A SPECTRUM OF IMMUNITY IN TUBERCULOSIS

by

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To my wife Chineze

and children Chidi and Chinedu

for their tolerance

and

to my teacher and mentor, Professor B. Osuntokun,

for his very kind support

"If the importance of a disease for mankind is measured from the number of fatalities which are due to it, then tuberculosis must be considered much more important than those most feared infectious diseases, plague, cholera, and the like. Statistics have shown that 1/7 of all humans die of tuberculosis.....

On the basis of my extensive observations, I consider it as proven that in all tuberculous conditions of man and animals there exists a characteristic bacterium which I have designated as the tubercle bacillus, which has specific properties which allow it to be distinguished from all other microorganisms .....

All of these facts taken together lead to the conclusion that the bacilli which are present in the tuberculous substances not only accompany the tuberculosis process, but are the cause of it. In the bacillus we have, therefore, the actual tubercle virus."

Robert Koch, 1882.

(from Classics in infectious diseases. The aetiology of tuberculosis. Robert Koch, Berlin, Germany, 1882. Rev Infect Dis 1982;4:1270-1274)

"In all tubercles two processes go on : the one - caseation - destructive and dangerous; and the other - sclerosis - conservative and healing. The ultimate result in a given case depends upon the capabilities of the body to restrict and limit the growth of the bacilli. There are tissue-soils in which the bacilli are, in all probability, killed at once. - the seed has fallen by the wayside. There are others in which the lodgement is gained and more or less damage done, but finally the day is with the conservative, protecting forces, - the seed has fallen upon stony ground. There are tissue-soils in which the bacilli grow luxuriantly; caseation and softening, not limitation and sclerosis, prevail, and the day is with the invaders - the seed has fallen upon good ground."

Sir William Osler, 1906

(from Huber JB. Consumption. Philadelphia and London, Lippincott Co, 1906;71)

".....If the tubercle bacillus were to disappear from the face of the earth it would be necessary to create it afresh - in order to serve as an immunological tool."

D'Arcy Hart, 1948

(from Hart PD. Statement of the questions. Ann NY Acad Sci 1948; 154:1-7)

ABSTRACT

The variable clinical presentation of tuberculosis would suggest a spectrum of host immune responses to the disease. To investigate this hypothesis, 30 hospitalised patients aged 19 to 61 years (mean 34.4 years) with bacteriologically-proven tuberculosis were studied prospectively clinically and with a representative range of *in vivo* and *in vitro* tests of immune function. 73% of patients had pulmonary disease, 70% originated from the Indian Subcontinent, and 50% were vegetarians. Responses were compared with those of healthy controls carefully matched for age, sex, ethnic group and diet.

Before treatment, patients were profoundly malnourished and had significant anaemia, hyponatraemia, hypoalbuminaemia, neutrophil leucocytosis, lymphocytopenia (predominantly helper T-cell), monocytosis, elevation of sedimentation rate, serum alkaline phosphatase, total globulins and immunoglobulins G and A.

Accelerated skin reactions (6-8 hrs) to intradermal tuberculin-purified protein derivative were significantly more frequent, and 48 hr skin reactions were larger in the patients. Even allowing for the booster effect of repeated tuberculin testing seen in controls, chemotherapy was associated with an increase in tuberculin reactivity.

Peripheral blood mononuclear cells from patients were less responsive *in vitro* to purified protein derivative, but not to potent mitogens. No inhibitory effect was observed when normal mononuclear cells were cultured in patients' sera.

Migration of mononuclear cells, judged by mean random locomotion and casein-stimulated chemotaxis *in vitro* was impaired but no differences were observed between patients and controls in spontaneous and interferon-augmented Natural Killer cytotoxicity.

No significant differences were found between patients and controls in titres of interferon-α induced by Newcastle Disease Virus. However, mononuclear cells from a subset of nine patients produced relatively low titres of Interferon-γ in response to purified protein derivative. In comparison with other patients, this group was characterised by lower interferon-α and interferon-γ responses to virus and mitogens respectively, relative anergy to tuberculin skin testing, depressed *in vitro* mononuclear cell proliferative responses to purified protein derivative, and neutrophil leucocytosis. Two patients, both alcoholic male Caucasians with extensive pulmonary disease, developed acute respiratory failure within two weeks of starting chemotherapy. One of them died. In all other patients, the initially abnormal measurements returned to normal limits for controls during chemotherapy, except that protein malnutrition persisted

for at least as long as the nine-month regimen of treatment.

It is proposed that patients be categorised into five groups according to their reactivity to tuberculin-purified protein derivative in the accelerated and delayed skin tests, mononuclear cell proliferation and lymphokine (Interferon-γ) production assays. Responses to purified protein derivative were graduated and ranged from a state of hyperesensitivity (Group 1) to one of anergy (Group 5) in a continuous downward gradation. These immunological patterns showed only a weak association with nutritional status, but no association with age, radiological extent or duration of disease, serum antibodies, cell counts or erythrocyte sedimentation rate.

These studies indicate a spectrum of immunological responses in human tuberculosis, analogous to leprosy, but exclusive of a form equivalent to chronic lepromatous leprosy.

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Certification by Supervisor

The initial idea of this thesis and its development originated from the author. The clinical and laboratory work described in it was carried out personally by Dr James Onwubalili, and materials recorded therein resulted from research carried out by him in the Division of Communicable Diseases, Clinical Research Centre, Harrow, under supervision by Dr D A J Tyrrell and Dr G M Scott.

Assistance was given as follows. Haematological tests were performed by Mrs Linda Vass and Ms Wendy Wood of the Haematology Department. Biochemical measurements were carried out in Clinical Chemistry, serum immunoglobulin assays in the Division of Immunological Medicine, and radioactive counting in the Radioisotopes Division of Northwick Park Hospital and the Clinical Research Centre. The author separated and stained the cells for fluorescence-activated cell sorting, but their enumeration was performed by Dr Andrew Edwards of the Division of Immunological Medicine. Interferon-α and -γ assays were carried out jointly by Dr G M Scott, Ms J A Robinson and the author.

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Abbreviations, Glossary and Definitions

AMC - mid-triceps arm muscle circumference

AMP - adenosine monophosphate

B-cell - bone marrow-derived lymphocyte

BCG - bacille de Calmette et Guerin

CMI - cell-mediated immunity

CPM - counts per minute

DTH - delayed-type hypersensitivity

DMSO - dimethyl sulphoxide

E-rosetting - forming rosettes with sheep erythrocytes

EAC-rosetting - forming rosettes with sheep erythrocytes coated with antibody and complement

ELISA - enzyme-linked immunosorbent assay

ESR - erythrocyte sedimentation rate

FCS - fetal calf serum

FITC - fluorescein isothiocyanate

Hb - haemoglobin

HLA - human leucocyte antigen

H_2O_2 - hydrogen peroxide

id - intradermal

IFN - interferon

MAF - macrophage activating factor

MCF - macrophage chemotactic factor

MIF - macrophage migration inhibitory factor

MRC - Medical Research Council (Great Britain)

NDV - Newcastle Disease Virus

NK - natural killer

PBMC - peripheral blood mononuclear cells

PBS - phosphate-buffered saline

PPD - purified protein derivative of *M. tuberculosis*

RPMI - Ros well Park Memorial Institute

RUL - right upper lobe (of lung)

SD - standard deviation

SEA - Staphylococcal Enterotoxin A

SFT - mid-triceps skinfold thickness

T-cell - thymus-derived lymphocyte

T_{CTL} - cytotoxic T-lymphocyte

T_{DH} - delayed hypersensitivity T-lymphocyte

T_H - helper T-lymphocyte

T_M - memory T-lymphocyte

T_s - suppressor T-lymphocyte

TB - tuberculosis

TIBC - total iron binding capacity

TPA - tetrahydrophorbyl acetate

TU - tuberculin units

UK - United Kingdon

W/H^2 - weight-for-height squared

WBC - white blood cells

CHAPTER ONE - INTRODUCTION

Robert Koch's discovery and study of Mycobacterium tuberculosis (Koch, 1882) established the causative agent of tuberculosis, led to the development of an effective vaccine (bacille de Calmette et Guerin, BCG), suggested the use of tuberculin as a diagnostic tool and laid the foundations of modern immunology. Furthermore, it provided the rational basis for the development of a cure for tuberculosis: from the days of tuberculin therapy, gold therapy, sanatorium isolation and artificial pneumothoraces, until the advent of effective chemotherapy was signalled by the discovery of streptomycin in 1944.

One hundred and two years have passed since Koch's discovery, yet tuberculosis remains a major public health problem throughout the world. Its global prevalence is currently estimated to be 15-20 million cases, with an annual incidence of about 10 million and at least three million deaths each year (WHO Memorandum, 1982). Although its incidence has declined considerably in economically advanced countries, tuberculosis has not been eradicated. In 1980 almost 30,000 new cases were reported to the United States Centers for Disease Control (Morbidity Mortality Weekly Report, 1982) and there were approximately 7,000 cases in the United Kingdom (McNicol, 1983) with a surprising mortality of almost 10% in each country.

Since infection with M. tuberculosis is transmitted from man to man with no important animal and environmental reservoirs, and drug therapy is rapidly effective at reducing infectivity, control of tuberculosis appears to be a reasonable goal. However, tuberculosis remains a major public health problem because of two fundamental practical problems : finding the cases, and applying treatment. Many of the obstacles to progress in the Third World are cultural, political and economic, rather than simply medical. Childhood immunization with BCG is still not universal practice, and the protective effect of this vaccine has been shown to be highly variable (Luelmo, 1982). High prevalence of infectious disease with overcrowding, poverty and malnutrition predispose the young child to early infection. Most infectious persons remain undiscovered, and many who are discovered remain infectious owing to the lack of or inefficient use of highly effective drugs. So much chemotherapy is still required reliably to achieve sterilization of a tuberculous infection - a minimum of six months' treatment even with the most potent rifampicin- and pyrazinamide-containing regimens (Mitchison, 1980; Fox, 1981) - that it is beyond the available financial resources on the scale needed in the countries most affected.

In the United Kingdom (UK) and other developed countries, reactivation of infection after many years of dormancy in the tissues, and late diagnosis, still constitute major problems (Fox, 1981). However, poor patient compliance has been and remains the principal cause of treatment failure

worldwide (Fox, 1983). The development of therapeutic strategies that would further reduce the length of treatment and also eliminate dormant organisms would therefore be a major advance towards the eradication of tuberculosis.

In pursuit of this goal, the use of an immunopharmacological agent as a possible adjunct to chemotherapy has recently aroused interest (Chan, Lee and Simone, 1976; Yaseen, Thowaini, Al-Tawil and Jazrawi, 1980; Singh, Kumar, Malaviya and Kumar, 1981; Abstracts of the International Society for Immunopharmacology, 1982). However, the deficiencies in our knowledge of the host-parasite relationship and the mechanisms of immunity in tuberculosis have hindered progress in this important area. These aspects of the infection must be better understood before an improved therapeutic strategy can be evolved. The need to expand immunological research and knowledge on tuberculosis using modern techniques was highlighted in a recent WHO Memorandum (1982). Discussion leading to that document was instigated by the magnitude of the problem of tuberculosis and uncertainty as to the efficacy of current control measures, in the light of the apparent failure of BCG vaccination in the largest-ever field trial in South India (WHO Memorandum, 1979).

For example, we still do not fully understand the mechanisms of killing or inhibition of M. tuberculosis, the mechanism of bacillary persistence in tissues during and after "adequate" chemotherapy, and what causes subclinical

infection in some individuals to change into overt disease. The complexity of lymphocyte-macrophage interactions in cell-mediated immunity is now becoming understood, but it is not yet known which antigens, T-cells, lymphokines or macrophage antimicrobial mechanisms are most critical for antimycobacterial immunity.

Although some studies have been done, the changes that occur in cell-mediated and humoral immunity during the course of tuberculosis and its treatment have not been fully investigated. In this respect, the occurrence of drug-unrelated "reactions", sometimes fatal, during chemotherapy has recently attracted attention (Ellis and Webb, 1983). Physicians with experience in treating tuberculosis are aware of such unexplained phenomena as fever, gland enlargement or tenderness, new lesions (e.g. choroid and brain tubercles), ureteric obstruction, acute psychosis and respiratory distress following the initiation of drug treatment. It is possible that these represent increased delayed hypersensitivity reactions to masses of dead bacilli, but this pattern of response to treatment is by no means consistent. The host-parasite relationship appears to vary widely from one individual to another, and this variation is evident in all aspects of the disease - clinical presentation, severity of disease, and response to treatment. In the pre-antibiotic era, the mortality from overt tuberculosis was estimated at 50%. In addition, the response

to primary infection is rarely fatal; whereas some patients develop fulminant miliary disease, others have short-lived focal lesions.

In the UK, tuberculosis now occurs predominantly among immigrants of Indian Subcontinent (ISC) origin. They have more pulmonary and extrapulmonary disease, commonly caused by relatively less virulent strains of M. tuberculosis (Subbaiah, Mitchison and Selkon, 1960; Mitchison, Selkon and Lloyd, 1963; Bates and Mitchison, 1969; Grange, Abor, Allon, Mitchison, Mikhail, McSwiggan and Collins, 1977).

The clinical spectrum observed in tuberculosis would suggest that there is a spectrum of immunological responses to the disease, analogous to that described in leprosy (Ridley and Joplin 1966), which is not dependent on variablility in the organism, but on the variability of the host's immune response. In this study, I have set out to investigate and define this immunological spectrum in a group of patients with active tuberculosis. However, in order to begin to comprehend the pathological consequences of infection with M. tuberculosis it is essential to consider the nature of the bacterium and its components, the manner in which they evoke their effects, and the nature of the host's defensive response against this agent.

A. The paraeite

1. Impermeable cell wall

M. tuberculosis possesses a highly complex lipoidal cell wall which renders the organism impermeable, protecting it from drugs, humoral bactericidins and other adverse agencies found in phagocytes (Goren and Brennan, 1979). The mycobacterial cell wall is difficult for macrophages to digest and eliminate, thus cell wall antigens persist in the infected cells long after the bacilli are dead (Kriog and Meyers, 1979). The cell wall components account for its acid-alcohol fastness, and contribute to other properties discussed below.

2. Virulence

Immunologically activated macrophages are the major cells involved in control of the spread of M. tuberculosis (Lurie, 1964). Although rapidly phagocytosed by macrophages, virulent bacilli are able to replicate within these cells or to persist in a dormant state for prolonged periods (Fenner, Martin and Pierce, 1949). The subcellular mechanisms that enable tubercle bacilli to resist intracellular killing and survive in the otherwise destructive environment of the macrophage are not completely understood, but must involve subversion of the normal killing mechanisms of this cell. Several virulence factors have been described.

a) Prevention of phagosome-lysosome fusion

Normally, micro-organisms are ingested by phagocytes to form phagosomes; enzyme-rich cytoplasmic granules (lysosomes) then fuse with phagosomes and release their enzymes to digest the micro-organisms. Following phagocytosis by macrophages, tubercle bacilli avoid exposure to lysosomal digestive enzymes by inhibiting phagosome-lysosome fusion (Armstrong and Hart, 1971; Hart, Armstrong, Brown and Draper, 1972; Lowrie, Aber and Jackett, 1979). However, when the bacilli are dead, or are coated with antibody, the phagosomes and lysosomes fuse without difficulty, suggesting that viable tubercle bacilli produce a substance that inhibits fusion (Armstrong and Hart, 1975; Lowrie, Jackett, Aber and Carrol, 1980). Attention has recently focussed on four possible candidate products:

(1) Sulphatides:

Sulphated glycolipids of M. tuberculosis applied either as emulsion, or coated on ingested yeasts, prevent phagosome-lysosome fusion in cultured macrophages (Goren, Hart, Young and Armstrong, 1976). Virulent strains of M. tuberculosis produce more sulphatides in their cell wall than attenuated strains, and elaboration of sulphatides in culture correlates with relative order of virulence for many strains (Goren, Brockl and Schaefer, 1974: Grange, Aber, Allen, Mitchison and Goren, 1978). This suggests that cell wall sulphatides may promote intracellular survival and growth by antagonizing phagosome-lysosome membrane fusion,

but it is not clear why dead M. tuberculosis which have not lost their sulphatide, do not inhibit fusion.

(ii) Cyclic AMP:

Cyclic adenosine 3':5'monophosphate is known to inhibit phagosome-lysosome fusion in phagocytic cells (Weismann, Goldstein, Hoffstein, Chauvet and Robineaux, 1975). Lowrie and associates (1979) showed that infection of macrophages with live M. microti and M. bovis BCG is associated with an elevation of cyclic AMP levels and inhibition of phagosome-lysosome fusion. Dead bacteria, inert particles and live M. lepraemurium, and Salmonella typhimurium do not elevate cyclic AMP levels nor inhibit fusion (Carrol, Jackett, Aber and Lowrie, 1979). It is not known whether cyclic AMP found in infected macrophages is synthesised by the bacteria, the macrophages or both, but these observations suggest that fusion inhibition in tuberculous mycobacterial infection may be mediated by cyclic AMP.

(iii) Polyglutamic acids:

Polyglutamic acids are also inhibitors of phagosome-lysosome fusion and are produced in cell walls of virulent M. tuberculosis (Goren et al, 1976).

(iv) Ammonia:

Ammonia is another known inhibitor of phagosome-lysosome fusion (Gordon, Hart and Young, 1980) produced by M. tuberculosis in culture media, and possibly in

macrophages.

Despite these observations, it is not known whether avoidance of lysosomal enzymes by inhibiting fusion is an important phenomenon since promoting fusion in vitro has inconsistent antimycobacterial effects, and it is not yet established if or to what extent fusion is actually inhibited in vivo (Lowrie, 1983).

b) Resistance to lysosomal enzymes

M. tuberculosis can resist digestion by lysosomal enzymes. When antibody-coated tubercle bacilli are phagocytized by mouse peritoneal macrophages, phagosome-lysosome fusion occurs, but the bacilli remain viable within the phagosome-lysosomes (Armstrong and Hart, 1975). The basis for this resistance may be that the cell wall glycolipid C-mycoside forms a chemical shield around the organism, as suggested for *M. lepra* (Draper, 1982).

c) Resistance to hydrogen peroxide

The nature of the killing mechanism within the phagosome-lysosome is unknown; however, there are indications that hydrogen peroxide (H_2O_2) and other oxygen-dependent radicals are involved. The myeloperoxidase-halide system is the most effective enzyme system known to kill M. tuberculosis in human cells (Jackett, Aber and Lowrie, 1978). H_2O_2 is produced by activated macrophages, and resistance to H_2O_2 is a virulence determinant for M. tuberculosis (Mitchison et al, 1963).

When guinea pigs are infected with isoniazid-resistant, H_2O_2-susceptible mutants, the mutants are killed or inhibited more than their parent strains (Jackett, Aber, Mitchison and Lowrie, 1981). This observation suggests that H_2O_2 is generated in significant amounts in the environment of the tubercle bacilli within tissues. Because the mutants are killed more effectively in vaccinated animals than in non-vaccinated controls, perhaps H_2O_2 is generated in greater amounts in acquired immunity. The peroxidase content of macrophages is relatively low, but Jackett and co-workers (1980) have shown that catalase can replace peroxidase and kill tubercle bacilli in vitro.

Nevertheless, it is unlikely that macrophage peroxide alone is a comprehensive explanation for defence against M. tuberculosis (Lowrie, 1983). Such an antimicrobial system is very oxygen-dependent and is probably ineffective within the closed granulomata that develop in tuberculosis. Indeed, the development of tuberculous infection is impaired under conditions where oxygen availability is restricted (Chandler, Allison, Margolis and Gerszten, 1965), and it is unlikely that this can be accounted for entirely by the dependence of tubercle bacillus metabolism on oxygen (Lowrie, 1983).

d) The toxic cord factor

Koch first observed that M. tuberculosis grows in culture in the form of serpentine cords which consist of bacilli in close parallel arrangement. Cording is now known to be a property restricted to virulent strains, caused by a

cell wall mycolic acid ester of trehaloee (Block, 1950) which is highly toxic for mouse liver mitochondria (Kato, 1968) and possibly for human macrophages.

3. Adjuvant activity

Mycobacteria greatly potentiate the immune response to unrelated antigens administered simultaneously. The observation that organisms presented in a water-in-oil emulsion can evoke a far greater adjuvant response than the same quantity of organisms in saline led Julee Freund (1956) to develop the adjuvant composition that bears his name. Freund's complete adjuvant is an emulsion of mineral oil in water into which killed M. tuberculosis is incorporated. Intact mycobacterial cells and several cell wall components possess adjuvant activity. These include peptidoglycolipids (Waxes D), muramyl dipeptides, cord factor, and water-soluble adjuvants (Goren and Brennan, 1979).

There is little agreement about the way in which adjuvants potentiate the immune response. Various mechanisms suggested include formation of a slow-release antigen-depot at the injection site (Freund, 1956), action on lysosomal membranes (Spitznagel and Allison, 1970), provision of nucleic acids for dividing lymphocytes (Schmidtke and Johnson, 1971) and lymphocyte trapping at the injection site (Frost and Lance, 1973). Rook (1977) postulated that adjuvant activity may be mediated through a non-specific mitogenic effect on lymphocytes, especially B cells, and may represent a primitive recognition system, present before the

evolution of the specific immune system, which could respond to certain common bacterial components.

4. Immunogenicity, granuloma formation, macrophage activation

Various mycobacterial components (a ribosomal fraction, an RNA fraction and viable whole cells) are potent stimulators (immunogens) of a specific immune response of the host against the organism (Youmans, 1979). Mycobacterial components also stimulate the accumulation of macrophages around the organism (granulomatous response) and their activation. The induction of specific immunity , granuloma formation and macrophage activation constitute the major responses that are concerned with resistance to tuberculous infection in man (Youmans, 1978). The mechanisms and consequences of these protective responses are explained later in this chapter.

5. Suppressor substances

Two cell wall polysaccharides of M. tuberculosis have been shown to suppress immunologic responses in vitro - arabinomannan (Ellner and Daniel, 1979) and arabinogalactan (Kleinhenz, Ellner and Daniel, 1979). It is possible that these substances play a role in the genesis of tuberculin anergy often seen in severe progressive tuberculosis.

B. The host

Successful resistance in tuberculosis is determined by the balance between various virulence factors of the organism discussed above, and the competence of the immune response of the host. Thus, if the host response is adequate the organism is successfully resisted and contained. Inadequate host response allows unrestricted bacillary proliferation with production of a large quantity of toxic components. These may induce a high level of hypersensitivity and tissue damage. Continued bacillary replication will eventually induce suppressor cells and anergy which are associated with a poor prognosis (Chaparas, 1982). The mechanisms of host resistance, and various host factors that affect prognosis, will now be discussed.

1. Natural history

The natural history of tuberculosis is now well understood. The development of tuberculosis is a two-stage process: first, an infection must become established, and secondly, that infection must progress to active disease. Therefore, the risk of developing tuberculosis depends on two separable sets of risks: the risk of infection, and the risk of developing disease after infection (Comstock, 1975). This distinction is important because tuberculosis differs from most other bacterial diseases in one respect: the resistance which develops after successful recovery from primary infection is usually not capable of eradicating all the organisms. As a consequence, an unknown but significant

proportion of infected persons (perhaps all tuberculin reactors) remain at risk of reactivation for the rest of their lives. Although this risk is highest shortly after primary infection, the subsequent lifetime risk remains great because of the net effect of a low risk operating over a long period. This means that the incubation period of tuberculosis varies from a few weeks to a lifetime, in marked contrast to most other communicable diseases where the incubation period is fixed within a relatively short and discrete interval after exposure to the agent. Both stages of tuberculosis can be accurately identified - the acquisition of infection by tuberculin testing, and the development of active disease by bacteriologic studies.

a) Risk of infection

The risk factors for primary infection with M. tuberculosis are mainly extrinsic - principally related to the probability of contact with an infectious case, the duration and intensity of exposure, the likelihood of airborne transmission, and the degree of infectiousness of that case. These determinants are intimately associated with concomitants of low socioeconomic status and poverty, such as overcrowding, inadequate ventilation and hygiene, and malnutrition which correlate with a high prevalence of infectious cases in the community. By far the most important determinant of whether or not a contact becomes infected is severity of disease and sputum positivity of the source case (Chan and Dyerly, 1964). Degree of crowding, measured as

persons per room, and intimacy of exposure are also important, but family income *per se* appears to be unimportant. Factors determining infectiousness are the number of organisms expectorated in the sputum, frequency of cough, and extent of disease (Loudon and Spohn, 1969). On the basis of tuberculin skin reactivity, about 50% of the world's population has had primary infection (Daniel, 1982). In countries with a high prevalence of tuberculosis the uninfected population is mainly the young, while in low prevalence countries most people of all ages remain uninfected. It is estimated that between five and 15% of infected persons subsequently develop overt tuberculosis (Comstock and Edwards, 1975).

The institution of adequate chemotherapy renders patients non-infectious very quickly (Rouillon, Perdrizet and Parrot, 1976), much sooner than it takes for sputum to convert from positive to negative by staining or culture. Indeed, chemotherapy is as effective as hospital isolation in breaking infectious contact. This was convincingly demonstrated by the Madras Trial of hospital versus outpatient treatment of infectious tuberculosis, in which no evidence could be found of an increased attack rate among household contacts of patients whose entire treatment was given at home (Kamat, Dawson, Dovadatta, Fox, Janardhanam, Radhakrishna, Ramakrishnan, Somasundaram, Stott and Velu, 1966). The principal finding of this and other studies of infectiousness of tuberculosis in relation to treatment was that family contacts were exposed and infected before

diagnosis of the index case, and that the subsequent risk was miniscule. This has been taken to mean that the index cases were no longer infectious after initiation of chemotherapy. However, it should be considered that they may still be infectious to new uninfected contacts albeit for a short period. That treated patients cease to be infectious before viable tubercle bacilli cease to appear in sputum can be related to a number of factors: increased concentration of drugs on tubercle bacilli as droplet nuclei evaporate, diminution in cough frequency (Sultan, Nyka, Mills, O'Grady, Wells and Riley, 1960; Loudon and Spohn, 1969), and the relatively low infectiousness of tuberculosis (Comstock, 1975).

b) Risk of disease after infection

Once primary infection with M. tuberculosis has occurred, the probability of developing manifest disease varies greatly among persons and groups of persons. The risk factors for developing disease after primary infection are intrinsic: age, sex, genetic susceptibility, body build, and host resistance (Comstock, 1980).

(i) Age and sex:

The incidence of tuberculosis varies with age and sex depending on whether the disease occurs in high- or low-prevalence populations. In high-prevalence populations there is a high incidence of primary disease in infancy and early childhood, followed by a second peak (presumably of

reactivation tuberculosis) in late adolescence and early adult life (Comstock, Livesay and Woolpert, 1974). The first peak reflects unavoidable close contact with infectious family members and is usually attributed to an inability of infants to resist tuberculosis owing to immunological immaturity, an opinion strengthened by the high frequency of haematogenous dissemination at this age. However, since the risk of tuberculosis is greatest during the first year following infection, regardless of age (Comstock et al, 1974), the high incidence in infants may not be attributable to infancy per se, but to the fact that infections at this age must be recent by definition (Comstock, 1975).

