy, dual therapy with dapsone and rifampicin, and clofazimine monotherapy.

OTHER CLINICAL FEATURES ASSOCIATED WITH INCREASED ACTIVITY OF LEPROSY (BL AND LL)

The complaint of 'rheumatism' either preceded or accompanied the increased activity of the disease in more than half of the patients. Erythema nodosum

leprosum (ENL) preceded or accompanied the increased activity of the disease in one-half of the patients, occurring for the first time during pregnancy in most cases. In contrast reversal reaction was seen postpartum when recovery of CMI would be expected to occur. New nerve enlargement was observed in one-third of the patients, and neuritis (due to ENL or reversal reaction) was observed in more than half of the patients who had increased activity of the disease, including those who had only a transient rise in BI during pregnancy. Neuritis is discussed in detail elsewhere.[14]

Twenty-eight patients who were considered to have evidence of dapsone resistance are discussed elsewhere.[15]

RELAPSE IN ASSOCIATION WITH DOWNGRADING PHENOMENA (BL AND LL)

Six patients (all BL) downgraded from BL to LL, 5 during pregnancy and 1 during lactation. They were diagnosed on histology, but 3 also had clinical evidence of relapse due to dapsone resistance. In addition 3 relapse patients showed, for the first time, the histological features of polar lepromatous leprosy (LL_p)[16] during the third trimestre or immediately after delivery. After delivery 3 of the patients who had downgraded to LL upgraded to BL or BT.

Discussion

Protection and survival of the foetus as an allograft is the result of adaptive maternal responses to pregnancy including transient suppression of CMI.[17] Suggestive evidence for this is the increased survival time of adult skin homografts on pregnant hosts, especially during the third trimestre, compared with non-pregnant hosts;[18] the depression of tuberculin sensitivity in the third trimestre of pregnancy;[19] the increased severity of certain viral diseases during pregnancy[20–22] and amelioration of diseases such as rheumatoid arthritis,[23–24] ulcerative colitis[25] and sarcoidosis[26] during pregnancy with deterioration postpartum. The pregnancy-associated alterations of these conditions pertain to cell-mediated immune reactions.[27].

Host resistance to mycobacterial disease is dependent on CMI and can be measured *in vitro* by the lymphocyte transformation test (LTT). Results of such tests, using phytohaemagglutinin (PHA) and purified protein derivative of tubercle (PPD), indicate suppression of CMI during pregnancy which ceases at delivery or shortly afterwards.[28–31] It is possible that pregnancy-associated α-macroglobulin plays a part in this process.[32] In pregnant leprosy patients it is likely that plasma contains suppressive factors in addition to those normally associated with pregnancy, as plasma from mothers with

leprosy had a greater inhibitory effect on their babies' LLT than plasma from healthy mothers.[33]

Before the era of chemotherapy it was observed that there was a sex difference in the mortality from tuberculosis: according to the United States Census Bureau Statistics there was a consistently higher death rate in females aged 15–25 years of age from 1900 to 1942.[34] It was well recognized that pregnancy had an adverse effect on tuberculosis. In many cases the first sign of tuberculosis was observed soon after parturition and where tuberculosis was already established mortality was increased during later pregnancy and the puerperium,[35–39] although with proper sanitorium care throughout pregnancy the danger was greatly diminished if not avoided.[40] A similar adverse effect of pregnancy on tuberculosis was observed in cattle[41] and experimental animals.[42]

In leprosy the overall prevalence in men is greater than in women. However, women appear to develop the disease at an earlier age than men. For instance, among leprosy patients in India 50% of the women had developed leprosy by the age of 20 years, compared with 30% of men.[2] In Ethiopia as many as 75% of female patients in the studies of the Medical Research Council Leprosy Project had developed leprosy by the age of 20 (M E Duncan & J M H Pearson, unpublished observations). It is tempting to link this early onset with an increased risk of infection and rate of evolution associated with increased endocrine activity during puberty and suppression of CMI in frequent pregnancies during the late teens.

In leprosy where the host resistance is dependent chiefly on CMI, one would expect pregnancy to be associated with (i) the first appearance of leprosy; (ii) relapse in cured patients; and (iii) increased activity of the disease with a tendency to shift towards the lepromatous end of the spectrum and increase in bacillary load. These features were all seen in our study.

(i) NEW CASES

In Addis Ababa the new case rate for the city is 1 per 3,000 population; in the villages surrounding the Leprosy Hospital the rate is higher, 1 per 1,000 population (0.1%). It is therefore significant that of 33 women observed during 36 pregnancies, 2 (5.6%) showed the first sign of disease during the third trimestre or early lactation. Our observation confirmed earlier reports.[1–4, 6–7, 43] Women already infected with *Mycobacterium leprae* and incubating the disease show overt leprosy in late pregnancy or early lactation as a result of decreased host resistance of pregnancy.[1, 7, 43]

(ii) RELAPSE OF 'CURED' PATIENTS

The relapse rate in patients with cured TT and BT leprosy in Ethiopia has been reported[44] as 5% per annum. A considerable number of patients relapsed because they had been misclassified as BT rather than BB or BL, and thus had

received inadequate treatment prior to stopping therapy. Our observation that 9 'cured' TT and BT patients relapsed with active leprosy (3 as BL and 6 as BT) confirms the above findings. While the original clinical diagnosis had not been in doubt in any of our cases and all were BI negative, none had had histological confirmation. In pregnant women the skin lesions may not be typical of either BT or BL leprosy, thus causing difficulties in clinical classification as happened with two of the patients who relapsed in our study (Table 4). Ideally (to ensure adequate treatment), histological confirmation and classification is recommended in all patients especially women presenting with overt leprosy in association with pregnancy or lactation. It is also possible that the initial 'BT' classification in our patients was correct but that 3 of these women who were all parous had downgraded to BL during a previous pregnancy.

Nerve damage was a feature of relapse in BT/RFC patients, 4 out of 8 had nerve damage early. This is the same as is found in early active tuberculoid leprosy (JMH Pearson, unpublished observation). The observation of Naafs (B Naafs, personal communication) that 'rheumatism' was a symptom of relapse in these patients was confirmed in this study although we found it was more consistently a symptom of 'late silent neuritis'.

It has been suggested that pregnancy be regarded as a test of cure of leprosy.[43] Seven of the 9 women who relapsed with active leprosy did so during the first pregnancy after stopping treatment and 2 relapsed in the second pregnancy after stopping treatment (Table 4). Thus one pregnancy cannot be regarded as a test of cure and we recommend that all women with 'cured leprosy' who have stopped therapy, be carefully assessed during and after all subsequent pregnancies if late nerve damage is to be avoided.

(iii) INCREASED ACTIVITY OF LEPROSY WITH A TENDENCY TO SHIFT TOWARDS THE LEPROMATOUS END OF THE SPECTRUM AND INCREASE IN BACILLARY LOAD

We observed increased activity of their leprosy in just under half of the patients with active TT or BT leprosy (8/18) and in rather more than half (38/71) of the BL and LL patients who were followed up. The very high rate of relapse or deterioration of leprosy status, half of which appeared to be a transient phenomenon, would undoubtedly have been overlooked had these patients not been assessed frequently with the use of routine skin smears and biopsies even in the absence of skin lesions. The importance of carrying out routine skin smears at regular intervals cannot be overemphasized, as it is only by so doing that relapse can be detected early.[45–46]

The increased activity of the patient's leprosy recorded in 17 of the 89 patients was diagnosed on the basis of a rise in BI and/or MI or on increased activity at the histological level in women who did not at any time during the study show new or active skin lesions. The timing and transient nature of this

phenomenon was of interest in that it was related to the third trimestre of pregnancy when CMI would be maximally suppressed. A similar observation was made by Browne who refers to a transient non-significant rise in BI which he attributes to hormonal disturbances of pregnancy.[47] However, by having the opportunity to follow up these Ethiopian patients we found that 6 out of 16 lepromatous women who had a transient rise in BI during pregnancy developed the clinical picture of dapsone resistance during the next 15 months (4 with new nerve damage) and 9 others developed new nerve damage during lactation. Thus we feel that a transient rise in BI during pregnancy can no longer be considered as insignificant.

The conversion to BI positive with increase in size and number of new lesions in BT patients, the tendency to downgrade from BL to LL during pregnancy with upgrading following delivery, and the onset of leprosy with reversal reaction during early lactation[8] are evidence of the increased instability of women with leprosy during pregnancy, especially those classified as borderline.

While further investigation is required to elucidate the mechanisms of the adverse effect of pregnancy on leprosy, the practical implications which should be made widely known to all leprosy workers are:

1. The pregnant woman, because of physiological suppression of CMI most marked during the third trimestre, is especially at risk. If she is a known leprosy contact incubating leprosy, she is most likely to show overt disease either in late pregnancy or during lactation when it may well be complicated by reaction. 'Cured' BT patients run the risk of relapsing with active disease, and in patients receiving treatment for leprosy there is a 50% chance of the disease being aggravated with a shift towards the lepromatous end of the spectrum, increased bacillary load, subsequent risk of ENL and in the puerperium reversal reaction possibly with severe nerve damage.
2. In relapsing RFC patients and those who are developing dapsone-resistant leprosy, with multiplication of viable bacilli during pregnancy, there is a real risk that the foetus may be infected *in utero*[48–49] and go on to clinical leprosy in early childhood; furthermore the woman herself will become a risk to her household and the community as she is likely to be infectious. We therefore recommend:

(I) *Health education.* Incorporation of this knowledge into health education of women in the reproductive age group. At the same time advice on family planning should be given so that as far as possible pregnancies can be postponed until after the leprosy is well under control.

(II) *Increased surveillance.*

(i) For women with active leprosy: increased surveillance during pregnancy, (a) to ensure a maximal patient compliance, if possible substituting parenteral for oral dapsone therapy during the first

trimestre if emesis gravidarum is troublesome; (b) routine assessments with skin smears and biopsies, as possible, during the second and third trimestre and at 3 and 6 months postpartum, by which time most relapses should have occurred.

(ii) For women with cured leprosy (TT and BT/RFC): clinical assessment with particular attention to peripheral nerves during pregnancy and at 6, 12 and 18 months postpartum. Additional tests, namely skin biopsy, nerve biopsy (if possible), VMT, ST or EMG when relapse is suspected but clinical findings are not diagnostic.

(iii) For healthy contacts, especially of infectious cases: assessment during the third trimestre of pregnancy and postpartum, ideally at 3 and 6 months.

(iv) For the child born to a woman who has had an active relapse during pregnancy there is risk of clinical leprosy in early childhood. This is likely to be of the indeterminate type and self healing, particularly in the very young child, and probably occurs more frequently than realized hitherto. Regular inspection at child health care clinics when weighing and measuring the child, naked, provides diagnostic opportunities. A history of lactation should be obtained as anti-leprosy drugs are transmitted through the mother's milk. (This will be discussed more fully elsewhere.)[50]

(III) *Additional anti-leprosy drugs.* The question of additional anti-leprosy drugs during pregnancy and lactation requires careful thought and is discussed elsewhere.[15] To prevent relapse in TT and BT/RFC patients, we underline the recommendations made by Touw-Langendijk and Naafs[44] that very careful review of the patient's initial diagnosis, treatment, progress and date of leprosy 'inactivity' be made before the patient is RFC. We add to this a recommendation that no female patient be RFC either during pregnancy or within a year of delivery; all pregnancies and dates of delivery should be noted in the patient's records. These two recommendations would reduce the number of patients being RFC prematurely. For those already RFC and becoming pregnant, increased surveillance during and after pregnancy and an awareness of the risk of relapse to allow early diagnosis is probably preferable to blind treatment of RFC patients for an empirical period of time during and after pregnancy, which could mask clinical relapse.

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The association of pregnancy and leprosy

II. Pregnancy in dapsone-resistant leprosy

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Summary Sixty-seven women with lepromatous leprosy were studied during 70 pregnancies and followed up during lactation; 6 patients were already dapsone resistant and an additional 4 were receiving dapsone 100 mg daily under trial conditions for suspected dapsone resistance. During the study 28 patients including the 4 already suspected of having dapsone resistance relapsed with probable dapsone resistance. While failure in patient compliance was thought to be important in some cases, recurrent pregnancies, by providing periods of physiological suppression of cell-mediated immunity, could well be the factor in causing the progression of dapsone resistance among women.

Introduction

Dapsone-resistant leprosy has become a major problem in Ethiopia. The incidence among patients with lepromatous leprosy in the Addis Ababa area in the period 1973–77 was about 3% per annum[1] and a high prevalence of primary dapsone resistance has also been reported.[2]

The factors contributing to the development of drug-resistant leprosy are probably much the same as those in tuberculosis. Inadequate dosage, irregular treatment and above all prolonged monotherapy all played a part in the causation of the Ethiopian epidemic of dapsone-resistant leprosy. However, it is possible that other factors, such as the immunosuppression associated with

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pregnancy, could play a part in determining the time at which incipient dapsone-resistant leprosy becomes clinically manifest.

Even in untreated lepromatous leprosy host factors are present which make some contribution towards controlling the infection. Thus, it is almost universally observed that such patients have only some 10% of solidly staining, presumed viable bacilli, the remainder are for the most part fragmented and non-viable, and therefore some host mechanism must be responsible for this process. Such a host factor must either prolong the generation time or (more likely) slow down the increase in bacillary load by killing a considerable proportion of bacilli without assistance by chemotherapy. If this control were lessened, for example as part of the process of immunosuppression during pregnancy, it could be expected that relapse or deterioration of untreated patients would be associated with pregnancy. Similarly, a subpopulation of dapsone resistant *Mycobacterium leprae* might be expected to show a rapid increase in numbers under these conditions, even if a patient were receiving monotherapy with dapsone.

This paper reports the results of a prospective study of the effects of pregnancy on lepromatous leprosy.

Patients and methods

The patients were all Ethiopian women of the low socio-economic class who lived in the villages surrounding the Addis Ababa Leprosy Hospital. There were 67 women (35 classified as having borderline lepromatous leprosy, BL and 32 with lepromatous leprosy, LL)[3] studied throughout 70 pregnancies. They were all receiving outpatient treatment for leprosy and were first seen and taken into this study when they presented themselves at the Hospital Antenatal Clinic. Selection of patients was based on their willingness to participate in the study, to deliver their babies in hospital rather than at home and to be seen with their babies for regular assessment including blood tests for a period of up to 2 years during lactation. Intake of patients was staggered over 12 months.

Assessment of leprosy was made during pregnancy and after delivery at 6-month intervals whenever possible. This included inspection of skin lesions, clinical drawings, palpation of nerves and regional lymph nodes, slit skin smears and biopsies; full details are recorded elsewhere.[4] When a patient was suspected of having developed dapsone-resistant leprosy, a biopsy of an active skin lesion, with a positive morphological index (MI), was taken and tested for dapsone resistance in all 11 cases by the mouse foot-pad technique.[5, 6]

Resistance to dapsone was defined as multiplication of *M. leprae* in mouse foot pads at a concentration of dapsone 0.0001% or more in the diet.[6]

Results

Sixty-seven patients were included in the study (35 were BL, 32 LL); 3 of them were followed through 2 pregnancies. At the start of the study 6 patients were already diagnosed as dapsone resistant, and were taking clofazimine or rifampicin plus thiacetazone and dapsone. An additional 4 patients were suspected of dapsone resistance, and were receiving dapsone 100 mg daily under trial conditions with the maximum possible supervision and frequent assessments. Thus the initial prevalence of proven and suspected dapsone-resistant leprosy was 10/70, 14%; that is, much the same as the general prevalence among lepromatous patients at that time. The remaining patients were receiving dapsone 100 mg daily under routine outpatient clinic supervision.

During the course of the study an additional 24 patients showed clinical and/or bacteriological or histological deterioration despite apparently continuing to take dapsone, and were therefore considered to have prima-facie evidence of dapsone-resistant leprosy. In the majority of cases the diagnosis was clinical; the patients showed new active skin nodules, in which the BI and MI were raised. However, nearly half of the patients (10/24) gave indications of relapse on routine skin smears and/or biopsies before new skin lesions became evident, and an additional 3 cases showed definite relapse on smears/biopsies when there was only minimal clinical evidence of relapse. A striking feature of clinical relapse in these patients was the rapidity of development and increase in number of skin lesions after routine smears gave indications of relapse. The most rapid deterioration occurred toward the end of pregnancy when 7/24 women showed marked clinical deterioration during a 3-month period including part or all of the third trimestre. An additional 5 patients showed moderately rapid deterioration starting in the third trimestre and extending into the first 6 months of lactation.

It was not possible to perform mouse foot-pad tests for dapsone resistance in all cases. In some cases, because of shortage of mice, foot-pad tests were only done with DDS in low dietary concentrations. There were 4 'technical failures' on account of delays in the biopsied material reaching the laboratory; in these cases repeat biopsies were not carried out as alternative dual therapy had been instituted on account of rapid deterioration during pregnancy. Four of the 6 patients, already designated as DDS resistant, had this confirmed by mouse tests prior to their present pregnancies. Results of the 7 patients tested successfully *during* the study are shown in Table 1. Three of these (Nos. 1, 2 and 7) were from the 4 patients suspected of being DDS resistant, but improving on dapsone 100 mg daily monotherapy under trial conditions prior to pregnancy; the remaining 4 were from the 24 patients relapsing for the first time during pregnancy. Of the patients whose detailed results are known 11/11 were resistant at 0.0001% DDS in diet, 6/8 were resistant at 0.001% DDS in diet and 6/6 were sensitive at 0.01% DDS in diet. None of the patients tested was proved dapsone sensitive.

Table 1. Results of mouse foot-pad assessment of dapsone sensitivity

No. of Patients	Patient No.	Concentration of DDS in Mouse Diet: 0.01% (Human Equivalent Dose 100 mg)	0.001% (10 mg)	0.0001% (1 mg)	Conclusion
Tested during study	7	1	ND	+	+	R
		2	0	+	+	R
		3	0	+	+	R
		4	0	+	+	R
		5	0	ND	+	R
		6	0	0	+	R
		7	0	0	+	R

ND = Not done.
R = Resistant.
\+ = Growth of bacilli.
0 = No growth of bacilli.

The timing of relapse is shown in Table 2. Half the patients (14/28) relapsed clinically in the third trimestre (in some cases a rising BI was detected earlier in the pregnancy) and most of the remainder within 6 months after delivery.

In addition to these patients, all 4 patients already suspected of dapsone resistance and being treated under trial conditions showed further deterioration during this study (3 in the third trimestre or lactation, 1 at the end of the first trimestre).

The complaint of 'rheumatism' was commonly associated with relapse, it preceded relapse in about half the patients, and was almost always a complaint at the time of relapse. ENL and neuritis preceded relapse in about half the patients (but were not uncommon in smear positive cases who did not relapse). At the time when relapse became clinically evident ENL was observed in half (14/28) of the patients. ENL was much more common than might be expected in BL patients, being recorded during pregnancy in 25% (9/36) of them: ENL was seen more frequently in BL patients who relapsed (33%: 5/15) than in BL patients who did not relapse (19%: 4/21).

Three BL patients downgraded to LL in association with relapse due to probable dapsone resistance; 2 downgraded during the third trimestre of pregnancy, 1 at 6 months postpartum. The diagnosis was made on histological grounds. The 2 patients who downgraded during pregnancy at the time of clinical relapse, together with another patient (initially classified LL) who had also relapsed during the third trimestre of pregnancy, all upgraded during lactation (1 to BL, 1 to BB/BL and 1 to BB/BT). The upgrading reaction was associated with new nerve enlargement or frank neuritis in all 3 cases and was confirmed by histology. The upgrading reaction was most marked at 6 months postpartum when all 3 women were still lactating and amenorrhoeic. In addition, and as reported elsewhere,[4] 3 patients downgraded to polar lepromatous

Table 2. Timing of clinical relapse due to probable dapsone resistance

Classification of mother's leprosy		BL	LL	Total
No. of pregnancies studied		36	34	70
No. of relapses		15	13	28
Timing of clinical relapse:				
Pregnancy trimestre	– first	2	–	2
	– second	1	2	3
	– third	8	6	14
Lactation (3-month periods)	0–3	2	2	4
	4–6	1	1	2
	7–9	–	–	–
	10–12	1	1	2
	13–15	–	–	–
	16–18	–	–	–
	19–21	–	1	1
	22–24	–	–	–

leprosy (LL_p)[7] showing for the first time, the histological features of LL_p during late pregnancy or the puerperium in association with relapse.

