

A PROSPECTIVE STUDY OF BORDERLINE
LEPROSY REACTIONS

by

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1977.

And it came to pass, when he was in a certain city, behold a man full of leprosy: who seeing Jesus fell on his face, and besought him, saying Lord, if Thou wilt, Thou canst make me clean. And he put forth his hand, and touched him, saying, I will: be thou clean. And immediately the leprosy departed from him.

Luke 5, 12 - 13.



Frontispiece "The nerve destruction with consequent deformity, which is the most important complication of leprosy usually occurs during borderline leprosy reactions."

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SUMMARY

SUMMARY

Leprosy is still one of the most important diseases in the world today: it afflicts 15 million people and is the most important cause of deformity in man. The causative organism, Mycobacterium leprae (M. leprae) has an affinity for skin and nerve and is remarkably non-toxic; therefore, most of the clinical manifestations are due to the host response. The nerve destruction with consequent deformity, which is the most important complication of the disease usually occurs during borderline leprosy reactions. Surprisingly there has been little research into this type of reaction, though it seems likely that they result from an increase in cell-mediated immunological reactivity.

This thesis describes a prospective study of 83 patients who attended the Addis Ababa Leprosy Hospital with borderline leprosy, the purpose of which was to clarify the mechanisms involved in borderline leprosy reactions. Seventeen of the 83 patients developed reactions during the period of follow up (between one and two years) and the clinical, histological and immunological findings were compared in this group of patients with those patients who did not develop reactions.

It was observed that in the reaction patients there was a significant rise in lymphocyte transformation (LT) responses to both 'whole' and 'sonicated' preparations of M. leprae, confirming that these reactions are due to an increase in cell-mediated immunity to mycobacterial antigens. However it was found that in those patients who presented with reactions involving the skin alone,

there was a very marked rise in the responses to whole M. leprae (and a smaller rise in response to sonicated M. leprae) whereas those who had reactions involving nerve alone had a rise in LT responses only to sonicated M. leprae. Those with involvement of skin and nerve in the reaction had a marked rise in responses to both antigens. The reason for this remains obscure, but it seems likely that bacillary membrane antigens are associated with 'skin' reactions, and cytoplasmic antigens with 'nerve' reactions; it is possible that this results from different exposure of antigens in skin and in nerve.

There were two other findings of considerable importance related to the mechanisms which might be involved in reactions. Firstly, bacilli were often found in dermal and peripheral nerves without surrounding chronic inflammatory infiltrate, though there was marked granulomatous response in skin. It seems likely that this is of importance as sudden 'immune recognition' of bacilli in nerve tissue might account for the increased cell-mediated reactivity that occurs in this type of reaction. Secondly, autologous plasma, which is normally suppressive to LT responses to phytohaemagglutinin in leprosy, developed an augmenting effect during reaction. It is possible that plasma factors normally act as a form of 'brake' mechanism preventing delayed hypersensitivity reactions but during reaction this effect is lost.

Probably the most important observation of the study in the short term was the effect of dapsone on reactions. It has always been taught that dapsone should

be given initially at low dosage in an attempt to prevent reactions: however, there have been no published reports to support this hypothesis. In a study of 68 patients it was found that of those receiving dapsone 5 mg daily, 11 patients developed reactions whereas only 3 of those receiving 50 mg daily did so. This would suggest that dapsone in higher dosage does not predispose to borderline leprosy reactions, and indeed, may prevent them.

When those patients who did not develop reactions were studied it was found that those with borderline tuberculoid (BT) leprosy had significantly higher LT responses to whole and sonicated M. leprae than those with borderline lepromatous (BL) leprosy. However, there was considerable variation in both groups. Those patients with inflamed skin lesions had higher responses in both BT and BL patients than those with non-inflamed lesions, and indeed, those with BL leprosy and inflamed skin lesions had higher responses than those with BT leprosy and non-inflamed lesions. This would suggest that the LT test in leprosy reflects the degree of delayed hypersensitivity rather than that of protective immunity, and that as in tuberculosis the two phenomena are not necessarily related.

Thus some of the mechanisms involved in borderline leprosy reactions and the immune response in leprosy have been clarified, and it is hoped that as a result of this study reactions in some patients will be prevented. It is also hoped that the encouraging results of the study will stimulate further research into these reactions to minimise the morbidity of leprosy in the future.

INTRODUCTION

- i. General introduction.
- ii. Mycobacterium leprae. Properties of the bacillus.
- iii. Treatment of leprosy.
- iv. Spectrum of leprosy. Clinical and histological aspects.
- v. The immune responses in leprosy and their relationship to the clinical and histological spectrum.
- vi. Reactions in leprosy.
 - a) Previous clinical and histological observations.
 - b) Previous immunological research.
- vii. Proposed objectives of study.

1. GENERAL INTRODUCTION

Leprosy is still one of the most important diseases in the world today. It is estimated to affect 15 million people, and is the most important cause of deformity throughout the world. However, it is now mainly confined to the tropics, whereas a millennium ago it was worldwide.

It is still a major problem in Africa, but in many countries there have been effective leprosy control programmes which have ameliorated the situation. However this has not been so in Ethiopia, where leprosy control was not instituted till 14 years ago. Ethiopia, though it probably has the oldest culture in "black" Africa, is one of the poorest and most primitive countries in the continent. This is partly due to its Christian-based culture, as the people were driven into the mountains of the Ethiopian plateau with the upsurge in Mohammedanism and they have consequently remained isolated from the rest of the world, in such inhospitable terrain that communications have been extremely difficult. In addition, the country has never been colonised (apart from the period 1935-41 following the Italian invasion of Ethiopia from Eritrea) and so the country has never benefited from outside influence, as have the other East African countries.

Partly as a result of the lack of previous leprosy control programmes, Ethiopia has become a centre of major importance in research and instruction on the disease, the leprosy hospital in Addis Ababa embracing the Armauer Hansen Research Institute, a sophisticated immunology laboratory financed by Scandinavia and the All Africa

Rehabilitation and Training Centre (ALERT). I was sent to the Addis Ababa Leprosy Hospital in January 1974 to work as a member of the Medical Research Council Leprosy Project for 2 years, on secondment from the Laboratory for Leprosy and Mycobacterial Diseases, National Institute for Medical Research, Mill Hill, London, and the main purpose for my presence in Ethiopia was to carry out a study of borderline leprosy reactions. (Borderline leprosy is the commonest type of leprosy there, roughly 75% of patients having that classification.) This was a collaborative study comparing the clinical, the histological and immunological features of this kind of reaction, and my main collaboration was with Dr. G. Bjune, a basic immunologist working in the Armauer Hansen Research Institute.

ii. MYCOBACTERIUM LEPRAE - PROPERTIES OF THE BACILLUS

Mycobacterium leprae (M. leprae), the causative organism of the disease was originally described by Hansen (1874): it has a number of special characteristics.

1. It is an obligate intracellular organism, being found in the skin predominantly in macrophages, and in peripheral and dermal nerve in Schwann cells.
2. It has an affinity for skin and nerve: involvement of peripheral nerve causes the deformity so notorious in this disease.
3. It has a longer generation time than any other bacterium, estimated as 12 - 13 days in the

logarithmic phase of growth in mice (Shepard and McRae, 1965).

4. It has not to date been cultured on an artificial medium, despite several claims to the contrary. This has considerably hampered leprosy research. Indeed it was only as late as 1960 that Shepard (1960) achieved multiplication of the organism in the mouse footpad. Following this, mouse models have been used extensively to study various aspects of the disease, including immunopathology, (Rees, 1971). Latterly Kircheimer and Storrs (1971) have used the armadillo as an animal model for lepromatous leprosy, and this is now proving of great benefit as a source of very large numbers of bacilli for experimental work.
5. It is an organism with a remarkable lack of toxicity (Godal et al, 1974). Thus most manifestations of leprosy are due to the host's immunological response. Lepromatous leprosy patients may be seen in the clinic without a single clinical manifestation, though they may harbour 10^{13} bacilli within the body tissues. This fact gives added importance to leprosy in the field of research, as a model of disease due to the host response.
6. It is an organism of high infectivity, contrary to previous belief. Godal and Negassi (1973) in a study of staff contacts in the Addis Ababa Leprosy Hospital demonstrated that these contacts developed high responses in the lymphocyte transformation (LT) test using M. leprae as antigen, thus suggesting that

most develop subclinical infection, a situation analagous to that in tuberculosis.

This being so, the method of spread previously assumed to be skin-to-skin clearly lay open to doubt in this non-ulcerative disease where the organism is probably non-motile (c.f. primary yaws). A number of studies have shown recently that the most likely spread of the organism is from nose to nose (Shepard, 1962; Goodwin, 1967; Pedley, 1973; Davey and Rees, 1974; Rees and Meade, 1974.) It is interesting to note that this confirmed work of almost a century ago by Leloir (1886) and Schaffer (1898). The "nose blow" is now used as a method of importance in assessing the infectivity of patients.

Congenital leprosy has never been described, which is surprising in that mothers with leprosy may have 10^{13} bacilli within the body tissues, with a constant bacillaemia of up to 10^6 bacilli (Drutz et al, 1972). Barnetson et al (1976) have shown that this may be partly explained by the fact that where the mother possesses immunity to the bacillus, the foetus also develops this immunity possibly due to transplacental passage of a sub-lymphocyte factor akin to 'transfer factor'.

iii. TREATMENT OF LEPROSY

Prior to 1941, there was no effective treatment for leprosy: a number of preparations were used, including

hydnocarpus oil, though with very limited success. In 1941, the treatment of the disease was revolutionised by the introduction of the sulphones in leprosy. Dapsone (diphenyl diamino-sulphone) proved to be the most effective antileprosy drug (Lowe, 1950) and has retained its place as the primary therapeutic agent till the present day, though it may have to be replaced or given as a drug combination in view of increasing drug resistance (Pearson et al, 1975; Pearson et al, in press). It has the advantages that it is inexpensive, an attribute of major importance in tropical medicine, and in the dosages required is relatively non-toxic. Few alternative drugs are available: they include clofazamine, and such anti-tuberculous drugs as thiacetazone, streptomycin, ethionamide and rifampicin.

Most authorities however have taught that dapsone plays an important part in precipitating hypersensitivity reactions (Jopling, 1964; W.H.O., 1966; Leiker, 1971; Bryceson and Pfalzgraff, 1973). However there is little evidence that this is true either for borderline leprosy reactions or for erythema nodosum leprosum (ENL). Lowe (1950) described how nerves became smaller and less tender when the patient received treatment with dapsone and in none was neuritis aggravated. Two further studies by Lewis et al (1957) and Prasad (1971) which compared "high" and "low" dosage regimes in borderline tuberculoid leprosy patients showed no difference in the incidence of reactions between the two groups. Similarly in ENL, Pearson and Helmy (1973) and Waters and Helmy (1974) could find no

evidence that dapsone caused or seriously worsened the reaction.

iv. SPECTRUM OF LEPROSY - CLINICAL AND HISTOLOGICAL ASPECTS

Leprosy is a classical example of a disease which demonstrates a spectrum both clinically and histologically. This does not depend on variability in the properties of the bacillus, but rather on the host response.

Danielssen and Boeck (1847) originally described two forms of leprosy "anaesthetic", subsequently termed "tuberculoid", and "nodular" subsequently termed "lepromatous". Surprisingly the concept of "borderline" leprosy - an intermediate form between these two extremes - took 100 years to formulate. Initially, Wade (1952) put forward a suggested comprehensive system of classification which included borderline leprosy and it was decided on a formal basis that this should be adopted by the 6th International Congress of Leprology in Madrid 1953. In 1966 Ridley and Jopling (1966) introduced a five group classification, splitting borderline leprosy into borderline tuberculoid (BT), borderline (BB), and borderline lepromatous (BL) leprosy. Only then was the classification crystallised: previously many BT patients were classified as tuberculoid, and BL as lepromatous. A later modification by Ridley and Waters (1969) defined a group between BL and LL termed lepromatous indefinite (LI). Thus the full spectrum of leprosy was characterised clinically and histologically.

The spectrum also correlated to some degree with the

patients' immune defences. Tuberculoid (TT) leprosy is characterised clinically by localization of the disease, patients present with an anaesthetic anhidrotic hypopigmented area in the skin, with perhaps a related enlarged nerve. Histologically the picture is that of epithelioid cell granuloma in the skin, with areas of epithelioid cells and giant cells surrounded by cuffs of lymphocytes. This process usually affects the full thickness of the dermis with infiltration of the subepidermal zone, and erosion of the epidermis. Bacilli are rarely found, though occasionally they may be seen in dermal nerve or muscle; thus the immune response seems to be comparatively efficient.

At the other end of the spectrum lies lepromatous (LL) leprosy, and this is characterised by lack of immune responsiveness. Thus the disease is generalised, and clinically there may be diffuse hypopigmentation with nodules in the skin and diffuse thickening of the peripheral nerves (though this may be a minor feature). Nodules are not only found in the skin, but also in the mouth, nose and larynx. Histologically the picture is that of a histiocytic infiltrate throughout the dermis, though not infiltrating the epidermis: there is no evidence of epithelioid cell formation and within the histiocytes, which have a "foamy" appearance, there are numerous bacilli, a proportion of which are intact (suggestive of viability), though a majority appear granular and fragmented.

In borderline leprosy a mixture of these two extremes is seen both clinically and histologically. An important

characteristic of borderline leprosy is its unstable state, with a tendency to progress to one end of the spectrum or the other. Those with untreated leprosy tend to progress towards the lepromatous end of the spectrum, and those receiving treatment progress towards the tuberculoid end of the spectrum. Those in the middle of the spectrum are the most unstable group of all.

In BT leprosy, the disease is more widespread than in tuberculoid. The patient has numerous hypopigmented areas which are anaesthetic, with diffuse nerve enlargement, and commonly resultant deformity from nerve destruction. Also characteristically in this type of leprosy the hypopigmented areas may become swollen and erythematous, with associated nerve swelling and tenderness. Histologically, BT leprosy resembles TT leprosy with epithelioid-cell granuloma formation, though the subepidermal zone tends to be spared and giant cells are more numerous. Bacilli are rarely found.

BL leprosy presents a more lepromatous picture clinically. Hypopigmentation is very diffuse and the skin lesions are not well demarcated as they are in BT leprosy. There may be nodules particularly on the limbs and on the face, within the hypopigmented areas. Nerve enlargement is diffuse and deformity is again common. Histologically, the infiltrate within the dermis is histiocytic with few lymphocytes (generally), and bacilli are present within the histiocytes, though less numerous than in lepromatous leprosy.

Borderline leprosy (BB) is a highly unstable stage,

with features clinically and histologically of both BT and BL leprosy. Patients usually develop inflammation of their hypopigmented lesions and this process is often very active. A very characteristic sign of this type of leprosy is the "ring lesion" where there is an area of normal skin within the inflamed lesion. Histologically there is epithelioid cell granuloma formation, with a moderate number of bacilli present. Langhans giant cells are absent and lymphocytes may be scanty.

One further group also deserves mention; these are patients with "indeterminate leprosy". This group is something of a "rag bag", including those with very early leprosy who have not yet developed the characteristic histology of the disease. However, some BT patients as diagnosed clinically, have an indeterminate histology on skin biopsy, though biopsy of a peripheral nerve may demonstrate typical epithelioid cell granuloma.

v. THE IMMUNE RESPONSES IN LEPROSY AND THEIR RELATIONSHIP TO THE CLINICAL AND HISTOLOGICAL SPECTRUM

During the immunology "explosion" of the last decade, many papers on the immunology of leprosy have been written, and leprosy has thus become a model of the human host response to chronic infection. The non-toxicity of the bacillus makes immunological study of this disease doubly interesting, as most of the clinical manifestations of the disease are due to the host's immune response.

It is well recognised that the human host immune

responses may be split into two components: cell mediated immunity and humoral immunity. In cell mediated immunity the lymphocytes are dependent on a maturation stage in the thymus, and they are thus known as T lymphocytes. However humoral-immune responses are mediated by a different class of lymphocytes; in chickens these have been shown to be dependent for maturation on the Bursa of Fabricius and they are thus known as B lymphocytes, although no equivalent to this organ has been described in man. As might be expected, there is considerable interplay between these two immune systems, and it is becoming increasingly apparent that humoral immunity is important in diseases which were thought only to provoke a cell mediated response, and vice versa.

(1) CELL MEDIATED IMMUNITY (C.M.I.)

It has long been known that immunity to intercellular parasites such as the leprosy bacillus is mainly dependent on cell mediated immune mechanisms rather than serum antibodies (Mackaness and Blanden, 1967), and thus on T lymphocytes. However, the T lymphocytes are not usually capable of destroying the parasites directly (Tripathy and Mackaness, 1969; McGregor and Koster, 1971); their function is to release lymphokines, including macrophage migration inhibition factor, macrophage activation factor and specific macrophage-arming factor, which inhibit macrophage migration (David, 1966) and activate the surrounding macrophages (Mackaness, 1967). This results in a number of changes in properties of the macrophage including

increased pinocytosis, increased phagocytosis and enhanced bacteriostasis, enabling more efficient destruction of the bacillus.

Responses to Antigens

Assessment of the cell mediated immune response to M. leprae may be carried out by in vivo or in vitro tests.

a) In vivo: the lepromin test has been in use since 1919 (Mitsuda, 1919) but to date has proved of only very limited use as a diagnostic test. The reagent is prepared from infected human tissue and consists of autoclaved leprosy bacilli contaminated with some tissue elements (Rees, 1964). Injected intradermally the suspension may elicit an early reaction (Fernandez) after 48-72 hours, equivalent to the "Tuberculin test" and a late reaction (Mitsuda) which reaches its maximum 4 weeks after injection and is equivalent to the Kveim test.

It has proved of very limited diagnostic value, as in studies of healthy controls in non-leprosy-endemic areas, a proportion of those tested have had positive results. This is almost certainly due to cross-reactivity with other mycobacteria (Rees, 1964; Gohman-Yahr et al, 1968) and this may be supplemented by the host skin elements present in the lepromin (Rees, 1964). However, it has recently been shown by Myrvang et al (1975) that provided the test is standardised, the early reaction may be of epidemiological value in detecting leprosy contacts with evidence (obtained by in vitro tests) of subclinical

infection.

The significance of the late reaction is still unknown. De Souza and De Souza (1948) suggested that the Fernandez reaction might demonstrate the "allergic condition" of the patient, and the Mitsuda reaction his "immune status". This hypothesis is still tenable (Myrvang et al, 1973). It is interesting to note that even 30 years ago hypersensitivity and protective immunity in leprosy were considered by some as separate entities.

b) In vitro: recently a number of tests have been established by which it is possible to monitor lymphocyte function in vitro. They include (i) the lymphocyte transformation (LT) test and (ii) the leucocyte migration inhibition (LMI) test, both of which have been used in leprosy. The immunological bases for these tests are as follows:-

- (i) LT test (Ling, 1968). It is well established that T lymphocytes carry receptors for antigen on their surface. When exposed to an antigen which can interact with the receptors, the lymphocytes will enlarge and start to divide. This lymphocyte "transformation" may be monitored in vitro by counting the number of morphologically transformed cells, or measuring the associated DNA synthesis by uptake of radioactive thymidine.
- (ii) LMI test (David et al, 1964) One of the results of T cell stimulation is the liberation of lymphokines. This production and release of lymphokines can be monitored in vitro by various tests, but the most

useful test using this principle is the leucocyte migration inhibition test. However this test has lost some of its popularity as B lymphocytes may also produce macrophage inhibition factor, and results are often poorly reproducible.

Both these tests have been used to assess the cell-mediated immune response of leprosy patients, using M. leprae as the antigen. (Particular attention has been paid to correlating LT test results and the classification of the disease, and to demonstrating changes associated with reactions. They have also been used to detect subclinical infection of leprosy and to characterise the defect of C.M.I. in lepromatous leprosy.

DEFINITION OF IMMUNOLOGICAL SPECTRUM IN LEPROSY

Myrvang et al (1973) studied the immune responsiveness to M. leprae and other mycobacterial antigens throughout the clinical and histopathological spectrum of leprosy by using the Lepromin test, the LT test and LMI test in 135 patients. By all these methods of investigation, a continuous decrease was shown from strong responses in the tuberculoid patients to virtually negative responses in the LL group; and there was a good correlation between the in vitro methods and the lepromin skin test. However, there was considerable variation in each group, and all histologically indeterminate patients were exempted from the study. These patients were the subject of a later paper.

The immune response to mycobacterium tuberculosis

bovis, strain Bacille Calmette Guerin (BCG) and "purified protein derivative" (PPD) in those patients studied, decreased only slightly towards the lepromatous pole of the spectrum, confirming the high degree of specificity of the defect in lepromatous leprosy.

Their findings have given strong support for the concept of a host-determined, immunological disease spectrum as suggested by the 'Ridley-Jopling spectrum' (Ridley and Jopling, 1966).

They also studied a group of indeterminate patients, based on histopathological diagnosis (Myrvang et al, 1973). (It is important to note here that the definition of indeterminate leprosy varies quite markedly between the clinical and the histopathological spectrum, and this is especially so in Ethiopia where many patients with widespread hypopigmentation suggestive of BT leprosy may give an indeterminate histology when their skin is biopsied, though nerve biopsy may show typical epithelioid cell granuloma.) In their series, they studied 31 histologically classified indeterminate leprosy patients, of whom 14 were also clinically indeterminate, the others being classified as TT or BT. Those who were indeterminate both histologically and clinically had no response with in vivo or in vitro tests: however, those with a clinical diagnosis of TT or BT had a variable response, one or two giving quite strongly positive results.

The reason for the apparent inability of lepromatous patients to destroy the bacillus is not fully understood. Most studies of macrophage function in these patients

have not shown any defect (Godal and Rees, 1970; Drutz and Cline, 1970; Samuel et al, 1973; Drutz et al, 1974; Convit et al, 1974). It is likely therefore that there is a specific defect in lepromatous patients' T lymphocytes, and this has been borne out by a number of studies. Godal et al (1971, 1972) have shown that though LL patients have negative responses with the LT test using M. leprae as antigen, they may have strongly positive responses to BCG and PPD, thus underlining the specificity of the defect. Furthermore, this defect seems to be lifelong, as LL patients failed to respond to M. leprae even after 10 years of anti-leprosy treatment. (This might preclude the possibility of the effect being due to T lymphocyte trapping in lymph nodes due to overloading with persistent antigen as suggested by Rook (1975).) They concluded that in these patients there was immunological tolerance to the bacillus, though the reason for this was unclear.

RESPONSES TO MITOGENS

It is well recognised that small lymphocytes can be transformed by non-specific substances termed mitogens. These are plant lectins which interact with carbohydrate receptors on lymphocytes. One example of a mitogen which stimulates T lymphocytes to transform is phytohaemagglutinin (PHA) which is extracted from the red kidney bean, *Phaseolus vulgaris* and this can be used to some extent to measure the immune capacity of the patient. However, the range of responses in the normal population is so great that cell-mediated deficiency has to be very marked before it

can be detected by this method.

There have been a number of studies of lymphocyte responses to PHA in leprosy patients, though results have been contradictory. A majority of authors are however agreed that PHA responses are sub-normal in lepromatous patients (Rodriguez Paradisi et al, 1968; Dierks and Shepard, 1968; Bullock and Fasal, 1971; Han et al, 1971; Wong et al, 1971; Mehra et al, 1972). A number of papers have also suggested that PHA responses in tuberculoid patients are also decreased, though less markedly (Dierks and Shepard, 1968; Bullock and Fasal, 1971; Han et al, 1971; Wong et al, 1971). Others have not been able to confirm a decreased PHA response in leprosy patients (Sheagren et al, 1969; Nelson et al, 1971; Rea et al, 1976).

There are a number of possible reasons for these controversial findings. The methodology varied considerably in these studies and in some the LT test was carried out in the presence of autologous plasma or serum, which has been shown to depress responses in some leprosy patients (Nelson et al, 1971). (These authors also found considerable racial differences in responses.) Another important variable was the patients' therapy when tested: some were receiving treatment with dapsone or other anti-leprosy drugs, whilst others were tested before initiation of treatment. Mehra et al (1972) found that patients with lepromatous leprosy had reduced responses prior to treatment, but these returned to normal after dapsone therapy. A further important factor which might modify PHA responses is the reduction of circulating T cells

which has been reported in lepromatous patients by Dwyer et al (1973), and Mendes et al (1974); this might also account to some degree for the difference between lepromatous and tuberculoid patients.

(2) HUMORAL IMMUNITY IN LEPROSY

Although the main defence against intra-cellular organisms such as M. leprae is through cell-mediated mechanisms, humoral antibodies are produced against the organism. Their function in the pathogenesis of leprosy is unknown, and the only consequence of their presence demonstrated to date is the occurrence of erythema nodosum leprosum associated with immune complex formation.

There have been several studies of serum immunoglobulins (Ig) in leprosy. In studies of IgG, IgA and IgM there have been varying results. Sirisinha et al (1972) demonstrated that all these immunoglobulins were significantly higher in leprosy patients, whether tuberculoid or lepromatous, than in a normal control population. Bullock et al (1970) however found that only lepromatous leprosy patients had significantly raised levels of these immunoglobulins, when compared with normal controls, and Sheagren et al (1969) demonstrated similar results in lepromatous patients though the raised Ig levels were confined to IgG and IgA. Of particular interest for the purpose of this thesis is the study of Srivastava et al (1975), as it was performed on leprosy patients in Ethiopia. They found that compared with healthy controls IgG, IgA and

IgM were all elevated in lepromatous patients, whereas only IgG and IgM were elevated in tuberculoid patients. Malaviya et al (1972) on the other hand could not demonstrate any difference between leprosy patients (lepromatous or tuberculoid) and normal controls.

There have also been several studies of specific anti-mycobacterial antibodies, which are found in the IgG and IgM classes. They are much more common at the lepromatous end of the spectrum, where they are found in nearly 100% of patients, though they are also present in a small proportion of patients with tuberculoid leprosy (Navalkar et al, 1965; Ulrich et al, 1969; Myrvang et al, 1974; Kronvall et al, 1975). Similarly the number of demonstrable antibodies is greater in patients towards the lepromatous end of the spectrum.

The incidence of auto-antibodies has also been studied in leprosy patients. Bonomo (1965), Matthews and Trautman (1965), Malaviya et al (1972) and Petchclai et al (1973) all found that leprosy patients had a higher incidence of auto-antibodies (including anti-nuclear antibodies, rheumatoid factor, antithyroglobulin antibody) than did normal controls, and this was particularly true in lepromatous patients. Kreisler et al (1975) also demonstrated cold lymphocytotoxins in 50% of leprosy patients, most of whom were lepromatous. Finally Wright et al (1975) demonstrated that 40% of lepromatous and 20% of tuberculoid patients have circulating anti-neural antibodies. It had previously been suggested that nerve damage in leprosy might be caused by similar mechanisms to those causing

experimental allergic neuritis (Waksman and Adams, 1955).

vi. REACTIONS IN LEPROSY

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

1. Borderline leprosy reaction ("reversal reaction")
2. Erythema nodosum leprosum.

As will be seen it is likely that the former is an example of delayed-hypersensitivity reaction (Coombs and Gell type IV hypersensitivity reaction), and the latter is an example of reaction due to immune complex formation (Coombs and Gell type III hypersensitivity reaction)(Coombs and Gell, 1963).

1. Borderline leprosy reactions present characteristically with swelling and erythema of previously hypopigmented skin lesions and appearance of new lesions and on occasion with severe oedema of the face and limbs. Usually a marked feature of this type of reaction is neuritis with swelling and tenderness of the peripheral nerves (on occasion reaction may present with neuritis and no alteration of skin lesions). If this neuritis is untreated, then permanent nerve damage due to granuloma formation and consequent deformity is likely to result: neuritis in borderline leprosy reactions is the most important complication of the disease. Histologically there is an increase in epithelioid cell

granuloma formation both in skin and nerve, and in skin there is a tendency to develop more tuberculoid features. (Thus the term "reversal reaction", as there is an apparent reversal of the tendency of untreated borderline leprosy to become more lepromatous).

2. Erythema nodosum leprosum (ENL) occurs only in patients with lepromatous leprosy. They develop tender erythematous swollen skin lesions which tend to be smaller than those in borderline leprosy reactions, and more transient. Common concomitant manifestations are fever, neuralgia and arthralgia. Less commonly they may also have severe continuous neuritis, arthritis, orchitis, irido-cyclitis and features of glomerulonephritis, with a clinical picture suggestive of serum sickness. The histology is typical of lepromatous leprosy, with the addition of foci of polymorphonuclear leucocytes, and evidence of vasculitis in some cases.

a) PREVIOUS CLINICAL AND HISTOLOGICAL OBSERVATIONS

There are five accounts of importance and each reflects the confusion that has existed as to the classification of reactions.

(1) Hansen and Looft (1895)

Hansen and Looft (1895) described the occurrence of "eruptions" in their treatise on leprosy, and from their description both main types of reaction may have been implicated. However it is clear that many of those who had reactions had erythema nodosum leprosum, especially

as they describe in these patients concomitant iridocyclitis and orchitis.

They described a very varied duration and presentation of these "eruptions". Some had fever and malaise, and those with frequent reactions tended to have a poor prognosis. In some the skin lesions ulcerated and nerves became scarred with resultant anaesthesia. In some the "leprous affections" disappeared and the patient was healed from his leprosy though "this was not frequent". Confirmation of this was obtained at autopsy.

(2) Campos and de Souza (1954)

Probably the next important description of reactions in leprosy was that by Campos and de Souza (1954), (the first 50 years of the 20th century seem to be in general a "leprological dark age"). Their paper highlighted the state of confusion which existed at the time in regard to reactional states. However it is clear that they classified ENL in its own separate group, being apart from all other reactional states. The paper describes the reactional states which occur in tuberculoid and borderline leprosy, and it is probable that these would all come under the definition of borderline leprosy reaction in present day nomenclature.

They classified these reactions in 3 groups:

(i) Tuberculoid reactions. In the acute exacerbation occurring in the circinate variety of tuberculoid leprosy, pre-existing lesions became active, and somewhat increased in size, and new lesions might appear. Involvement of nerves was frequent though general disturbance was not caused.

The patients were bacteriologically negative and their lepromin test remained positive. Histologically there was tuberculoid granuloma and oedema.

(ii) Reactional tuberculoid (RTL). An acute condition characterised by the appearance of lesions of polymorphous aspect. On occasion these were the initial manifestations of the disease, though usually it constituted a development from indeterminate leprosy to tuberculoid. Clinically the lesions consisted of "tubercules", "nodular plaques" or "erythrodermic exanthoses". This state might progress to cure, or conversely towards the lepromatous end of the scale. The lesions were erythematous or erythematoviolaceous, and well demarcated from the surrounding skin. Involvement of the face, palmar and plantar surfaces was particularly characteristic. Smears were almost always positive, progressing to negativity. The lepromin test was usually negative initially, progressing to positivity. Histologically this type of reactional state was characterised by the presence of epithelioid cells, and intercellular and intracellular oedema. Giant cells were present in a variable number. Lymphocytes were usually present - without the usual tendency to form cuffs around the "epithelioid nodules".

(iii) Borderline group. Clinically this type of reactional state resembled RTL but the lesions were a slightly different colour - more yellowish, having a rather "rusty" colour. There were large plaques and nodules of varied sizes with borders sometimes well-defined and sometimes indefinite. These might embrace

(as a relapse eruption) an area where there was previously a reactional lesion. The new lesion surrounded the site of the old one and therefore its inside edge was well defined. Smears were consistently positive, and the lepromin test was negative. The histology of the lesions was difficult to interpret, being an intermediate stage between RTL and lepromatous leprosy. In general there was epithelioid cell granuloma, but bacilli were numerous. There might be some lymphocytes, but there were no giant cells.

It is probable in retrospect that these authors were describing the same immunological phenomenon as it presented throughout the borderline spectrum. Their description however of this picture is extremely accurate though the involvement of nerves in these reactions is barely mentioned.

(3) Tajiri (1955)

The other important account of that time was that of Tajiri (1955) who recognised that reactions could occur in both tuberculoid and lepromatous patients.

In discussing reactions in tuberculoid patients he emphasised that he was not considering such things as acute neuritic syndromes in neural cases but noted that 2 forms of reaction were recognised: reaction in established T cases and RTL. These followed the definition of Campos and de Souza (1954).

In lepromatous leprosy, reactions also occurred and these he classified into 3 groups:

(i) Acute lepromatous infiltration or "reactivation"

in which there was an active aggravation of infiltrations on nodules caused by an increase in histiocytes bearing bacilli. This was mainly a histopathological condition and was probably equivalent to the "exacerbation nodules" described by Ridley (1969).

(ii) Erythema nodosum leprosum, which he reported to be more frequent with the introduction of sulphones. These reactions were characterised by pain and other clinical manifestations and did not appear to be related to the course of the disease.

(iii) Acute infiltration syndrome. These occurred abruptly in lepromatous cases, sometimes at an early stage of the disease but more frequently after a prolonged period of "regression and resorption" of the disease without augmentation and aggravation of the pre-existing lepromatous lesions.

They were characterised mainly by an erysipelas-like eruption with hyperaemic lesions, with limited or extensive oedema associated. The face might become swollen overnight and there might be concomitant fever. Smears demonstrate bacilli, but fewer than one would expect in a lepromatous case. The lepromin test (Mitsuda) was converted from negative to positive during the reaction, and remained positive for some time subsequently. Histologically the lesions demonstrated an infiltration of lymphocytes and epithelioid cells, sometimes with giant cells and plasma cells. Prognostically there was variation. They might proceed to healing, or they might become lepromin negative again with the re-appearance of lepromatous nodules. Some

patients also suffered severe nerve damage.

It is probable from this account that the latter group were borderline leprosy reactions occurring in BL patients. Tajiri's account is thus similar to that of Campos and de Souza but they introduce the concept of "exacerbation nodules" as an extra group.

(4) Ridley (1969)

Probably the most important descriptive paper on reactions was that by Ridley (1969). However, it has been outdated to some extent by recent immunological research. Ridley recognised 4 types of leprosy reactions:

- (i) Downgrading reactions in borderline leprosy
- (ii) Reversal reactions in borderline leprosy
- (iii) Exacerbation nodules in lepromatous leprosy
- (iv) Erythema nodosum leprosum in lepromatous leprosy

Reactions in borderline leprosy. Downgrading and reversal reactions were considered under the same heading and the author conceded that they had similar clinical features with erythema, oedema of nerves and nerve involvement, though the former resulted in loss of immunity, and the latter in gain of immunity. Thus downgrading reactions tended to occur in the near-tuberculoid and borderline patients and in patients who were not receiving treatment, and reversal reactions tended to occur in near-lepromatous and borderline patients in patients on treatment.

However histologically there were differences when the reaction process was followed through sequentially. Both were characterised by intracellular and extracellular

oedema, but in reversal reactions the granuloma tended to increase in volume and become more typically epithelioid cell granuloma whereas in downgrading reactions there was a loss of focalisation of the granuloma with a subsequent increase in the number of bacilli in each section.

Reactions in lepromatous leprosy. Erythema nodosum leprosum differed from other reactions as ENL occurred not in major skin lesions but in small clinically inapparent lesions where there were few bacilli. However, the centre of reaction always appeared to coincide with the bacterial focus. The presence of polymorphs was the essential and predominant feature of the early stage of the reaction lesions and there was much cellular degeneration. Later there might be significant numbers of lymphocytes; vasculitis was prominent in some.

He described exacerbation nodules as more of academic interest than of practical importance. They occurred in patients with very active LL, usually in relapse. One lesion became exceptionally large and loaded with many times more bacilli than most other lesions: microscopic examination demonstrated heavy polymorph infiltration and a picture very similar to that in ENL.

(5) Waters et al (1971)

Finally, Waters et al (1971) described a study of the histology of lymph nodes in 15 patients with borderline leprosy reactions. (The histological appearance of the lymph nodes across the leprosy spectrum had previously been described by Turk and Waters (1971) who found that in lepromatous patients the paracortical areas were infiltrated

by undifferentiated cells of the histiocyte-macrophage series which failed to eliminate mycobacteria: as resistance to infection increased across the leprosy spectrum histiocytes became more differentiated eventually appearing epithelioid, and this was paralleled by increasing numbers of lymphocytes in the paracortical areas.) In general the lymph nodes of patients in reaction did not differ significantly from patients with the same classification in the spectrum without reaction, but in 2 patients with BL/LI leprosy partial repopulation of the paracortical areas with lymphocytes was seen, suggestive of regeneration of the cell-mediated immune response in these patients, the features becoming more suggestive of tuberculoid leprosy: this correlated with the bacteriological and histological observations.

From these accounts it was possible to surmise that there were two main types of reaction, borderline leprosy reactions, and erythema nodosum leprosum, though their pathogenesis remained unclear. However, this has been clarified to a considerable extent by subsequent immunological investigations.

b) PREVIOUS IMMUNOLOGICAL RESEARCH

Borderline leprosy reactions

Prior to our study there was only one important publication on the mechanisms of reactions in borderline tuberculoid leprosy, that of Godal et al (1973), which they described as a preliminary report. They studied 10 BT patients with reactions, using the lymphocyte transformation

test, the leucocyte migration inhibition test, and skin testing with lepromin. Seven patients had no previous treatment, and their reactions were the clinical presentation of their disease, each having erythematous swollen skin lesions which had appeared 2 - 7 months before they were examined in hospital. The other 3 patients had well established leprosy, two of these being on low doses of dapsone for about 1 year before the commencement of their reactions (low dosage dapsone was at that time recommended in an attempt to prevent reactions.) In addition, they studied 3 other patients with clinical and bacteriological evidence (increase in bacterial index) of movement from BT to BL leprosy; these patients were considered to be "downgrading", though there were no clinical manifestations of reaction.

Those patients with reaction, whether receiving treatment or not, had a strongly positive LT test and LMI test during the reaction, whether they were on treatment or not. Those who had evidence of "downgrading" had negative LT tests. They therefore concluded that patients whether receiving treatment or not, and whether BT or BL, who presented with signs of reaction were all having "reversal reaction" with greatly increased LT and LMI responses to M. leprae. They conceded that some patients if left untreated might subsequently become lepromatous and thus appear to have downgraded. They emphasised both that it would be paradoxical for a decrease in immunity to provoke an acute reaction, and also that the 3 patients who were downgrading were not in reaction and showed very different

immunological findings. They thus favoured the view that all borderline reactions were "reversal reactions", though the final outcome post-reaction might be "upgrading" or "downgrading". The important contribution of this paper is to separate the concept of "reaction" from that of "upgrading" or "downgrading".

Erythema nodosum leprosum

In contrast to borderline leprosy reactions, there has been a considerable amount of immunological research in ENL.

Wemambu et al (1969) first demonstrated deposits of immunoglobulin complement and soluble mycobacterial antigens in the skin lesions of ENL and concluded from this that ENL was an example of the Arthus phenomenon, due to immune complex formation.

Circulating immune complexes were also detected by Moran et al (1972), Rojas-Espinosa et al (1972), and Gelber et al (1974) though they were found also in patients with uncomplicated lepromatous leprosy. Moran et al (1972) and Gelber et al (1974) found that the levels of immune complexes circulating were higher in those with ENL, and it was suggested that the clinical manifestations of ENL were probably due to immune complex deposition.

However a recent paper by Bjorvatn et al (1976) has suggested that the skin manifestations of ENL may be due to extravascular formation of immune complexes, as no significant correlation between serum $^{125}\text{I}C1q$ binding activity and the C3d level in the serum was found, though patients with ENL and uncomplicated lepromatous leprosy all had evidence of circulating immune complexes.

This is an interesting observation, because the histology of ENL is not always typical of vasculitis as there are often dense sheets of polymorphs presenting a picture similar to that seen in experimental animals when complexes are inoculated directly into the skin (W.E. Parish, personal communication). It is likely however that circulating immune complexes are deposited at times, and this could account for some of the more unusual complications such as nephritis, where immune complex deposition has been shown to occur (Tin Schwe, 1972).

vii PROPOSED OBJECTIVES OF STUDY

It was planned to perform a prospective study of about 100 patients with borderline leprosy (of whom about 25% would be expected to develop reaction) in an attempt to clarify the mechanisms involved in borderline leprosy reactions. This was to be achieved by correlation of the clinical and histological observations with the immunological results.

It was considered important to confirm the report of Godal et al (1973) that patients in reaction had high LT responses to whole M. leprae, which suggested an increase in cell-mediated immune reactivity. It was also important to establish that these high responses coincided with the reaction, and were not present previously in these patients. By using "whole M. leprae" and "sonicated M. leprae" as antigens in the LT test it was hoped to define to some extent the nature of the mycobacterial antigens involved

in reaction.

The triggering mechanism for borderline leprosy reactions has not been elicited, but it was suggested by Godal et al (1973) that infection with other mycobacteria might be responsible. For this reason, LT tests to BCG and Mycobacterium duvalii should be performed also.

It was felt that it would be useful to study the LT responses to PHA in relation to reaction, and in particular to compare the responses in a standard serum pool with those in autologous plasma, as plasma factors might play some part in the reaction.

There had been no research into the role of humoral immunity, if any, in borderline leprosy reactions. In view of the numerous reports demonstrating T and B lymphocyte co-operation in experimental animals, it was considered important to estimate the levels of serum immunoglobulins and to detect the presence of antimycobacterial antibodies in the patients who developed reaction.

It had been suggested that the nerve destruction which follows these reactions might be due to auto-immune phenomena in a manner analagous to experimental allergic neuritis (Waksman and Adams, 1955) though there had been no research to support this hypothesis. For this reason, a study of auto-antibodies (anti-nuclear antibodies, anti-lymphocyte antibodies and neural auto-antibodies) should be included in the trial.

Finally the role of treatment in these reactions had to be elicited. It had always been taught that the dosage of dapsone was an important factor in triggering reactions,

though there was no good evidence for this in the literature. Patients were thus to be treated with two different regimes, half on dapsone 50mg daily, and half on dapsone 5mg daily on a random basis, in an attempt to clarify the situation.

PATIENTS AND METHODSPATIENTSINVESTIGATIONS

- a) Initial investigations.
- b) Subsequent follow-up investigations.
- c) Investigations during reactions.
- d) Investigations subsequent to reaction.

TREATMENTMETHODSIMMUNOLOGICAL TESTS

- a) Lymphocyte transformation tests.
- b) Serum immunoglobulin estimations.
- c) Detection of immunoglobulin G heterogeneity by agarose gel electrophoresis.
- d) Detection of anti-mycobacterial antibodies by crossed immunoelectrophoresis.
- e) Crossed immunoelectrophoretic analysis of immunoglobulin G.
- f) Detection of anti-nuclear antibodies.
- g) Detection of anti-lymphocyte antibodies.
- h) Detection of neural auto-antibodies.

STATISTICAL ANALYSIS

PATIENTS

Eighty-three patients attending the Addis Ababa Leprosy Hospital were included in the study; 47 were male and 36 female and their ages ranged from 12 to 61 years.

All had a clinical diagnosis of borderline leprosy, and this was supported by histological examination of a skin biopsy from an "active" lesion, though in some BT patients the histology was that of "indeterminate" leprosy.

Each patient was interviewed by the Medico-Social Unit of the Hospital, and the study included only those deemed to be registerable for treatment in Addis Ababa.

INVESTIGATIONS

- a) Initial investigations on entry to the study.