Tuberculosis of adolescence and young adulthood is more common in females (Comstock, Forebes and Hammes, 1967). In low-prevalence populations the incidence of tuberculosis increases with advancing age, and it is tempting to attribute this to a progressive loss of immunological responsiveness. Although there is some evidence for a progressive decline of lymphocyte reactivity during aging (Wekster and Hutteroth, 1974), there is also evidence to suggest that cell-mediated immunological memory remains intact (Waldorf, Willkens and Decker, 1968). It may seem presumptuous, therefore, to attribute the higher incidence of tuberculosis in the aged solely to loss of immunological competence. Disease in the older population may represent late endogenous reactivation of primary infections acquired in the 1920s and 1930s when tuberculosis was common in developed countries (Frost, 1939; Mitchell, 1963).

(11) Genetic markers:

Genetic variation in susceptibility to tuberculosis has been most convincingly documented in rabbits, in which low- and high-resistance lines have been bred (Lurie, 1964). Although it is more difficult to establish in man because of the importance of environmental influences, evidence from studies of community groups (Large, 1964) and twins (Kallman and Reisner, 1943; Comstock, 1978) strongly support a role for genetic factors in the susceptibility of humans to tuberculosis.

There is growing evidence for Human Leucocyte Antigen (HLA) control of resistance and susceptibility to tuberculosis. Various groups of workers have found associations between susceptibility to tuberculosis and HLA-B8 (Selby, Barnard, Buehler, Crumley, Larsen and Marshall, 1978), HLA BW15 (Al Arif, Goldstein, Affronti and Janicki, 1979), HLA-BW49 (Chukanova, 1981; Khomenko, 1981), HLA-DR2 (Singh, Mehra, Dingley, Pande and Vaidya, 1983) and HLA-BW35 (Jiang, An, Sun, Mittal and Lee, 1983). Moreover, 87 individuals are hyporesponsive to tuberculin skin testing after BCG treatment of malignant melanoma (Buckley, White and Siegler, 1977). However, as is usual in this field, other workers (Rosenthal, Scholtz, Klimmek, Albert and Blaha, 1973; Cox, Arnold, Cook and Lundberg, 1982) were unable to find any significant differences in HLA phenotype frequency between patients with tuberculosis and healthy controls.

Evidence has accumulated for genetic control of the immune response of man to a number of other infectious agents, including *M. leprae* (DeVries, Nijenhuis, Lui, Fat and Vanrood, 1976), vaccinia virus (DeVries, Kreeftenberg, Loggen and Vanrood, 1977), streptococci (Greenberg, Gray and Yunis, 1975) and Clostridium tetani (Sasazuki, Kohno, Iwamoto, Tanimura and Naito, 1978).

Studies of ABO blood groups in tuberculosis have yielded conflicting results. Halber and Hirszfeld (1926) found no relationship, but Saha and co-workers (1968) and Jain (1970) reported increased resistance with group O, and increased susceptibility with group AB. Also, Berezovskii, Kogan and Mostovoy (1981) found an increased frequency of groups B and AB among patients with active disease. Further studies are needed to confirm these findings, but it is unlikely that HLA typing and blood grouping will prove valuable markers for diagnosis or for high risk.

Rich (1951) found evidence for a high resistance among Jews and a high susceptibility among Blacks. Comstock (1964) found similarly that tuberculosis of adolescence and young adulthood was more common among negroes than whites. In the UK, the average tuberculosis notification rate for subjects of Indian Subcontinent (ISC) origin was 35 times higher than that for the white population, and 12 times higher than for the West Indian population (MRC Report, 1982a). Also, children of ISC origin born in and resident in the UK were 20 times more likely to develop tuberculosis than white children

(MRC Report, 1982b). However, evidence for a racial or ethnic factor remains inconclusive, as it is often impossible to separate environmental factors. In none of the studies could a complete adjustment have been made for the effects of all the variables under investigation.

(iii) Body build:

There is indirect evidence to show that undernutrition favours reactivation of latent tuberculosis. Tuberculosis case rates among 28,478 tuberculin-positive US Navy recruits were three times higher among men who were at least 10% underweight for height than among those who were at least 10% overweight (Edwards, Livesay, Acquaviva and Palmer, 1971). A similar association of high risk with leanness as assessed by skin-fold thickness has been reported by Comstock (1975). Furthermore, the induction of marked weight loss following jejunoileal bypass operations for obesity appears to be the prerequisite for the increased risk for tuberculosis associated with this operation (Snider, 1982). It is probable that undernutrition causes both leanness and susceptibility to tuberculosis.

(iv) Host resistance:

For reasons not yet fully explained, the following situations increase the liability of the host for endogenous reactivation of tuberculosis: malnutrition, uraemia, labile diabetes (Boucot, Dillon and Cooper, 1952; Silver and Oscarsson, 1958), silicosis (James, 1954), leukaemia,

lymphomas, alcoholism, drug addiction, steroid therapy (Garland, 1951; Johnson and Davey, 1954) and immunosuppressive therapy (Lichtenstein and Macgregor, 1983). These are conditions associated with impairment of cell-mediated immunity, which predispose the host to endogenous 'reawakening' of dormant tubercle bacilli. Gastrointestinal resection is also reported to predispose to reactivation (Thorn and Brookes, 1956; Frucht, Kunkel and Spiro, 1957), but this has never been established in properly controlled trials. The risk may relate to leanness related to longstanding peptic ulceration or even concomitant alcoholism, rather than to gastric resection *per se* (Thorn and Brookes, 1956), but the evidence is inconclusive.

The vast majority of individuals with overt tuberculosis do not have any obvious predisposing cause. It is not clear what causes a quiescent primary focus to reactivate in five to 15% of all infected persons. The cause of disease reactivation probably lies in the complexity of interactions between the lymphocyte, lymphokine and macrophage - the three key effectors of host resistance - and the tubercle bacillus.

2. Response to infection

Koch showed that prior exposure of a susceptible animal to tubercle bacilli not only protected it from death but also rendered it hypersensitive to a second challenge with virulent tubercle bacilli. These early observations led to the development of BCG immunization and tuberculin testing for hypersensitivity, and stimulated further investigation

into the nature of the host response in tuberculosis. When a person is infected with virulent M. tuberculosis, the following immunological responses occur: First, ANTIBODIES are produced against a variety of mycobacterial antigens (Daniel and Janicki, 1978; Youmans, 1978). Secondly, HYPERSENSITIVITY to tuberculoproteins develops (Koch, 1882; Lester and Atwell, 1958). Thirdly, specific IMMUNITY to reinfection with M. tuberculosis is induced (Youmans, 1978).

a) Antibodies

Antibodies are produced by an infected host against various mycobacterial protein, lipid and polysaccharide antigens. The precise role of this antibody response in host resistance is unknown, but both positive and negative influences are possible. The finding that antibody-coated M. tuberculosis does not inhibit phagosome-lysosome fusion (Lowrie et al, 1979) suggests that opsonization of tubercle bacilli may render them incapable of avoiding exposure to lysosomal digestive enzymes of macrophages. On the other hand, antibodies may enhance tuberculous infection by interfering with T-cell function in the same way that antibodies can enhance tumours (Hoppner, 1972). Furthermore, Rees (1976) has suggested that antibodies (or immune complexes) may suppress lysosomal enzymes in phagocytic cells. However, the evidence in support of these hypotheses remains tenuous.

b) Delayed hypersensitivity

Infection with tubercle bacilli is invariably accompanied by a general state of tissue hypersensitivity to tuberculin, and the development of a positive tuberculin skin test represents one of the first indications of contact with thie organism (Lester and Atwell, 1958). Delayed-type hypersensitivity (DTH) is mediated by specifically sensitized T-lymphocytes which secrete lymphokines upon exposure to tubercle bacilli. Some lymphokines attract macrophages to the site and cause them to aggregate to form the characteristic induration palpable at 48 to 72 hours. Although first noted by Koch in 1881, the nature of the association between DTH and acquired immunity is still controversial. One view is that they are different expressions of the cellular immune response to M. tuberculosis, and the skin reaction is a peripheral manifestation of cellular events that control the infection (Mackaness, 1967). This view is supported by their temporal association and the fact that both are mediated by sensitized T-lymphocytes. Another view is that immunity and hypersensitivity are coincidental, and that far from being an expression of a common mechanism, DTH is destructive and detrimental to immunity (Rich, 1951). This is supported by the absence of a direct relationship between the two phenomena. However, DTH cannot always be measured accurately; a negative tuberculin skin test does not always denote its absence.

The tuberculin skin tests detect DTH and distinguish infected from non-infected persons. The Mantoux test is superior to multiple puncture devices because it is quantitative and therefore offers the opportunity for discrimination between sensitivity to M. tuberculosis and atypical mycobacteria. A positive result consists of a palpable induration measuring 6mm or more at 48 to 72 hours. following intradermal (id) injection of 0.1 ml of Purified Protein Derivative (PPD). Most patients give a reaction of 15 to 20 mm. but a few give reactions less than 10 mm or more than 25 mm. The test is not specific owing to cross-reactivity with non-tuberculous mycobacteria which occure to varying degrees in different parts of the world. In general, most small reactions (less than 6 mm) to 1 Tuberculin Unit (TU) of PPD are due to atypical mycobacterial infections (Chaparas, 1982).

c) Tuberculo-immunity

The most important immune response to infection with M. tuberculosis is a specific T-cell mediated immunity that involves sensitized T-cells, lymphokines and macrophages. The important non-specific defence mechanisms - macrophage activation and granuloma formation - act in concert with specific immunity to promote the resolution of a tuberculous infection. These three are the major responses of man that are concerned with host resistance to infection with M. tuberculosis (Youmans, 1979).

(1) Specific T-cell immunity:

Early workers observed that, unlike other infections such as diphtheria, passive immunization with convalescent serum did not protect against tuberculosis (Baldwin, Kinghorn and Allon, 1905). This observation led to the concept of cell-mediated immunity (CMI), which postulated that immunity was vested in phagocytic cells rather than serum. The first illustration of this fact came from Lurie (1942) who showed that mononuclear phagocytes from immunized animals inhibited intracellular multiplication of virulent tubercle bacilli compared to macrophages from non-immunized animals. Immune serum added to immune-activated macrophages yielded results similar to normal serum added to immune macrophages, suggesting that protection was mediated by immune macrophages. These findings were later confirmed by Suter (1953) and Mackaness (1954). In his elegant transfer experiments Mackaness (1964) subsequently showed that immune lymphocytes - but not macrophages or serum - from a tuberculous mouse could protect a normal recipient against infection with tubercle bacilli given alone or with listeria, but not with infection against listeria given alone (Fig. 1). Thus, the triggering of a specific secondary immune response to the tubercle bacillus endowed the animal with a simultaneous but transient non-specific resistance to listeria, another unrelated obligate intracellular parasite. There is ~~co-pelling~~ evidence that these lymphocytes are T-cells. They are sensitive to anti-Thy 1 antibodies and complement (Lefford, McGregor and Mackaness, 1973), and

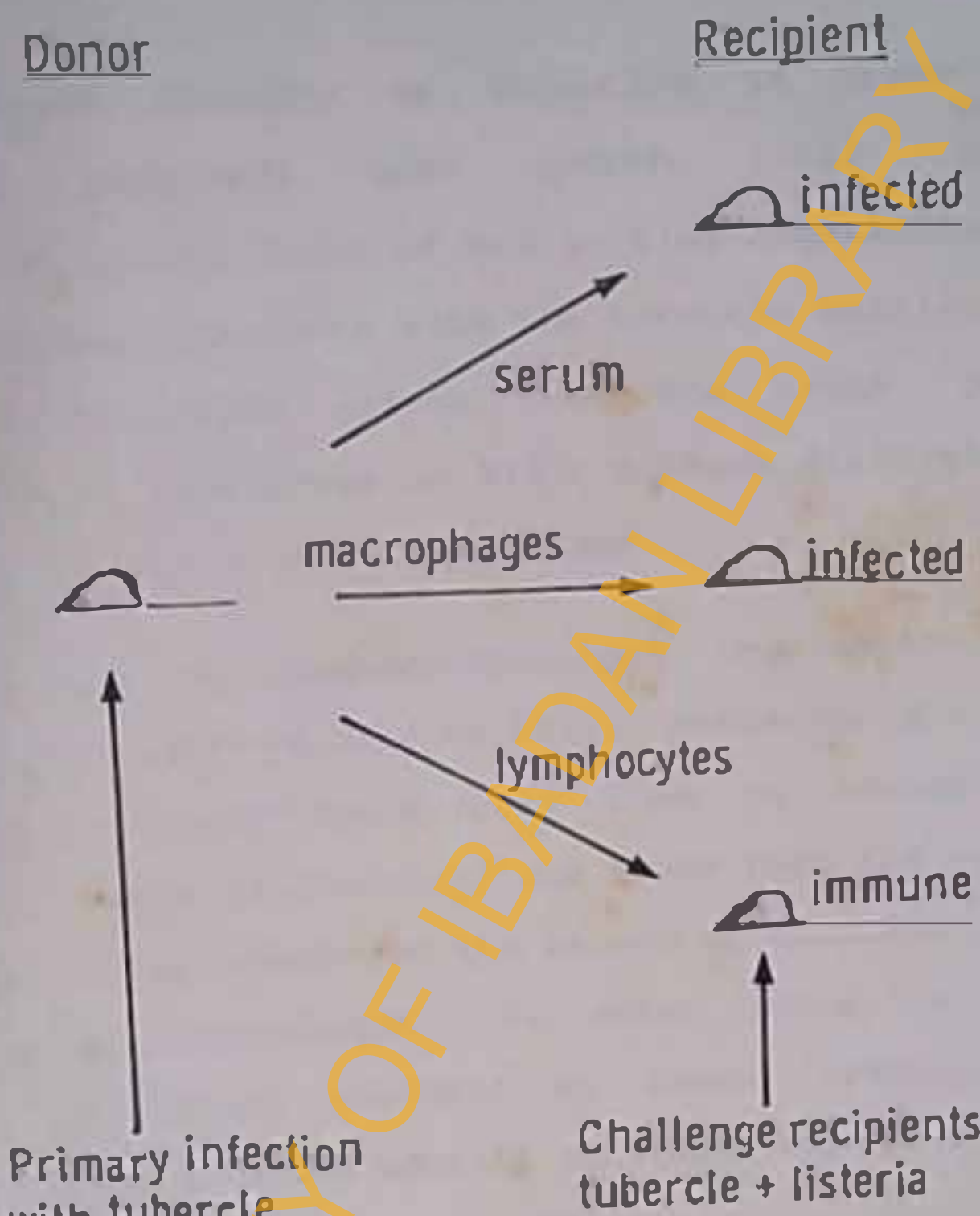

Fig 1: Transfer of specific and non-specific immunity in tuberculosis (Mackaness, 1964). Lymphocytes - but not macrophages or serum - from a mouse infected with tubercle bacilli protected a non-immune recipient against tubercle bacilli and listeria organisms given simultaneously. The recipient was not immune to listeria given alone (courtesy of Professor I. Roitt).

anti-tuberculosis immunity is defective in thymectomized (T-lymphocyte deprived) mice (North, 1973). Specific protection lies at the level of the initial interaction of a specific T-lymphocyte clone with the tubercle bacillus; the non-specific protection arises from the newly acquired ability of the macrophage to kill, without discrimination, almost any organism it has phagocytised.

But how does the lymphocyte/antigen interaction confer on the macrophage the ability to kill? Patterson and Youmans (1970) cultured splenic lymphocytes from an immune animal with viable tubercle bacilli, and found that the cell-free culture supernatant inhibited the growth of tubercle bacilli within normal macrophages. In other words, a soluble mediator (lymphokine) produced by immune lymphocytes on stimulation with tubercle bacilli activates the macrophage to become non-specifically bactericidal.

A hypothesis for the sequence of events in the expression of specific immunity in tuberculosis is now accepted (Youmans, 1979) (Fig. 2). Inhaled bacilli are transported via alveolar lymphatics to regional lymph nodes, the spleen and other lymphoid organs. Antigen-presenting cells, including dendritic macrophages, phagocytise, process and present the antigen (tubercle bacilli) using their immune associated (Ia) surface antigen, to immunocompetent B- and T-lymphocytes (Rosenthal, Lipsky and Shevach, 1975) which initiate humoral and cell-mediated responses respectively. B-cells produce mycobacterial antibodies, sometimes with T-cell help

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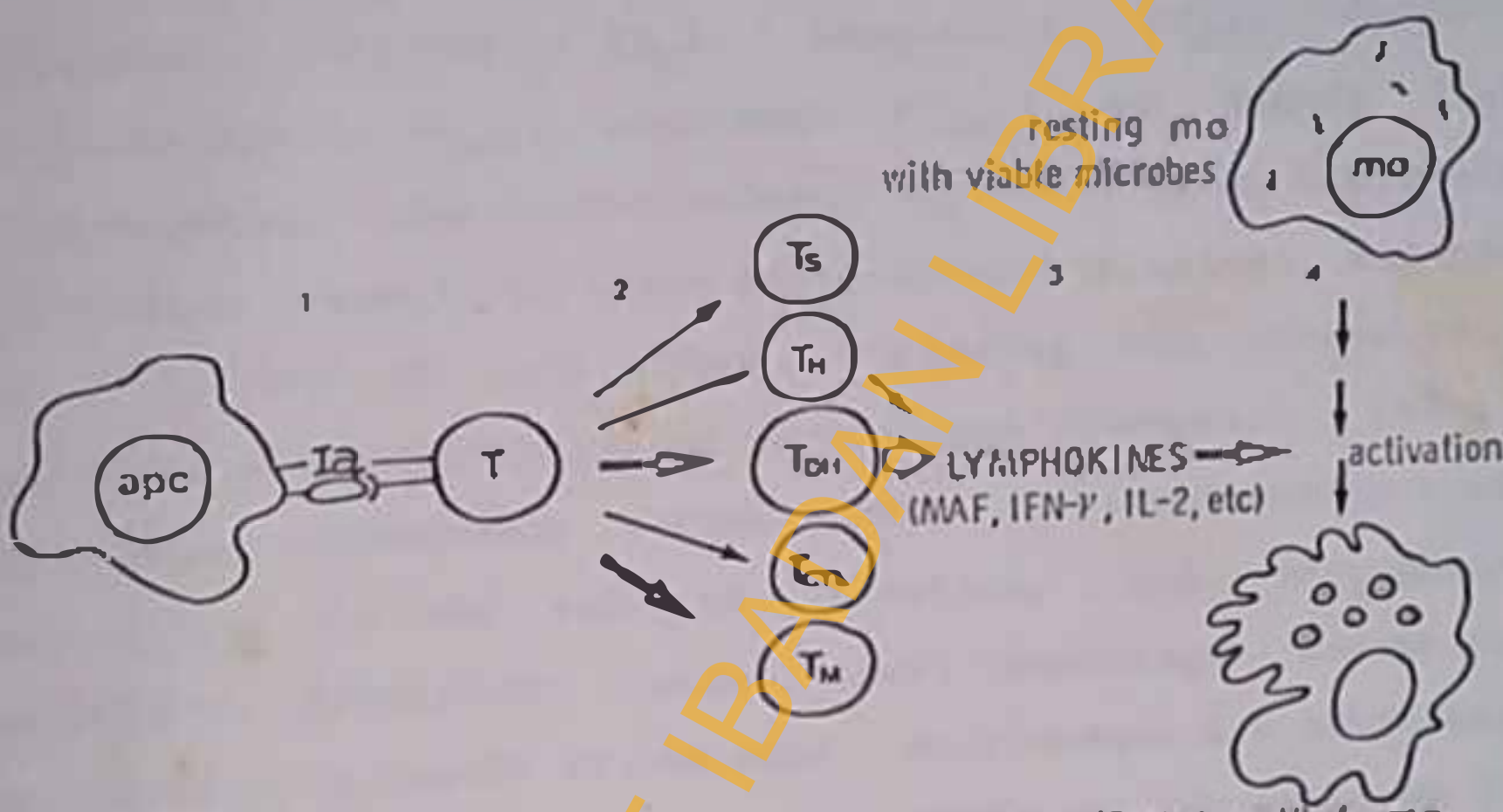

Fig 2: The pathway of cell-mediated immunity to *M. tuberculosis*. Antigen-stimulated T-cells(1) differentiate into effectors(2) some of which release soluble mediators(3). These lymphokines cause activation of macrophages (mø)(4) which develop the ability to inhibit the organism (apc = antigen-presenting cell; Ia = immuno-associated antigen; T_S, T_H, T_{DH}, T_{CTL} and T_M = suppressor, helper, delayed hypersensitivity, cytotoxic and memory T-cells; MAF = macrophage activating factor; IFN-γ = Interferon-gamma; IL-2 = Interleukin-2).

(T-dependent). In turn, T-cells differentiate into specialised effector cells which are named according to their functions: helper (T_H), suppressor (T_S), delayed hypersensitivity (T_{DH}), cytotoxic (T_{CTL}) and memory (T_M) T-lymphocytes. Antigen-stimulated T_{DH} cells (phenotypically T_H) secrete lymphokines which have marked biological effects on a variety of cell types, including the attraction, aggregation and activation of macrophages (Campbell, 1976). Macrophage chemotactic factor (MCF) initiates movement of macrophages to the site of infection, and macrophage migration inhibitory factor (MIF) encourages migrating macrophages to remain at the site. Macrophages are activated by macrophage activating factor (MAF) and immune (type 2) interferon (IFN-γ). Activated B-cells may also produce lymphokines (Yoshida, Sonozaki and Cohen, 1973; Rocklin, MacDermott, Chess, Schloseman and David, 1974; Mackler, Altman, Rosenstreich and Oppenheim, 1974; Wahl, Wilton, Rosenstreich and Oppenheim, 1975). Although *in vitro* assays exist for some of these biological activities, individual lymphokines are notoriously difficult to isolate; therefore it is often not clear whether an observed effect is caused by one or a mixture of these substances.

(ii) Macrophage activation:

There is evidence that macrophages exposed *in vivo* to virulent tubercle bacilli become activated, and acquire a greater capacity to destroy or limit the growth of mycobacteria and other unrelated organisms (Dannenberg, 1968;

Dannenberg, Ando and Shima 1972; Walker and Lowrie, 1981). Macrophage activation or "arming" involves a variety of morphologic and metabolic changes which lead to increases in surface adherence (Nathan, Karnovsky and David, 1971), ruffled membrane movement (Nathan et al, 1971), membrane adenylate cyclase activity (Remold-O'Donnell and Remold, 1974), phagocytic activity (Nathan et al, 1971), pinocytic activity (Meade, Lachmann and Brenner, 1974), hexose monophosphate oxidation (Nathan et al, 1971), protein synthesis (Pantalone and Page, 1977), collagenase secretion (Wahl, Wahl, Mergerhagen and Martin, 1975), complement C2 production (Littman and Ruddy, 1977), lysosomal enzyme activity (Pantalone and Page, 1977), tumoricidal (Piessens, Churchill and David, 1975) and microbicidal (Patterson and Youmans, 1970) capacity. Two microbicidal mechanisms are "switched on". Oxygen-dependent mechanisms lead to generation of superoxide, peroxide, hydroxyl and singlet oxygen radicals, while oxygen-independent agencies involve lysozyme, acid hydrolases and cationic proteases (Babior, 1978; Klebanoff, 1980; Nathan, 1983,). Macrophages are activated either immunologically through lymphokines, or directly by bacterial exotoxin, inert materials and a variety of mycobacterial cell wall substances (Youmans, 1979). Although immune-activated macrophages have the capacity to kill mycobacteria (Walker and Lowrie, 1981), and it is probable that they do so in vivo, it is as yet unproven. Patterson and colleagues (1970) have suggested that in vivo, activated macrophages at best achieve bacteriostasis rather

than killing, although numerous tubercle bacilli die. This view would explain the chronic nature of tuberculous infection, and bacillary persistence after suppression of active clinical infection by drug therapy (Youmans, 1978). Youmans (1978) postulated that the major function of chemotherapy is to allow the diseased host time to develop a potent and effective specific immunity that effectively retards the multiplication of the invading microorganism.

(iii) Granuloma formation:

The accumulation of macrophages at the site of infection is a characteristic feature of tuberculosis. The granulomatous response is stimulated either directly by granulomagenic cell-wall substances of tubercle bacilli or immunologically by lymphokines as already explained. T-cells and macrophages predominate, although B cells and neutrophils may also be present. Macrophages continue their usual affector and effector roles, and activated macrophages may transform into epithelioid and giant cells. High macrophage toxicity, caused by tubercle bacilli, results in a "high-turnover granuloma" in which the high death rate is compensated by macrophage proliferation and recruitment from blood monocytes. Dannenberg, Ando, Shima and Tsuda (1975) showed that the young macrophages within the periphery of a granuloma meet one of three possible fates: they are killed upon ingestion of tubercle bacilli and deposited in the caseous core, or they leave the granuloma via the lymphatics, or they become activated by lymphokines and by ingestion and

digestion of necrotic material. Prevailing adverse conditions in the granuloma, namely low pH, poor supply of oxygen and nutrients, and the activity of activated macrophages may be sufficient to bring about resolution of the infection.

C. Immunological profile in tuberculosis

1) Humoral

Patients with tuberculosis usually have significant polyclonal hypergammaglobulinaemia most marked in the IgG and IgA classes (Faulkner, Carpenter and Patnode, 1967; Buckley and Dorsey, 1970; Grange, Gibson and Nasseau, 1980). This probably represents a non-specific stimulation of immunoglobulin synthesis (Motieka, 1974) caused by the mitogenic activity of circulating tuberculin-like proteins produced *in vivo* by viable M. tuberculosis. Tuberculin-PPD has been shown to be a B-cell mitogen in mice and guinea pigs (Sultzer and Nilsson, 1972), although it also acts on specifically sensitized T-cells. Antibodies specific to various mycobacterial antigens are also produced, especially in the IgG and IgA classes, but less so in the IgM class. Specific antibodies are of course not considered to be primarily responsible for recovery from tuberculosis. Initiation of chemotherapy appeared to increase the levels of specific antibodies in some studies (Nicholls and Horsfield, 1976; Kaplan and Chase, 1980) but not others (Mitchison, Aber, Ahmed, Allen and Devi, 1977). Levels tend to fall slowly with increasing length of time after treatment.

a) Serologic diagnosis of tuberculosis

The antibody response to infection with M. tuberculosis has been extensively studied for over 85 years, with a view to developing a simple and convenient serodiagnostic test for

tuberculosis. Such a test would be invaluable for the patient with smear-negative extra-pulmonary disease in whom bacteriological proof is difficult to obtain. Unfortunately all tests so far have been complicated by problems of reproducibility, sensitivity and specificity. Many patients produce antibodies to a wide range of antigens of M. tuberculosis, specific and non-specific. Also, the response to the same antigen may vary from host to host, owing to such factors as genetic control of antigen processing by macrophages, T-cell function, antigen load, and priming from previous contact with environmental mycobacteria.

Since the earliest test was introduced by Arloing and Courmont in 1898 (30% false-positive rate), important improvements have been achieved by means of haemagglutination (Middlebrook and Dubos, 1948), gel diffusion (Parlett and Youmans, 1959), bentonite flocculation (Wallace, Diena, Greenberg and Jessamine, 1966), fluorescent antibody (Nassau and Merrick, 1970), radioimmunoassay (Nassau, Parsons and Johnson, 1975), enzyme-linked immunosorbent assay (ELISA) (Nassau, Parsons and Johnson, 1976; Grange et al, 1980; Reggiardo, Vasquez and Schnaper, 1980), and monoclonal antibodies (Hewitt, Coates, Hitchinson and Ivanyi, 1982). Using an agglutination assay, Nicholls (1975) found significant elevations of antibodies to phenol-killed M. tuberculosis in 87% of patients with active disease and 0% of healthy controls, while Hitchison and co-workers (1977) found significant antibody levels in 70% of patients but also

in 59% of tuberculin-positive family contacts. Carr and colleagues (1980), using quantitative radioimmunoassay, detected antibodies to BCG in 67% of patients and 31% of persons with healed tuberculosis. By a similar method, Winters and Cox (1981) found no significant differences in specific IgG levels against five mycobacterial antigens between persons with active and healed tuberculosis, whereas as many as 27% of tuberculin-positive persons and 10% of tuberculin-negative persons had significant elevations in antibody levels.