During the course of the study it became evident that there was an unexpectedly high incidence of probable dapsone-resistant leprosy associated with pregnancy. Therefore, case records of women already diagnosed as suffering from dapsone-resistant leprosy were reviewed, and the patients interviewed. An obstetrical history was obtained from 42 patients; 36 of them had had children after starting anti-leprosy treatment, of whom 31 first noticed new relapse nodules during pregnancy or soon after delivery or after a spontaneous abortion. Only 5 relapsed independently of pregnancy. The patients themselves were well aware that pregnancy had made their leprosy worse.

Discussion

Emergence of dapsone-resistant leprosy occurs more frequently when the dosage of dapsone is low or irregular; thus it will be associated with poor-patient compliance in taking dapsone regularly. Studies in Ethiopia have indicated that outpatients swallow approximately half the dapsone issued to them.[8] This is the usual finding in such studies, though figures as high as 89% have been reported.[9]

In the study we are reporting, less than 10% of women stated they had stopped taking dapsone for a few weeks during the first trimestre on account of emesis gravidarum. They all stated they had resumed treatment during the second trimestre. The rest of the women said they never stopped taking dapsone. Furthermore, it appeared to be generally believed that dapsone (unlike some other drugs) would not harm the foetus. The degree of patient–

doctor contact was high, and we considered it probable that the women took treatment regularly. However, from the results of the mouse foot-pad tests (Table 1) there is evidence that patients 2–7 could not have been taking dapsone regularly or fairly regularly, otherwise the lower level of dapsone-resistant mutants would have been killed. The failure of compliance demonstrated by these patients is disturbing. Nevertheless, it follows the pattern of other diseases; attempts to improve compliance by educating diabetic, hypertensive or tuberculosis patients about the importance of regular treatment have all failed.[10]

In Ethiopia, where dapsone resistance has become a major problem, dapsone resistance at a concentration of dapsone 0.0001% in the diet is referred to as low-grade resistance and has been shown to respond, for a period of up to 4 years, to treatment with dapsone 100 mg daily.[11] But as these patients harbour a number of more highly resistant dapsone mutants[12] in time resistance to higher dosage of dapsone emerges in a stepwise fashion.[11, 12] This is in contrast with the single-step emergence of resistance to rifampicin.[13] Recurrent pregnancies by providing periods of physiological suppression of CMI could well be a factor in contributing to the progression of dapsone resistance among women.

The suppression of CMI during pregnancy is also probably responsible for the extremely rapid deterioration observed during the third trimestre of pregnancy – 3 to 6 months for half of our patients compared with 12 months for clinical relapse in a male patient under closely controlled conditions.[14] Downgrading and upgrading in association with relapse, occurring during pregnancy and lactation respectively is further evidence of the increased immunological instability associated with pregnancy.

The association of pregnancy and the emergence of dapsone-resistant leprosy is clear from the obstetrical histories of women already diagnosed as having developed dapsone-resistant leprosy. It is fully confirmed by this prospective study. Indeed, the difficulty is not to establish the relationship but to account for the excessively high incidence in the trial patients during the study period. Possible sources of error include:

(1) Selection of patients. Although to the best of our knowledge no special selection of patients occurred, it is possible that patients who were already feeling that all was not well regarding their leprosy opted to be in the study, thus applying some degree of self-selection.
(2) Overdiagnosis of relapse. This is not a serious possibility. The clinical and laboratory findings supported each other in most cases, as most of the patients showing (at first) only laboratory evidence of relapse, had relapsed clinically by the end of the study.
(3) Overdiagnosis of resistance. This again is unlikely. Four of the 6 patients already dapsone resistant (following relapse in a previous pregnancy) and

7 patients in the present study were tested by mouse foot pad tests and none showed dapsone-sensitive bacilli.

(4) One possibility is that in the early stages of emergence of dapsone-resistant leprosy the clinical signs are labile, and that relapse lesions might resolve between pregnancies, the condition progressing in a stepwise fashion. The relatively short period of this study prevents any definite conclusion but when last seen only 3 of the patients suspected of dapsone-resistant leprosy, but not tested in mice, were still improving on dapsone monotherapy.

Practical applications

There are 3 important areas of application of these findings to the practical management of women with lepromatous leprosy.

(1) The possibility of giving supplementary chemotherapy in effective dosage during pregnancy and lactation might be considered: this would aim both to prevent the emergence of dapsone-resistant leprosy and also to lessen the risk of infecting the baby before and after delivery. Clofazimine (in the dosage of 100 mg at least 3 times a week) for 1 year starting at the beginning of the second trimestre would probably be the most suitable drug for the purpose, and would have the additional advantage of possibly reducing the amount of ENL occurring during pregnancy and lactation.

(2) There is a clear risk that pregnancy will make leprosy worse; patients frequently develop ENL and neuritis, which could be damaging even in the absence of dapsone resistance. It would be reasonable to advise women with lepromatous leprosy to limit the size of their families by whatever means are locally acceptable. However, it should be remembered that the role of exogenous oestrogens (such as are found in the contraceptive 'pill') in the causation of relapse is as yet unknown, and use of oral contraceptives should be carefully monitored. Personal interviews with patients who had suffered relapse or reaction in association with pregnancy not only revealed that the patients were aware of the adverse effect of pregnancy on leprosy, but in addition many patients volunteered the information that they wished for no more than 1 or 2 children at most.

(3) Should any of the children of women with dapsone-resistant leprosy develop leprosy at an early age, it is highly likely that they will have dapsone-resistant leprosy – and hence would require alternative therapy.

Acknowledgements

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IgA and IgM Antibodies against *Mycobacterium leprae* in Cord Sera and in Patients with Leprosy: An Indicator of Intrauterine Infection in Leprosy

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Melsom, R., Harboe, M., Duncan, M. E. & Bergsvik, H. IgA and IgM Antibodies against *Mycobacterium leprae* in Cord Sera and in Patients with Leprosy: An indicator of Intrauterine Infection in Leprosy. *Scand. J. Immunol.* 14, 345–354, 1981.

A solid-phase radioimmunoassay was developed for demonstration and quantification of IgA and IgM anti-*M. leprae* antibodies. IgA and IgM anti-*M. leprae* antibodies were demonstrated in a lepromatous serum pool, in various amounts in individual patients with lepromatous leprosy, and in lower concentration in tuberculoid leprosy and non-leprosy controls. IgA and IgM anti-*M. leprae* antibodies were demonstrated in cord sera from babies of mothers with leprosy. The reliability of fetal IgA and IgM antibody synthesis as an indicator of intrauterine infection in leprosy is discussed.

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The humoral immune response in leprosy has been less studied recently than cellular immune reactions. Many authors have reported on antibodies against various mycobacterial antigens in leprosy [2, 17], but information on their immunoglobulin class is very limited [20]. No quantitative data are available on production of IgA antibodies towards *M. leprae*. The IgM response is also poorly documented. So far, IgM antibodies towards *M. leprae* have only been demonstrated as part of the antibody response towards one antigenic component of the bacillus, *M. leprae* antigen 7 [20].

Antibodies of the IgA and IgM classes are particularly valuable as indicators of stimulation of the immune system *in utero*.

The purpose of the present work was to develop a solid radioimmunoassay (SRIA) for demonstration and quantification of IgA and IgM antibodies against *M. leprae* [16]. Normal rabbit immunoglobulins contain anti-mycobacterial antibodies. Purified, labelled anti-IgA and anti-IgM antibodies therefore had to be used to obtain a sensitive and specific assay.

The technique was first used for assay of IgA and IgM antibodies against *M. leprae* in sera taken from adult female leprosy patients at delivery.

It has been shown previously that babies of mothers with active lepromatous leprosy have an increased concentration of IgA in cord serum [21]. Since IgA and IgM do not cross the placenta, this is an indication of fetal immunoglobulin production [33] and possibly of intrauterine infection with *M. leprae*. The SRIA was therefore used to measure IgA and IgM antibodies against *M. leprae* in cord sera from babies of mothers with various forms of leprosy.

MATERIALS AND METHODS

Patients

Forty-four pregnant women were selected for this study. All of them attended the outpatient clinic at the Addis Ababa Leprosy Hospital. They were clinically and histologically classified according to the extended Ridley-Jopling scale [24, 29, 30]. Most of the patients in this study are identical with the

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patients used in previous investigations [21, 22]. The group of tuberculoid leprosy and non-leprosy controls consist of some of the individuals previously reported on, whereas the group of pregnant women with active lepromatous leprosy has been expanded. This group of women, who are expected to have a prolonged bacteraemia during pregnancy with up to 10^5 *M. laprae* bacilli/ml blood [7, 32], was thereby increased. All the women in this study were Ethiopians living around Addis Ababa under poor socioeconomic conditions. As in the previous study [21], the patients were divided as follows.

Group 1 (LL-BL, BI^+). This group consisted of 22 mothers with active lepromatous leprosy (LL, LI and BL). They all had a positive bacterial index with acid-fast bacteria in one or several skin smears taken from six different sites, and they all received 100 mg dapsone (DDS) daily. Two patients received 20–30 mg prednisolone daily owing to severe reactions, and one patient received rifampicin in addition.

Group 2 (LL-BL, BI^-). This group consisted of seven mothers with inactive lepromatous leprosy with a negative bacterial index; that is, no acid-fast bacilli had been found in any of the skin smears taken during the last 2 years before this study. Six of these patients received, and had received for more than 5 years prior to this study, 50–100 mg DDS daily. One patient had stopped treatment and was BI^- at the start of the study.

Group 3 (BT-TT).This group consisted of six patients with tuberculoid leprosy; three patients received 50–100 mg DDS daily, and in the remaining three patients DDS had been withdrawn after more than 5 years of regular treatment.

Group 4 (NL). This consisted of nine mothers without any clinical signs of leprosy but with the same socioeconomic background as the leprosy patients.

Lepromatous leprosy serum pool (*LSP*). The pool comprised sera from 40 patients with lepromatous leprosy who were either newly diagnosed or had been treated with DDS for less than 6 months.

The serum samples were obtained from venous blood, stored at −20°C, and some of them were freeze-dried before transportation to Norway. Before being tested for antibodies against *M. leprae*, these sera were reconstituted with distilled water containing 0.01% NaN_3 as preservative. They were subsequently stored at 4°C. The other sera were stored at −20°C and transported to Norway on dry ice. They were stored at −20°C after arrival until used.

Purification and testing of anti-human IgA and anti-human IgM

Rabbit anti-human IgA and anti-human IgM immunoglobulins (DAKO Immunoglobulins a/s, Copenhagen, Denmark) were labelled with ^{125}I by electrolytic iodination as described previously [12]. Of the labelled immunoglobulins 10–15% reacted with IgA and IgM. By crossed radioimmunoelectrophoresis it was further demonstrated that both preparations contained antibodies against several *M. leprae* antigens. Pure anti-IgA and anti-IgM were therefore isolated from the rabbit immunoglobulin preparations by immunoabsorption.

IgA was isolated from human colostrum by A.-B. Otnæss by precipitation with ammonium sulphate and chromatography on an Ultragel AcA column [25]. The IgA preparation contained less than 0.1% IgM and IgG and was coupled to cyanogen-bromide-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) [19]. Labelled rabbit anti-human IgA immunoglobulin was adsorbed on the Sepharose-insolubilized IgA and eluted by 1M glycine–HCl buffer of pH 2.8.

The activity of labelled anti-human IgA immunoglobulin and of isolated anti-human IgA antibodies was determined by SRIA in the following manner: purified IgA was diluted to 10^{-3} g/l in phosphate-buffered saline (PBS) *not* containing any other protein, and 150 µl samples were added to 70 × 11 mm polystyrol test tubes (Nunc, Kamstrup, Denmark). The tubes were left for 3 h at 20°C and then for at least 24 h at 4°C before use. The fluid was removed by suction and the tubes left at 37°C for 2 h to dry. Thereafter, 100 µl of either ^{125}I-labelled anti-IgA immunoglobulin or ^{125}I-labelled purified anti-IgA antibody were added to the coated tubes. The tubes were incubated for 2 h at 20°C and for 20 h at 4°C. They were washed twice in PBS containing 0.2% Tween 20, sucked dry, and counted for radioactivity in a Selektronik gammacounter, model 45-22 (Selektronik a/s, Hørsholm, Denmark). The amount of radioactivity bound to IgA on the wall of the test tube increased from 15% of labelled anti-IgA-containing immunoglobulin to 55% of the labelled anti-IgA antibody. Less than 5% of the labelled anti-IgA antibody was bound to uncoated tubes or tubes coated with human IgM or IgG.

IgM was purified from serum of a patient (Ka) with Waldenström's macroglobulinaemia by euglobulin precipitation and gel chromatography on Sephadex G-200 [13]. The preparation contained less than 0.1% IgG, and IgA could not be detected by single radial diffusion. Coupling to Sepharose, isolation of labelled anti-IgM, and testing for reactivity with IgM were done as for IgA.

The amount of radioactivity bound to IgM on the wall of the test tubes increased from 12% of anti-IgM-containing immunoglobulin to 82% of the isolated labelled anti-IgM antibody preparation. Less than 5% of the labelled anti-IgM antibody preparation was bound to uncoated tubes and tubes coated with IgA and IgG.

Coating of tubes with M. leprae *sonicate and assay of anti-*M. leprae *antibodies*

Mycobacterium leprae bacilli purified from infected armadillo liver [6] were obtained from Dr R. J. W. Rees, London. The bacilli (50 mg dry weight) were suspended in 12 ml 0.9% NaCl and left overnight. The bacilli were then sonified for 10 min on an ice bath with a Branson B-12 Sonifier (Branson Sonic Power Co., Danbury, Conn., USA) at 80 W. The supernatant was collected after centrifugation for 15 min at 20,000 *g* at 4°C [20]. This sonified *M. leprae* preparation had a protein concentration of 0.45 g/l and was diluted to 4.5×10^{-4} g/l in PBS *not* containing any protein. Samples of 150 µl of this preparation were added to Nunc polystyrol test tubes. The

tubes were incubated at 20°C for 3 h and subsequently for at least 24 h at 4°C before use. The test tubes were sucked empty and dried for 2 h at 37°C. Sera to be assayed for antibody activity were diluted 10^{-1}, 10^{-2} and 10^{-3} in PBS containing 1% Tween 20, 0.2% BSA and 0.002% NaN_3. Of each of these dilutions, 100 μl were added to the *M. leprae*-coated tubes and left for 20 h at 4°C. They were then sucked empty and washed twice with PBS containing 0.2% Tween 20 and finally in PBS containing 1% Tween 20 and 0.2% BSA. Again, the tubes were sucked dry, and either 100 μl of purified labelled anti-human IgA or anti-IgM were added. The tubes were left for 2 h at 20°C and 20 h at 4°C, washed twice in PBS containing 0.1% Tween 20, and sucked dry, and the amount of radioactivity bound was determined in the gamma counter.

Statistical calculations

For calculation of the statistical significance of difference between the groups, Wilcoxon's modified ranking test was used [5].

RESULTS

IgA and IgM antibodies against M. leprae *in LSP*

Figure 1 shows the results obtained when tenfold serial dilutions of LSP were tested for IgA and IgM antibodies against *M. leprae* sonicate.

The largest amount of anti-IgA, about 30% of the total amount added, was bound to the tubes previously incubated with LSP diluted 10^{-1}, whereas 50% of the added anti-IgM was bound to the tubes incubated with LSP diluted 10^{-1} and 10^{-2}. The amount of radioactivity bound decreased upon dilution of the lepromatous serum, and significant binding was found up to a dilution of 10^{-3} in the assay for IgA antibodies and up to a dilution of 10^{-4} in the IgM antibody assay. When the labelled anti-IgA- or anti-IgM-containing preparations were used, maximal binding of both preparations was about 10%; the titration curves were much less steep, thus providing a considerably less sensitive assay, as shown in Fig. 1.

When tenfold serial dilutions of LSP were added to uncoated tubes, less than 0.1% of purified anti-IgA or anti-IgM was bound. The same result was obtained when anti-IgA or anti-IgM was added directly to sonified *M. leprae*-coated tubes. PBS with 1% Tween 20 and 0.2% BSA had to be used as dilution fluid and blocking agent to prevent unspecific binding of antibodies to the tubes.

To obtain additional evidence that binding of labelled anti-IgA and anti-IgM was due to

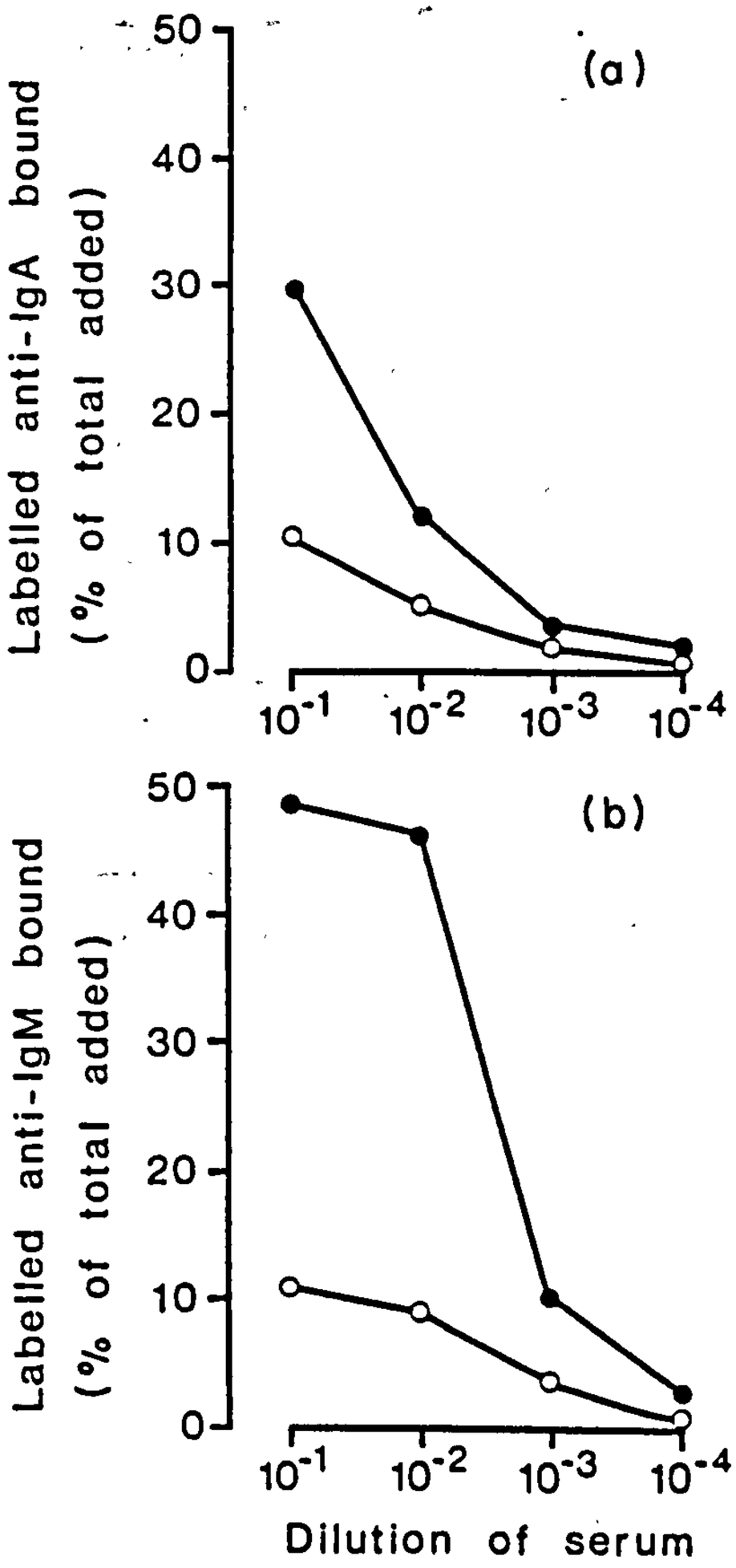


FIG. 1. (a) Shows IgA antibody activity against *M. leprae* in tenfold serial dilutions of lepromatous serum pool (LSP), where (●—●) represents the assay using labelled anti-human IgA antibodies and (○—○) with the use of a labelled anti-IgA-containing immunoglobulin preparation. (b) shows IgM antibody activity against *M. leprae* in serial tenfold dilution of LSP. Procedures and designations as for IgA (a).