CLINICAL AND HISTOLOGICAL

1. History and Examination. A detailed history was taken, and a general medical examination was carried out.

Clinical drawings of the extent and type of skin lesions were made and the erythema and oedema of lesions were graded on arbitrary scales (Table 1). Photographs of the patients' skin lesions were also taken.

A detailed neurological examination was performed, and note was taken of the presence of enlarged and tender peripheral nerves.

TABLE 1 Scale for clinical grading of erythema and oedema of the skin lesions.

| Degree recorded | Appearance of lesions | |
|-----------------|---|--|
| | Erythema | Oedema |
| 0 | Hypopigmented | Flat |
| (+) | Slight erythema | Slightly raised edges |
| + | Marked erythema | Whole lesion raised |
| ++ | Marked erythema of lesions and the surrounding skin | Gross swelling and oedema, also affecting the surrounding skin |

2. Voluntary muscle tests (Goodwin, 1968) were carried out in the physiotherapy department to test the following nerves:
- (a) Ulnar nerves by testing: abductor digiti minimi, 1st dorsal interosseus, and 3rd and 4th lumbrical muscles.
 - (b) Median nerves by testing: abductor pollicis brevis, opponens pollicis and 1st and 2nd lumbrical muscles.
 - (c) Radial nerves by testing: extensor carpi radialis longus and brevis, extensor carpi ulnaris, extensor digitorum communis, extensor indices and extensor digiti minimi muscles.
 - (d) Facial nerves by testing: orbicularis oculi.
 - (e) Lateral popliteal nerves by testing: tibialis anterior, extensor digitorum longus, extensor hallucis longus and peroneus longus and brevis muscles.

The power of these muscles was graded on a 6 point (0 - 5) scale as follows:- 0 = no power, 1 = flicker of movement, 2 = movement unopposed, 3 = movement against gravity, 4 = slight weakness, 5 = full power.

3. Nerve conduction studies of the ulnar and median nerves were performed in those patients who would agree to the test (carried out by Dr. Naafs). It was considered that motor nerve conduction was abnormally slow below 55 m/sec. The methodology was described by Naafs et al (1976).

4. Skin smears for bacterial index (BI) and morphological index (MI) from at least 4 sites.
5. Biopsy of active skin lesion for accurate classification, and detailed histological examination with Haematoxylin and Eosin (H. & E.) and Triff (a mixture of Trichrome and Fite-Farraco stains which demonstrates both acid fast material and cellular structure)(Wheeler et al, 1965) stains were carried out. Biopsy specimens were halved, so that Dr. D.S. Ridley could give an independent histological classification on each patient.
6. Biopsy of one radial cutaneous nerve was carried out in a majority of patients. Histological specimens were stained with H. & E. and Triff stains.
7. Full blood counts, chest X-ray and urinalysis were performed to exclude other significant disease. (If these studies demonstrated the presence of other significant disease, the patient was not included in the trial).

IMMUNOLOGICAL

Blood was collected by venesection into 2 syringes where possible. The first syringe (30ml) contained 10I.U. of heparin (without preservative) per ml of blood, and this was used for the lymphocyte studies. The second syringe (20ml) contained no anticoagulant and was used for serum studies. In some where it was impossible to collect a second aliquot of blood, the plasma remaining

from the lymphocyte studies was converted to serum by incubation with 1% polybrene (heparin antagonist).

Studies on lymphocytes.

1. Lymphocyte transformation tests with M. leprae (whole and sonicated), whole BCG and sonicated M. duvalii used as antigens.
2. Lymphocyte transformation tests with PHA in standard serum, and autologous plasma.

Studies on serum

1. Serum immunoglobulins G, A and M.
2. Anti-mycobacterial antibodies.
3. Anti-nuclear factor (IgG).
4. Anti-lymphocyte antibodies.
5. Neural auto-antibodies.

b) SUBSEQUENT FOLLOW UP AND INVESTIGATIONS

CLINICAL

Each patient was reviewed by myself monthly or two-monthly. At review, a history of symptomatology was taken, and a medical examination performed, taking account of changes in skin lesions and enlargement and tenderness of peripheral nerves. Voluntary muscle testing was carried out each time in the physiotherapy department, by the same tester. At six-monthly intervals skin smears for BI and MI were repeated.

IMMUNOLOGICAL

Lymphocyte transformation tests and serum immunoglobulins were repeated at intervals of between 1 and 6 months

throughout the study. The frequency of testing varied on 2 counts:

- (a) Changing clinical picture in the patient, for example those with erythematous lesions, or nerve tenderness would be tested more frequently than those who were relatively "inactive".
- (b) The reliability and punctuality of the patient. LT tests had to be performed on a set day, and relatively early in the day.

c) INVESTIGATIONS DURING REACTION

Patients were informed of the symptoms of "borderline leprosy reactions" and encouraged throughout the trial to attend for review between appointments if they developed relevant symptoms: increasing erythema and oedema of skin lesions and/or severe pains and tenderness of the peripheral nerves.

On development of reaction, the patient was admitted to the Hospital under my care.

The following investigations were carried out:

Clinical and Histological

1. General medical examination, including drawings and photographs. The erythema and oedema of skin lesions were graded on arbitrary scales as previously described. Nerve involvement in the reaction was assessed by 3 criteria, at least 2 of which had to be satisfied for nerve involvement to be considered to be marked -
 - (a) clinical assessment of nerve tenderness and swelling, recorded as absent (0), slight (\pm), or marked (+);
 - (b) motor nerve conduction studies of

the ulnar and median nerves (below 55m/sec was regarded as abnormal); (c) voluntary muscle tests (sustained fall in muscle power in two or more muscles supplied by nerve indicated deterioration (+); lesser changes (0) were disregarded.

2. Skin smears from at least 4 sites for BI and MI.
3. Biopsy of an active skin lesion, for diagnosis of reaction, classification at the time, and detailed histological examination. The stains used were H. & E. and Triff, as before.
4. Biopsy of radial cutaneous nerve, usually the contralateral to the nerve biopsied at the inception of the study. Stains - H. & E. and Triff.

Immunological

The immunological studies during reaction were the same as those at the beginning of the trial. They were repeated weekly whilst the patient was in hospital during the reaction period.

d) INVESTIGATIONS SUBSEQUENT TO REACTION

Patients were seen monthly after reaction, or more frequently if their condition warranted it. Particular note was made in the history of occurrence of limb pains, weakness or changes in sensation, and on examination tenderness and enlargement of peripheral nerves were recorded. VMTS were carried out as before. A post-reaction skin biopsy was carried out 6 months to a year after reaction.

Lymphocyte studies were performed at intervals of 1 - 3 months after reaction, particularly when the patients' steroid therapy was stopped. Blood for serum studies was also taken at the cessation of steroid treatment.

TREATMENT

Antibacterial treatment

Following the initial investigations patients were started on treatment with dapsone. The first 15 patients on the trial were treated with dapsone 50mg daily orally. The remaining 68 received treatment with dapsone 5mg or 50mg daily orally on a random basis: 34 received 5mg and 34, 50mg daily. The tablets were issued monthly (or two monthly) when the patient was reviewed.

On review, patients were asked when they last took their tablets, and a specimen of urine was collected for estimation of the urine dapsone/creatinine ratio (Ellard et al, 1974) to assess regularity of therapy.

Treatment of reaction

Patients who developed reaction were treated initially with prednisolone 20-40mg daily, and the dosage was decreased as the clinical condition allowed. For symptomatic relief of their symptoms they received paracetamol for analgesia, and trimeprazine for sedation.

METHODSIMMUNOLOGICAL TESTS(a) LYMPHOCYTE TRANSFORMATION TESTS

The lymphocyte transformation test relies on the fact that when T lymphocytes are stimulated by mitogens, non-specifically, or antigens to which they have been specifically sensitized, they undergo mitosis. In doing so there is a marked increase in DNA synthesis, and consequently in thymidine incorporation. Thymidine may be labelled with tritium, and the radioactive uptake by lymphocytes during transformation may be measured in a scintillation counter.

LT testing was performed by a micro method in this study according to the technique described by Closs (1975) with a few modifications. Venous blood (30ml) was drawn into a disposable syringe containing 10I.U. of heparin per ml of blood (heparin without preservative: Ulleval Apotek, Oslo, Norway). The blood was mixed 2:1 with 0.9% saline and then layered onto Ficoll/Isopaque, density 1.077 (10 parts of 33.9% Isopaque [Nyegaard & Co A/S, Oslo, Norway] plus 24 parts of 9% Ficoll [Pharmacia AB, Uppsala, Sweden]) in 10ml centrifuge tubes according to the method originally described by Boyum (1968). The tubes were then centrifuged at 400 g, and the plasma/saline layer removed. This was kept at 0°C for use in those LT tests requiring autologous plasma, or stored at -20°C for conversion to serum with 1% polybrene in those patients where separate serum samples were not obtained. The lymphocyte layer was then removed from each tube and the lymphocytes washed twice with

Hanks balanced salt solution (Hanks BSS, Flow Laboratories, Irvine, Scotland). They were then resuspended in Hanks BSS at a concentration of 5×10^6 cells/ml.

The lymphocytes (25 μ l cell suspension) were cultured in triplicate in 200 μ l culture medium and 25 μ l antigen or mitogen in plastic trays for tissue culture (IS-FB-96-TC, Limbro Chemical Co., Newhaven Connecticut, USA). The culture medium consisted of TC 199 with Earles salts (Flow Laboratories, Irvine, Scotland) containing 50I.U. penicillin/ml and 50 μ g streptomycin/ml (BDH Chemicals Ltd., Poole, England) and 10% human AB serum. (This serum was a pool from healthy controls from a leprosy-non-endemic area). When LT responses to PHA were performed their responses in 10% human AB serum were compared with those in 10% autologous plasma. The trays were incubated at 37 $^{\circ}$ C in an atmosphere containing 5% CO₂ and 100% humidity. Control cultures without antigen or mitogen were also included in the trays. Mitogen stimulated cultures were harvested on the fourth day, whereas antigen stimulated cultures were harvested on the seventh day of culture. (Pilot studies had shown that when an optimal concentration of stimulant was used the peak response was obtained at those times.)

The cells were labelled with 0.5 μ Ci tritiated thymidine (3H-TdR) (Specific activity 2Ci/mmol: Radiochemical Centre, Amersham, England) 16 hours before termination of the cultures.

The trays were either harvested immediately or frozen and kept at -20 $^{\circ}$ C for some days. By using a specially

constructed harvesting device, the cultures were transferred by suction, 6 at a time onto glass fibre filters (Gelman type A, Gelman Instrument Co., Ann Arbor, Michigan, USA). Each well was then washed through with distilled water to complete the transfer of cells to the filters. The filters were transferred to counting vials and dried at 60°C for 1 hour.

5ml toluene based scintillator (5g 2,5-diphenyloxazole and 0.15g 1,4 bis (2) - (5-phenyloxazolyl) benzene (Koch Light Laboratories Ltd., Buckinghamshire, England) per litre of toluene) was added to each counting vial before estimation of ³H-TdR uptake in an Intertechnique SL31 scintillation counter (Intertechnique, Plaisire, France).

The recorded values for lymphocyte transformation by antigens or PHA were calculated as the mean of stimulated triplicate cultures, with the mean of unstimulated triplicates (i.e. those wells on the tray with no antigen or mitogen added) subtracted. The responses to antigens recorded in the text were the peak responses in the dose-response curve of antigen concentrations.

PREPARATION OF ANTIGENS AND MITOGEN FOR LT TESTS

ANTIGENS

- (i) M. leprae. The same antigen preparations were used in all tests and were derived from a large subcutaneous histoid nodule (Wade, 1963) from a patient with lepromatous leprosy. The nodule was cut finely with scissors in a Petri dish, and ground in a glass homogeniser in ice cold phosphate-buffered saline (PBS),

pH 7.2, with 1% human serum albumin (HSA) (AB Kabi, Stockholm, Sweden). The homogenate was centrifuged at 150g for 5 minutes. The supernatant was again centrifuged at 2500g for 1 hour and the pellet washed twice in cold PBS with HSA. After resuspension the volume was adjusted to 1×10^9 acid fast bacilli (AFB) per ml in PBS-HSA. The batch was subdivided into small aliquots and stored at -70°C until required. This preparation was termed "whole washed" M. leprae.

Half of the batch was ultrasonicated in a Branson Sonifier B-12 (Branson Sonic Power Co., Danbury, Connecticut, USA) with 80watt output on the medium-sized tip for 1 hour, until no acid fast bacilli (AFB) remained. This preparation was termed "sonicated" M. leprae and was subdivided and frozen as described for "whole washed" M. leprae.

In all tests both M. leprae antigen preparations were added at three concentrations standardised to the bacillary content of the original homogenate: that is 10^9 , 10^8 and 10^7 bacilli/ml.

(ii) Mycobacterium tuberculosis bovis, strain BCG (BCG).

LT responses to BCG in all patients were also estimated. The whole antigen preparation was used because it was felt that membrane antigens of BCG were more important to assess previous sensitisation to M. tuberculosis than cytoplasmic antigens which would be represented in the sonicated preparation, (T. Godal, personal communication).

Dried BCG vaccine (Glaxo, Greenford, England) was

resuspended in 0.9% saline to give a concentration of 10^9 AFB/ml and this suspension was kept at -20°C until required. It was added to the lymphocyte cultures at concentrations 10^9 , 10^8 and 10^7 bacilli/ml.

(iii) Mycobacterium duvalii (M. duvalii).

LT responses to M. duvalii were also assessed.

M. duvalii is a fast-growing scotochromogenic organism, of which to date only 4 strains have been cultured, all originating from cases of lepromatous leprosy. The organisms have not been reported from studies of environmental mycobacteria, suggesting that natural acquisition of protection from leprosy is unlikely to be common. M. duvalii is similar to M. leprae antigenically, and lepromatous tissues may be particularly prone to colonisation with M. duvalii as a consequence of cross-protection provided by the patients' specific inability to destroy M. leprae.

The LT test was carried out with the sonicated preparation, which contained both membrane and cytoplasmic antigens. The M. duvalii strain was obtained from Dr. J. Stanford, Middlesex Hospital, London, England and cultured on Sauton medium solidified with 1.5% agar. The bacilli were harvested in the log phase, washed three times in 0.9% saline and resuspended at a concentration of 10^9 bacilli/ml in saline. This preparation was then subjected to ultrasonication in the Branson Sonifier B12 for 1 hour after which time no AFB remained in the suspension. This suspension was termed "sonicated" M. duvalii, and added to lymphocyte cultures at equivalent concentrations of 10^9 , 10^8 and 10^7 bacilli/ml.



MITOGENPhytohaemagglutinin (PHA)

PHA (Reagent grade, Wellcome, Beckenham, England) was stored at -20°C until use. Lymphocytes were cultured with a final concentration of 10^{-2} from stock, which was found to be optimal in previous experiments.

(b) SERUM IMMUNOGLOBULIN ESTIMATIONS

Serum IgG, IgA and IgM were quantified by the radial immuno-diffusion method described by Mancini et al (1965). In this test, antisera to the Igs are incorporated into a thin layer of agarose, and the serum samples containing the Igs, which act as antigens, are placed in wells cut into the agarose. As the antigen diffuses radially, a ring of precipitation forms around the well and moves outwards, eventually becoming stationary at equivalence. At equivalence the diameter of the ring is related to the antigen concentration in the well. Using standard antigen concentrations run simultaneously a calibration curve may be constructed to determine unknown concentrations of the same antigen.

Serum specimens from patients were taken when patients were first seen (baseline) and at intervals during the trial. (In some instances Ig determinations were carried out using plasma from heparinised blood, diluted 2:1 in saline as for lymphocyte separation. Appropriate correction for dilution was made in these cases.) Those patients who developed reactions had serum specimens taken during

reaction (at initiation of steroid therapy), and post-reaction (on completion of their steroid therapy)). The serum specimens were stored at -70°C until assayed. (One baseline serum sample of a "reaction" patient was lost).

Serum IgG, IgA and IgM levels were measured by addition of 7 μl diluted serum (dilutions: IgG, 1:50; IgA, 1:10; IgM, 1:5) to the wells in the agarose diffusion plates (2% agarose in barbital buffer (sodium barbital 200gm, barbital 40gm, sodium azide 10gm, in distilled water, to a total volume of 10 litres)) containing specific antisera to these immunoglobulins (Dakopatts A/S, Copenhagen, Denmark) and compared with antigen reference standards obtained commercially (Behring-werke Standard Serum 974H (containing 1380mg/100ml IgG, 250mg/100ml IgA and 108mg/100ml IgM), Behring-werke A.G. Marburg/Lahn, Germany).

(c) DETECTION OF IMMUNOGLOBULIN G HETEROGENEITY BY AGAROSE GEL ELECTROPHORESIS

This test depends on the fact that protein molecules bear different electrical charges. Thus subjected to an electric field serum, proteins will separate in agarose gels into albumin, α_1 , α_2 , β and γ globulins. Most humoral antibodies in leprosy belong to the IgG class: IgG forms an electrophoretically heterogeneous group and gross changes in the polyclonal Ig pattern may be detected by this method (Laurell, 1972). Serum from 6 reaction patients was tested at baseline, during reaction and post-reaction (on stopping steroid therapy).

Electrophoresis plates (size, 205 x 110 x 1.5mm) were

filmed with 1% agarose (Litex, Glostrup, Denmark) in barbital buffer (pH 8.6) and slits were made in the gel at one edge. Patients' serum was diluted 1:1 in 0.1% bromophenol blue in barbitone buffer, and 7 μ l placed into each slit with a micro-burette syringe (Hamilton, Micromesure, B.V., Hague, Holland). Electrophoresis was performed in electrophoresis apparatus as described by Laurell (1972) with a potential difference of 3v/cm for a period of 3 hours. After that time saturated picric acid solution was added for 15 minutes, and then the excess of the solution removed with 96% ethanol; the gel was dried, and stained with Coomassie brilliant blue in a dye tray.

(d) DETECTION OF ANTI-MYCOBACTERIAL ANTIBODIES BY CROSSED IMMUNO-ELECTROPHORESIS

In this method, first described by Laurell (1965) and modified by Axelsen et al (1974), two steps are involved: first mycobacterial antigens (from sonicated M. leprae) are separated by means of agarose gel electrophoresis; the second step is electrophoresis in a dimension at right angles to the first dimension, forcing the separated mycobacterial antigens into a bed of human serum. The precipitin lines so formed in the agarose enable detection and identification of the antibodies in the patients' serum. The serum of all patients in the study was tested initially for antimycobacterial antibodies, and the serum of those who developed reaction was tested also during reaction and post-reaction, on completion of steroid therapy.

The first dimension plate was prepared by dissolving agarose 1% in barbital buffer, pH 8.6, and pouring 20ml onto an electrophoresis plate. Holes were punched at intervals along one edge of the plate, and 8 μ l of antigen (soluble preparation of M. leprae prepared by ultrasonication in a Branson Sonifier B12, with insoluble residues removed by centrifugation) was added to each hole with a micro-burette syringe (Hamilton, Micromesure BV, Hague, Holland). The plate was then placed in the electrophoresis apparatus with the holes containing antigen adjacent to the cathode, and electrophoresis carried out for 2 hours with a potential difference of 5v/cm. After that time the agarose of the first dimension plate was cut into 2cm strips in a direction parallel to that of the electrophoresis and added to the second dimension plate, which had been filmed with 20% serum in 1% agarose (from each patient separately) with the first dimension strip adjacent to the cathode, the two being separated by a strip containing 2ml barbital buffer in 1% agarose. Electrophoresis in the second dimension was continued for 20 hours with a potential difference of 3v/cm. The plate was then immersed in PBS containing 0.02% sodium azide (PBSA) for 24 hours, rinsed in distilled water, dried, stained with Coomassie brilliant blue and then destained in a methanol/acetic acid mixture.

(e) CROSSED IMMUNOELECTROPHORETIC ANALYSIS OF IMMUNOGLOBULIN G

A prerequisite for quantitative immunoelectrophoresis is that the antigen migrates at a pH at which the antibodies

do not migrate. In agarose gel, native antibodies are non-migrating at pH 8.6, as the tendency for anodal migration at this pH is largely counterbalanced by the endosmotic flow which is directed against the cathode. It has been shown by Bjerrum et al (1973) that antibodies which do not move electrophoretically at a pH between 8.6 and 4.5 may be prepared for immuno-electrophoresis by carbamylation: in this process amino groups are converted to carbamylamino groups by reaction with cyanate. These groups are virtually non-basic and so the iso-electric point of the antibodies will be lowered. Provided the carbamylation is carried out under carefully selected conditions, the antibody titre and the avidity are not affected to any appreciable degree.

Thus using carbamylated rabbit anti-serum, qualitative and quantitative analysis of IgG in human patients can be carried out. Bjerrum et al (1973) showed that to make the electrophoretically heterogenous serum IgG population migrate in the same direction in agarose gel electrophoresis, the pH must be lowered to 5.0. Consequently to quantitate all classes of native IgG by immunoelectrophoresis an antiglobulin with a migration rate of zero at pH 5.0 is required, and as rabbit antibodies are non-migrating at pH 8.6, the iso-electric point has to be drastically lowered.

Most antimycobacterial antibodies in humans belong to the class IgG. The above principle was used on the serum of 6 reaction patients at baseline, during reaction and post-reaction, to assess whether the rise in IgG during

reaction was monoclonal, due to formation of new specific antibody, or polyclonal, due to a non-specific increase in antibody formation.

10ml of rabbit anti-IgG antiserum was mixed with 10ml saturated ammonium sulphate in PBSA. The precipitate was recovered by centrifugation at 7700g and this was washed 4 times, and then dissolved in 6ml PBSA in dialysis tubes. Dialysis was continued for 48 hours, with 2 changes of PBSA during that period. Carbamylation was carried out with 160mg potassium cyanate incubated at 45°C for 4 hours, and following this dialysis with PBSA was repeated for 48 hours. The contents of the dialysis tube were then ultracentrifuged at 80,000g in a Beckman Spinco ultracentrifuge and the supernatant collected and stored at -20°C.

The first dimension of immunoelectrophoretic analysis of patients' serum was performed with a potential difference of 5v/cm for 3 hours. The second dimension of electrophoresis which caused the patients' serum to migrate into the zone of carbamylated rabbit anti-human globulin was carried out with a potential difference of 3v/cm, though anode and cathode were reversed (as human IgG migrates towards the cathode at pH 5.0). The gel was then pressed, washed, dried, stained and destained as described previously.

(f) DETECTION OF ANTI-NUCLEAR ANTIBODIES

Antibodies reacting with components of nuclei can be most readily detected by indirect immunofluorescence, using a variety of tissues. Anti-nuclear antibodies may be of the

IgG, IgA and IgM classes though it is usually the IgG antibodies which are clinically significant. In this test, mouse liver was used as substrate and IgG anti-nuclear antibodies were sought using the method originally described by Holborow et al (1957). The serum of all patients was tested initially for anti-nuclear antibodies, and in the patients who developed reaction the test was repeated on serum taken during reaction and post-reaction.

Frozen sections of mouse liver were dried in air on slides. 25 μ l of patients' serum diluted 1:5 in PBSA, was dropped onto each section and the preparation incubated in a moist chamber for 45 minutes. After washing 3 times in PBSA, 25 μ l of a 10% dilution of anti-human IgG (State Bacteriological Laboratories, Stockholm, Sweden) labelled with fluorescein isothiocyanate was dropped onto each section, and the preparation was again washed with PBSA, and dried in air. The sections were mounted in PBS-glycerine and the presence of anti-nuclear antibodies assessed by fluorescent staining of the liver nuclei in comparison with positive control serum using a Leitz Orthoplan Fluorescence microscope. The positive control serum was obtained commercially (State Bacteriological Laboratories, Stockholm, Sweden) and diluted 1:6 and 1:22 in PBSA.

(g) DETECTION OF ANTI-LYMPHOCYTE ANTIBODIES

The presence of anti-lymphocyte antibodies can be detected by a cytotoxicity test, using human lymphocytes

in the presence of complement. The method used was a micro-method, originally described by Terasaki and McClelland (1964) and modified by Axelsson (1975). Serum from 16 of the reaction patients was tested at baseline, during reaction and post-reaction, having been stored at -20°C .

Paraffin oil was dropped into rings of a micro tray (Moller Coats A/S, Moss, Norway), and $1\mu\text{l}$ of serial doubling dilutions of serum added to each ring with a micro-burette syringe (Hamilton, Micromesure, Hague, Holland). To this was added $1\mu\text{l}$ of human lymphocytes (concentration: 2×10^6 cells/ml in Hanks BSS (Flow Laboratories, Irvine, Scotland)) and the tray incubated for 30 minutes at 20°C . Then $1\mu\text{l}$ of guinea pig complement was added and the mixture incubated for 10 minutes at 20°C . After this time $1\mu\text{l}$ EDTA (final concentration: $0.4\mu\text{g/ml}$) was added and the mixture incubated at 20°C for 10 minutes. Finally $1\mu\text{l}$ 2% Trypan blue in saline was added and the trays incubated for 30 minutes at 20°C .

The degree of cytotoxicity of patients' serum was assessed by counting 200 lymphocytes with an inverted microscope and estimating the percentage of cells which had absorbed Trypan blue. These were compared with positive control serum (rabbit anti-lymphocyte antiserum), and negative control serum from the healthy lymphocyte donor.

(h) DETECTION OF NEURAL AUTO-ANTIBODIES

This test was kindly carried out by Dr. D.J.M. Wright, Charing Cross Hospital, London, on reaction patients' serum at baseline, during and post-reaction by the method

described by Wright et al (1975).

STATISTICAL ANALYSIS

Non-parametric methods of statistical analysis were used for all results of the study, except where otherwise stated. (Where results are shown in scattergrams in the text, medians are indicated.) Statistical significance of differences between groups was estimated with the Wilcoxon Matched-Pairs-Signed-Ranks test when observations were paired, and by the Wilcoxon Rank-Sum test when observations were not paired (Wilcoxon, 1945). These tests were used throughout, except where otherwise stated. Differences were considered significant when the probability of their occurring by chance was less than 5% ($p < 0.05$).

OBSERVATIONS AND RESULTS

CLINICAL OBSERVATIONS

1. REACTION PATIENTS

- (a) CLINICAL FEATURES OF REACTION
 - (i) Clinical presentation
 - (ii) Timing of reaction after initiation of treatment with dapsone
 - (iii) Duration of reaction and prednisolone therapy
- (b) DAPSONE DOSAGE AND INCIDENCE OF REACTION

2. ALL PATIENTS IN STUDY

- (a) Importance of intermittent "limb pains" as a symptom of disease activity
- (b) Regularity of dapsone intake by patients

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HISTOLOGICAL OBSERVATIONS

- (a) Histology of skin and nerve in reaction patients
- (b) Variation between initial clinical and histological diagnosis
- (c) Disparity of bacilli in skin and nerve

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IMMUNOLOGICAL RESULTS(a) LT RESPONSES TO ANTIGENS

1. Reaction patients
2. Initial responses to M. leprae in all patients
3. Non reaction patients

(b) LT RESPONSES TO MITOGEN (PHA)

1. Non reaction patients
2. Reaction patients

(c) SERUM IMMUNOGLOBULINS

1. Reaction patients
2. Non reaction patients

(d) ANTI-MYCOBACTERIAL ANTIBODIES

1. Reaction patients
2. Non reaction patients

(e) ANTI-NUCLEAR ANTIBODIES(f) ANTI-LYMPHOCYTE ANTIBODIES(g) NEURAL AUTO-ANTIBODIES

CLINICAL OBSERVATIONS1. REACTION PATIENTS(a) CLINICAL FEATURES OF REACTION(i) Clinical presentation

Of the 83 patients in the study 17 developed reaction. However it was noted that there was considerable variability in clinical presentation:

- (1) Six developed reactions with skin involvement only, with only slight tenderness of nerves (if any) and no deterioration in VMTS or nerve conduction velocities:- "SKIN REACTIONS".
- (2) Five had erythema and oedema of skin lesions together with marked swelling and tenderness of nerves, with evidence of deterioration of VMTS and nerve conduction velocities as previously defined:- "MIXED REACTIONS".
- (3) Six had no evidence of change in skin lesions, but developed severe tenderness and swelling of their nerves with deterioration of VMTS and nerve conduction velocities:- "NERVE REACTIONS".

The clinical manifestations in these patients are summarised in TABLE 2. Five of the patients with skin reactions were BT, and one BB. Four of the patients with mixed reactions were BT, and one BB. Three of the patients with nerve reactions were BT, and three BL. It was thus seen that BT patients tended to develop skin and mixed reactions, and BL patients to develop nerve reactions. A patient with skin reaction is shown in Fig. 1, and with nerve reaction in Fig. 2.

TABLE 2 Clinical manifestations in 17 patients in reaction

| Case No | Clinical classification | Clinical assessment | | | | | Reaction |
|---------|-------------------------|---------------------|-------|----|---|-------|----------|
| | | Skin | Nerve | | | | |
| | | | C | N | M | | |
| 1 | BT | 2+ | 0 | ND | 0 | Skin | |
| 2 | BT | 2+ | 0 | 0 | 0 | Skin | |
| 3 | BT | 2+ | 0 | 0 | 0 | Skin | |
| 4 | BT | 2+ | 0 | 0 | 0 | Skin | |
| 5 | BT | 2+ | ± | 0 | 0 | Skin | |
| 6 | BB | 2+ | + | 0 | 0 | Skin | |
| 7 | BT | 2+ | + | + | + | Mixed | |
| 8 | BT | 2+ | + | ND | + | Mixed | |
| 9 | BT | + | + | ND | + | Mixed | |
| 10 | BB | 2+ | + | + | + | Mixed | |
| 11 | BT | 2+ | + | + | 0 | Mixed | |
| 12 | BT | 0 | + | + | + | Nerve | |
| 13 | BL | 0 | + | + | + | Nerve | |
| 14 | BT | 0 | + | + | + | Nerve | |
| 15 | BL | 0 | + | + | + | Nerve | |
| 16 | BT | 0 | + | + | + | Nerve | |
| 17 | BL | 0 | + | ND | + | Nerve | |

C = Clinical assessment

ND = Not done

M = Voluntary muscle testing

N = Motor nerve conduction velocity studies

SKIN REACTIONS: the BT patients who developed skin reactions tended to do so slowly, with a gradual increase in the erythema and swelling of previously hypopigmented lesions. This period varied between 1 month and 1 year. In the BB patient progress from initial symptoms of the disease to reaction occurred within a month. (The BB patient with a mixed reaction also developed the reaction quickly - within 2 months of initial clinical manifestations.)

MIXED REACTIONS: four of the patients who developed reaction developed erythema and swelling of their skin lesions first, followed by neuritis. In the remaining patient who initially had BT leprosy clinically, with an indeterminate histological picture, new erythematous lesions developed together with neuritis.

NERVE REACTIONS: all occurred in BT patients with indeterminate histology, or in patients with BL leprosy. (One of the latter had the histology of LI leprosy.) The development of reaction was relatively sudden in all patients, with a crescendo of limb pains and deterioration of voluntary muscle tests, particularly of the ulnar and median nerves, accompanied by deterioration of nerve conduction velocities of the ulnar and median nerves. In 1 patient, nerve reaction followed an episode of ENL.

(ii) Timing of reaction after initiation of treatment with dapsone

Of the 17 patients, 6 developed reaction during the first month of treatment with dapsone, and a further 5

during the second month of treatment. Four developed reaction in the period 2 - 6 months after commencement of treatment and only 2 of the 17 patients developed reaction after that time, at 7 months and 9 months (though the latter patient had only had very intermittent treatment due to irregular re-attendance at the hospital.) There was no evidence that skin reactions developed earlier than nerve reactions, or vice versa.

(iii) Duration of reaction and duration of prednisolone therapy

The period of reaction was very variable, though nerve reactions tended to be more transient than skin reactions. In mixed reactions neuritis tended to be ameliorated with prednisolone therapy more quickly than erythema and swelling of skin lesions.

Patients required treatment with prednisolone for a period varying between 2 months and 15 months. They required an initial dosage of between 20 and 40mg daily to control the reaction. No patient developed permanent deformity as a result of reaction whilst in the study, provided they were treated with prednisolone immediately. (Two patients who developed ulnar paralysis as a result of borderline leprosy reaction were away from Addis Ababa at the time and thus could not be treated with prednisolone immediately.)

(b) DAPSONE DOSAGE AND INCIDENCE OF REACTION IN FINAL 68 PATIENTS

Of those receiving dapsone 50mg daily, 26 had BT leprosy and 8 BL: 18 were male and 16 female. Of those receiving 5 mg daily, 24 had BT leprosy, 3 BB and 7 BL: 22 were male and 12 female. Borderline leprosy reactions developed in 14 of the 68 patients, whose dosage of dapsone had been randomised. Eleven of the patients were receiving 5mg of dapsone daily, and 3 were receiving 50mg (see Table 3). There is a significant difference between the 2 groups ($p < 0.025$) using the Chi-square test. Ten of the group receiving 50mg daily dose had erythematous skin lesions and reactions might have been expected to develop in these patients: however reactions developed in only 2. Of the group receiving dapsone 5mg daily, 9 had erythematous skin lesions and reactions developed in 8.

TABLE 3 Clinical data of 68 patients treated with dapsone 5mg or 50mg daily

| Clinical data | Number of patients receiving treatment with dapsone | | | |
|--|---|-------------|------------|-------------|
| | 5mg daily | | 50mg daily | |
| | Reaction | No Reaction | Reaction | No Reaction |
| Skin lesions "inflamed" at start of trial | 8 | 1 | 2 | 8 |
| Skin lesions "quiescent" at start of trial | 3 | 22 | 1 | 23 |
| TOTAL | 11 | 23 | 3 | 31 |

2. ALL PATIENTS IN STUDY

(a) Importance of intermittent "limb pains" as a symptom of disease activity

A majority of patients in this study presented themselves for treatment because of "limb pains" probably associated with neuritis. Only 5 patients tolerated these pains for longer than a year, though many had observed hypopigmented lesions on their skins for much longer (up to 14 years).

Following treatment the incidence of limb pains became less as time progressed, only 7 patients having pains at the end of 1 year, and 4 having pains by the end of the trial (the period varied between 1 and 2 years). It was noted that in the 68 patients who had the dosage of their dapsone randomised, fewer patients on 50mg daily had pains throughout the trial (see Fig. 3).

(b) Regularity of dapsone intake by patients

Estimation of the dapsone/creatinine (D/C) ratio in the urine was carried out when each patient was reviewed.

From those patients who were taking dapsone 50mg, it was estimated that 36% of observations were low (D/C ratio < 30), of which 8% were very low (D/C ratio < 10). (Those patients taking dapsone 5mg daily all had much lower D/C ratios due to the low drug dosage: it was therefore impossible to assess regularity of drug ingestion in these patients.) None of them however gave evidence of taking higher than prescribed dosage.

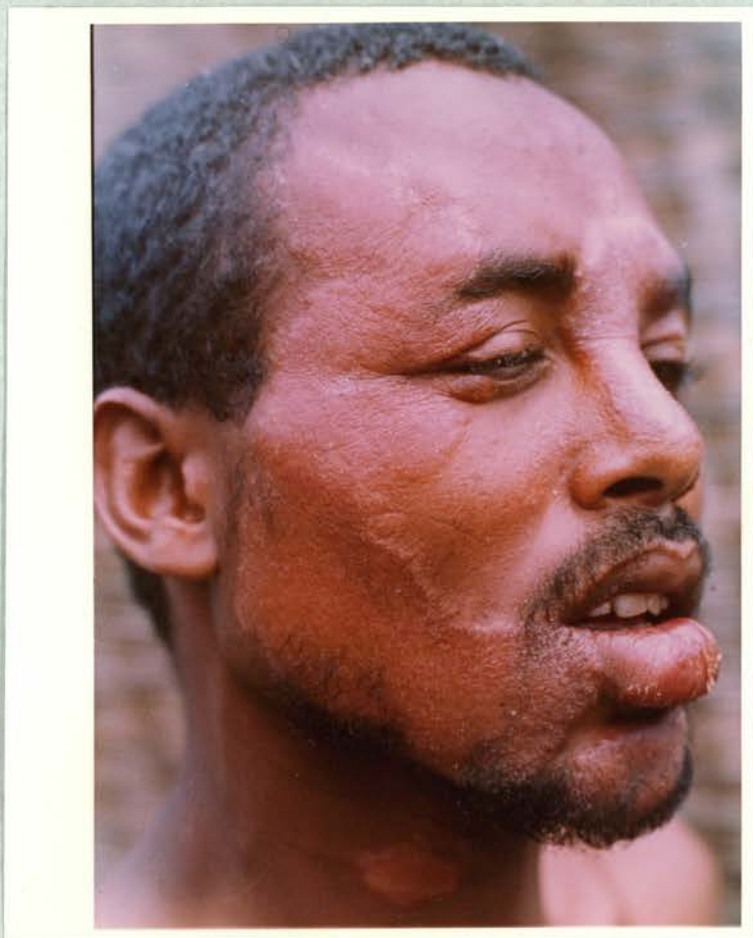


Fig. 1 Patient with BT leprosy with a "skin reaction". He had marked swelling and erythema of skin lesions but no neuritis.

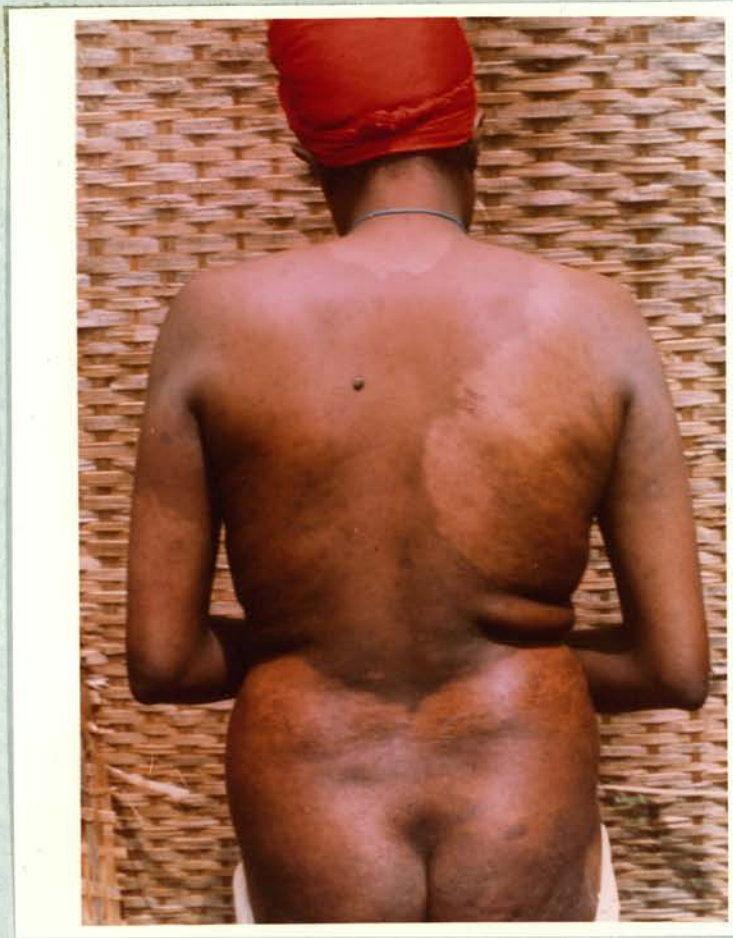


Fig. 2 Patient with BT leprosy with a "nerve reaction". She had severe generalised neuritis, with tenderness and swelling of peripheral nerves, but no change in her hypopigmented skin lesions.

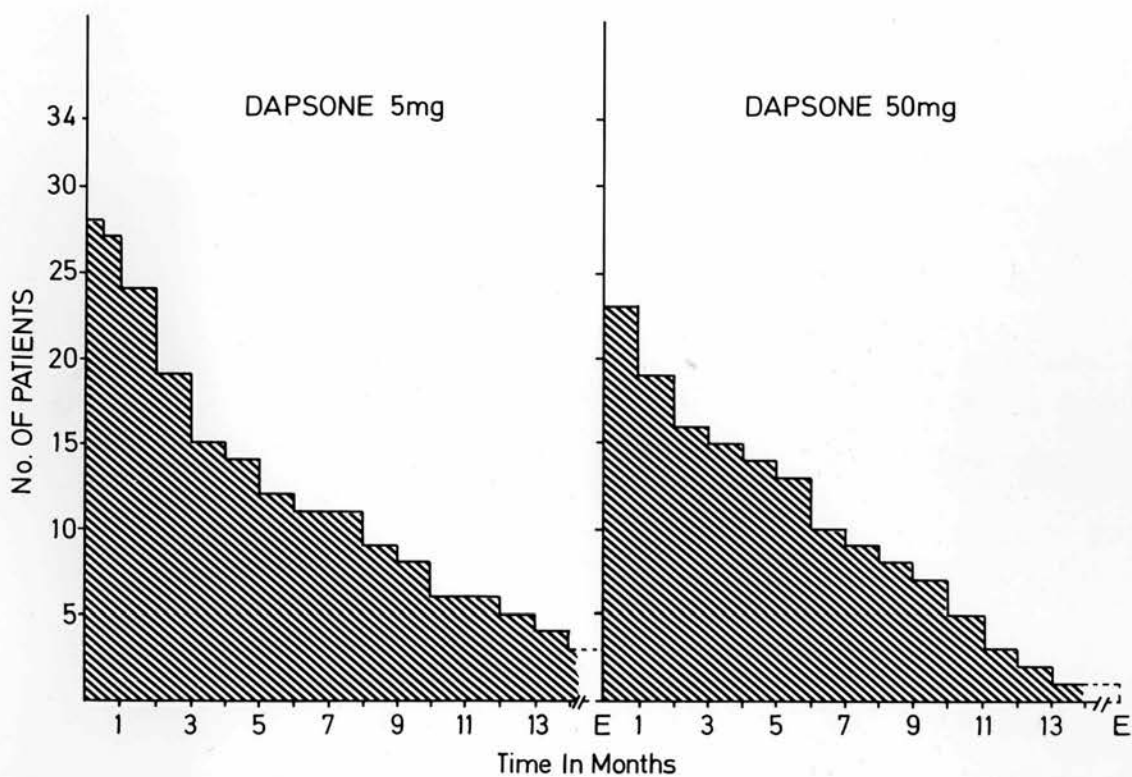


Fig. 3 Duration of limb pains due to neuritis following commencement of treatment of dapsone 5mg or 50mg in the 68 patients included in the study of the comparison of dapsone dosage to the incidence of reaction.

(E = termination of study).

HISTOLOGICAL OBSERVATIONS(a) Histology of skin and nerve in reaction patients

Of those 17 patients who developed borderline reactions, 12 were BT clinically, 2 were BB and 3 BL. Histological examination of initial skin biopsies confirmed the clinical diagnosis in each case though one of the BL patients was graded LI, and 4 of the BT patients had an indeterminate histology.

When the patients developed reaction, those with "skin" and "mixed" reactions all developed a more tuberculoid picture in skin biopsies, with increase in volume of epithelioid cell granuloma, infiltration of the subepidermal zone and erosion of the epidermis. Three of these "upgraded" histologically, 2 from BT to TT and 1 from BB to BT. In 8 of these there were large dermal nerves deeper in the dermis within inflammatory infiltrate, suggesting that they were actively involved in the reaction process. In those with nerve reactions, there was no change in the histology of skin lesions (though one patient showed evidence of the ENL which preceded his borderline leprosy reaction), but biopsy of peripheral nerve showed dense infiltration in each case, with some epithelioid cell granuloma formation usually related to perineurium.