Grange et al (1980), using an ELISA method, found higher levels of IgG to soluble antigens of M. tuberculosis in 75% of patients with active disease; the levels of IgA and IgM antibodies were much less discriminative. However, they also found significant antibody activity in healthy controls and in patients with sarcoidosis and Crohn's disease! When they applied this method to a further study (Kardjito, Handoyo and Grange, 1982) in which due attention was paid to the skin test status of their controls, only 52% of patients had significant antibody elevations when compared with tuberculin-positive controls. Using ELISA to detect anti-PPD antibodies, Viljanen, Eskola and Tala (1982), Radin, Zeiss and Phair (1983) and Kalish, Radin, Phair, Levitz, Zeiss and Metzger (1983) have recently confirmed that the best serologic discrimination between patients and healthy tuberculin reactors, or other patients with diseases mimicking tuberculosis, is found with IgG antibodies.

Purified antigens have given better discrimination. Naseau and colleagues (1976), using ELISA, reported antibodies to a soluble filtrate antigen of *M. tuberculosis* in 80% of patients and 8% of tuberculin-positive healthy controls. By the same method, Reggiardo et al (1980) found antibodies to five purified mycobacterial glycolipids in 95.5% of patients and 3 to 4% of healthy controls.

Monoclonal antibodies have also been applied to serodiagnosis of tuberculosis. Competitive inhibition by test serum of the binding of radiolabelled monoclonal antibodies to *M. tuberculosis* yielded results (71% positive among 14 patients) which were no better than other methods (Hewitt et al, 1982).

The combined results of these studies have established that there is still no satisfactory serodiagnostic test for tuberculosis. The main problems are as follows:

False negatives: Between 20 to 25% of patients do not have detectable antibodies to mycobacterial antigens. It is highly improbable that such patients do not make specific antibodies at all. It is possible that in such patients, antibody is already bound to mycobacterial antigens in vivo, leaving only small undetectable amounts in the circulation. In this regard, Carr and co-workers (1980) and Johnson, McNicol, Burton-Kee and Mowbray (1981) have found immune complexes in sera from 68% and 56% respectively of patients with active tuberculosis. Furthermore, Carr and his colleagues observed an inverse relationship between levels of

mycobacterial antibodies and immune complexes, which would be in accord with this proposition.

False positives: A significant number of healthy tuberculin skin test-positive and -negative subjects have demonstrable mycobacterial antibodies. This may be explained by subclinical infection with either M. tuberculosis or cross-reacting ubiquitous mycobacteria (Minden, McClatchy, Cooper, Bardana and Farr, 1971), or by previous BCG immunization (Wallace, Diena, Jessamine and Greenberg, 1967). Also, although clinical cure of tuberculosis is usually associated with a decline in antibody levels with increasing length of time after treatment, serum antibodies may remain detectable for years after treatment (Kalish et al, 1983). Persistent serum antibody may be related to the fact that viable tubercle bacilli persist in tissues for long periods after suppression of active infection (Youmans, 1978). Slow release of antigen from sites of persistence would provide an intermittent boost for antibody production.

Multiple assays with highly purified antigens seem the most promising diagnostic test for the future. With the introduction of monoclonal antibodies it should now be possible to isolate and purify the relevant antigens of M. tuberculosis, and apply such pure antigens to the most sensitive techniques in current use, namely ELISA and radioimmunoassay.

2. Cellular

a) Tuberculin skin test

Skin reactivity to tuberculin-PPD is of limited diagnostic value in active tuberculosis, since it is often positive in the absence of disease (healthy sensitised persons), and occasionally negative in the presence of active disease. While nearly all patients with active tuberculosis reacted to tuberculin in the first comprehensive studies (Purcolow, Howell and Nelson, 1942; Lester and Atwell, 1958), currently available reagents are not so sensitive. More recent reports indicate that up to 20% of patients failed to react to 5TU PPD (Holden, Dublin and Diamond, 1971; Browder and Griffon, 1972; Hyde, 1972; McMurray and Echeverri, 1978; Nash and Douglas, 1980). Anergy, defined as negative skin test reactions to 250 TU PPD, has been reported in up to 10% of patients by some (McMurray and Echeverri, 1978; Nash and Douglas, 1980) but in less than 2% of patients by other workers (Kent and Schwartz, 1967; Zeitz, Oetrow and van Axedel, 1974). Patients with miliary, fulminant or chronic long-standing tuberculosis are more likely to have negative tuberculin skin tests (Celb, Leffler, Brewer, Mascatellov and Lyons, 1973; Grieco and Chmel, 1974; Sahu and Neff, 1974; Uberoi, Malaviya, Chattopadhyay, Kuman and Srivinas, 1975; Ali, Puri, Chandra and Chandra, 1983), suggesting a correlation between anergy and extent or severity of disease.

However, anergy is occasionally seen in less ill patients with apparently localised disease (Kent and Schwartz, 1967; Holden et al, 1971; Schacter, 1972; McMurray and Echeverri, 1978). The state of anergy may relate to the type, dose, method of application, and potency of the antigens (Edwards, 1972) or to host factors such as malnutrition (Harrison, Tugwell and Pawcett, 1975; Bhatnagar, Malaviya, Narayanan, Rajgopalan, Kumar and Bharadwaj, 1977), age (Girard, Paychere, Cuevas and Fernandes, 1977), concomitant disease and drug therapy known to depress cell-mediated immunity (Johnson, Maibach and Salmon, 1971; Starr and Berkovich, 1974; Hillinger and Herzig, 1978).

Most patients who fail to react to PPD are also unresponsive to other recall antigens (McMurray and Echeverri, 1978). Malaviya and colleagues (1975) found that fewer than 40% of patients with untreated pulmonary tuberculosis could be sensitized to dinitrochlorobenzene (DNCB), a hapten used experimentally to induce delayed hypersensitivity. It would appear that as tuberculous infection progresses untreated, tuberculin hypersensitivity (acquired early in the disease) is gradually lost, and this state of skin test anergy persists throughout the infection (Rooney, Crocco and Kramer, 1976). Tuberculin anergy may imply a poor prognosis, especially in advanced disease (McMurray, 1980), but as a rule anergy disappears with successful treatment of tuberculosis (Uberoi et al, 1975; Rooney et al, 1976).

b) Lymphocytes

i) Lymphocyte count

Advanced tuberculosis is often associated with an absolute lymphocytopenia affecting predominantly T-cells (Zwolinski, Wieczorek and Skibinski, 1976; Shima, Takenada, Ando and Tokuomi, 1976; Bhatnagar et al, 1977; Al-Tawil and Thewaini, 1978; Skvor and Trnka, 1979; Katz, Goldstein and Fauci, 1979; Humber, Nsanzumuhire, Aluoch, Webster, Aber, Mitchison, Girling and Nunn, 1980; Prabhu and Reddy, 1983). Within this decreased T-cell population Katz and colleagues (1979) found a relative increase in T-cells with receptors for the Fc portion of IgG (T_G, suppressor) and a proportional decrease in T-cells with IgM receptors (T_M, helper) (Moretta, Webb, Grossi, Lydyard and Cooper, 1977). The depressed total lymphocyte, total T and helper T-lymphocyte counts returned to normal during treatment, suggesting that this finding was an effect rather than a predisposing cause of the infection.

In contrast, some have found normal (Williams, Debord, Mellbye, Messner and Lindstrom, 1973; Gatner and Anderson, 1980; Hsieh and Chong, 1981) and increased (Kventy, 1977) T-cell counts in patients with active tuberculosis. Also, various workers have reported increased (Hsieh and Chong, 1981; Chapras, 1982) and normal (Williams et al, 1973; Bhatnagar et al, 1977; Al-Tawil and Thewaini, 1978; Gatner and Anderson, 1980) B-cell counts in active disease.

The reasons for these discrepancies are not clear. These investigators all studied patients with proven tuberculosis using similar techniques: sheep erythrocyte (E)-rosetting for T-cells (except Williams and colleagues who used an anti-thymocyte serum) and direct immunofluorescence with fluorescein isothiocyanate (FITC)-conjugated antibody for B-cells (except Hsieh et al, 1981 and Gather et al, 1980, who used sheep erythrocyte - antibody - complement (EAC) - rosetting). The differences may be due to technical problems, or may reflect peculiarities of the different ethnic groups, or disease severity.

(ii) Lymphocyte proliferation

A critical function of lymphocytes in the development of CMI is their ability to proliferate in response to stimulation by bacterial antigens and mitogenic bacterial products. The cells respond by transforming into lymphoblasts: lymphocytes which were previously metabolically inactive start synthesising DNA, RNA and proteins at a greatly increased rate; they enlarge and usually divide. This process may be measured by labelling with precursors of DNA, RNA or protein synthesis (e.g. ^{3}H-thymidine), or simply by microscopic observation.

In vitro studies of lymphocyte proliferation in tuberculosis have yielded conflicting data. After Shrock (1963) and Pearmain, Lycette and Fitzgerald (1963) independently showed that PPD transformed lymphocytes in culture, early reports had indicated that lymphocytes from

only skin test-positive patients were transformed by PPD. However, it has become clear that lymphocytes from skin test-negative patients sometimes proliferate, whereas those from skin test-positive patients sometimes fail to proliferate in response to PPD (Gump, Fekety, Urbanetti and Nosenzo, 1967; Aspegren and Roreman, 1964). One study based on morphologic criteria demonstrated PPD-induced lymphocyte proliferation in seven skin test-negative tuberculous patients (Smith and Reichmann, 1972). When ^{3}H-thymidine incorporation was used as an index of transformation, various groups of investigators reported a good correlation between in vitro lymphocyte transformation and in vivo skin test reactivity to PPD (Kerby, 1968; Hinz, Daniel and Baum, 1970; Miller and Jones, 1973; Zeitz et al, 1974). In general, patients who are anergic on skin testing also display anergy in vitro when their lymphocytes are stimulated with PPD. Many such patients have disseminated disease, although there are a few who are clinically indistinguishable from others with good skin responses. Even when in vitro lymphocyte function is apparently preserved in tuberculosis, the progressive enhancement of lymphocyte reactivity during the course of therapy is consistent with earlier transient suppression of delayed hypersensitivity (Goldstein, Ang, Foellmer and Jelicki, 1976).

c) Lymphokines

(i) Lymphokine production

Lymphokines are non-immunoglobulin products of antigen-activated lymphocytes which have marked biologic effects on lymphocytes, macrophages, eosinophils, basophils and neutrophils (Rocklin, Bondtzon and Greineder, 1980). In tuberculosis, there is evidence that lymphokines are essential in the genesis of CMI: some induce the accumulation and activation of macrophages, which develop the ability to kill or inhibit the organism (North, 1974). Some lymphokines mediate tuberculin hypersensitivity which follows tuberculous infection. Various authors have reported deficient activity of leucocyte migration inhibitory factor (LIF) in response to PPD in some patients with tuberculosis (Bhatnagar et al, 1977; Lenzini, Rottoli and Rottoli, 1977; Gatner et al, 1980) and that this function correlates well with tuberculin skin reactivity (Bhatnagar et al, 1977; Lenzini et al, 1977) and inversely with extent of disease (Trnka and Skvor, 1979). However, dissociations occur in active disease (Rieger, Trnka and Skvor, 1979). Joffe and Rabson (1981) found suppressor T-cells which inhibited LIF production but not blastogenesis in patients with tuberculous meningitis. Their data support the consensus view that in vitro production of some lymphokines is independent of cell proliferation (Goroki, Dupont, Hansen and Good, 1975). In healthy subjects, there is an association between dermal sensitivity, MIF and LIF production, and lymphocyte transformation (Astor, Spitler, Frick and Fudenberg, 1973; Hoffman, Spitler, Hsu and Fudenberg, 1975), although

dissociations also occur (Thomas, Clemente and Grzybowski, 1977).

(ii) Interferon

Interferons (IFNs) constitute a family of proteins of molecular weights about 20,000, discovered by Isaacs and Lindenmann (1957), which are secreted by nucleated cells when they are stimulated by viruses, double-stranded RNA, bacteria, foreign cells, macromolecules and various other compounds. The secreted IFNs are able to stimulate other cells to produce intracellular proteins which inhibit virus multiplication. IFNs act locally and at distant sites, being secreted in the blood stream. IFNs also regulate the immune response, cell growth and other cell functions. Three broad types of ~~human~~ IFNs are presently distinguished on the basis of antigenic differences (Committee on IFN Nomenclature, 1980). The predominant family derived by stimulating buffy coat leucocytes with virus is designated IFN-α. It consists of more than 20 closely-related proteins with 166 amino acids. Fibroblasts in culture produce a similar molecule, IFN-β. T-lymphocytes in culture produce a different glycosylated molecule in response to immune stimuli (mitogen and antigen) and designated IFN-γ. IFNs α and β are generally stable at pH2 whilst γ is inactivated. A recently described species of IFN-α associated with autoimmune disease is pH2 labile (Preble, Black, Friedman, Klippel and Vilcek, 1982). IFN-γ is not neutralized by antibodies raised against IFNs α and β. All IFNs exert three main biologic effects in

man : virus replication inhibition, cell growth inhibition and immune modulation. Viruses and tumour cells induce mainly IFN-α (Wheelock, 1966; Timonen, Sakeela, Virtanen and Cantell, 1980), while IFN-γ is the major product when human lymphocytes are stimulated with mitogens and antigens (Epstein, Cline and Merigan, 1971, 1972; Osborne, Georgiades and Johnson, 1980; Langford, Weigent, Georgiades, Johnson and Stanton, 1981). IFN-γ is therefore a lymphokine.

Although IFNs are probably important in early responses to virus infections (Creeser, Maury, Kress, Blangy and Manoury, 1979; Isaacs, Clarke, Tyrrell, Webster and Valman, 1981), their role in bacterial infections such as tuberculosis is less clear. Some observations suggest that IFNs - immune interferon (IFN-γ) in particular - may be important in host defence against tuberculosis. First, immune-specific induction of IFN in vitro (Glasgow, 1966; Green, Cooperband and Kibrick, 1969) and in vivo (Stinebring and Absher, 1970) suggest that IFNs could be mediators of CMI. Secondly, IFN-γ, a lymphokine, is readily induced in vitro and in vivo in immune animals and man by mycobacterial antigens such as tuberculin-derived PPD (Green et al, 1969; Epstein et al, 1971; Stinebring and Absher, 1970) and live BCG (Salvin, Youngner and Leder, 1973). Thirdly, all IFNs 'activate' mononuclear phagocytes (Moore, 1983). IFNs also affect delayed hypersensitivity in vivo, cell surface antigen expression, antibody production and lymphocyte blastogenesis (Moore, 1983), all of which processes participate in the host response to tuberculous infection. It may be that IFNs

induced in vivo in response to infection with tubercle bacilli activate macrophages to phagocytize and kill bacilli, and so limit the progress of the infection.

d) Monocytes and macrophages

(i) Monocyte count

It has been recognised for nearly 60 years that blood monocyte counts rise in parallel with the activity of tuberculous infection (Cunningham, Sabin, Sugiyma and Kindwall, 1925; Doan and Wiseman, 1934). These early observations suggested that the macrophages and epithelioid cells of tuberculous granulomas might arise from blood monocytes. The majority of circulating monocytes replace macrophages killed by tubercle bacilli, giving rise to increased monocyte consumption, enhanced monocyte production and promature release of monocytes from the marrow. Hyperstimulation and hyperproliferation of monocytes represent an adaptive compensatory mechanism in active tuberculosis which is the classic "high turnover" granuloma (Schmitt, Mouret and Stix, 1977). Monocytosis is reversed during the course of therapy when tubercle bacilli are killed and there is a fall in macrophage toxicity and monocyte consumption. There is evidence that monocytes may contribute to anergy in some tuberculous patients : adherent cell depletion significantly enhanced the in vitro responsiveness of peripheral blood mononuclear cells to PPD (Ellner, 1978). However, while monocytosis occurred only in anergic patients in Ellner's series, Hsieh et al (1981) found it predominantly in tuberculin reactors.

(ii) Monocyte/macrophage activation

Schmidt and Douglas (1977) have shown that circulating peripheral blood monocytes from patients with active tuberculosis are activated as evidenced by increased monocyte IgG and C3 surface receptor activity. Other monocyte alterations consistent with activation have also been reported: increased glycolysis and hexose monophosphate shunt activity (Para, Sagone, Balcerzak and Lobuglio, 1972; Kitahara, Eyre and Hill, 1979), increased chemiluminescence on exposure to opsonised bacteria and yeast (Kitahara et al, 1979), and enhanced killing of Staphylococcus aureus (King, Barn and Lobuglio, 1975). In contrast, Cruchaud, Girard and Mitoglou (1977) reported reduced monocyte bactericidal capacity for Staph. aureus in severe tuberculosis.

(iii) Monocyte chemotaxis

Meltzer, Jones and Boetcher (1975) and Poplack, Sher, Chaparas and Blaese (1976) showed that peritoneal macrophages from mice infected intraperitoneally with BCG exhibited an enhanced responsiveness to chemotactic stimuli. In 1979, Kitahara and colleagues demonstrated increased monocyte chemotaxis in a heterogeneous group of patients with inflammatory diseases, including tuberculosis. In contrast, Campbell (1979) and Nielsen, Bennedsen, Larsen, Rhodes and Viskum (1982) reported defective monocyte chemotaxis in patients with active pulmonary tuberculosis, apparently due to a heat-stable anti-chemotactic factor in their plasma.

(iv) Monocyte phagocytosis

Again, studies of pinocytic and phagocytic activities of monocytes in tuberculosis have provided conflicting results. Whereas Urbanitz, Gregoritz, Fechnor and Gross (1974) and Cruchaud et al (1977) found depression, Nielsen and colleagues (1982) reported normal, and Kitahara et al (1979) found enhanced phagocytic capacity.

e) Killor cells

In vitro cytotoxicity of tumour cells may be mediated by one of three types of effector cells: cytotoxic T-lymphocytes (T_{CTL}) which are immunologically induced, killer (K) cells which are one cell-type responsible for antibody-dependent cell-mediated cytotoxicity (ADCC), and natural killer (NK) cells which are neither induced immunologically nor functionally dependent on antibody. The roles of these cells in antituberculous immunity are not known. NK cells are a population of large granular lymphocytes from normal nonimmune donors with the capacity to recognize and rapidly lyse, within hours, tumour cells and virus-infected cells - a unique ability that endows them with the potential of in vivo immunosurveillance against tumour growth (Kiessling, Klein and Wigzell, 1975). There is evidence to suggest that NK cells are also important in natural resistance against virus (Ching and Lopez, 1979) and other microbial (Reder and Press, 1982) infections. Some observations suggest that the NK

system may play a role in tuberculosis. BCG immunization activates macrophages (Hibbs, 1973; Germain, Williams and Benacerraf, 1975), induces NK cells and augments NK activity in mice (Wolfe, Tracey and Henney, 1976) and humans (Saal, Riethmuller, Eiber, Hadam, Ehinger and Schneider, 1977), probably by inducing interferon. Also, activated macrophages are essential for the NK augmentation induced by BCG (Tracey, 1979), and low-normal NK reactors are less reactive to BCG in vitro than those with high or normal NK activity (Mandeville and Rocheleau, 1983).

D. Summary of immune defects in tuberculosis

The studies described suggest that patients with active tuberculosis exhibit a wide range of functional defects in vivo and in vitro. These include overproduction of immunoglobulins, alterations in absolute counts and proportions of T-lymphocytes, abnormalities in cutaneous delayed hypersensitivity reactions to specific and non-specific antigens, and defects in mitogen- and antigen-induced lymphocyte proliferation and inflammatory mediator production. Increased monocyte consumption may stimulate peripheral blood monocytosis. The monocytes probably circulate in an activated state, although their responsiveness to chemotactic stimuli may be impaired.

No single basis for these defects is known, but their apparent reversal during treatment suggests that they result from rather than predispose to active tuberculous infection. However, many patients with active tuberculosis have apparently normal immunological responses and in those with abnormalities, the range of abnormal immune responses and the degree of individual alterations are extremely variable. What factors determine the occurrence, nature and degree of immunological alterations in an individual patient? There is evidence that the paramount factor is the host-parasite interaction, rather than variation in pathogenicity of individual infecting mycobacteria. Therefore, it is likely

that each host reponde differently, resulting in the varied clinical picture. In this study I have set out to explore the range of different host reponses and examine its implications for the control of this disease.

2. Aims of the present study: A spectrum of immunity in tuberculosis

1. To investigate the presenting clinical features, nutritional state and immunological functions of a group of hospitalised patients with active tuberculosis, comparing responses with those in apparently healthy controls matched for age, sex, ethnic group and diet.

2. To observe changes in nutritional statue and immunological functions in tuberculosis patients with drug therapy, comparing them with the same controls.

3. To investigate possible correlations among initial clinical, nutritional and immunological features of tuberculosis patients and any unusual responses to treatment.

My hypothesis is that there is a spectrum of immunological responses to tuberculosis which is reflected in its clinical behaviour and natural history. The results of this study may lead to a better understanding of host resistance in this disease, identify some host factors which affect prognosis and stimulate further research towards the development of effective immunotherapeutic interventions which may eventually lead to control of the disease. Patients who may respond well or poorly to such therapeutic strategies may also be identified.

CHAPTER TWO - SUBJECTS, MATERIALS AND METHODS

A. Study population:

Consenting adult patients (19-61 years) with newly diagnosed tuberculosis, hospitalised in Northwick Park and Colindale Hospitals, were studied prospectively. Diagnosis was based on the demonstration of acid-fast bacilli (by Auramine or Ziehl-Neelsen stains) in sputum, cerebrospinal fluid, pleural fluid or histology specimens, and was eventually confirmed by positive cultures on Lowenstein-Jensen egg slopes. Most patients lived in the Harrow, Brent or Edgware/Hendon Health Districts of North-West London and were referred by General Practitioners, or admitted through the Hospital Accident and Emergency Department. A few patients were referred direct from Heathrow Airport because they were found to have abnormal chest X-ray films on arrival. The Borough of Brent has a large immigrant population and the highest notification rate for tuberculosis in England and Wales : 114.3, compared to 35 (Greater London), 23.7 (West Midlands) and 5.2 (Anglia) per 100,000 of the population per annum (MRC Report, 1982a).

Patients were not studied if they were pregnant, had received anti-tuberculous treatment previously, had co-existing systemic diseases or were receiving medication with corticosteroids, cytotoxics or other drugs known to affect the immune system. Controls were drawn from healthy

staff of the Hospitals, the Clinical Research Centre, Harrow and members of Harrow Community. They were eligible for entry if they had no symptoms suggestive of any illness, were not pregnant, had not received anti-tuberculous treatment previously, were not receiving medication with immunosuppressive drugs, and had a normal haemoglobin and erythrocyte sedimentation rate (ESR).

Patients and controls were entered in pairs, matched for sex, age (within five years), ethnic origin (Indian Subcontinent, Caucasian or African) and diet (vegetarian or non-vegetarian). All 30 controls had received BCG immunization in infancy, childhood or adolescence. The Indian Subcontinent (ISC) ethnic group consisted of patients and controls originating from India, Pakistan, Bangladesh or East Africa. The Caucasian group consisted of white patients and controls from Europe, North America or Australasia. The African ethnic group was constituted by negroid patients and controls originating from Africa. There were no Carribbean or American Negroes in this study. In most cases the control had been born in the same geographical region (defined above) as the patient. This was considered of great importance with regard to ISC patients. Controls and patients were also broadly matched for socio-economic background.

B. Ethical Committee Submission:

The protocol was submitted to Ethical Committees and approved by the Harrow District (E.C. 827), Brent Health Authority (E.C. 220) and Edgware/Hendon Health District.

C. Clinical assessment:

1. History and examination

At presentation, a full history was obtained from every subject (see Study Proforma, Appendix 2, p.260), and every patient was examined to ascertain the site and extent of tuberculous disease and exclude other obvious pathology.

2. Anthropometric indices:

a) Weight and height:

With shoes and outer clothing removed, each subject had height (H) in cm and weight (W) in kg measured, from which a weight-corrected-for-height-squared (W/H^2) index was calculated.

b) Mid-triceps skinfold thickness:

A fold of skin plus subcutaneous tissue without underlying muscle was grasped between finger and thumb at the midpoint of the right upper arm over the triceps muscle. The crest of the fold was parallel to the long axis of the arm. The mid-triceps skinfold thickness (SFT) was measured with a Harpenden caliper (Holtain Ltd, Crymych, Pembrokeshire), without releasing the finger-thumb grasp. The caliper was read (mm) after three to five seconds of caliper pressure.

c) Mid-upper arm circumference:

With the subject standing erect, the upper arm length

was measured from the acromion to the olecranon processes, using a tape measure, and the midpoint marked on the skin. With the arm hanging relaxed at the side, the horizontal mid-upper arm circumference (MAC) was measured (cm) without skin distortion.

Arm muscle circumference (AMC) was derived from the formula, AMC (cm) = MAC (cm) - 0.314 x SFT (mm). All anthropometric indices were measured in all subjects before treatment, and in those who were accessible at six months and 12 months afterwards.

3. X-ray:

The extent of pulmonary disease and the presence or absence of cavitation were assessed from a full-size postero-anterior chest X-ray taken on admission, according to the Hong Kong study criteria of the MRC (1981). A single assessor (Dr F Mitchell), a consultant Radiologist at Northwick Park Hospital with no knowledge of the clinical features or test results of individual patients, scored each chest X-ray blind in one session. Controls did not have a chest X-ray, as this was considered unethical.

4. Basic laboratory data

Blood samples were taken from all subjects before treatment was instituted, and from those who were accessible at six and 12 months afterwards. Full blood count (FBC),

total and differential white blood-cell counts (WBC), and ESR (Westergren) were performed by one appointed technician within the routine haematology service at Northwick Park Hospital. PBC was measured by Coulter counter and differential WBC by visual counting (500 cells) on a blood smear stained with May-Grünwald Giemsa. Immunoglobulin levels were estimated in the Division of Immunological Medicine with a Hyland laser nephelometer PDQ system. Relevant serum biochemical values (see Study Proforma, Appendix 2, p.260) were determined in the routine Clinical Chemistry laboratory service using Autoanalyzers.

E. Mantoux test:

Mantoux tests were performed (by the investigator only) on all subjects on admission into the study, and on those available at six and 12 months afterwards. 0.1 ml of tuberculin PPD (Evans Medical Ltd, Greenford, Middx) containing 1 tuberculin unit (TU) was injected intradermally into the inner surface of either forearm so that a wheal formed. The subject was asked not to scratch the site. The area of induration was defined by palpation and measured at 6,12,24,48 and 72 hours in two perpendicular diameters using a calibrated tape. The average reading (mm) was recorded. This method gave a representative reading for asymmetrical reactions (Sokal, 1975). Erythema alone, or a mean diameter of induration less than 6 mm at 72 hours was regarded as a negative reaction, and the injection was repeated on the

other arm using 10 TU PPD in 0.1 ml. All subjects were tested by one observer to eliminate inter-observer error, which is common in Mantoux test reading (Beerman, Kleinman, Glyer and Lacroix, 1964). In preliminary tests, the use of "Sellotape" tracings or the ball-point technique (Sokal, 1975) to delineate induration was not found to be any more accurate than simple palpation by the same observer.