reaction with IgA and IgM antibodies in LSP bound to *M. leprae* sonicate on the test tube wall during the first reaction step, inhibition experiments were carried out as illustrated in Fig. 2. Addition of IgG did not inhibit binding of labelled anti-IgA or anti-IgM. The anti-IgA

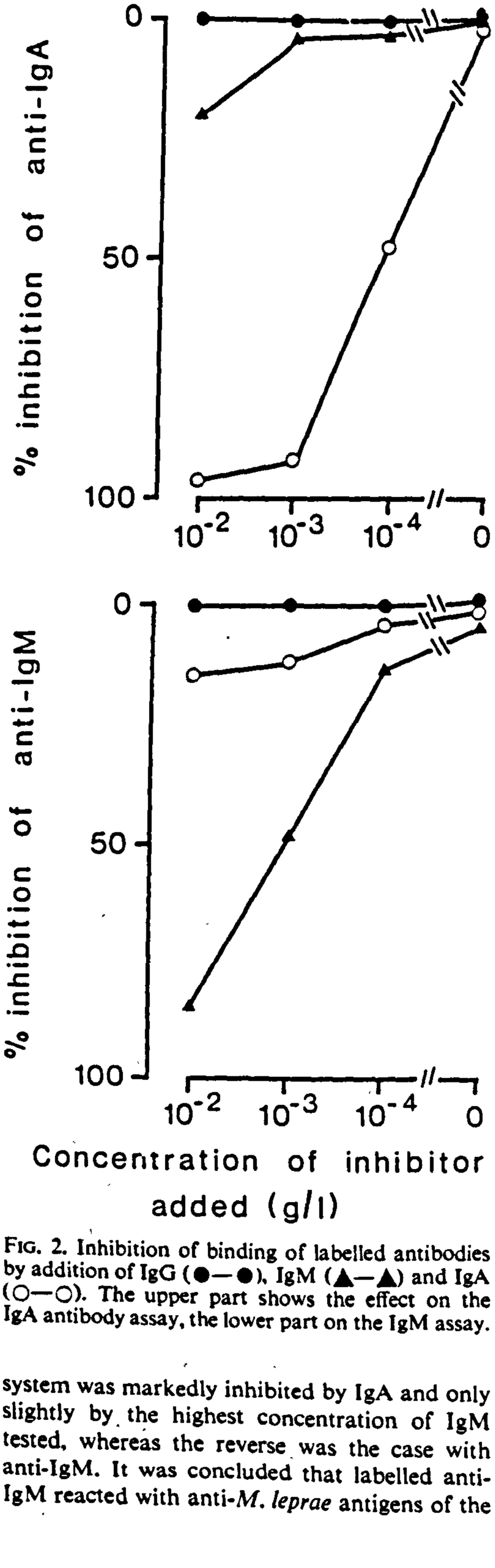


FIG. 2. Inhibition of binding of labelled antibodies by addition of IgG (●—●), IgM (▲—▲) and IgA (○—○). The upper part shows the effect on the IgA antibody assay, the lower part on the IgM assay.

system was markedly inhibited by IgA and only slightly by the highest concentration of IgM tested, whereas the reverse was the case with anti-IgM. It was concluded that labelled anti-IgM reacted with anti-*M. leprae* antigens of the IgM class, whereas labelled anti-IgA reacted with anti-*M. leprae* antibodies of IgA class.

*IgA and IgM anti-*M. leprae *antibodies in maternal sera*

Figure 3 shows the IgA anti-*M. leprae* antibody concentration in maternal sera. On the basis of the findings illustrated in Fig. 1, the sera were tested in dilutions of 10^{-1}, 10^{-2} and 10^{-3}. Each point represents one individual. The IgA or IgM anti-*M. leprae* antibody activity for each individual serum was calculated from the results obtained at three different dilutions: 10^{-1}, 10^{-2} and 10^{-3} for the IgA assay and 10^{-2}, 10^{-3} and 10^{-4} for the IgM assay. The results from these dilutions were compared with the results obtained with 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} dilutions of LSP, which were included in each set of experiments. A standard curve was made from the results with LSP, and the dilution of test serum, which was positioned on the steepest part of the standard curve, was used to calculate

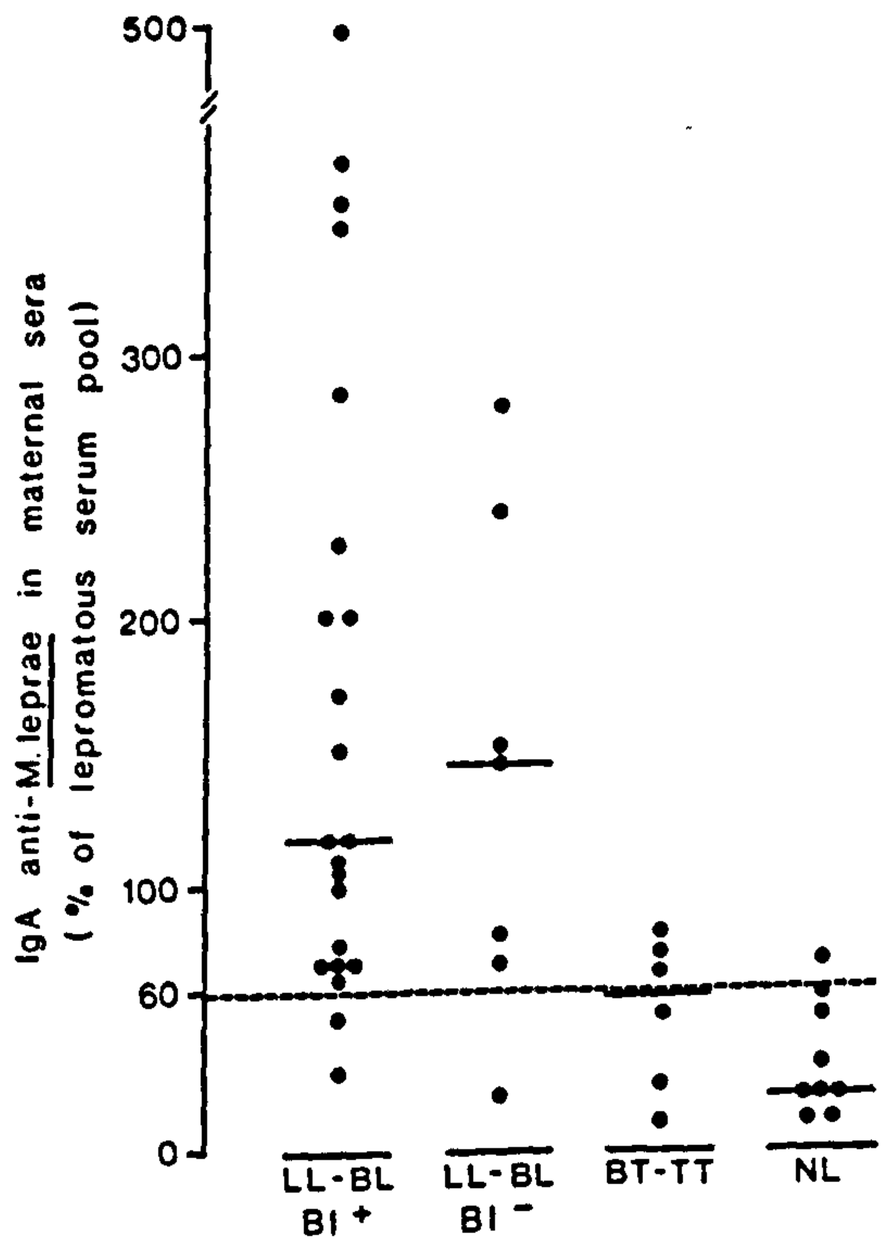


FIG. 3. IgA anti-*M. leprae* antibodies in maternal sera at delivery. Each point represents one individual, and the amount is expressed as a percentage of the IgA antibody activity against *M. leprae* in lepromatous serum pool. The horizontal bars represent the median values.

the antibody activity in percentage of LSP activity. A value of 100% of LSP means that the same number of counts was obtained with the serum diluted 10^{-2} as with LSP diluted 10^{-2}. 10% of LSP means the same number of counts was obtained with the test serum diluted 10^{-1} as in the 10^{-2} dilution of LSP, and, finally, 1000% means the same number of counts was obtained with the 10^{-3} dilution of the test serum as with the 10^{-2} dilution of LSP. The calculations were made on a semi-logarithmic paper, recording the standard curve of LSP with percentage on the ordinate and the number of counts on the abscissa.

IgA antibodies against *M. leprae* antigens were found in all the patients examined. In the patients with active lepromatous leprosy, the concentration varied markedly, from 30 to 500% of the concentration of LSP, the median concentration being 117%. The variation in the smaller group of patients with inactive lepromatous leprosy was 20–280%, with a median value of 140%. The variation of IgA antibody concentration in patients with tuberculoid leprosy was 10–80%, with a median value of 40%, whereas the non-leprosy group had a median value of 20%, with a variation of 10–70% of the concentration in LSP.

Sera from all except 1 of the individuals in the non-leprosy group contained less than 60% of the amount of IgA antibodies in LSP; 3 out of 7 patients with tuberculoid leprosy had a concentration above 60%; and 6 out of 7 patients with inactive lepromatous leprosy and 22 out of 24 patients with active lepromatous leprosy had a concentration above 60% of LSP.

Figure 4 shows anti-*M. leprae* antibodies of the IgM class. LSP gave almost identical counts in the 10^{-1} and 10^{-2} dilutions in the IgM anti-*M. leprae* assay, as can be seen in Fig. 1. To obtain an assay suitable for demonstration of variation in IgM antibody content in the individual sera, they were tested in dilutions of 10^{-2}, 10^{-3} and 10^{-4}. Each point in Fig. 4 represents one serum, and the activity is expressed as a percentage of LSP IgM anti-*M. leprae* antibody activity, as in Fig. 3.

IgM antibodies against *M. leprae* were present in all the sera examined. Again, the lepromatous leprosy groups had the highest concentration of antibodies. The median value was 140%, with a variation between 10 and 350% in active lepromatous leprosy, and the median value was

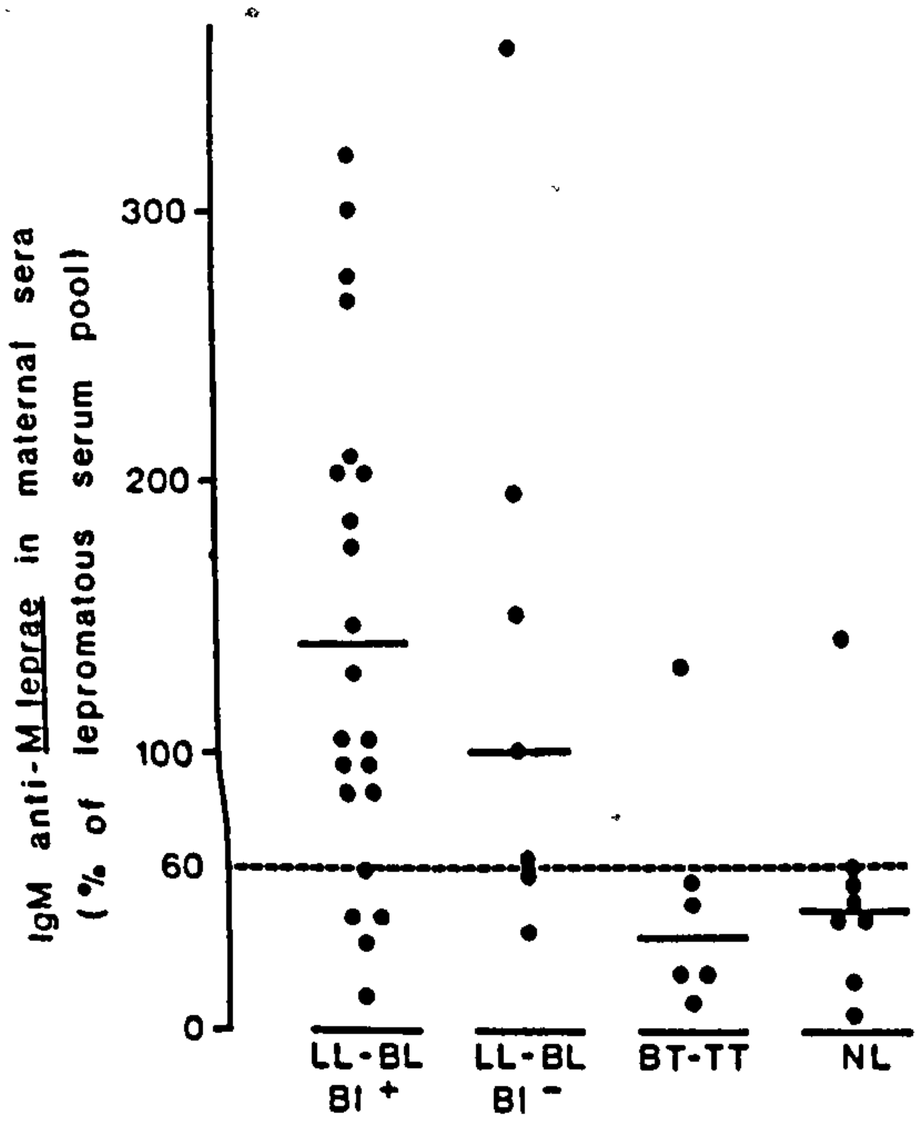


FIG. 4. IgM anti-*M. leprae* antibodies in the same sera as in Fig. 3.

100% with a variation between 40 and 150% in inactive lepromatous leprosy. For tuberculoid leprosy the median value was 35%, with a variation between 10 and 130%, and for the non-leprosy group the median value was 40%, with a variation between 7 and 140%.

Although there was a marked difference in concentration of IgM anti-*M. leprae* antibodies in individual lepromatous sera, the antibody concentration was strikingly higher in this group than in tuberculoid leprosy. Figure 4 shows that 24 out of 31 lepromatous sera (77%) contained more than 60% of the amount in LSP, whereas only one out of six tuberculoid leprosy sera was above this value.

*IgA and IgM anti-*M. leprae *antibodies in cord sera*

Figure 5 shows the amount of IgA antibodies towards *M. leprae* antigens in cord sera taken from the babies of the mothers described above. The cord sera were tested in a dilution of 10^{-1}. Seven of the cord sera from babies of the 22 mothers with active lepromatous leprosy contained IgA antibodies against *M. leprae* antigens, indicated on the figure as percentage of IgA anti-*M. leprae* activity in LSP. No IgA antibodies against *M. leprae* could be demonstrated

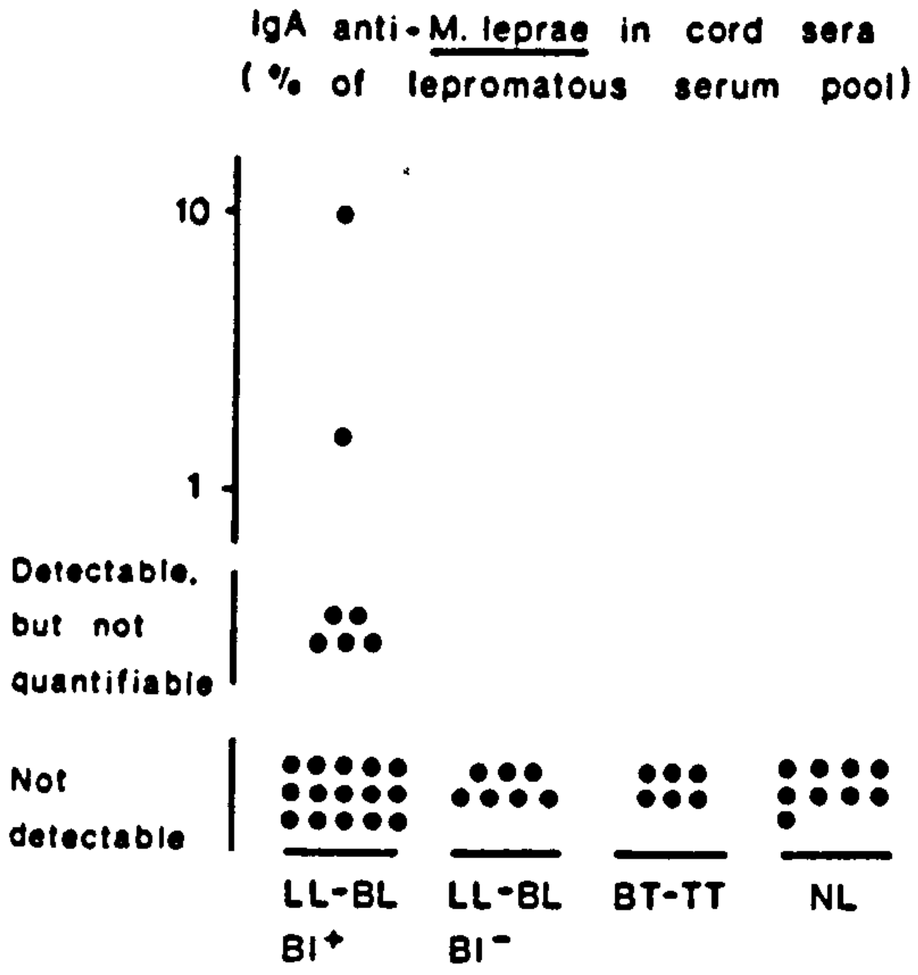


FIG. 5. IgA anti-*M. leprae* in cord sera diluted 10^{-1}. The amount is expressed as a percentage of IgA antibody activity in lepromatous serum pool.

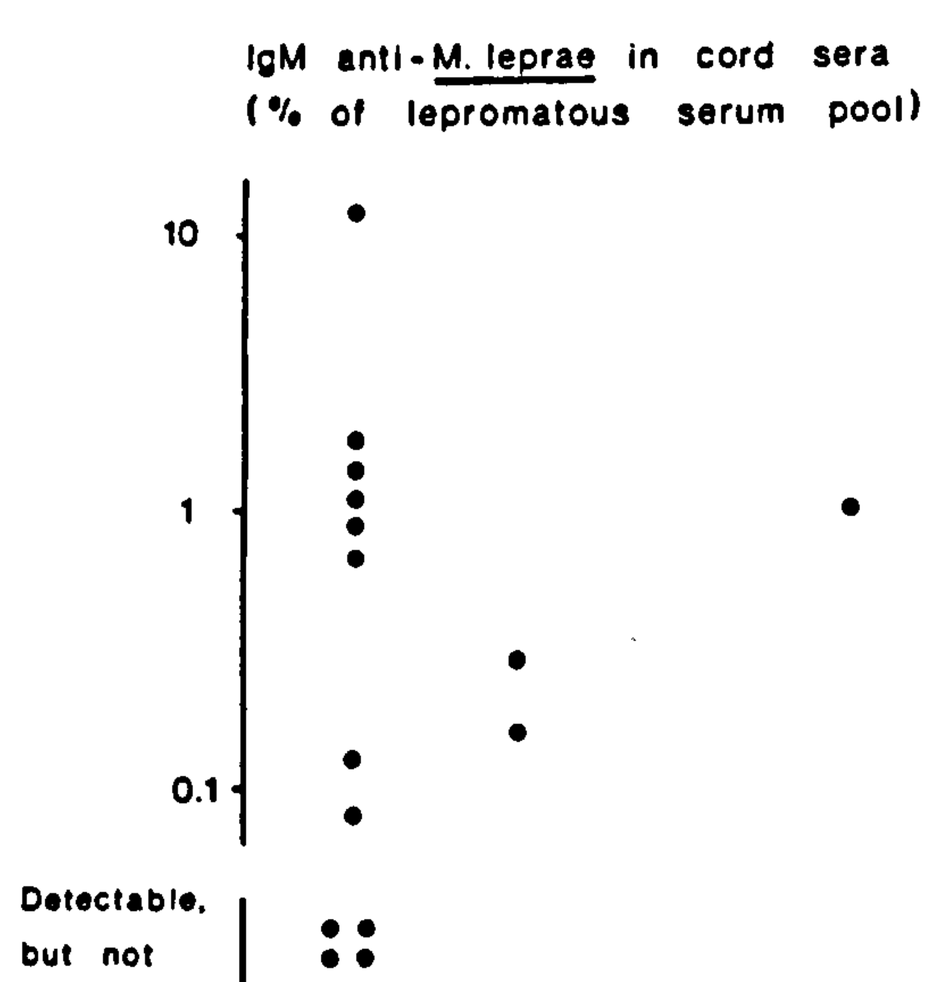


FIG. 6. IgM anti-*M. leprae* antibodies in the same cord sera as in Fig. 5.

in cord sera from the babies of the 22 mothers in the three other groups.