Skin biopsies were repeated between 6 months and 1 year after reaction, and in each case the histological appearance had become more indeterminate.

(b) Variation between initial clinical and histological diagnosis

When the 83 patients were classified clinically 59 were BT, 3 BB and 21 BL and this was confirmed histologically on skin biopsy. The BT group was further split according to histological findings into TT, BT and Indeterminate (Idt) subgroups, and the BL group into BL and LI subgroups. (See Table 4)

TABLE 4 Number of patients in the study which fall into the different groups of the leprosy spectrum when classified according to clinical and histological criteria respectively.

| Classification | |
|----------------|---------------------------|
| Clinical | Histopathological |
| BT 59 | TT 10 BT 25 Idt. 24 |
| BB 3 | BB 3 |
| BL 21 | BL 9 LI 12 |

Twenty-four patients, though BT clinically, had an Idt histology: 22 of them had nerve biopsies, 16 of which showed typical BT histology with epithelioid cell granuloma; 6 of them also contained demonstrable AFBs.

It was therefore seen that both BT and BL groups demonstrated a histological spectrum. This was particularly

true of the BT patients: those with TT histology (see Fig. 4) who clinically had erythematous skin lesions composing one end of the spectrum, and those with Idt histology with linear subepidermal infiltration (see Fig. 5), and featureless hypopigmented skin lesions the other. It was found that there was a significant difference in the responses to whole and sonicated M. leprae between these two groups (see page 81). There was no significant difference between those with BL and LI histology in the responses to these antigens.

(c) Disparity of bacilli in skin and nerve

In all BL patients, AFBs were seen in both dermal infiltration, dermal nerves and peripheral nerve.

In the BT and BB patients there was often a marked disparity between the presence of AFBs in skin infiltrate and nerve tissue. Of 43 patients (BT and BB) who had skin and nerve biopsies 3 had AFBs in the dermal infiltrate, though 14 had bacilli in the dermal nerves and 13 in peripheral nerves.

The most prominent example of this bacillary disparity between skin and nerve tissue is shown in Figs. 6 and 7. In skin there is a dense round cell infiltrate with one granular AFB within a histiocyte; however in nerve there are many AFBs within the Schwann cells and there is no surrounding infiltrate.

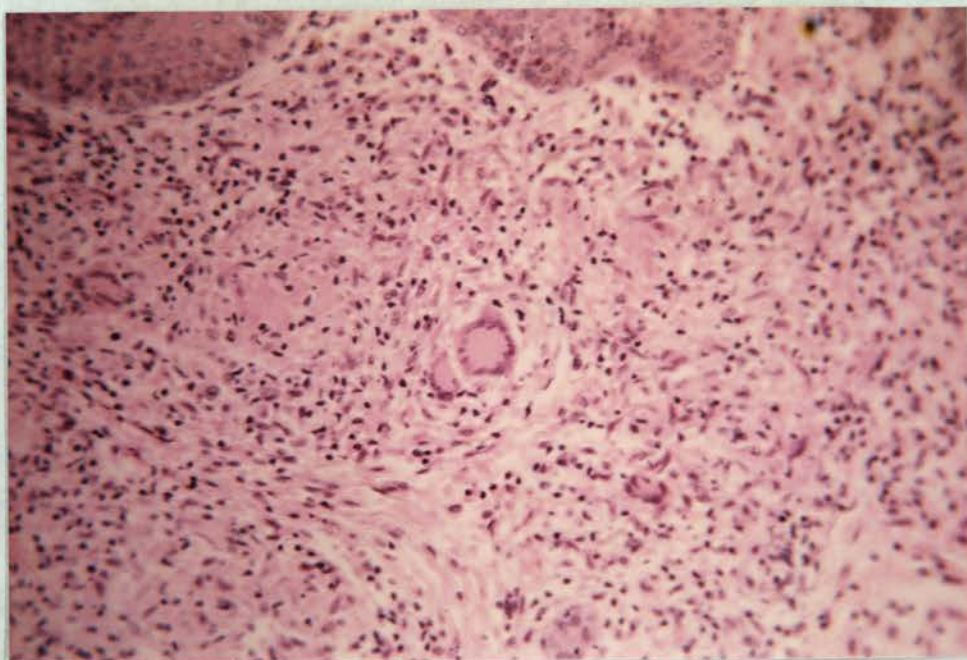


Fig. 4 BT case clinically with erythematous lesions. Dense epithelioid cell granuloma is seen throughout the dermis, with infiltration of the subepidermal region and erosion of the epidermis. Haematoxylin and eosin x 200

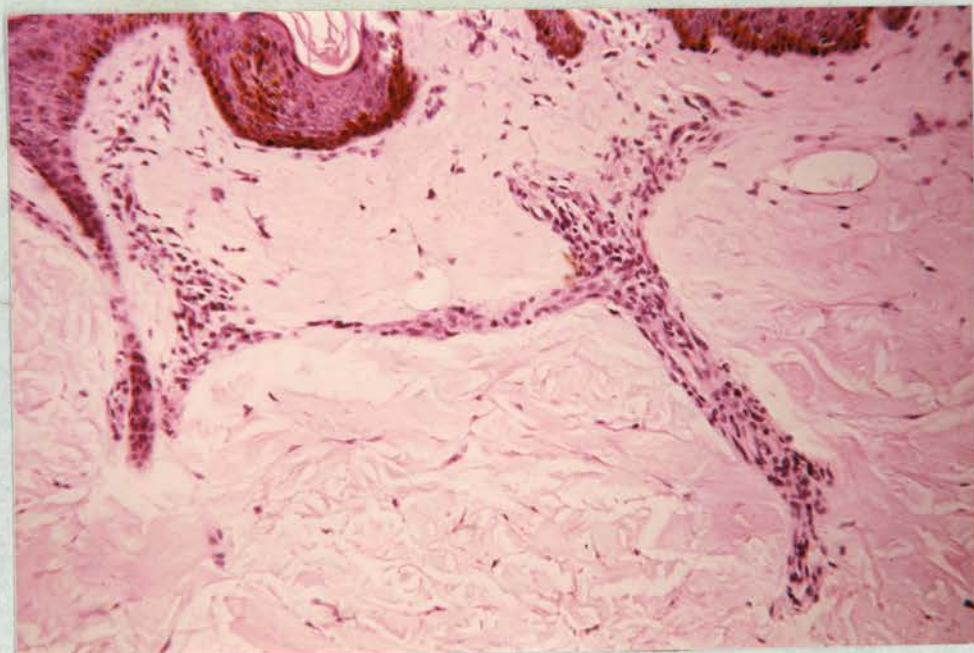


Fig. 5 BT case clinically with hypopigmented lesions. Scanty linear subepidermal infiltrate, without epithelioid cell granuloma formation. Haematoxylin and eosin x 200.

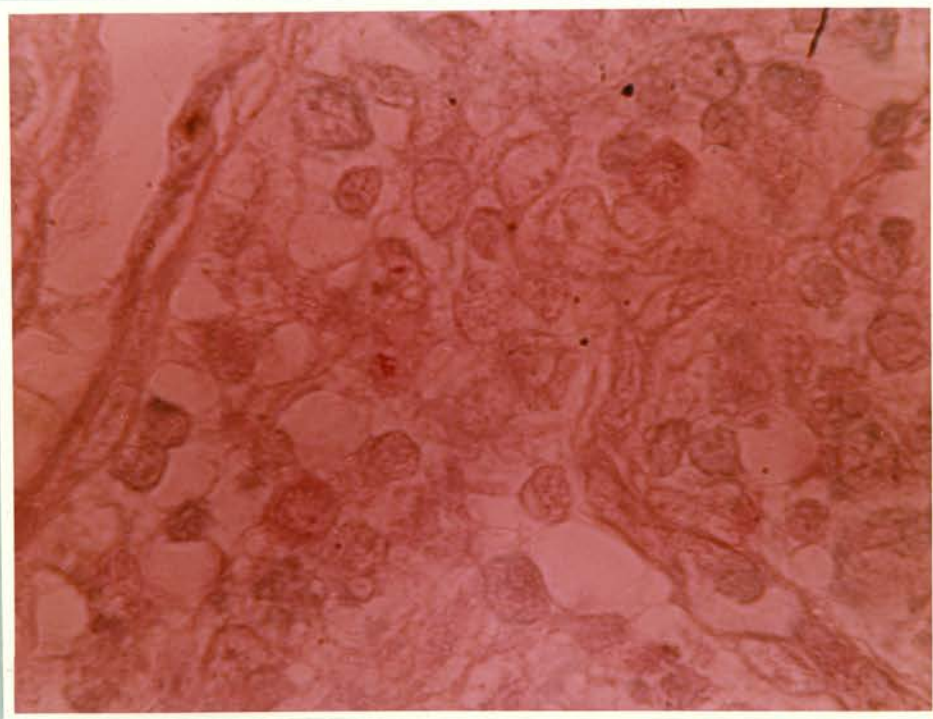


Fig. 6 BB patient. Skin biopsy demonstrating granuloma. Fragmented AFB within a histiocyte. Triff stain x 500.

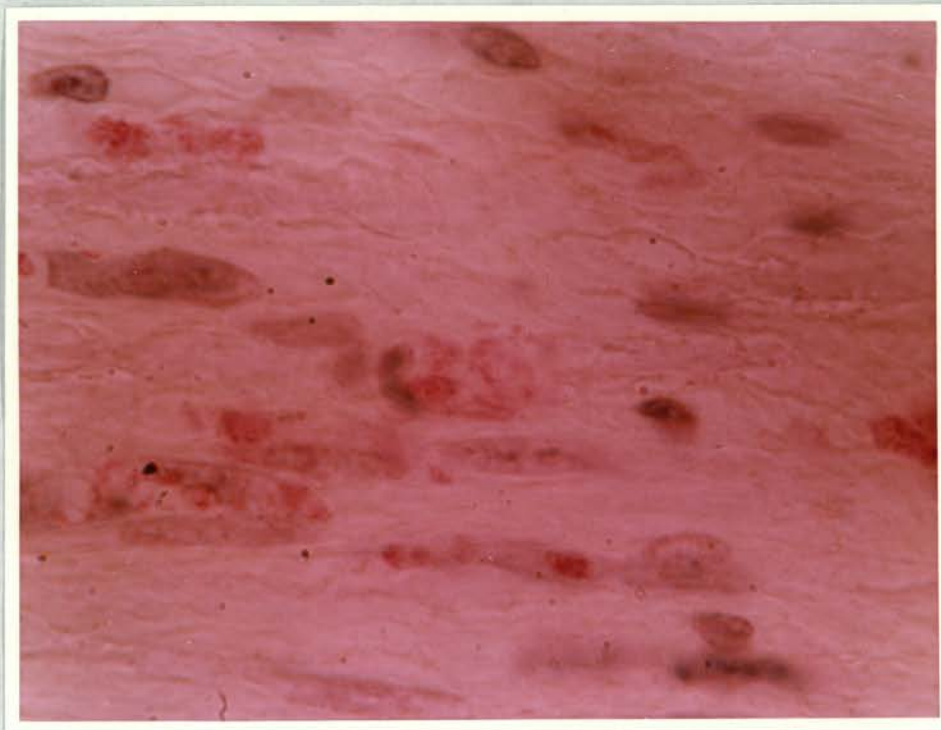


Fig. 7 Same BB patient. Biopsy of radial cutaneous nerve, with many AFBs within Schwann cells, and no infiltrate. Triff stain x 500.

IMMUNOLOGICAL RESULTS(a) LT RESPONSES TO ANTIGENS1. REACTION PATIENTS(a) M. leprae (Whole and sonicated)

The LT responses to whole and sonicated M. leprae in the 17 reaction patients during the pre-reaction, reaction and post-reaction (up to 12 months) periods are shown in Figs. 8 & 9. (Each LT response is represented by a dot at the appropriate time in relation to reaction.) It is seen that there is a marked increase in the LT responses to both whole and sonicated M. leprae during the period of reaction.

The LT responses to whole and sonicated M. leprae at "baseline" (on initiation of patient's treatment with dapsone), "during reaction" (on initiation of treatment with prednisolone) and "post-reaction" (on cessation of prednisolone therapy) in the 17 reaction patients are shown in Figs. 10 & 11. Unfortunately 1 baseline blood specimen was lost.

With whole M. leprae there was a significant rise for the group ($p < 0.05$) in LT responses from baseline (median: 670CPM) to reaction values (median: 1890CPM). There was also a significant fall ($p < 0.01$) from reaction to post-reaction values (median: 60CPM).

With sonicated M. leprae there was a significant rise ($p < 0.01$) in the responses from baseline (median: 580CPM) to reaction values (median: 2090CPM). There was also a significant fall ($p < 0.01$) from reaction

to post-reaction values (median: 70CPM).

Clinical presentation of reaction and LT responses to whole and sonicated *M. leprae*

Of the 17 patients, 6 had "skin" reactions, 6 had "nerve" reactions and 5 had "mixed" reactions. It was seen that in all 6 who had skin reactions there was a very marked rise in the LT responses to whole *M. leprae* during reaction and in 5 of them there was a lesser rise in LT responses to sonicated *M. leprae* which paralleled the rise with the former antigen (see Fig. 12). In 5 of the 6 who had nerve reactions there was a rise in LT responses to sonicated *M. leprae* but no concomitant rise to whole *M. leprae* (see Fig. 13). In those with mixed reactions, 3 of the 5 had marked rise with both antigens (see Fig. 14). The LT responses, correlated with the clinical presentation of reaction are summarised in Table 5: the peak responses (gross responses) during the clinical period of reaction are shown in each patient. (Gross responses were recorded in this instance, to ensure that all results were greater than zero, and thus ratios could be calculated.) The ratio of LT responses to whole *M. leprae* to LT responses to sonicated *M. leprae* are shown in Fig. 15. There is a highly significant difference between the 3 groups of patients ($p < 0.001$) using the non-parametric test of Kruskal and Wallis for independent samples (Kruskal, 1952).

TABLE 5 Clinical manifestations related to peak LT responses (gross responses) during reaction, in the 17 patients

| Case No | Clinical classification | LT (counts min) | | | Ratio of whole to sonicated M.leprae |
|---------|-------------------------|-----------------|----------------|--------------------|--------------------------------------|
| | | Reaction | Whole M.leprae | Sonicated M.leprae | |
| 1 | BT | Skin | 13,861 | 1,770 | 7.83 |
| 2 | BT | Skin | 19,654 | 5,574 | 3.53 |
| 3 | BT | Skin | 4,800 | 1,500 | 3.20 |
| 4 | BT | Skin | 5,300 | 2,353 | 2.25 |
| 5 | BT | Skin | 7,195 | 4,434 | 1.62 |
| 6 | BB | Skin | 7,396 | 6,123 | 1.21 |
| 7 | BT | Mixed | 12,089 | 10,031 | 1.21 |
| 8 | BT | Mixed | 9,050 | 9,950 | 0.91 |
| 9 | BT | Mixed | 1,515 | 2,001 | 0.76 |
| 10 | BB | Mixed | 3,763 | 5,032 | 0.75 |
| 11 | BT | Mixed | 1,086 | 1,494 | 0.73 |
| 12 | BT | Nerve | 359 | 357 | 1.01 |
| 13 | BL | Nerve | 5,187 | 6,669 | 0.78 |
| 14 | BT | Nerve | 1,626 | 4,749 | 0.34 |
| 15 | BL | Nerve | 2,156 | 6,873 | 0.31 |
| 16 | BT | Nerve | 1,086 | 3,693 | 0.29 |
| 17 | BL | Nerve | 261 | 1,136 | 0.23 |

(b) BCG (Whole)

The LT responses to whole BCG in the 17 reaction patients during the pre-reaction, reaction and post-reaction periods are shown in Fig. 16. There was a rise in LT responses in 7 of the patients during reaction but there was no significant difference between baseline values (median: 460CPM) and reaction values (median: 530CPM) for the group. There was also no significant difference between reaction and post-reaction values (median: 240CPM).

The 3 very high LT responses during the post-reaction period belonged to the same patient (No. 42).

(c) M. duvalii (Sonicated)

The LT responses to sonicated M. duvalii in the 17 reaction patients during the pre-reaction, reaction and post-reaction periods are shown in Fig. 17. There was a rise in LT responses in 9 patients during the reaction period (median: 940CPM) when compared with baseline (median: 350CPM) but there was no significant rise for the group. However there was a significant fall ($p < 0.05$) to post-reaction values (median: 180CPM).

2. INITIAL LT RESPONSES TO M. LEPRAE IN ALL PATIENTSCorrelation of LT responses to M. leprae with clinical diagnosis

The initial LT responses to whole M. leprae in 76 of the patients are shown in Fig. 18. (Seven were lost due to technical failures of the test.) Fifty-two of these were clinically BT, 3 BB and 21 BL. It was seen

that though the responses in BT patients (median: 550CPM) were significantly higher ($p < 0.05$) than those in BL patients, (median: 220 CPM) there was considerable variation of responses in each group. Though there were only 3 BB patients, the responses of each were higher than the median BT responses.

The initial LT responses to sonicated M. leprae in the same 76 patients are shown in Fig. 19. It was seen that the responses in BT patients (median: 590CPM) were again significantly higher ($p < 0.01$) than the BL patients (median: 110CPM); as with whole M. leprae there was considerable variation in LT responses in the two groups.

Correlation of LT responses to M. leprae with histological diagnosis

When the initial LT responses to whole and sonicated M. leprae were correlated with the histological classification, it was found that those who were clinically BT, but had histology of TT leprosy had significantly higher responses with both antigens (medians: 1630 and 1040CPM) than those with BT (medians: 500 and 490CPM) or Idt (medians: 440 and 330CPM) leprosy histologically. ($p < 0.01$ for both)(See Fig. 20)

There was no difference in LT responses between patients with BL leprosy histologically, and those who were LI.

3. NON REACTION PATIENTS

1. M. leprae (Whole and sonicated)

The LT responses to whole M. leprae in the 66 patients who did not develop reaction are shown in Fig. 21. As with the initial LT responses, there was considerable variation in LT responses, related to the presence of inflammation in skin lesions. Those with inflamed skin lesions (BT, BB and BL) had higher responses than those with non inflamed (silent) skin lesions (see below). However there were 4 exceptions to this. Four patients with "silent" skin lesions had very high responses: the responses in these patients are those joined by lines.

The LT responses to sonicated M. leprae in these 66 patients are shown in Fig. 22. Again, there was considerable variation in LT responses, those with inflamed skin lesions having higher responses than those with silent lesions. However 3 of the 4 patients with silent lesions who had very high responses to whole M. leprae also had very high responses to sonicated M. leprae: again the responses in these patients are those joined by lines.

Correlation of LT responses to clinical inflammation of skin lesions

The LT responses to whole M. leprae comparing those with inflamed skin lesions and those with silent skin lesions in the BT and BL patients are shown in Fig. 23. (The responses shown are those throughout the period of the study: when a patient's inflamed skin lesions became silent, they are included amongst the "silent" group.) In both

BT and BL patients there was a significant difference between those with inflamed lesions (medians: 650 and 650CPM respectively) and those with silent lesions (medians: 270 and 130CPM respectively). ($p < 0.01$ for BT patients, $p < 0.01$ for BL patients.) Also those with BL leprosy with inflamed lesions (median: 650CPM) had higher LT responses than the BT patients with silent lesions (median: 270CPM), though the difference did not reach significance.

The LT responses to sonicated M. leprae comparing patients with inflamed skin lesions and those with silent lesions in BT and BL groups are shown in Fig. 24. In both BT and BL patients there was a significant difference between those with inflamed lesions (medians: 430 and 280CPM respectively) and those with silent lesions (medians: 180 and 30CPM), ($p < 0.01$ for both BT and BL patients). Also the LT responses in the BL patients with inflamed lesions were higher than those BT patients with silent lesions, though the difference was not significant.

2. BCG (Whole)

25% patients in the study had consistently high responses to BCG, suggesting previous infection with tuberculosis. There was however quite marked variation in responses from time to time: LT responses to BCG were often increased when responses to M. leprae antigens were increased, (such as in reaction patients) suggesting cross reactivity.

3. M. Duvalii (Sonicated)

The LT responses to M. duvalii were also very variable, and usually paralleled high LT responses with the M. leprae and BCG antigens, suggesting cross reactivity.

(b) LT RESPONSES TO MITOGEN (PHA)

1. NON REACTION PATIENTS

Though LT responses to PHA in standard serum were performed on all patients at baseline and throughout the trial, LT responses to PHA in autologous plasma were not carried out routinely till 5 months after the trial had commenced. Thus PHA responses in autologous plasma were only performed at baseline in the last 33 patients in the study.

The baseline responses to PHA in standard serum and autologous plasma in 23 of these patients (the reaction patients and those with methodological failures were excluded) are shown in Fig. 25. The responses in autologous plasma (median: 18,700) were lower than those in standard serum (median: 23,900), though this difference is not significant. There was no difference in PHA responses between BT and BL patients.

The LT responses to PHA in standard serum at baseline were compared with those after the patients had received 6 months treatment with dapsone 5mg daily and dapsone 50mg daily respectively (see Fig. 26). There was a significant difference ($p < 0.01$ for both) between the post-treatment responses and the baseline responses (median: 24,200)

for both that group taking 5mg dapsone daily (median: 46,700CPM) and that taking 50mg dapsone daily (median: 64,700CPM). Though the responses to PHA were higher in those taking dapsone 50mg daily than in those taking 5mg daily, the difference between the 2 groups was not significant.

The LT responses to PHA in autologous plasma at baseline were also compared with those after 6 months treatment with dapsone 5mg and 50mg daily (see Fig. 27). There was a significant difference ($p < 0.05$) between the post-treatment responses and the baseline responses (median: 18,500) for those patients taking dapsone 50mg daily (median: 42,000CPM): there was also a difference in PHA responses in those patients taking dapsone 5mg daily, but it did not reach significance. Again the responses to PHA were higher in those taking dapsone 50mg daily than those taking 5mg daily but the difference between the two groups was not significant.

2. REACTION PATIENTS

Plasma from the 17 patients who developed reactions together with 7 patients (4 BT, 1 BB, 2 BL) not included in the prospective study produced a similar degree of suppression of PHA responses both before and after reaction, while by contrast for the period related to the reaction they augmented the responses. The duration of the augmenting effect of plasma and its degree showed great individual variation, as did the intensity and duration of the clinical reaction. In 10 of the patients we were

able to perform several tests before, during and after the reaction (Fig. 28). When the effect of autologous plasma from BT patients on the lymphocyte responses to PHA (recorded as a percentage of the responses in standard serum) was compared before, during and after reaction, a significant difference appeared (Fig. 29). The median value before reaction was 52%, during reaction 128% and after reaction 57% ($p < 0.01$ for both rise and fall).

The augmenting effect of plasma from BL patients appeared later than that from BT patients. Post-reactional values recorded up to 2 months after the clinical reaction had subsided, were higher (median: 90%) than the reactional values (median: 36%), though this did not reach significance.

The changes in the effect of plasma on PHA responses during the course of reversal reactions were not a direct effect of steroid treatment. Reactional BT patients on steroid treatment had a median response of 126% and post-reactional ones 52.5%, which were not significantly different from patients in the same groups without such treatment (125% and 57% respectively).

The effect of plasma in each case from reactional patients, taken before, during and after reaction, was tested simultaneously on autologous lymphocyte responses and lymphocytes from an unrelated individual. The relationship of the effect of reactional patients' plasma on autologous and homologous lymphocytes is shown in Fig. 30. From this it is seen that the plasma had the same effect on both types of test cells. Correlation coefficient, $r = 0.81$ ($p < 0.001$).

The PHA responses of the reaction patients were compared with those of 19 other patients in the study who had non-inflamed skin lesions and no neuritis, and thus their leprosy was relatively "inactive" and in these there was a significant difference ($p < 0.01$) when their lymphocytes were cultured in standard serum (median: 48,200CPM) compared with culture in autologous plasma (median: 34,700CPM). The degree of inhibition by autologous plasma remained fairly constant in the 9 clinically stable males, while the 10 stable females showed considerable variation from test to test (Fig. 31).

(c) SERUM IMMUNOGLOBULINS

1. REACTION PATIENTS

Serum IgG

The serum IgG levels at baseline, during reaction and post-reaction (post steroids) are shown in Fig. 32.

There was a significant rise ($p < 0.01$) in IgG levels from baseline (median: 2,216 mg%) to reaction values (median: 2,615 mg%) and there was a significant fall ($p < 0.01$) to post-reaction levels (median: 2,099 mg%).

Serum IgA

The serum IgA levels at baseline, during reaction and post-reaction are shown in Fig. 33.

There was a significant rise ($p < 0.01$) in IgA levels from baseline (median: 206 mg%) to reaction values (median: 253 mg%) and there was a significant fall ($p < 0.01$) to post-reaction levels (median: 218 mg%).

Serum IgM

The serum IgM levels at baseline, during reaction and post-reaction are shown in Fig. 34.

There was a significant rise ($p < 0.01$) in IgM levels from baseline (median: 98 mg%) to reaction values (median: 127 mg%), but the fall to post-reaction levels (median: 114 mg%) was not significant.

2. NON REACTION PATIENTS

All patients in the study had blood for serum studies taken initially, and at intervals during the trial. Most patients had between 2 and 3 samples tested for serum Igs each, over the period of the trial.

The initial serum levels of IgG, IgA and IgM all demonstrated a considerable variation. The ranges and medians for each Ig in the non-reaction patients were as follows:

- (a) IgG: range 1065 - 4925 mg%; median 2,662 mg%
- (b) IgA: range 39 - 596 mg%; median 252 mg%
- (c) IgM: range 42 - 477 mg%; median 150 mg%

There was also considerable variation in the serum Igs in the post-treatment serum samples in each patient, but no evidence that treatment for the duration of the study (1 - 2 years) had any effect in raising or lowering the patients' immunoglobulins.

(d) ANTI-MYCOBACTERIAL ANTIBODIES

1. REACTION PATIENTS

The following investigations were carried out at baseline, during reaction and post-reaction on 6 of the 17 reaction patients.

- (a) Agarose gel electrophoresis for detection of IgG heterogeneity.
- (b) Crossed immunoelectrophoretic analysis of patients' IgG carried out at pH 5.0 using carbamylated ammonium sulphate fractionated gamma globulin from rabbit anti-human IgG anti-serum.
- (c) Crossed immunoelectrophoretic analysis of antimycobacterial antibodies using sonicated M. leprae as antigen.

Agarose gel electrophoresis, and crossed immunoelectrophoresis of these sera showed a polyclonal increase in gamma globulins and IgG respectively. No monoclonal components were detected. (An example of the crossed immunoelectrophoresis in 1 of these patients is shown in Fig. 35.) Crossed immunoelectrophoretic analysis of antimycobacterial antibodies also showed no change between baseline, reaction and post-reaction sera in each case. In particular there was no rise in titres of existing antimycobacterial antibodies and no evidence of formation of new antibodies during reaction (changes of 30% can be detected using this technique semiquantitatively (Kronvall et al, 1975)). Of the reaction patients, 9 had antimycobacterial antibodies demonstrated (5 of the 12 BT patients [average: 0.67

antibodies/patient], 1 of the 2 BB patients [average: 0.5 antibodies/patient] and all 3 BL patients [average: 2 antibodies/patient]).

2. NON REACTION PATIENTS

Crossed immunoelectrophoretic analysis was performed on the initial serum samples of 50 patients. Six of 34 BT patients (including those with Idt histology) had antimycobacterial antibodies demonstrated (average: 0.26 antibodies/patient), the 1 BB patient had 2 antimycobacterial antibodies and 14 of 15 BL patients had antimycobacterial antibodies (average: 2.27 antibodies/patient).

(e) ANTI-NUCLEAR ANTIBODIES

No anti-nuclear (IgG) antibodies were found in any of the reaction patients at baseline, during reaction or post-reaction. Two non reaction patients had antibodies present on initial testing of serum.

(f) ANTI-LYMPHOCYTE ANTIBODIES

No anti-lymphocyte antibodies were found in any of the reaction patients at baseline, during reaction or post-reaction.

(g) NEURAL AUTO-ANTIBODIES

Three of the patients who developed reaction had neural auto-antibodies demonstrated. There was no evidence of formation of fresh neural auto-antibodies during reaction in any of the patients.

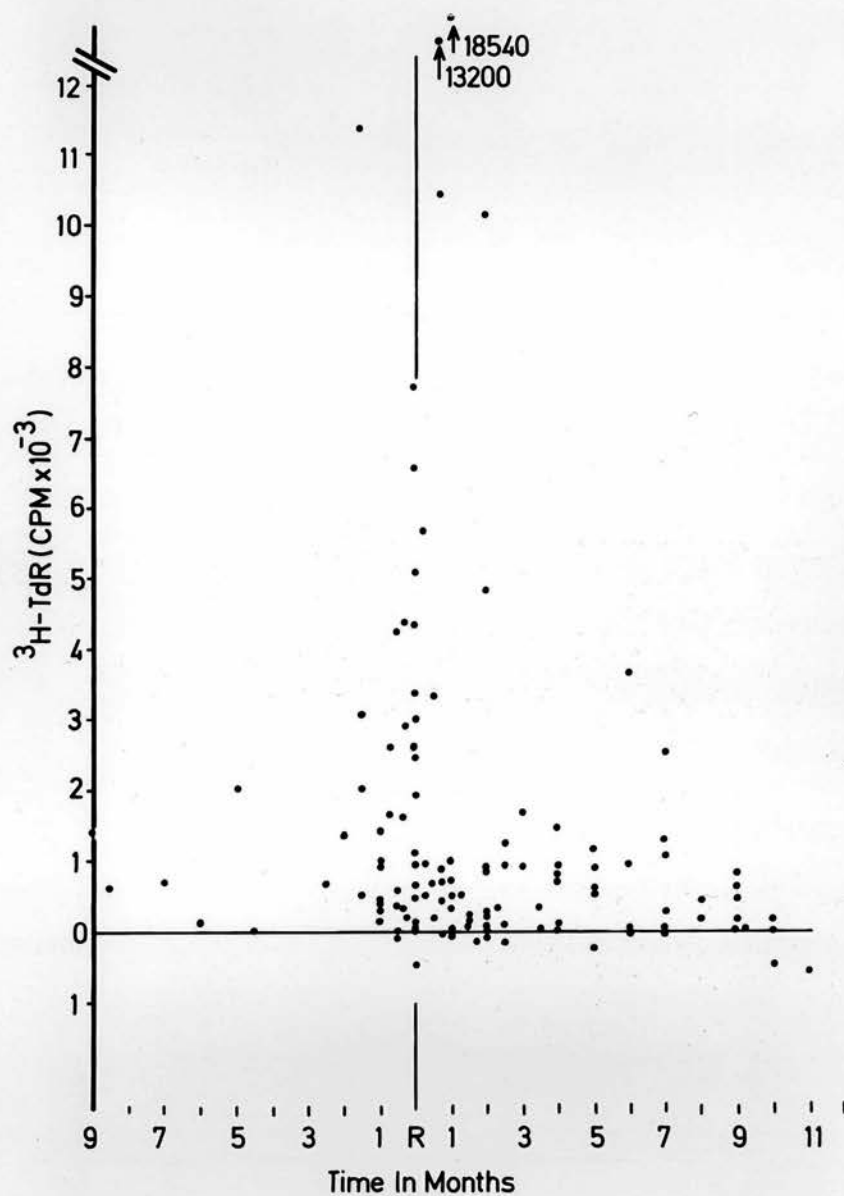


Fig. 8 LT responses to whole *M. leprae* in the 17 reaction patients in the pre-reaction period, during reaction (R) and post-reaction.

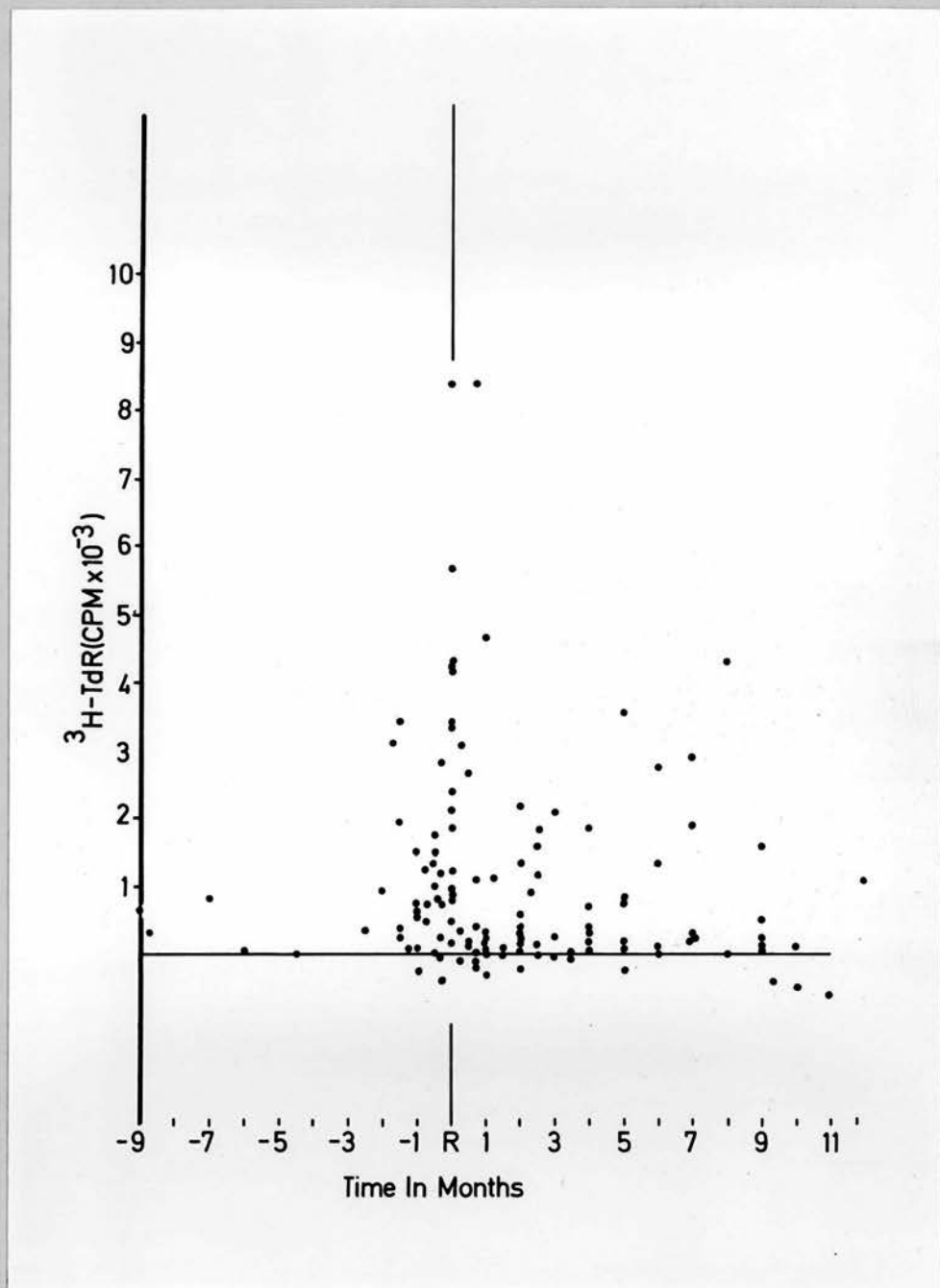


Fig. 9 LT responses to sonicated *M. leprae* in the 17 reaction patients, in the pre-reaction period, during reaction (R) and post-reaction.

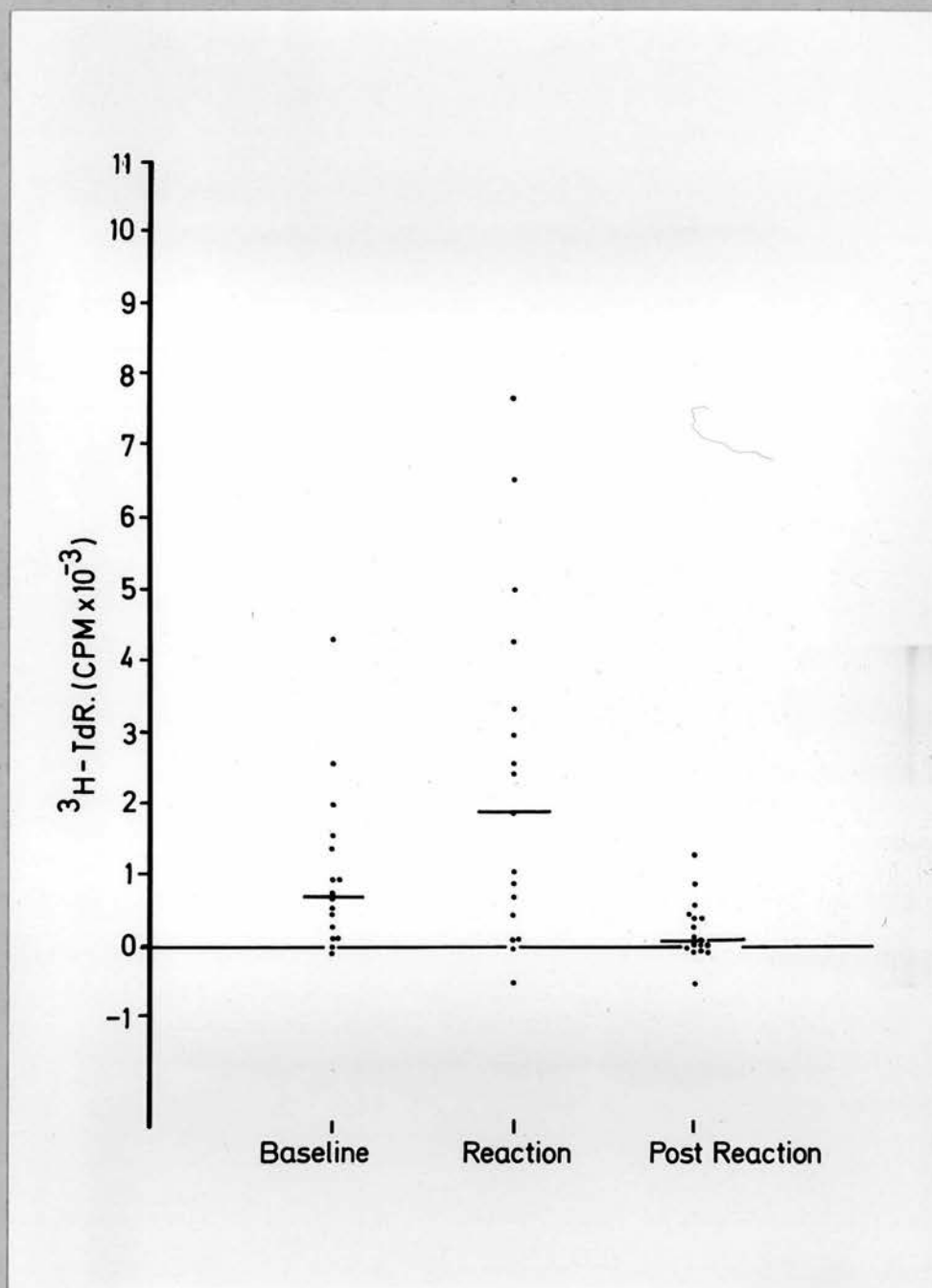


Fig. 10 LT responses to whole M. leprae in the 17 reaction patients at baseline, during reaction, and post-reaction.

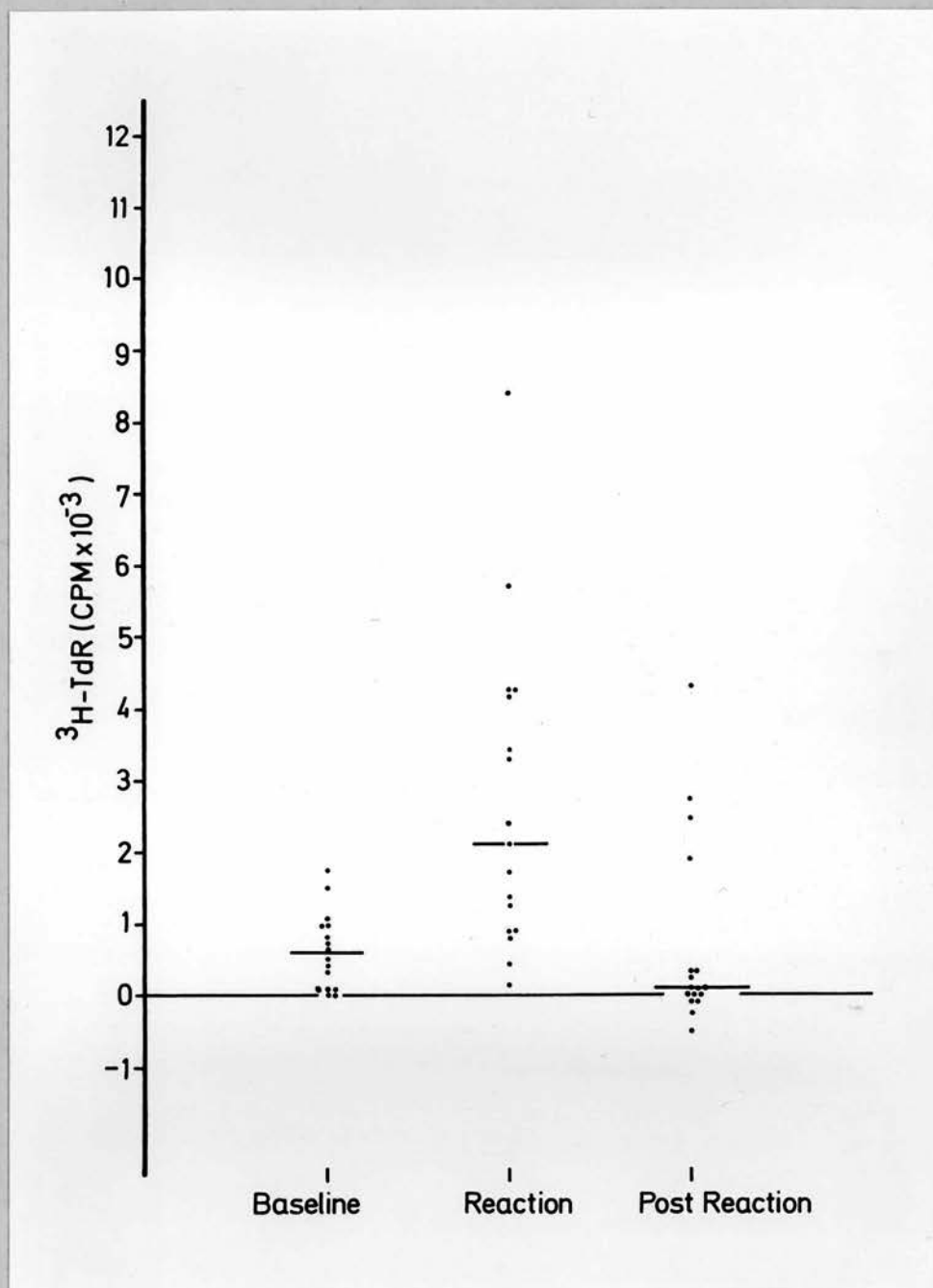


Fig. 11 LT responses to sonicated M. leprae, in the 17 reaction patients, at baseline, during reaction and post-reaction.