F. Isolation of mononuclear cells:

Defibrinated peripheral blood (20 ml) was diluted 1:2 with bicarbonate-buffered RPMI 1640 (Moore, Gerner and Franklin, 1967) and separated on Ficoll-Paque (Pharmacia) at 2,400 rpm (400 g) for 20 minutes (Boyum, 1968). The interface cells were removed, washed three times and resuspended in bicarbonate-buffered (13 mM) RPMI 1640 supplemented with L-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 µg/ml) and 10% heat-inactivated fetal calf serum (56°C x 30 mins) (FCS, Flow Laboratories) (hereafter called medium). The viable mononuclear cell count was determined in a Neubauer haemocytometer by trypan blue (0.1%) dye exclusion (see Cell Counting, Appendix 3a, p. 262) and adjusted to four working concentrations: 8, 4, 2 and 1 million cells/ml medium. An aliquot was frozen down in liquid nitrogen (see Cell Freezing, Appendix 3b, p. 263).

C. Enumeration of lymphocyte subsets:

Mononuclear cells were thawed out from liquid nitrogen (see Cell Thawing, Appendix 3c, p.263), washed three times and resuspended at 2 x 10^7 viable cells/ml in RPMI supplemented with 2% FCS plus 0.02% sodium azide. The numbers and proportions of total T, helper/inducer T(T_H), suppressor/cytotoxic T(T_S) and B lymphocyte phenotypes were defined by means of immunofluorescence analysis using monoclonal antibodies Leu 4 (or 1), 3a, 2a and 12 (Becton Dickinson, Sunnyvale, Ca) respectively. Equal volumes (2 ml) of the mononuclear cell suspension and each monoclonal antibody were incubated on ice for 30 mins, washed three times, and then incubated for another 30 mins with fluorescein isothiocyanate-coupled rabbit anti-mouse immunoglobulin (FITC-RaMIg 10 mg/ml, Nordic Immunological Labs, Maidenhead, Berks) at a final concentration of 1:200. Immunofluorescence analysis was performed by Dr Andrew Edwards of the Division of Immunological Medicine, using a fluorescence-activated cell sorter, FACS II (Becton Dickinson) under standardised conditions for this machine (Loken and Herzenberg, 1975).

H. Cytotoxicity assay:

1. Spontaneous cytotoxicity

Cultured cells from the K562 erythroleukaemic cell line (kindly supplied by Dr Colin Sanderson, National Institute for Medical Research, Mill Hill, London) grown in medium were

used as target cells. The line was routinely subcultured 18-24 hours before an assay. 5×10^6 target cells in 100-200 µl of medium were incubated with occasional mixing with 3.7 MBq of sodium-^{51}chromate (Amersham International UK, Code CJS4) for 1h at 37°C. The ^{51}Cr-labelled K562 target cells were washed three times. Viability was determined by trypan blue exclusion, and the cells resuspended in medium at 1×10^5 viable cells per ml. Triplicate cultures were set up in LP2 tubes containing 100 µl of target cell suspension plus 100 µl of each concentration of mononuclear cells to give three effector:target cell ratios - 40:1, 20:1 and 10:1. Triplicate cultures for minimum and maximum lysis were also set up with 100 µl of K562 suspension plus 100 µl of medium or 100 µl of 10% Triton X-100 (BDH Chemicals) respectively. The cultures were incubated for 4 hours at 37°C in a humidified atmosphere containing 5% CO_2:95% air. The contents of each tube were mixed thoroughly then centrifuged for 10 mins at 500 rpm. 100 µl of supernatant were removed from each tube into a fresh LP2 and radioactivity was determined in a gamma counter (Wallac CTL 300/1000). The percentage specific ^{51}Cr release (% cytotoxicity) was calculated from the formula (E-S)/(M-S) x 100, where E is counts per minute (cpm) in experimental tube, S is cpm spontaneous isotope release (minimum lysis), and M is cpm maximal isotope release effected by triton lysis. Dose-response values were plotted for each individual as % cytotoxicity against effector:target (E:T) cell ratio.

2. IFN-augmented cytotoxicity:

Parallel assays were set up for IFN-augmented cytotoxicity after mononuclear cells were treated with monoclonal antibody-purified leucocyte IFN (MK2-IFN), kindly supplied by Professor K Cantell, Helsinki, and Dr D S Secher, MRC Laboratory of Molecular Biology, Cambridge. 4×10^6 mononuclear cells were incubated for 1 hour at 37°C with 1000 IU IFN/ml in a humidified 5% CO_2-air atmosphere. The cells were washed three times, counted, and viability determined by trypan-blue exclusion. Cell counts were adjusted and mononuclear cell/K562 cultures set up in triplicate as above.

I. Mononuclear cell proliferation assay:

1) Principle:

The proliferative responses of peripheral blood mononuclear cells (PBMC) to tuberculin antigen and mitogens were measured using incorporation of the radiolabelled DNA precursor, ^{3}H-thymidine. PPD (Evans), also a T-dependent polyclonal B-cell activator (Sultzer and Nilsson, 1972; Ringden, Rynnel-Dagoo, Kunori, Smith, Hammaretrom, Freijd and Moller, 1979), was used as antigen, while a mixture of the potent T-cell stimulants Staphylococcal Enterotoxin A (SEA, Division of Microbiology, Food and Drug Administration, Cincinnati, Ohio) plus Tetrahydrophorbyl Acetate (TPA, Sigma) was used as mitogen (Langford, Stanton and Johnson, 1978). Optimal ^{3}H-thymidine uptake in response to PPD occurs at 4-6 days (Epstein et al, 1971), and in response to SEA at 3-4 days (Langford et al, 1978). However, recent evidence

suggests that data must be obtained from a range of cell concentrations, culture periods and antigen or mitogen concentrations, in order to measure properly the proliferative capacity of PBMC (Knight and Farrant, 1978; Knight, Harding, Burman, O'Brien and Farrant, 1979; Farrant and Knight, 1979). A simple and convenient way of doing the large number of tests required with only a limited number of cells is to culture the cells on the meniscus of 20 µl hanging droplets in inverted Terasaki plates (NUNC) (Farrant and Knight, 1979; O'Brien, Knight, Quick, Moore and Platt, 1979). A harvester for such cultures has been designed, allowing the rapid transfer of washed, acid-precipitable cell fractions into scintillation vials for measurement of incorporation of ^{3}H-thymidine into DNA (O'Brien et al, 1979). In this study, ^{3}H-thymidine uptake was measured in triplicate wells with one mitogen, three antigen and three cell concentrations over three culture periods. For every subject, 27 responses were obtained to antigen and nine responses to mitogen on each occasion of testing. The overall maximum response was selected for analysis.

2. Cell culture:

The wells of sterile Terasaki microtiter plates were filled in triplicate with 20 µl aliquots of 4, 2 and 1 x 10^{6} viable mononuclear cells/ml medium. A repeating dispenser Hamilton syringe was used to add to each well either medium, 4 ng BCA plus 200 ng TPA, or 100, 10 or 1 unit PPD. The plates were inverted over a support of petri dishes above a

bath of sterile saline in a plastic sandwich box, and incubated for 3, 4 or 5 days in humidified 5% CO_2:95% air at 37°C (fig. 3).

3. Measurement of ^{3}H-thymidine incorporation:

On the day of harvest 1 µl methyl-tritiated thymidine (0.1 µg/well, specific activity 5.5 GBq/mmol, Amersham TRA 120, diluted in cold thymidine, see Thymidine dilution, Appendix 3d, p.263) was added to each well, incubated for 2h and harvested onto individual filter discs (TiterTek No. 78-105-05). The discs were washed with excess 5% Trichloroacetic acid and finally with methanol (O'Brien et al, 1979). Dried discs containing ^{3}H incorporated into acid-precipitated DNA were counted in 1 ml of Nuclear Enterprises 260 scintillation fluid using a Wallac LKB liquid scintillation counter. Responses were recorded as counts/min (cpm) of tritium per well. Non-stimulated control samples often exceeded 200 cpm and results were expressed as means with 95% confidence limits without correction for this background.

4. Effect of "tuberculous" serum on antigen- and mitogen-induced mononuclear cell proliferation:

To investigate the effect of serum from patients with tuberculosis, additional cultures of PBMC suspended in RPMI 1640 medium were set up in parallel, supplemented either with

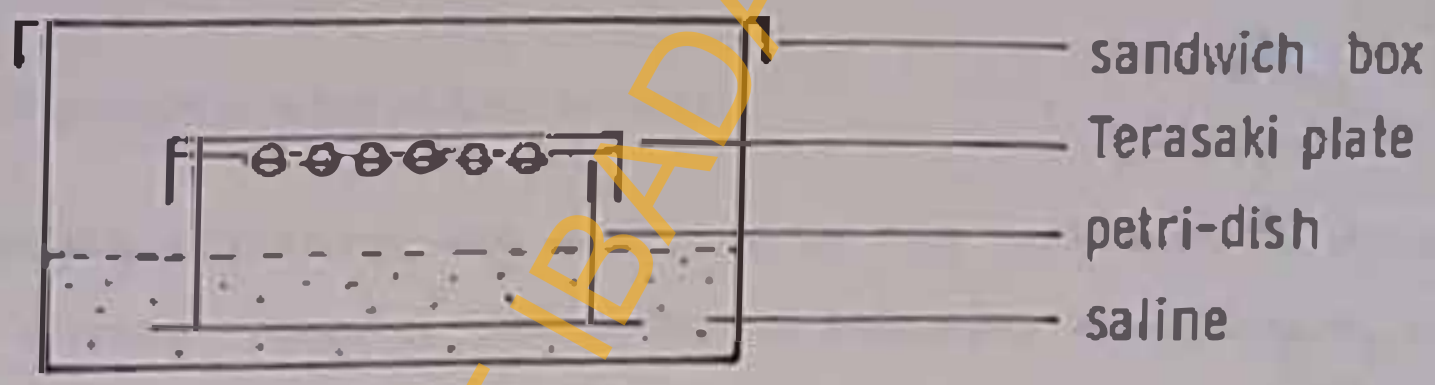

Fig 3: Cross-section of a Terasaki plate during culture of mononuclear cells for interferon induction or proliferation assay.

10% heat-inactivated patient's serum or with 10% heat-inactivated pooled control human serum. Patient's serum was obtained by centrifuging clotted blood at 3000 rpm for 10 mins. Control human serum was pooled from 12 healthy donors (four Caucasians, four Asians and four Africans), centrifuged and stored in aliquots at -70°C. A single batch was used in all the experiments.

J. Monocyte migration assay:

Monocyte migration was measured by the raft modification of the Boyden chamber (Addison and Babbage, 1976) which is based on the ability of monocytes to migrate across a cellulose membrane, at random or in response to a chemotactic stimulus.

A Whartman filter paper soaked in RPMI 1640 without serum (for Random Locomotion, RL) or 2% casein solution in RPMI 1640 (for Chemotaxis, CTX) (Wilkinson, 1982) was placed over the bottom of a petri dish. Air bubbles were stroked away and a cellulose membrane (Sartorius, Gottingen, FRG) of 8 μ pore size was placed on each filter paper. This quickly soaked up medium or casein. 2×10^6 PBMC/ml in RPMI 1640 without serum were dispensed into the 8mm plastic caps (capacity 0.25 ml) of LP3 tubes (Luckham Ltd, Sussex) to fill the caps without meniscus. The caps were carefully inverted over the wet cellulose membrane and the petri dish covered and incubated at 37°C for 1 hour in humidified 5% CO_2:95% air. The caps were discarded and the membranes washed with

saline, fixed with propan-2-ol (BDH Chemicals, Poole), stained with Harris Haematoxylin (Raymond A Lamb, London) and cleared and mounted in cedar wood oil (BDH Chemicals) (see monocyte migration Assay, Appendix 3e, p.264). Each membrane was examined under x40 objective, the leading monocyte identified by morphology, and its distance (μ) from base-line measured using the focussing micrometer (the leading front method). Readings were taken from five fields on each membrane and results expressed as the mean. Particular care was taken to identify the front-runner cell (as a monocyte) since neutrophils migrate faster than monocytes (Hoffman, Spitler, Hsu and Fudenberg, 1975, 1976). However, relatively few neutrophils (less than 2%) contaminate PBMC preparations.

K. Interferon production:

1. Interferon-α induction and assay:

Inducer: Newcastle Disease Virus (Wheelock, 1966; Rubinstein, Rubinstein, Familletti, Miller, Waldman and Pestka, 1979).

Newcastle Disease Virus (NDV) B1 strain, was harvested from the allantoic sacs of 10-11 day old hens' eggs after incubation for 48 hours, diluted with phosphate-buffered saline (PBS), and titered as haemagglutination units (HAU) using washed day-old chick red blood cells (Flow Labs). 60,000 PBMC in 10 μl medium were incubated in Terasaki wells with 0.64 HAU of NDV in 10 μl of saline, at 37°C for 24 hours

in a humidified 5% CO_2:air atmosphere (fig. 3). The cultures from five wells were pooled, diluted 10-fold with complete PBS plus 0.5% bovine serum albumin plus 0.05% sodium azide, and assayed in duplicate by a sandwich immunoradiometric technique employing a polyclonal sheep anti-human IFN-α bound to a solid phase (plastic bead), and an iodinated monoclonal antibody ^{125}I-MK2 (Celltech Ltd, Slough) (Secher, 1981) (see Interferon-α assay, Appendix 3f, p.264). The counts per minute (cpm) bound to the solid phase for unknowns were compared with a curve derived for varying concentrations of the MRC Standard 69/19B. The lower limit of sensitivity was 50 u/ml.

2. Interferon-γ induction and assay:

Inducers: SEA + TPA, PPD.

SEA is an excellent inducer of IFN-γ (Johnson, Stanton and Baron, 1977; Langford, Stanton and Johnson, 1978; Wilkinson and Morris, 1983). Certain tumour promoters such as the phorbol ester TPA, which themselves induce small amounts of IFN-γ, act synergistically with SEA and other T-cell mitogens to increase IFN-γ yields up to 30-fold (Vilcek, gulea, Vovovitz and Yip, 1980; Yip, Pang, Urban and Vilcek, 1981a; Yip, Pang, Oppenheim, Nadibar, Hentikson, Zerobecky-Eckhardt and Vilcek, 1981b; Wilkinson and Morris, 1983). It has also been shown that leucocytes from immune donors produce IFN-γ when treated with PPD (Green et al, 1969; Epstein et al, 1971).

Bioassay of IFN-γ is based on the knowledge that some viruses produce damage to cells which can be seen in unstained cultures at low magnification. This cytopathic effect of virus is inhibited if the cultures are pretreated with sufficient IFN (Ho and Enders, 1959).

In a pilot study, varying concentrations of SEA plus TPA were tested with varying mononuclear cell concentrations in Terasaki plates. 80,000 PBMC in 20 µl medium incubated with 4 ng SEA plus 200ng TPA for three days at 37°C in humidified 5% CO_2:95% air gave optimal yields of IFN-γ of 10^4u/ml among healthy volunteers. To test for "immune-specific" IFN-γ, PBMC at 80,000 cells in 20 µl medium were incubated with 100, 10 and 1 U PPD for five days, under similar conditions. Only cultures stimulated with 100 U PPD were assayed. IFN-γ was measured in pools of five Terasaki wells (total volume 100 µl) by observing the dilution of each unknown which, after overnight incubation, caused 50% inhibition of cytopathic effect (cpe) of Semliki Forest Virus (120 TCID50) (kindly supplied by Professor D.C. Burke, Warwick University), in confluent human epithelial (Hep-2) cells (Flow Labs) (see Interferon-γ assay, Appendix 3g, p.265). A laboratory IFN standard derived by the incubation of buffy coat cells from one volunteer with phytohaemagglutinin previously gave a mean titre of 3.03 ± 0.31 $\log_{10}$ u/ml in 12 assays on "WISH" human amnion cells, and 1.95 ± 0.19 $\log_{10}$ u/ml in human foreskin fibroblasts. For the purpose of standardisation of assays, this was designated 1000 u/ml and included on each plate for comparison with unknowns. Cytopathic effect developed over

48-72 h after addition of virus and was read macroscopically and microscopically after staining with 0.1% crystal violet in 20% ethyl alcohol. All samples were assayed in duplicate, patients with controls, and in nine assays the lower limits of detection varied from 37 to 111 u/ml of the laboratory standard.

L. Chemotherapy regimen and follow-up:

After initial base-line investigations, patients were treated with a standard regimen which consisted of Rifampicin, Isoniazid and Ethambutol for two months, continuing with Rifampicin and Isoniazid for a further seven months (pulmonary) (British Thoracic and Tuberculosis Association Report, 1976) or 16 months (extra-pulmonary). Measurements were repeated in those who were available at six and 12 months after initiation of treatment (Table 1).

M. Statistical analysis:

All data were tested for normality of distribution using the Shapiro-Francia test (1972), and if necessary, transformed to achieve a Gaussian distribution. (See Details of Statistical Methods, Appendix 4a and 4b, p.267) For untransformed data, the variability of the observations was expressed as standard deviation (SD), in preference to the smaller standard error (SE) which, less usefully, indicates merely the precision of estimation of the mean. Transformed data were summarised as mean and 95% confidence intervals (see Details of Statistical methods,

TABLE 1

Investigations carried out before, during and after nine months of chemotherapy in patients with tuberculosis and matched controls

	On admission	At 6 months	At 12 months
1. Clinical assessment:			
- History and examination	•	•	•
- Chest X-ray (patients only)	•		
2. Nutritional and metabolic assessment:			
- Anthropometry (W/H, skinfold, arm muscle circumference)	•	•	•
- Haematology (Hb, WBC, differentials)	•	•	•
- Biochemistry (sodium, urea, proteins, albumin, calcium, asp. trans, alk phos, iron, TIBC)	•	•	•
3. Immunological assessment:	•	•	•
- ESR	•	•	•
- Total globulins and immunoglobulins	•	•	•
- Mantoux reaction at 6,12,24,48 & 72 h	•		
- Enumeration of lymphocyte subsets			
- Macrophage cell proliferation to mitogens and antigens	•	•	
- Macrophage cell production of IFN-α and IFN-γ	•	•	
- Natural killer (NK) cell activity:	•	•	
- spontaneous	•		
- lymphokine-activated	•		
- Monocyte random locomotion and chemotaxis	•		

Appendix 4c, p.269). The results from matched groups of patients and controls were compared by paired t-tests, whereas unequal groups were compared by pooled t-tests.

CHAPTER 3 - RESULTS

A. Study Population (Table 2)

Age was found to follow a log-normal distribution. The ages of 30 patients ranged from 19 to 61 years (mean 34.4, 95% confidence limits 30.1 - 39.4), compared with 20 to 62 years (mean 33.0, 95% confidence limits 29.5 - 37.1) among 30 matched controls. Sixteen patients (53%) were aged 35 years or less and 20 (67%) were males. Twenty-one (70%) were of Indian Subcontinent (ISC) origin. Only eight patients (27%) were Caucasians and one (3%) was African. Twenty-four patients (80%), including all 21 of ISC origin, were born outside the UK, with a median duration of residence of 60 months (range 0 - 264). In comparison, 22 controls (73%) born outside the UK had a median duration of residence of 96 months (range 24 - 216), P=0.69. Six immigrant patients (20%) were diagnosed and referred direct from the medical unit at Heathrow Airport (zero duration of residence in the UK); four of them (13%) had no symptoms. Twelve patients born abroad (48%) had tuberculosis detected within 60 months of their immigration to the UK (although it is possible that some had undetected active disease on arrival). Fifteen patients (50%) and 14 matched controls (47%) were vegetarians.

TABLE 2

Summary of matching characteristics of patients and controls

		Tuberculosis (n = 30)	Controls (n = 30)
Age (yrs) :	range	19 - 61	20 - 62
	mean (95% limits)	34.4 (30.1 - 38.4)	33.0 (29.5 - 37.1)
Sex :	male	20	20
	female	10	10
Ethnic group:	Indian Subcontinent (ISC)	21	21
	Caucasian	8	8
	African	1	1
Diet :	vegetarian	15	14
	non-vegetarian	15	16
Number born outside UK :		24	22
If born abroad, months in UK before patient's diagnosis:	range	*0 - 264	24 - 216
	median	60	96
	lower quartile	*0	78
	upper quartile	120	144

*0 = diagnosed on arrival at Heathrow Airport

8. Clinical features and laboratory data

Twenty-two patients (73%) had lung disease only, while two (7%) had each of meningeal, miliary, glandular (one cervical, one axillary) and bone (one spinal, one tibial) tuberculosis (Table 3). One patient with meningitis and another with glandular disease also had lung lesions. Seven of the eight patients (88%) with extrapulmonary disease were of ISC origin. All four asymptomatic patients had pulmonary disease: one had extensive disease (involving an area greater than the right upper lobe, RUL), while two had cavitation. The commonest symptoms among 20 symptomatic patients with pulmonary or miliary tuberculosis were weight loss (14 patients), feverishness (14), cough with sputum (13), night sweats (12), dyspnoea (7), malaise (5), pleuritic pain (5) and haemoptysis (3), ranging in duration from one to 24 weeks. Both patients with tuberculous meningitis had systemic symptoms (weight loss, night sweats, feverishness), but in addition had neck stiffness, headache and confusion of less than 14 days' duration; one also had fits and ataxia. The two patients with glandular disease complained of gland swelling, but only one had local pain and systemic symptoms. Both patients with bone disease had bone pain, local swelling and systemic upset. Haemoptysis and dyspnoea (except in patients with pleural effusions) were associated with more severe disease. Of 22 patients with pulmonary disease, nine (41%), including the two patients with miliary tuberculosis, had extensive lesions. Seven (32%) had cavitation and four (18%) had pleural effusions.

TABLE 3

Clinical features of patient population

Case no.	Sex	Age (yrs)	Ethnic group	Site of disease	Duration (wks)	Extent (grade)
1	M	26	Cauc	Pul	2	3
2	M	58	Cauc	Pul	4	4
3	M	21	ISC	Pul	4	4
4	F	24	ISC	Pul	8	2
5	M	25	ISC	Men + Pul	2	3
6	M	47	ISC	Pul	6	-
7	M	42	Cauc	Mil	2	5
8	F	29	ISC	Mil	4	5
9	F	52	ISC	Pul	No symptoms	5*
10	F	30	ISC	Men	1	-
11	M	40	ISC	Pul	7	5*
12	M	54	Cauc	Pul	13	5
13	M	19	ISC	Pul	24	5*
14	M	31	Cauc	Pul + eff	16	5
15	F	22	ISC	Pul	8	4*
16	F	41	ISC	Gland	3	-
17	M	34	ISC	Pul	1	4
18	M	35	ISC	Pul + eff	4	-
19	M	23	Afr	Pul	3	5*
20	M	40	ISC	Pul + eff	3	4
21	F	19	ISC	Pul	No symptoms	3
22	F	61	ISC	Bone	24	-
23	M	35	ISC	Bone	3	-
24	F	57	ISC	Gland + Pul	2	2
25	M	49	Cauc	Pul	1	4
26	M	[illegible]	ISC	Pul	7	4
27	M	[illegible]	ISC	Pul + eff	1	2
28	M	[illegible]	Cauc	Pul	No symptoms	4
29	M	[illegible]	Cauc	Pul	14	5*
30	F	[illegible]	ISC	Pul	No symptoms	4*

Ethnic group: Cauc = Caucasian; ISC = Indian Subcontinent; Afr = African

Site of disease: Pul = Pulmonary; Men = Meningeal; Mil = Miliary; eff = effusion.

Extent (grade) of lung disease [illegible]

[illegible]

[illegible]

* = [illegible]

- = [illegible]

1. Anthropometric indices

a) Weight-for-height-squared, W/H^2

On admission, tuberculosis patients had a significantly lower mean W/H^2 which gradually increased towards normal (control) values during treatment

Values for W/H^2 followed a normal distribution, ranging from 13.7 to 27.2 (mean 19.3, 1SD 3.7) among 30 patients, and from 17.7 to 27.2 (mean 22.2 1SD 2.6) among 30 controls, P=0.001 (Fig. 4). After six months of chemotherapy, 21 patients had a mean W/H^2 of 21.0 (3.5) compared with 22.2 (2.7) for 21 controls, P=0.30 At 12 months the mean W/H^2 for 15 patients (21.1, 1SD 4.1) was also comparable to that for 15 controls (22.1, 1SD 2.6), P=0.89. The maximum rate of recovery of body weight occurred in the first six months of treatment (Fig. 5).

b) Skinfold thickness (SFT)

The mean SFT of tuberculosis patients was significantly lower than that of controls, but normal values were restored during treatment.

Values for SFT required square-root transformations initially, but were normal at six months and log-normal at 12 months (Fig. 6). Initial mean SFT among 26 patients (7.81 mm, 95% limits 5.83-10.1) was significantly less than that for 28 controls (12.08 mm, 95% limits 10.01-14.4), P=0.02.

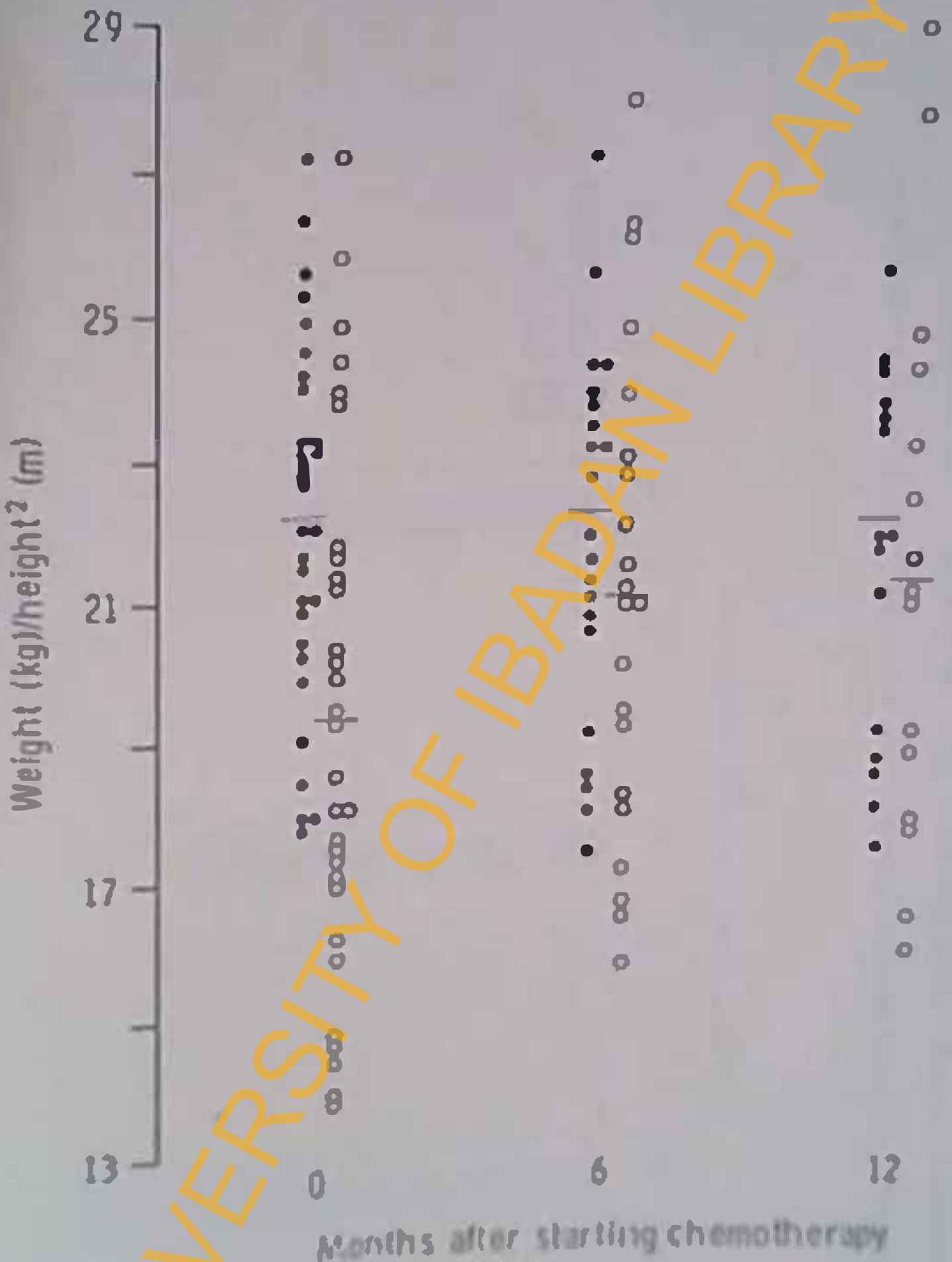

Fig 4: Distribution of weight-for-height squared (W/H²) among matched healthy controls (•) and tuberculosis patients (o).