The presence of IgM antibodies against *M. leprae* antigens in cord sera is shown in Fig. 6. Twelve of 22 cord sera from babies of mothers with active lepromatous leprosy contained IgM antibodies towards *M. leprae* when tested in a dilution of 10^{-1}. The seven cord sera with IgA anti-*M. leprae* also contained IgM anti-*M. leprae* antibodies. The results are indicated in Fig. 6 as a percentage of the IgM anti-*M. leprae* activity in LSP. Two cord sera from babies of the nine mothers with inactive lepromatous leprosy contained IgM anti-*M. leprae* antibodies. No IgM antibodies towards *M. leprae* could be detected in cord sera from babies of the seven mothers with tuberculoid leprosy. In the non-leprosy control group, IgM antibodies against *M. leprae* were found in one of the nine cord sera tested.

DISCUSSION

The SRIA system was developed to obtain a sensitive assay for anti-*M. leprae* antibodies in various immunoglobulin classes in leprosy sera, with particular emphasis on IgA and IgM antibodies. These two classes are of main interest in attempts to demonstrate antibody formation by the fetus as an indicator of intrauterine infection.

To obtain a sensitive assay and to avoid interference of anti-mycobacterial antibodies present in the rabbit immunoglobulin preparations used, labelled anti-IgA and anti-IgM were isolated by an immunosorbent technique. This proved to be essential to obtain an assay of sufficient sensitivity and specificity.

The binding of labelled anti-IgA and anti-IgM antibodies could be almost completely inhibited by human IgA or IgM, respectively, as demonstrated in Fig. 2, whereas poor inhibition was obtained by IgM or IgG in the IgA antibody assay and by IgA or IgG for the IgM antibody assay. This was taken as additional evidence that we demonstrated IgA and IgM antibodies against *M. leprae* antigens in sera from leprosy patients.

Rheumatoid factors (RF) occur in increased amounts in sera of patients with rheumatoid arthritis and in chronic infections like leprosy [8, 15]. Antibodies of IgM class against maternal

IgG have also been demonstrated in cord sera [27]. Since a major part of RF is IgM protein with anti-IgG activity [15], it might cause false-positive reactions in the IgM SRIA with sera containing high titres of RF and anti-*M. leprae* antibodies in only the IgG class. This prediction was confirmed in model experiments with a purified monoclonal IgM RF. The amount of RF needed to obtain a false-positive reaction in our SRIA was distinctly greater than the amount occurring in LSP. Furthermore, the IgM and the IgG fractions of LSP were separated by density gradient ultracentrifugation [10]. Only the IgM-containing fractions contained IgM anti-*M. leprae* antibodies detectable by the SRIA for this immunoglobulin class, and the amount detected did *not* increase upon addition of the IgG fraction of LSP. Two cord sera with detectable IgM antibodies against *M. leprae* antigens were similarly investigated after separation of the IgG fractions from the IgM fractions by density ultracentrifugation. Again, the amount of IgM anti-*M. leprae* antibodies did not increase after addition of the IgG fractions. In these two cord sera the concentration of maternal IgG antibodies against *M. leprae* antigens was considerably higher than the concentration of fetal IgM antibodies against *M. leprae*.

M. leprae sonicate was used for coating of the test tubes. The sonicate contained several distinct antigenic components detectable by crossed immunoelectrophoresis [4]. The fine specificity of the IgA and IgM antibodies detected by the present SRIA system has yet to be determined. This would require inhibition experiments with purified antigenic components of *M. leprae*.

This work demonstrates IgM antibodies against *M. leprae* antigen. In LSP we could demonstrate and quantitate such antibodies down to a dilution of 10^{-4}. IgM antibodies against *M. leprae* antigens could be quantitated in a dilution of 10^{-3} or 10^{-4} of the sera taken from the mothers. This work therefore confirms and extends our previous demonstration of IgM antibodies against *M. leprae* antigen 7 by radioimmunoassay (RIA) [20].

We could demonstrate IgA antibodies against *M. leprae* in LSP diluted 10^{-3}, and we could quantitate such IgA antibodies in maternal sera diluted 10^{-2}. As far as we are aware, this is the first time that IgA antibodies against *M. leprae* antigens have been demonstrated and quantified. The significance of such IgA antibodies in leprosy is unknown.

The wide variation in IgA and IgM anti-*M. leprae* antibody content in sera from patients with similar clinical classification corresponds to previous findings in RIA for antibodies against bacillus Calmette-Guérin antigen 60 [11] and *M. leprae* antigen 7 [23, 35]. But the difference in amount of both IgA and IgM anti-*M. leprae* antibodies between lepromatous leprosy and tuberculoid leprosy is striking in the present SRIA (cf. Figs. 3 and 4). The difference was statistically significant ($P < 0.01$). The number of sera is small, and they originate from selected patients, i.e. women at delivery, but the results obtained so far indicate that the present SRIA system may be developed into a serodiagnostic test for lepromatous leprosy. To substantiate this and that the difference between the groups is greater than with RIA for anti-*M. leprae* 7 antibodies requires tests on a larger number of sera from leprosy patients and normal controls.

Cord sera from babies of mothers with active lepromatous leprosy have previously been shown to contain a significantly increased concentration of IgA [21]. In the present investigation IgA anti-*M. leprae* antibodies were demonstrated in the cord sera of 7 out of 22 babies of mothers with active lepromatous leprosy but not in any of the 22 cord sera in the other groups. Further, we have demonstrated on average five times higher concentration of IgA anti-*M. leprae* antibodies in sera taken from 3- to 6-month-old babies of lepromatous leprosy mothers compared with babies of tuberculoid leprosy mothers and non-leprosy mothers (Melsom *et al.*, data to be published).

IgM anti-*M. leprae* antibodies were demonstrated in 12 out of 22 cord sera from babies of mothers with active lepromatous leprosy (Fig. 6). The seven cord sera with IgA anti-*M. leprae* antibodies also contained IgM anti-*M. leprae* antibodies.

Fetal production of IgM antibodies towards infectious agents have been described in congenital infections, e.g. syphilis [9], rubella [1] and toxoplasmosis [28]. This is a better indicator of congenital infection than the demonstration of increased concentration of IgM proteins in the cord sera [21]. False-negative reactions have been described in syphilis [27] and toxoplasmosis [28]. Absence of IgM antibodies in

cord sera is thus no reliable indicator of absence or presence of a particular congenital infection. By contrast, demonstration of fetal antibody production is considered a positive indicator of intrauterine infection. False-positive reactions have also been observed, e.g. in toxoplasmosis [28]. In each infection, the mechanism(s) of false-positive reactions should be carefully explored since their elimination is essential for development of valuable and specific diagnostic procedures for congenital infections. The properties of the anti-IgM preparation used are essential. In one study of toxoplasmosis [28], one anti-human IgM preparation gave false-positive reactions while another gave no false-positive reaction. The purity of the antibody preparation is an important feature. Admixture of maternal blood during sampling must be carefully avoided both during sampling and during delivery.

We found no evidence to indicate that the positive findings in the IgA and IgM assay for anti-*M. leprae* antibodies were due to false-positive reactions except in one case discussed below.

Two cord sera from babies of mothers with 'inactive lepromatous leprosy' contained IgM anti-*M. leprae* antibodies. Of the mothers originally classified as having inactive lepromatous leprosy, 35% have since relapsed, with new clinical signs of leprosy and reoccurrence of a positive bacterial index during pregnancy or within a few months after delivery of the baby in the present study. These women were reclassified and included in the active lepromatous leprosy group. It is likely, owing to the slow multiplication rate of *M. leprae*, that several of the women still classified as having inactive lepromatous leprosy may have had a relapse without overt clinical signs of the disease during pregnancy. These women would probably have a bacteraemia during the initial stage of the subclinical reactivation phase, and the fetus may thereby have been exposed to *M. leprae* antigens.

IgM anti-*M. leprae* antibodies were demonstrated in the cord serum of one baby of a non-leprous mother (cf. Fig. 6). During the course of the study and sampling, two women classified as non-leprosy controls developed clinical signs of leprosy and had to be excluded from the series. The non-leprous mothers lived in areas with a high incidence of leprosy, many of them having leprosy patients among their relatives. The non-leprous mother of the baby with IgM anti-*M. leprae* antibodies had high concentrations of IgA and IgM anti-*M. leprae* antibodies in her serum, and she might have had leprosy in the stage before development of clinical symptoms. This baby also had high concentrations of IgA and IgM in the cord serum, which indicates placental leakage with transfer of maternal plasma proteins into the fetal circulation. Since we quantitated and demonstrated IgM anti-*M. leprae* antibodies in a 10^{-4} dilution of LSP compared with IgA anti-*M. leprae* antibodies in a 10^{-3} dilution, a small placental leakage might cause a positive finding in SRIA for IgM antibodies against *M. leprae* due to the presence of maternal IgM in the cord serum. A considerably larger leakage is probably needed to result in a positive test for IgA anti-*M. leprae* antibodies in the cord serum. None of the other babies had such high concentrations of both IgA and IgM in the cord serum as this baby. Admixture of maternal immunoglobulin may thus explain this false-positive result.

In her thesis, Valla [34] demonstrated *M. leprae* in one placenta from a woman with lepromatous leprosy. In the same work she refers to earlier work by Vietnamese authors who have demonstrated acid-fast bacilli in cord blood from babies of leprosy mothers. Studies of other mycobacterial infections have established that mycobacteria may cross the placenta. In the case of congenital tuberculosis, infection has been ascribed to transplacental infection, ingestion of infected amniotic fluid, or infection from the birth canal during delivery [14]. The localization of the tuberculous granulomas is an important indicator of the infectious route. Owing to the particular anatomical features of the fetal circulation, occurrence of the primary granulomas in the liver points to placental transmission of *M. tuberculosis*, and this has been demonstrated in several cases [18, 26].

Occurrence of both IgA and IgM anti-*M. leprae* antibodies in the same cord sera indicates foetal antibody synthesis induced by stimulation from mothers with leprosy and *M. leprae* bacteraemia during pregnancy. The stimulus is most likely due to transfer of either *M. leprae* antigens or live bacilli. Distinction between these alternatives is important. We favour the second alternative, since synthesis of anti-*M. leprae* antibodies continues during the first months of life in many babies of mothers with highly bacilliferous leprosy [22]. The clinical conse-

quences of this early stimulation of the immune system and possible intrauterine infection with *M. leprae* should be carefully studied.

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Estrogen Excretion in Pregnant Women with Leprosy: Evidence of Diminished Fetoplacental Function

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Estrogen excretion was assayed in 64 women with leprosy and 15 healthy control women. The mean estrogen excretion was lower in women with leprosy than in controls and the incidence of subnormal estrogen values was higher in the leprosy patients than in the controls. There was an association between infant birth weight and frequency of subnormal estrogen excretion. These features were most marked in women with lepromatous leprosy and are further evidence of diminished fetoplacental function in women with leprosy. ***(Obstet Gynecol 60:82, 1982)***

The quantity of estrogen excreted daily in the urine in late pregnancy is widely accepted as an accurate index of fetoplacental function and provides a valuable indication of impending intrauterine death from placental insufficiency.[1] Urinary estrogen, predominantly estriol, is critically dependent on production of precursors (androgen sulfates) by the fetal adrenal glands and on the ability of the placenta to convert these precursors to estrogen through a complex series of reactions.[2]

Maternal complications such as hypertension and preeclampsia are often associated with low estrogen excretion, particularly when these sufficiently disturb placental function so as to cause growth retardation. Other maternal conditions not induced by pregnancy appear to have little influence on estrogen excretion, although anemia, including thalassemia, is associated with a high incidence of subnormal estrogen excretion.[3] Poor maternal nutrition and high altitude have also been associated with low estrogen excretion, possibly due to fetal undernutrition in patients living in poor social and economic conditions.[4,5]

Leprosy is a chronic disease caused by a virtually nontoxic organism. Clinical manifestations of the disease can be defined clinically, histopathologically, and immunologically.[6] In tuberculoid leprosy the cell-mediated immune response is high, whereas lepromatous leprosy involves low cellular immunity. Between these polar forms of leprosy lies the borderline group. It has previously been noted[7] that women with leprosy who become pregnant give birth to lower birth weight infants and have smaller placentas than healthy subjects from the same population. It was therefore decided to assess estrogen excretion in some of these women as another index of fetoplacental function. This study reports associations between urinary estrogen excretion, birth weight, and placental weights in Ethiopian women with leprosy and in those free from the disease.

Patients and Methods

Sixty-four patients with leprosy and 15 healthy subjects without leprosy, who served as controls, took part in this study, which formed part of a larger prospective investigation of the effects of leprosy on pregnancy. All the women were Ethiopians of low socioeconomic class, living in the villages surrounding the Addis Ababa Leprosy Hospital at an altitude of 2340 m above sea level. The modes of life of the leprosy patients and the control group were comparable. Income per capita per month was difficult to evaluate, as many earned a livelihood by subsistence farming, making handicrafts and local beer, or by begging alms. Most of the healthy subjects were married to leprosy patients. The average income per family in both the leprous and control groups was estimated at less than $25 (U. S. currency) per month. Life was physically demanding, as water was carried from standpipes in the village streets, firewood was carried from the forest, and transport was by foot, as few could afford

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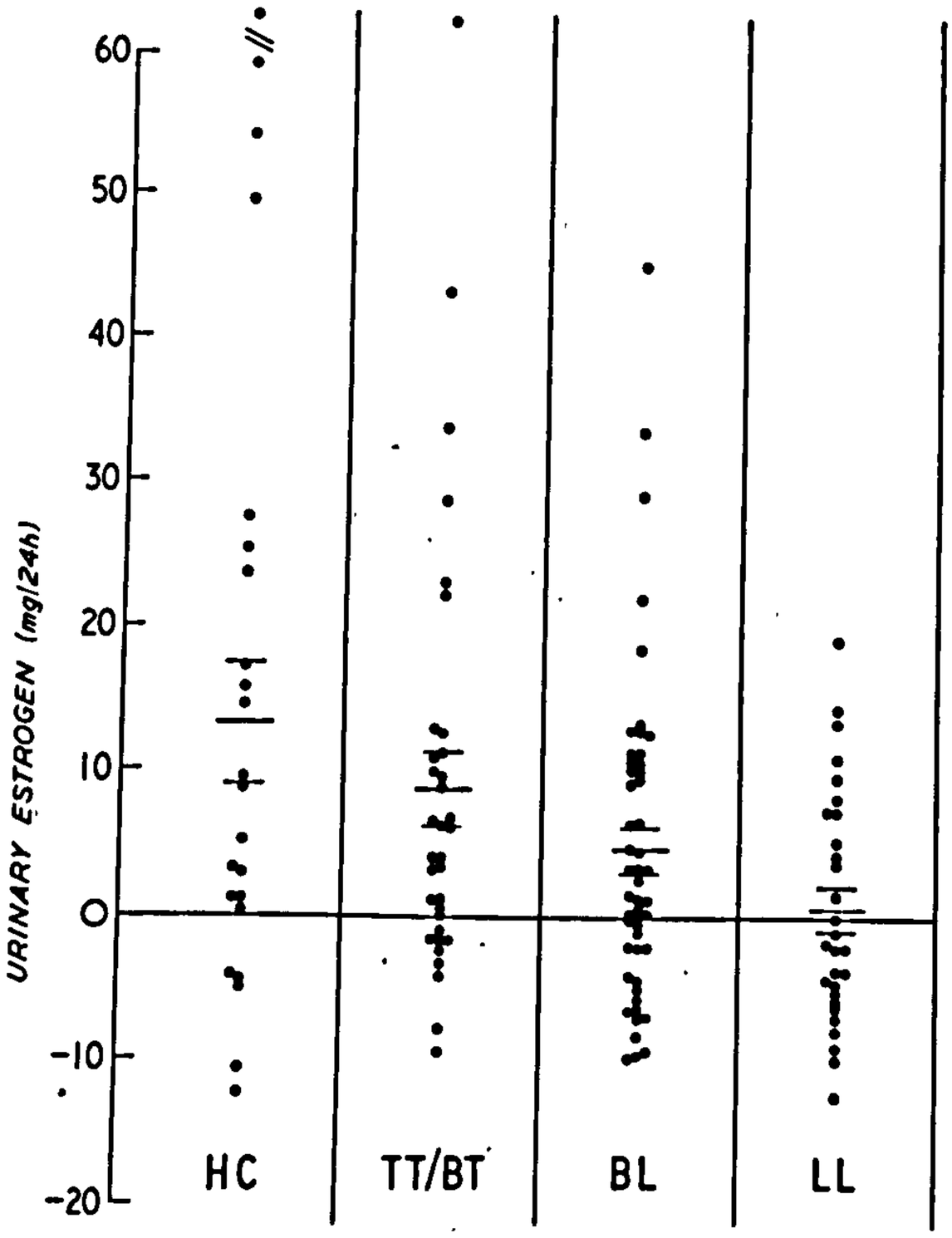


Figure 1. Estrogen excretion according to the clinical classification of the mother. Values are plotted (in mg/24 hr) relative to the lower limit of normal excretion in European women for the same period of gestation. (The means and standard error of the mean are indicated. HC = healthy controls; TT/BT = tuberculoid and borderline tuberculoid leprosy; BL = borderline lepromatous leprosy; LL = lepromatous leprosy.)

bus fares. The women with leprosy and the healthy subjects ate an average of 2 meals per day. The diet contained adequate amounts of second-class protein and iron; caloric intake was estimated at 1700 calories per day and meat was eaten only 4 times a year on feast days.

Selection of Patients

Patients were first seen and admitted to the study when they attended the hospital antenatal clinic that supplied care to patients with leprosy and to the wives of leprosy patients and hospital staff. Selection of the patients was based on their willingness to attend the special investigation clinics set up for this study, to be seen regularly during pregnancy, to deliver their babies in a delivery center or hospital rather than at home, and to attend regular follow-up sessions with the baby for up to 2 years post partum. All patients were told that there would be frequent investigations including blood tests. Apart from ensuring that there were comparable numbers within each group of women studied, no special selection of patients was made. Recruiting of patients was made over a 12-month period. Full details of the nutritional status and the obstetric care[7] as well as assessment of leprosy status[8] are described elsewhere.

Patients with leprosy were classified according to Ridley and Jopling[9] into 3 groups: tuberculoid and borderline tuberculoid leprosy (20 patients); borderline lepromatous leprosy (27 patients); and lepromatous leprosy (17 patients). Thirteen patients with tuberculoid and borderline tuberculoid leprosy and 2 with borderline lepromatous leprosy were assumed to be cured and had stopped treatment. The remaining patients with leprosy received dapsone monotherapy (50 to 100 mg daily); 4 (1 with borderline lepromatous leprosy and 2 with lepromatous leprosy) also received clofazimine (100 mg 3 times each week) or rifampin and thiambutosine for dapsone-resistant leprosy. No patients in these groups received corticosteriod therapy.

The 15 patients without leprosy were apparently healthy women from local villages who attended the Leprosy Hospital for their antenatal care.

All patients followed traditional laws of purification and their dates of last menstruation were well established in most cases. There were no cases of antepartum hemorrhage, abruptio placentae, or acute hypertension of pregnancy. The results therefore refer to women with singleton pregnancies essentially free from obstetric complications.