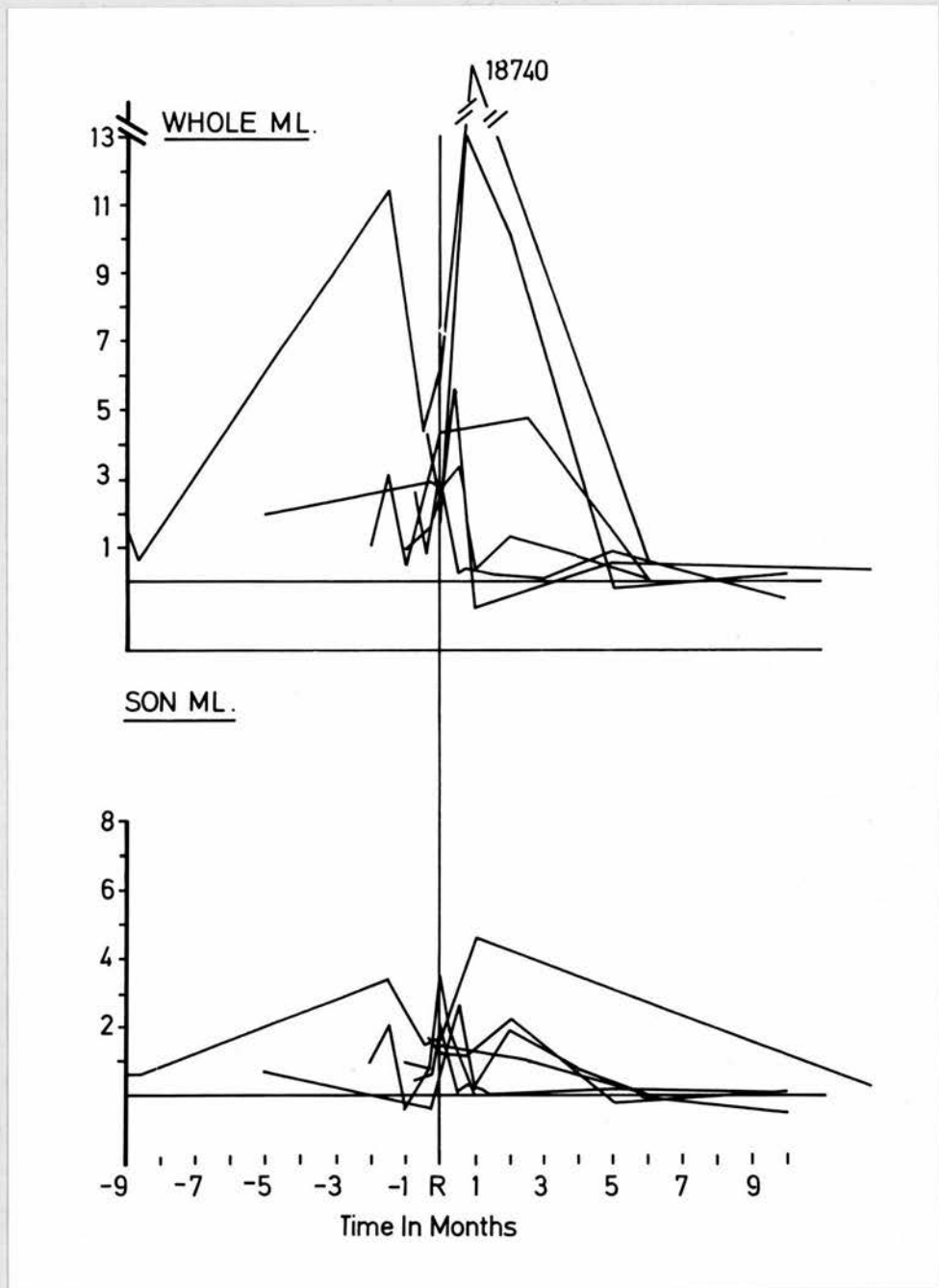


Fig. 12 LT responses to whole M. leprae and sonicated M. leprae in patients with 'skin' reactions. (R = time of reaction).

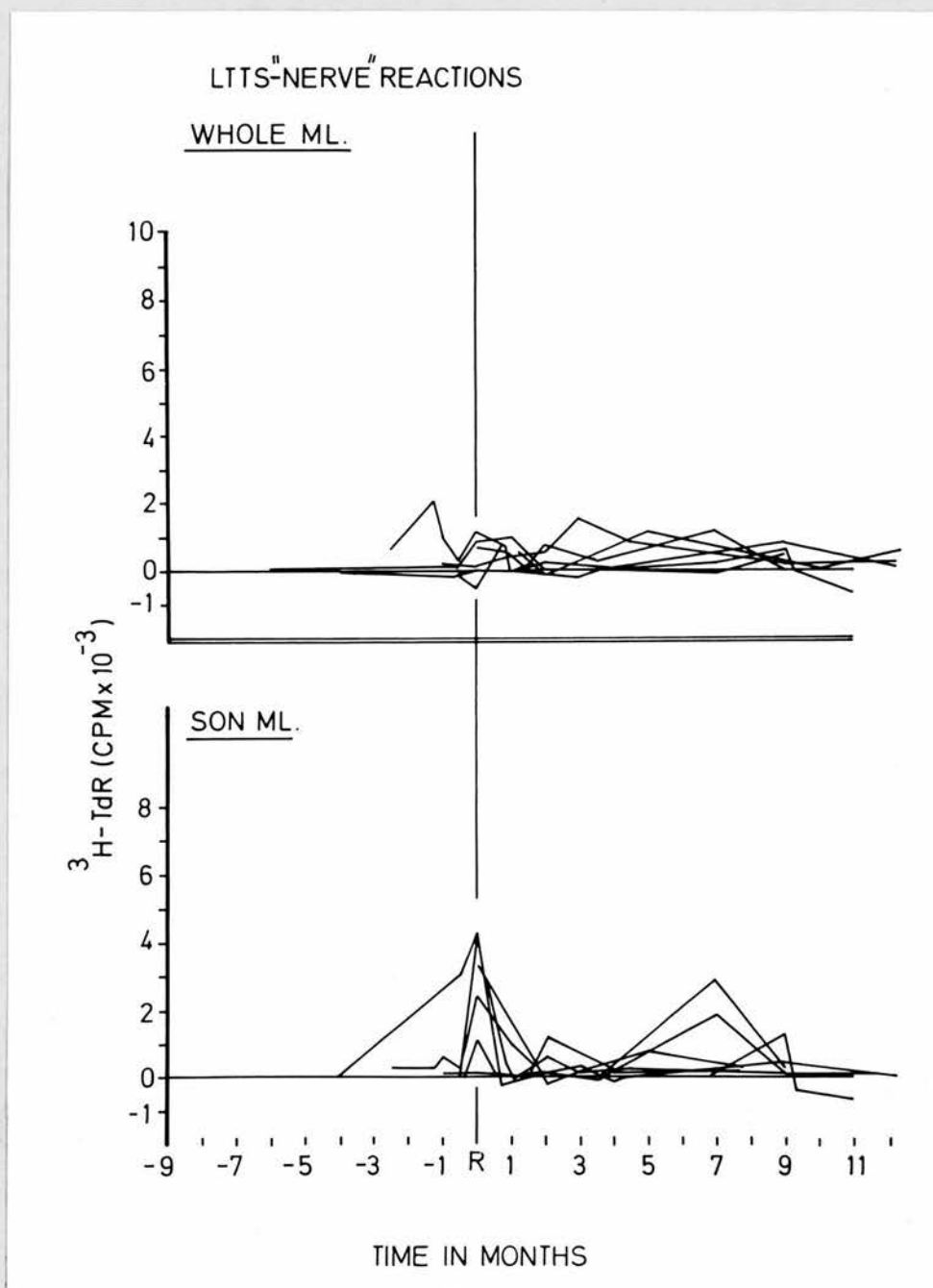


Fig. 13 LT responses to whole M. leprae and sonicated M. leprae in patients with 'nerve' reactions. The apparent second peak in LT responses to sonicated M. leprae at 7 months in one patient was probably due to concomitant very high LT responses with BCG (No. 42)
(R = time of reaction)

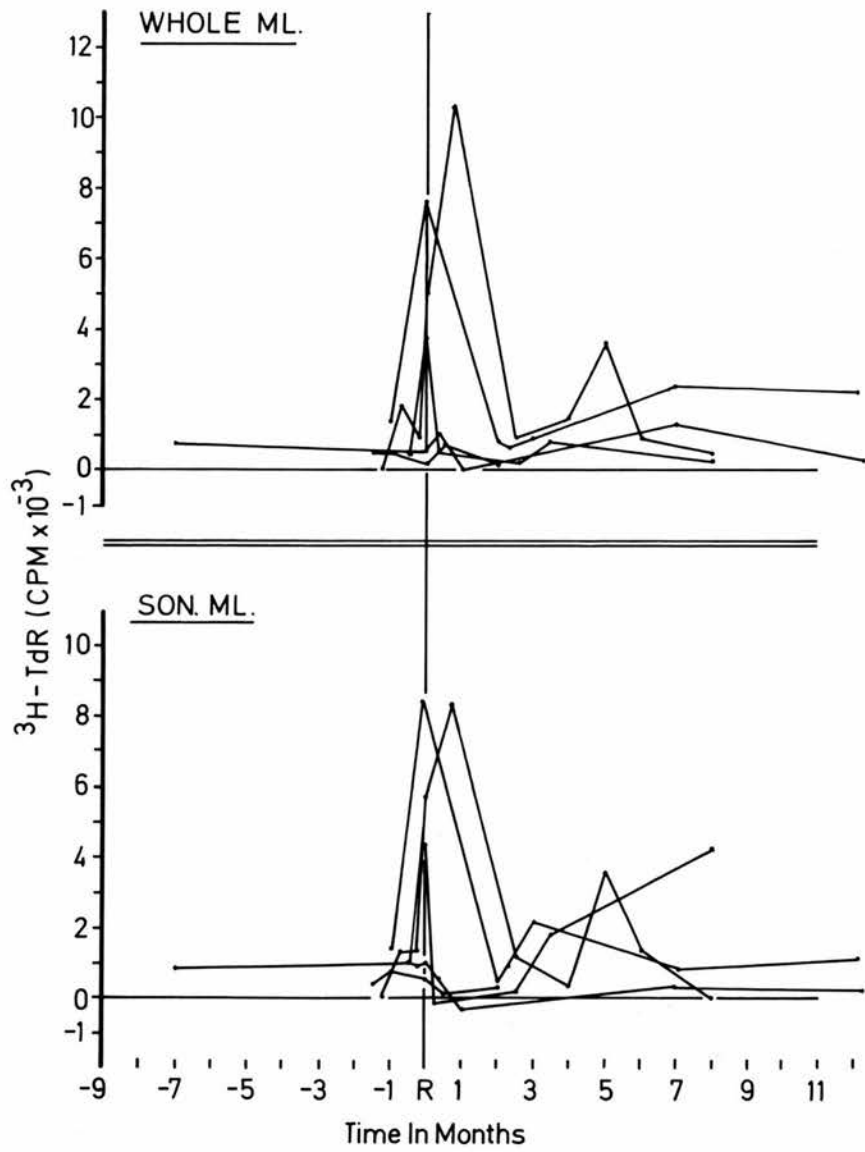


Fig. 14 LT responses to whole M. leprae, and sonicated M. leprae in patients with 'mixed' reactions. (R = time of reaction)

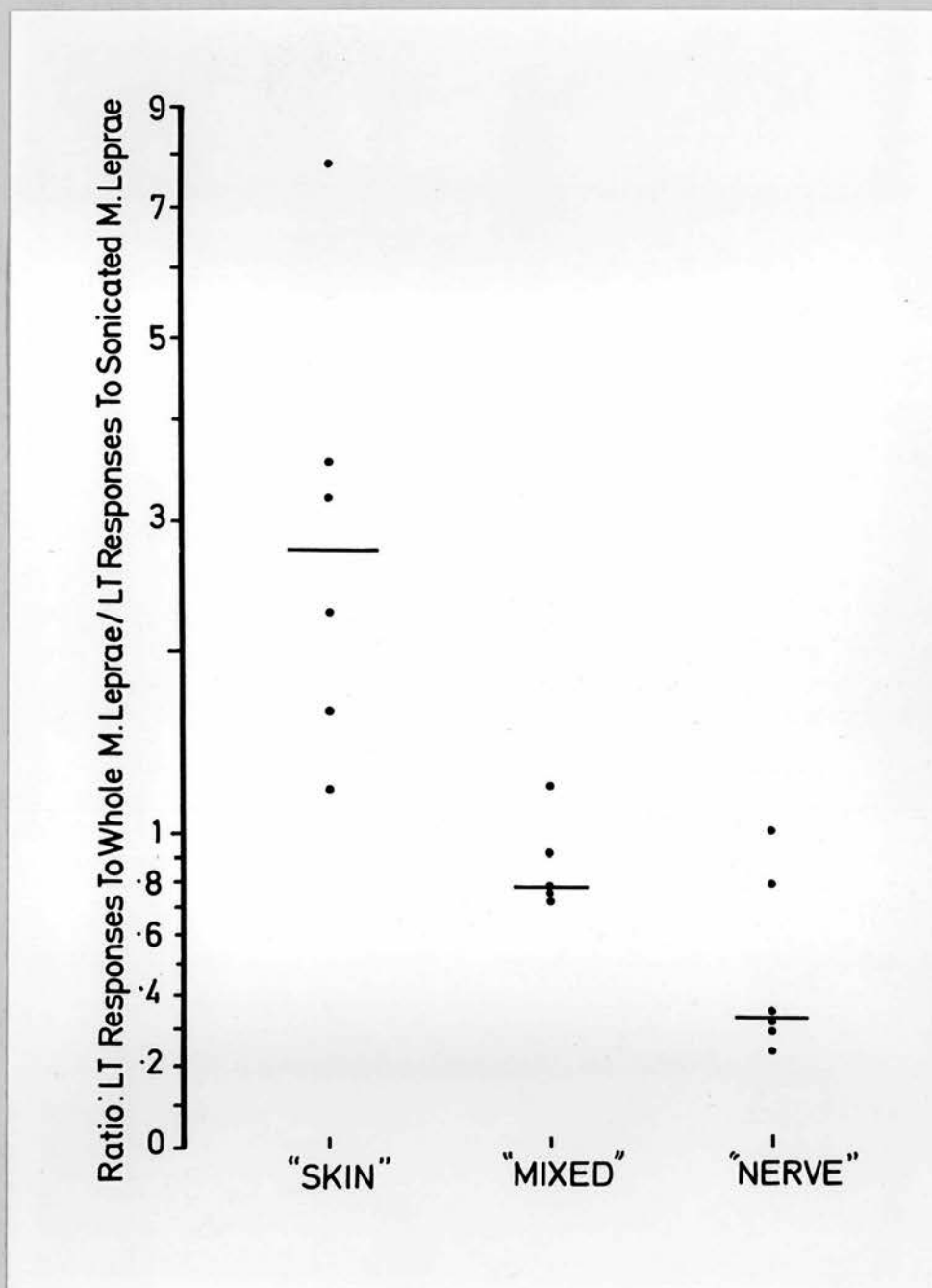


Fig. 15 Ratio: LT responses to whole *M. leprae* / LT responses to sonicated *M. leprae* in the 17 patients presenting with 'skin', 'mixed' and 'nerve' reactions.

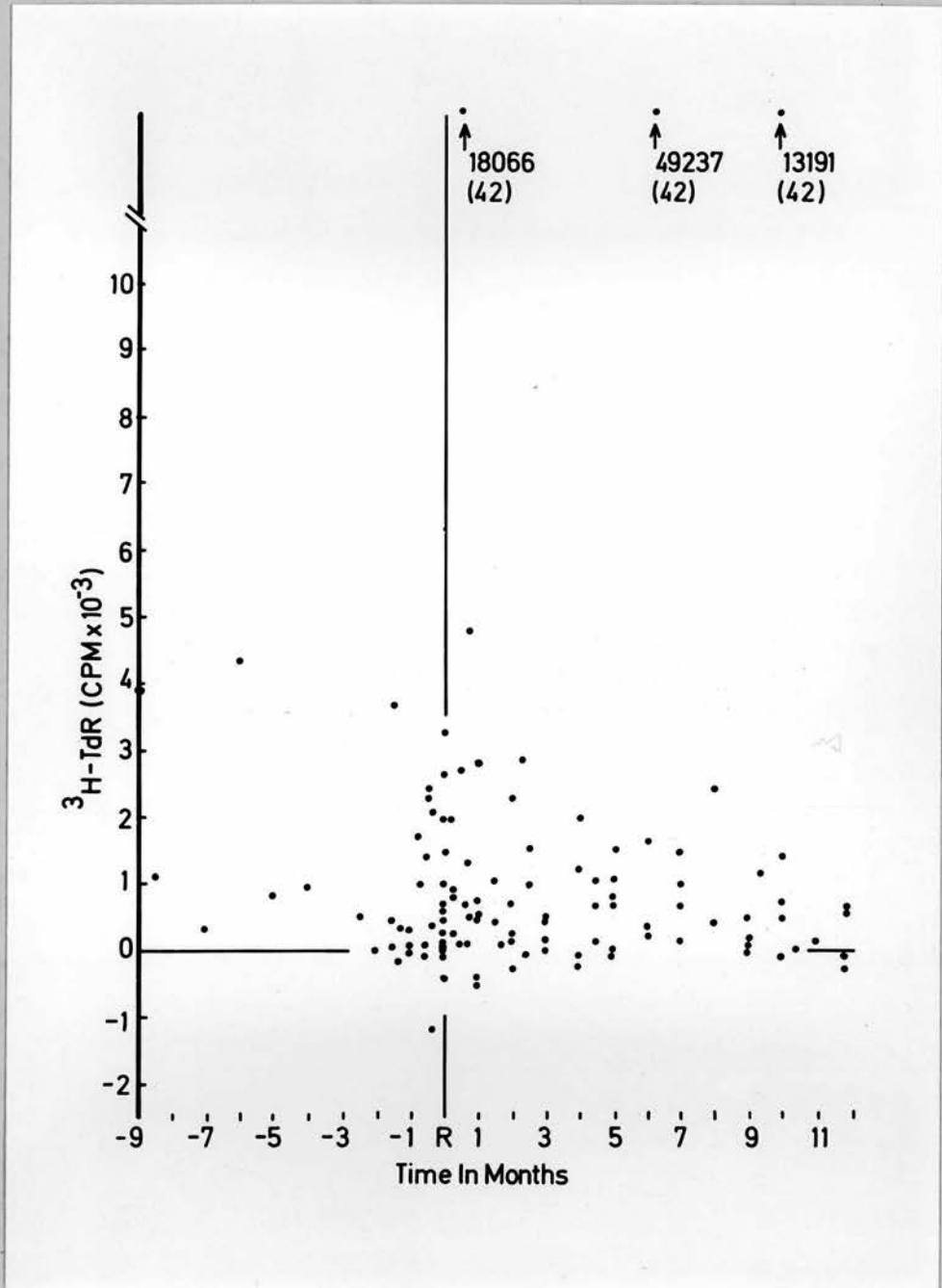


Fig. 16 LT responses to whole BCG in the 17 reaction patients.
(R = time of reaction)

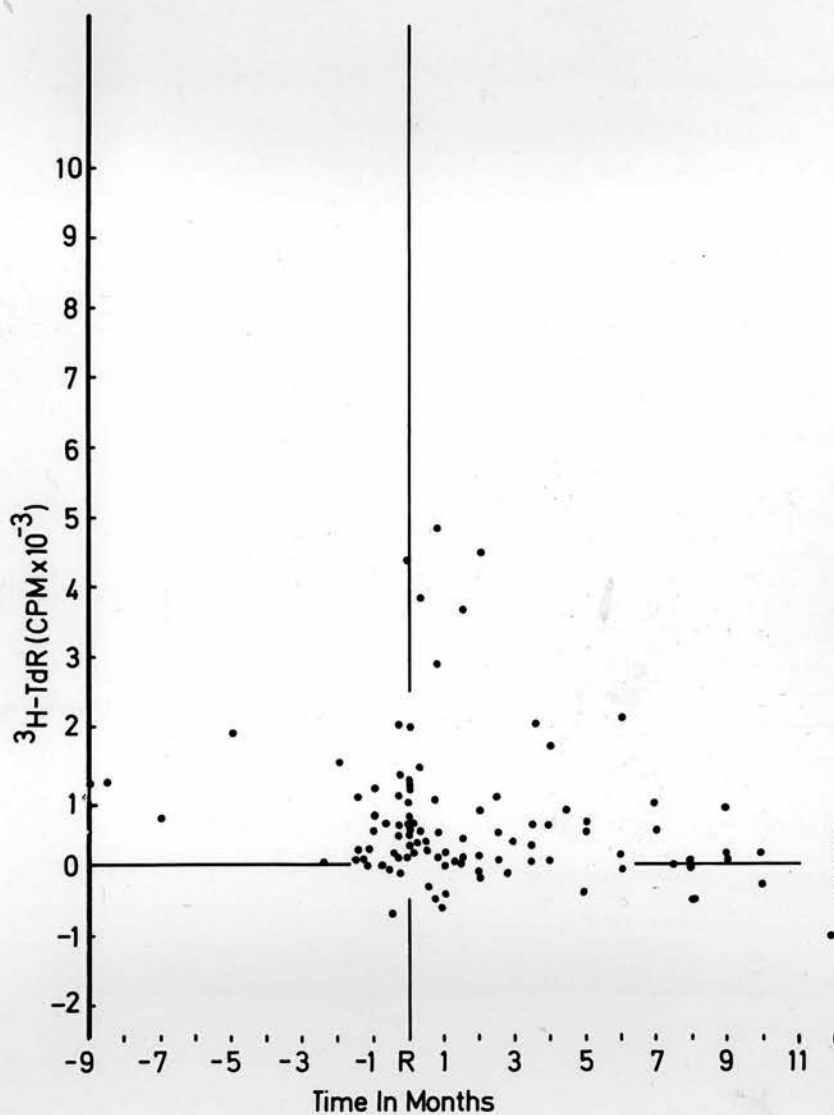


Fig. 17 IT responses to sonicated M. duvalii
in the 17 reaction patients.
(R = time of reaction)

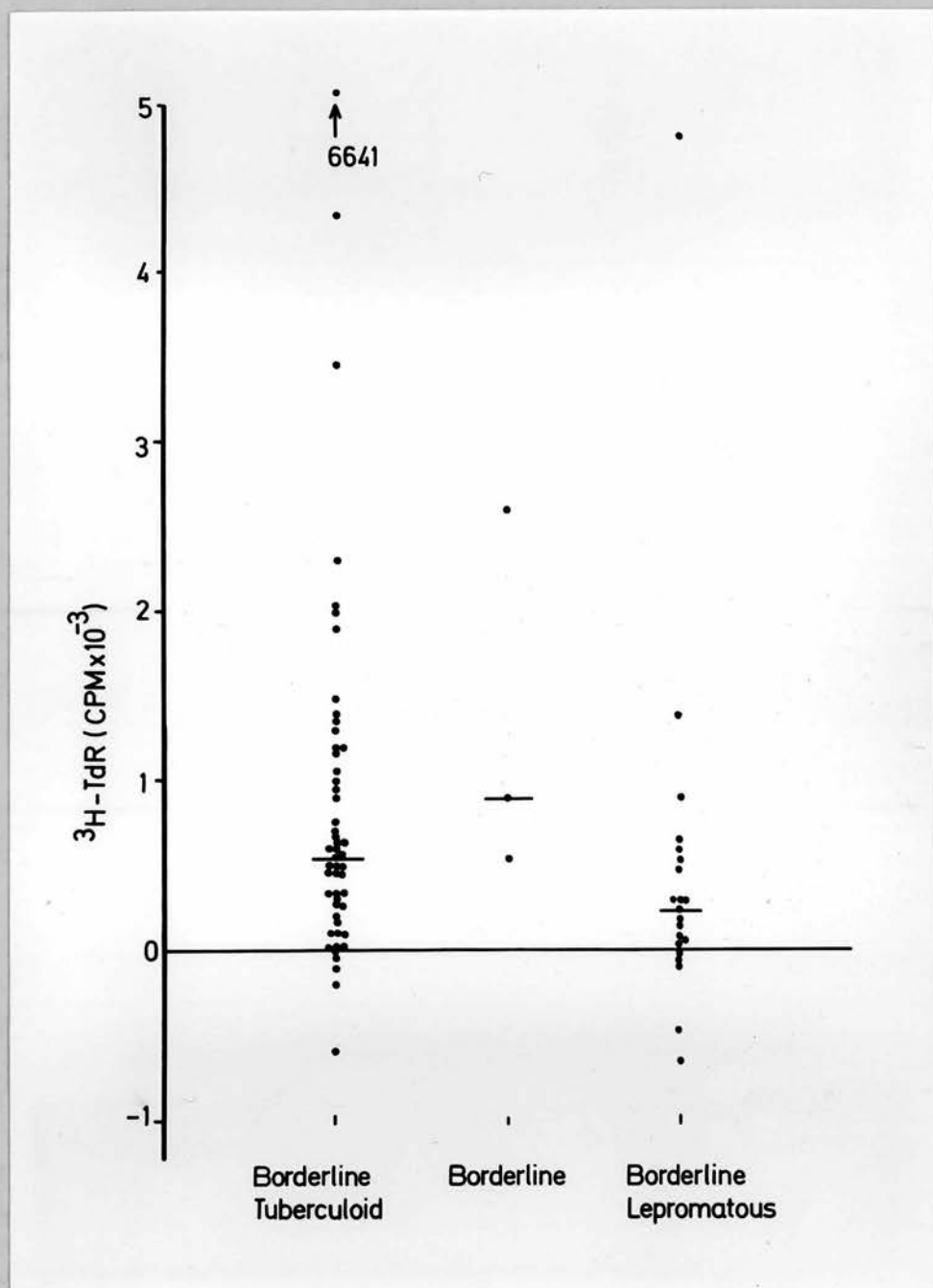


Fig. 18 Initial LT responses to whole M. leprae in all patients (7 were methodological failures, and thus these results are omitted).

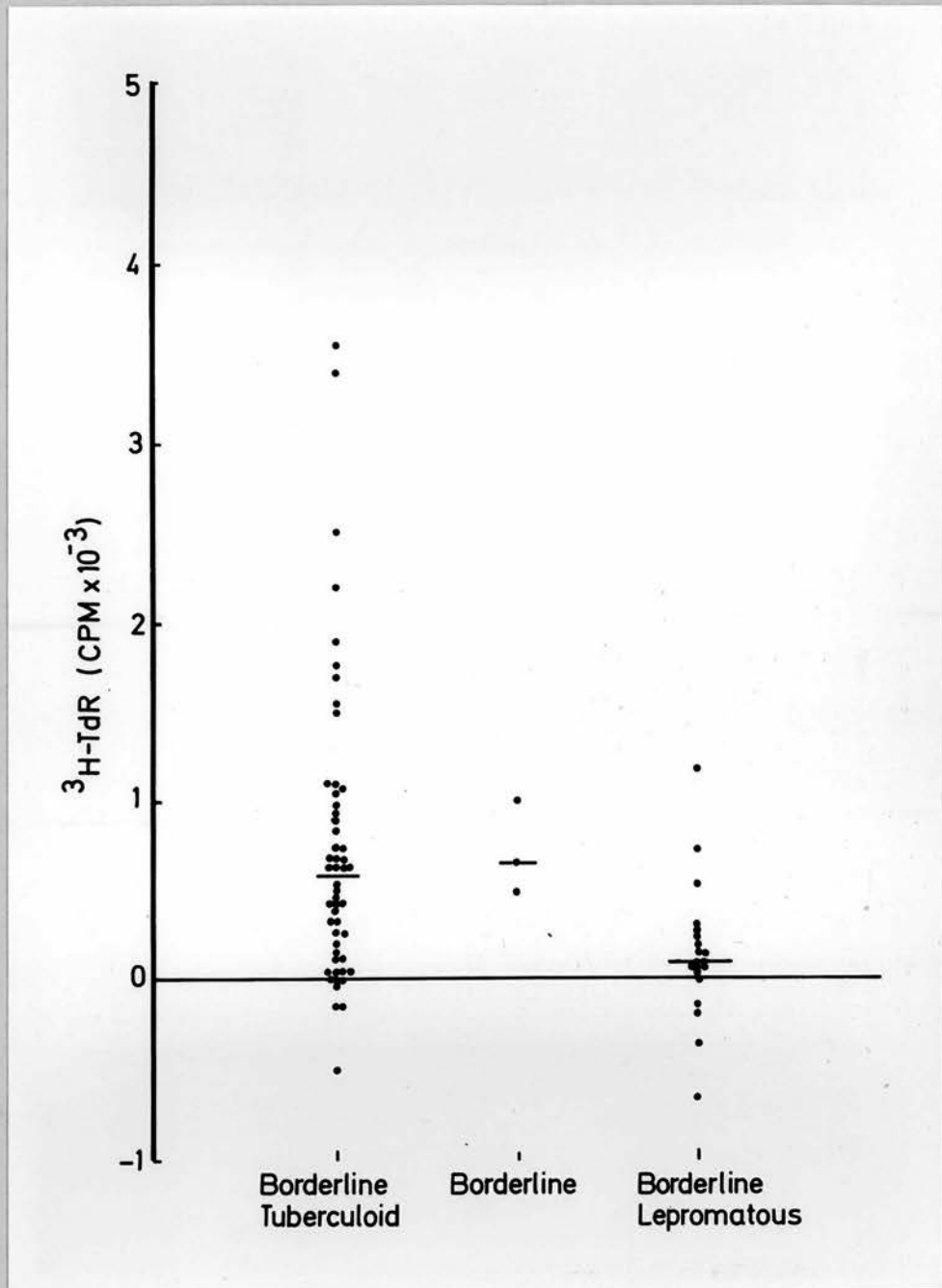


Fig. 19 Initial $L3$ responses to sonicated *M. leprae* in all patients (7 were methodological failures, and thus these results are omitted).

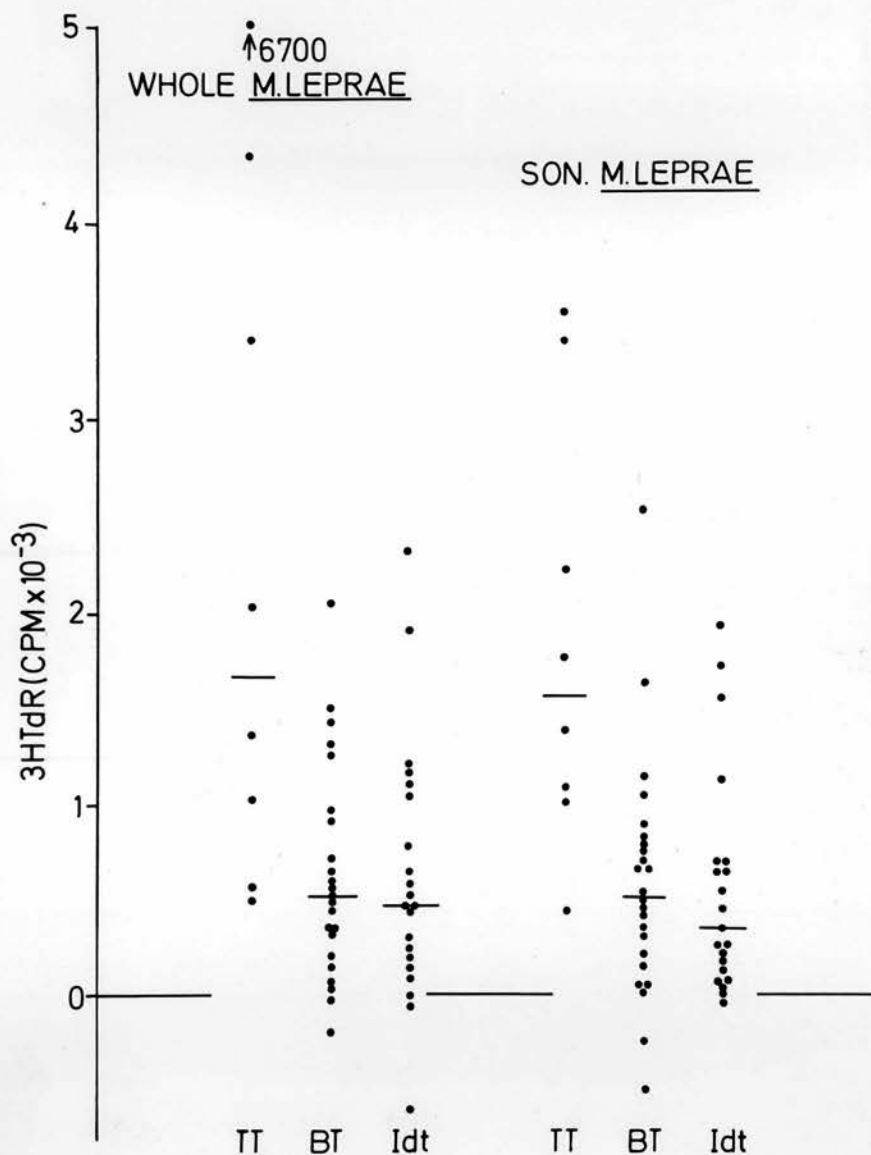


Fig. 20 Correlation of initial LT responses to whole M. leprae and sonicated M. leprae to the histological diagnosis in those patients who were clinically borderline tuberculoid.
(TT = tuberculoid, BT = borderline tuberculoid, Idt = indeterminate leprosy)

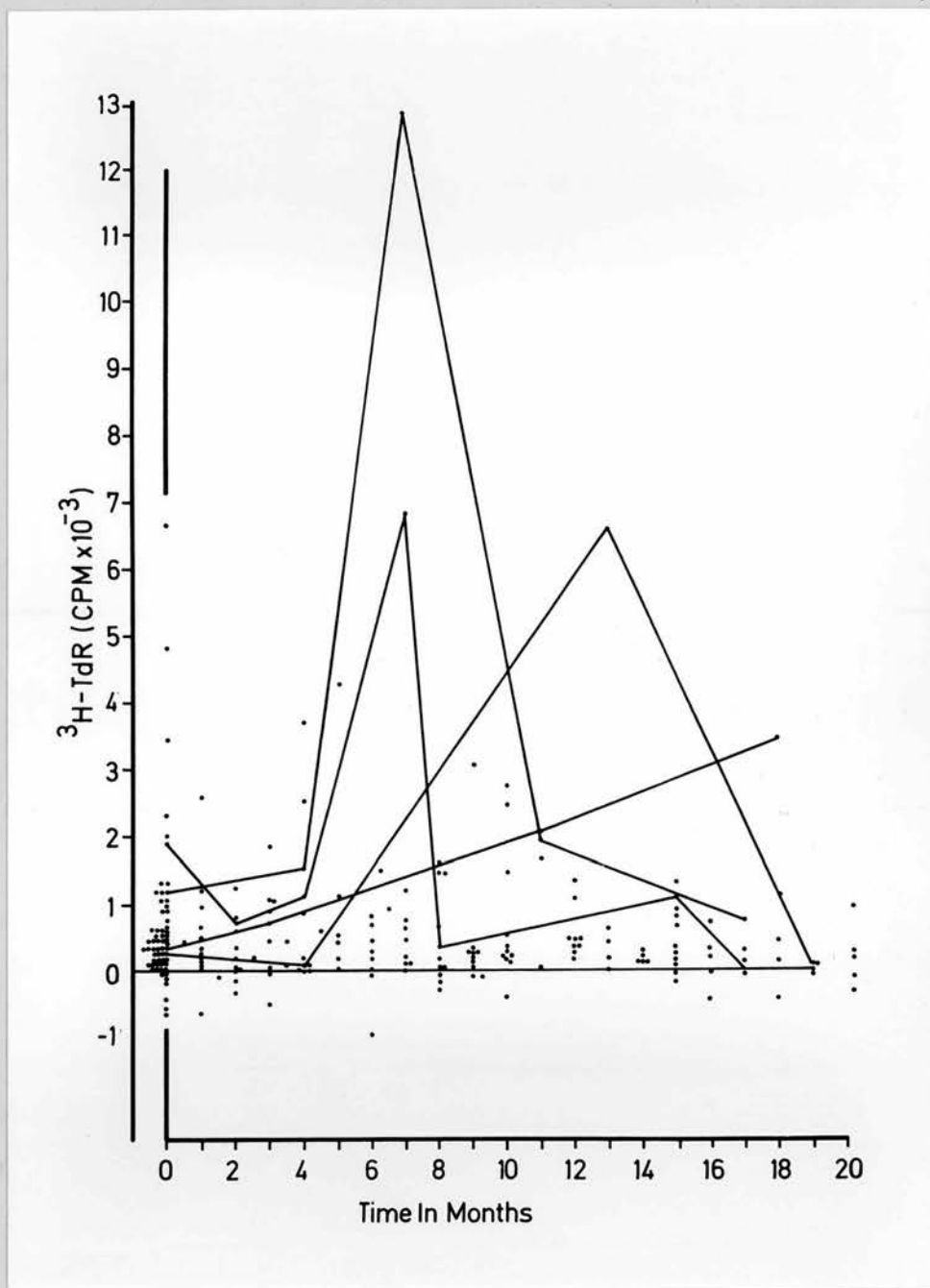


Fig. 21 LT responses to whole *M. leprae* in those patients who did not develop reaction. (Dots joined by lines are 4 patients with 'silent' skin lesions who had unexpectedly high LT responses).

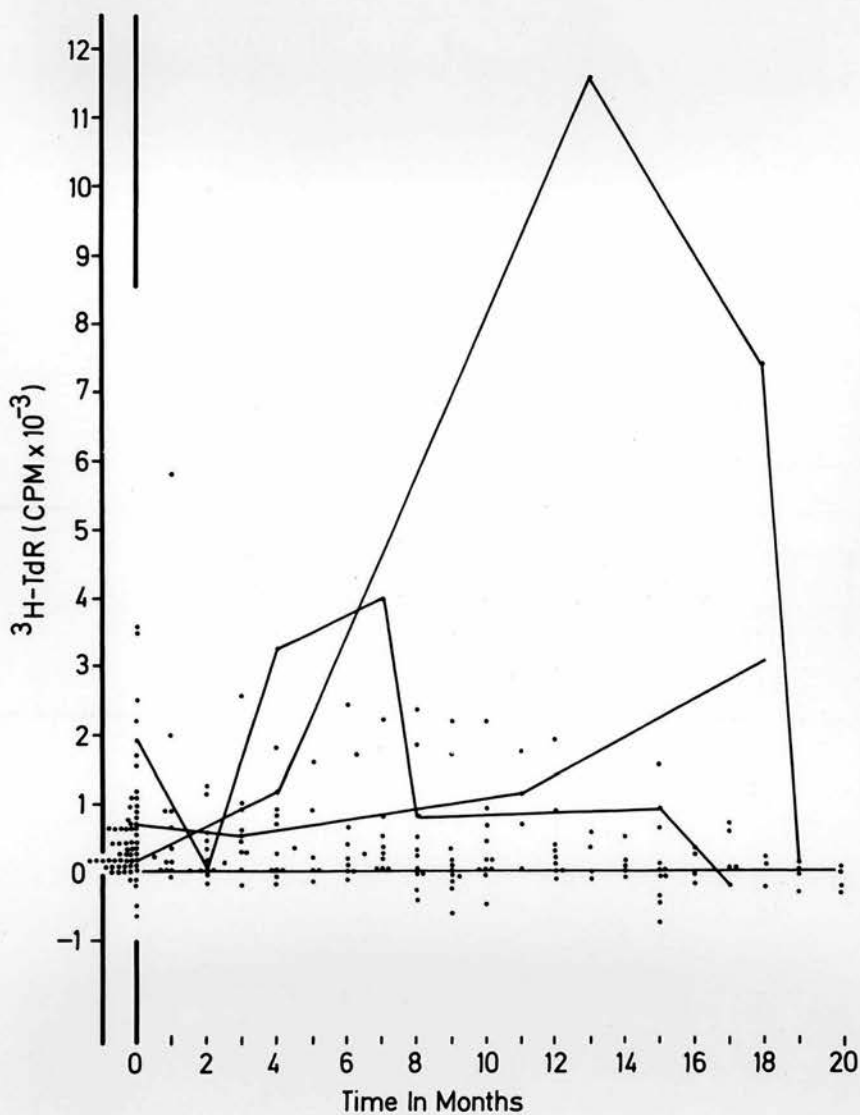


Fig. 22 LT responses to sonicated M. leprae in those patients who did not develop reaction. (Dots joined by lines are 3 of the 4 patients in Fig. who also had unexpectedly high LT responses to sonicated M. leprae).

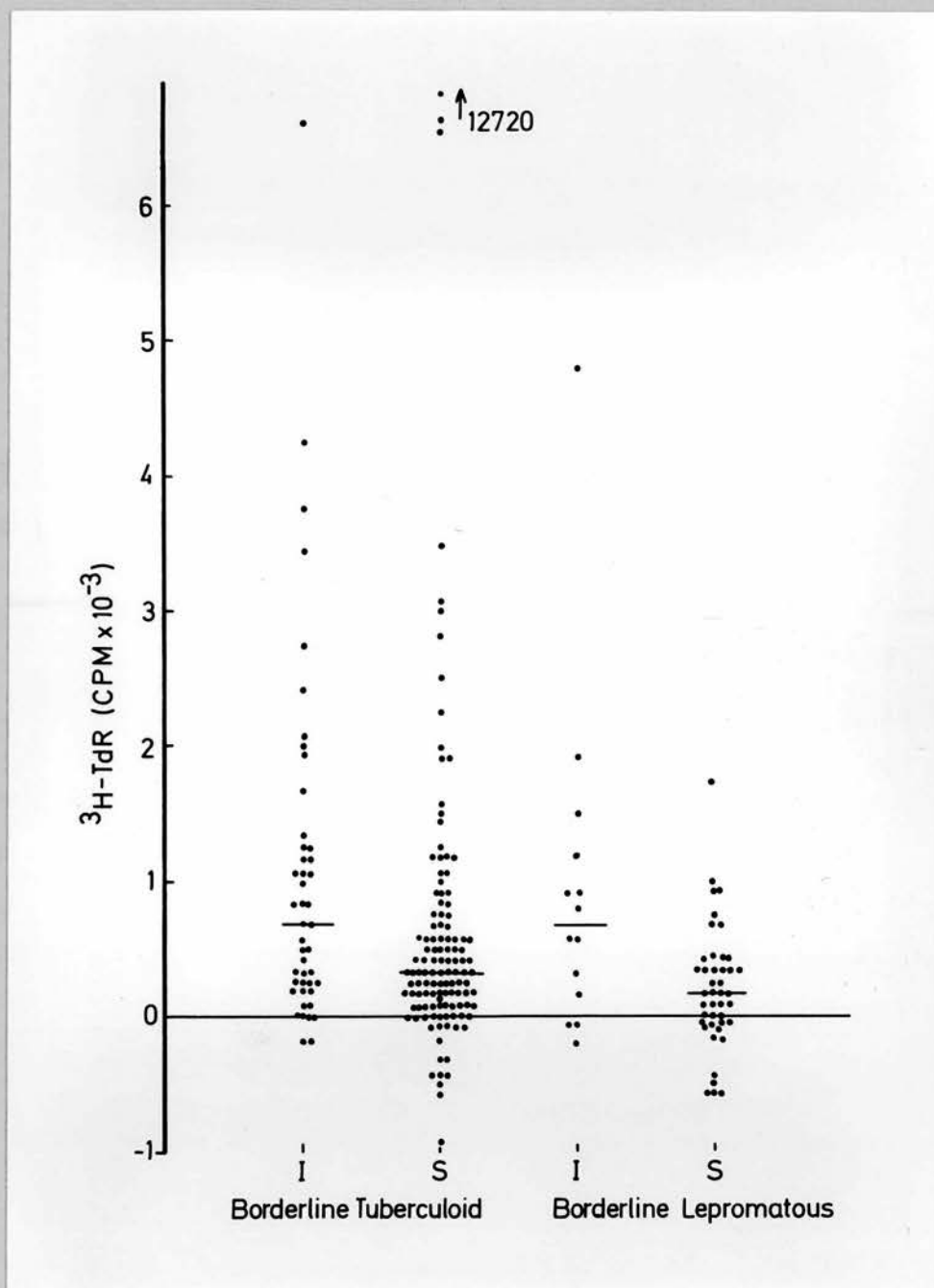


Fig. 23 LT responses to whole *M. leprae* in those patients who did not develop reaction (all LT responses); patients were classified on the basis of 'inflamed' (I) skin lesions or 'silent' (S) lesions.