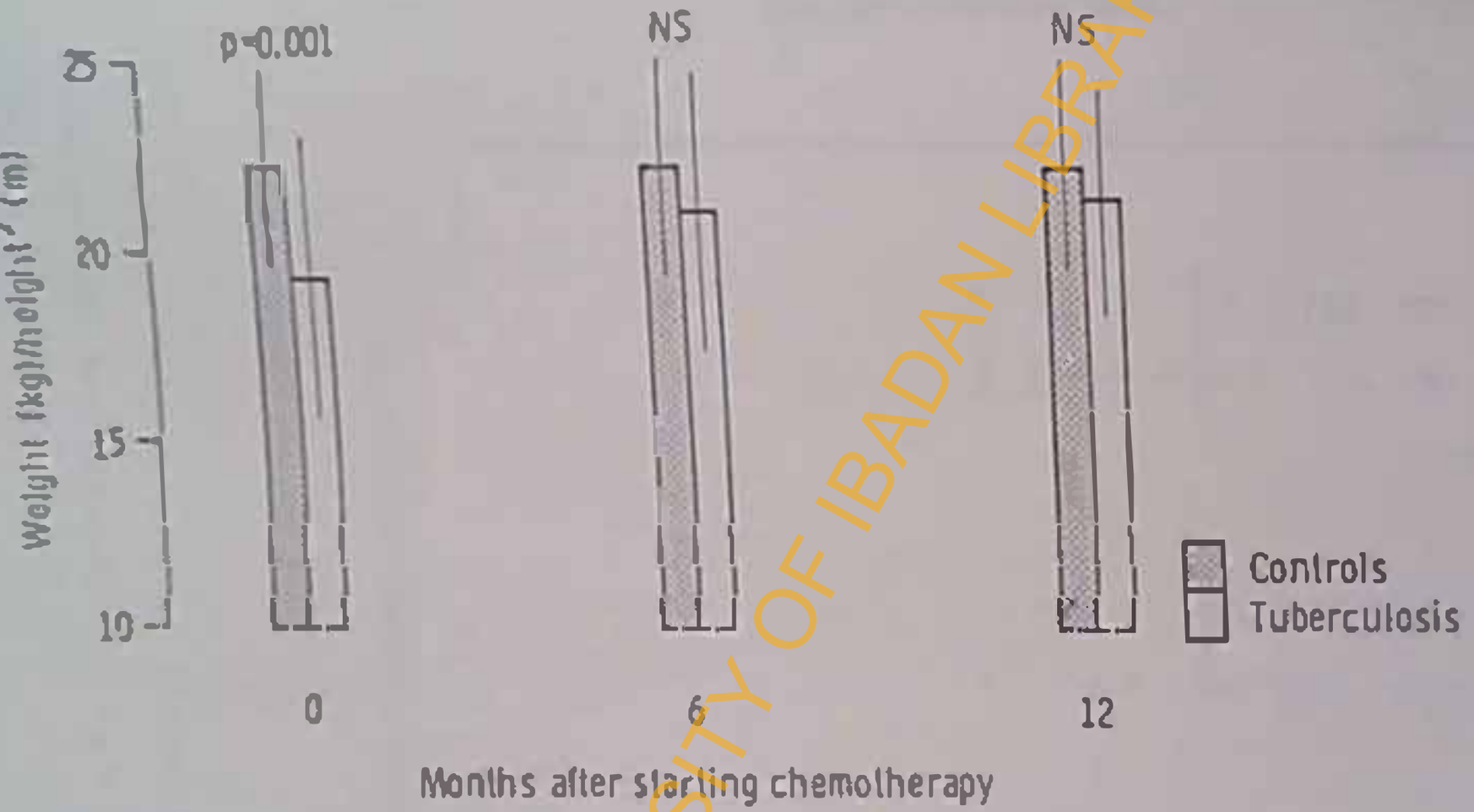

Fig 5: Changes in mean weight-for-height squared (W/H^2) of tuberculosis patients during chemotherapy. 2SD indicated. (n, controls: patients = 30:30, 21:21 and 15:15 at 0, 6 and 12 months respectively)

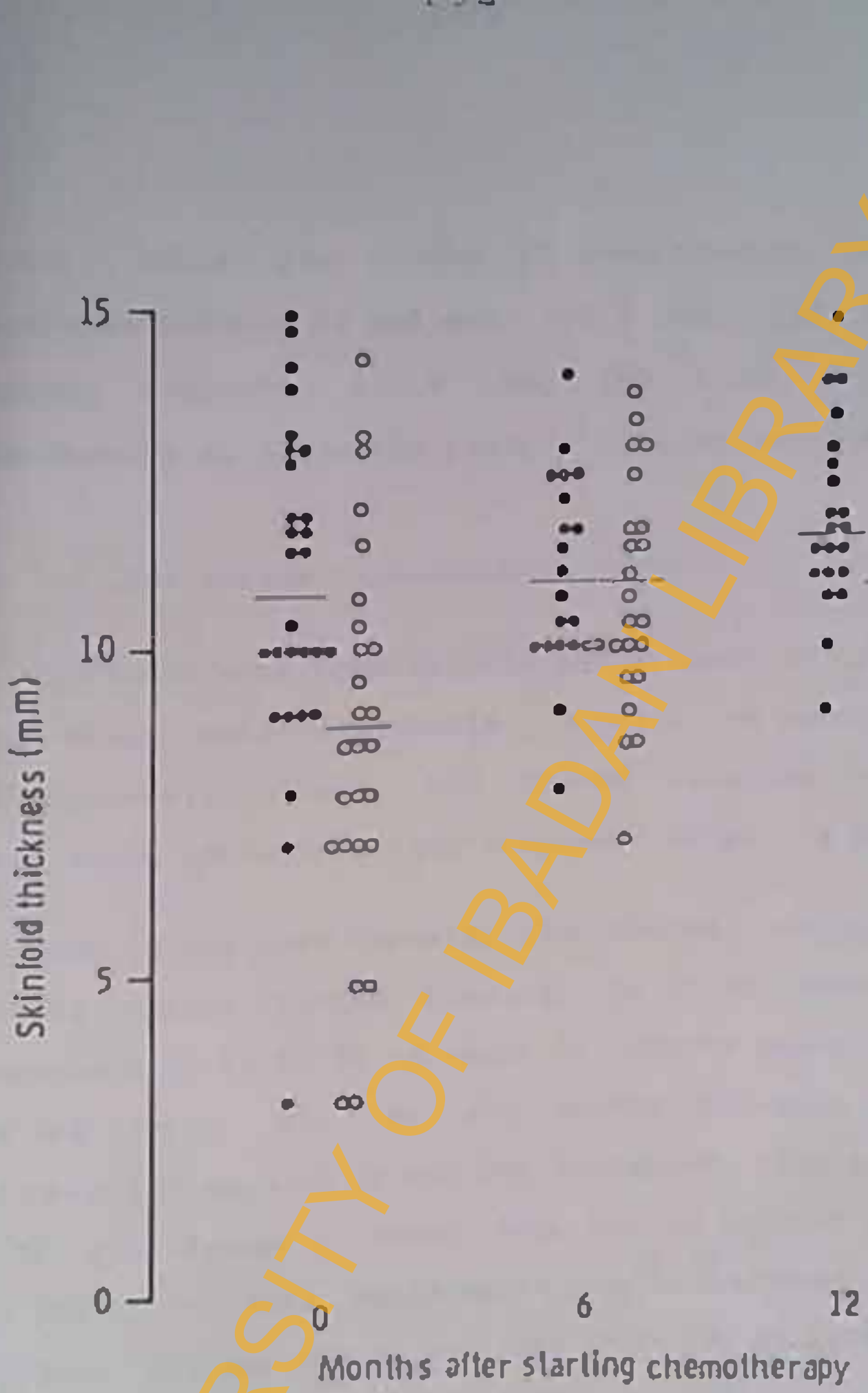

Fig 6: Distribution of mid-triceps skinfold thickness among matched healthy controls (•) and tuberculosis patients (o).

However, after six months of chemotherapy, mean SFTs were comparable between 22 patients (12.9 mm, 1SD 5.2) and 21 control subjects (12.8 mm, 1SD 3.8), P=1.0. Repeat measurements at 12 months yielded similar results (Fig. 7).

c) Arm muscle circumference (AMC)

Patients with tuberculosis had a lower initial mean AMC than their matched controls. However, in contrast to other anthropometric indices, AMC values remained significantly lower among patients during treatment (Figs. 8 and 9)

AMC values were normally distributed. Among 26 patients initial values ranged from 17 to 27 cm (mean 21, 1SD 3), compared with 19 to 30 cm (mean 24, 1SD 3) among 27 controls, P=0.001 (Fig. 8). At six months, the mean AMC among 22 patients (22 cm, 1SD 3) had not increased (P>0.5), and was still significantly lower than for 25 matched controls (24 cm, 1SD 3) P=0.004. Again at 12 months the mean AMC of 14 patients (21 cm, 1SD 3) was less than for 23 controls tested (24 cm, 1SD 3), P=0.023 (Fig. 9).

2. Haemoglobin (normal range for laboratory = 12.0-17.0 g/dl):

Patients were admitted with significantly lower haemoglobin (Hb) values than controls, but normal values were restored during treatment (Fig. 10).

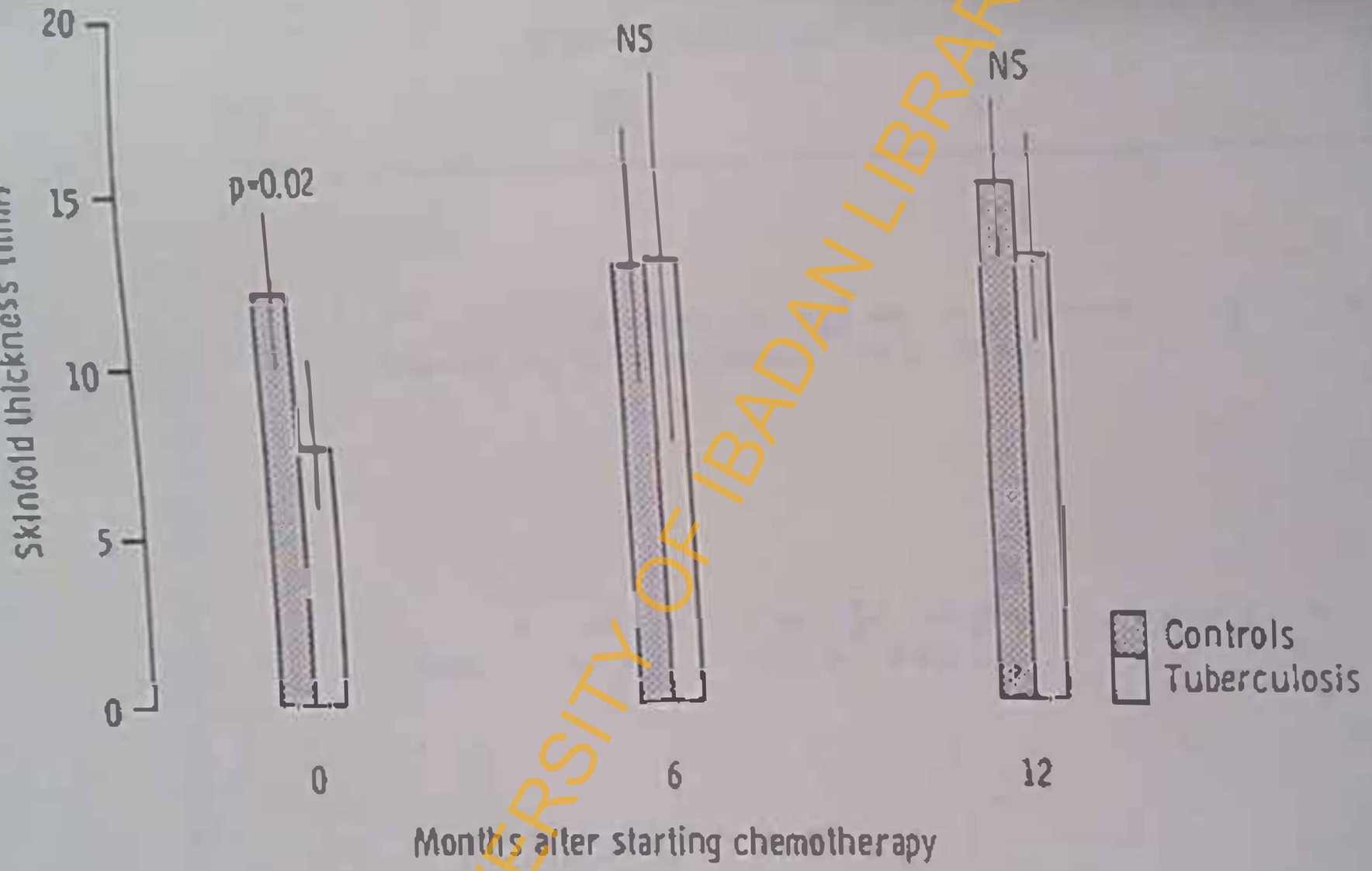

Fig 7: Changes in mean mid-triceps skinfold thickness of tuberculosis patients during chemotherapy. 2 SD or 95% confidence intervals indicated. (n, controls:patients = 28:26, 21:22 and 21:15 at 0, 6 and 12 months respectively)

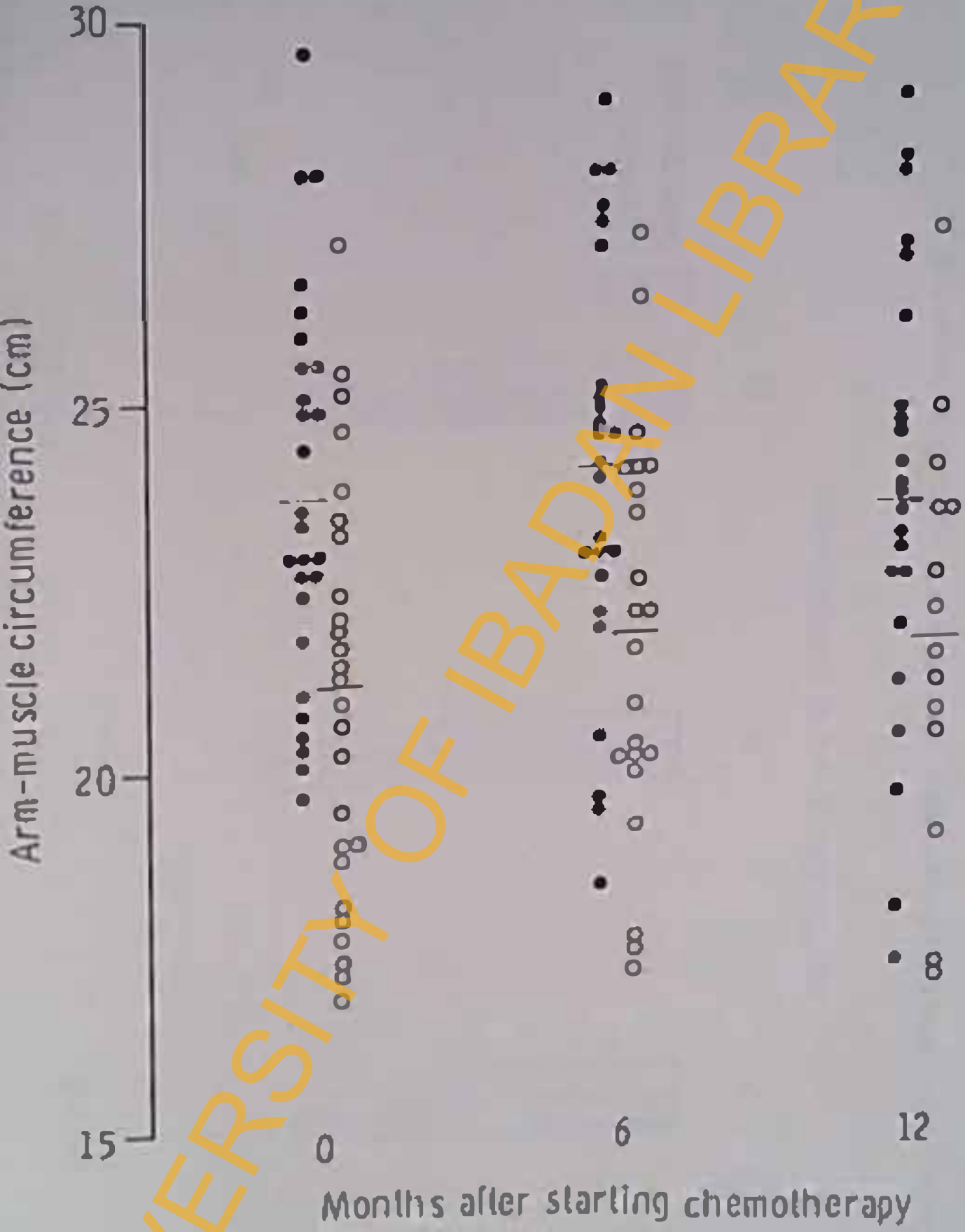

Fig 8: Distribution of mid-triceps arm-muscle circumference among matched healthy controls (•) and tuberculosis patients (o).

Fig 9: Changes in mean mid-triceps arm-muscle circumference of tuberculosis patients during chemotherapy. 2 SD or 95% confidence limits indicated. (n, controls:patients = 27:26, 25:22 and 23:14 at 0, 6 and 12 months respectively)

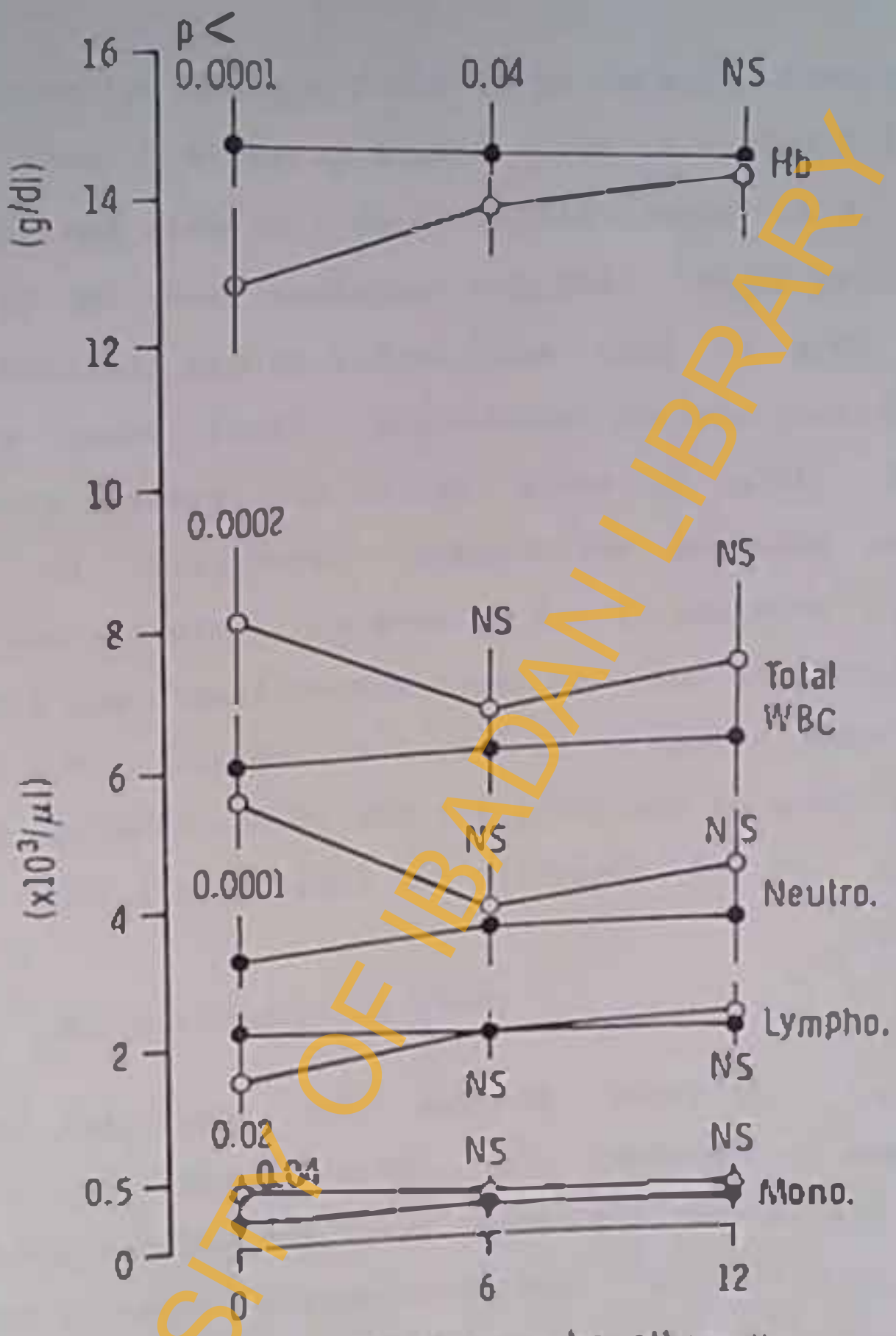

Fig 10: Changes in mean haemoglobin and blood cell counts of matchod healthy controls (●——●) and tuberculosis patients (○——○) during chemotherapy. 2SD or 95% confidence limits indicated. (n, controls:patients = 28:28, 19:15 and 14:11 at 0, 6 and 12 months respectively, except for lymphocyte counts where n = 27:25 before treatment)

Values for Hb were found to be normally distributed, and ranged from 7.9 to 17.3 g/dl (mean 12.9, 1SD 2.1) among 28 patients, and from 13.0 to 17.1 g/dl (mean 14.8, 1SD 1.2) among 28 matched controls, P<0.0001. Eight patients (29%) but no controls had Hb values less than 12 g/dl (P=0.003, Fisher's exact test). One patient and one control subject, both heavy smokers, had values above 17 g/dl. After six months of treatment, tuberculosis patients were still relatively anaemic: the mean Hb for 15 patients (13.9, 1SD 1.5 g/dl) was significantly lower than for 19 controls (14.6, 1SD 1.4 g/dl), P=0.04. However, by 12 months, mean Hb values for 11 patients (14.3, 1SD 1.8 g/dl) and 14 control subjects (14.5, 1SD 1.5 g/dl) were not different, P=0.84.