All patients were admitted to the hospital for each 24-hour urine collection. Urine was collected over sodium azide (5 g), the 24-hour volume recorded, and a portion stored at 4C. The samples were heated at 65C for 1 hour to destroy any pathogenic viruses and were then transported to Leeds, England, packed in solid CO_2. After thawing, urinary estrogen level was determined by a continuous flow method using a fluorimetric end point.[10] Measurements were made retrospectively and the results were not available for obstetric management.

The weights of infants and placentas were recorded for 58 of the women. The remaining 21 were unable to travel to the hospital for delivery because of the curfew or preferred to deliver at home.

Results

Estrogen Excretion

Estrogen excretions between 32 and 40 weeks' gestation in patients and control subjects were assessed

Table 1. Mean Estrogen Excretion Relative to Reference Value for Gestation and Incidence of Subnormal Values in Pregnant Ethiopian Women with Leprosy

Leprosy classification of the mother	No. of patients	No. of assays	Mean estrogen excretion (mg/24 hr relative to reference value) ± SEM	Proportion of subnormal values (as percentage of all assays)
Tuberculoid and borderline tuberculoid	20	34	8.8 ± 2.6	26
Borderline lepromatous	27	50	4.4 ± 1.6	36
Lepromatous	17	29	0.6 ± 1.5	59
Healthy control	15	23	13.2 ± 4.2	22

SEM = standard error of the mean.

with reference to the lower limit of normal in European women for the corresponding gestational period.[11] For statistical analysis of differences between the groups, the Student *t* test was used after logarithmic conversion, log [(100 + x)]. The values shown in Figure 1 represent urinary estrogen values (mg/24 hr) greater or less than the corresponding lower limit of normal excretion for European women at a comparable gestational age. It can be seen (Table 1) that the mean estrogen excretion was lowest in women with lepromatous leprosy and highest among the healthy control group. Similarly, the incidence of subnormal values was lowest among the control subjects and highest among the patients with lepromatous leprosy.

Birth Weights, Placental Weights, and Placental Coefficients

Birth weights and placental weights were known for 58 of the 79 pregnancies studied (9 of the 15 healthy control subjects and 49 of the 64 patients with leprosy). The remaining patients delivered at home either by choice or before transfer to the hospital could be arranged. Mean birth weights, placental weights, and placental coefficients for each of the 4 groups of subjects are shown in Table 2. There is a significant difference ($P < .001$, Mann Whitney U test) between mean birth weights of the healthy control and the lepromatous leprosy groups. The difference between mean placental weights of the 2 groups is even more striking ($P < .001$). Mean birth weights and placental weights for the tuberculoid and borderline lepromatous leprosy groups were intermediate between these extremes.

Table 2. Infant Birth Weight, Placental Weight, and Coefficient of Singleton Term Deliveries in Women with Leprosy

Leprosy classification of the mother	Birth weight (g)	Placental weight (g)	Placental coefficient
Tuberculoid and borderline tuberculoid	3100 ± 78.1 (18)	575 ± 52.0 (16)	0.18 ± 0.007 (16)
Borderline lepromatous	2982 ± 92.6 (22)	540 ± 36.4 (17)	0.18 ± 0.009 (17)
Lepromatous	2522 ± 103.0 (9)	339 ± 18.2 (7)	0.14 ± 0.005 (7)
Healthy control subjects	3252 ± 142.8 (9)	613 ± 50.1 (6)	0.19 ± 0.004 (6)

Values are mean ± standard error of the mean; number of observations are in parentheses.

Association Between Birth Weight and Frequency of Subnormal Estrogen Excretion

Birth weights of all infants were assessed relative to the data of Lubchenco et al,[12] which refers to subjects accustomed to comparable altitudes. Table 3 shows that the proportion of women who gave birth to infants weighing below the tenth centile was much higher among the lepromatous leprosy group than among the healthy controls. Similarly, the proportion

Table 3. Incidence of Low Birth Weight* and Subnormal Estrogen Excretion in Pregnant Ethiopian Women with Leprosy

Leprosy classification of the mother	No. of patients	No. with low estrogen in birth weights > 10%*	No. with low estrogen in birth weights < 10%*
Tuberculoid and borderline tuberculoid	18	4/17	1/1
Borderline lepromatous	22	5/18	3/4
Lepromatous	9	2/5	3/4
Healthy control subjects	9	1/9†	0/0‡
Total	58	12/49(24%)	7/9(78%)

* Below Lubchenco's tenth centile.

† Numerator is number of patients with low estrogen values of those (denominator) who delivered infants larger than the tenth centile.

‡ Numerator is number of patients with low estrogen values of those (denominator) who delivered infants smaller than the tenth centile.

of women with low estrogen values was greatest among the women with low birth weight infants.

Condition of the Infants at Birth

Despite the relatively poor social and economic conditions of the women considered in this study, there were only 2 cases of perinatal death. The condition of the infants at birth in each group of women is described below:

Healthy control women. There were no perinatal deaths nor operative deliveries for fetal hypoxia in this group. Apgar scores of 10 were recorded for all 8 infants on whom this measurement was made.

Tuberculoid and borderline tuberculoid group. Of the 20 infants delivered in this group, 1 required resuscitation after delivery by cesarean section for cephalopelvic disproportion and 1 died of pneumonia at 7 days. All other infants did well.

Borderline lepromatous leprosy group. Three of the 27 infants delivered in this group showed signs of hypoxia in labor. There was 1 neonatal death in an infant delivered at home.

Lepromatous leprosy group. All 17 infants were in good condition at delivery except 1 who died immediately after delivery at home and 1 who suffered from acute hypoxia associated with prolonged labor.

Discussion

This survey has reinforced the earlier finding[7] that among the population of patients examined, women with lepromatous leprosy have lower birth weight infants and placentas smaller for gestational age than does a comparable control group of healthy women. Women with borderline lepromatous leprosy or tuberculoid leprosy fall into intermediate categories.

The smaller infants and placentas among lepromatous leprosy patients are associated with a higher incidence of low estrogen excretion values relative to those of the healthy group. For example, in women with lepromatous leprosy 59% of values were lower than the reference values, whereas 36% of values were lower in women with borderline lepromatous leprosy, 26% in women with tuberculoid and borderline tuberculoid leprosy, and only 22% in healthy controls.

Treatment with penicillin-related antibiotics or with high doses of corticosteroids is known to diminish urinary estrogen excretion without risk to the fetus. Such causes of low estrogen excretion were not present in the patients whose estrogen excretion is shown in Figure 1 and Table 2. Dapsone was used to treat patients with all classifications of leprosy and hence is an unlikely cause of low estrogen values in women with lepromatous leprosy.

High altitude and poor nutrition have been associated, at least indirectly, with low estrogen excretion in pregnancy.[4,5] These factors affected both leprosy patients and the control group of healthy subjects.

Thus, drugs, social conditions, altitude, and perinatal morbidity and mortality can reasonably be excluded as causes of the higher incidence of low estrogen excretion in the pregnant women with leprosy. This high incidence is presumably associated with the lower mean birth weight and placental weight in the lepromatous leprosy group, relative to the controls, and provides another indication of disturbed fetoplacental function in leprosy.

Recent work has demonstrated that low birth weight is associated with low blood pressure in obstetrically normal women. Moreover, it now seems that many of the effects of undernutrition are mediated through low maternal blood pressure and reduced uteroplacental perfusion. The authors therefore reviewed the blood pressure recordings in the 4 groups of women but found no significant differences in either the peak systolic and diastolic blood pressures or the means of the systolic and diastolic blood pressures in each trimester. A hemodynamic cause of low birth weight in the present patients is therefore excluded.

A dependence of estrogen excretion on fetal weight as reflected by birth weight has been recorded by several authors. A relationship was shown between estriol excretion and subsequent delivery of infants weighing more than 3.6 kg or less than 2.7 kg,[13] and weak correlations between estrogen excretion and birth and placental weights were recognized.[14] Also estrogen excretion was noted to be proportional to birth weight, placental weight, and placental coefficient in prolonged pregnancies without other obstetric complications.[15] Furthermore, there is a marked similarity during pregnancy between the mean daily estrogen excretion and fetal and placental weights for normal populations.[16]

The cause of the low estrogen excretions in lepromatous women remains obscure. A diminished supply of androgen sulfate precursors must be considered. There is an association between small fetal adrenal glands and subnormal estrogen excretion in anencephaly.[17] Autopsy reports on infants born to women with lepromatous leprosy were not available, but 1 infant born to a woman with borderline lepromatous leprosy was found to have small adrenals. It is possible that some degree of hypoplasia of the fetal zone of the fetal adrenal may exist in the pregnancies complicated by lepromatous leprosy.

Enzymes located in the placenta play essential roles in converting the androgen sulfates to estrogen. Therefore, some degree of placental inefficiency in this

regard may occur in lepromatous leprosy. In the lepromatous patient the placenta appears disproportionately small, even in those pregnancies with infants with low birth weights. Usually, in retarded fetal growth the placental coefficient is equal to that in pregnancies with well-grown infants,[18] and so there appears to be an extra degree of inhibition of placental growth in pregnancies complicated by lepromatous leprosy. Such an abnormality may arise from the depressed cell-mediated immunity recognized in lepromatous leprosy.[19,20]

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Neuritis in Pregnancy and Lactation[1]

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Mycobacterium leprae, the causative organism of leprosy, has the unusual property of entering peripheral nerves and multiplying within their Schwann cells. The intraneural inflammation which is elicited by this process accounts for the development of nerve damage in leprosy. In tuberculoid leprosy an inflammatory response can be elicited by low concentrations of mycobacterial antigen; but in lepromatous leprosy considerable numbers of bacilli can be seen within otherwise rather normal looking nerves. However, because of the slow multiplication rate of *M. leprae*, nerve damage progresses rather slowly in all types of untreated leprosy if the immune response is stable, and some recovery of nerve function can be expected under normal chemotherapy.

When the course of leprosy (whether untreated or under chemotherapy) is characterized by an unstable host parasite relationship, sudden episodes of increased inflammation, known as reactions, occur. These reactions can be caused by disturbances of cell-mediated immunity (reversal reaction [RR], Type 1 reaction) or of humoral immunity (erythema nodosum leprosum [ENL], Type 2 reaction); the neuritis associated with reactions is caused by intraneural reaction. Nerves can be damaged both acutely and extensively during reactions, and steroid treatment is often required to prevent irreversible damage.

Thus leprosy is a disease in which most of the clinical manifestations are brought about by the immunological response of the host. The "immunological instability" of pregnancy might therefore be expected to be associated with reactions and neuritis in leprosy patients. Such association has been reported by a number of authors [3, 6, 7, 8, 9, 10, 13, 14, 15, 16]. However, all the information available at present has come from retrospective studies and reviews of case histories. No prospective studies have apparently been undertaken, and therefore the risk of neuritis which leprosy patients undergo by becoming pregnant has not been appreciated. This paper describes the association of neuritis with pregnancy as observed in a prospective study of Ethiopian leprosy patients.

PATIENTS AND METHODS

The patients were all Ethiopian women from the low socioeconomic class, of whom 96% lived in the villages surrounding the leprosy hospital. They were being treated for leprosy at the hospital outpatient clinics and were first seen for this study when they presented at the hospital antenatal clinic. Selection of patients was based on their willingness to participate in the study, to deliver their babies in the hospital rather than at home, and to be seen with their babies for regular assessment for a period of up to 2 years during lactation. Intake of patients was staggered over 12 months.

Classification and treatment of mothers. One hundred and forty-six women were studied during and after 153 pregnancies. There were 115 women with leprosy (119 pregnancies) and 31 healthy women with 34 pregnancies. The 115 women with leprosy were classified as follows using the scale of Ridley and Jopling [12]:

Tuberculoid and borderline-tuberculoid leprosy (TT/BT) ... 39 (40 pregnancies)
Borderline lepromatous leprosy (BL) ... 44 (45 pregnancies)
Lepromatous leprosy (LL) ... 32 (34 pregnancies)

Patients studied twice, because of having

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a second pregnancy during the study period, are considered as two patients.

Eighty-seven patients were receiving treatment with dapsone monotherapy (50–100 mg daily). Twenty-six patients, 1 BL, the rest BT or TT, were believed to have been cured, had stopped treatment, and had been "released from control" (RFC). Three of these BT/RFC patients who relapsed with active BL leprosy during the third trimester of pregnancy ([4]) were reclassified as BL and are included in the BL group. Six patients (2 BL, 4 LL) had developed dapsone resistant leprosy and were receiving clofazimine (4 patients, all LL, 5 pregnancies) or rifampin plus thiambutosine (2 patients, both BL).

Assessment of patients. Assessment of the patients' leprosy was made during pregnancy and after delivery at 6-month intervals whenever possible. Details of the patients' complaints, state of health, and drug treatment were recorded. Examination included inspection and palpation of the skin, clinical drawings, palpation of nerves and regional lymph nodes, slit skin smears, and biopsies.

Voluntary muscle power tests (VMT) were performed by standard methods and the muscle power graded on a 0–5 scale ([5]). Sensory skin tests (SST) were performed on the palms of the hands (using a stiff nylon bristle) and the soles of the feet (using a ballpoint pen tip); sufficient pressure was applied to indent the skin slightly. Standard sites (at least five for the area of distribution of each nerve) were stimulated and the result of each recorded as "felt" or "not felt."

Motor nerve conduction velocity (NCV) was measured in a few patients to determine whether nerve damage was long standing or of recent onset.

The patients' hospital case records were examined and data abstracted regarding leprosy status (clinical relapse, slit skin smear results and biopsy reports), frequency and type of reaction and assessment of nerve damage (VMT, SST and NCV) prior to admission to the study. Particular attention was paid to the three month period immediately preceding pregnancy. This was expected to provide a baseline figure for complications in Ethiopian women.

Definitions of neuritis. Neuritis in leprosy is usually defined as "pain and/or tenderness of nerves" ([11]). In this paper we define "overt neuritis" as pain and/or tenderness of nerve. In addition to pain and/or tenderness of nerves, all our patients with overt neuritis, except 9, also showed evidence of simultaneous impairment of nerve function. We define "silent neuritis" as impairment of sensory and/or motor function without nerve pain or tenderness.

Motor function was recorded as impaired if there was loss of 2 or more points (on the 0–5 scale) in 2 separate muscles within the same nerve distribution. (In three episodes of neuritis nerve damage was recorded when the motor deficit, though only 1 point, included almost all the tested muscles.) In the presence of obvious clinical changes (such as facial paralysis or "tic" of the facial muscles; wasting of the intrinsic muscles of the hand; "curving" of the fourth and fifth fingers; claw hand or foot drop) formal VMTs were sometimes omitted. These are reported as "clinical assessments."

Impairment of sensory function was recorded if loss of sensation had occurred in at least two test sites within the distribution of a single nerve. In a few patients with extensive anesthesia with anhidrosis, formal SSTs were omitted; they are reported as "clinical assessments."

RESULTS

There was a total of 85 episodes of neuritis during pregnancy and lactation. In 11 episodes no nerve damage ensued. In the remaining 74 episodes, 29 showed pure motor loss, 12 pure sensory loss and 33 mixed loss. By contrast, only two women (1 BT, 1 BL) had neuritis during the 3 month period immediately preceding pregnancy. However, both episodes of neuritis followed a previous pregnancy.

The general severity of the nerve damage, taking all types of leprosy together, is shown in Tables 1 and 2. The majority of patients with sensory loss were severely affected, whereas the motor deficits were in most cases mild. The apparently greater vulnerability of sensory nerves may indicate that damage occurred at both dermal nerve and nerve trunk levels. The danger that insidious silent neuritis will cause severe sensory loss is well shown. Neuritis

TABLE 1. *Severity of motor damage incurred during each episode of neuritis.*

Type of neuritis		Degree of nerve damage[a]	
		Mild	Severe
Overt	1 nerve only	7	1
	many nerves	11	6
Silent	1 nerve only	4	1
	many nerves	17	15
Total		39	23

[a] Mild damage = loss of 1 or 2 VMT grades in 1 or more nerves, or NCV evidence only. Severe damage = loss of 3 or more VMT grades in 1 or more nerves, or clinical assessment only.

TABLE 2. *Severity of sensory damage incurred during each episode of neuritis.*

Type of neuritis		Degree of nerve damage[a]	
		Mild	Severe
Overt	1 nerve only	1	2
	many nerves	3	6
Silent	1 nerve only	4	2
	many nerves	6	21
Total		14	31

[a] Mild damage = loss of 1 or 2 sensory test areas in 1 or more nerves. Severe damage = loss of 3 or more sensory test areas in 1 or more nerves, or clinical assessment only.

affecting multiple nerves was usually more damaging than when only one nerve was affected.

Some indication of the severity of the neuritis may also be derived from the treatment that was employed. A total of 52 patients developed neuritis. However, in 14 cases the diagnosis (silent neuritis) was made only at the time of final assessment. Of the remaining 38 patients, 26 required treatment with corticosteroids during the study period.

The number of patients studied, and the proportion developing neuritis during the study period, are shown in Table 3. There was considerable risk of neuritis in all types of leprosy. The timing of the episodes of neuritis is shown in Figure 1. BL cases were almost free of neuritis during pregnancy but were greatly at risk in the first 6–9 months after delivery. In BT and TT cases, neuritis was less common and showed little relationship to the events of pregnancy.

Overt neuritis was usually associated with skin reaction, deterioration of the leprosy condition (due to relapse or dapsone resistance), or both. Early clinical deterioration not infrequently presented as apparent ENL (Type 2) reaction (Fig. 2c), and clinically puzzling mixtures of Type 1 and Type 2 reactions were occasionally encountered (Fig. 2b).

In general, overt neuritis was chiefly encountered just before delivery and during the 9–12 months postpartum. Silent neuritis, on the other hand, occurred at all stages (Fig. 2a), but became the predominant problem from about 6–9 months postpartum.

Healthy contacts (HC). One healthy contact got overt neuritis. She complained of "rheumatism" and was found to have enlarged nerves at 10 weeks postpartum. At 6 months postpartum she had tender nerves with loss of motor and sensory function. Biopsy showed active BL leprosy. She is included in the BL group.

Tuberculoid leprosy (TT and BT). Of 25 patients initially classified as TT and BT/RFC (including the three who relapsed as BL during the study period), eight patients had 12 episodes of neuritis in association with relapse.

TABLE 3. *Occurrence of neuritis among leprosy patients during pregnancy and lactation.*

Classification of leprosy	No. of patients studied	No. with neuritis	No. of episodes of neuritis	No. of episodes per patient
TT and BT	40	16 (40%)	24	0.6
TT and BT/RFC	22	6 (27%)	9	0.4
TT and BT/Active	18	10 (56%)	15	0.8
BL[a]	45	21 (47%)	35	0.8
LL	34	15 (44%)	26	0.8
Total	119	52 (44%)	85	0.7

[a] This group includes 3 patients (7 episodes of neuritis) originally classified as BT/RFC who relapsed with active BL leprosy during the study period.