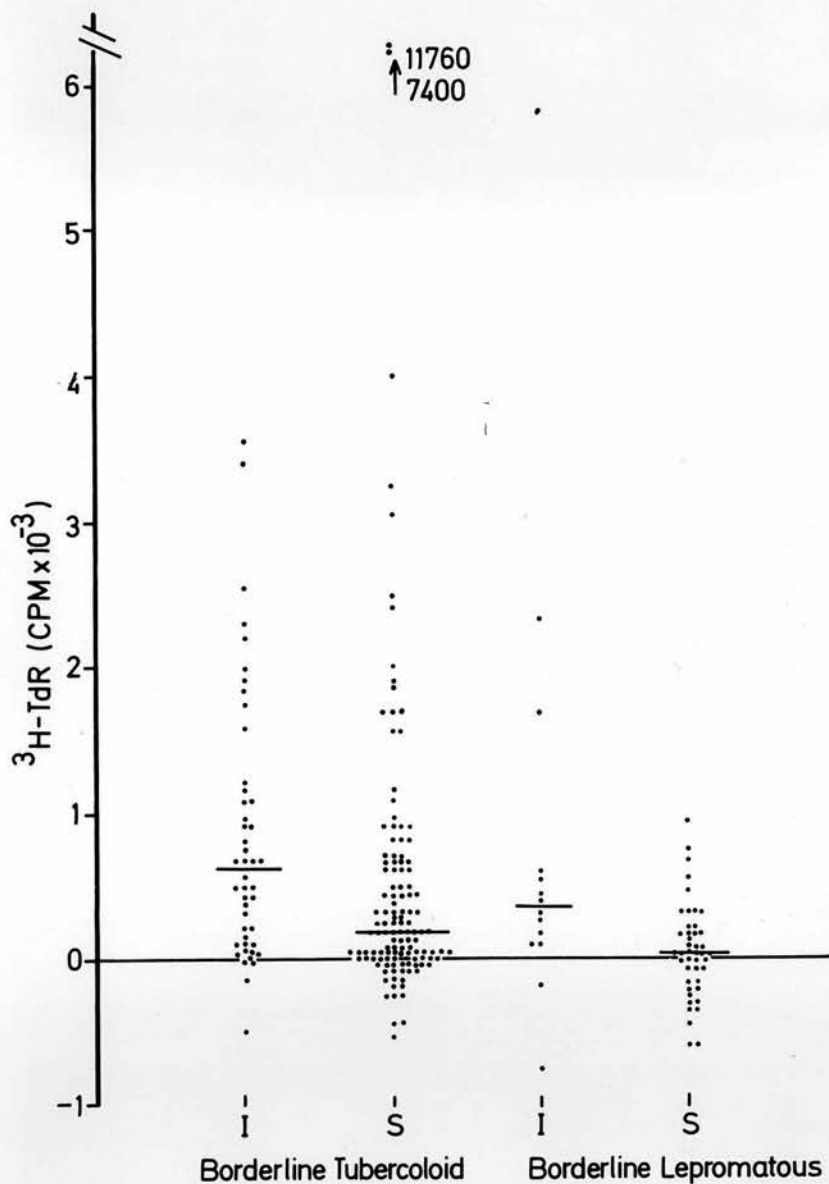


Fig. 24 LT responses to sonicated *M. leprae* in those patients who did not develop reaction (all LT responses); patients were classified on the basis of 'inflamed' (I) skin lesions or 'silent' (S) lesions.

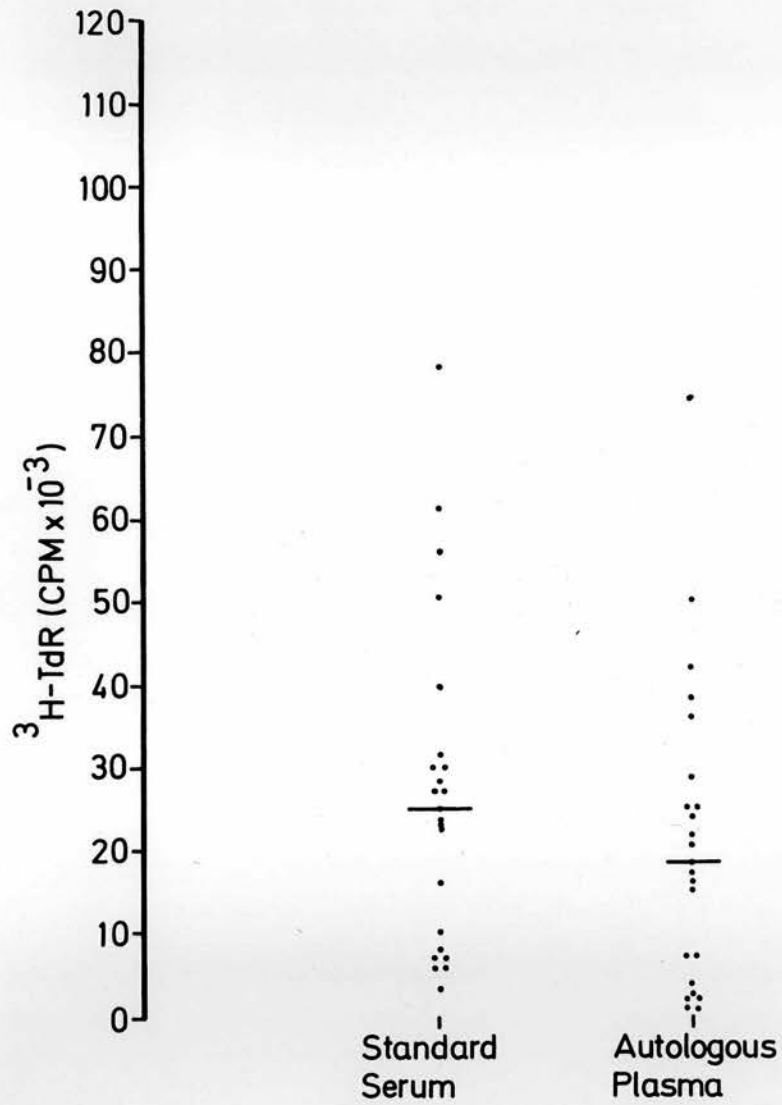


Fig. 25 LT responses to PHA in 23 "non reaction" patients comparing the initial responses in standard serum and autologous plasma.

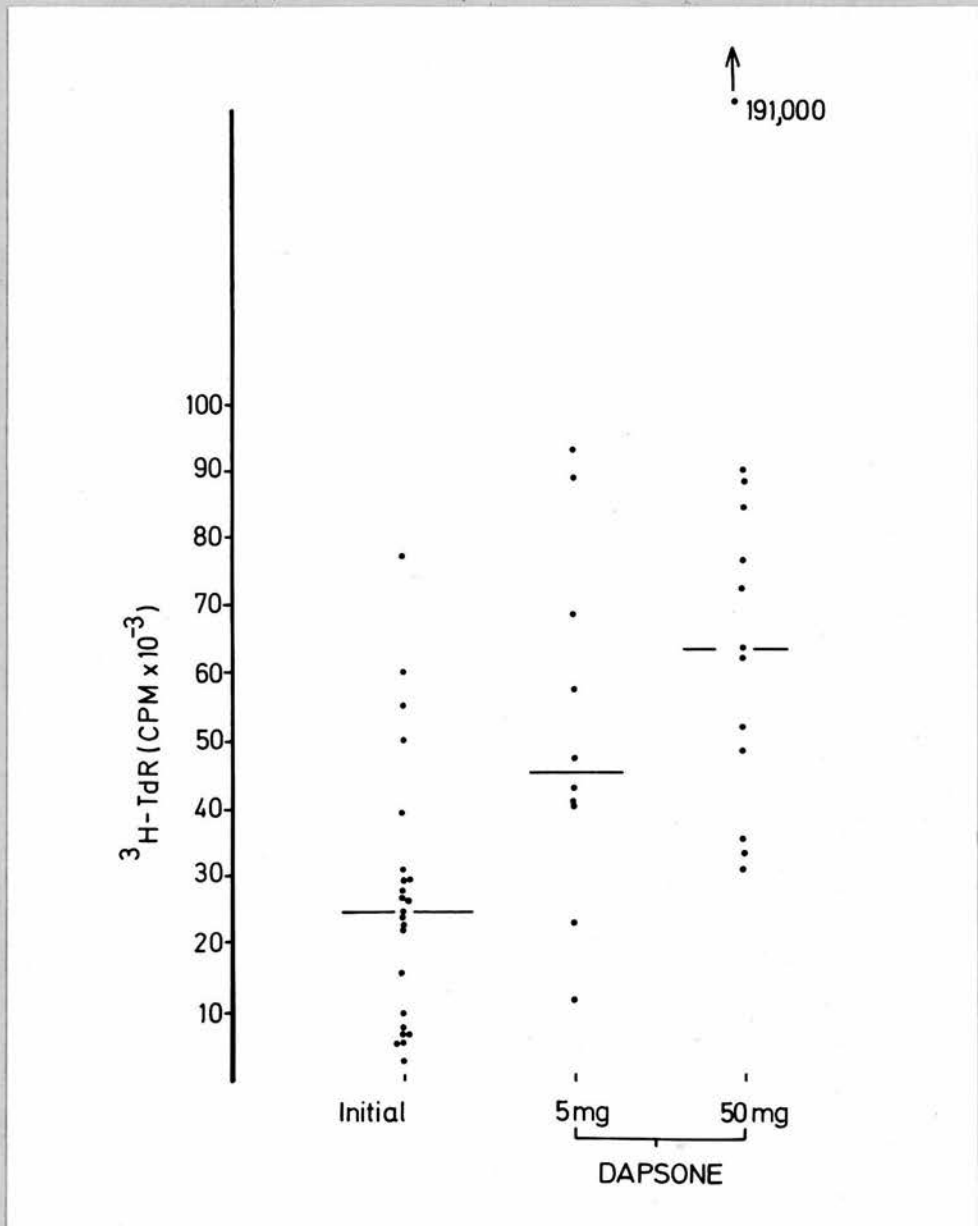


Fig. 26 LT responses to PHA in standard serum in 23 patients who did not develop reaction, before commencing treatment and after 6 months treatment with dapsone 5mg or 50mg daily.

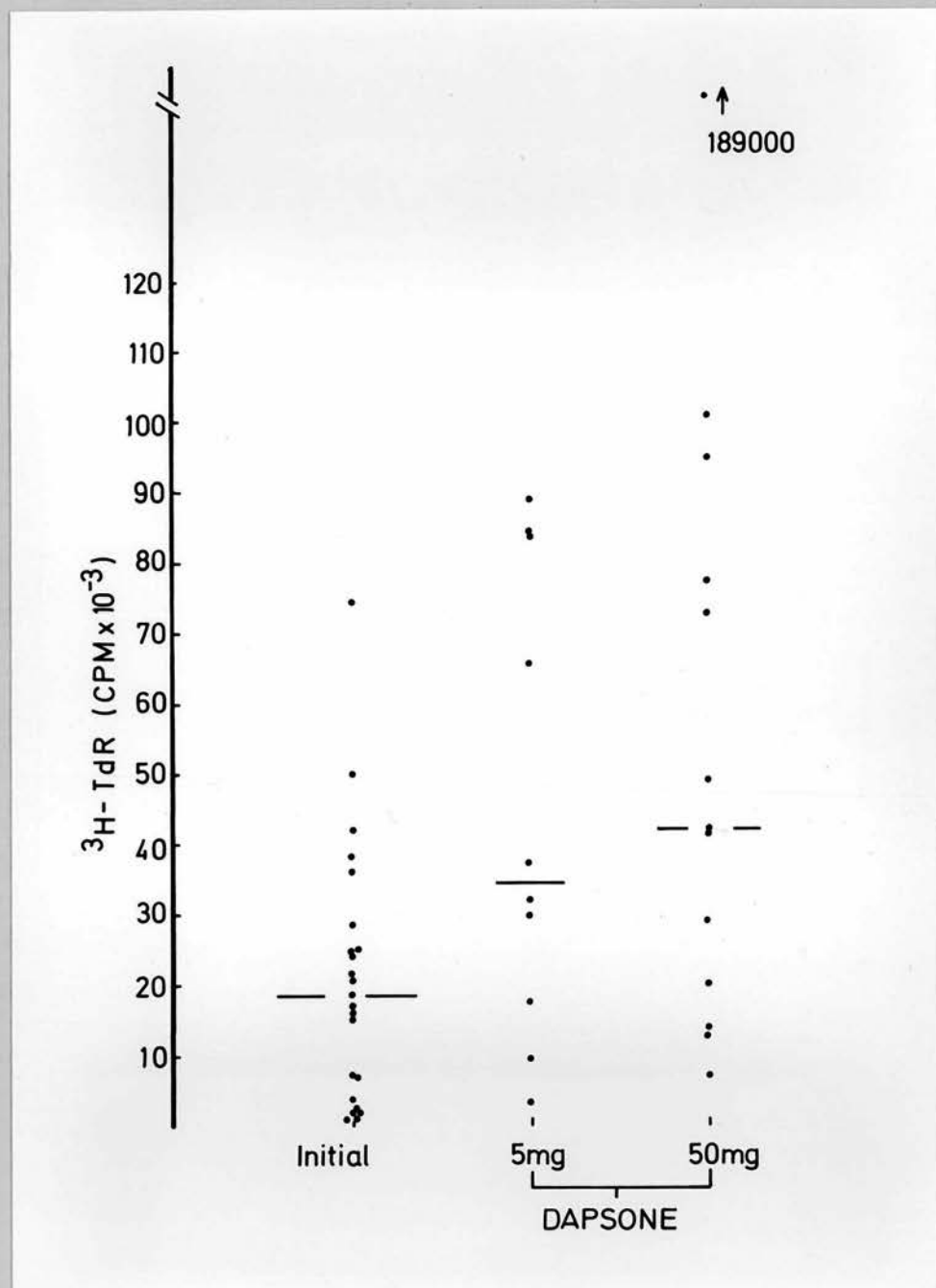


Fig. 27 LT responses to PHA in autologous plasma in 23 patients who did not develop reaction, before commencing treatment and after 6 months treatment with dapsone 5mg or 50mg daily.

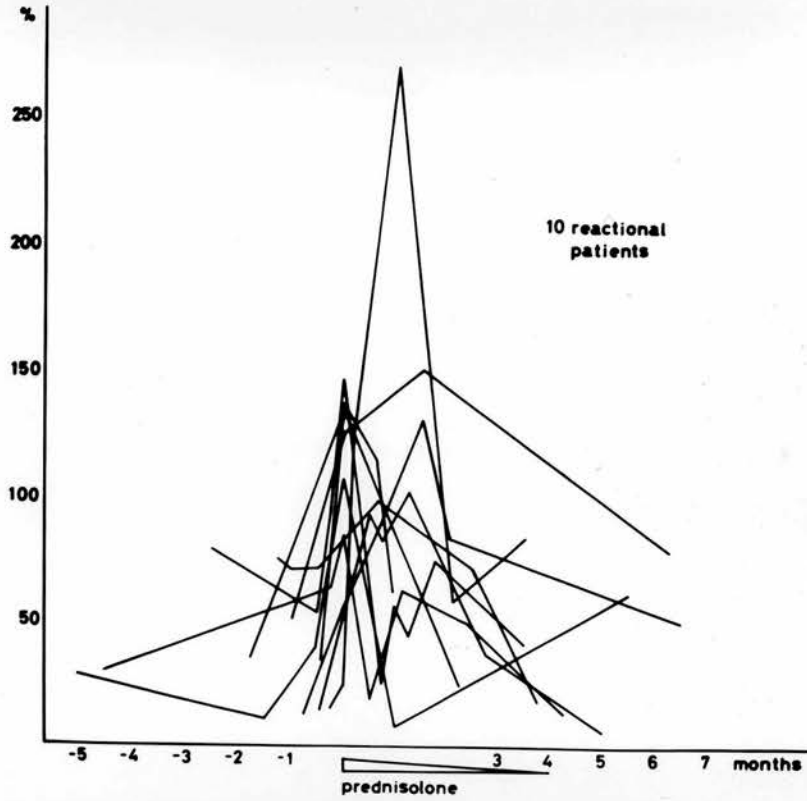


Fig. 28 Effect of autologous plasma on LT responses to PHA, recorded as a percentage of the parallel responses in standard serum, in 10 of the patients who developed reaction.

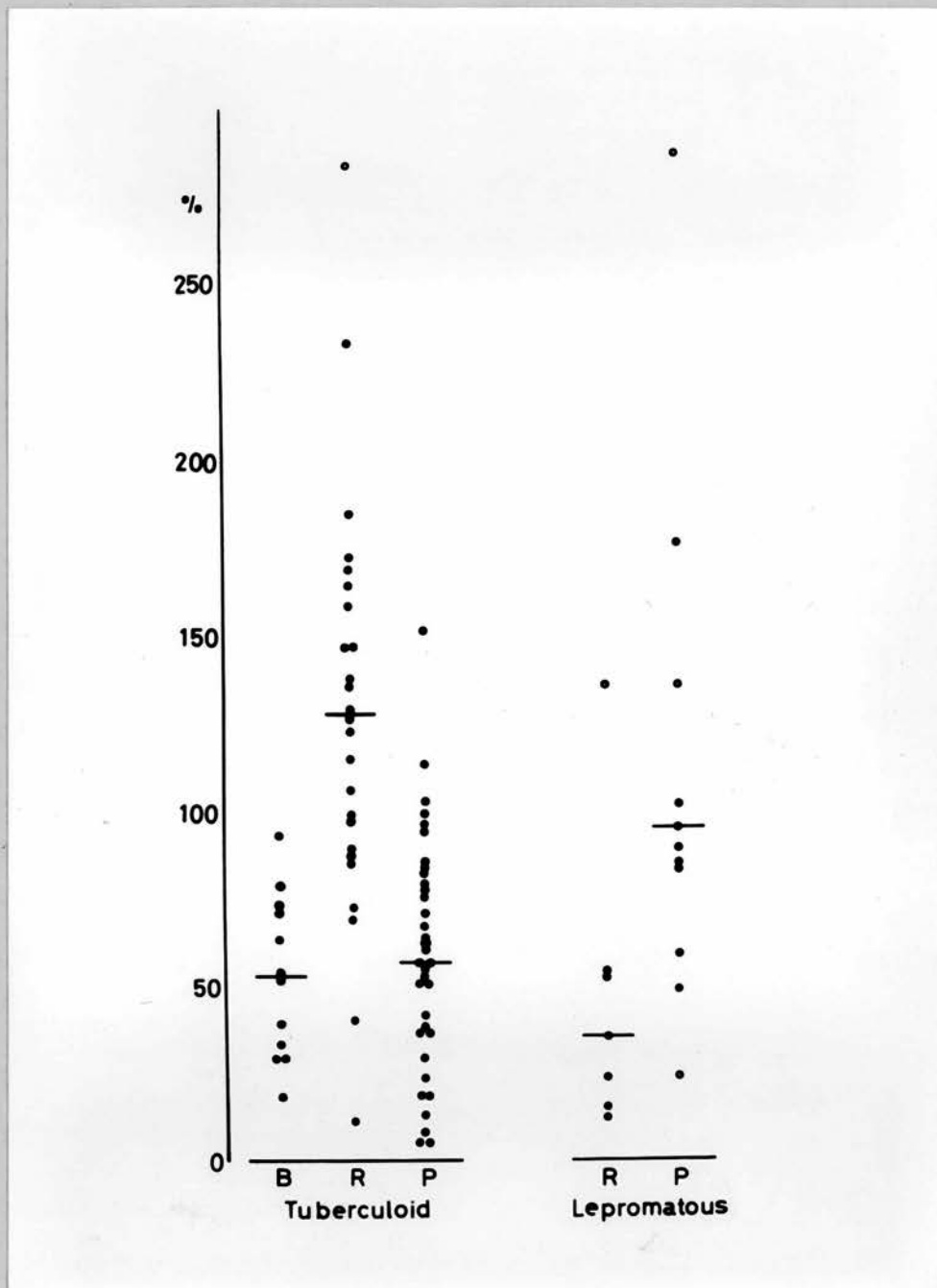


Fig. 29 Effect of autologous plasma on LT responses to PHA recorded as a percentage of the parallel responses before reaction (B), during reaction (R) and post-reaction (P). Comparison of LT responses in 16 BT patients and 5 BL patients who developed reaction (Some patients had their PHA responses measured on 2 or more occasions during the reaction and post-reaction periods).

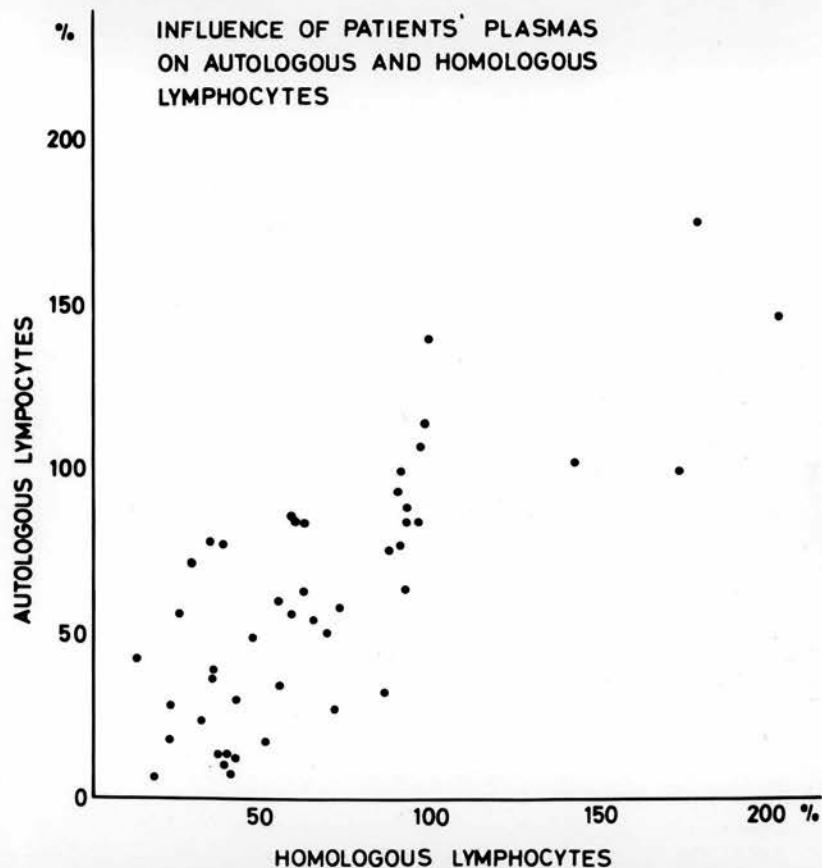


Fig. 30 Effect of reactional patients' plasma on LT responses to PHA, recorded as a percentage of the parallel responses in standard serum: correlation between the effect on autologous lymphocytes and lymphocytes from unrelated individuals.

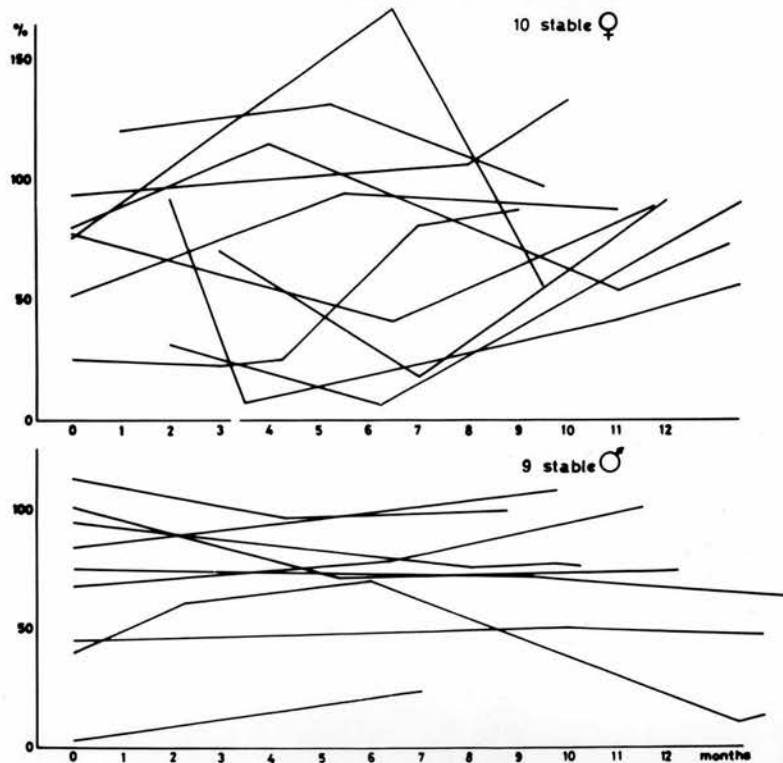


Fig. 31 Effect of autologous plasma on the responses to PHA, recorded as a percentage of parallel responses in standard serum, in 10 clinically stable females (♀) and 9 stable males (♂) with leprosy.

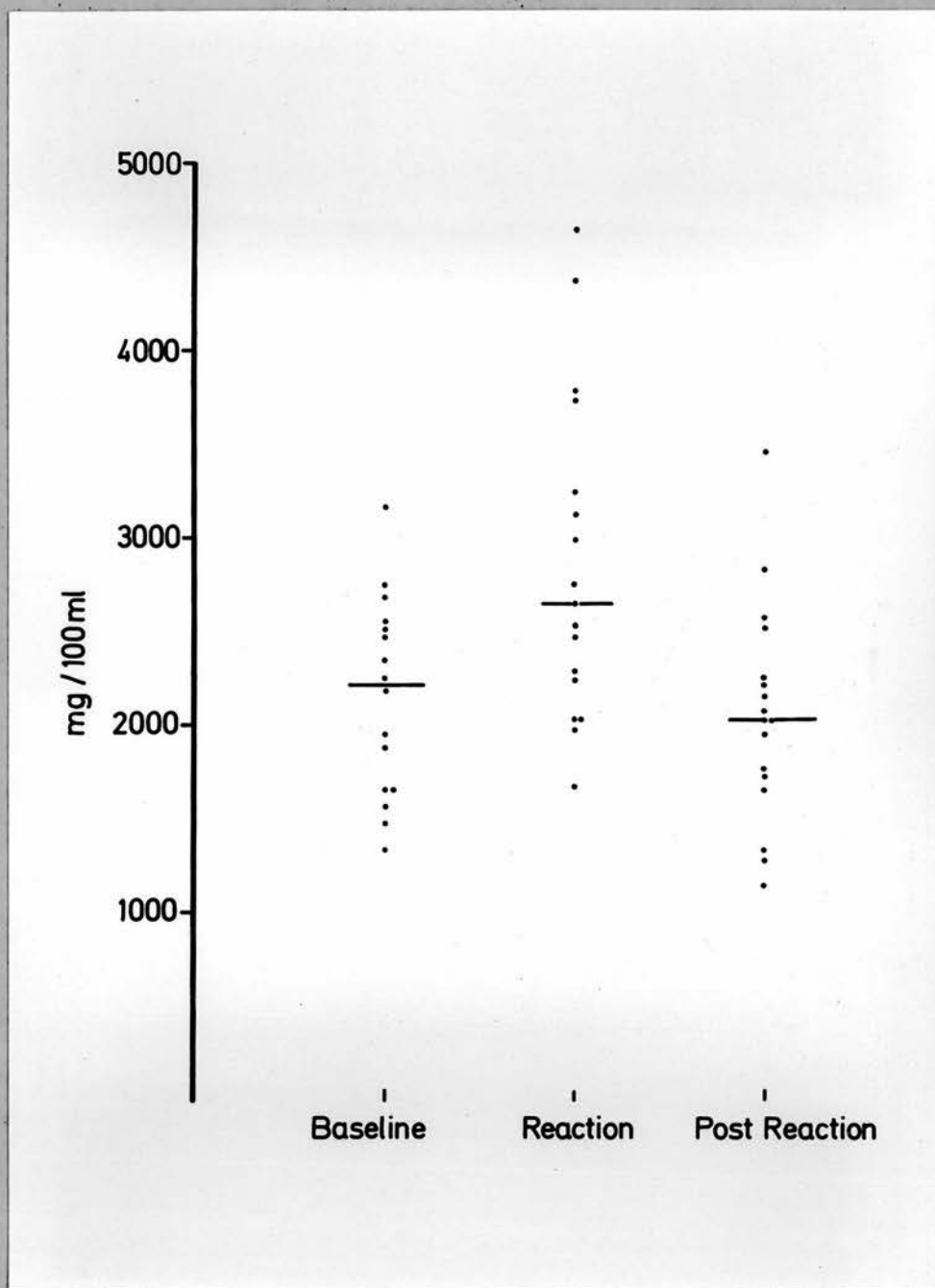


Fig. 32 Serum levels of immunoglobulin G at baseline, during reaction and post-reaction in the 17 reaction patients.

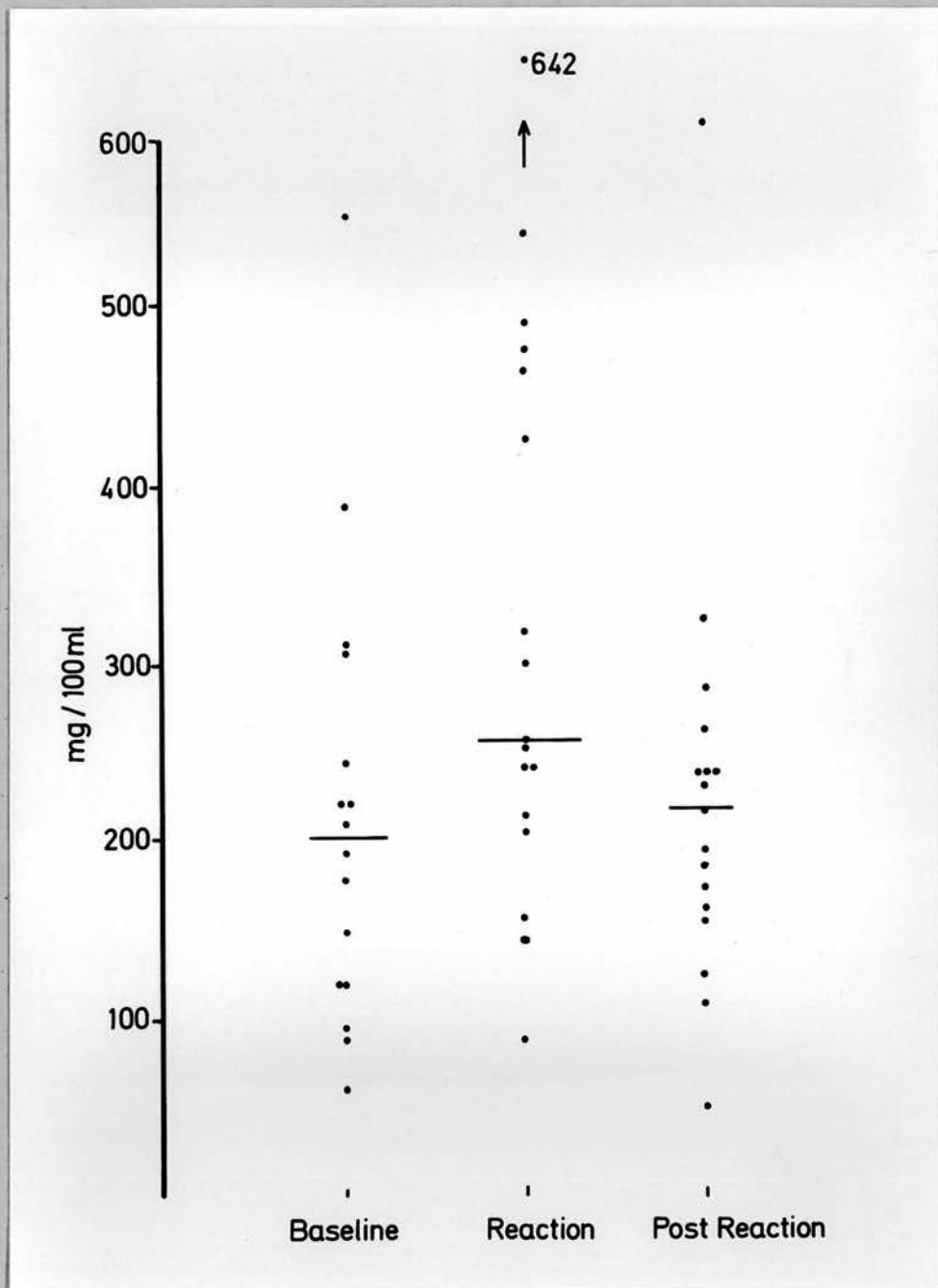


Fig. 33 Serum levels of immunoglobulin A at baseline, during reaction and post-reaction in the 17 reaction patients.

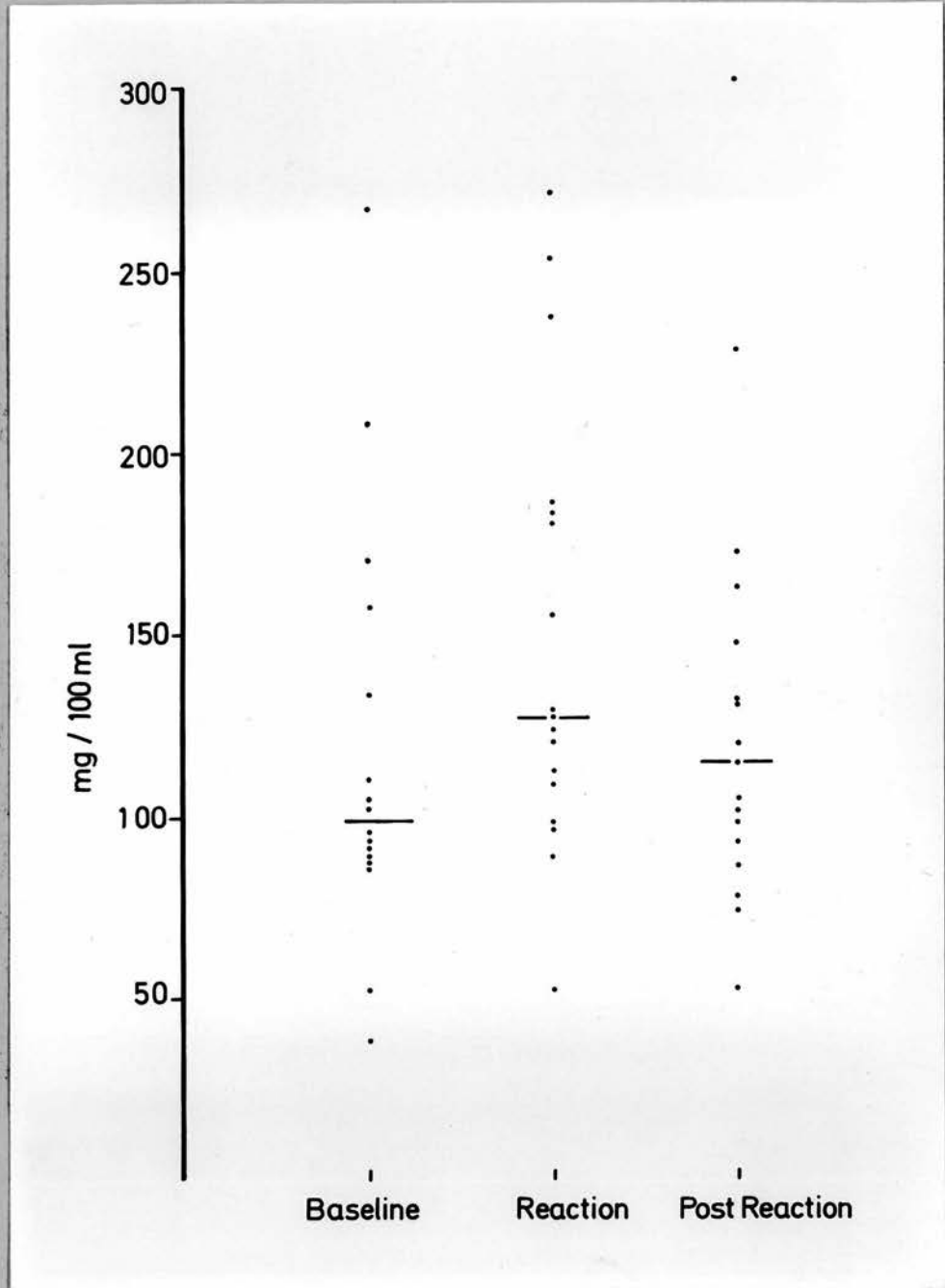


Fig. 34 Serum levels of immunoglobulin M at baseline, during reaction and post-reaction in the 17 reaction patients.

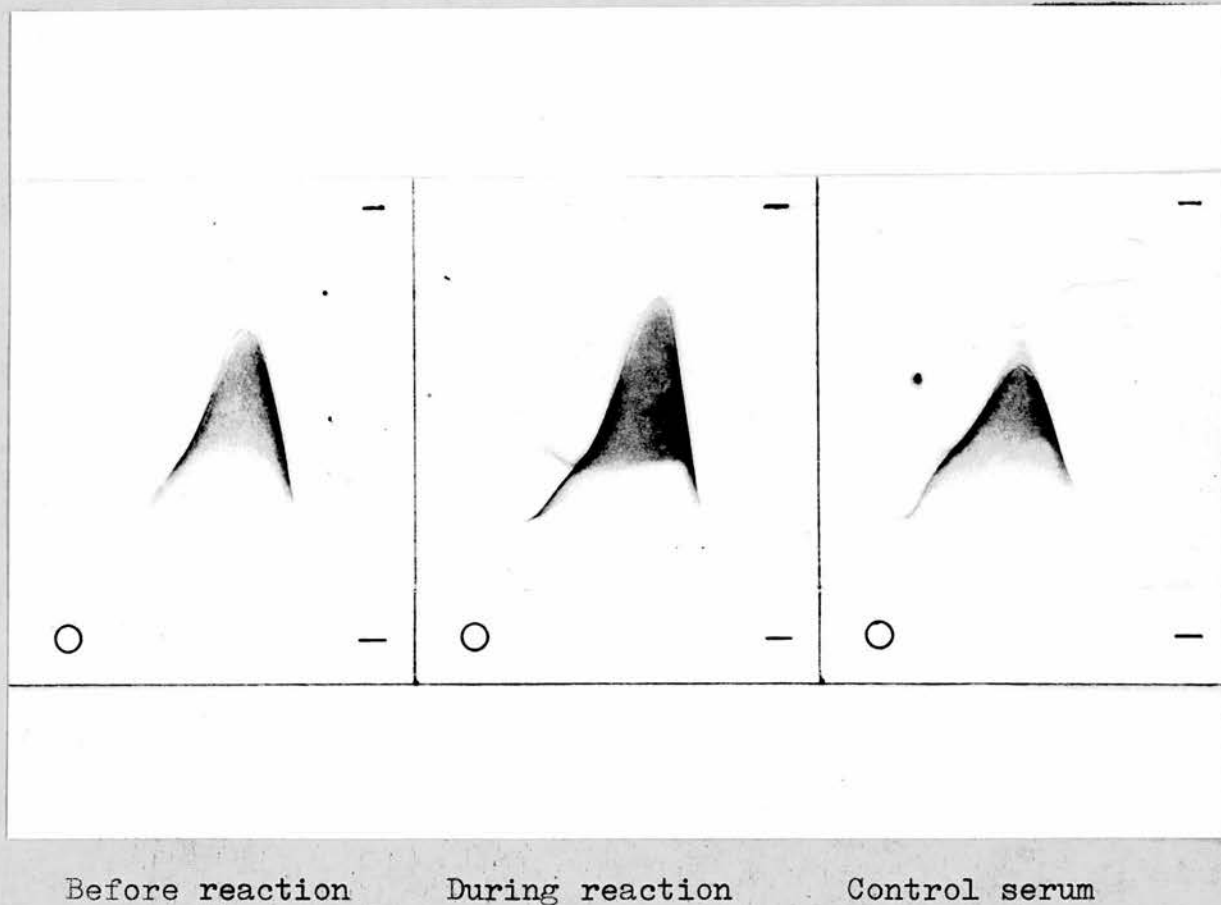


Fig. 35 Crossed immunoelectrophoretic analysis of IgG in one patient who developed reaction carried out at pH 5.0, using ammonium-sulphate-fractionated gamma globulin from anti-human IgG antiserum.

DISCUSSION

1. MECHANISMS OF BORDERLINE LEPROSY REACTIONS
(CORRELATION OF CLINICAL AND HISTOLOGICAL
OBSERVATIONS AND IMMUNOLOGICAL RESULTS)
 - (a) CELL MEDIATED IMMUNE RESPONSES
 - * i. LT responses to antigens
 - ii. LT responses to phytohaemagglutinin
 - (b) HUMORAL IMMUNE RESPONSES
 - i. Serum immunoglobulins and anti-mycobacterial antibodies
 - * ii. Auto-antibodies
2. RELATIONSHIP OF DAPSONE DOSAGE TO INCIDENCE OF BORDERLINE LEPROSY REACTIONS
3. THE SPECTRUM OF LEPROSY - HYPERSENSITIVITY AND PROTECTIVE IMMUNITY IN LEPROSY
4. OTHER OBSERVATIONS
5. CONCLUSIONS AND WORK FOR THE FUTURE

1. MECHANISMS OF BORDERLINE LEPROSY REACTIONS

(Correlation of clinical and histological observations, and immunological results.)

(a) CELL-MEDIATED IMMUNE RESPONSES

i. LT responses to antigens

(a) M. leprae

The prospective study of the 17 patients who developed borderline leprosy reactions showed an appreciable rise in LT responses to antigens of M. leprae during the reaction and confirmed the observations of Godal et al (1973), that the mechanism of these reactions is an increase in cell-mediated immunological reactivity. (Of the 3 patients in whom there was no increase in LT responses during reaction, the lack of response in 2 was probably due to technical failure of the test, and in the other was due to the fact that the reaction peak was missed when she was away from Addis Ababa.)