3. White blood cells (WBC)

In comparison with matched controls, tuberculosis patients had higher total WBC, neutrophil, monocyte and eosinophil counts, and lower lymphocyte counts, all of which returned to normal during treatment (Fig. 10).

a) Total WBC (normal range: 4-11 x $10^3/\mu l$):

These were also found to be normally distributed. Twenty-eight patients had WBC values ranging from 4.7 to 13.5 x $10^3/\mu l$ (mean 8.1, 1SD 2.2) compared with 4.2 to 9.7 x $10^3/\mu l$ (mean 6.1, 1SD 1.4) for 28 matched controls, P=0.0002. Four patients (14%) but no control subject had WBC counts above 11 x $10^3/ul$. Over the treatment period there was a

drop in total WBC counts of most patients so that at six months the mean total WBC count among 15 patients (6.8, 1SD 1.7 $10^3/\mu l$) was not different from that of 19 controls (6.2, 1SD 1.5 x $10^3/\mu l$), P-0.5. Similarly, at 12 months the mean count for 11 patients (7.4, 1SD 2.2 x $10^3/\mu l$) was comparable to that of 14 control subjects (6.3, 1SD 1.8 x $10^3/\mu l$), P-0.31.

b) Neutrophils (normal range: 2200-7000/μl):

These were initially log-normally distributed, but subsequently normally distributed. Values for 28 patients ranged from 3,249 to 10,375/μl (mean 5,568, 95% limits 4,931-6,288) compared with 1,554 to 5,402/μl (mean 3,320, 95% limits 2,853-3,864) for 28 matched controls, P<0.0001. Six patients (21%) but no controls had neutrophil counts above 7000/μl (P-0.01, Fisher's exact test) on admission to the study. Mean neutrophil counts were not different at six months between 15 patients (3886, 1SD 1287/μl) and 19 controls (3641, 1SD 1338/μl), and at 12 months between 11 patients (4423 1SD 1754/μl) and 14 controls (3700, 1SD 1415/μl). Figure 10 shows that the fall in the neutrophil counts of tuberculosis patients during chemotherapy occurred in parallel with the fall in their total WBC counts.

c) Lymphocytes (normal range: 1300-3000/µl):

Lymphocyte counts followed a normal distribution on all three occasions of testing. Twenty-five patients had initial values ranging from 332 to 2988/µl (mean 1535, 1SD 756), compared to 1261 to 3570/µl (mean 2187, 1SD 606) for 27 matched control subjects, P=0.02 (Fig. 11). Nine patients (32%), as against two controls (7%), had lymphocyte counts below 1300/µl (P=0.013, Fisher's exact test). Mean lymphocyte counts were not different at six months between 15 patients (2076, 1SD 638/µl) and 19 controls (2048, 1SD 695/µl) P=0.69, and at 12 months between 11 patients (2209, 1SD 862/µl) and 14 controls (1973, 1SD 731/µl), P=0.47. The initially low lymphocyte counts of tuberculosis patients rose within a few days of starting chemotherapy. Of seven patients with initial lymphocytopenia, consistently normal counts were restored within seven days of treatment in five, and within 14 days in the other two (Fig. 12).

d) Monocytes (normal range: 100-800/µl):

Monocyte counts required square-root transformation for normal distribution, except at six months when they were normally distributed. Initial values for 28 patients ranged from 47 to 1458/µl (mean 405, 95% limits, 286-545), compared to 0 to 836/µl (mean 253, 95% limits 185-331) for 38 controls, P=0.016. Four patients (14%) and only one control (6%) had monocyte counts above 800/µl (P=0.11, Fisher's exact test). Mean monocyte counts were not different at six months between 15 patients (304, 1SD 219/µl) and 19 controls (358 1SD 319/µl), P=0.59 and at 12 months between 11 patients

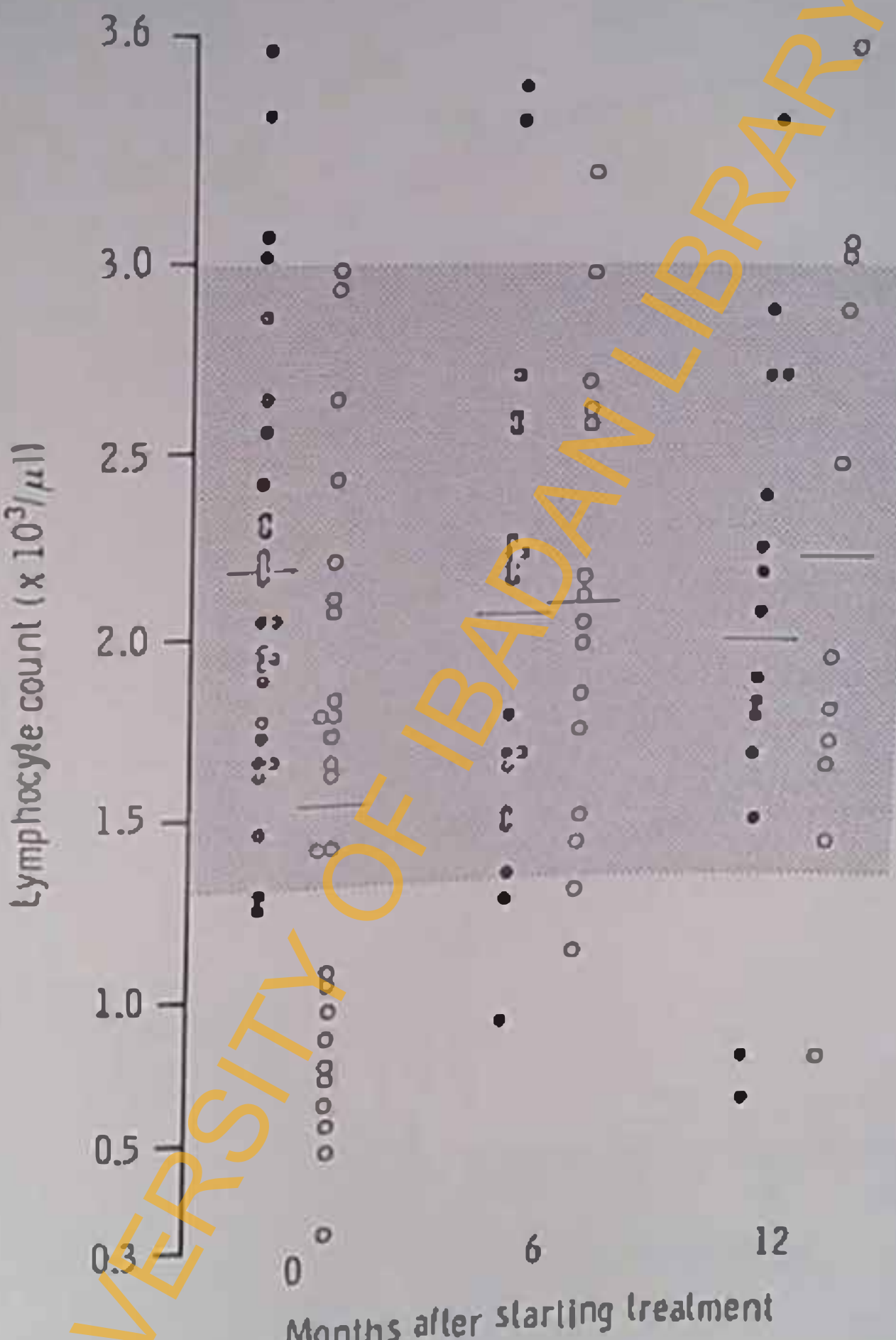

Fig 11: Distribution of peripheral blood lymphocyte counts among matched healthy controls (•) and tuberculosis patients (o). Shaded area represents the normal laboratory range (1300-3000/μl)

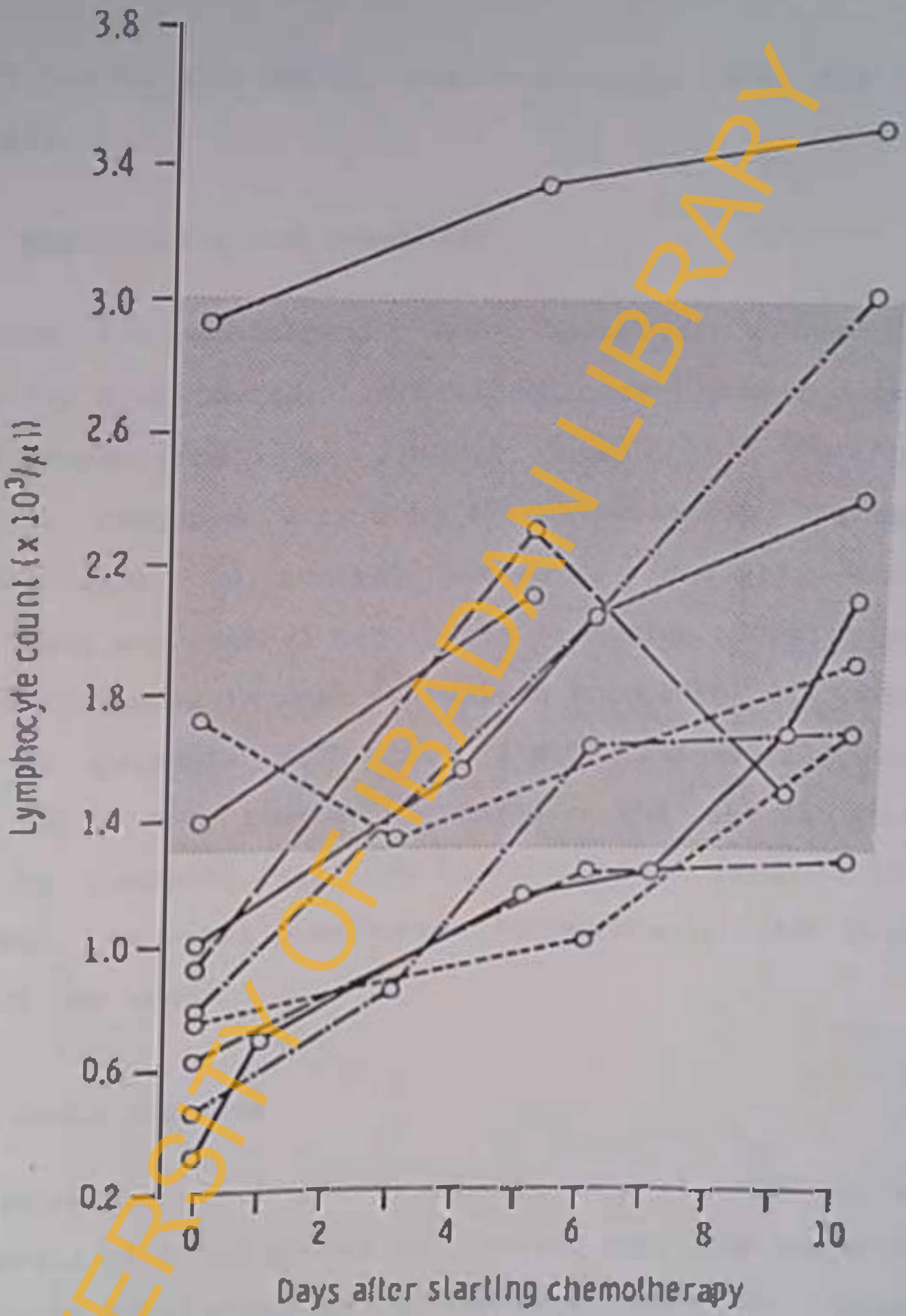

Fig 12: Early changes in peripheral blood lymphocyte counts following initiation of anti-tuberculosis chemotherapy in ten patients. Shaded area represents the normal laboratory range (1300-3000/µl)

(348, 95% limits 171-588/μl) and 14 controls (250, 95% limits 170-345/μl).

e) Eosinophils and basophils

Values for eosinophil and basophil counts were log-normally distributed. Initial eosinophil counts among 28 patients ranged from 0 to 2350/μl (mean 211, 95% limits 128-346/μl) compared with 0 to 472/μl (mean 105, 95% limits 74-148/μl) among 28 control subjects, P=0.017. Three patients and one control had counts exceeding 400/μl. There were no differences in mean eosinophil counts at six months between 15 patients (330, 95% limits 147-585/μl) and 19 controls (150, 95% limits 87-229/μl), and at 12 months between 11 patients (412, 1SD 134/μl) and 14 controls (200, 1SD 135/μl). Basophil counts were comparable in both groups throughout the study.

4. Serum proteins

Patients had significantly lower levels of albumin and higher levels of total globulins, IgG and IgA, than controls, but levels of total protein and IgM were comparable. Except for serum albumin, all values returned to normal during treatment (Fig. 13).

Values for total protein, albumin and globulin were normally distributed.

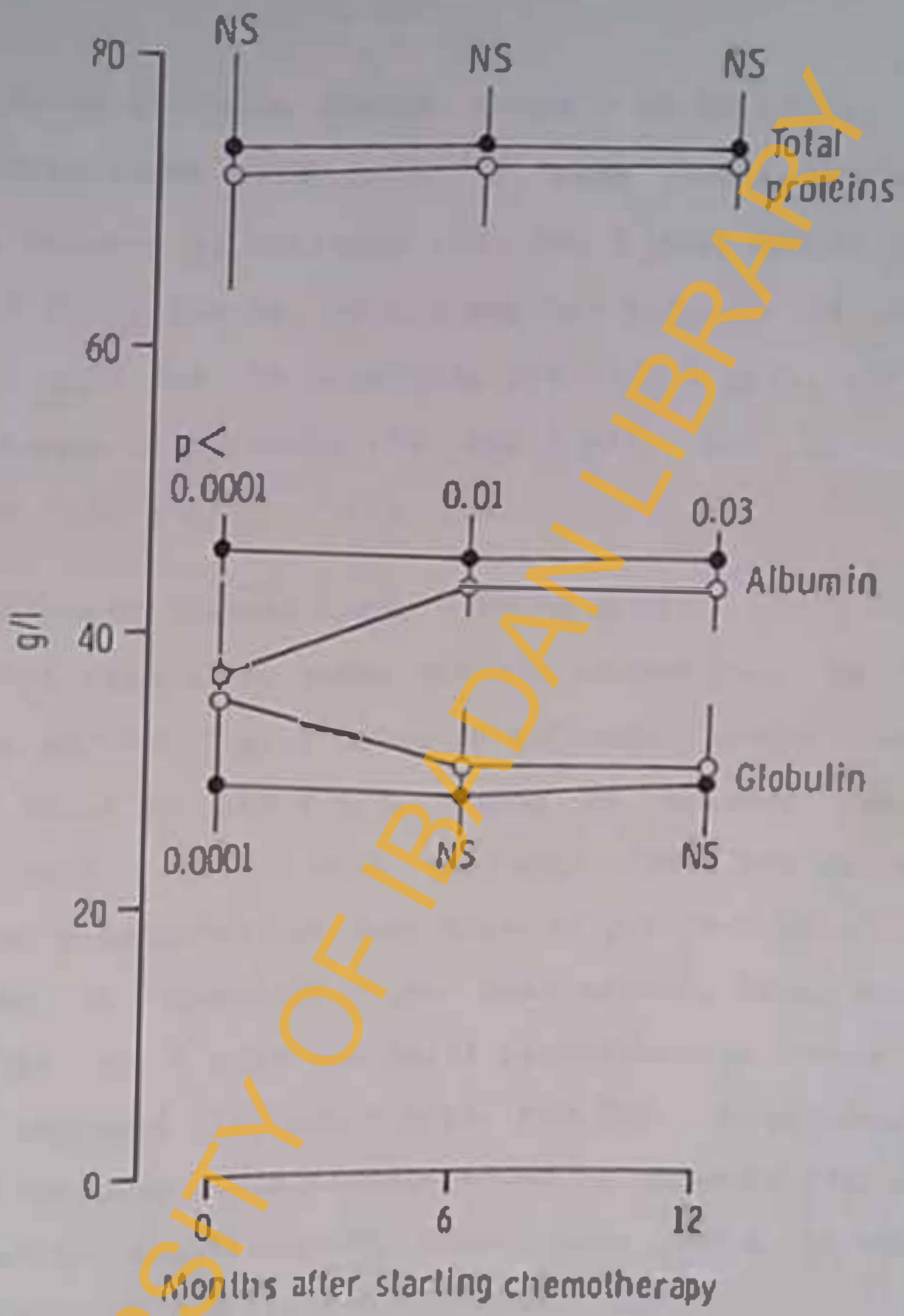

Fig 13: Changes in mean serum proteins of matched healthy controls (●——●) and tuberculosis patients (○——○). 2SD indicated. (n, controls:patients = 28:28, 19:15 and 18:12 at 0, 6 and 12 months respectively)

a) Total proteins (normal range - 60-80 g/l):

No differences were seen in mean total proteins on admission between 28 patients (72, 1SD 8 g/l) and 28 controls (74, 1SD 3 g/l), P-0.14, at six months between 15 patients (73, 1SD 4 g/l) and 19 controls (74, 1SD 4 g/l), and at 12 months between 12 patients (73, 1SD 2 g/l) and 18 controls tested (74, 1SD 4 g/l). (Fig. 13).

b) Albumin (normal range - 30-50 g/l):

Initial values for serum albumin ranged from 18 to 47 g/l (mean 37, 1SD 7 g/l) among 28 patients, compared with 41 to 51 g/l (mean 46, 1SD 2 g/l) among 28 matched controls, P<0.0001 (Fig. 14). Four patients (14%) but no control subject had albumin values less than 30 g/l (P-0.06). After six months of treatment, the mean albumin level among 15 patients (43, 1SD 2 g/l) was still significantly lower than among 19 controls (45, 1SD 3 g/l), P-0.013. Surprisingly at 12 months the mean serum albumin among 12 patients (43, 1SD 3 g/l) remained significantly lower than among 18 matched controls (45, 1SD 2 g/l), P-0.025 (Fig. 13).

c) Globulin (normal range - 20-35 g/l):

Initial serum globulin levels among 28 patients ranged from 26 to 49 g/l (mean 35, 1SD 6), compared with 23 to 34 g/l (mean 29, 1SD 3) among 28 controls, P<0.0001 (Fig. 15). A total of 11 patients (39%) but no controls had values greater than 35 g/l (P-0.001, Fisher's exact test). Mean globulin levels were not different at six months between 15

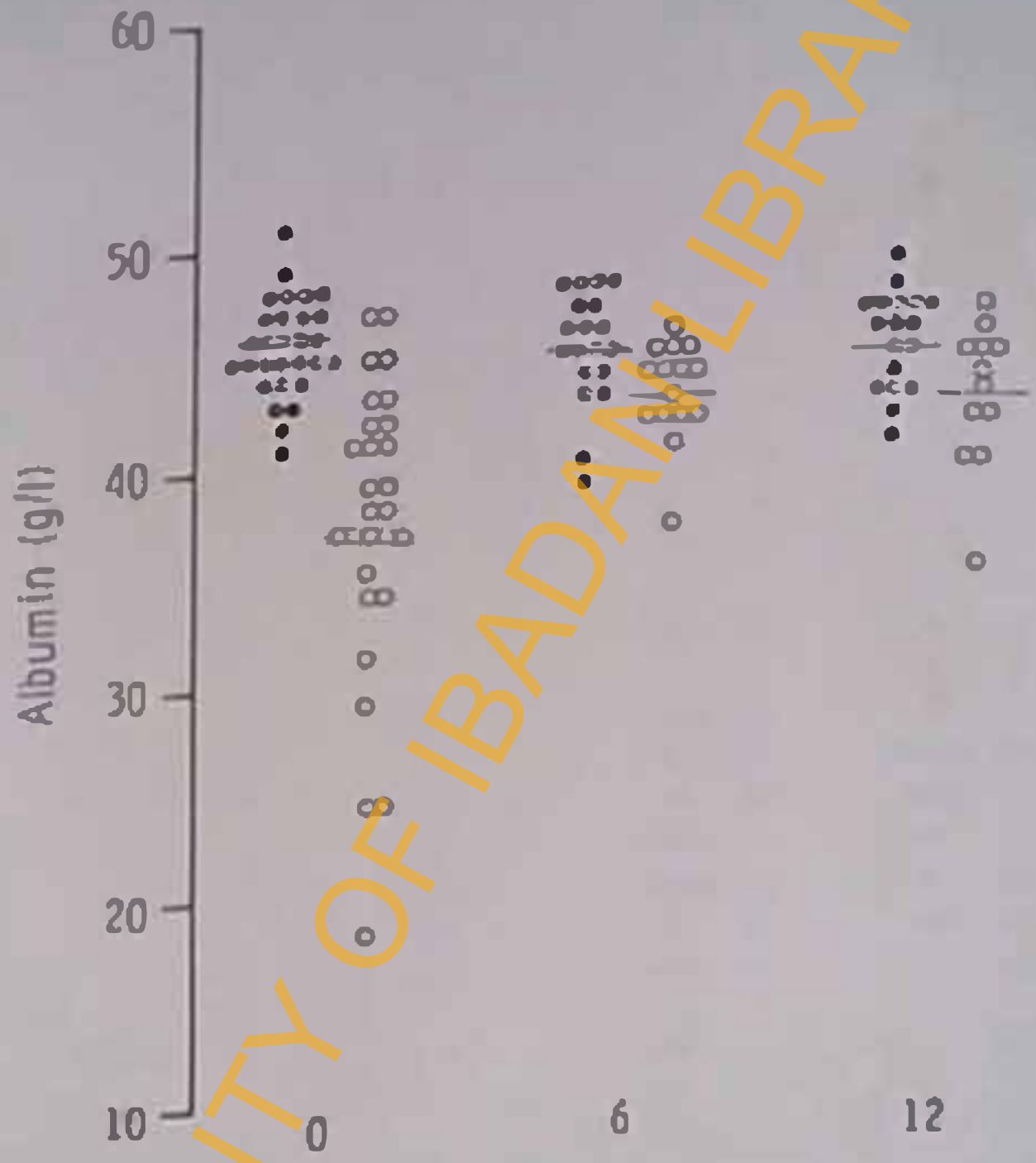

Fig 14: Distribution of serum albumin levels of matched healthy controls (•) and tuberculosis patients (o).

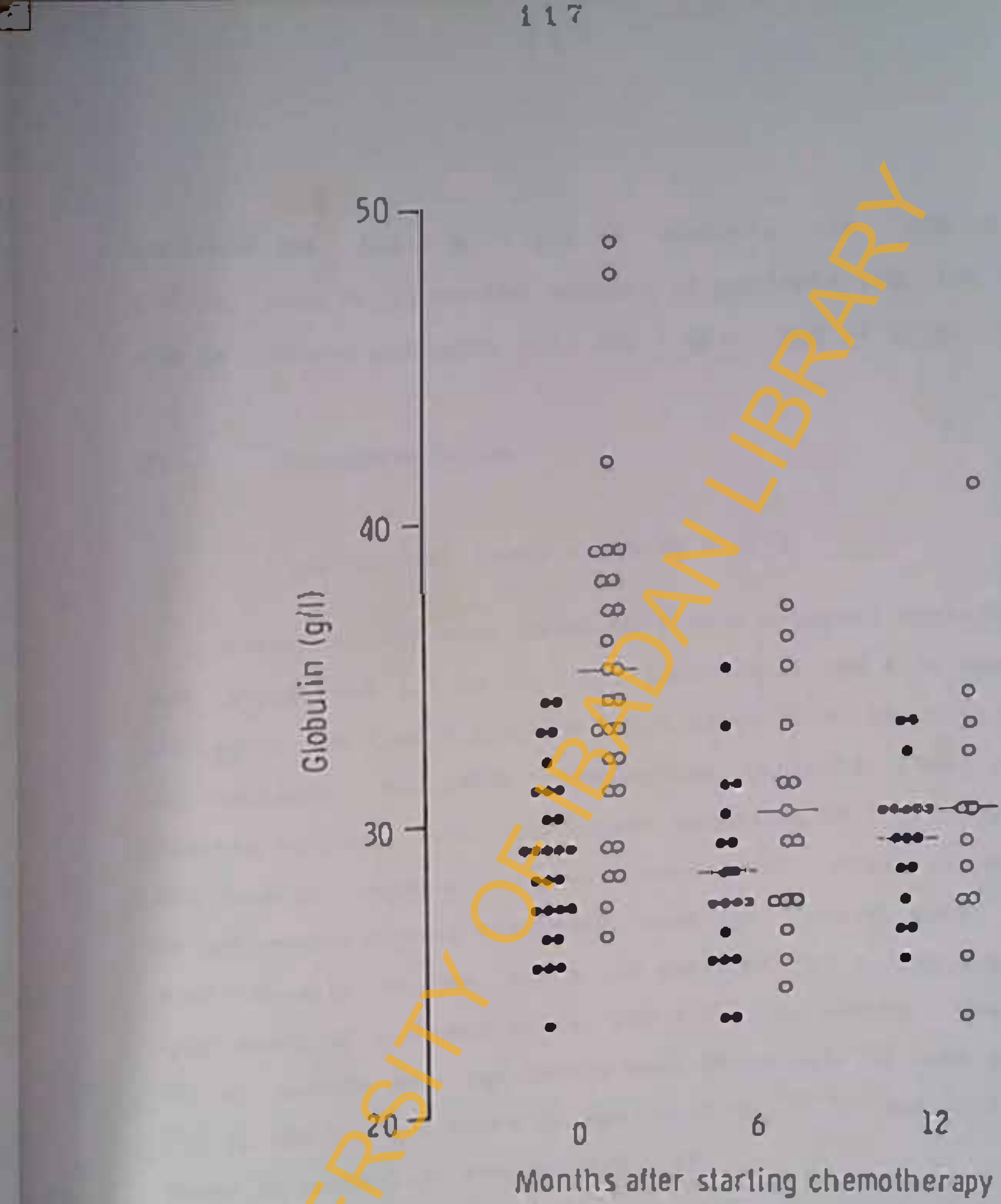

Fig 15: Distribution of serum globulin levels of matched healthy controls (•) and tuberculosis patients (o).

patients (30, 1SD 4 g/l) and 19 controls (28, 1SD 3 g/l) P-0.25, and at 12 months between 12 patients (30, 1SD 5 g/l) and 18 control subjects (29, 1SD 3 g/l), P-0.64 (Fig. 13).

d) Immunoglobulins

(i) IgG (normal range - 4.8-10.7 g/l):

Levels of IgG were found to follow a normal distribution and ranged from 6.7 to 27.8 g/l (mean 14.5, 1SD 4.7) among 28 patients, and from 7.1 to 14.3 g/l (mean 10.9, 1SD 1.9) among 28 controls, P-0.0005. Twenty-two patients (76%) and 15 control subjects (54%) had levels exceeding 10.7 g/l (P>0.1, chi-squared test with Yates' correction). After six months of anti-tuberculosis treatment, mean IgG levels were still significantly higher among 16 patients (13.7, 1SD 2.1 g/l) than among 19 controls (11.4, 1SD 1.9 g/l), P-0.04. However, at 12 months mean IgG levels were comparable in both groups (13.1, 1SD 3.0 g/l among 11 patients, and 12.2, 1SD 2.3 g/l among 18 controls), P-0.56 (Fig. 16).

(ii) IgA (normal range - 0.5-3.7 g/l):

Levels of IgA in patients were log-normal in distribution initially, but subsequently were normally distributed. Among 28 patients initial values ranged from 1.0 to 6.2 g/l (mean 2.6, 95% limits 2.2-3.1 g/l), compared with 0.6 to 4.5 g/l (mean 2.0, 95% limits 1.6-2.5 g/l) among 28 controls, P-0.04. Four patients (14%) and one control

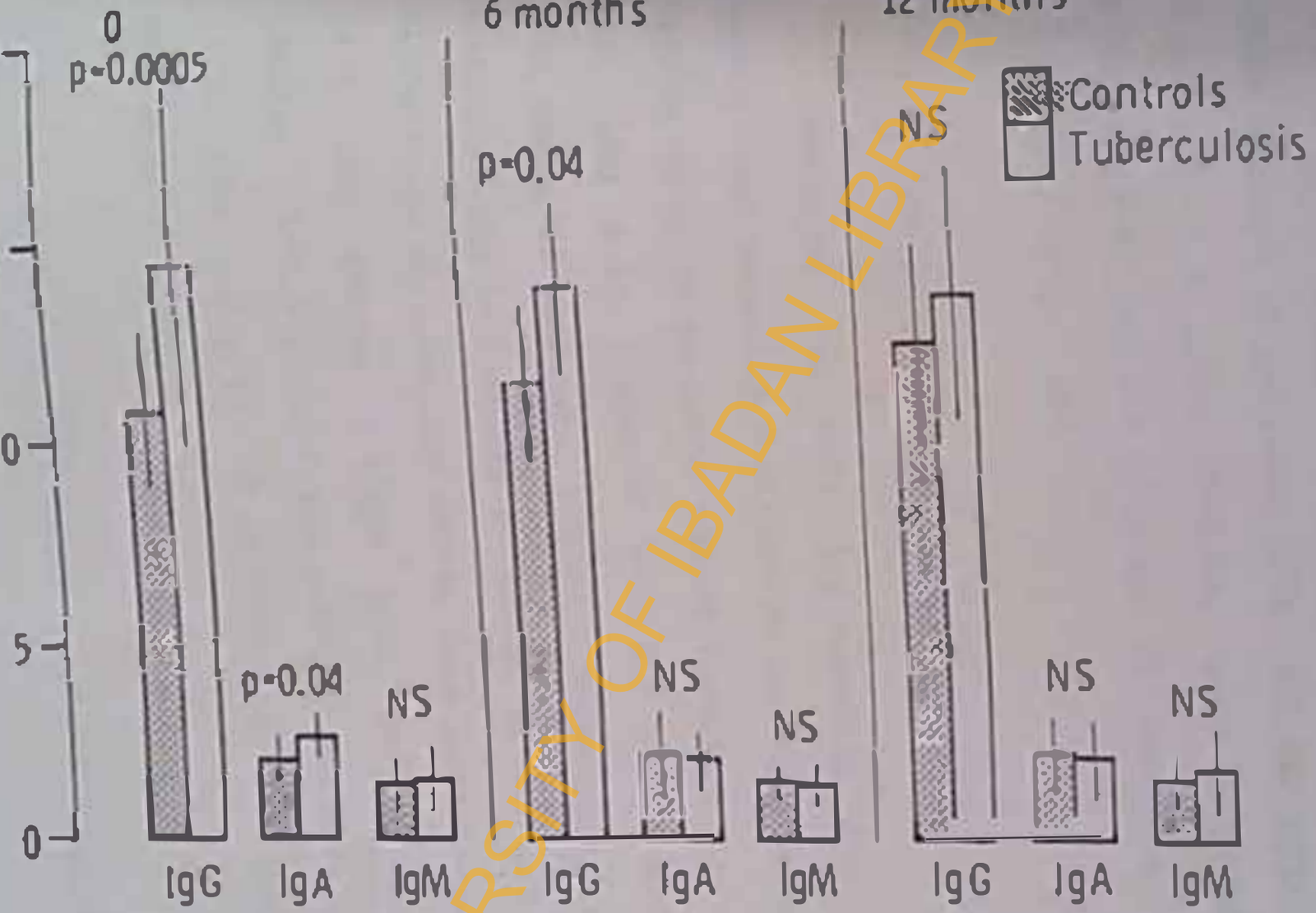

Fig 16: Changes in mean serum immunoglobulins of tuberculosis patients during chemotherapy. 2 SD or 95% confidence limits indicated. (n, controls:patients = 28:28, 19:16 and 18:11 at 0, 6 and 12 months respectively)

(4%) had IgA levels above 3.7 g/l (P=0.16, Fisher's exact test). No differences were seen in mean IgA levels at six months between 16 patients (2.0, 1SD 0.7 g/l) and 19 controls (2.1, 1SD 1.1 g/l), P=0.78, and at 12 months between 11 patients (2.0, 1SD 0.9 g/l) and 18 controls (2.1, 1SD 0.8 g/l), P=0.5 (Fig. 16).