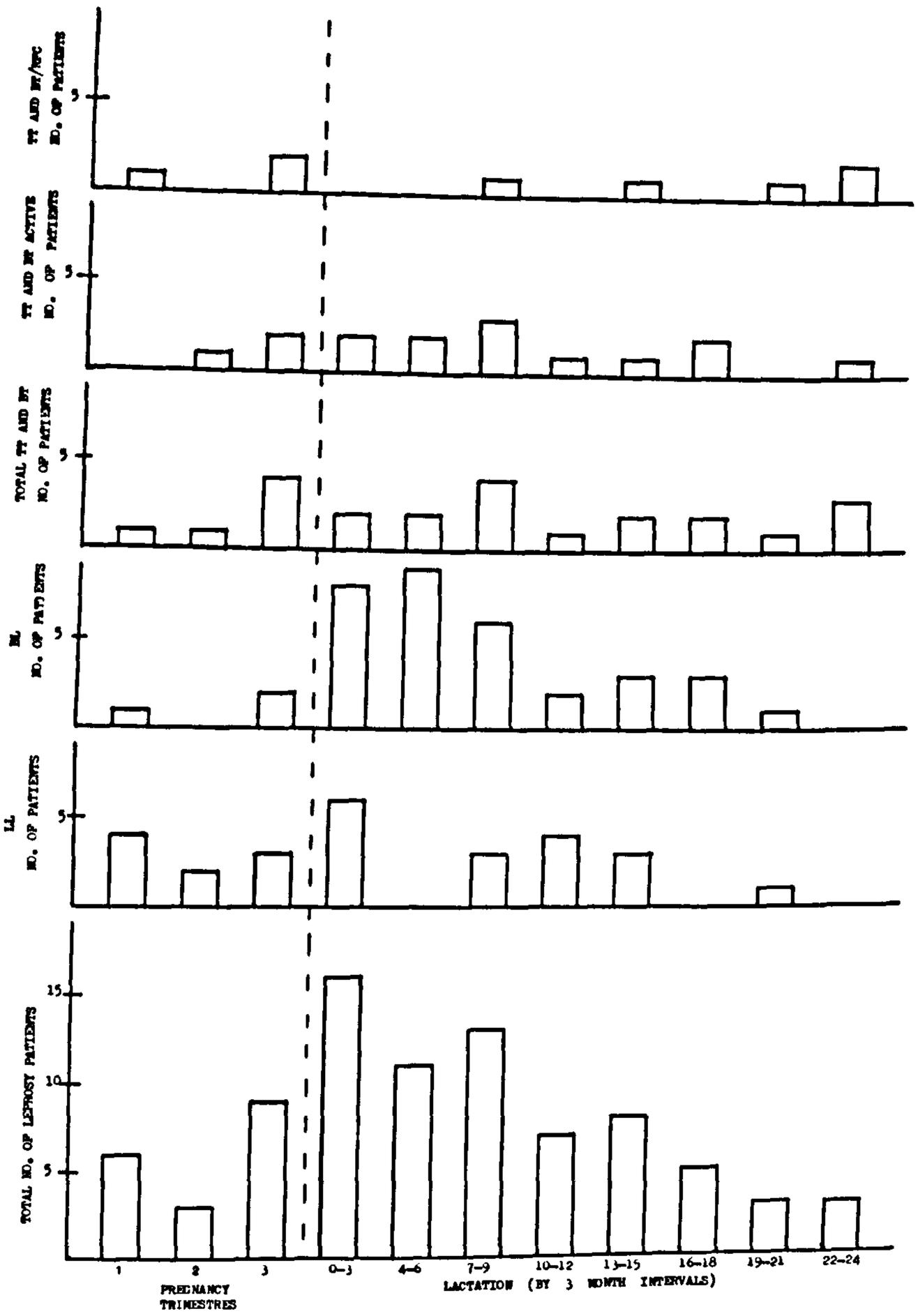


FIG. 1. Timing of episodes of neuritis during pregnancy and lactation according to the clinical classification of the patients at the outset of the study.

Sixteen out of 40 patients had 24 episodes of neuritis in association with pregnancy or lactation. Five patients had neuritis (six episodes) during pregnancy. These five patients, all of whom were either newly diagnosed or relapsed cases, had silent neuritis which was diagnosed early, by clinical or physiological testing, after Type 1 reaction was observed in the skin. Six of eight episodes of neuritis observed during pregnancy or the puerperium were in association with Type 1 reaction of the skin lesions. One patient only, a newly diagnosed case, developed "classical" overt neuritis at 6 weeks postpartum. The remaining cases of neuritis occurring during lactation were all silent, the majority being preceded by the complaint of "rheumatism" (generalized aches and pains) and new, non-tender, nerve enlargement.

Lepromatous leprosy (LL). Fifteen out of 33 patients had 26 episodes of neuritis. In

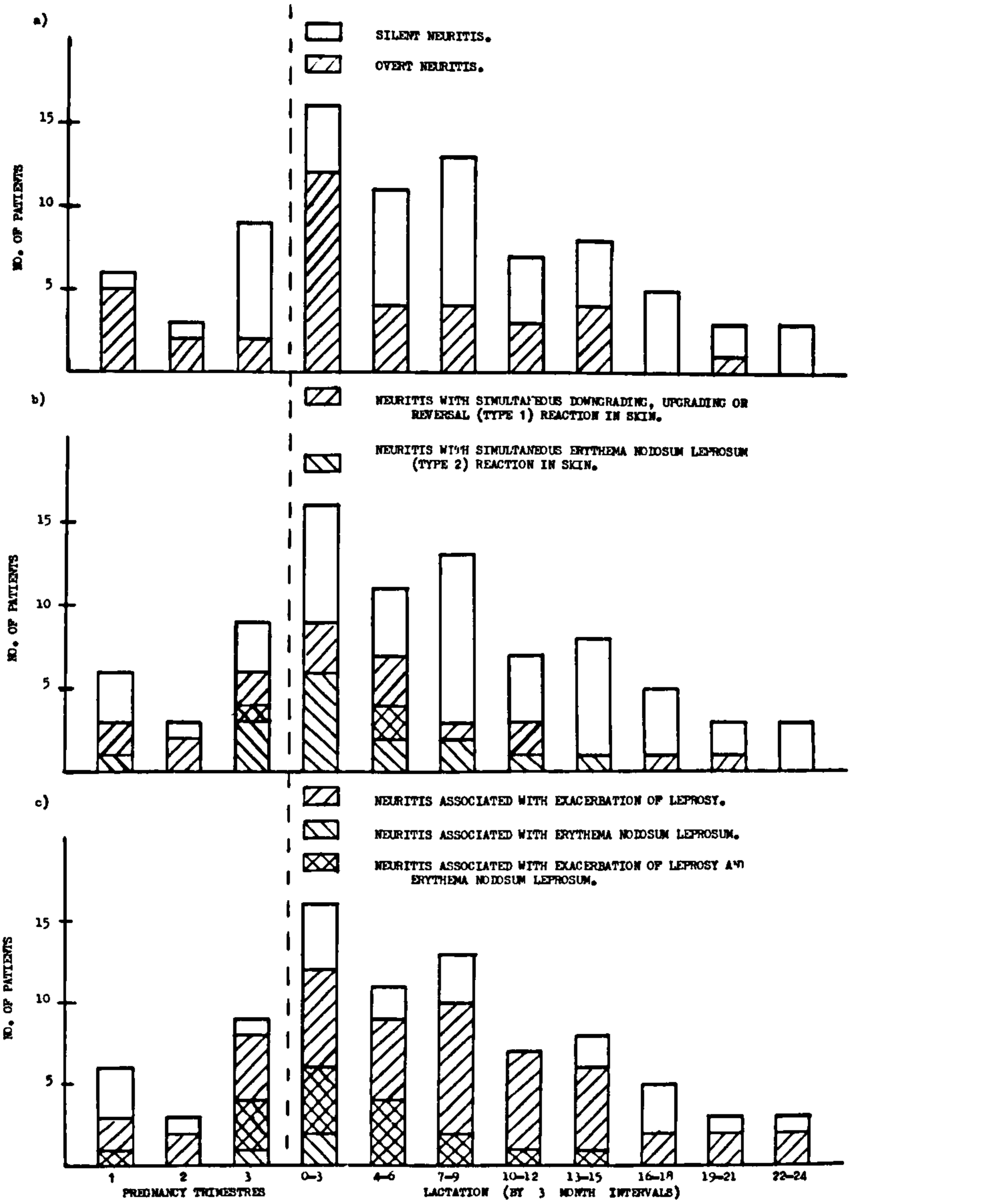


FIG. 2. Clinical aspects of neuritis in association with pregnancy and lactation. a) Timing of silent and overt neuritis; b) Timing of neuritis in association with simultaneous Type 1 and Type 2 lepra reactions; c) Association of neuritis with exacerbation of leprosy and Type 2 reaction.

11 cases there were also mixed ENL and clinical deterioration; two patients showed deterioration without reaction and two had ENL only. In most cases, ENL preceded the appearance of neuritis.

Fifteen episodes of neuritis were observed during pregnancy and the first 3 months of lactation; 12 were overt, 3 silent. Eleven episodes of neuritis occurred later in lactation, six of which were silent and usually preceded by the complaint of "rheumatism" and the finding of newly enlarged non-tender nerves. Nerve conduction velocity was measured in two cases (at 12 months postpartum) and indicated active nerve damage rather than slow residual fibrosis.

Borderline lepromatous leprosy (BL). Twenty-one out of 45 patients had 35 episodes of neuritis; only three episodes oc-

curred during pregnancy. The neuritis was almost always associated with deterioration of the leprosy (15 cases) or reaction (ENL, 8 cases; reversal reaction, 7 cases), and many patients showed both reaction and deterioration.

Overt neuritis (13 patients) was associated with reaction (9 cases) and/or clinical deterioration (8 cases); 14 of 19 episodes occurred during the first 12 months after delivery. Silent neuritis, on the other hand, was associated with reaction in only four cases and with clinical deterioration in 12 cases. It sometimes continued until 2 years after delivery and was in 11 cases still present at the patient's final assessment. As in LL cases, silent neuritis was usually preceded by the complaint of "rheumatism" and the finding of nerve enlargement. Nerve conduction velocity studies performed on three patients 12–15 months after delivery showed evidence of active demyelination.

Treatment of neuritis. Twenty-six patients were treated with corticosteroids. Not all had completed their courses of treatment during the study period, but at the time of their final assessments 9 of 26 (35%) showed improvement (using the reverse of the criteria for deterioration) both clinically and on VMT and SST. Twelve patients with neuritis did not receive corticosteroids; only two (17%) improved.

DISCUSSION

These findings are summarized simply. Nearly half of the Ethiopian women with leprosy who were studied suffered from deterioration of nerve function during a single pregnancy and/or during lactation. All patients, including those with tuberculoid leprosy treated for some years and apparently "cured" (RFC), were at risk. Insidious silent neuritis, leading to sensory and motor nerve damage during lactation, was a particularly dangerous and hitherto undescribed risk of pregnancy.

Overt neuritis is usually associated with reaction in leprosy and is a manifestation of the reactional process as it occurs within nerves. Thus in borderline and tuberculoid leprosy there is initial edema of the intraneural granuloma, and subsequently increased granuloma formation as the immune response to *M. leprae* progresses, so that Schwann cells are progressively replaced by the epithelioid granuloma. The patients most at risk were those classified as BL, although newly diagnosed or relapsing BT or TT cases could also be affected. The neuritis usually started after delivery, thus coinciding with recovery of cell-mediated immunity (CMI) after the immunosuppression of normal pregnancy. We have shown elsewhere that there is some increased bacillary multiplication during pregnancy, in some cases as a transient phenomenon ([4]) due to lowered host CMI and possibly also reluctance of women to take any medication, including antileprosy drugs, during pregnancy. Peripheral nerve is a partially immunologically privileged site ([17]), and the immunosuppression of normal pregnancy might therefore be particularly influential.

The description given by Rose and McDougall ([13]) of adverse reactions following pregnancy in untreated patients with "dimorphous" leprosy gives us the picture of evolution of leprosy during late pregnancy or early lactation as a result of suppressed CMI and occurrence of skin and nerve reactions during lactation due to the recovery of CMI after delivery.

In lepromatous leprosy overt neuritis probably represents intraneural ENL. This reaction requires the presence of *M. leprae* within the nerve and appears to be more common and severe when there is a high concentration of intraneural antigen. Thus bacillary multiplication unchecked by chemotherapy (as when a patient is developing dapsone resistant leprosy) or CMI (suppressed during pregnancy) is a likely precipitating factor that applies to many cases in this series. Neuritis in these patients was likely to occur at any stage of pregnancy or lactation.

While any form of neuritis is of concern to both the patient and the leprosy worker, overt neuritis is more readily diagnosed and therefore treated, and hence is the lesser evil for the lactating mother. Silent neuritis with its slow, insidious progress means that permanent nerve damage can occur before the patient, or leprosy worker, is aware that anything is wrong. The diagnosis of silent neuritis was made possible in this study by regular sensory and motor function tests; however, among our patients persistent

"rheumatic pains" were frequently associated with silent neuritis and indicated that something was wrong. This symptom and its significance will be described elsewhere.

The etiology of silent neuritis remains uncertain. One obvious possibility is that it is due to late fibrosis in nerves that have previously been damaged by intraneural granulomata. In our study, however, "new active demyelination" was reported in several patients with silent neuritis during lactation. Segmental demyelination at sites where bacilli were not present has been demonstrated in all types of leprosy (1). However, the apparent localization of nerve damage to sites where they are particularly vulnerable to physical changes in their environment makes autoimmune demyelination unlikely as a cause of silent neuritis.

Silent neuritis appears to cause more damage to sensory nerves than to motor nerves. This suggests that the process may cause damage at both dermal nerve and nerve trunk levels. The reversibility with corticosteroid treatment implies an immunological cause. This possibility is supported by the work of Bullock on trapping of sensitized lymphocytes (2), but since little is known of immunological or endocrine function in women who prolong lactation for 2 or more years, it is difficult to discuss possible immune mechanisms. Further studies, including nerve biopsies, will be required to elucidate the mechanisms of neuritis in lactating women.

The implications of this study are clear. Women with leprosy (even apparently cured) run a serious risk of deterioration of nerve function when they become pregnant. They may develop overt neuritis, or an insidious silent neuritis; in either case, regular tests of nerve function are required to demonstrate nerve damage and follow the response to treatment. With training this sort of management could be provided in the context of a "vertical" leprosy control program; it will be more difficult to ensure proper care in integrated primary health care programs. Health education to make women with leprosy aware that pregnancy can seriously damage their health must be undertaken, and may well be the correct long-term response to the problem of neuritis in pregnancy and lactation.

SUMMARY

One hundred and forty-six women were studied during and after 153 pregnancies (31 healthy contacts: 34 pregnancies; 115 leprosy patients: 119 pregnancies). One healthy contact and 51 leprosy patients developed neuritis during the study period. All leprosy patients, including those who were considered to be cured and had stopped treatment, were at risk. Neuritis was accompanied by Type 1 and Type 2 lepra skin reactions and/or deterioration of the patients' leprosy status; this was particularly the case when neuritis was associated with nerve pain or tenderness (overt neuritis). Neuritis without nerve pain or tenderness (silent neuritis), preceded by the complaint of "rheumatism" and the clinical finding of enlarged peripheral nerves, was seen more frequently than overt neuritis (48:37 episodes). Insidious silent neuritis with loss of sensory and motor function during lactation was a particularly dangerous and hitherto undescribed risk of pregnancy.

RESUMEN

Se estudiaron 146 mujeres durante y después de 153 embarazos (31 contactos sanos: 34 embarazos; 115 pacientes con lepra: 119 embarazos). Uno de los contactos sanos y 51 pacientes con lepra desarrollaron neuritis durante el periodo de estudio. Todas las pacientes con lepra, incluyendo a aquellas que se habían considerado curadas y que por ésto habían suspendido su tratamiento, estuvieron bajo el riesgo de desarrollar neuritis. La neuritis estuvo acompañada de reacciones dérmicas del tipo 1 e del tipo 2, con o sin deterioro del estado leproso de las pacientes; este fue particularmente el caso cuando la neuritis estuvo asociada con dolor de nervios o con gran sensibilidad de los mismos (neuritis abierta). La neuritis sin los síntomas anteriores (neuritis silenciosa), precedida por molestias de "reumatismo" y agrandamiento de nervios periféricos, fue observada con más frecuencia que la neuritis abierta (48:37 episodios). La neuritis silenciosa e insidiosa con pérdida de las funciones motora y sensorial durante la lactancia fue uno de los riesgos particularmente peligrosos (hasta ahora no descritos) del embarazo.

RÉSUMÉ

On a étudié cent quarante-six femmes, pendant et après 153 grossesses (31 contacts non malades, correspondant à 34 grossesses, 115 malades de la lèpre, représentant au total 119 grossesses). Au cours de la période d'étude, un contact sain et 51 malades de la lèpre ont développé une névrite. Tous les malades de la lèpre, y compris ceux qui étaient considérés comme guéris et avaient interrompu le traitement, étaient ex-

posés au risque. La névrite était accompagnée par des réactions lépreuses cutanées de type 1 et de type 2, ainsi que par une détérioration de l'état des malades en ce qui concerne leur maladie lépreuse; ces deux manifestations pouvaient coexister. Cette association était particulièrement notée dans les cas où la névrite était associée avec des douleurs nerveuses ou une sensibilité des nerfs (névrite ouverte). La névrite sans douleurs nerveuses ou sensibilité des nerfs (névrite silencieuse), précédée par des plaintes de "rhumatismes," et par l'observation clinique d'un épaississement des nerfs périphériques, a été constatée plus fréquemment que la névrite ouverte (48 épisodes sur 37). Une névrite silencieuse insidieuse, avec perte des fonctions motrices sensorielles, au cours de la lactation, s'est révélée particulièrement dangereuse; il s'agit là d'un risque de la grossesse qui n'avait pas été décrit jusqu'à présent.

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IgA, IgM and IgG anti-*M. leprae* antibodies in babies of leprosy mothers during the first 2 years of life

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SUMMARY

IgA, IgM and IgG anti-*M. leprae* antibody activity was estimated by solid phase radioimmunoassay in repeated serum samples from cord sera to sera taken 2 years after birth from 29 babies of mothers with lepromatous leprosy (Group 1) and 16 babies of mothers with tuberculoid leprosy and non-leprosy control mothers (Group 2). IgA anti-*M. leprae* antibody activity could be detected in 30% and IgM anti-*M. leprae* antibody activity in 50% of cord sera from Group 1, but not in any of the cord sera from Group 2. After birth, there was a significantly higher increase of IgA and IgM anti-*M. leprae* antibody activity in sera taken 3–6 months after birth from babies of Group 1 compared to Group 2, but the IgA and IgM activity in sera taken after 6 months of age showed the same increase in the two groups. IgG anti-*M. leprae* antibody activity showed a marked decrease in sera from both Groups 1 and 2 taken 3–6 and 6–9 months after birth compared to the activity in the cord sera. No increase of the IgG activity could be demonstrated even in sera taken 15–24 months after birth in any of the two groups. These findings are discussed in relation to possible transfer of *M. leprae* bacilli across the placenta, the influence of *M. leprae* and other mycobacteria exposure on the antibody activity, the poor IgG anti-*M. leprae* antibody response and subclinical leprosy infection in babies exposed to leprosy below 2 years of age.

INTRODUCTION

A solid phase radioimmunoassay (sRIA) was developed for demonstration and quantification of IgA and IgM antibodies against *M. leprae* antigens in cord sera from babies of leprosy mothers and sera from leprosy patients (Melsom *et al.*, 1981). IgA and IgM anti-*M. Leprae* antibodies could be demonstrated in 1/3 of the cord sera from babies of mothers with active lepromatous leprosy, whereas no corresponding antibodies could be demonstrated in sera from babies of mothers with tuberculoid leprosy or no clinical signs of this disease. These findings indicate that the immune system of these babies was stimulated *in utero* by transfer of *M. leprae* antigens or live bacilli from mothers with active multibacillary leprosy. Significantly higher concentrations of IgA and IgM anti-*M. leprae* antibody were demonstrated in sera from lepromatous leprosy patients compared to tuberculoid leprosy patients and non-leprosy controls. These findings indicate that immunoglobulin class specific assays for anti-*M. leprae* antibodies might lead to the development of a sero-diagnostic test for lepromatous leprosy (Melsom *et al.*, 1981).

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Serum samples were taken at repeated intervals up to the age of 2 years from the babies of this study (Melsom *et al.*, 1981). The present paper describes the extension of demonstration of IgG anti-*M. leprae* antibodies by sRIA and the quantification of IgA, IgM and IgG anti-*M. leprae* antibodies in sera from babies of leprosy and non-leprosy mothers from birth until 2 years of age. It therefore compares the formation of antibodies of the IgA, IgM and IgG classes against *M. leprae* in babies who may have been exposed to *M. leprae* both *in utero* and after birth with antibody formation in babies exposed to *M. leprae* only after birth. Some of the babies were BCG vaccinated during the period of study. The influence of exposure to other saprophytic mycobacteria, BCG and *M. leprae* on the antibody response of the babies will be discussed.

MATERIALS AND METHODS

A lepromatous serum pool (LSP) was prepared by pooling sera from 40 patients with lepromatous (LL or BL) leprosy. They were either newly diagnosed or had been on anti-leprosy treatment for less than 6 months. This pool was identical to the LSP pool used in the previous study (Melsom *et al.*, 1981) demonstrating IgA and IgM anti-*M. leprae* antibodies in cord sera of babies of lepromatous leprosy mothers, and it was used as a 'standard' throughout the present investigation.