The problems of discerning histologically between "downgrading" and "reversal" reactions except by serial biopsies have been emphasised by Ridley (1969): and in this series all reactions were classified as "reversal", although a number occurred in patients who had hardly started treatment, and might therefore have been expected to be "downgrading". It must be concluded that in borderline leprosy there is only one type of reaction, immunologically characterised by increased LT responses to M. leprae, and histologically by

evidence of delayed hypersensitivity reaction, whether in skin or nerve. This is not to say that "downgrading" as a process is non-existent: indeed it is well documented. But the reaction that can accompany downgrading should be regarded as a transient pause in the process, or as an immunologically irrelevant episode, rather than as aetiologically concerned with the process. The same may be true for upgrading: it is yet to be proved that reversal reactions permanently affect the immunological status of the patient. Probably the only way the question could be tested would be to compare relapse rates of matched patients who did or did not develop reversal reactions.

It is surprising that in a chronic infection which provokes a cell-mediated immune response that a sudden increase in delayed hypersensitivity should occur at all. It would be expected that the host-parasite relationship would be in balance, unless new antigens were introduced. We have confirmed the observations of Muir and Chatterji (1936), and Weddell and Pearson (1975) that many leprosy bacilli are often found in Schwann cells of nerve in BT and BB patients, whereas they are not found in the skin. The same phenomenon has been noted in BL patients after several months of treatment. It seems likely that borderline leprosy reactions result from "immune recognition" of antigen lying in the nerve tissue, which would be equivalent to introduction of new antigen.

If bacilli lie "concealed" within Schwann cells, it is likely that only soluble (probably cytoplasmic) antigens are able to escape to the surface of the Schwann cell or

out of it and so be "recognised". In the early phases of a reaction, therefore, responses to cytoplasmic (sonicated) M. leprae might be expected to increase. There is some indication that this is so. When LT tests in patients with erythematous skin lesions (in the pre-reaction phase) are compared with those with hypopigmented macules, the difference is shown more markedly with sonicated preparations of M. leprae.

The same argument may be applied to nerve reaction, in which the LT responses are only directed at sonicated M. leprae. In these cases the reaction process was always aborted by steroid treatment at an early stage. It is likely therefore that in nerve reaction the majority of the bacillary-loaded Schwann cells remained intact, at least sufficiently so that the soluble cytoplasmic antigens provided the antigenic stimulus for the reaction. The failure under these conditions for reaction to develop in the skin may be quantitative; too little antigen escaped from the dermal nerves to stimulate reaction. In patients developing nerve reaction the "trigger" mechanism is uncertain. But it has been shown by Pearson (1975) that, in patients under treatment, bacillary material is redistributed within nerves so as to be concentrated around intraneural blood vessels and in the subperineurial zone: in this way higher concentrations of antigen might accumulate in those parts of the nerve where it would be more readily recognised.

In skin reactions the increase in LT responses was much greater, and also more prolonged. This may be

accounted for by the bulk of tissue involved, which is greater even in mild skin reaction than in severe nerve reaction. However, the major immunological difference was that in skin reactions the responses directed at whole M. leprae (i.e. membrane antigens) increased, as well as those to sonicated M. leprae. This difference can most readily be explained by intraneural granuloma formation: this was commonly seen in dermal nerves in skin reaction. The Schwann cell destruction associated with granuloma formation would be likely to expose surface antigens of M. leprae, and so increase LT responses to whole M. leprae. (This contrasts with the situation in nerve reactions where patients failed to develop permanent nerve damage indicating that there was no significant intraneural granuloma formation in these cases.) In addition the increase in bacillary exposure and destruction would further increase responses to sonicated M. leprae: this was demonstrated in some patients who only developed skin reactions.

In mixed reactions, there was a marked rise in LT responses to both whole and sonicated M. leprae, as might be suggested by the findings in skin and nerve reactions. The nerves were often affected some weeks after skin reaction had begun to develop, and this might imply that cytoplasmic antigens released during the "skin phase" resulted in immune recognition of bacilli lying in the Schwann cells of peripheral nerve, due to an increase in circulating T lymphocytes sensitised to these antigens.

(b) BCG and M. Duvalii

Godal et al (1973) suggested that borderline leprosy reactions might be triggered by contact with other mycobacterial disease, such as tuberculosis or infection with anonymous mycobacteria. We therefore studied the LT responses to BCG and M. duvalii in our patients. There was no evidence of increased LT responses to these antigens prior to reaction, though there was a rise in the LT responses in some patients to both antigens during reaction. It is probable that this was due to some degree of cross-reactivity. One patient (no. 42) developed very high responses to BCG after reaction, though he had no evidence of clinical tuberculosis at that time. His LT responses to M. leprae were also higher concomitantly.

ii. LT responses to phytohaemagglutinin

The observation that LT responses to PHA during or after reaction are augmented by autologous plasma instead of the usual suppression by these factors may be of great importance. The reason for the emergence of hypersensitivity from a state of comparative non-reactivity is still unclear. One possibility, however, is that reaction occurs when there is a breakdown of control mechanisms which normally regulate CMI responses and prevent them from becoming overactive and harmful. A factor in plasma might play an integral part in that control mechanism.

Factors in the blood inhibiting lymphocyte responses to PHA have been demonstrated in a number of conditions where there is prolonged stimulation of the immune system; e.g. in leprosy by Nelson et al (1971), in recurrent infections in childhood by Fitzgerald and Hosking (1976), in various neoplasms by Sucio-Foca et al (1973) and in pregnancy by Gatti (1971).

Patients with borderline leprosy often show surprisingly little inflammatory response, despite the presence of bacilli in the tissues for many years. Plasma from such patients caused inhibition of lymphocyte responses to PHA compared with the responses in standard serum. In those patients with BT leprosy who developed a reaction this inhibitory effect disappeared during the reaction and was replaced by an augmenting effect of plasma on lymphocyte response. However in those with BL leprosy the augmenting effect of plasma appeared later than those with BT leprosy, though within 3 months after the reaction.

That both the augmentation and inhibition of lymphocyte responses were due to factors in the plasma and not to a change in lymphocyte receptors, is supported by the observation that the plasma had the same effect on both homologous and autologous lymphocytes. Control experiments with different concentrations of PHA gave no indication of a changed sensitivity of lymphocytes during reaction nor was there any significant change in the actual height of the PHA responses in standard serum in relation to reaction. So the augmentation of responses by autologous plasma during reaction was not due to a change in the lymphocytes!

responsiveness to PHA.

Although male patients with stable leprosy had a constant degree of inhibition during the period of observation, the effect of plasma from stable female patients on PHA responses showed great variation from test to test. Further studies on normal individuals (G. Bjune, personal communication) have shown that females have pronounced variations of plasma effect on the PHA response related to the menstrual cycle. The magnitude of these variations considerably surpasses the basic level of suppression due to the leprosy infection.

The finding in BL patients that the period of augmentation by autologous plasma was delayed in relation to the onset of clinical reaction, could indicate that the plasma factor(s) were secondary to the reaction. However, it is probable that the plasma factor(s) described here will initially occupy lymphocyte receptors and exert their effect there, before they appear free in the blood. Therefore the delayed appearance in the blood of the augmentary effect does not necessarily negate their primary importance in transforming the CMI response from a well controlled fight against bacillary multiplication to an overactive and self-destructive hypersensitivity reaction.

(b) HUMORAL IMMUNE RESPONSES

i. Serum immunoglobulins and anti-mycobacterial antibodies

The finding of significant alterations in immunoglobulins during borderline leprosy reactions raises the question of whether they themselves are the cause of the reaction, or associated with the cause directly, or whether they are merely secondary incidental findings caused by the reaction.

The results indicate that there is a non-specific, polyclonal rise in Ig levels rather than specific increases in anti-mycobacterial antibodies or formation of auto-antibodies or tissue destruction during the reaction. Such changes are likely to be secondary. They would not be expected to influence T-cell activation by M. leprae antigens or to be caused by their exposure.

That the increases in Ig levels are apparently not due to M. leprae antigen stimulation calls for an alternative explanation. Several studies have demonstrated that proliferating, non-secreting B cells can be converted into antibody producers by a factor or factors produced by activated T cells. (Dutton et al, 1971; Schimpl and Wecker, 1972; Watson, 1973; Amerding and Katz, 1974; Davie and Paul, 1974.) In borderline leprosy reactions there is a marked increase in the number of circulating T lymphocytes sensitised to antigens of the leprosy bacillus. These lymphocytes, when activated in the widespread lesions,

might stimulate the production of serum Igs by non specific action on proliferating B lymphocytes. This is supported by the finding that the rise in IgM during reaction was proportionately much less than that in IgG or IgA, and there was no significant fall after reaction. This is in agreement with the findings of Davie and Paul (1974), who noted a relative T-cell independence of IgM antibodies in experimental animals. The rise in Igs in this study would thus be an effect rather than a cause of the reaction.

ii. Anti-nuclear antibodies, anti-lymphocyte antibodies and neural auto-antibodies

It has been suggested that peripheral nerve injury during borderline leprosy reactions might be due to auto-immune phenomena in a manner analagous to experimental allergic neuritis (Waksman and Adams, 1955). The reaction patients in our study were tested for ANAs,ALAs and neural auto-antibodies before, during and after reaction but without positive findings. There was thus no suggestion from these tests that auto-immunity played any part in these reactions. Moreover the histopathological changes seen in nerve biopsies were quite unlike those in auto-immune neuritis.

2. RELATIONSHIP OF DAPSONE DOSAGE TO INCIDENCE OF BORDERLINE LEPROSY REACTIONS

Of 34 patients taking 5mg dapsone daily, 11 developed borderline leprosy reactions whereas only 3 taking 50mg

dapsone daily developed reaction. These results indicate that patients (particularly those with erythematous swollen skin lesions) receiving low dosage of dapsone (5mg daily) are more likely to develop borderline leprosy reaction than those 50mg daily from the start of treatment. This finding contradicts current teaching, and is probably the most important observation of the study, in the short term. In the 2 previous studies by Lewis et al (1957) and Prasad (1971) which compared "high" and "low" dosage regimens and found no difference in the incidence of reaction between the two groups. The "low" dosage was, except for the initial 2 - 3 weeks equivalent to 50mg daily or more.

In this series, as is usual, reaction developed in most patients (11 of 17) within the first 2 months of being started on dapsone treatment. The reason for this is obscure, as dapsone is a bacteriostatic drug and the multiplication time of the bacillus about 14 days; thus, any direct effect of the drug on the bacillus with exposure of new antigens seems unlikely. It is possible though that many patients initially attended hospital for treatment because of the prodromal symptoms of reaction. If this is so, dapsone 5mg daily may be regarded as not affecting the natural history of the reaction whereas 50mg resulted in suppression. The influence of dapsone on the immune system is unknown. It appears to have some immunological activity e.g. it suppressed the Arthus reaction in guinea pigs (Thompson and Souhami, 1975), and this may account for its therapeutic success in dermatological conditions such as dermatitis

herpetiformis and leucocytoclastic vasculitis. Beiguelman and Pisani (1974) suggested that dapsone may also suppress cell mediated immunity.

The results of this study are inconsistent with these suggestions, for the LT responses to PHA were higher in patients receiving treatment than before treatment. This rise, however, developed slowly: probably too slowly to affect reactions in the first month or two of treatment. Also it was somewhat greater in patients receiving dapsone 50mg daily; whereas those on 5mg daily were more liable to develop reaction. Since the minimal inhibitory dose of dapsone is about 1mg daily (Waters et al, 1968) prevention of reactions is unlikely to be the result of variation in anti-bacterial activity. The mechanism by which dapsone in higher dosage seems to suppress reactions remains obscure.

The finding, however, is of considerable practical importance. Current drug regimens are unduly complicated for field workers and patients. Their design was based on the misconception that low dosage of dapsone would prevent or mitigate reactions. It has previously been shown by Pearson and Helmy (1973) that even stopping dapsone completely does not significantly ameliorate ENL, and this study indicates that dapsone in higher dosage may be beneficial in borderline leprosy reactions. We believe therefore that all patients with leprosy should be treated with dapsone 1 - 2mg per Kg daily from the start of therapy, and that treatment should be continued uninterrupted during reactions. If this policy is implemented many reactions will be prevented, and effective treatment for

reactions that do develop will not be delayed by ineffectual manipulation of dapsone dosage.

3. THE SPECTRUM OF LEPROSY. HYPERSENSITIVITY AND PROTECTIVE IMMUNITY IN LEPROSY

It has been shown that the histopathological changes in reversal reaction are diagnostic of Coombs and Gell Type IV reaction, and the immunological tests described indicate that, when skin is involved in the reaction, the membrane antigens of M. leprae are mainly associated with the reaction. Loss of a humoral inhibiting factor could also play an important role in the pathogenesis of the reaction. The relationship between LT responses and the spectrum of leprosy, however, is much less precise, and the average responses shown by Myrvang et al (1973) conceal a very great range of results for each type of leprosy. The results of this study confirm this (Figs. 18, 19) in the borderline range of leprosy. Clearly the LT test, as used in this study, is a poor assessment of host resistance during the disease.

How, then, is host resistance to be defined. In practice, 2 methods can be employed. One is the ability of the patient under effective treatment to dispose of disintegrating bacillary material. A rough indication of this can be attained by following the fall of the Bacterial Index during treatment. However, this process is markedly hastened when patients develop reversal reaction: that is to say, it is influenced by hypersensitivity phenomena.

The other indication of host resistance is the ability of the patient to limit his bacillary load. Thus resistance is defined in terms of the spectrum of leprosy, and can be investigated by studying the histopathology of the untreated disease. When this is done, the disparity between LT tests and resistance is clearly shown.

In the BT patients, LT responses to M. leprae were lower when the histopathological features consisted of non-specific round cell infiltration in the dermis. High LT responses correlated with the numbers of epithelioid and giant cells (i.e. with hypersensitivity) which in turn was commonly associated with erythematous raised lesions. But the average duration of the disease in the "indeterminate" histology group (3.8 years) was greater than in the histologically determinate (BT or TT) cases (2.2 years). This indicated that the disease had been "contained" for longer, and therefore the host resistance was higher, in the low LT test group.

Also in "indeterminate leprosy" (diagnosed both clinically and histologically) many lesions are self healing; host resistance should therefore usually be high. But LT tests are almost always low (Myrvang et al, 1973) and there is no histological evidence of hypersensitivity.

There is therefore clear evidence that LT test results using M. leprae (as prepared in this study) as antigen are related to hypersensitivity phenomena rather than to host resistance. The marked though transient increases seen during reactions, which are known to be

hypersensitivity phenomena, confirm this conclusion.

There are a number of possible explanations of this apparent paradox (that high resistance tends to be associated with low LT responses). They include:-

(a) Technical failures of the test. This seems unlikely here. Each patient in this study was tested with 3 different concentrations of 2 different preparations of M. leprae. Differences in the dose eliciting a peak response should therefore not affect the percentage of responders. Good responses to PHA, as well as to antigens like BCG and M. duvalii prove the ability of the cells to respond in the actual experiment. Most patients were also tested more than once, and if clinically stable during the period of observation, the LT responses were also stable.

(b) The wrong antigens were used. CMI in leprosy could have specificity for different antigens of M. leprae. In a given preparation of bacilli not all antigens are necessarily equally well exposed. Thus lymphocytes which perform well in vivo might show no LT response in vitro.

(c) The wrong lymphocytes were used. The number of specifically sensitized lymphocytes in the peripheral blood represents a balance between their production and their trapping in the lesions (Thestrup-Pedersen, 1974); small numbers in the circulation need not imply inefficient formation. The potential of the patient's immunity is difficult to estimate when no standard stimulus exists in vivo.

Elucidation of this situation is clearly still required. But it is clear that the dissociation between resistance and hypersensitivity which has long been recognised in the field of tuberculosis (Rich and McCordock, 1929) must now be accepted as also important in leprosy. There are a number of theoretical explanations:-

(i) The antigens stimulating high LT responses are irrelevant for resistance, possibly because they are exposed only by dead bacilli (Youmans, 1975).

(ii) Differences in the presentation of antigen, especially between presentation in Schwann cells and macrophages, could explain why bacilli can stay undetected in one tissue, while giving rise to vigorous reactions in another. The findings in reversal reactions indicate that antigens are presented differently in the two cell types.

(iii) Even in untreated patients most M. leprae are non-viable. It is possible that only antigens produced by living bacilli and which can escape from the host cell are responsible for immune resistance to the infection.

The results of this study offer some clues as to the nature of the antigen(s) involved in infection immunity: in previously untreated patients the difference in response between BT and BL cases was greater with sonicated than whole washed M. leprae (Figs. 18, 19), suggesting that a cytoplasmic antigen is involved. However, it must be emphasised that the difference was not marked. At present,

it appears, the LT test is of limited value in the assessment of host resistance during the disease.

4. OTHER OBSERVATIONS

1. Incidence of "limb" pains

Pain is an important symptom of leprosy as an indication of disease activity. This may seem a facile observation, but it is not given emphasis in textbooks on leprosy. A number of patients had hypopigmented lesions for many years (up to 14 years) but presented soon after they developed "limb pains" due to neuritis. Only 5 of the patients who had had pains prior to clinical presentation, had them for longer than 1 year.

After commencement of treatment, these pains disappeared in a vast majority within the first year of treatment. Three of the 4 patients who were having pains at the end of the study had had "nerve reactions" and thus might be expected to have more prolonged neuritis than those who had not had reactions.

A recent study at the Addis Ababa Leprosy Hospital (B. Naafs, personal communication) showed that those patients who had been "released from control" having had what was felt to be adequate therapy for their leprosy, (the duration of treatment depending on the type of leprosy) and relapsed, tended to present with limb pains.

It is likely that any patient who continues to have pains for longer than 1 year after commencement of treatment may not be taking his therapy. Three of the

patients who were having pains at the end of the trial (including the 1 non-reaction patient who had consistently low D/C ratios) were taking dapsonc 5mg daily.

2. Regularity of dapsonc intake

Patients' urine was tested for the dapsonc/creatinine ratio at each review. It was found that overall 36% of observations were low, of those taking dapsonc 50mg daily. In only 8% of observations was the D/C ratio less than 10, implying that dapsonc had not been ingested for a number of days. Very often, low observations fitted with the patient's story of their tablets having become crushed or destroyed by the rain. The patients in the study took their dapsonc more consistently than those reported by Low and Pearson (1974) and this might be expected, as they were receiving more attention than the normal patients; in particular they were being reviewed by a doctor monthly (or two-monthly) rather than 6 monthly.

3. Serum immunoglobulin levels in all patients in the study

Though there was considerable variation in serum levels of IgG, IgA and IgM, there was no significant difference in those patients with BT leprosy and those patients with BL leprosy. This variation in Ig levels has been noted in other developing countries (McFarlane, 1966; Hobbs, 1971) and presumably reflects the greater immunological challenge provided by the environment.

5. CONCLUSIONS AND WORK FOR THE FUTURE

Borderline leprosy reactions are the most important complication of leprosy infection. Our study has clarified some of the mechanisms involved but it is important that the work is continued in certain directions:

1. We have confirmed that borderline leprosy reactions are a type of delayed hypersensitivity reaction. It seems likely that different presentations of the reaction when they affect skin or nerve are associated with the release of different antigens. The mechanism of this requires further study.
2. It is possible that these reactions are related to the presence of "plasma factors" which though normally suppressive to the cell-mediated responses, during reaction develop augmentary properties. It is important that the nature of these plasma factors is ascertained, and that a primary role in the reaction is confirmed.
3. It seems likely that the intrinsic mechanism of borderline leprosy reactions is immune recognition of bacilli lying sequestered in Schwann cells of peripheral and dermal nerves. This is a fascinating phenomenon which deserves further study.
4. Serum immunoglobulins G, A and M all rise during reaction. We have shown that this is likely to be a secondary phenomenon due to T-lymphocyte sensitization of B lymphocytes. It is pleasing that on occasion

work demonstrated in experimental animals can be confirmed in human disease.

5. We have shown that contrary to previous teaching, dapsons given in higher dosage does not predispose patients to borderline leprosy reactions. Our results suggest in fact that higher dosages of dapsons prevent reactions, and it is important that this be confirmed by an independent group, and the mechanisms of prevention fully investigated.

6. A great majority of patients develop reactions during the first 6 months treatment. It may be beneficial to give borderline leprosy patients a low dose of steroid prophylactically during the first 6 months of review in an attempt to further prevent reactions. A trial designed to test this hypothesis has been instituted in the Addis Ababa Leprosy Hospital.

7. The triggering mechanism for borderline leprosy reactions has not yet been elicited. Four of our patients had unexpectedly high responses to antigens of M. leprae and high responses are often seen in contacts of leprosy patients. It is possible that further contact with lepromatous patients may induce an increase in circulating T-lymphocytes sensitised to M. leprae, even in patients who already have the disease and this could trigger reactions. This possibility should be examined further.

8. The LT test in leprosy measures parameters of hypersensitivity rather than immune protection against the bacillus. It may be that different bacterial antigens are involved in the 2 phenomena and this will clearly need further investigation.

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APPENDIX

(Published papers)

Antigenic heterogeneity in patients with reactions in borderline leprosy

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Summary

Fifteen patients with borderline leprosy who developed "reversal" reactions were studied from the inception of treatment. Thirteen showed an appreciable increase in lymphocyte transformation (LT) when preparations of *Mycobacterium leprae* were used as antigen. The LT responses to either "whole" or "sonicated" preparations of the bacillus in these 15 patients and in nine others also showed a reaction correlated with the clinical presentation. Those with skin disease predominating in the reaction showed an appreciable increase in LT when whole *M leprae* was used as antigen. Those with nerve disease predominating showed an increase with sonicated *M leprae*. In those with both skin and nerve disease there was an increase with both antigen preparations. The ratios of the LT test results (whole to sonicated *M leprae*) showed highly significant differences between the three groups.

Introduction

Mycobacterium leprae, the causative organism of leprosy, is an obligate intracellular parasite with an affinity for skin and nerve. It is remarkably non-toxic—patients may have 10^{12} – 10^{13} bacilli in the body without systemic illness—and most of the clinical manifestations of the disease result from the host response. The most important cause of deformity in leprosy is nerve damage due to hypersensitivity to bacilli lying in the nerves. Such damage may result from two different mechanisms, both termed "reactions."

"Reversal" reactions, which occur in borderline leprosy, are probably due to a delayed hypersensitivity reaction (Coombs and cell type IV hypersensitivity reaction), as there is usually a dense lymphocytic infiltration in histological specimens of skin and nerve and an appreciable increase in lymphocyte transformation (LT) when "whole" *M leprae* is used as antigen.¹

"Erythema nodosum leprosum," which occurs in lepromatous leprosy and is associated with immune complex formation,² is an example of a Coombs and Gell type III hypersensitivity reaction. Nerve damage rarely results from this type of reaction unless it recurs frequently.

Patients with reversal reactions may vary considerably in their clinical presentation and may be divided into three groups—(1) those with skin hypersensitivity predominating, in which there is erythema and oedema of the skin lesions without obvious disease of the nerves; (2) those with nerve hypersensitivity

predominating, in which there is swelling and tenderness of the nerves but no change in the hypopigmented skin lesions; and (3) those with both skin and nerve hypersensitivity.

We describe here 24 patients who developed reversal reactions.

Patients and methods

Fifteen patients were included in a prospective study of the immunology of borderline leprosy; pretreatment assessments (including LT responses) were available. Nine further patients were first seen at the start of their reactions. All were classified clinically and histologically according to the Ridley-Jopling scale³; 16 were borderline tuberculoid, two borderline, and six borderline lepromatous. Ten of the patients were male and 14 female, and their ages ranged from 12 to 41 years.

All of the patients were on dapsone, which was continued in unaltered dosage throughout the study. Reactions were treated with prednisolone. The initial dosage was 30–40 mg daily, which was gradually reduced over one to nine months depending on the duration and severity of the reaction.

ASSESSMENTS DURING REACTION

The following assessments were made repeatedly during the reactions and in the post-reaction periods, which varied from one to nine months.

Skin disease was rated arbitrarily as 0 to 3+ according to the degree of erythema and oedema of skin lesions.

Nerve disease was assessed by three criteria, at least two of which had to be satisfied for nerve involvement to be considered to be marked—(a) clinical assessment of nerve tenderness and swelling, recorded as absent (0), slight (\pm), or marked (+); (b) motor nerve conduction studies of the ulnar and median nerves (below 55 m/s was regarded as abnormal); (c) voluntary muscle tests⁴ (sustained fall in muscle power in two or more muscle groups supplied by nerve indicated deterioration (+); lesser changes (0) were disregarded).

LT tests were performed at least twice during the reactions, whole and "sonicated" preparations of *M leprae* being used as antigens, and phytohaemagglutinin being used to show cell viability.

Whenever the LT tests were performed dose-response curves were drawn for each antigen and the maximum values for the two preparations of *M leprae* added together. The concentrations with the highest total, representing the peak of the reaction, are those reported here. Similarly, assessments of skin and nerve disease are the maximum figures recorded.

PREPARATION OF ANTIGENS

The same antigen preparations were used in all tests and were derived from a large subcutaneous nodule excised from a patient with lepromatous leprosy. The nodule was homogenised and the bacilli were washed in phosphate-buffered saline at pH 7.2. The suspension was stored at -20°C . This preparation is referred to as whole *M leprae*. The bacilli in part of the same suspension were exposed to sonic vibration until no acid-fast bacilli remained. This preparation is referred to as sonicated, and was also stored at -20°C .

TECHNIQUE OF LT TEST

In all tests both *M leprae* antigen preparations were used at three concentrations standardised to the bacillary content of the original homogenate—that is, 10^6 , 10^7 , and 10^8 bacilli/ml. A micromethod was used. Peripheral blood lymphocytes were separated on Ficoll Isopaque, washed three times, and cultured in tissue culture medium 199 (Gibco) at a concentration of 10^6 /ml. Triplicate cultures were

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incubated with the appropriate antigen preparation in 5% CO₂ in air for seven days at 37°C and 100% humidity. Control cultures without antigen added were also included in the tray. Sixteen hours before harvesting, 0.5 µCi tritiated thymidine (³H-TdR; specific activity 2 Ci/mmol) was added to each well. The contents of the wells were harvested on to glass-fibre filters and the ³H-TdR uptake was recorded as counts/min using a liquid scintillation counter.

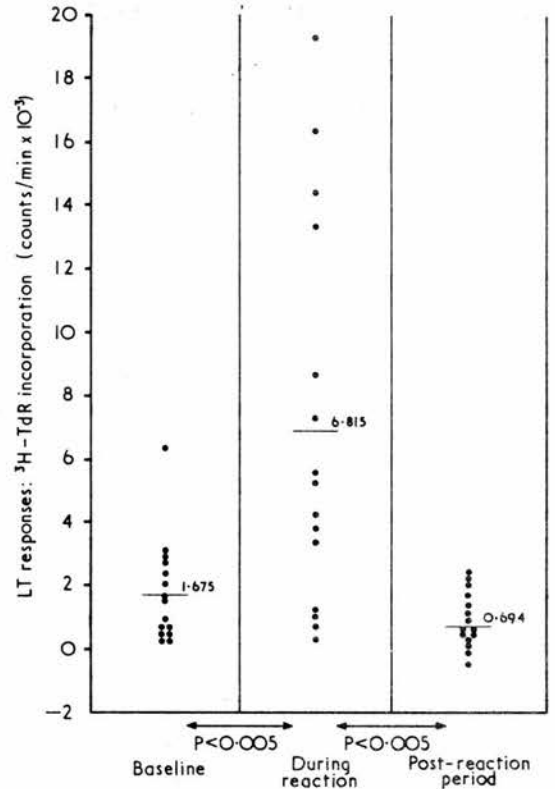
Results

The LT responses (with control values subtracted) in the 15 patients studied from the inception of antileprosy treatment at baseline (before treatment), during a reaction, and in the post-reaction period are shown in the fig. Thirteen of the patients showed an appreciable increase in response with the two antigen preparations during a reaction, with a comparable fall in the post-reaction period. Both the rise and fall were highly significant (P < 0.005; Student's *t* test).

The rise in LT response was due, in different patients, to increased responses to whole or sonicated preparations of *M leprae* or both. When the total LT responses in these patients when in reaction, and in the nine further patients who were also in reaction though they had not been followed from the inception of treatment, were studied the clinical presentation was found to correlate with the rise in LT with the two different antigen preparations. In the 10 patients judged to have a skin reaction clinically there was an appreciable increase in LT when whole *M leprae* was used but a smaller or negligible increase when sonicated *M leprae* was used. In those judged to have a nerve reaction the reverse was true. Those with mixed reactions showed a high LT response with both antigen preparations. The table gives a summary of these results, together with the LT values expressed as a ratio of whole to sonicated *M leprae* (total responses). The mean ratios for the patients with skin, mixed, and nerve reactions were 2.91, 0.94, and 0.50 respectively. Statistical significance was estimated with the non-parametric test of Kruskal and Wallis⁵ for independent samples, the differences between the three groups being highly significant (P < 0.001).

Discussion

The prospective study on the 15 borderline leprosy patients showed an appreciable increase in LT responses to antigens of *M leprae* during reversal reactions, and confirmed that the mechanism of these reactions is an increase in cell-mediated immunological reactivity. The results of LT tests in all 24 patients in reaction showed that nerve involvement and skin disease are associated with responses to different antigens. The



LT responses in 15 patients before (baseline), during, and after reaction. Responses to whole and sonicated *M leprae* are added, with control values subtracted.

failure of whole washed bacilli to elicit a high response in nerve reactions suggests that cytoplasmic antigens play an important part in this condition. In skin reactions the increased response is chiefly directed at whole washed bacilli, indicating that surface antigens are more important. The smaller response to sonicated preparations (in which surface antigens are present) may be due to dilution affect.

The mechanism of nerve reactions may therefore be as follows. In some borderline tuberculoid leprosy patients immunological competence enough to recognise and destroy the leprosy bacilli, many bacilli may be present in the nerve but few if any may

Clinical manifestations related to LT responses in 24 patients in reaction

| Case No | Biopsy classification* | Clinical assessment | | | | Reaction | LT (counts/min) | | Ratio of whole to sonicated <i>M leprae</i> |
|---------|------------------------|---------------------|--------|----|----|----------|-----------------------|---------------------------|---|
| | | Skin | Nerve† | | | | Whole <i>M leprae</i> | Sonicated <i>M leprae</i> | |
| | | | C | N | M | | | | |
| 1 | BT | 2+ | 0 | ND | 0 | Skin | 13 861 | 1770 | 7.83 |
| 2 | BT | 2+ | 0 | 0 | 0 | Skin | 11 799 | 2353 | 5.01 |
| 3 | BT | 3+ | ± | 0 | 0 | Skin | 4306 | 950 | 4.53 |
| 4 | BT | 2+ | ± | 0 | 0 | Skin | 2665 | 1029 | 2.59 |
| 5 | BB | 3+ | 0 | 0 | ND | Skin | 6493 | 3462 | 1.88 |
| 6 | BT | 2+ | 0 | 0 | 0 | Skin | 7022 | 3751 | 1.87 |
| 7 | BT | 2+ | ± | 0 | 0 | Skin | 7195 | 4434 | 1.62 |
| 8 | BL | 2+ | 0 | 0 | 0 | Skin | 4196 | 3014 | 1.39 |
| 9 | BB | 2+ | + | 0 | 0 | Skin | 7396 | 6123 | 1.21 |
| 10 | BL | 2+ | ± | 0 | 0 | Skin | 2925 | 2484 | 1.18 |
| 11 | BT | 2+ | + | + | + | Mixed | 12 089 | 10 031 | 1.21 |
| 12 | BT | 2+ | ± | + | + | Mixed | 3450 | 3174 | 1.09 |
| 13 | BT | + | + | + | + | Mixed | 1550 | 1658 | 0.93 |
| 14 | BT | 2+ | + | ND | + | Mixed | 9050 | 9950 | 0.91 |
| 15 | BT | 2+ | + | + | + | Mixed | 3763 | 5032 | 0.75 |
| 16 | BT | 2+ | + | + | 0 | Mixed | 1086 | 1494 | 0.73 |
| 17 | BT | 0 | + | + | + | Nerve | 359 | 357 | 1.01 |
| 18 | BL | 0 | + | + | + | Nerve | 5187 | 6669 | 0.78 |
| 19 | BT | 0 | + | ND | + | Nerve | 1232 | 1967 | 0.63 |
| 20 | BL | 0 | + | ND | + | Nerve | 827 | 1831 | 0.45 |
| 21 | BT | 0 | + | + | + | Nerve | 1626 | 4749 | 0.34 |
| 22 | BL | 0 | + | + | + | Nerve | 2156 | 6873 | 0.31 |
| 23 | BT | 0 | + | + | + | Nerve | 1086 | 3693 | 0.29 |
| 24 | BL | 0 | + | ND | + | Nerve | 261 | 1136 | 0.23 |

*Ridley-Jopling scale. † BT = Borderline tuberculoid. BB = Borderline. BL = Borderline lepromatous. †C = Clinical assessment. N = Motor nerve conduction velocity studies. M = Voluntary muscle testing. ND = Not done.

ent in the skin.* The same phenomenon has been noted in borderline lepromatous patients, particularly after one or two courses of treatment. This is presumably because Schwann cells, in which most of the bacilli are contained, are relatively long-lived. In this situation probably the surface antigens of the bacillus are rarely exposed; soluble cytoplasmic antigens are more likely to be exposed but they are probably released slowly, and few lymphocytes are sensitised. In reactions there appears to be a sudden increase in the release of these cytoplasmic antigens, though the trigger mechanism is unknown. Subsequent destruction of the Schwann cells may then lead to increased exposure of bacillary surface antigens, and it was noted that in seven of the eight patients with nerve reactions there was a rise in skin responses when whole *M leprae* was used as antigen in the skin tests after the reaction began.

In skin reactions the LT results indicate that there may be an increased exposure of bacillary surface antigens. In borderline tuberculoid leprosy few bacilli are present in the skin and they are predominantly found in the dermal nerves. It appears that lymphocytes and macrophages in the skin suddenly recognise bacilli in the dermal nerves, with destruction of the Schwann cells and consequent exposure of surface antigens. This hypothesis is borne out by the observation that in eight out of 10 patients with skin or mixed reactions who had skin biopsies during reaction large nerves were present containing numerous bacilli within granuloma.

In mixed reactions hypersensitivity reaction in nerves often occurs weeks after the reaction in skin. This may be because some cytoplasmic antigens are released during the "skin phase" with increased lymphocyte sensitisation to these antigens, leading to increased recognition of such antigens elsewhere—particularly in nerves.

Although the trigger mechanism for these reactions remains unknown, it has been suggested that coincidental exposure to other mycobacteria¹ may be a cause. There is no good evidence for this, however, and in those of our patients on whom LT tests were performed using BCG and *M duvalii* as antigens during reactions there was no consistent concomitant rise in responses.

Reversal reactions are of great importance as they may lead to nerve destruction almost overnight. Unfortunately this usually occurs when the patient is receiving antileprosy treatment, the more so if he has been diagnosed at an early stage of the disease, before nerve damage has occurred. It is important to elucidate the precipitating factors that cause these reactions, and when this is achieved it may be possible to prevent them.

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sensitisation could be detected at twelve months. However, when a group of patients were re-patch tested four or more months after sensitisation and one D.N.C.B. patch test, full migration inhibition was restored (fig. 3).

Discussion

This simple technique enables sensitivity to D.N.C.B. to be monitored in-vitro. Since it can be quantitated, it could prove useful in monitoring the small changes of C.M.I. which occur in immunosuppression, in augmentation of the immune response in immunotherapy, or in study of the immunological response to surgery, radiotherapy, or chemotherapy. Monitoring of drug dosage in immunosuppression for transplantation might be made more rational. The lymphocyte transformation method² for detection of D.N.C.B. sensitisation takes five days to complete, whereas the migration test can be read at 24 hours. If the migration inhibition test described reflects the overall state of the patients' C.M.I. then it will be useful as a rapid monitor of C.M.I.

Inhibition of migration by the conjugate waned with time but could be restored by patch testing. To prevent the decline in inhibition by the D.N.C.B./R.B.C. conjugate, skin tests for sensitivity should be repeated every three months.

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EVIDENCE FOR PREVENTION OF BORDERLINE LEPROSY REACTIONS BY DAPSONE

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Summary 68 patients were included in a prospective study of the treatment of borderline leprosy. 34 were treated with dapsone 5 mg daily, and 34 with 50 mg daily. Reversal reactions developed in 11 of those on 5 mg daily and in 3 of those on 50 mg daily. The statistically significant difference between the two treatment groups indicates that, contrary to previous teaching, dapsone given in higher dosage does not predispose patients to reversal reactions and indeed may prevent them.

Introduction

DEFORMITY in leprosy usually results from nerve damage caused by delayed hypersensitivity reactions termed "reversal reactions" which occur in borderline leprosy. During these reactions there is an apparent reversal of the tendency of untreated borderline leprosy to become more lepromatous, and the histological features become more tuberculoid. When this occurs there is a pronounced increase in the erythema of skin lesions and swelling and tenderness of the peripheral nerves. In severe cases there may be fever, malaise, and generalised

oedema. During the weeks preceding a reaction there are often prodromal symptoms, including vague aches and pains and paræsthesiæ. Lymphocyte transformation tests, in which whole *Mycobacterium lepræ* is used as antigen are strongly positive during reversal reactions^{1 2} suggesting an increase in cell-mediated immunity to the bacillus, possibly triggered by recognition of "hidden" antigen in Schwann cells.²

Reversal reactions can cause such sudden and severe nerve damage that their prevention would be a major advance in the management of leprosy. The risk of reversal reactions is thought to be reduced when dapsone treatment is initiated at a low dosage rather than at a high dosage.³⁻⁶ There are, however, no published reports to support (or refute) this hypothesis; and in particular the incidence of reactions in patients treated with dapsone in "orthodox" dosage (1-2 mg per kg) or the very low dosage which is now often advised has not been studied. We describe a prospective study of the effect of very low dapsone dosage on the incidence of reversal reactions in borderline leprosy.

Patients and Methods

68 patients with borderline leprosy who attended the Addis Ababa Leprosy Hospital, Ethiopia, were studied for 6 months to 2 years after dapsone treatment started. 52 patients were borderline tuberculoid cases, 3 were borderline cases, and 13 were borderline lepromatous cases as judged by the Ridley Jopling clinical and histological scale.⁷ There were 38 males and 30 females aged from thirteen to sixty-one. In each case clinical examination, chest X-ray, full blood-count, and urinalysis were performed to exclude the presence of other important disease.

Therapy was given on an outpatient basis, and patients were randomly allocated to treatment with 5 mg or 50 mg of dapsone daily. There were 34 patients in each group. Treatment was continued at an unaltered dosage throughout the study, and urine specimens were tested monthly for dapsone⁸ to assess regularity of intake. In those in whom reactions developed, the diagnosis was made on clinical grounds and confirmed in each case by a pronounced increase in the strength of response to whole and sonicated preparations of *M. lepræ* in lymphocyte transformation tests.²

Results

Reversal reactions developed in 14 of the 68 patients 1-9 months after dapsone treatment started. 11 of these patients were receiving 5 mg of dapsone daily and 3 were receiving 50 mg (see accompanying table) ($P < 0.025$). 10 of the group on the 50 mg daily dose had erythematous skin lesions and reactions might have been expected to develop in these patients; however, reactions developed in only two. Of the group on dapsone 5 mg

CLINICAL DATA OF 68 PATIENTS TREATED WITH 5 mg OR 50 mg OF DAPSONE DAILY

| Clinical data | No. of patients receiving treatment with dapsone | | | |
|--|--|-------------|-------------|-------------|
| | 5 mg daily | | 50 mg daily | |
| | Reaction | No reaction | Reaction | No reaction |
| Skin lesions active at start of trial | 8 | 1 | 2 | 8 |
| Skin lesions quiescent at start of trial | 3 | 22 | 1 | 23 |
| Total | 11 | 23 | 3 | 31 |

daily, 9 had erythematous skin lesions, and reactions developed in 8.

Discussion

These results indicate that patients (particularly those with erythematous swollen skin lesions) receiving low dosage of dapsone (5 mg daily) are more likely to develop reversal reactions than those on 50 mg daily from the start of treatment. This finding contradicts current teaching. Two previous studies^{9,10} have compared "high" and "low" dosage regimens, but in both of them the "low" dosage was, except for the initial 2-3 weeks, equivalent to 50 mg daily or more. This probably accounts for the failure of both studies to demonstrate any difference in the incidence of reactions and neuritis with different dosages of dapsone.

In this series, as is usual, a reaction developed in most patients within the first 3 months of being started on dapsone treatment. It seems likely, however, that many patients initially attended hospital for treatment because of the prodromal symptoms of reaction. If this is so, dapsone 5 mg daily may be regarded as not affecting the natural history of the reaction, whereas 50 mg daily resulted in suppression.

The mechanism by which dapsone prevents reactions remains uncertain. Since the minimal inhibitory dose of dapsone is about 1 mg daily,¹¹ prevention of reactions is unlikely to be the result of variation in antibacterial activity. Dapsone appears to have some immunological activity—e.g., it suppressed the Arthus reaction in guineapigs¹²—and this may account for its therapeutic success in dermatological conditions such as dermatitis herpetiformis and leucocytoclastic vasculitis. Beiguelman and Pisani¹³ suggested that dapsone may also suppress cell-mediated immunity, but in our patients there was no significant difference in lymphocyte transformation responses to phytohemagglutinin in those receiving 5 mg dapsone daily and those receiving 50 mg.

Current drug regimens are unduly complicated for field workers and patients. Their design was based on the misconception that low dosage of dapsone would prevent or mitigate reactions. It has previously been shown¹⁴ that even stopping dapsone completely does not ameliorate erythema nodosum leprosum (the other type of leprosy reaction, which occurs in lepromatous leprosy); and our results indicate that dapsone in higher dosage may be beneficial in reversal reactions. We believe that all patients with leprosy should be treated with dapsone 1-2 mg/kg daily from the start of therapy, and that treatment should be continued uninterrupted during reactions. If this policy is implemented, many reactions will be prevented, and effective treatment for reactions that do develop will not be delayed by ineffectual manipulation of dapsone dosage.

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References continued at foot of next column.

Preliminary Communication

TREATMENT OF LAMELLAR ICHTHYOSIS AND OTHER KERATINISING DERMATOSES WITH AN ORAL SYNTHETIC RETINOIC

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Summary Thirteen patients with keratinising dermatoses were treated for 2-17 weeks with oral 13-*cis* retinoic acid. There was near complete clearing of the skin lesions beginning within 2 weeks of starting treatment in all five patients with lamellar ichthyosis (including two cases of non-bullous congenital ichthyosiform erythroderma), in two of the three patients with Darier's disease, and in one patient with pityriasis rubra pilaris. The patients with psoriasis and nævus comedonicus did not improve. The main form of toxicity was cheilitis. These results indicate that 13-*cis* retinoic acid may be more effective and is less toxic than naturally occurring retinoic acid (all-*trans* vitamin A acid), and that the synthetic retinoids may represent a potent new class of drugs in the treatment of cutaneous disease.

INTRODUCTION

VITAMIN A, and more recently retinoic acid (all-*trans* vitamin A acid), has been used either systemically or topically to treat keratinising disorders of the skin.¹ Therapeutic responses have been inconsistent and limited by either systemic toxicity or local irritation when applied topically. Retinoic acid has proved to be more potent than vitamin A in the treatment of these conditions. However, the hypervitaminosis-A syndrome induced by systemic retinoic acid has prompted the search for synthetic derivatives with similar or greater therapeutic activity which are less toxic. Preliminary data in animals and man have indicated that 13-*cis* retinoic acid may be as potent and less toxic than the naturally occurring all-*trans* retinoic acid.²

MATERIALS AND METHODS

Drugs.—13-*cis* retinoic acid (Ro-43780, Hoffmann-La Roche, Nutley, New Jersey) was administered as 10 mg and 20 mg capsules.