(iii) IgM (normal range = 0.4-2.5 g/l):

Levels of IgM were log-normally distributed at six months but normally distributed on other occasions. Initial values among 28 patients ranged from 0.4 to 3.6 g/l (mean 1.5, 1SD 0.8) compared with 0.3 to 2.8 g/l (mean 1.4, 1SD 0.6) among 28 controls, P=0.55. Three patients (10%) and two controls (7%) had values in excess of 2.5 g/l. Similarly, no differences in mean IgM levels were seen between 16 patients (1.3, 95% limits 0.9-1.9 g/l) and 19 controls (1.4, 95% limits, 1.1-1.8 g/l) at six months, and between 11 patients (1.7, 1SD 1.0 g/l) and 18 controls (1.5, 1SD 0.6 g/l) at 12 months (Fig. 16).

5. Sodium (normal range - 136-147 mmol/l):

Initial levels of serum sodium were significantly lower in patients than controls, but returned to normal during treatment (Fig.17).

Surprisingly, serum sodium levels did not follow a Gaussian distribution after several transformations, and were therefore compared using a non-parametric test. Among 26

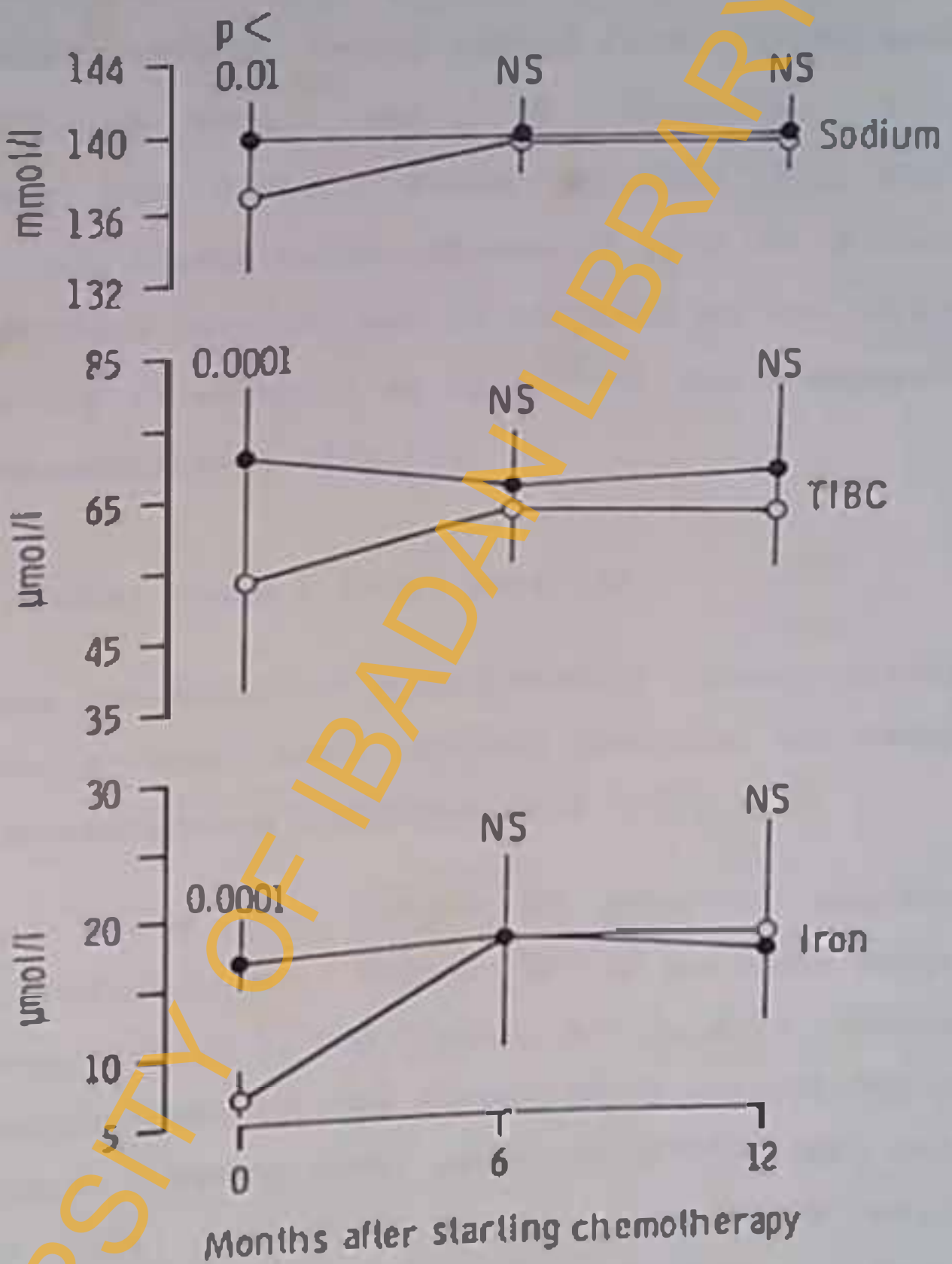

Fig 17: Changes in mean serum levels of sodium, total iron binding capacity (TIBC) and iron of matched healthy controls (●——●) and tuberculosis patients (o——o) during chemotherapy. 2SD or 95% confidence limits indicated. (n, controls:patients = 26:26, 19:15 and 17:12 at 0, 6 and 12 months respectively)

patients, initial sodium levels ranged from 122-142 mmol/l compared to 136-144 mmol/l among 26 controls, P<0.01 (Wilcoxon's rank sum test). Seven patients (27%) but no control subject had levels below 135 mmol/l (P=0.01, Fisher's exact test). Fifteen patients and 19 controls at six months, and 12 patients and 17 controls at 12 months had comparable serum sodium concentrations (Fig.17).

6. Iron (normal range - 12-27 µmol/l):

Tuberculosis patients had significantly lower initial serum iron levels than their matched controls, but normal values were restored during treatment (Fig. 17).

The initial serum iron levels in patients required square-root transformation. Values for 26 patients ranged from 3 to 18 µmol/l (mean 7, 95% limits, 6-9 µmol/l) compared with 4 to 28 µmol/l (mean 17, 95% limits 15-20 µmol/l) for 26 controls, P<0.0001. Twenty-four patients (92%) but only three controls (12%) had iron levels less than 12 µmol/l (P<0.0001, chi-squared test with Yates' correction). There were no differences in mean serum iron at six months between 15 patients (18, SD 8 µmol/l) and 19 controls (18, SD 6 µmol/l), and at 12 months between 12 patients (18, SD 8 µmol/l) and 17 controls (17, SD 5 µmol/l).

7. Total iron binding capacity (TIBC, normal range - 40-75 µmol/l):

Tuberculosis patients also had lower initial levels of TIBC than controls, which increased to normal during treatment (Fig. 17)

TIBC values were normally distributed. Among 26 patients TIBC ranged from 15 to 84 µmol/l (mean 54, 1SD 16) compared to 50 to 90 µmol/l (mean 71, 1SD 10) among 26 control subjects, P<0.0001. Six patients (23%) and no control had values less than 40 µmol/l (P=0.01, Fisher's exact probability test). After six months of therapy mean TIBC values were comparable between 15 patients (63, 1SD 8 µmol/l) and 19 controls (66, 1SD, 8 µmol/l), P=0.17. Also at 12 months no difference was seen between 12 patients (62, 1SD 8 µmol/l) and 17 controls (68, 1SD 12 µmol/l), P=0.39.

8. Alkaline phosphatase (normal range - 80-280 iu/l)

Patients had a higher mean level of alkaline phosphatase than controls but normal levels were restored during chemotherapy.

Initial levels were log-normally distributed, and ranged from 68 to 999 iu/l (mean 246, 95% limits 201-301) among 26 patients compared with 58 to 231 iu/l (mean 150, 95% limits 133-170) among 26 controls, P<0.001. Ten patients (38%) but no controls had levels above 280 iu/l (P=0.001, Fisher's exact probability test). No differences were seen in mean alkaline phosphatase values at six months between 15 patients (208, 1SD 126 iu/l) and 18 controls (154, 1SD 36 iu/l)

P=0.16, and at 12 months between 12 patients (196, 1SD 83 iu/l) and 17 controls (145, 1SD 30 iu/l) P=0.10.

9. Aspartate transaminase (AST, normal range - 10-35 iu/l):

AST values of patients were found to be log-normally distributed, except at 12 months. No differences were observed between the mean AST values of patients and controls on admission (22, 95% limits 17-30 and 20, 95% limits 18-22 iu/l, respectively, P=0.57), at six months (22, 95% limits 19-27 and 21, 95% limits 17-25 iu/l respectively, P=0.35), and at 12 months (21, 1SD 4 and 23 1SD 8 iu/l respectively, P=0.28).

10. Erythrocyte sedimentation rate (ESR, Westergren, normal range - 0-7 mm/hr):

The mean ESR of tuberculosis patients was much higher than control values, rose further in the first few weeks of treatment, but fell to normal levels by six months (Figs. 18 and 19).

ESR values were log-normally distributed and ranged from 7 to 140 mm/hr (mean 37, 95% limits 26-51 mm/hr) among 28 patients compared with 1 to 10 mm/hr (mean 3, 95% limits 2-4 mm/hr) among 27 matched controls, P<0.001. Twenty-six Patients (93%) but only three healthy controls (11%) had ESR values above 7 mm/hr (P<0.001, chi-squared test with Yates' correction). Chemotherapy was associated, in eight out of

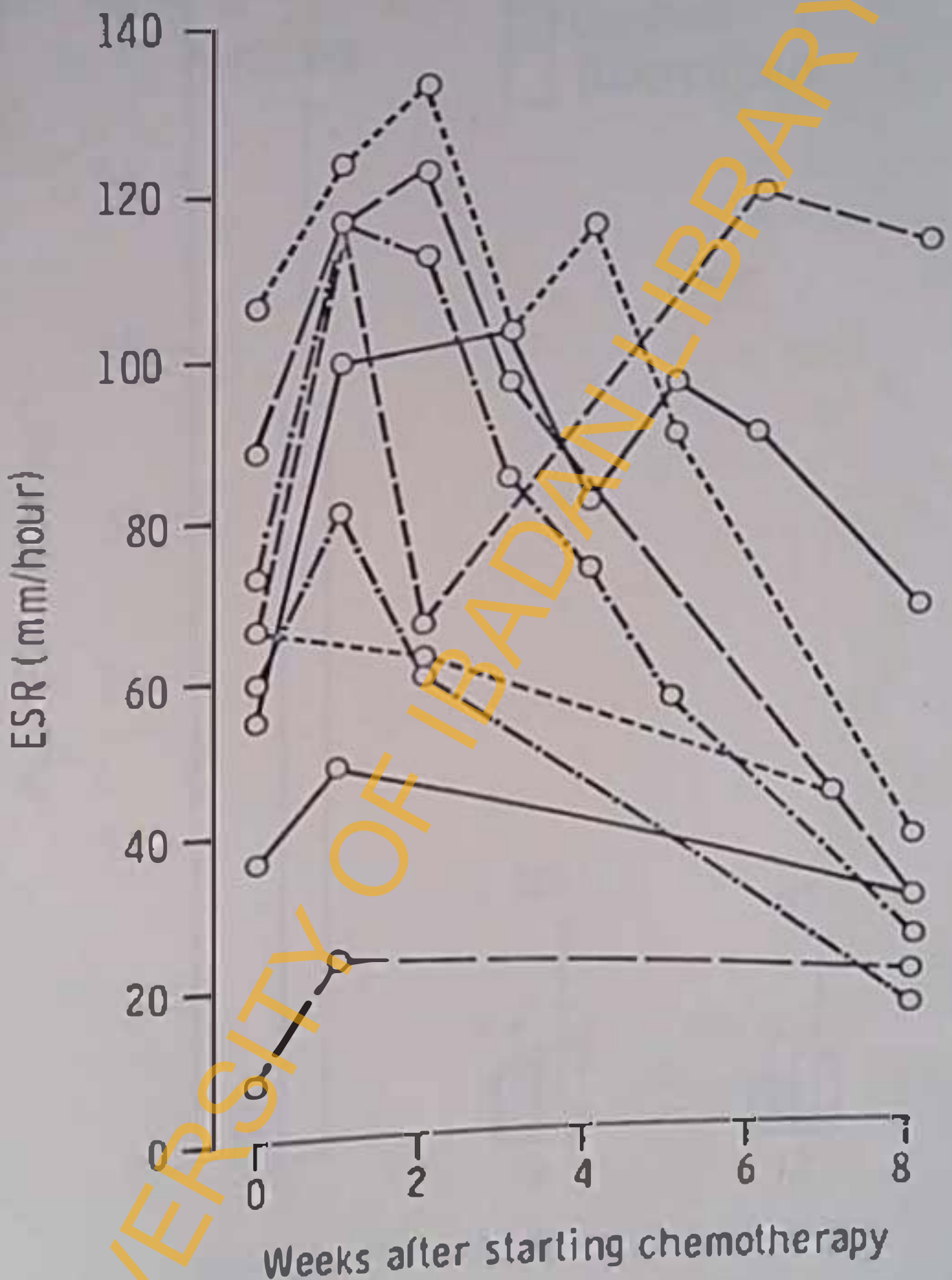

Fig 18: Early changes in erythrocyte sedimentation rates (ESR) of nine patients following initiation of chemotherapy.

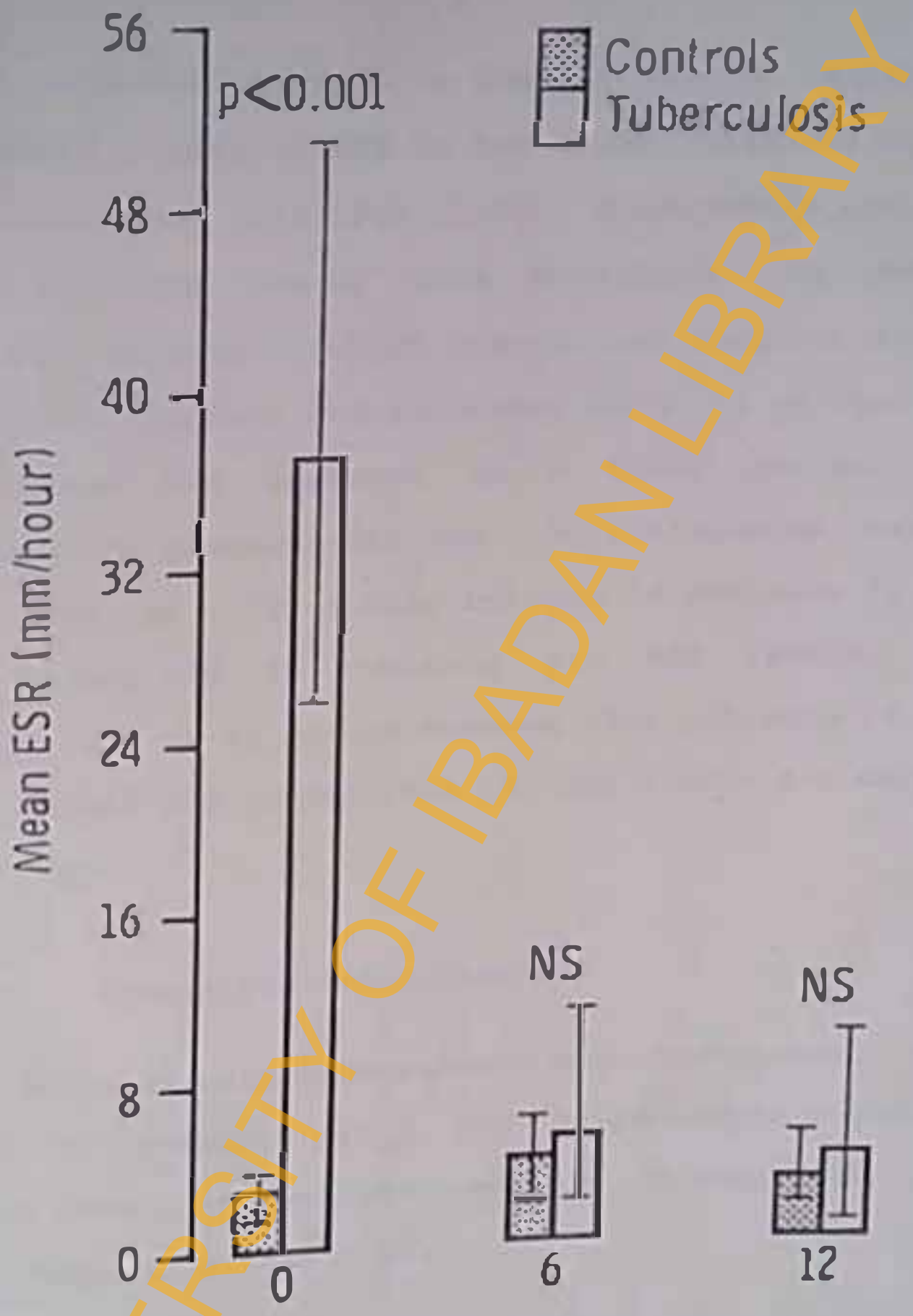

Fig 19: Changes in mean erythrocyte sedimentation rate (ESR) of tuberculosis patients during chemotherapy. 95% confidence limits indicated. (n, controls:patients = 27:28, 20:14 and 15:9 at 0, 6 and 12 months respectively)

nine patients studied in detail, with an initial sharp rise in ESR to a peak at one to two weeks, followed by a gradual and continuous fall (Fig. 18). A secondary rise occurred in three patients, two of whom developed complications. One patient developed sudden unexplained respiratory distress at two weeks, another had pulmonary embolism at four weeks, but no reason was apparent in a third patient who had a tuberculous pleural effusion. No differences were seen in mean ESR at six months between 14 patients (5, 95% limits 2-11 mm/hr) and 20 controls (4, 95% limits, 2-6 mm/hr) P-0.23, and at 12 months between nine patients (4, 95% limits 1-10 mm/hr) and 15 controls (3, 95% limits 2-5 mm/hr), P-0.55 Fig. 19).

C. Lymphocyte subpopulations

Patients with tuberculosis had significantly lower mean total T, T-helper (T_H) and B-lymphocyte peripheral blood counts than their matched controls. T-suppressor (T_S) counts were comparable (Fig. 20).

Among 13 patients and 12 matched controls, total T, T_H, T_S and B lymphocyte phenotypes were defined with fluorescein-conjugated monoclonal antibodies. T_H and T_S counts, and T_H/T_S ratios were derived from the absolute lymphocyte counts. All variables examined required base-ten logarithmic transformations for Gaussian distribution, and comparisons were made between the groups by pooled t tests. Lymphocyte counts (by morphology) among these 13 patients

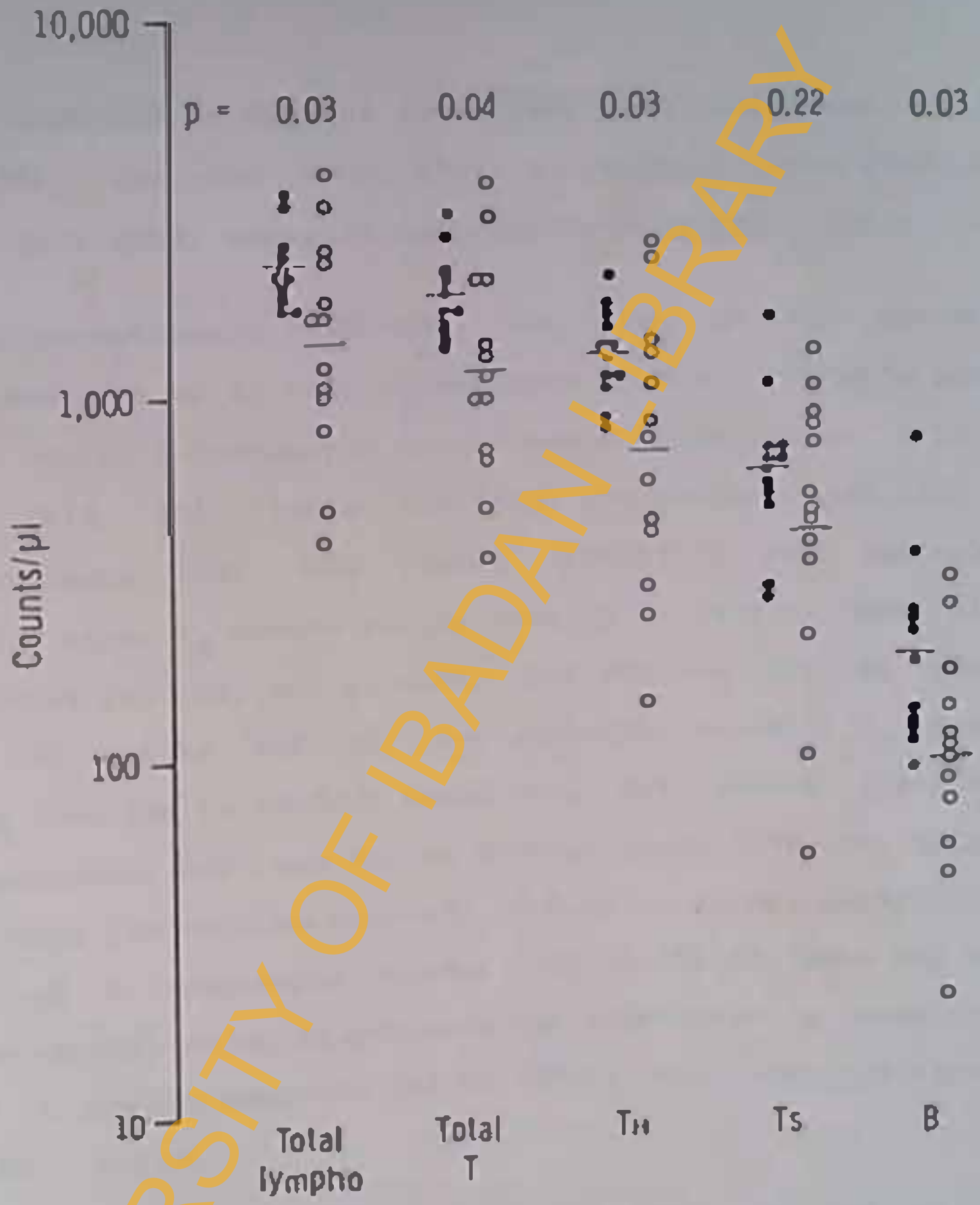

Fig 20: Absolute numbers (/µl) of circulating lymphocytes, total T (Leu 1/4 +), suppressor/cytotoxic T (T_S, Leu 2a +), helper/inducer T (T_H, Leu 3a +) and total B (Leu 12 +) lymphocytes in the peripheral blood of matched healthy controls (•) and tuberculosis patients (o).

ranged from 405 to 4120/μl (mean 1407, 95% confidence limits 925-2139), compared with 1776 to 3570/μl (mean 2282, 95% limits 1975-2639) among 12 controls, P-0.03 Fig. 20).

Lymphocytopenia (<1300/μl) was seen in six patients (46%) and none of 12 control subjects (P-0.01, Fisher's exact test). Total T-lymphocyte counts ranged from 356 to 3832/μl (mean 1171, 95% limits 768-1785) for patients and 1365 to 3106/μl (mean 1885, 95% limits 1606-2212) for controls, P-0.04. Also, T_S counts ranged from 49 to 1318/μl (mean 411, 95% limits 231-732) for patients, and 267 to 1607/μl (mean 603, 95% limits 443-822) for controls, P-0.22. T_H counts ranged from 138 to 2544/μl (mean 685, 95% limits 402-1167) for patients, and from 825 to 2103/μl (mean 1279, 95% limits 1067-1532) for matched controls, P-0.03. Surprisingly, the range of B lymphocyte counts (20 to 301/μl, mean 93, 95% limits 58-150) among 12 patients was significantly lower than among 11 control subjects (89 to 750/μl, mean 184, 95% limits 120-282), P-0.03.

Decreased T_H/T_S ratios (<1.2) were seen in two of 13 patients (15%) and in one of 12 controls (8%). The mean T_H/T_S ratio was 1.67 (95% limits 0.98-2.82) for patients, compared with 2.12 (95% limits 1.51-2.98) for controls, P-0.42. Both patients with decreased T_H/T_S ratios also had absolute lymphocytopenia, which implies decreased T_H cells. In the course of chemotherapy, a gradual increase in T_H/T_S ration (and lymphocyte counts) was observed in both patients: patient 1 - from 0.19 (total lymphocyte count, TLC 1062/μl)

to 0.20 (TLC 1206 /μl) at four weeks, and to 0.74 (TLC 2407/μl) at 14 weeks; patient 2 - from 0.66 (TLC 1003/μl) to 0.72 (TLC 1969/μl) at two weeks, and to 0.98 (TLC 1482/μl) at seven weeks (Table 4). In contrast, the control subject with a low T_H/T_S ratio (0.96) had an increased TLC (3570/μl), implying an increase in T_S cells. He was perfectly healthy and non homosexual, but reported an attack of chickenpox four to six weeks previously.

D. Tuberculin skin reactions

Tuberculosis patients had significantly higher proportions of early (6 hr) reactions and larger reaction sizes to 1TU PPD than matched healthy controls. Repeat testing during treatment showed a significant increase in the proportions of patients giving both early and delayed (48 hr) reactions, the latter to 100%, and the absolute reaction sizes. In contrast, repeat testing of controls increased the proportions only of controls giving early (but not of those giving delayed) reactions, and the absolute reaction sizes.

Twenty-eight patients and 26 matched controls were skin-tested with 1TU, and if negative 10TU PPD, and reactions were read at 6,12,24,48 and 72 hrs. All values for diameter of induration were normally distributed, except at 48 hrs when base-ten logarithmic transformations were necessary. Comparisons of proportions between the groups were performed using the chi-squared test with Yates' correction for discontinuity (Armitage 1971). Where comparisons of

TABLE 4

T-helper/suppressor ratios in 12 normal controls and 13 tuberculosis patients

Case No.	T-helper/suppressor ratio	
	Controls	Patient
22	2.04	2.71
26	3.03	1.91
27	1.85	3.04
29	6.25	2.60
31	1.71	0.19
32	1.47	0.64
33	0.96	2.52
34	1.67	2.13
35	2.44	1.64
36	0.83	1.60
37	1.21	1.78
38	1.64	1.59
39	-	1.87
Repeats		
31 (4 wks later)	-	0.20
31 (10 wks later)	-	0.74
32 (2 wks later)	-	0.73
32 (7 wks later)	-	0.88

proportions obtained on different occasions within a group were performed with McNemar's test (Armitage 1971). Similarly, reaction sizes between unequal groups were compared by pooled t tests, while reaction sizes obtained on different occasions in the same individuals within a group were compared by paired t-tests.