Patient sera. Repeated serum samples were taken from 45 babies from birth to 2 years of age. The babies were divided into two groups according to the leprosy status of their mothers. All came from the same socio-economic class.

Group 1 consisted of 29 babies of mothers with lepromatous (LL or BL) leprosy. Twenty-five of these babies were BCG vaccinated at different times after birth. The remaining four babies were not BCG vaccinated. Twenty-five of the mothers had active lepromatous leprosy with *M. leprae* present in one or several skin smears, while four of the mothers had 'inactive' or quiescent lepromatous leprosy with no acid fast bacilli in skin smears taken during or after pregnancy.

Group 2 consisted of 16 babies. Eleven of the babies were born of mothers with paucibacillary borderline tuberculoid (BT) leprosy. Seven of these were BCG vaccinated at different times after birth, while four were not BCG vaccinated. The remaining five babies were born of mothers with no clinical signs of leprosy; three of them were BCG vaccinated.

BCG vaccination. This was performed by intradermal inoculation of 0·1 ml of Glaxo BCG vaccine.

Purified freeze-dried *M. leprae* were obtained from Dr R.J.W. Rees, London through the WHO Immunology of Leprosy (IMMLEP) program. The bacilli were isolated from liver tissue of *M. leprae* inoculated armadillos as previously described (Draper, 1976).

Solid phase radioimmunoassay (sRIA). This was carried out as previously described (Melsom *et al.*, 1981). In short, Nunc polystyrol test tubes were coated with sonicated *M. leprae* bacilli. Dilutions of LSP or patient sera were added into the tubes which were incubated and washed before addition of labelled rabbit anti-human IgA, IgM or IgG antibodies. After suitable incubation, the test tubes were again washed, and finally counted in a gammacounter. The labelled rabbit anti-human IgG antibodies had to be purified as well as the rabbit anti-human IgA and IgM antibodies. The last two preparations were made as previously described (Melsom *et al.*, 1981).

Preparation of labelled anti-IgG. The rabbit anti-human IgG immunoglobulin (Dako Immunoglobulins, Copenhagen, Denmark) was labelled with ^{125}I as previously described (Harboe & Følling, 1974), and the labelled anti-IgG antibodies were further purified by immunoabsorption. Human IgG (Kabi, Stockholm, Sweden) containing more than 95% IgG was coupled to cyanogen bromide activated Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) as described by March, Parikh & Cuatrecasa (1974). Ninety percent of the added protein was bound to the gel. The gel was washed, the labelled anti-IgG immunoglobulin was added, and after subsequent washing of the gel, the anti-IgG antibodies were eluted with 1 M glycine-HCl buffer pH 2·8 as previously described (Melsom *et al.*, 1981).

The effect of the purification procedure was tested by sRIA using test tubes coated with human IgG, IgM or IgA in the same way as for labelled anti-IgA and anti-IgM antibody preparations (Melsom *et al.*, 1981). The amount of radioactivity bound to the wall of IgG coated test tubes

increased from 12% of the labelled anti-IgG containing immunoglobulin to 80% of the isolated labelled anti-IgG antibody preparation. Less than 2% of the labelled purified anti-IgG antibodies were bound to uncoated test tubes or test tubes coated with human IgA or IgM proteins.

Statistical methods. Wilcoxon's ranking test was used for the calculation of the statistical significance of differences between groups (Diem, 1962).

Calculation of the results. The patient sera were tested in three dilutions, 1×10^{-1}, 1×10^{-2} and 1×10^{-3} for the three assays. The LSP was run in parallel in dilutions 1×10^{-1}, 1×10^{-2}, 1×10^{-3} and 1×10^{-4}, and a standard curve was made on a semi-logarithmic paper with the dilutions of LSP on the ordinate and the number of counts on the abscissa recording the results obtained with LSP in each set of experiments. For example, IgG anti-*M. leprae* antibody activity was calculated by the following method: antibody activity in patient serum corresponding to 1% of LSP means that the same number of counts was obtained with 1×10^{-1} dilution of the patient serum as with LSP diluted 1×10^{-3}, 10% of LSP means the same number of counts with 1×10^{-2} dilution of patient serum as with 1×10^{-3} dilution of LSP, and finally 100% of LSP means that the same number of counts was obtained with the patient serum diluted 1×10^{-3} as with LSP diluted 1×10^{-3}. The steepest part of the standard curve (the largest difference in number of counts between two neighboring dilutions of LSP) was usually between 1×10^{-2} and 1×10^{-3} dilution of LSP. The dilution of patient serum lying on this part of the standard curve was used for calculation of the percentage of IgG anti-*M. leprae* antibody activity. The two other dilutions were used as controls. The IgA and IgM anti-*M. leprae* antibody activity was calculated by a similar method.

RESULTS

Fig. 1 shows the median increase in IgA and IgM anti-*M. leprae* antibody activity and the median decrease of IgG anti-*M. leprae* activity in serum samples obtained at birth compared with samples obtained repeatedly during the first 2 years of life from babies of mothers with lepromatous leprosy.

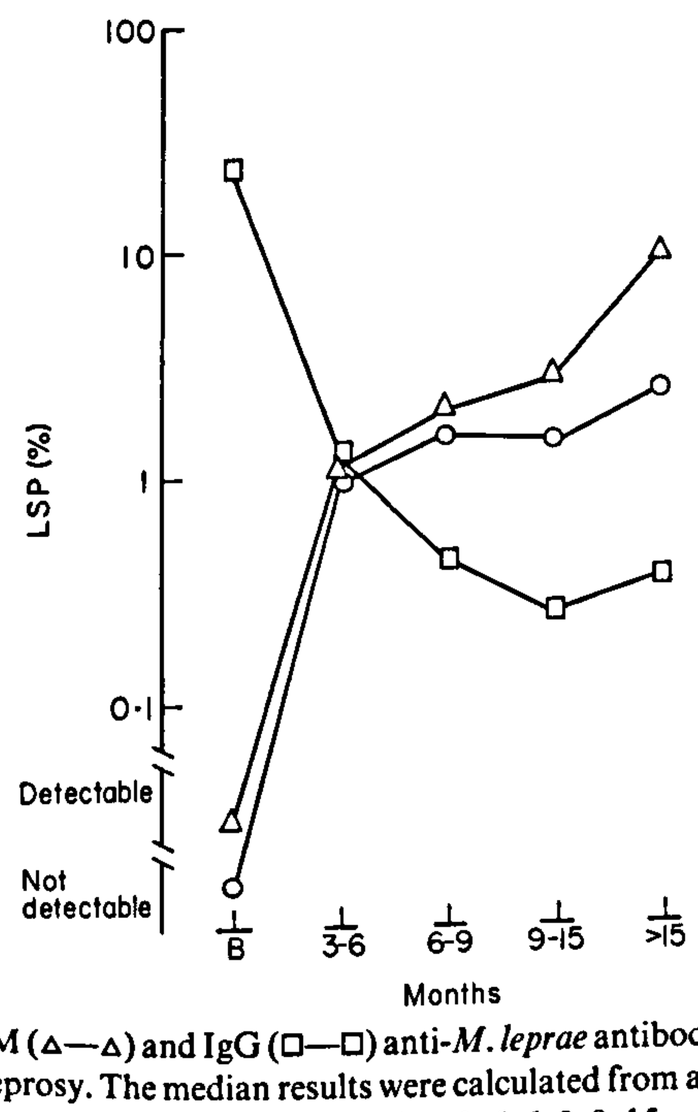


Fig. 1. Median IgA (○—○), IgM (△—△) and IgG (□—□) anti-*M. leprae* antibody activity in sera from 29 babies of mothers with lepromatous leprosy. The median results were calculated from all the individual results after the sera had been allocated to five groups, cord sera and sera taken 3–6, 6–9, 9–15 and 15–24 months after birth. The results are expressed as percentage of IgA, IgM or IgG anti-*M. leprae* antibody activity in a lepromatous serum pool (LSP).

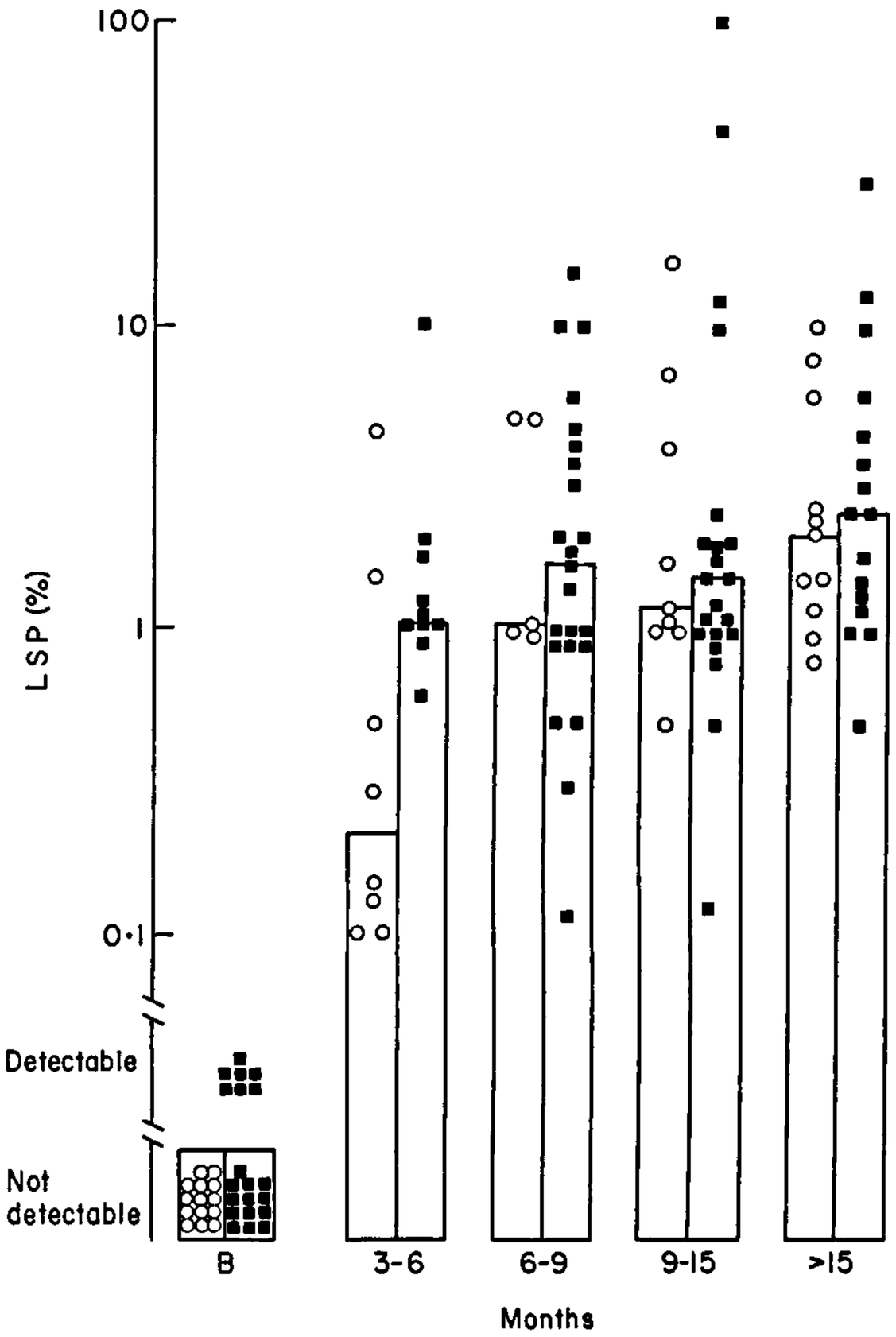


Fig. 2. IgA anti-*M. leprae* antibody activity in individual sera from babies of mothers with lepromatous leprosy (■) and in sera from babies of tuberculoid leprosy mothers and non-leprosy control mothers (○). Otherwise as for Fig. 1.

IgA antibody activity increased, on average, from being undetectable in cord serum to 1% of LSP at 3–6 months, 1·6% at 6–9 months, 1·5% at 9–15 months and 2·5% of LSP in sera taken between 15 and 24 months of age.

IgM antibody activity increased from being just detectable in cord serum to 1·2% of LSP at 3–6 months, 2·0% at 6–9 months, 2·8% at 9–15 months and 10% of LSP in sera taken between 15 and 24 months of age.

IgG anti-*M. leprae* antibody activity decreased from a median value of 24% of LSP at birth to 1·1% at 3–6 months, 0·4% at 6–9 months, 0·25% at 9–15 months and finally showing a slight increase to 0·35% of LSP in sera taken between 15 and 24 months of age.

Fig. 2 shows the distribution of the IgA anti-*M. leprae* antibody activity in individual sera from babies of lepromatous leprosy mothers (Group 1) recorded as closed squares and in sera from babies of tuberculoid and non-leprosy mothers (Group 2) recorded as open circles. IgA anti-*M. leprae* antibody activity was demonstrated in seven of 20 cord sera from babies of lepromatous leprosy mothers, while none of the cord sera from babies of Group 2 showed detectable IgA anti-*M. leprae* antibody.

After birth, the median IgA anti-*M. leprae* antibody activity increased from 1% of LSP in sera taken at 3–6 months of age to 2·5% of LSP in sera taken between 15 and 24 months of age. Similar figures for sera from babies of Group 2 were 0·24% of LSP at 3–6 months increasing to 2·1% of LSP

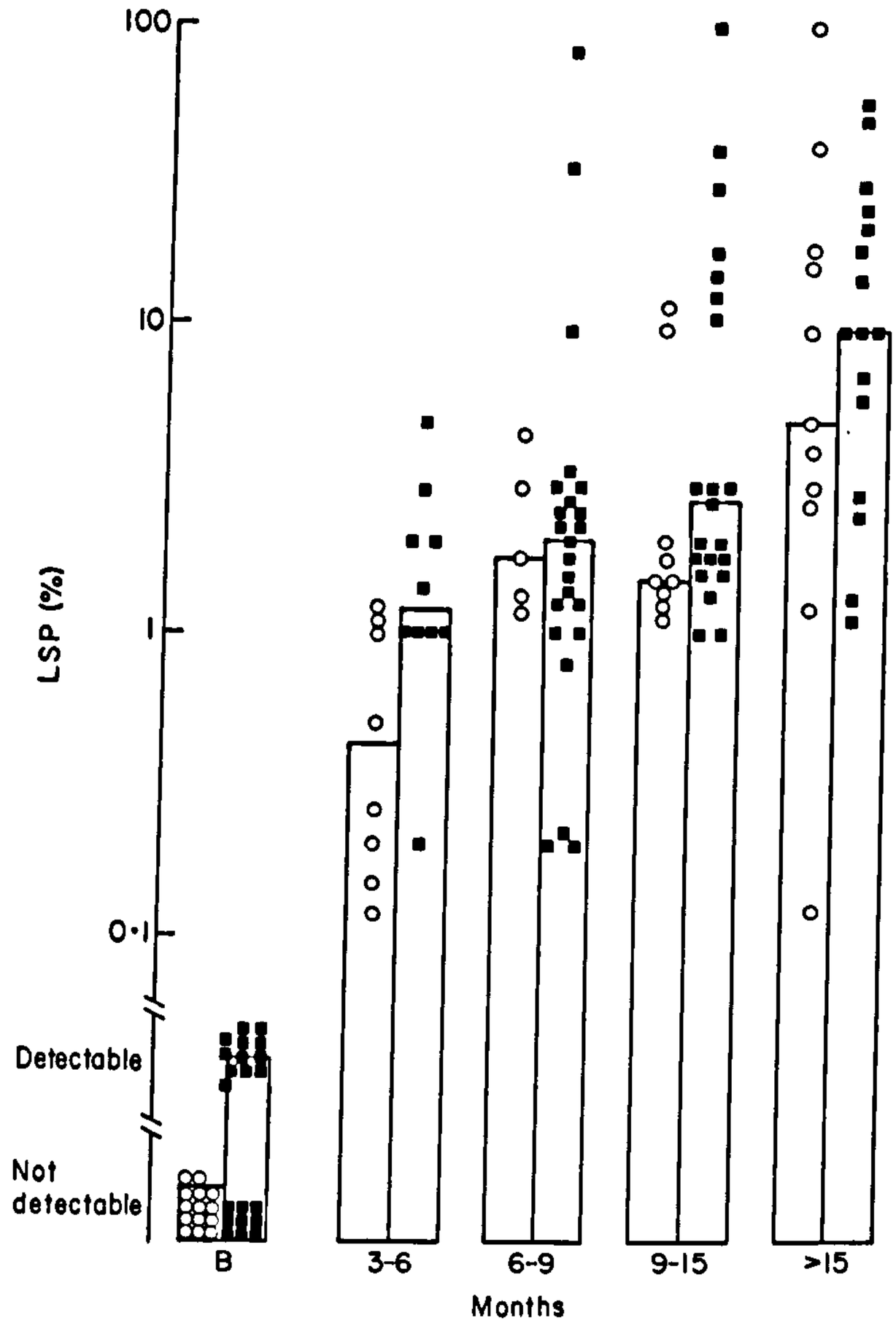


Fig. 3. IgM anti-*M. leprae* antibody activity, otherwise as for Fig. 2.

in sera taken between 15 and 24 months of age. The IgA anti-*M. leprae* antibody activity in sera taken at 3–6 months of age from babies of Group 1 was significantly higher than the antibody activity in sera from babies of Group 2 ($P=0{\cdot}01$). The differences in IgA antibody activity between babies of Group 1 and Group 2 were not significant in the three other age groups studied.

The IgM anti-*M. leprae* antibody activity in the same sera is shown in Fig. 3. In babies of Group 1, 11 of 20 cord sera contained detectable IgM anti-*M. leprae* antibodies. All of the seven sera with detectable IgA anti-*M. leprae* antibodies also contained IgM anti-*M. leprae* antibodies. No IgM anti-*M. leprae* antibodies were detected in cord sera from babies of Group 2. At 3–6 months of age, the median IgM anti-*M. leprae* antibody concentration in sera of babies of Group 1 had increased to 1·2% of LSP whereas the corresponding figure in babies of Group 2 was 0·4% of LSP. This difference was statistically significant ($P=0{\cdot}01$). The differences in IgM antibody activity between babies of Group 1 and Group 2 were not significant in the three other age groups studied.

The IgG anti-*M. leprae* antibody activity is shown in Fig. 4. The median IgG anti-*M. leprae* antibody activity in cord sera from babies of lepromatous leprosy mothers was 24% of LSP whereas the median IgG antibody activity in babies of Group 2 was 5% of LSP. This difference was not statistically significant. In both groups, the antibody activity decreased at 3 months and further at 6–9 months of age, and the difference in antibody activity between Group 1 and 2 was again not statistically significant. In the 9–15 months age group, the median IgG anti-*M. leprae* antibody activity was identical in babies of Groups 1 and 2.