Patients.—Thirteen patients with chronic, treatment-resistant, keratinising dermatoses were studied (see accompanying

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Does Nonspecific T-Lymphocyte Stimulation of B Lymphocytes Occur During Reversal Reaction in Borderline Leprosy?

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Serum immunoglobulins G, A, and M were estimated in 14 patients with borderline cases of leprosy at commencement of treatment and subsequently when they developed 'reversal reaction'. There was a significant increase in all immunoglobulin levels during the reaction, with a subsequent fall; the postreaction values for IgG and IgA were below the base-line figures. Additional investigations in six patients indicated that the rise was a nonspecific one, not brought about by an increase in antimycobacterial antibodies. It seems likely that the rise in immunoglobulins during reaction is due to nonspecific T-lymphocyte stimulation of B lymphocytes.

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Infection immunity to intracellular parasites such as *Mycobacterium leprae* (*M. leprae*) is dependent on cell-mediated immune mechanisms rather than circulating antibodies (7, 10, 12, 22, 24). However, humoral antibodies are also produced in leprosy, particularly at the lepromatous end of the spectrum (1, 3, 15, 21, 23, 30). Their function is unknown, and the only clinical consequence of their presence demonstrated to date is 'erythema nodosum leprosum' associated with circulating (20) and tissue deposition of immune complexes (11, 28, 33).

M. leprae has many unique features. In particular, it has an affinity for skin and nerve, and it is remarkably nontoxic, so that most of the clinical manifestations are due to the host responses. In patients with borderline

cases of leprosy it is this cell-mediated response that causes nerve damage and consequent deformity. The process can occur as part of the natural history of the disease, but more sudden and severe nerve damage occurs during the course of 'reversal reactions'. During these reactions there is an apparent reversal of the tendency of untreated borderline leprosy to become more lepromatous, and the histological features become more tuberculoid. When this occurs, there is a very marked increase in the oedema and erythema of skin lesions, with concomitant swelling and tenderness of the peripheral nerves. In severe cases there may be fever, malaise, and generalized oedema. Lymphocyte transformation tests using whole *M. leprae* as antigen are strongly positive during reaction (5, 13), suggesting an increase

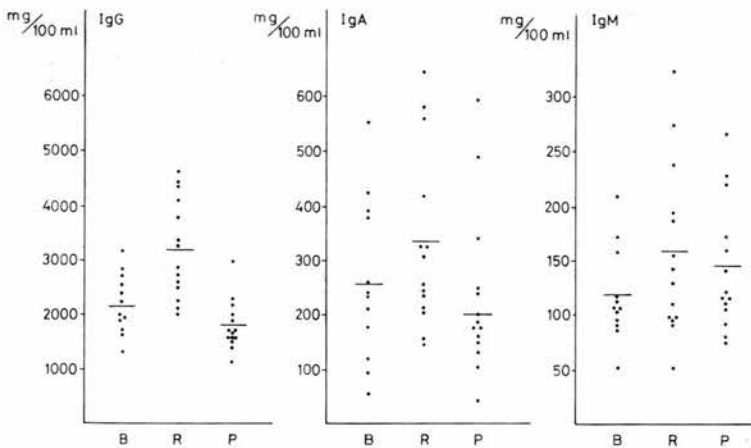


Fig. 1. Serum levels of immunoglobulins G, A, and M at base line (B), during reaction (R), and after reaction (P).

in cell-mediated immunity to the bacillus, possibly triggered by recognition of 'hidden' antigen in Schwann's cells (5).

There have been several studies of serum immunoglobulins in leprosy (6, 18, 27, 29, 31). Sirisinha et al. (29) demonstrated that IgG, IgA, and IgM levels were significantly higher in leprosy patients, whether tuberculoid or lepromatous, than in a normal control population. Bullock et al. (6), however, found that only lepromatous leprosy patients had significantly raised levels of these Igs when compared with normal controls. There has to date been no study published of serum Ig levels in patients who developed reversal reactions.

This paper describes a prospective study of 14 patients who developed reversal reactions and who were followed from the commencement of treatment.

MATERIALS AND METHODS

Fourteen adult Ethiopians with borderline cases of leprosy who developed reversal reactions were studied. Twelve were borderline tuberculoid (BT) and two borderline lepromatous (BL), as classified histologically according to the Ridley-Jopling scale (25). Six were men and eight women, with ages ranging between 20 and 55 years.

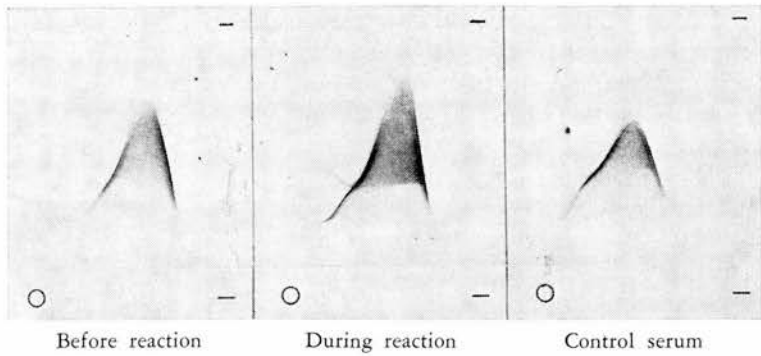
Each patient was examined and investigated to ensure that there was no other significant disease present. To this end, a full blood count, chest X ray, and urinalysis were carried out both initially and later when the patient developed a reaction.

Blood specimens for immunoglobulin determinations were taken at the following times: (a) base line, at commencement of treatment, (b) during reaction, and (c) after reaction—a period that varied from 1 to 9 months after reaction. Two of the base-line serum samples were lost before analysis.

Blood specimens were refrigerated immediately after collection. Sera were separated and frozen within 24 hr and kept frozen at -20°C until assayed. Quantification of IgG, IgA, and IgM was performed by means of agar diffusion plates containing specific antisera (Dakopatts A/S, Denmark), as described by Mancini et al. (19), and compared with antigen reference standards that were obtained commercially (Behringwerke, Standard Serum 974H). In some instances immunoglobulin determinations were carried out using plasma from heparinized blood, diluted 2:1 in saline as for lymphocyte separation. Appropriate correction for dilution was made in these cases.

The following investigations were also carried out on base-line and reaction serum samples from 6 of the 14 patients who developed

Fig. 2. Crossed immunoelectrophoretic analysis of IgG in one patient who developed reversal reaction. Carried out at pH 5.0, using carbamylated ammonium-sulphate-fractionated gamma globulin from anti-human IgG antiserum.



reversal reaction: (a) agarose gel electrophoresis (17) for analysis of serum proteins; (b) crossed immunoelectrophoretic analysis of human IgG (16) carried out at pH 5.0 using carbamylated ammonium-sulphate-fractionated gamma globulin from rabbit anti-human IgG antiserum (4); and (c) crossed immunoelectrophoresis for analysis of antimycobacterial antibodies, using 'sonicated' *M. leprae* as antigen (14, 15).

RESULTS

Serum immunoglobulin levels in the 14 reaction patients at base line and during and after reaction are shown in Fig. 1. There were significant rises in the mean values of each class of immunoglobulin during reaction (IgG, $P < 0.005$; IgA, $P < 0.005$; IgM, $P < 0.025$), using the Student's *t* test for statistical analysis. After the reaction, however, there was a significant fall in IgG and IgA only (IgG, $P < 0.001$; IgA, $P < 0.001$).

Agarose gel electrophoresis and crossed immunoelectrophoresis of sera from 6 of the 14 patients who developed reversal reactions showed a polyclonal increase in gamma globulins and IgG, respectively; no monoclonal components were detected. (An example of the crossed immunoelectrophoresis in one of these patients is shown in Fig. 2.) Crossed immunoelectrophoretic analysis of antimycobacterial antibodies also showed no change between base-line and reaction sera in each case. In particular, there was no rise in titres of existing antimycobacterial antibodies and no

evidence of formation of new antibodies during reaction in patients with no antibodies detected in base-line serum samples. (Titre changes of 30% can be detected using this technique semiquantitatively (15).)

DISCUSSION

The finding of significant alterations in immunoglobulins during reversal reactions raises the question of whether they themselves are the cause of the reaction or associated with the cause directly, or whether they are merely secondary, incidental findings, caused by the reaction.

Reversal reactions are characterized histopathologically as delayed-type hypersensitivity reactions and in lymphocyte transformation tests *in vitro* by very high responses to whole washed *M. leprae* as the antigen. Extensive T-cell proliferation and activation clearly underline the inflammation of reversal reaction; the primary cause, however, is unclear, although exposure of previously 'hidden' mycobacterial antigen is the most likely explanation.

The findings of this study, however, make it unlikely that this is the cause of the immunoglobulin changes. The results indicate that there is a nonspecific, polyclonal rise in immunoglobulin levels rather than specific increases in antimycobacterial antibodies or formation of antitissue antibodies on tissue destruction during the reaction. Such changes are likely to be secondary; they would not be expected to influence T-cell activation by *M. leprae* antigens or to be caused by their exposure.

That the increases in immunoglobulin levels are apparently not due to *M. leprae* antigen stimulation calls for an alternative explanation. Several studies have demonstrated that proliferating, nonsecreting B cells can be converted into antibody producers by a factor or factors produced by activated T cells (2, 8, 9, 26, 32). In reversal reactions there is a marked increase in the number of circulating T lymphocytes sensitized to antigens of the leprosy bacillus. These lymphocytes, when activated in the widespread lesions, might stimulate the production of serum immunoglobulins by non-specific action on proliferating B lymphocytes. This is supported by the finding that, although there was a highly significant rise in IgG and IgA during reaction, the rise in IgM was much less significant and there was no significant fall after reaction. This is in agreement with the findings of Davie & Paul (8), who noted a relative T-cell independence of IgM antibodies in experimental animals.

The rise in immunoglobulins in this study would be an effect rather than a cause of the reversal reaction.

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Lymphocyte transformation test in leprosy; correlation of the response with inflammation of lesions

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SUMMARY

Lymphocyte transformation tests (LTT) using 'whole washed' and 'sonicated' preparations of *Mycobacterium leprae* (*M. leprae*) as antigen were studied in eighty-one patients with borderline leprosy. The results were correlated with the histological and the clinical pictures.

There was a good correlation with the histological spectrum, LTT responses generally being higher in the borderline tuberculoid leprosy patients and lower in the borderline lepromatous. However, considerable variation was noted in each group of the borderline leprosy spectrum, and it was found that this was due in part to the degree of inflammation in the skin. Thus those with 'inflamed' skin lesions had higher responses than those with 'silent' lesions, and even those with borderline lepromatous leprosy with inflamed lesions had higher responses than those with borderline tuberculoid leprosy whose lesions were silent. Those who had reversal reactions, where inflammation is very marked, had very high LTT responses which fell with treatment of the reaction with steroids.

It thus appears that the LTT in leprosy is influenced by the occurrence of hypersensitivity reactions as well as by the patient's ability to resist bacillary multiplication.

INTRODUCTION

Leprosy is probably the best example of a disease which has a clinical spectrum due to the host response (Ridley & Jopling, 1966). The high resistant form, tuberculoid leprosy (TT), presents with a few well-defined skin lesions that histologically resemble delayed-type hypersensitivity granulomata. Bacilli are rarely found. In the low resistant form, lepromatous leprosy (LL), the lesions are multiple and diffuse, and consist histologically of histiocytes containing large numbers of leprosy bacilli (*M. leprae*). Between these two extremes (polar forms) a continuous clinical and histopathological range, termed borderline leprosy, is seen; patients in this range can be classified as mid-range (BB) or near the lepromatous (BL) or tuberculoid (BT) poles.

The skin lesions of patients with borderline leprosy can vary in appearance from time to time, both in treated and untreated cases: they may be hypopigmented macules, or they may be erythematous and oedematous nodules and plaques. Sometimes inflammation is severe and acute, and this phenomenon is termed a 'reversal reaction' (Fernandez *et al.*, 1962; Ridley, 1969). The histological counterpart of this is a development of more tuberculoid features, and a relatively rapid elimination of bacilli.

The lymphocyte transformation test, using peripheral blood lymphocytes from leprosy patients, and whole washed *M. leprae* as antigen, gives a spectrum of responses corresponding to the clinical and histopathological spectrum of leprosy, though considerable variation in each group has been noted (Myrvang *et al.*, 1972). Because of this correspondence, the LTT has been assumed to measure the host cell-mediated immunity (CMI) responsible for resistance to the infection. However, a more detailed

histological study of the cases reported by Myrvang *et al.* revealed that the LTT was correlated with the proliferative and destructive aspects of inflammation but not with the number of lymphocytes in skin lesions, though the presence of many lymphocytes enhances the prognosis in some respects and is a factor in classification (Ridley, 1974).

In tuberculosis (both experimental and human) it has been shown that delayed hypersensitivity responses to intradermal challenge with purified protein derivative (PPD) do not parallel immunity to the infection (Reggiardo & Middlebrook, 1974; Knight Shapiro, Harding & Smith, 1974; Medical Research Council, 1963). It seems likely that this is also the case in leprosy, where similar hypersensitivity phenomena could account for the variations in LTT responses noted by Myrvang *et al.* (1972), and also for the high responses found in reversal reactions (Godal *et al.*, 1973; Barnetson *et al.*, 1975).

This paper describes a study of LTTs in correlation with the clinical and histopathological features in eighty-one patients with borderline leprosy.

PATIENTS AND METHODS

The study includes eighty-one previously untreated leprosy patients who attended the routine diagnostic clinic of the All Africa Leprosy and Rehabilitation Training Centre (ALERT) in Addis Ababa. There were thirty-six females and forty-five males; their ages ranged from 13 to 61 years. In all cases clinical examination, chest X-rays, blood counts and urine analysis were performed to exclude the presence of other significant disease.

Assessment of leprosy. The following assessments were undertaken.

Skin involvement. Clinical drawings on body charts recorded the sites and extent of the skin lesions. The erythema and oedema of lesions were graded on arbitrary scales (Table 1). Drawings were supplemented by photographs of individual lesions.

TABLE 1. Scale for clinical grading of erythema and oedema of the skin lesions

| Degree recorded | Appearance of lesions | |
|-----------------|---|--|
| | Erythema | Oedema |
| 0 | Hypopigmented | Flat |
| (+) | Slight erythema | Slightly raised edges |
| + | Marked erythema | Whole lesion raised |
| ++ | Marked erythema of lesions and the surrounding skin | Gross swelling and oedema also of the surrounding skin |

Nerve involvement. Enlargement and/or tenderness of peripheral nerves at specific sites was recorded. In addition, motor nerve function was assessed by voluntary muscle testing (Goodwin, 1968) and by electromyography.

Skin smears for acid-fast bacilli. Skin smears were taken from six sites, stained with Ziehl-Neelsen's stain (Baker, 1967), and the bacteriological and morphological indices recorded (Ridley, 1964).

Skin histology. Biopsy of a representative skin lesion was performed for independent histological classification according to the modified Ridley-Jopling scale (Ridley & Jopling, 1966; Ridley & Waters, 1969). In many cases (and in all patients whose histological classification was reported as Indeterminate) biopsy of the radial cutaneous nerve was also undertaken.

In addition to the routine classification, each biopsy was given an arbitrary rating of a number of histological features (Table 2). The presence of other findings (such as polymorphs and plasma cells) was also recorded.

Lymphocyte transformation test (LTT). LTTs were performed before treatment was initiated (see below).

Clinical management and serial tests. After initial assessments all patients received treatment with dapsone, which was continued in unaltered dosage throughout the study. Clinical assessments, voluntary muscle testing and electromyography were repeated periodically, including all occasions when LTTs were performed. Skin smears were repeated 6-monthly. When patients developed reactions, biopsies and other tests were repeated, and treatment initiated with prednisolone 30-40 mg daily; this dose was gradually reduced over a period of 1-9 months according to the duration and severity of the reaction.

TABLE 2. Scale for microscopic grading of single features of the histology of skin lesions

| Histological parameter | Degree recorded | | | | |
|-----------------------------------|-------------------------------------|--|---|---|---------------------------|
| | 0 | ± | + | ++ | +++ |
| No. of lymphocytes | None | Scanty, diffuse | Sufficient to produce some clumps | Fairly abundant | Dense accumulations |
| No. of epithelioid cells | None | Small foci of a few cells only | A definite granuloma | Large granuloma | — |
| No. of giant cells | None | Scanty and undifferentiated | Several undifferentiated or one large Langhans cell | A few large Langhans cells | Many large Langhans cells |
| Infiltration of subepidermal zone | None | Infiltration with incomplete occupation | Granuloma extends up to epidermis | — | — |
| Erosion of the epidermis | None | Infiltration into epidermis but no destruction | A patch of partial destruction | Complete destruction up into granular layer | — |
| Enlarged dermal nerves | All diameters less than 180 μ m | — | Diameter more than 180 μ m | — | — |
| Histological activity | Clear edge of the granuloma | A few spurs or a few infiltrating cells around the granuloma | Strong spur formation | Satellite foci of the granuloma | — |
| Oedema | No oedema | Dilatation of lymphatics or mild extracellular oedema | Marked extracellular oedema | Severe extracellular oedema and intracellular vacuoles, disruption of the granuloma | — |
| Dermal reaction | Normal fibroblasts | Some hyperplasia of fibroblasts | Marked hyperplasia, swelling of elastic fibres and oedema of dermis | Serious disruption of dermis followed by widespread fibrosis | — |

Lymphocyte transformation test. Lymphocyte cultivation. LTT was performed according to the technique of Closs (1975), with a few modifications.

Lymphocytes separated from peripheral blood by centrifugation on a layer of Ficoll-Isopaque (Böyum, 1968) were cultivated in Linbro tissue culture trays (IS-FB-96-TC, Linbro Chemical Company, New Haven, Connecticut) for 7 days in the presence of antigen. Lymphocytes were cultured in triplicate with a minimum of four concentrations of each antigen and two or more triplicates were left without antigen (control). Tritium labelled thymidine was added 16 hr before harvest of the cultures. Cells were harvested on glass fibre filters, washed in distilled water and counted in a liquid scintillation counter (SL-31, Intertechnique, Plaisire, France).

The reagents employed were: Ficoll (Pharmacia, Uppsala, Sweden); Isopaque (Nyegaard & Company, Oslo, Norway); Hanks's balanced salt solution (Flow Laboratories, Irvine, Scotland); medium TC 199 with Earle's salts (Flow Laboratories); penicillin-streptomycin for TC (BDH Chemicals Ltd, Poole, England); glass fibre filters, type A (Gelman Instrument Company, Ann Arbor, Michigan, U.S.A.); toluene, PA grade (March, Dharmstad, West Germany); 2,5-Diphenyloxazole (Koch-Light Laboratories Ltd, Bucks., England); 1,4-Bis(2)-(5-phenyloxazolyl) benzene (Koch-Light Laboratories).

Preparation of antigens. Leprosy bacilli prepared from a single subcutaneous nodule of a non-treated lepromatous leprosy patient were used as stimulants for the cultures. The nodule was immediately cut finely with scissors in a Petri dish. It was then ground in a glass homogenizer in ice cold phosphate-buffered saline (PBS), pH 7.2, with 1% of human serum albumin (HSA) (AB Kabi, Stockholm, Sweden). Homogenates were centrifuged at 150 g for 5 min. The supernatants were again centrifuged at 2500 g for 1 hr and the pellets washed twice in cold PBS with 1% HSA. After resuspension the volume was adjusted to 1×10^9 acid-fast bacilli (AFB) per millilitre in PBS-HSA. The batch was subdivided into small aliquots and stored at -70°C until use. This preparation is termed 'whole washed' *M. leprae*.

Half of the batch was ultrasonicated in a Branson B-12 (Branson Sonic Power Company, Danbury, Connecticut) with 80 Watt output on the medium-sized tip for 1 hr, until no AFB remained. This preparation is termed 'sonicated' *M. leprae* and was subdivided and frozen as described for 'whole washed' *M. leprae*.

All patients were simultaneously tested with whole BCG bacilli (Dried B.C.G. vaccine, Glaxo Lab. Ltd, Greenford, England) and/or purified protein derivative, PDD (Statens Seruminstitut, Copenhagen, Denmark).

PHA stimulation. Cultures for stimulation with phytohaemagglutinin (PHA, Reagent grade, Wellcome Research Laboratories, Beckenham, Kent, England) were carried out in all patients in parallel with the antigen stimulation, to check culture conditions and cell viability. Experiments without stimulation by PHA were rejected.

Statistical analysis. The values recorded were the peak responses in the dose-response curve of antigen concentrations, with the control values (cultures without antigen) subtracted. Lymphocyte transformation results for groups of patients are given as medians with the variation expressed as the interquartile range covering the middle 50% of the observations. For comparison of groups of patients the Kolmogorov-Smirnov Two-Sample-Test is used (Siegel, 1956). This non-parametric test is applicable to populations of unequal size where the distribution of observations within the population is not known to be normal.

RESULTS

LTTs: correlation to histology

(a) *Initial LTTs and the histological classification.* The histological classification of the patients is shown in Table 3; the distribution is characteristic for Ethiopian patients with borderline leprosy. It will be

TABLE 3. Number of patients in the study which fall into the different groups of the leprosy spectrum when classified according to clinical and histological criteria respectively

| Classification | |
|----------------|-------------------|
| Clinical | Histopathological |
| BT 58 | TT 9 |
| | BT 25 |
| | Idt. 24 |
| BB 3 | BB 3 |
| BL 21 | BL 9 |
| | LI 12 |

Idt. = Indeterminate.

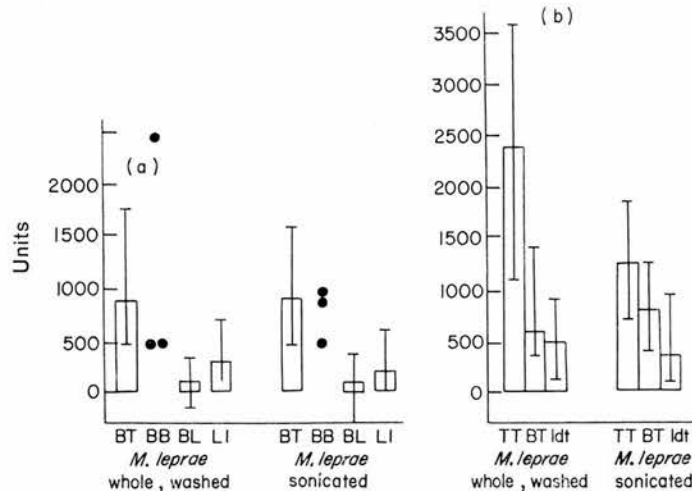


FIG. 1. Median LTT responses in patients in the different groups of the leprosy spectrum. (a) Responses throughout the borderline range; (b) responses throughout the range of BT leprosy alone.

noted that patients having clinical characteristics of BT leprosy could be subdivided (according to skin biopsies) into three groups: (1) twenty-four patients showed an indeterminate histology (but tuberculoid features were usually present in their nerve biopsies); (2) twenty-five patients had a classical BT histology; (3) nine patients had a histology of TT. Since all the 'indeterminate' cases in this study were clinically BT they are grouped as tuberculoid.

The correlation between the initial LTTs and the histological classification is shown in Fig. 1. Both 'whole washed' and 'sonicated' preparations of *M. leprae* showed significantly higher responses at the tuberculoid (BT, TT and Indeterminate) than at the lepromatous (BL, LI) end of the borderline range ($P < 0.001$ for both). The difference between TT and BT patients was also significant ($P < 0.005$ for both preparations), as was the difference between the histologically Indeterminate and 'determinate' (BT and TT) patients ($P < 0.001$ for both).

TABLE 4. Scoring of histological features correlated to LTT responses to 'whole washed' *M. leprae* in untreated tuberculoid and lepromatous patients. (For lepromatous patients none of the recorded parameters gave significant differences.)

| Histological factor | Degree recorded | Tuberculoid patients | | | Lepromatous patients | |
|---------------------------------------|-----------------|----------------------|---------------|----------------------------|----------------------|---------------|
| | | No. of observations | Median ct/min | Significance of difference | No. of observations | Median ct/min |
| No. of lymphocytes | 0 | 0 | — | $P > 0.1$ | 2 | 475 |
| | ± | 6 | 650 | | 9 | 120 |
| | + | 39 | 1100 | | 8 | 100 |
| | ++ | 23 | 1000 | | 6 | 550 |
| No. of epithelioid cells | 0 | 2 | 425 | $P < 0.05$ | 21 | 300 |
| | ± | 13 | 650 | | 2 | -425 |
| | + | 29 | 1000 | | 2 | 1000 |
| | ++ | 24 | 1400 | | 0 | — |
| No. of giant cells | 0 | 34 | 650 | $P < 0.005$ | 24 | 200 |
| | ± | 2 | 1400 | | 0 | — |
| | + | 15 | 1600 | | 1 | 1900 |
| | ++ | 17 | 1400 | | 0 | — |
| Infiltration of the subepidermal zone | 0 | 8 | 725 | $P < 0.001$ | 21 | 125 |
| | ± | 25 | 500 | | 0 | — |
| | + | 35 | 1825 | | 3 | 700 |
| Erosion of the epidermis | 0 | 48 | 650 | $P < 0.01$ | 24 | 275 |
| | ± | 9 | 1350 | | 0 | — |
| | + | 11 | 4300 | | 0 | — |
| Enlarged dermal nerves | 0 | 38 | 850 | $P < 0.05$ | 22 | 200 |
| | + | 18 | 1350 | | 1 | 1900 |
| Histological activity | 0 | 13 | 650 | $P < 0.05$ | 7 | 0 |
| | ± | 19 | 650 | | 1 | 300 |
| | + | 24 | 1350 | | 3 | 150 |
| | ++ | 8 | 1000 | | 11 | 200 |
| Oedema | 0 | 19 | 650 | $P < 0.1$ | 16 | 175 |
| | ± | 27 | 850 | | 5 | -75 |
| | + | 20 | 1500 | | 2 | 750 |
| Dermal reaction | 0 | 26 | 600 | $P < 0.01$ | 17 | 275 |
| | ± | 26 | 1150 | | 6 | -500 |
| | + | 13 | 2125 | | 0 | — |
| | ++ | 1 | 3000 | | 0 | — |

Obs.= observations. ct/min= Counts per minute. P = probability.

At the lepromatous end of the range LI patients showed slightly stronger LTT responses than BL patients ($P < 0.1$).

(b) *Correlation of LTT responses to histological features.* Particular study was made of the patients classified histologically as TT, BT and Indeterminate. Various features of the pre-treatment biopsy were graded (Table 4) and compared with the LTT response using 'whole washed' *M. leprae* as antigen.

In this group of patients LTT responses were correlated with most of the histological characteristics of tuberculoid leprosy, including the presence and maturation of epithelioid cells ($P < 0.05$), infiltration of the subepidermal zone ($P < 0.001$), and erosion of the epidermis ($P < 0.01$). In addition, 'histological activity', signs of spreading of the granuloma, showed higher responses ($P < 0.05$).

A further finding was that certain of the histological features of reaction (including interstitial oedema and dermal connective tissue reaction) could be present in patients who were clinically judged not to be in

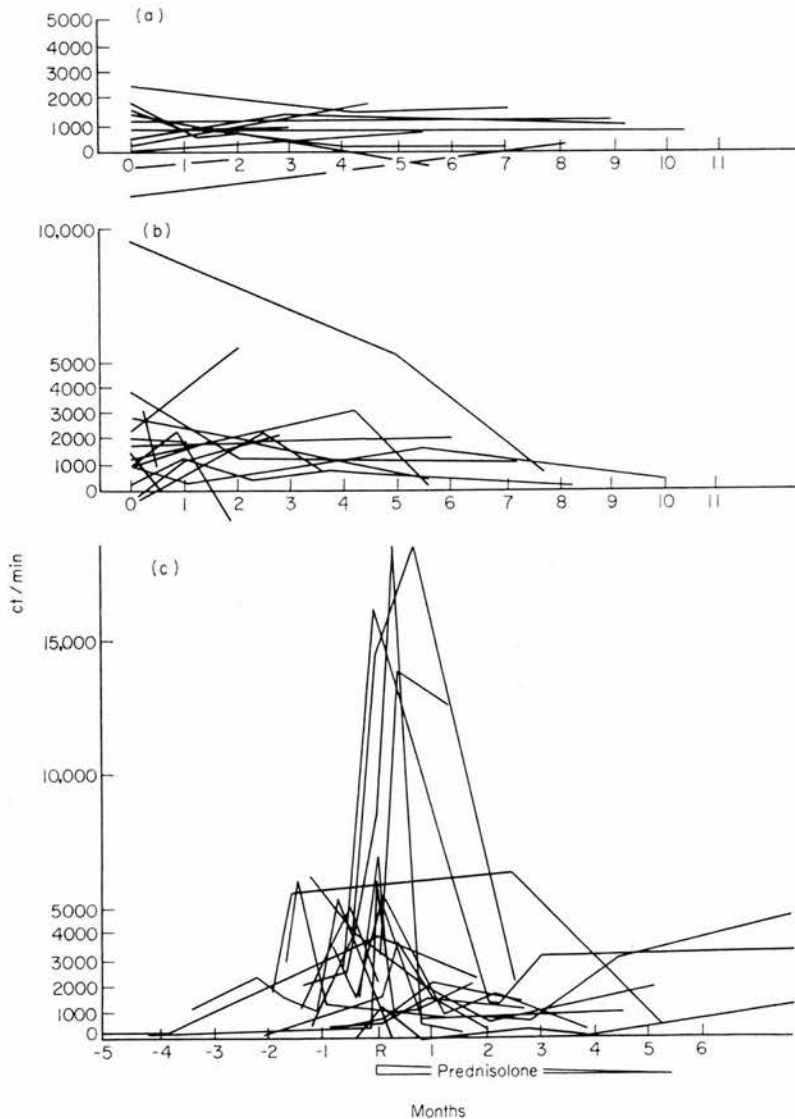


FIG. 2. Serial LTT responses during the time of observation correlated to the stability of clinical signs of inflammation in skin and/or nerves. Responses shown here are summed responses to 'whole washed' and 'sonicated' *M. leprae*. (a) Stable, non-reactive patients. (b) Labile, non-reactive patients. (c) Reactive patients.

reaction. Their presence, however, was associated with increased LTT responses (for oedema, $P < 0.1$; for dermal reaction, $P < 0.01$).

The presence of enlarged nerves in the biopsy (diameter more than 50% larger than the normal maximum of 125 μm) was strongly associated with high LTT responses ($P < 0.05$); responses below 400 ct/min were never recorded in these patients.

A negative finding was the absence of correlation between the number of lymphocytes in tuberculoid cases and LTT responses. However, when all patients were taken together, higher concentrations (+ and ++) of lymphocytes were associated with increased LTT responses ($P < 0.05$).

LTTs: correlation to clinical picture

(a) *LTTs and the stability of the clinical picture during treatment.* LTTs were performed more than once in forty-six cases, and the patients grouped according to the appearance of their skin lesions and status of their nerves. In fourteen cases both the clinical picture and the LTT results were stable (Fig. 2); in this figure LTT results are the summed values of responses to 'whole washed' and 'sonicated' preparations. In fifteen patients both showed considerable variations; often (but not invariably) high LTTs coincided with increased inflammation in skin and/or nerves. Seventeen patients with clinical and biopsy evidence of reversal reactions usually showed greatly increased LTTs during reaction (the peak of clinical reaction being defined as the time of initiating prednisolone treatment). In all cases the LTT values fell sharply after the termination of the reaction (Fig. 2).

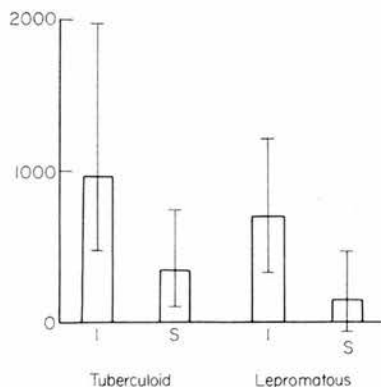


FIG. 3. LTT responses to 'whole washed' *M. leprae* according to the clinical appearance of the skin lesions. I=inflamed; S=silent.

(b) *LTTs and the amount of inflammation in the skin lesions.* Patients were grouped as 'Tuberculoid' (histologically BT, TT and Indeterminate) and 'Lepromatous' (BL and LI) and subdivided according to the appearance of their skin lesions at the time of each test. Patients in reaction were excluded. The average responses were much lower in the patients with 'silent' skin lesions (those receiving 0 gradings for both erythema and oedema) of both groups (Fig. 3). The differences were significant for both groups using 'whole washed' bacilli as antigen ($P < 0.001$ for tuberculoid and $P < 0.05$ for lepromatous patients). It was particularly remarkable that the 'inflamed' lepromatous group showed consistently higher readings than the 'silent' tuberculoid group ($P < 0.1$); the reverse of what should be expected according to their place in the histopathological spectrum. This is in keeping with the finding that the rise in LTT due to a reaction often outweighs a difference due to position in the spectrum.

DISCUSSION

The term 'resistance' in leprosy has never been accurately defined. However, in normal usage it is taken to mean:

(a) The ability of the host to limit the bacillary load of the body. Thus resistance is defined in terms of the spectrum of leprosy; tuberculoid patients having high resistance, lepromatous low. This definition is ultimately histopathological.

(b) The ability of the patient under effective treatment to dispose of disintegrating bacillary material. A rough indication of this can be attained by following the fall of the bacteriological index during treatment, and by this criterion a clear distinction is made between polar (LL), subpolar (LI) and borderline lepromatous (BL) cases. It is of little value, however, towards the tuberculoid end of the spectrum where bacilli are absent or scanty; and in this area histopathological classification is less precise than in lepromatous leprosy.

The development of the LTT and its application to leprosy led to the hope that this test might be a definitive assessment of host resistance. This study fully confirms the results of the previous study as regards the general correlation of some histological features with a high LTT result, and the lack of correlation between a high density of lymphocytes in the lesions and the LTT. This confirmation is of some interest because the present study has made use of a different (and more sensitive) method for the LTT, as well as different methods of preparation of *M. leprae* antigens. However, the present study has also emphasized the limitations rather than the value of the test.

(1) The rise in the LTT during reactions in borderline leprosy patients far outweighs the LTT response associated with uncomplicated tuberculoid leprosy. This applies to BB as well as BT cases, and there is a smaller though relatively high rise in patients who prior to the reaction are BL or LI. (It is true that in the present series all the reactions were of the reversal or upgrading type, which occur either in borderline patients on treatment or possibly in some early untreated BT patients. However, Godal *et al.* (1973) found a rise in the LTT even when the reacting patient was immunologically static or perhaps downgrading).

(2) Following the termination of the reaction, with steroid treatment, there was a fall in the LTT responses to baseline levels.

(3) There is some evidence that a high density of lymphocytes in lesions is associated with resistance to downgrading and perhaps a predisposition to upgrading by the patient, but not with the ability to dispose of *M. leprae* (Ridley, 1974). Lymphocytes in lesions do not correlate with the LTT. Thus BL patients, characterized by a high density of lymphocytes, give a lower LTT response than LI patients who show few lymphocytes, though BL patients have a better prognosis and are placed higher up the spectrum than LI. This suggests that the LTT does not simply reflect immune resistance.

(4) Patients with infiltrating granulomas (i.e. histological evidence of failure to contain the infection) tend to have higher LTTs than those with less active spread. The low LTT values of the clinically BT histologically indeterminate patients might be due either to the small size of the lesions (histologically), and their quiescence, or to the large number of lymphocytes and absence of granuloma. Similarly the high LTT responses given by patients with erythematous swollen lesions can probably be explained either by the state of activity of the infection, or by the patient being in the early stage of a reaction.

The issues raised by these findings can be resolved into two questions.

1. *Why do so many BT patients have low LTT responses?*

(a) *Methodological failures.* This seems unlikely here. Each patient in this study was tested with four different concentrations of two different preparations of *M. leprae* antigens. Differences in the dose eliciting a peak response should therefore not affect the percentage of responders. Good responses to PHA as well as to antigens like PPD and BCG prove the ability of the cells to respond in the actual experiment. Most patients were also tested more than once, and if clinically stable during the period of observation, they were also stable in LTT responses (Fig. 2).

(b) *The wrong antigens were used.* CMI in leprosy could have specificity for different antigens of *M. leprae*. In a given preparation of bacilli not all antigens might be equally well exposed. Thus lymphocytes which perform well *in vivo* might show no transformation response *in vitro*.

(c) *The wrong lymphocytes were used.* The number of specifically sensitized lymphocytes in the peripheral blood represents a balance between their production and their trapping in the lesions (Thestrup-Pedersen, 1974); small numbers in the circulation need not imply inefficient formation. The potential of the patient's immunity is difficult to estimate when no standard stimulus exists *in vivo*.

2. What factors are responsible for high LTT responses?

There appears to be a clear answer to this question, namely that LTT results are influenced by hypersensitivity phenomena.

(a) In untreated patients clinically classified as BT, those with higher LTTs are those which have histological features of delayed-type hypersensitivity.

(b) In both BT and BL patients (as clinically grouped) those in each group with erythematous swollen lesions tend to have higher LTT responses than those with silent looking lesions.

(c) In reversal reactions, which show both clinical and histological features of delayed-type hypersensitivity responses, there is a marked rise in LTT responses.

All these observations indicate that LTTs as performed in this study are affected more by hypersensitivity to bacillary antigens than by resistance to the infection.

A dissociation between hypersensitivity and resistance has long been recognized in the field of tuberculosis, and must now be accepted as also important in leprosy. There are a number of theoretical explanations:

(a) The antigens stimulating high LTT responses are irrelevant for resistance, possibly because they are exposed only by dead bacilli (Youmans, 1975).

(b) Differences in the presentation of antigen, and specially between presentation in Schwann cells and macrophages, could explain why bacilli can stay undetected in one tissue, while giving rise to vigorous reactions in another. The findings in reversal reactions indicate that antigens are presented differently in the two cell types. This heterogeneity of responses in reversal reaction is described fully elsewhere (Barnetson *et al.*, 1975).

(c) Even in untreated patients most *M. leprae* are non-viable. It is possible that only antigens produced by living bacilli and which can escape from the host cell are responsible for immune resistance to the infection.

The LTT has been used to characterize the defect in lepromatous leprosy (Godal *et al.*, 1971), to define the immunological spectrum (Myrvang *et al.*, 1972), and has been suggested as a tool to evaluate immune prophylaxis and immunotherapy in leprosy (Godal *et al.*, 1974). It is clear, however, that the interpretation of LTT results is by no means straightforward. Until the implications of the heterogeneity of the CMI response have been more thoroughly evaluated, the value of LTT to assess 'resistance' in leprosy will remain doubtful: nevertheless it has proved to be of service in identifying the histological features which are most precisely linked with hypersensitivity in leprosy.

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Plasma factors in delayed-type hypersensitivity

AUGMENTATION OF LYMPHOCYTE RESPONSES IN BORDERLINE LEPROSY REACTIONS

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SUMMARY

The phytohaemagglutinin-induced responses of lymphocytes were found to be inhibited by plasma from patients with leprosy when compared with their responses in pooled serum from healthy donors. When patients developed reversal reactions, the initial inhibitory effect of their plasma was replaced by an augmentary effect on the responses to phytohaemagglutinin. The period of augmentation coincided with that of the reversal reaction in patients with borderline tuberculoid leprosy, but was delayed in patients with borderline lepromatous leprosy. The plasma from each leprosy patient was also observed to have the same effect on lymphocytes from unrelated individuals, showing that the inhibition and augmentation were due to factors in the plasma and not to a change in lymphocyte receptors.

It is possible that the normal stable state of leprosy results from the presence of factors in plasma which act as a control mechanism, and that delayed hypersensitivity reactions may be caused by a breakdown of this control.

INTRODUCTION

Cell-mediated immune (CMI) responses are essential for resistance to various intracellular organisms and to neoplastic change in the tissues. Under certain circumstances, however, the CMI responses are not beneficial to the host, and they are then termed 'delayed hypersensitivity reactions' (Coombs and Gell type IV hypersensitivity reactions).

Possibly the most clear-cut clinical example of delayed-type hypersensitivity is the reversal reaction in borderline leprosy which often leads to permanent deformity due to granuloma formation in nerves. Ridley (1969) found the histology of these reactions typical of delayed-type hypersensitivity, and Godal *et al.* (1973), Barnetson *et al.* (1975), and Bjune *et al.* (1976), found a marked rise in *in vitro* lymphocyte transformation responses to antigens from *Mycobacterium leprae* during reaction.

The reason for the emergence of hypersensitivity from a state of comparative non-reactivity is still unclear. One possibility, however, is that reaction occurs when there is a breakdown of control mechanisms which normally regulate CMI responses and prevent them from becoming overactive and harmful.

Humoral factors influencing CMI responses can be studied *in vitro* using the lymphocyte transformation (LT) test. Phytohaemagglutinin (PHA) stimulates T lymphocytes predominantly and comparison of responses in autologous plasma and in standard serum may demonstrate any modifying effect of the patient's own plasma. In this investigation we have utilized such a system to demonstrate the effect of plasma factors on T-cell responses in patients with borderline leprosy who developed reversal reaction.

PATIENTS AND METHODS

Twenty-four patients with borderline leprosy who developed reversal reaction during their 1st year of treatment were studied. All patients were classified clinically and histologically according to the Ridley-Jopling scale (Ridley & Jopling, 1966). Sixteen were borderline tuberculoid (BT), four, borderline (BB) and four, borderline lepromatous (BL). Twelve of the patients were male, and twelve female; their ages ranged from 13-56 years. All patients received treatment with dapsone, which was continued in unaltered dosage throughout the study. When they developed reaction they were treated with prednisolone at an initial dosage of 30-40 mg daily, and this was gradually reduced over a period of 1-9 months depending on the duration and severity of the reaction. Clinical assessments and LT tests were carried out at intervals before, during and after the reaction. The degree of inflammation in skin lesions and nerves was carefully recorded and graded on an arbitrary scale as described earlier (Bjune *et al.*, 1976). When erythema and oedema involved normal skin around the lesions, and/or nerves were markedly tender and enlarged with electrophysiological and functional evidence of substantial acute involvement, the inflammation was considered a fully developed reaction. The diagnosis of reaction was confirmed by biopsy of a skin lesion and the radial cutaneous nerve.

Nineteen patients with borderline leprosy (BT:14, BL:5) matched for age, sex and duration of treatment who had quiescent hypopigmented skin lesions and no evidence of neuritis for at least 6 months, were used as controls. All tests and clinical assessments were carried out in the same way as for the reactional patients.

In forty-seven of the LT tests in reactional patients before, during and after the reaction, the effect of the patients' plasma was tested on lymphocytes from another individual (healthy or leprosy patient) in parallel with the autologous lymphocytes. The foreign lymphocytes and the patients' own cells were cultured identically on the same tray.

Lymphocyte transformation tests were carried out by a micro method as described by Closs (1975). Peripheral blood lymphocytes were separated by centrifugation on Ficoll-Isopaque (Nyegaard, Oslo, Norway) as described by Böyum (1968) and cultured in a concentration of 0.5×10^6 /ml. Stimulation with PHA (Reagent Grade, Wellcome, Beckenham) was carried out at a dilution of 10^{-2} from stock, found to be optimal in previous experiments. The standard serum was collected from twenty healthy donors from countries where leprosy was not endemic. It was filtered through a 0.45μ Millipore filter and stored in small aliquots at -70°C . The patients' plasma was recovered from the blood sample after the separation of cells, and was kept for not longer than 4 hr before being mixed with the cells for culture. Lymphocyte cultures were carried out in triplicate in medium TC 199 (Gibco, Glasgow) with 10% of either standard serum or patient's plasma on the same micro tray (Linbro, IS-FB-96-TC, New Haven, Connecticut). Control cultures without PHA added were also included in the tray. Lymphocyte cultures were incubated at 37°C , 100% humidity and 5% CO_2 for 96 hr. The cells were labelled with $0.5 \mu\text{Ci}$ of tritiated thymidine (specific activity, 2 Ci/mM; Radiochemical Centre, Amersham) 16 hr before they were harvested onto glass-fibre filters (Gelman, Ann Arbor, Michigan), washed with distilled water, dried and the thymidine uptake recorded as ct/min in a liquid scintillation counter (Inter-technique SL 31, Plaisire, France).

The recorded values for stimulation by PHA were calculated as the mean of stimulated triplicate cultures, with the mean of unstimulated triplicates subtracted. Statistical significance of differences between groups was estimated with the Wilcoxon matched-paired-signed-rank-test (W), or the Kolmogorov Smirnov two-sample-test (KS) when observations were not paired. Estimates of linear regression with statistical calculations for paired data were done on a Canon Canola F-20P computer.

RESULTS

The PHA-induced responses of lymphocytes from the nineteen control patients who had stable borderline leprosy gave a median value of 34,700 ct/min when cultured in 10% autologous plasma. This value was much lower than when the cells were cultured in 10% standard serum i.e. median: 48,200 ct/min ($P < 0.001$ (W)). The degree of inhibition by autologous plasma remained fairly constant in the nine clinically stable males, while the ten stable females showed considerable variation from test to test (Fig. 1).

Plasma from the twenty-four patients who developed reactions produced a similar degree of suppression of PHA responses both before and after reaction, while by contrast for the period related to the reaction they augmented the responses. The duration of the augmenting effect of plasma and its degree showed great individual variation, as did the intensity and duration of the clinical reaction. In ten of the patients we were able to perform several tests before, during and after the reaction (Fig. 2). When the effect of autologous plasma from BT patients on the lymphocyte responses to PHA (recorded as a percentage of the responses in standard serum) was compared before, during and after reaction, a highly significant difference appeared (Fig. 3). The median value before reaction was 52%, during reaction 128% and after reaction 57% ($P < 0.001$ (KS) for both rise and fall).

The augmenting effect of plasma from BL patients appeared later than that from BT patients. Post-reactional values recorded up to 2 months after the clinical reaction had subsided, were significantly

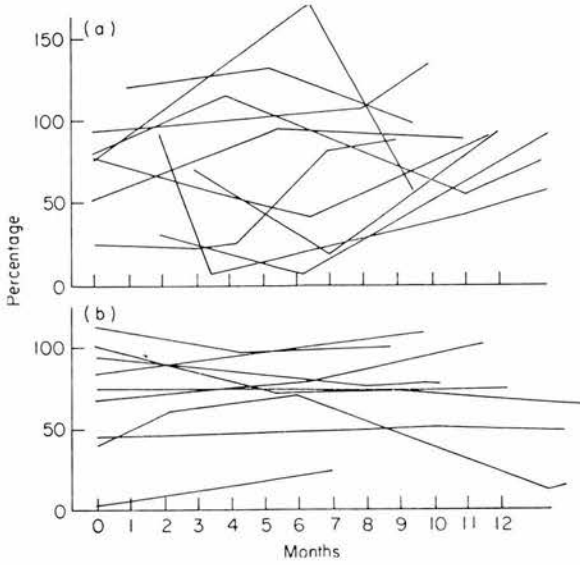


FIG. 1. Effect of autologous plasma on lymphocyte responses to PHA, recorded as a percentage of the parallel responses in standard serum. Variation with time in ten clinically stable females (a) and nine stable males (b).

higher (median: 90%) than the reactional values (median: 36%), ($P = 0.005$ (KS)). The degree of inhibition observed in stable BL patients (median: 61%) was not significantly different from the inhibition by plasma from BL patients in reaction.

The changes in the effect of plasma on PHA responses during the course of reversal reactions were not a direct effect of steroid treatment. Reactional BT patients on steroid treatment had a median response of 126% and postreactional ones 52.5%, which were not significantly different from patients in the same groups without such treatment (125% and 57% respectively).

The effect of plasma in each case from reactional patients, taken before, during and after reaction, was tested simultaneously on autologous lymphocyte responses and lymphocytes from an unrelated

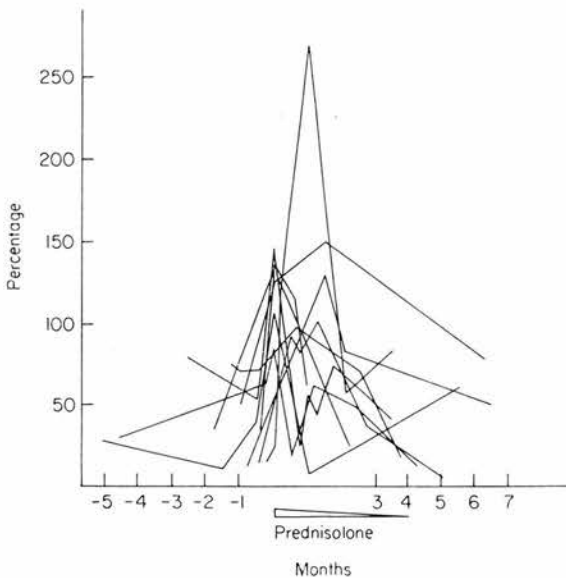


FIG. 2. Effect of autologous plasma on lymphocyte responses to PHA, recorded as a percentage of the parallel responses in standard serum. Variation with time in ten patients going through a reversal reaction. 0 = height of reaction clinically, when steroid treatment was initiated.

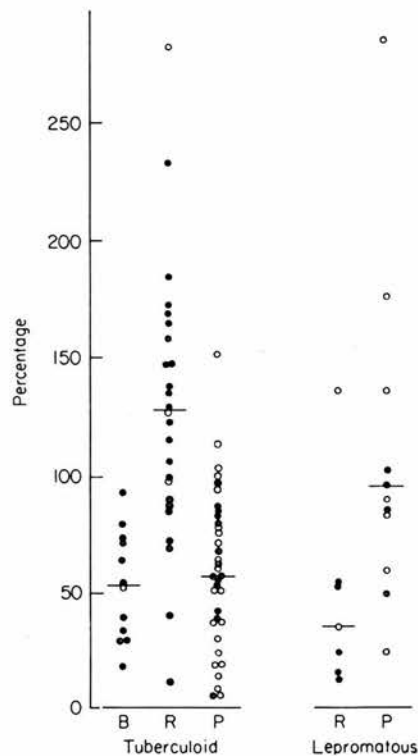


FIG. 3. Effect of autologous plasma on lymphocyte responses to PHA, recorded as a percentage of the parallel responses in standard serum. Comparison of responses before reaction (B), during reaction (R) and post reaction (P), in sixteen borderline tuberculoid and five borderline patients. (○) patients on steroid treatment; (●) patients on dapsone only; (—) median effect on the group.

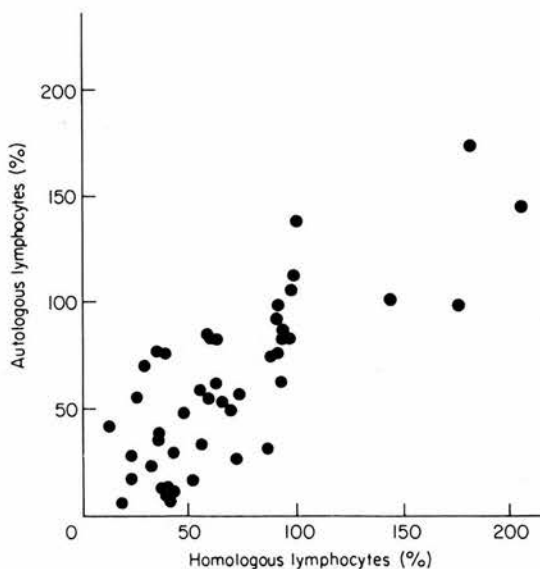


FIG. 4. Effect of reactional patients' plasma on lymphocyte responses to PHA, recorded as a percentage of the parallel responses in standard serum: correlation between the effect on autologous lymphocytes and lymphocytes from unrelated individuals (homologous). Correlation coefficient, $r = 0.81$ ($P < 0.001$).

individual. The relationship of the effect of reactional patients' plasma on autologous and homologous lymphocytes is shown in Fig. 4. From this it is seen that the plasma had the same effect on both types of test cells.

DISCUSSION

Factors in the blood inhibiting lymphocyte responses to PHA have been demonstrated in leprosy by Nelson *et al.* (1971), in recurrent infections in childhood by Fitzgerald & Hosking (1976), various neoplasms by Suciò-Foca *et al.* (1973), in pregnancy by Gatti (1971), and also in several other conditions. Common to many of these conditions is a prolonged stimulation of the immune system.

BT patients often show surprisingly little inflammatory response despite the presence of bacilli in the tissues for many years. Plasma from such patients caused inhibition of lymphocyte responses to PHA compared with the responses in standard serum (Fig. 1). In those patients with BT leprosy who developed a reversal reaction, this inhibitory effect disappeared during the reaction and was replaced by an augmenting effect of plasma on lymphocyte responses (Figs 2 and 3).

That both the augmentation and inhibition of lymphocyte responses were due to factors in the plasma and not to a change in lymphocyte receptors, is supported by the observation that the plasma had the same effect on both homologous and autologous lymphocytes (Fig. 4). Control experiments with different concentrations of PHA gave no indication of a changed sensitivity of lymphocytes during reaction nor was there any significant change in the actual height of the PHA responses in standard serum in relation to the reaction. So the augmentation of responses by autologous plasma during reaction was not due to a change in the lymphocytes' responsiveness to PHA.

Although clinically stable male patients had a constant degree of inhibition during the period of observation, the effect of plasma from stable female patients on PHA responses showed a great variation from test to test (Fig. 1). Further studies on normal individuals (Bjune, unpublished data) have shown that females have pronounced variations of plasma effect on the PHA response related to the menstrual cycle. The magnitude of these variations considerably surpass the basic level of suppression due to the leprosy infection.

It has been observed by Weddell & Pearson (1975) that bacilli can lie unrecognized in Schwann cells of dermal and peripheral nerves, while they are completely cleared from skin by macrophages. A reversal reaction could therefore be caused by a sudden detection of formerly hidden antigen as well as by a breakdown of control mechanisms possibly reflected by the plasma factor(s) described here.

The finding in BL patients that the period of augmenting effect of autologous plasma was delayed in relation to the onset of clinical reaction, could indicate that the plasma factor(s) are secondary to the reaction. However, it is probable that the plasma factor(s) described here will initially occupy lymphocyte receptors and exert their effect there, before they can appear free in the blood. Therefore the delayed appearance in the blood of the augmentary effect does not necessarily negate their primary importance in transforming the CMI response from a well controlled fight against bacillary multiplication to an overactive and self-destructive hypersensitivity reaction.

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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 response¹. But, no sudden changes in the immune mechan-

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 un sensitised mothers were not sensitised. We consider the likeliest explanation for our findings is the transplacental passage of a soluble lymphocyte factor.

Lymphocyte transformation tests (LTT) were carried out by a micromethod⁶ 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

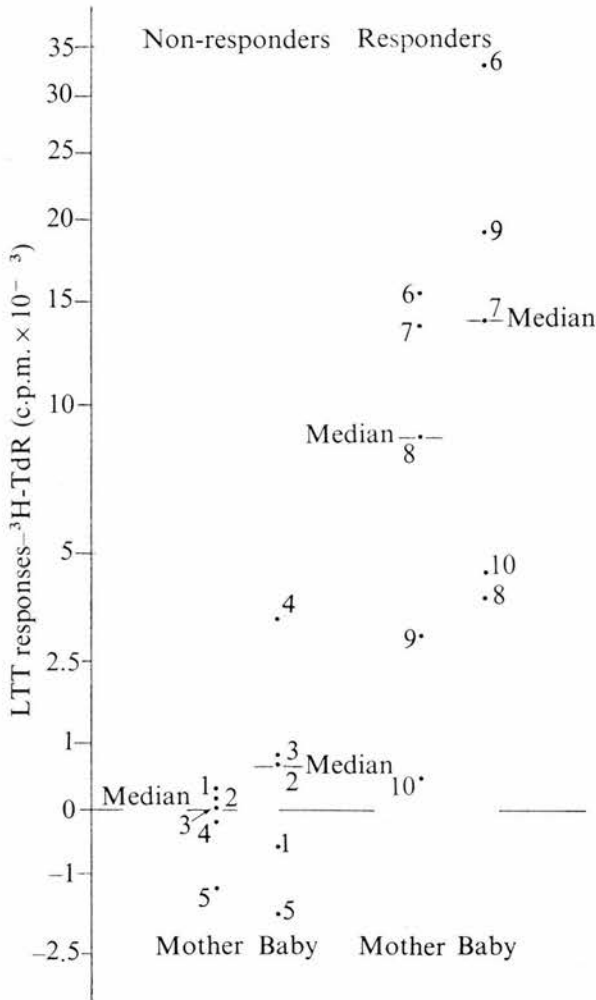


Fig. 1 LTT responses to whole *M. leprae* in mothers and neonates, comparing the five mothers with positive responses and the five mothers with negative responses (unstimulated control values are subtracted). ³H-TdR, Tritiated thymidine uptake.

isms 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 Caspary⁵ 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

Table 1 Median responses to antigens

| | <i>M. leprae</i> | BCG | PPD |
|---|------------------|-----|-------|
| Mothers with positive responses to <i>M. leprae</i> | 8,600 | 267 | 4,316 |
| Mothers with negative responses to <i>M. leprae</i> | -11 | 21 | -36 |
| Babies of mothers with positive responses to <i>M. leprae</i> | 13,658 | 995 | 3,654 |
| Babies of mothers with negative responses to <i>M. leprae</i> | 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.

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.

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 infection⁷. 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 tests⁸.

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 circulation⁹⁻¹², 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 factor¹³ 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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Immune complexes and complement hypercatabolism in patients with leprosy*

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SUMMARY

The occurrence of immune complexes in the serum and the level of the C3 breakdown product C3d in the plasma from patients with leprosy were studied by quantitative methods and the results were compared in various forms of the disease. These studies were performed on sixty-two samples from twenty-six patients. The serum ^{125}I -C1q binding activity was found to be increased by more than 2 s.d., as compared to the normal values, in most of the sera from patients with erythema nodosum leprosum (ENL) (80%) and uncomplicated lepromatous leprosy (82%), but also in the sera from patients with tuberculoid leprosy (58%). *In vitro* studies suggested that immune complexes involving mycobacterial antigens were present in leprosy sera. An increased C3d level (>2 s.d.) was also found in most of the plasma from patients with ENL (70%), but rarely in the plasma from patients with uncomplicated lepromatous leprosy (18%) and never in tuberculoid leprosy patients' plasma. The absence of a significant correlation between the ^{125}I -C1q binding activity and the C3d level in leprosy patients may suggest that extravascular immune complexes are involved in the complement activation occurring in ENL. The quantitation of C3d in plasma may be of some practical interest in the early diagnosis of ENL complications of leprosy.

INTRODUCTION

The various forms of leprosy represent a wide, continuous spectrum of clinical manifestations (Ridley & Jopling, 1966). Recent studies indicated that cellular and humoral immune reactions against *Mycobacterium leprae* may be involved in the development of some of the pathological manifestations (Godal, 1974). The formation of immune complexes may be expected in lepromatous leprosy from the concomitant occurrence of large amounts of mycobacteria and of corresponding antibodies. Indeed, there is some evidence of an involvement of immune complexes in the pathogenesis of erythema nodosum leprosum (ENL), mainly based on clinical and tissue studies. Certain disease manifestations of ENL, such as albuminuria or skin lesions, are similar to those encountered in serum sickness, or in the experimental Arthus reaction (Waters & Ridley, 1963). Deposits of immunoglobulins and C3, and sometimes mycobacterial antigens have been demonstrated by immunofluorescence in such ENL lesions (Wemambu *et al.*, 1969). The presence of immune complexes in circulating blood has also been suspected and serological studies have revealed the frequent occurrence in leprosy sera of substances which precipitate with C1q in agarose (Moran *et al.*, 1972; Rojas-Espinosa, Mendez-Navarrete & Estrada-Parra, 1972; Gelber *et al.*, 1974). However, the complement level has been consistently found to be normal or elevated (Saitz, Dierks & Shepard, 1968; Wemambu *et al.*, 1969; Malaviya *et al.*, 1972; Petchclai *et al.*, 1973; Gelber *et al.*, 1974).

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The purpose of the present work was to apply to the study of leprosy quantitative methods for the detection of immune complexes in serum and for the demonstration of complement activation products in plasma. The serological results were compared in the various forms of the disease and the correlation with the clinical activity of the disease in ENL was investigated. A radioimmunological method, the [125 I]C1q binding test, was used for the quantitation of immune complexes (Nydegger *et al.*, 1974; Zubler *et al.*, 1976a). Complement activation was studied by measuring the C3d levels with an immunochemical method (Perrin, Lambert & Miescher, 1975). It is known that activation of C3 results in a fragmentation of the native molecule (mol. wt 200,000) into C3b (mol. wt 190,000) and C3a (mol. wt 10,000). C3b is then cleaved by the C3b inactivator into C3c (mol. wt 150,000) and C3d (mol. wt 35,000). By measuring the C3 level by usual methods, one cannot recognize if an increased synthetic rate is masking an increased catabolism of the molecule. By selective quantitation of the small C3d fragment, a hypercatabolism of C3 may be demonstrated, independent of the C3 synthesis rate.

MATERIALS AND METHODS

Patients studied. Thirty-nine serum samples from thirteen patients with ENL (two serum samples or more per patient, obtained at intervals of 1 week or more); eleven sera from seven patients with uncomplicated lepromatous leprosy and twelve sera from six patients with tuberculoid leprosy were studied. The control groups included ten patients with active pulmonary tuberculosis and five clinically healthy individuals from Ethiopia. In addition, samples from thirty blood donors from Geneva were studied. Leprosy and tuberculosis patients were diagnosed and treated at the Addis Ababa Leprosy Hospital and Tuberculosis Center respectively. The age distribution of the different groups was similar, with means ranging from 23–27 years. All leprosy patients were under treatment with DDS or clofazimine. For the treatment of ENL, most patients received in addition a combination of two or more of the following drugs: stibophen, chloroquin, acetylsalicylic acid, chlorpromazine and prednisolone.

Collection and storage of serum and plasma samples. For collection of serum, blood was allowed to clot at ambient temperature for 1–2 hr, was then centrifuged at 1500 g for 15 min and the serum was stored at -70°C . To obtain plasma, blood was collected in plastic tubes containing EDTA (20 mM final concentration), centrifuged at 1500 g for 15 min and the supernatant was immediately stored at -70°C . Serum and plasma samples were shipped to Geneva on dry ice and were kept at -70°C for up to 3 months.

[125 I]C1 3 q binding test. The modified [125 I]C1q binding test for the detection of soluble immune complexes was used (Zubler *et al.*, 1976a): briefly, [125 I]C1q is mixed with native test serum and then free C1q is separated from C1q bound to complexes by a precipitation with polyethylene glycol (PEG). Results are expressed as percent [125 I]C1q precipitated as compared to the radioactivity precipitated with trichloroacetic acid (TCA) in control tubes. The mean of duplicated tests is calculated and represents the C1q binding activity (C1q-BA) of the sample. Sucrose gradient fractions have also been tested, and the specific C1q-BA in such gradient fractions was obtained after a correction for the non-specific C1q-BA observed in gradient fractions obtained with NHS (Zubler *et al.*, 1976b). In some experiments, the test was performed on sera or sucrose gradient fractions which had been incubated in presence of mycobacterial antigens. As controls, samples were tested after incubation in presence of 0.9% NaCl.

Complement studies. These were carried out on EDTA-plasma. Haemolytic activity (CH50) was quantitated in a continuous flow system (Nydegger *et al.*, 1972). C1q, C4, C3 and properdin factor B were quantitated by single radial immunodiffusion (Perrin, Lambert & Miescher, 1974). The C3 breakdown product, C3d, was quantitated in a two-step procedure (Perrin, Lambert & Miescher, 1975): in the first step, C3 and the high molecular weight fragment, C3c, were precipitated with PEG. In the second step, the C3d was measured in the PEG supernatant by single radial immunodiffusion using anti-C3d antigen antiserum. The values for CH 50, C1q, C4 and factor B were expressed in per cent of a normal plasma pool. The values for C3 and C3d were expressed in mg%.

Density gradient studies. Ultracentrifugations were performed with a SW65-Ti rotor in a Spinco L2-65B preparative ultracentrifuge. Samples of serum or PEG fraction obtained from serum (0.8 ml) were layered on 4.2-ml sucrose gradients, 10–40% (w/v) in 0.1 M borate, 0.1 M EDTA, pH 8.3, and centrifuged at 34,000 rev/min for 17 hr at 7°C . In some experiments, serum (0.4 ml) was first incubated in presence of mycobacterial antigens (0.8 ml); thereafter 1.2-ml portions were layered on 3.8-ml gradients and centrifuged at 43,000 rev/min for 13 hr at 7°C . The techniques used for the preparation of gradients, the collection of gradient fractions and for obtaining OD patterns from gradients with a u.v. flow cell, have been described (Zubler *et al.*, 1976b). IgG and IgM were used as 7s and 19s sedimentation markers respectively.

In some experiments, a PEG precipitation was done in order to concentrate the C1q binding material before ultracentrifugations (Zubler *et al.*, 1976b). For the present study, 1 vol. of serum was mixed with 4 vol. of a 4% (w/v) PEG (MW 6000) solution in borate-EDTA buffer, pH 8.3. The mixture was kept at 4°C for 1 hr, centrifuged at 2000 g for 30 min, the precipitate was homogenized and resuspended with borate-EDTA buffer in 1/3 or 1/6 of the original volume of serum.

Experiments with mycobacterial antigens. For such experiments, an antigen preparation of *M. leprae* from armadillo tissue, homogenized by sonication, was used at a protein concentration of 3 mg/ml (Kronvall *et al.*, 1976).

Statistical evaluation. Statistical evaluation was carried out according to Student's *t*-test and by linear regression analysis by the method of the least squares.

RESULTS

[¹²⁵I]C1q binding activity in serum samples

The [¹²⁵I]C1q binding activity (C1q-BA) was measured on thirty-nine serum samples from thirteen patients with ENL, eleven sera from seven patients with uncomplicated lepromatous leprosy (L. leprosy)

TABLE 1. [¹²⁵I]C1q binding activity and complement levels in patients with leprosy and in controls

| | Sera tested (no) | [¹²⁵ I]-C1q binding activity (%)* | Complement levels | | | | | |
|---------------------------|------------------|---|----------------------|---------------------|---------------------------|----------|-----------|-----------------------|
| | | | C1q (%) [†] | C4 (%) [†] | Factor B (%) [†] | C3 (mg%) | C3d (mg%) | CH50 (%) [†] |
| Normal values | | | | | | | | |
| (Blood donors, Geneva) | 30 | 7.0±2.5‡ | 100±18 | 100±21 | 100±14 | 135±21 | 0.8±0.5 | 100±15 |
| Ethiopian controls | 5 | 9.2±4.1 | 107±28 | 119±18 | 96±22 | 130±36 | 0.6±0.5 | 101±23 |
| Leprosy: ENL (total) | | | | | | | | |
| ENL acute | 12 | 18.7±8.7 | 127±29 | 112±36 | 128±39 | 160±48 | 2.3±0.8 | 111±27 |
| ENL convalescent | 12 | 20.6±6.3 | 112±29 | 115±42 | 163±44 | 188±58 | 2.4±0.4 | 115±39 |
| Lepromatous (without ENL) | 11 | 16.5±6.4 | 126±30 | 106±33 | 107±29 | 146±41 | 2.0±0.9 | 104±31 |
| Tuberculoid | 12 | 27.5±17.7 | 139±28 | 102±42 | 127±30 | 140±48 | 1.3±0.7 | 104±30 |
| Tuberculosis | 12 | 14.5±6.1 | 123±22 | 106±41 | 111±28 | 127±23 | 0.8±0.4 | 97±29 |
| Tuberculosis | 10 | 12.8±7.0 | 152±21 | 117±23 | 104±17 | 157±31 | 0.9±0.5 | 122±24 |

* Per cent radioactivity precipitated.

† Per cent of a normal plasma pool.

‡ These are mean values±1 s.d.

and twelve sera from six patients with tuberculoid leprosy (T. leprosy). For controls, thirty serum samples from blood donors from Geneva, five sera from healthy Ethiopians and ten sera from ten patients with active pulmonary tuberculosis (Tbc) who were also Ethiopian, were tested. The mean values for C1q-BA which have been found in these patient groups are shown on Table 1. As compared to the C1q-BA in the normal controls (blood donors), the mean C1q-BA was significantly increased in the sera

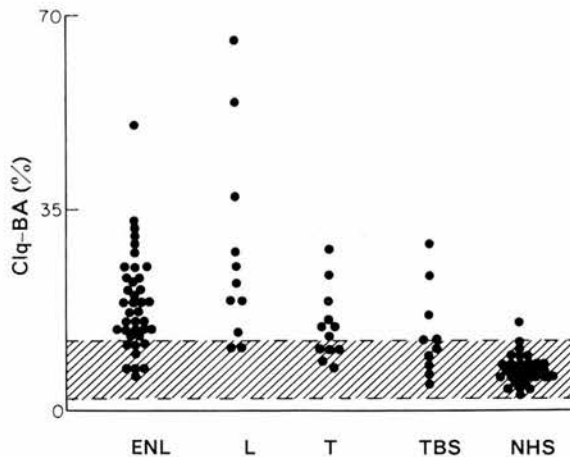


FIG. 1. [¹²⁵I]C1q binding activity (C1q-BA) in the serum from patients with erythema nodosum leprosum (ENL), uncomplicated lepromatous leprosy (L), tuberculoid leprosy (T), pulmonary tuberculosis (TBC) and from healthy blood donors (NHS). The normal range (±2 s.d.) for the C1q-BA is indicated.

from patients with ENL ($P < 0.001$), L. leprosy ($P < 0.005$) and T. leprosy ($P < 0.005$). The highest mean value was found in L. leprosy. No significant difference in C1q-BA was observed between the various leprosy patient groups. The mean C1q-BA was also increased in the Tbc patients ($P < 0.005$). Only in L. leprosy was the mean C1q-BA significantly higher when leprosy was compared to Tbc ($P < 0.05$). There was no significant difference between the mean C1q-BA in Ethiopian controls and that found in blood donors from Geneva. The values for C1q-BA in individual patient's sera are shown on Fig. 1. As compared to the normal controls, the C1q-BA was increased by more than 2 s.d. in 80% of ENL sera, 82% of L. leprosy sera, 58% of T. leprosy sera and 30% of Tbc sera.

Complement levels in plasma samples

Various complement parameters were measured in plasma samples obtained from patients who had sera tested. First, the total haemolytic complement activity (CH50) and the levels of C1q, C4, C3 and properdin factor B were quantitated. The mean values which were observed in the various patient groups

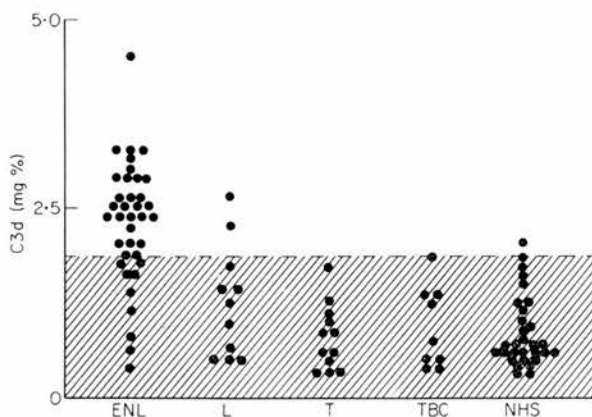


FIG. 2. C3d levels in the plasma from patients with erythema nodosum leprosum (ENL), uncomplicated lepromatous leprosy (L), tuberculoid leprosy (T), pulmonary tuberculosis (TBC) and from healthy blood donors (NHS). The normal range (± 2 s.d.) for the C3d level is indicated.

are shown on Table 1. As compared to the normal controls, all complement levels measured were increased to various extents in the leprosy and the Tbc patients. One should mention that the mean C1q levels were significantly increased in all these patient groups ($P < 0.01$). In individual patients, none of the complement values significantly correlated with the C1q-BA. Secondly, the C3d fragment of C3 was quantitated in the plasma samples. The mean C3d levels which were observed in the various patient groups are also shown on Table 1. The mean C3d level was significantly increased in ENL ($P < 0.001$), it was elevated at the limit of significance in L. leprosy ($P = 0.05$) and was not significantly different from the normal value in T. leprosy and Tbc. The mean C3d level was also significantly elevated in ENL when compared to L. and T. leprosy ($P < 0.001$). The C3d levels in individual patient's plasma are shown on Fig. 2. As compared to the normal control, the C3d levels were increased by more than 2 s.d. in 70% of ENL plasma, 18% of L. leprosy plasma and in none of the T. leprosy or Tbc plasma.

Correlation between the C1q-BA and the C3d level

By linear regression analysis, a significant correlation was found between the C1q-BA and the C3d level in patients with ENL, but the correlation coefficient was not very high ($r = 0.359$, $P < 0.05$). When studying all the patients with leprosy, the correlation between the C1q-BA and the C3d level was not significant ($r = 0.243$, $P > 0.05$ (Fig. 3).

C1q-BA, C3d level and clinical activity in ENL

From the patients with ENL, two to seven serum samples have been obtained at intervals of 1 week or

more for prospective follow up study. The C1q-BA and the C3d level have been compared to the clinical activity of the disease. From twelve ENL patients, serum samples have been obtained during both an acute bout of ENL and a period of convalescence. The C1q-BA and/or C3d levels were found to be lower during the convalescence in eight patients. However, the mean C1q-BA and C3d level were only insignificantly lower during the convalescence in comparison with the acute phase of ENL (Table 1). It should be noted that, in fact, the C1q-BA as well as the C3d level, remained rather constant within individual patients during the follow-up study. However, in a prolonged follow-up of one patient, the C1q-BA and the C3d level were regularly found to be decreased during periods of convalescence, as observed following three different bouts of ENL. All the other complement levels studied were lower during the convalescence. No conclusions could be drawn from this study with respect to the effects of various drug regimes.

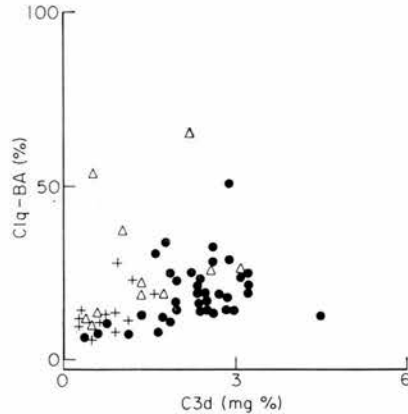


FIG. 3. Correlation between the [125 I]C1q binding activity (C1q-BA) and the C3d level in patients with leprosy: (●) erythema nodosum leprosum; (Δ) uncomplicated lepromatous leprosy; (+) tuberculoid leprosy.

Characterization of the C1q binding material in leprosy

In order to investigate the size of the C1q binding material, the sera from two patients with ENL and one patient with L. leprosy exhibiting an increased C1q-BA, were subjected to ultracentrifugation analysis, using sucrose density gradients (10–40% w/v). After ultracentrifugation, the C1q-BA was measured in each of seven serial gradient fractions. The C1q-BA was always encountered in gradient fractions 3 and 4 from the bottom of the tubes containing about 10–26s material (Fig. 4).

Some experiments were performed in order to investigate the nature of the C1q binding material in leprosy serum. The assumption was made that the increased C1q-BA in such serum may be related to the presence of immune complexes involving antigens from *M. leprae*. It is known that immune complexes in great antigen excess do not bind C1q efficiently. The C1q-BA was therefore studied to see whether it decreased after an incubation of leprosy sera in presence of an *M. leprae* antigen preparation (MI-ag). Sera were incubated overnight at 37°C in presence of either MI-ag or 0.9% NaCl for controls. The MI-ag protein concentration was 12 mg/ml serum. In experiments with three ENL and three L. leprosy sera, the C1q-BA was found to be increased after an incubation with MI-ag ($26 \pm 19\%$, mean C1q-BA \pm 1 s.d.) as compared to controls with NaCl ($17 \pm 18\%$). When T. leprosy sera, Tbc sera, normal human sera (NHS) or NHS containing various amounts of heat-aggregated human immunoglobulins (AHI) were used in these experiments, the C1q-BA was found to be the same (maximal difference of 2%) in samples with MI-ag as in controls with NaCl. The serum of one patient with ENL was subjected to ultracentrifugation analysis after an incubation with either MI-ag or NaCl. The results are shown on Fig. 5. When the serum was incubated with NaCl, the maximal C1q-BA was found in gradient fractions containing about 12–20s material; but when the serum was incubated with MI-ag, the maximal C1q-BA was found in gradient fractions containing smaller, 8–16s material. The total C1q-BA in all the fractions of the respective gradients was about the same after the incubation with either MI-ag or NaCl.

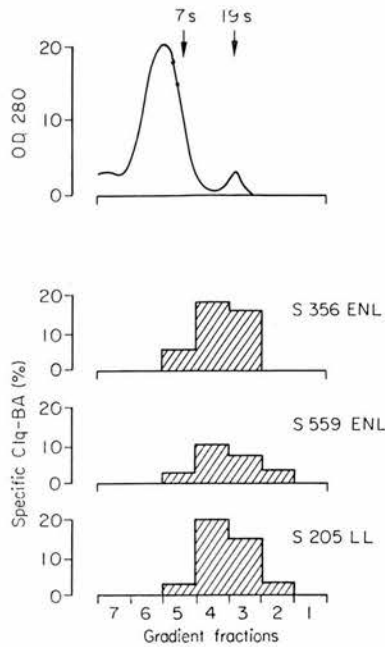


FIG. 4. Specific [125 I]C1q binding activity (C1q-BA) measured in individual sucrose gradient fractions obtained by separation of sera from patients with erythema nodosum leprosum (ENL) and uncomplicated lepromatous leprosy (LL): at the top of the figure the optical density (OD) pattern obtained with a serum and the positions of the 7s and 19s markers are indicated. C1q-BA is represented by the columns on the lower part of the figure.

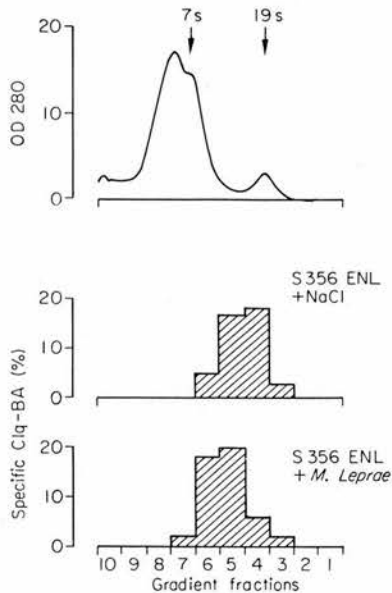


FIG. 5. Specific [125 I]C1q binding activity (C1q-BA) measured in individual sucrose gradient fractions obtained by separation of the serum from a patient with erythema nodosum leprosum (ENL) after an incubation of the serum with either an *M. leprae* antigen preparation or 0.9% NaCl for the control: at the top of the figure the optical density (OD) pattern obtained with the serum and the positions of the 7s and 19s markers are indicated, C1q-BA is represented by the columns on the lower part of the figure.

Since one possibility was that the increase in C1q-BA in ENL and L. leprosy sera observed in presence of MI-ag was the result of a reaction of MI-ag with free anti-mycobacterial antibodies, the C1q binding material was largely separated from free antibodies before investigating the effect of an incubation with MI-ag on the C1q binding activity. The C1q binding material from 2 ENL and one L. leprosy sera was partially purified and concentrated using PEG, and then fractionated on sucrose density gradients. This procedure led to a two to four times increased C1q-BA in 10–26s gradient fractions when compared to the C1q-BA in respective gradient fractions obtained with the original untreated sera. When such gradient fractions were incubated in presence of MI-ag, the C1q-BA was found to be strongly reduced as compared to the C1q-BA after incubation with NaCl. (Table 2).

TABLE 2. Effect of an incubation with *M. leprae* antigens on the [125 I]C1q binding activity of partially purified C1q binding material from leprosy sera

| Sera used for purification of C1q binding material | Specific [125 I]C1q binding activity (%) | |
|--|--|----------------------------|
| | When incubated with NaCl | When incubated with MI-ag* |
| S 356 ENL | 41† | 21 |
| S 559 ENL | 46 | 17 |
| S 205 L. leprosy | 49 | 26 |
| NHS | 0 (9.4‡) | 0 (11.2‡) |
| NHS+AH1 (0.2 mg/ml)¶ | 29 | 30 |

* Partially purified C1q binding material was incubated with *M. leprae* antigens (MI-ag) for 3 hr at 37°C; the MI-ag protein concentration was 4 mg/ml sucrose gradient fraction material.

† Mean values of duplicate experiments.

‡ Values for 'non-specific' [125 I]C1q precipitation in sucrose gradient fraction obtained with NHS, after an incubation with NaCl or MI-ag respectively.

¶ AH1 = Aggregated human immunoglobulins.

DISCUSSION

There is now good evidence that the immune reactions against *M. leprae* may play an important role in the pathogenesis of leprosy, while *M. leprae* itself exhibits little *in vivo* toxicity (Godal, 1974). Lepromatous leprosy is characterized by the concomitant occurrence of large amounts of *M. leprae* and of corresponding anti-mycobacterial antibodies (Kronvall *et al.*, 1976). Therefore, one might expect the formation of immune complexes as well as an activation of the complement system triggered by such immune complexes. Deposits of immunoglobulins and C3 have been detected by immunofluorescence in skin lesions (Wemambu *et al.*, 1969) and renal glomeruli (Shwe, 1972) in patients with L. leprosy or ENL. Furthermore, the existence of circulating immune complexes has been suggested by the finding of C1q precipitins in agarose (Agnello, Winchester & Kunkel, 1970) in the sera from patients with L. leprosy (Moran *et al.*, 1972; Rojas-Espinosa *et al.*, 1972; Gelber *et al.*, 1974). Some authors found a higher incidence of such C1q precipitins in agarose in ENL than in uncomplicated L. leprosy (Moran *et al.*, 1972; Gelber *et al.*, 1974). This observation was not confirmed using a quantitative estimation of immune complexes with the C1q binding radioassay. Indeed, in the present investigations, an increased [125 I]C1q binding activity (C1q-BA) was demonstrated frequently in the sera from patients with ENL and with uncomplicated L. leprosy, but was also found in sera from patients with T. leprosy.

Several data support the hypothesis that the detected [125 I]C1q binding material, which sedimented in a 10–25s range, consists of immune complexes involving *M. leprae* antigens. The decrease of the C1q-

BA of partially purified C1q binding material to about half of the control value after an incubation with *M. leprae* antigens (MI-ag) suggests that immune complexes involving such antigens were present in the sera. The MI-ag did not non-specifically interfere with the C1q binding test and the addition of MI-ag may lead to the formation of complexes with a higher antigen-antibody ratio, and with a lower efficiency to bind C1q (Nydegger *et al.*, 1974). Such experiments can only be carried out using partially purified C1q binding fractions, since it requires a preliminary separation of free antibodies which could form new complexes upon addition of MI-ag. Indeed, an increased C1q-BA results from the incubation of whole sera with MI-ag, probably indicating its reaction with an excess of anti-mycobacterial antibodies. The finding of a decreased sedimentation velocity of the C1q-BA after the incubation of a serum with MI-ag probably represents a decrease in the size of previously existing complexes upon addition of more antigen, as well as the formation of new, small complexes. Antigen-antibody systems other than those involving mycobacterial antigens may also be present in the leprosy sera. Bonomo & Dammacco (1970) found, in leprosy sera, mixed cryoglobulins with IgM exhibiting anti-IgG activity. In addition, although the interference of DNA and bacterial lipopolysaccharides is very limited in the [¹²⁵I]C1q binding test (Zubler *et al.*, 1976a), one cannot rule out that other biological substances which bind C1q may influence the present investigation. Moran *et al.* (1972) reported the possibility of a direct reaction of certain mycobacterial antigens with C1q in agarose.

In previous studies, normal or increased levels of all of the complement components studied have generally been observed (Saitz *et al.*, 1968; Wemambu *et al.*, 1969; Malaviya *et al.*, 1972; Petchclai *et al.*, 1973; Gelber *et al.*, 1974). In the present study, these findings have been generally confirmed. The increased levels of C1q, C3 and properdin factor B during ENL probably reflect an increased synthesis of these components in association with the inflammatory syndrome. Such modifications have been frequently observed in infectious diseases (Schur & Austen, 1968). However, the quantitation of breakdown products of complement components such as the C3d catabolic fragment of C3 has been shown to provide a possibility for the evaluation of complement activation independently of the synthetic rate of these components in human disease, such as systemic lupus erythematosus (SLE) and glomerulonephritis, as well as in *in vitro* complement activation studies (Perrin *et al.*, 1975). The usual haemolytic or immunochemical methods for measuring the plasma level of complement components provide only a static profile of the complement system and an increased synthetic rate can mask an increased catabolism. The finding of increased C3d levels in 70% of ENL plasma probably indicates an *in vivo* activation of C3. One should note that C3d levels were within the normal range in the control group of patients with tuberculosis although C3 plasma levels were as elevated as in ENL.

The generation of C3d in plasma may result from an activation of C3, a central component of the complement system, by the classical as well as by the alternative pathway, and either pathway may be activated by immunological or non-immunological mechanisms (Müller-Eberhard, 1974). In certain clinical conditions, such as SLE or rheumatoid arthritis, increased C1q-BA values in serum were frequently observed (Nydegger *et al.*, 1974; Zubler *et al.*, 1976a, b) and there is a very significant correlation between the C1q-BA and the C3d level (Lambert & Zubler 1976; Nydegger *et al.* 1976). This suggests that C1q binding complexes in the serum are involved in the complement activation. In the present study, only a poor correlation between the C1q-BA and the C3d level was found in ENL. In addition, while C3d levels are similar to those observed in SLE by Perrin *et al.* (1975), C1q-BA values are lower in ENL than in SLE. It is therefore not likely that the increased C3d levels in ENL were only due to complement activation by immune complexes present in the circulation. One should consider the possibility that extra-vascular immune complexes are involved in the complement activation occurring during ENL and that the C3d catabolic fragments detected in the plasma from patients with ENL would be diffusing from extra-vascular spaces. The results from tissue immunofluorescence studies by Wemambu *et al.* (1969) support such a hypothesis. In the light of the known biological effects of complement breakdown products (Cochrane & Koffler, 1973), the demonstration of a hypercatabolism of C3 in ENL suggests that an activation of the complement system may be involved in the pathogenesis of this clinical condition. Furthermore, the quantitation of C3d in plasma may be of some practical interest in the early diagnosis of ENL complications of leprosy.

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