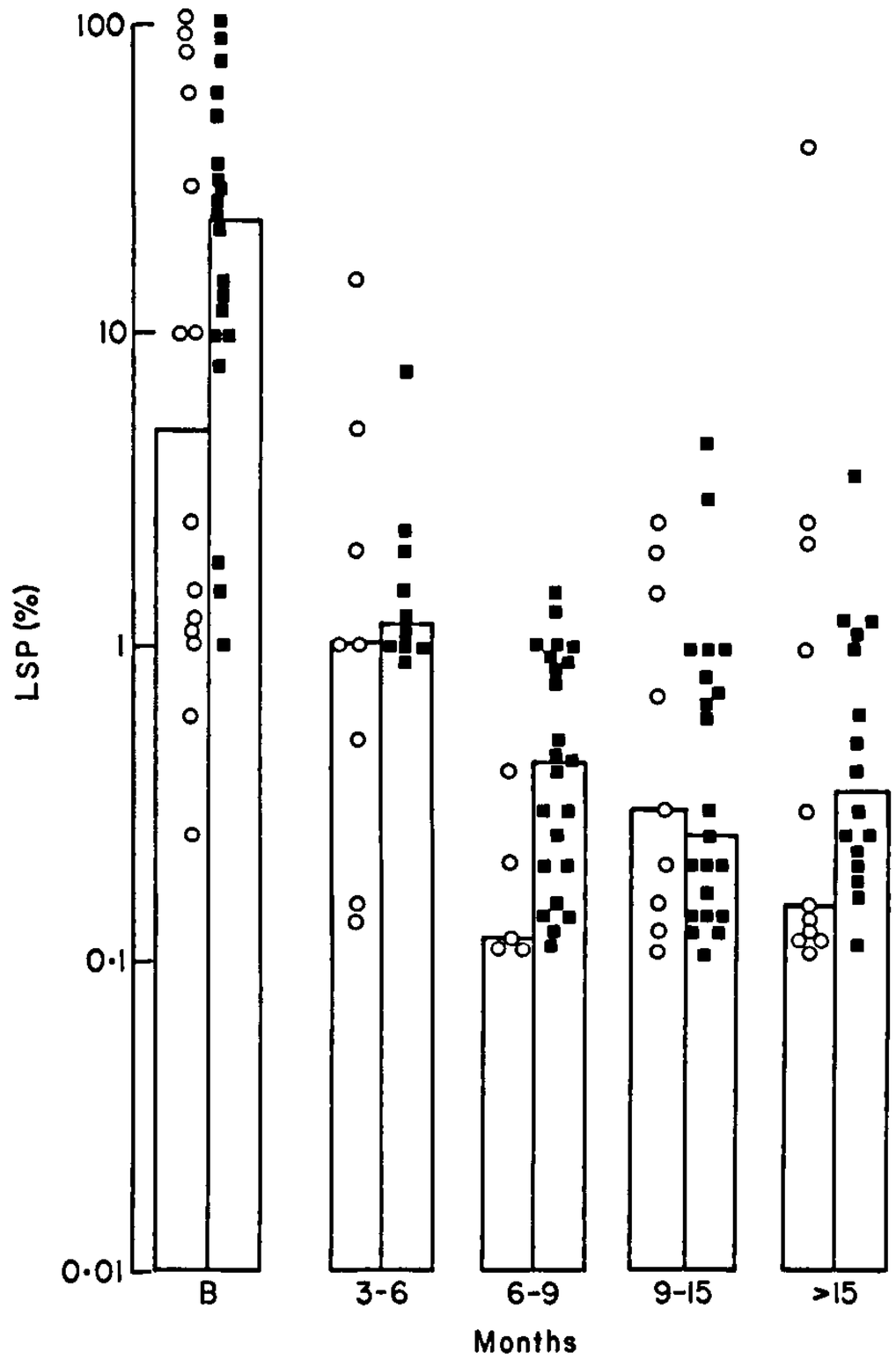


Fig. 4. IgG anti-*M. leprae* antibody activity, otherwise as for Fig. 2.

Fig. 5 compares the results obtained in this study to those of an earlier study describing the decline in concentration of antibodies against *M. leprae* antigen 7 from birth to 4 months of age. *M. leprae* antigen 7 is a cell wall antigen which cross-reacts with similar components in several mycobacterial species and *No cardia asteroides* (Harboe *et al.*, 1977). The quantification of antibodies against *M. leprae* antigen 7 was based upon a radioimmunoassay (RIA) using labelled *M. leprae* antigen 7 and protein A containing staphylococci to separate immunoglobulin-bound labelled *M. leprae* antigen 7 from free *M. leprae* antigen 7 (Melsom *et al.*, 1978). This RIA detects antibodies of the IgG1, IgG2 and IgG4 subclasses and reacts with about 30% of IgM in normal serum (Melsom & Duncan, 1980). The previous study showed a less than expected decline in the concentration of antibodies against *M. leprae* antigen 7 when comparing sera taken at birth with sera taken between 2 and 4 months of age from 10 of 20 babies of lepromatous leprosy mothers. By contrast, there was a normal decrease in the concentration of antibodies against *M. leprae* antigen 7 in similar sera taken from babies of non-leprosy and tuberculoid leprosy mothers (Melsom, Duncan & Bjune, 1980a). The expected decrease in antibodies against *M. leprae* antigen 7 was calculated on the basis of a normal half life of 28 days of maternal IgG in babies during their first four months of life (Gitlin & Gitlin, 1975). In Fig. 5 we have compared the increase in IgM anti-*M. leprae* antibody activity and the decrease in IgG anti-*M. leprae* antibodies with the decrease of antibodies against *M. leprae* antigen 7 in cord sera relative to sera taken 3 months later from 12 babies of lepromatous leprosy mothers. Sera from seven of these 12 babies showed an expected decrease in the

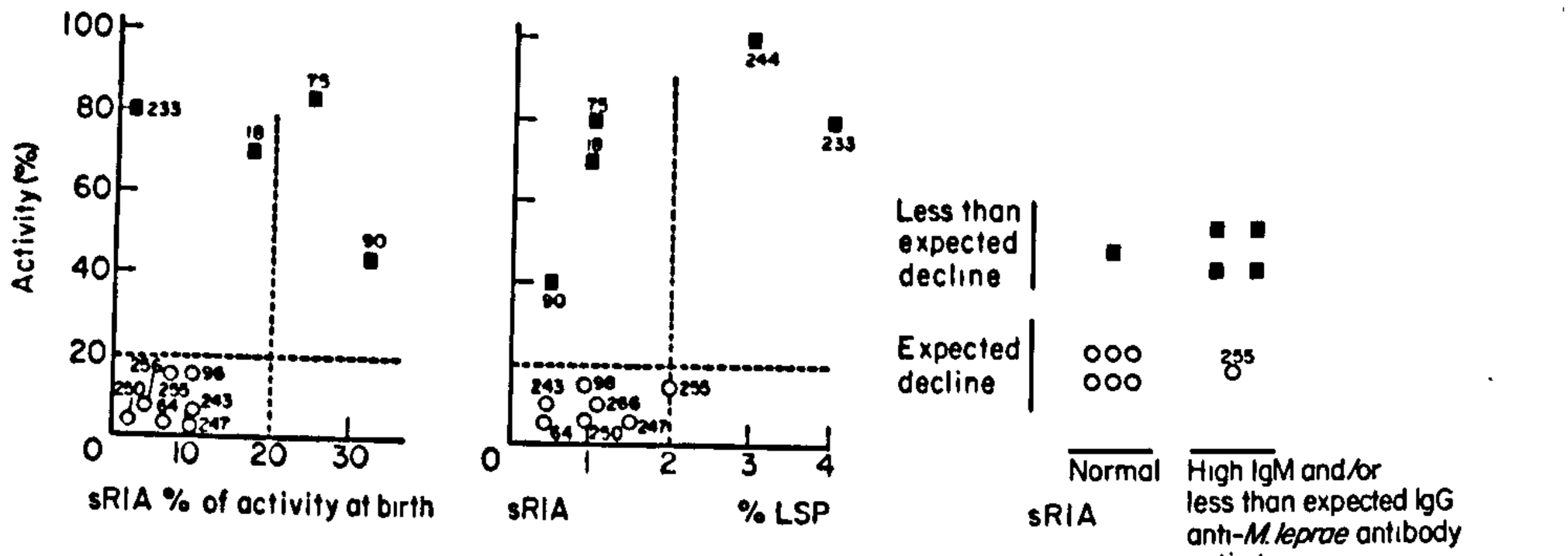


Fig. 5a. This shows the decline in concentration of antibodies against *M. leprae* antigen 7 (by RIA) on the ordinate and in IgG anti-*M. leprae* antibody activity (by sRIA) on the abscissa in individual sera taken three months after birth from babies of lepromatous leprosy mothers. The results are expressed as percentage of the activity found in the cord sera from the same babies. O represent the sera from babies with expected decline of antibody activity, and ■ sera from babies with less than expected decline of antibodies against *M. leprae* antigen 7 calculated from the catabolism of maternal IgG in the newborn baby. Values below the horizontal and to the left of the vertical stippled lines correspond to the expected catabolism of maternal IgG at about 3 months of age.

Fig. 5b. This shows the decline of antibodies against *M. leprae* antigen 7 as in frame A on the ordinate and the IgM anti-*M. leprae* antibody activity expressed as percentage of activity in LSP. The horizontal stippled line is as in frame A, and the vertical stipples line is set at twice the median value in all sera taken 3–6 months after birth from babies of lepromatous leprosy mothers. Values to the right of this line represent abnormally high IgM anti-*M. leprae* antibody activity.

Fig. 5c. This shows the combined results from frames a and b.

concentration of antibodies against antigen 7. Sera from six of these babies showed both an expected decrease in IgG anti-*M. leprae* antibody activity and a normal increase of IgM anti-*M. leprae* antibody activity measured by sRIA. By contrast, four of five sera taken at 3 months from babies showing a less than expected decrease of antibodies against *M. leprae* antigen 7 showed either a more than normal increase in IgM anti-*M. leprae* antibody activity or a less than normal decrease of IgG anti-*M. leprae* antibody activity measured by the presently described sRIA.

DISCUSSION

The specificity of the sRIA for demonstration and quantification of anti-*M. leprae* antibodies has been tested by inhibition experiments with isolated human IgG, IgA and IgM proteins. Antibodies of one particular class were inhibited by addition of protein belonging to this class but not by the two other immunoglobulin classes (Melsom *et al.*, 1981). The specificity is also evident from the pattern of antibody activity during the first months of life with decreasing concentration of IgG antibodies and a rapid and marked increase in IgA and IgM anti-*M. leprae* activity due to early production of antibodies of these immunoglobulin classes in the baby.

The babies were divided into two groups according to the type of leprosy of their mother. Group 1 consisted of babies of lepromatous leprosy mothers, a form of the disease often associated with marked bacteraemia (Drutz, Chen & Lu, 1972). Group 2 consisted of babies of mothers with paucibacillary tuberculoid leprosy and non-leprosy control mothers. Babies of Group 1 may have been exposed to leprosy bacilli *in utero* while it is unlikely that babies in Group 2 would be exposed to such bacilli *in utero* (Melsom *et al.*, 1981). The division into these two groups was made to study the effect of a possible fetal exposure to *M. leprae* compared with infection after birth. In previous investigations (Melsom *et al.*, 1980b), we divided the lepromatous leprosy mothers into an 'active' group consisting of individuals with a positive bacterial index and an 'inactive' group with no

acid-fast bacilli in skin smears taken during or shortly after pregnancy. During the study, it was disclosed that 30% of the 'inactive' lepromatous leprosy mothers experienced a reactivation of their leprosy during or shortly after the pregnancy and as a result had to be reclassified (Duncan *et al.*, 1981). It is possible that a larger number of these women had a reactivation of their lepromatous leprosy during pregnancy without overt clinical symptoms of leprosy and thus were not detected by standard tests. These patients would, most likely, have a bacteraemia during part of their pregnancy. In the present paper, we have therefore considered the babies of 'active' and 'inactive' lepromatous leprosy mothers as one group with the possibility of having been exposed to *M. leprae* before birth.

The solid phase RIA employs sonified *M. leprae* bacilli for coating the polystyrol test tubes. By crossed immunoelectrophoresis more than 20 distinct antigenic components have been demonstrated in concentrated *M. leprae* sonicates (Closs, Mshana & Harboe, 1979). Most of them cross-react with other mycobacteria (Closs *et al.*, 1979). Some of them may be *M. leprae* specific, but we have so far not been able to demonstrate *M. leprae* specific antigenic components. The relative amount and the identity of the antigenic components bound to the wall of the test tubes have not been determined so far. By the use of monospecific (Harboe, Closs & Deverill, 1976) and oligospecific antisera against mycobacterial components, it has been demonstrated that *M. leprae* antigen 7, which cross-reacts strongly with several other mycobacterial species and BCG antigen 60 (Harboe *et al.*, 1977), is bound to the polystyrol test tubes and that the sRIA demonstrates antibodies against other components of *M. leprae* in addition to anti-*M. leprae* 7 antibodies (Harboe, unpublished observations). Thus, the system mainly demonstrates cross-reacting antibodies and is not specific for *M. leprae*. Antibody production in the human towards other pathogenic mycobacteria, such as *M. tuberculosis*, and saprophytic mycobacteria would therefore lead to demonstration of IgA, IgM and/or IgG antibody activity against *M. leprae* in the present assay. Leprosy patients previously exposed to antigens cross-reacting with *M. leprae* will have a better production of antibodies to these cross-reacting antigens than antibodies to *M. leprae* specific determinants. An assay based upon antibodies against these cross-reacting antigens from *M. leprae*, might therefore be a more sensitive indicator of early *M. leprae* infection than an assay based upon antibodies against *M. leprae* specific determinants (Abrahams, 1970). Further work on the antibody response in humans against these cross-reacting antigens is very important in the development of a test for subclinical and early leprosy based upon antibody assay (Melsom *et al.*, 1982).

We have previously demonstrated a less than expected decline in anti-*M. leprae* antigen 7 antibody activity when comparing sera taken at birth with sera taken 2–4 months of age in 10 out of 20 babies of lepromatous leprosy mothers (Melsom *et al.*, 1980b). In the present investigation we found a correlation between decline in concentration of antibodies against *M. leprae* antigen 7 and the results in the sRIA for anti-*M. leprae* antibody activity against sonified *M. leprae*, as seen in Fig. 5. By two different methods it has thus been found that about half of the babies of mothers with active lepromatous leprosy show signs of anti-*M. leprae* antibody synthesis during the first months of life. IgM anti-*M. leprae* antibodies were demonstrated in 50% and both IgA and IgM antibodies in 30% of cord sera from babies of mothers with active lepromatous leprosy (Melsom *et al.*, 1981). These IgA and IgM anti-*M. leprae* antibodies in cord sera might be of maternal origin. In this case, the activity of these antibodies would have markedly decreased after birth due to rapid catabolism (Rowe, 1975). But our studies showed a clear increase of IgA and IgM anti-*M. leprae* antibody activity in sera taken within the first month after birth (data not shown). This indicates that the IgA and IgM anti-*M. leprae* antibodies are produced by the fetus and not transferred from the mother. These findings indicate that the stimulation of the immune system of these babies occurs both *in utero* and following birth and are best explained by passage of live *M. leprae* bacilli across the placenta with active proliferation of the bacilli in the fetus.

The difference between IgA and IgM anti-*M. leprae* antibody activity in sera from babies of Group 1 and Group 2 was statistically significant in the 3–6 months age group, but not in the later age groups (cf. Figs 2 and 3). The effect of priming during pregnancy (or immediately after birth) is thus more marked than the effect of later exposure to *M. leprae* or other mycobacteria during this period, whereas exposure to mycobacterial antigens in the external environment obviates the difference between Group 1 and 2 after 6 months. This may be due to exposure to *M. leprae* since the

importance of exposure to other individuals than the mother increases rapidly after birth under the socio–economic conditions of the individuals included in the present investigation. Cross-reacting antigens occur in saprophytic mycobacteria present in the environment (Daniel & Janicki, 1978; Beerwerth, Eysing & Kessel, 1979), but their role in induction of synthesis of anti-mycobacterial antibodies during the first year of life requires further study.

Most of the babies were BCG vaccinated. For technical reasons, BCG vaccination was performed at different ages, ranging from one week to 1½ years after birth. The data have been carefully analysed with regard to the effect of BCG vaccination on antibody activity. We could *not* demonstrate any effect in the form of increase or decrease in IgA, IgM or IgG anti-*M. leprae* antibody activity. Sampling was too infrequent to exclude that there might have been a (transient) effect of BCG vaccination on the antibody activity, but there was no evidence to indicate that BCG vaccination affected the general results of the present study.

In babies of Group 1, the IgG anti-*M. leprae* antibody activity decreased markedly from birth until 3–6 months of age. This decline corresponds to the normal catabolism of maternal IgG with a half-life of 28 days (Gitlin & Gitlin, 1975). The decline continued, but with less decrease until 9–15 months after birth. In sera taken later than 15 months after birth, the median IgG anti-*M. leprae* antibody activity was higher, but the difference was not statistically significant. The increase in IgA and IgM anti-*M. leprae* antibody activity indicates that there is a continuing antigenic stimulation from birth to 2 years of age, but this stimulation did not induce a significant production of IgG anti-*M. leprae* antibodies. This lack of IgG antibody production is difficult to explain, but a similar phenomenon has previously been demonstrated concerning antibody formation against an antigenic component of the *H. influenzae* cell wall, where a switch from the early IgM to IgG antibody synthesis did not occur until 18–24 months of age (Pabst & Kreth, 1980; Peltola *et al.*, 1977). The early increase in IgA and IgM anti-*M. leprae* antibodies appears to represent the demonstration of early synthesis of specific antibodies of their immunoglobulin classes corresponding to the well documented production of IgA and IgM proteins early after birth in humans (Stiehm, 1980).

Similar findings have been reported in a previous study of IgG, IgA and IgM anti-*M. leprae* antibodies in patients with lepromatous, tuberculoid and 'strictly indeterminate' leprosy compared with controls exposed to *M. leprae* but without clinical symptoms of the disease. The IgM anti-*M. leprae* activity was higher in indeterminate leprosy than in the control group with virtually no overlap. IgA anti-*M. leprae* was higher in indeterminate leprosy, but with considerable overlap with the controls. No difference between these two groups was found in the IgG anti-*M. leprae* assay (Melsom *et al.*, 1982).

Antibody formation during development of mycobacterial infection has also been studied in armadillos inoculated with *M. leprae*. Increased synthesis of anti-*M. leprae* 7 antibodies virtually only occurred in animals with active multiplication of bacilli leading to systemic mycobacterial infection. After a period with consistent low antibody activity, increased antibody synthesis became apparent 8–12 months after the inoculation (Harboe, 1981). The assay for anti-*M. leprae* 7 antibodies in armadillos is expected to detect mainly IgG antibodies. Application of assays specific for IgM and IgG anti-*M. leprae* antibodies respectively are planned to show if early production of IgM and later production of IgG anti-*M. leprae* antibodies is a feature also of this controlled experimental leprosy infection.

Epidemiological studies in the pre-sulphone era (Lara, 1946; Gomez, Basa & Nicolas, 1922) have shown that 25–50% of babies from families with lepromatous leprosy developed clinical signs of leprosy before 5 years of age. The majority of these lesions heal spontaneously (Lara & Nolasco, 1956). Little is known, however, of the long term effects of exposure to *M. leprae* in early childhood. By sRIA for IgA, IgM and IgG anti-*M. leprae* antibodies, direct evidence has been obtained for stimulation of the immune system of some babies of lepromatous leprosy mothers *in utero* and/or during the first 2 years of life (Melsom *et al.*, 1981). Two of the babies in the groups studied developed clinical signs of leprosy with histological confirmation before 17 months of age. Their lesions healed spontaneously, and clinical examination of 89 babies at 2½–4 years of age revealed no clinical signs of leprosy. We could demonstrate IgM anti-*M. leprae* antibody activity corresponding to more than 10% of the activity in LSP in sera taken after 9 months of age in both of these babies.

We found a similar high IgM anti-*M. leprae* antibody activity in sera from 14 of the 29 babies of mothers with lepromatous leprosy and in four of the 15 babies of tuberculoid leprosy mothers. The highest IgM anti-*M. leprae* antibody activity in a control group of healthy individuals exposed to *M. leprae* (Melsom *et al.*, 1982) was 30% of the activity in LSP. In the present study we found IgM anti-*M. leprae* antibody activity higher than 30% of LSP in sera from six babies (two of these with early signs of leprosy) of mothers with lepromatous leprosy and two babies of tuberculoid leprosy mothers. This indicates that these babies have been infected with *M. leprae* even though six of the eight had no clinical sign(s) of the disease. Most of these babies will probably control their subclinical leprosy infection without developing clinical signs of leprosy. This will be in agreement with the demonstration of self-healing in 75% of early childhood leprosy by Lara & Nolasco (1956).

Additional studies of anti-*M. leprae* antibodies and correlation of antibody activity with clinical symptoms and subsequent course are important to obtain additional information on the importance of early exposure to *M. leprae* and the value of antibody assays for diagnosis and evaluation of subclinical infection with *M. leprae* and indeterminate leprosy. Antibody assays may now represent an additional tool in the study of early leprosy and may point out factors responsible for healing and control of the infection known to occur in the majority of children with indeterminate leprosy (Lara & Nolasco, 1956; Cochrane, 1936).

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