1. Tuberculin reactions before treatment (Fig. 21):

Fourteen of 24 patients (58%) with active tuberculosis had reactions at 6 hrs to 1TU PPD compared with none of 24 matched controls, P<0.001. However, the mean size of induration at 6 hrs among reactors was only 5.1 (1 SD 1.4) mm. At 12 hrs, 17 patients (71%) reacted, compared with only 2 controls (8%), P<0.001, although the mean reaction sizes were comparable - 8.2 (1 SD 2.7) mm for patients, and 8.0 (1 SD 2.8) mm for controls. At 24 hrs, the proportions of positive reactions - 20 of 28 patients (71%) and 10 of 24 controls (42%) - were not different (P>0.05), but patients had a higher mean diameter of induration (12.8, 1 SD 3.3 mm) than controls (8.9, 1 SD 3.4 mm), P<0.05. The proportions reacting at 48 hrs were also similar: 21 of 28 patients (75%) and 16 of 26 controls (62%), P>0.5. However, the best discrimination in reaction sizes occurred at 48 hrs between skin test positive patients (17.5, 95% confidence limits 14.4-19.2 mm) and controls (12.8, 95% confidence limits 8.6-15.1 mm), P<0.05. No differences were seen at 72 hrs either in the proportions reacting to low dose tuberculin (79% and 62% respectively, P>0.1) or in the mean reaction sizes (14.0, 1 SD 4.2 mm and 12.0, 1 SD 4.7 mm respectively, P>0.1).

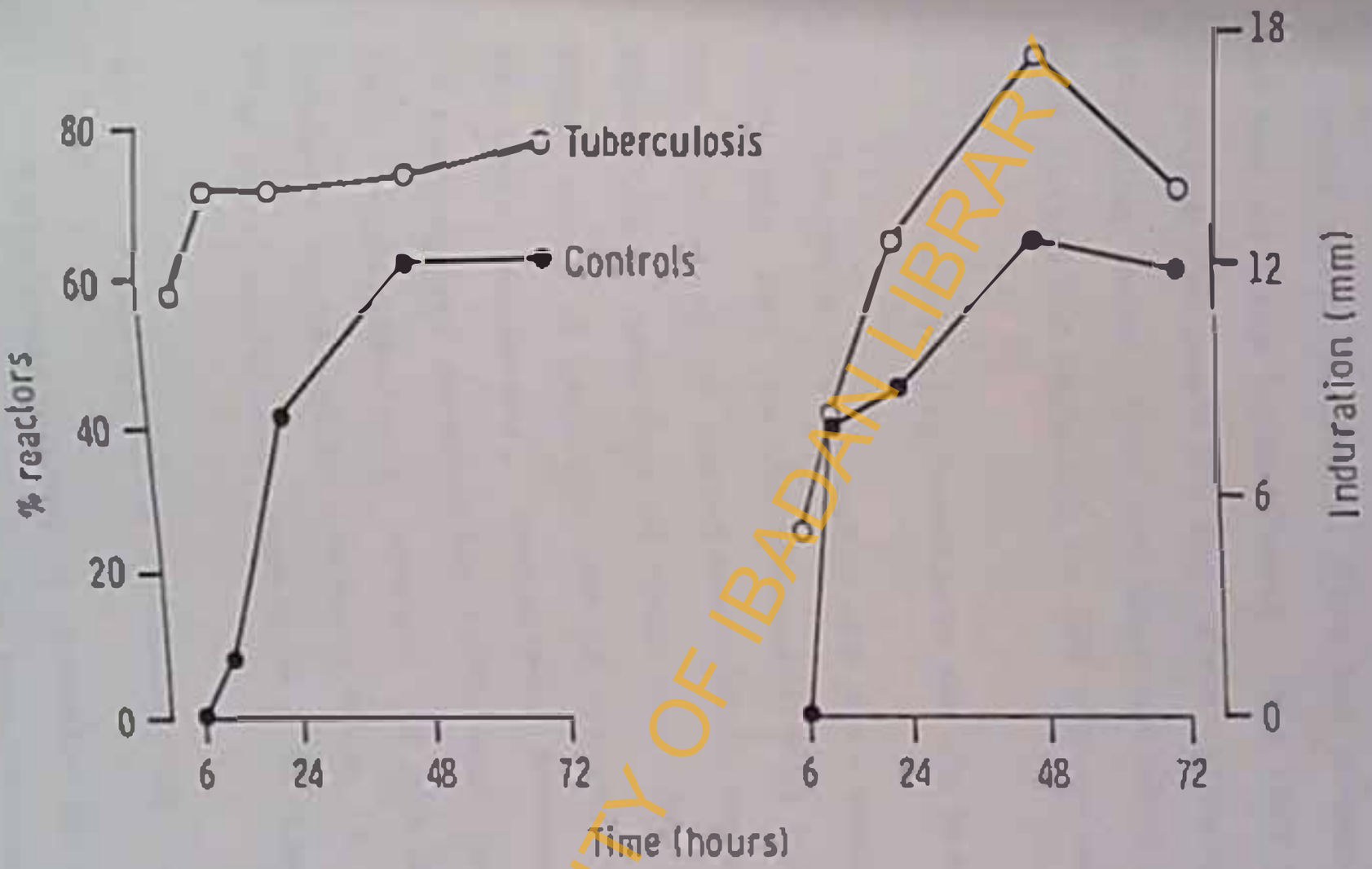

Fig 21: Time course of proportions (% reactors) and mean diameters of induration (cm) of subjects giving reactions to intradermal injection of 1TU PPD before chemotherapy. (n, controls:patients = 24:24, 24:24, 24:28, 26:28 and 26:28 at 6, 12, 24, 48 and 72 hrs respectively)

In all, seven of 28 patients (25%) and 10 of 26 matched controls (38%) were skin test negative to 1TU PPD at 72 hrs. Of these seven patients, five had pulmonary, one meningeal, and one miliary tuberculosis. All four patients with pleural effusions had positive low dose tuberculin reactions. On retesting with 10TU PPD four patients and five controls who were initially negative to 1TU PPD became skin test-positive.

2. Tuberculin reactions during treatment

Patients and controls who were available were re-tested with 1TU PPD (on the alternate arm) six and 12 months after the initiation of anti-tuberculosis chemotherapy. Fig. 22 shows the proportions of reactions, and Fig. 23 the reaction sizes among 10 patients and 18 controls who were tested on all three occasions. Comparisons between different occasions of testing are necessarily restricted to those subjects who were comprehensively studied on all three occasions. Some patients could not be followed up on all occasions because they were unavailable (moved away or died).

a) Proportions of reactors (Fig. 22)

A six month period of chemotherapy was associated with a significant increase in the proportions of patients reacting at 6 hrs (from 2 to 7 out of 10, P=0.03), 12 hrs (from 3 to 8 out of 10, P<0.05), 24 hrs (from 4 to 10 out of 10, P<0.05)

Fig 22: Time course of proportions (% reactors) of subjects giving reactions to intradermal injection of 1TU PPD at 0, 6 and 12 months following initiation of chemotherapy.

and 48 hrs (from 6 to 10 out of 10, P=0.046). Furthermore, all 10 tuberculosis patients became skin test-positive to 1TU PPD after six months of treatment and 90% gave early reactions at 6 hrs after 12 months of treatment (Fig. 22).

The proportions of reactors among 18 controls re-tested after six months also increased, from 0 to 4 (P>0.05) at 6 hrs, 1 to 7 (P<0.05) at 12 hrs, 7 to 9 (P>0.1) at 24 hrs, but decreased from 11 to 10 at 48 hrs and 72 hrs. These results were similar to those obtained from this group at 12 months.

b) Reaction sizes (Fig. 23)

The mean maximum diameters of induration always occurred at 48 hrs, and were comparable for 10 patients and 18 controls on all three occasions of testing: 15.0 (SD 3.4) against 11.4 (SD 5.1) mm, P=0.14 initially; 16.0 (4.4) against 17.2 (3.8) mm, P=0.52 at six months; 20.9 (5.6) against 18.3 (3.9) mm, P=0.25 at 12 months. Changes in mean reaction sizes following six months of treatment among patients were as follows: from 4.5 (2.1) to 10.4 (4.4) mm at 6 hrs, P=0.12; 6.7 (1.2) to 13.8 (3.8) mm at 12 hrs, P=0.013; and from 15.0 (3.4) to 16.0 (4.4) mm at 48 hrs, P=0.6. More striking changes from initial values were seen at 12 months: to 11.7 (5.2) mm at 6 hrs, P=0.09; to 14.7 (6.7) mm at 12 hrs, P=0.07; and to 20.9 (5.7) mm at 48 hrs, P=0.037. Even more dramatic changes in mean diameters of induration were noted among the 18 controls retested at six months: from 0 to 13.9 (6.6) mm at 6 hrs, P<0.001; from 11.4 (5.1) to 17.2 (3.8) mm at 48 hrs, P=0.008. Repeat testing of controls at 12 months gave similar results.

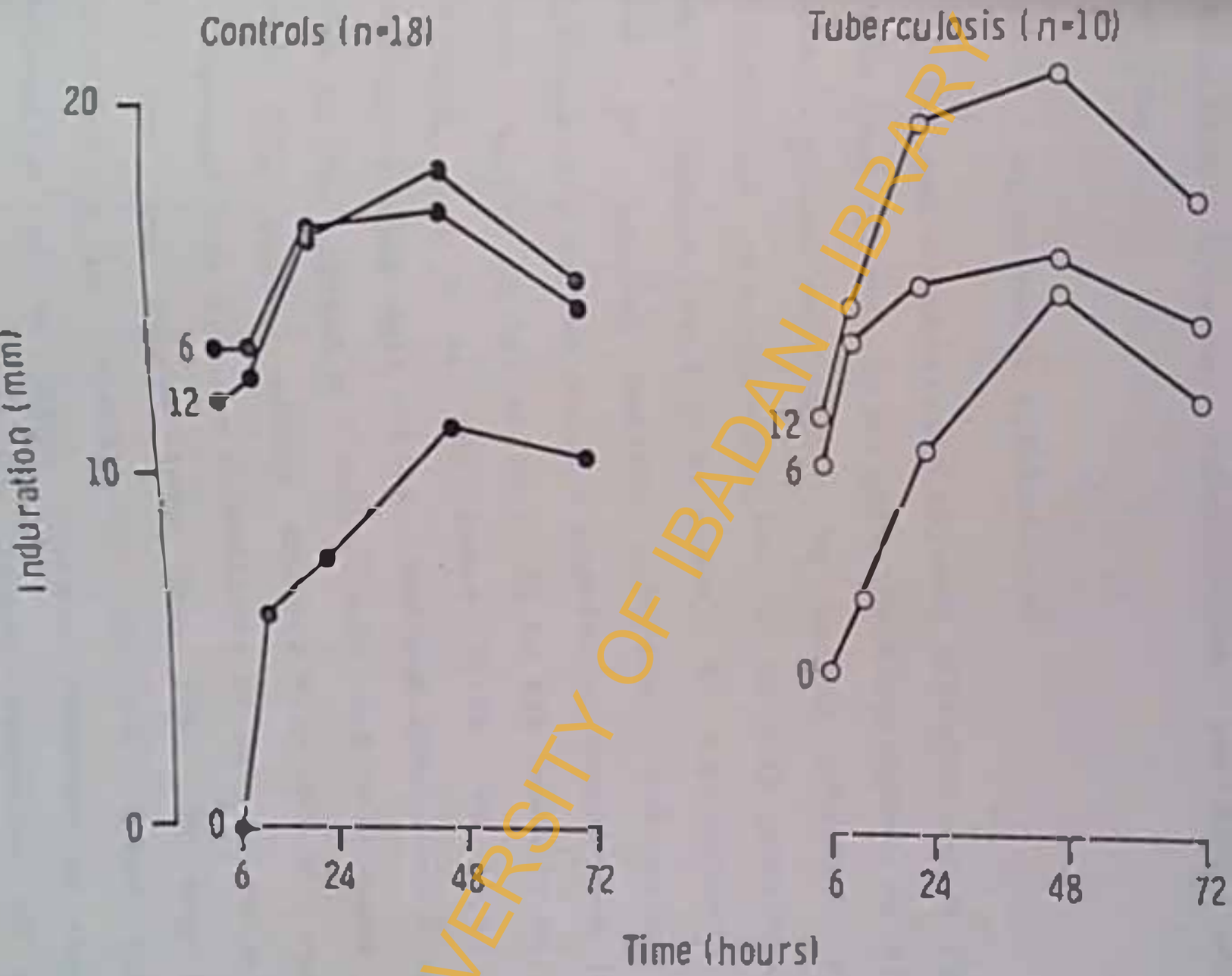

Fig 23: Time course of mean diameters of induration (mm) in subjects giving reactions to intradermal injection of 1TU PPD at 0, 6 and 12 months following initiation of chemotherapy.

E. Cytotoxicity

Levels of spontaneous and IFN-augmented NK activity were comparable between tuberculosis patients and healthy controls.

1. Spontaneous cytotoxicity

Percent cytotoxicity at each effector:target (E:T) cell ratio was found to be normally distributed, so differences between groups were tested by paired t-tests. Figure 24 illustrates the distribution of NK cell cytotoxicity in 70 healthy donors (aged 18-65 years), 27 tuberculosis patients and 27 matched healthy controls. Percentage specific cytotoxicity in the healthy population ranged from 18 to 71% (mean 41.6%, SD 12) at 40:1, 11 to 63% (mean 32.5%, SD 12.3) at 20:1, and 6 to 46% (mean 22.3%, SD 10.3) at 10:1 effector-target cell ratios. Maximum cytotoxicity was always seen at the highest E:T cell ratio and decreased linearly with this ratio, except at very high levels. Spontaneous background ^{51}Cr release in cultures of target cells without effectors was always less than 15%. At high levels of cytotoxicity the relationship was not linear but rather suggestive of an upper plateau response at variable E:T ratios. In addition, the linear responses of different individuals were significantly non-parallel, ($P<0.001$)

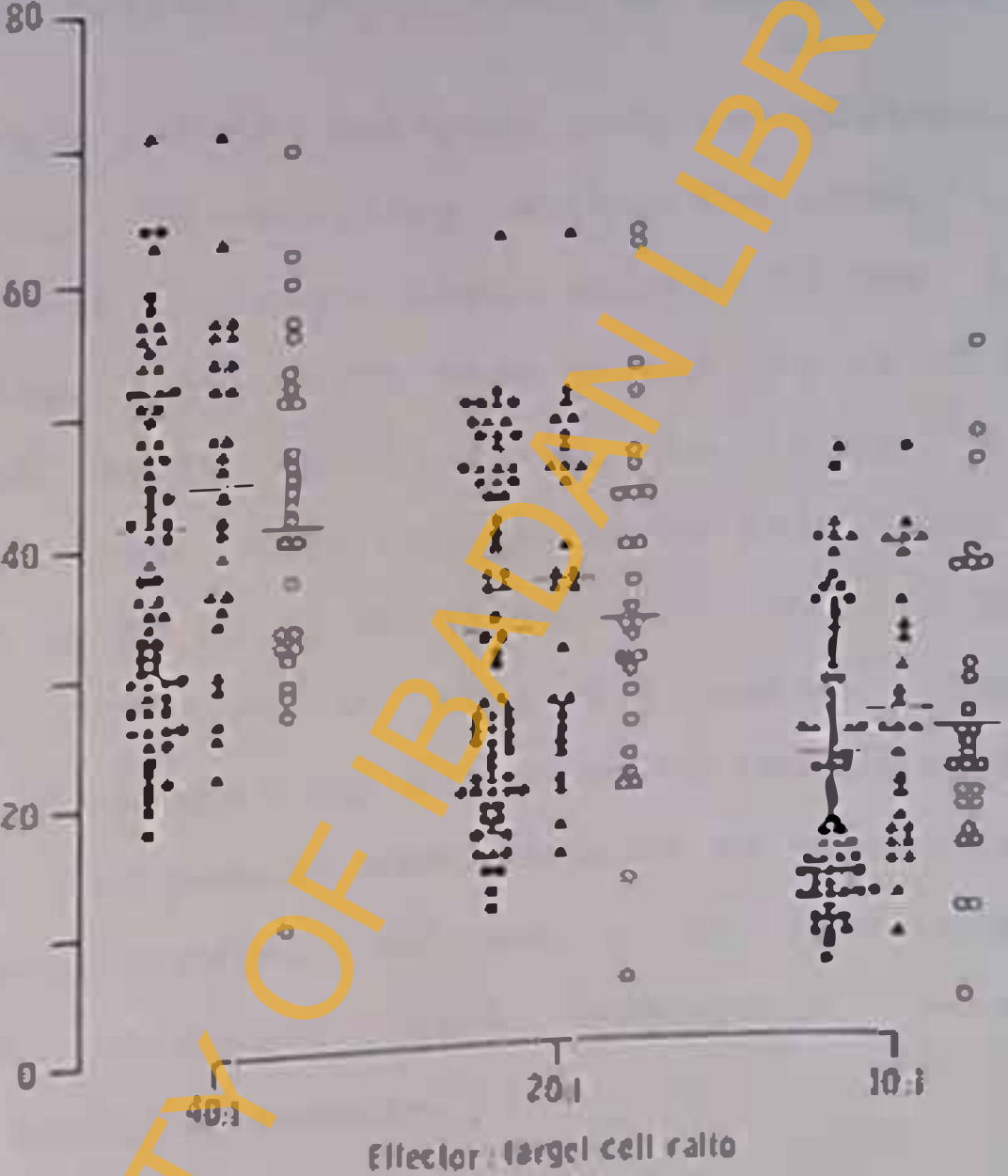

Fig 24: Distribution of Natural Killer (NK) cell activity (% cytotoxicity) in 70 normal adult donors (● ▲), 27 matched healthy controls (▲) and 27 untreated patients with tuberculosis (o).

(Armitage 1971), suggesting that lytic unit (LU) calculations (1 LU - the absolute number of effector cells required to yield a given percent lysis) would be invalid and inaccurate.

Twenty-six (of 27) patients with tuberculosis had levels of spontaneous NK activity within the normal range for 70 healthy controls including those matched to the tuberculosis patients (Fig. 24), with mean values (41.2% SD 12.3, 33.3% SD 13.0 and 24.2% SD 11.7 at the three E:T ratios respectively) that were not different from those of matched controls (44.5% SD 12.2, 36.4% SD 12.3 and 25.5% SD 10.4 respectively), P=0.39 at 40:1 E:T ratio. There were no differences in NK activity according to the site of disease. One patient had marked impairment of NK cytotoxicity - 10%, 5% and 3% respectively. He was a 54 year old severely malnourished alcoholic with widespread multibacillary tuberculous bronchopneumonia.

2. IFN-augmented cytotoxicity

Pre-treatment of their mononuclear cells with highly purified leucocyte IFN significantly enhanced NK cytotoxicity in 16 (of 18) patients and 15 (of 15) controls tested (Fig. 25). At 40:1 E:T cell ratio, the mean cytotoxicity was increased from 42.1 to 53.3% ($P<0.05$) in patients and from 51 to 63.1% ($P<0.05$) in controls.

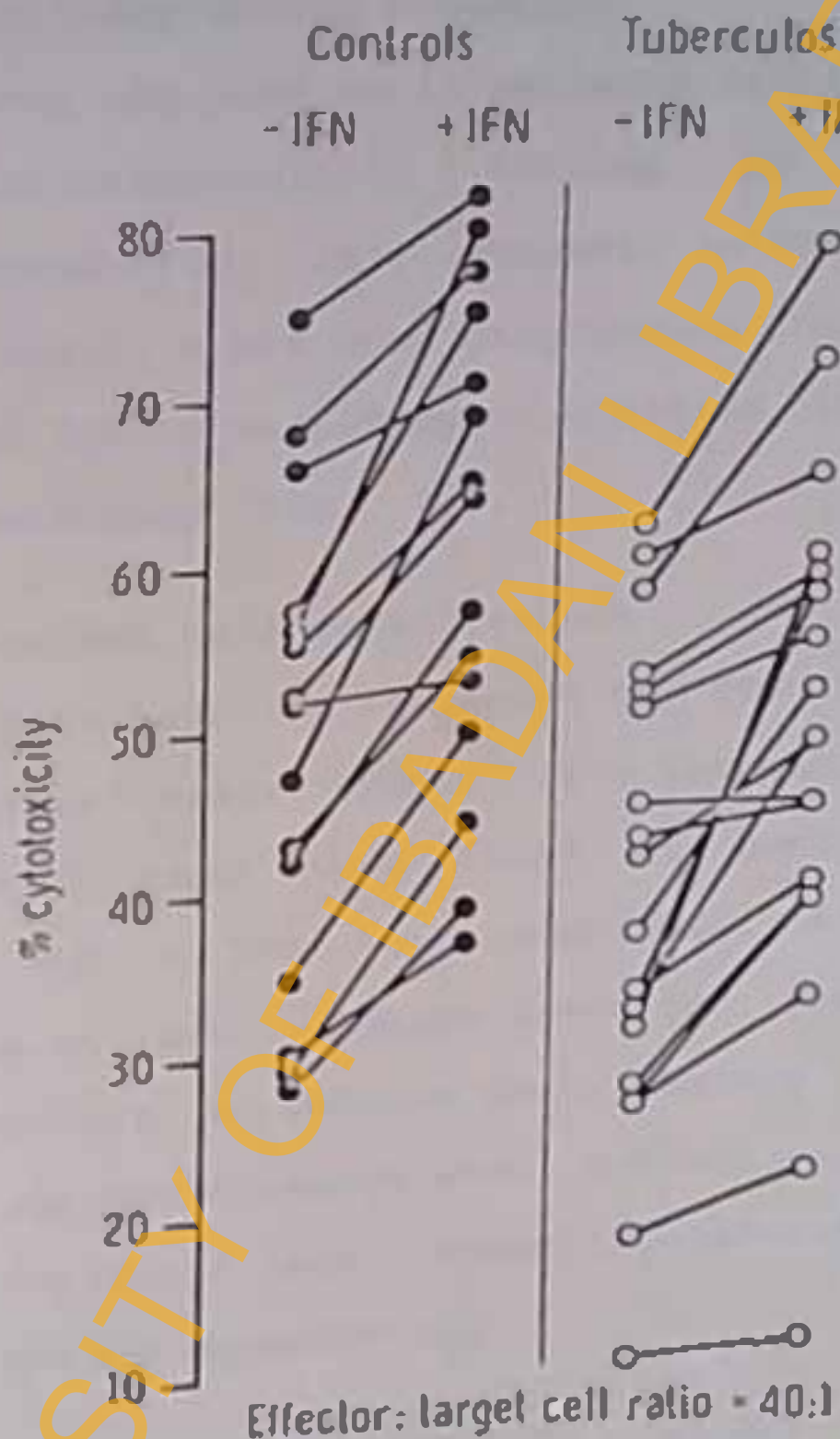

Fig 25: Effect of Interferon pretreatment (1000u/ml x 1hr at 37°C) of effector cells on NK cytotoxicity.

3. Cytotoxicity during treatment

Measurements repeated on 15 patients after six months of continuous anti-tuberculosis thorapy did not show any significant change (Fig. 26). However, in the patient with initial low levels, there were progressive increases in both spontaneous and IFN-augmented cytotoxicities throughout the period of chemotherapy (Fig. 27).

F. Mononuclear cell proliferation

In vitro proliferative responses to PPD of peripheral blood mononuclear cells (PBMC) from tuberculosis patients were significantly lower than those of controls, whereas responses to SEA + TPA were comparable. Also, PBMC from patients tended to require larger doses of PPD and longer periods of culture to achieve their maximum responses. No inhibitory effect was observed when mononuclear cells were cultured in patients' sera. Normal proliferative responses were restored during chemotherapy.

All values for ^{3}H-thymidine uptake (cpm) were transformed to square root to achieve a normal distribution.

1. In fetal calf serum (FCS)

a) Cells alone:

Culture of mononuclear cells in fetal calf serum without stimulants resulted in considerable proliferation. Thus, maximum ^{3}H-thymidine uptake obtained from unstimulated cells of 29 patients ranged from 28 to 752 cpm (mean 239 cpm, 95%

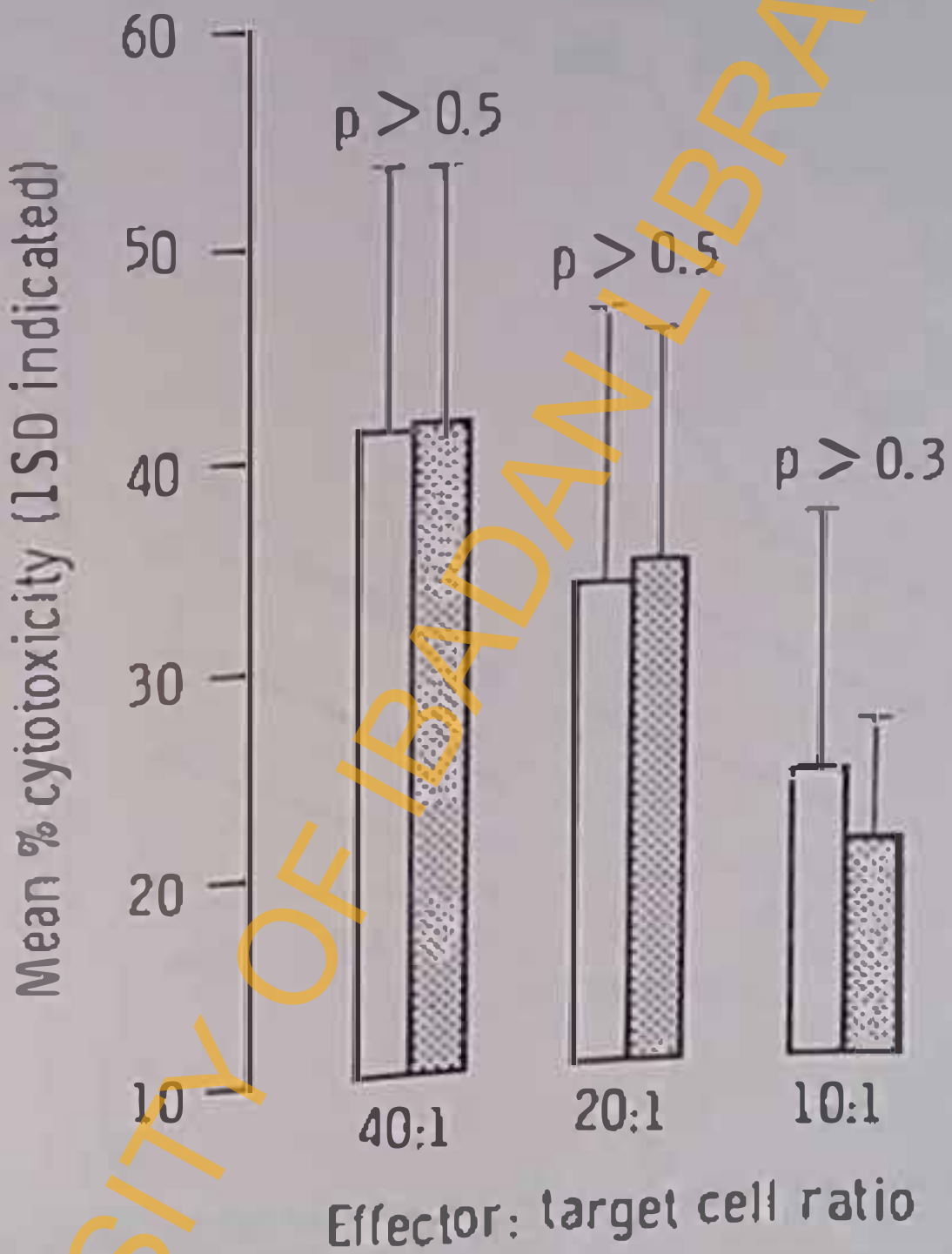

Fig 26: Mean (+ 1SD) of cytotoxicity for 27 tuberculosis patients before treatment (open bars) compared with 15 patients studied after six months of treatment (stippled bars).

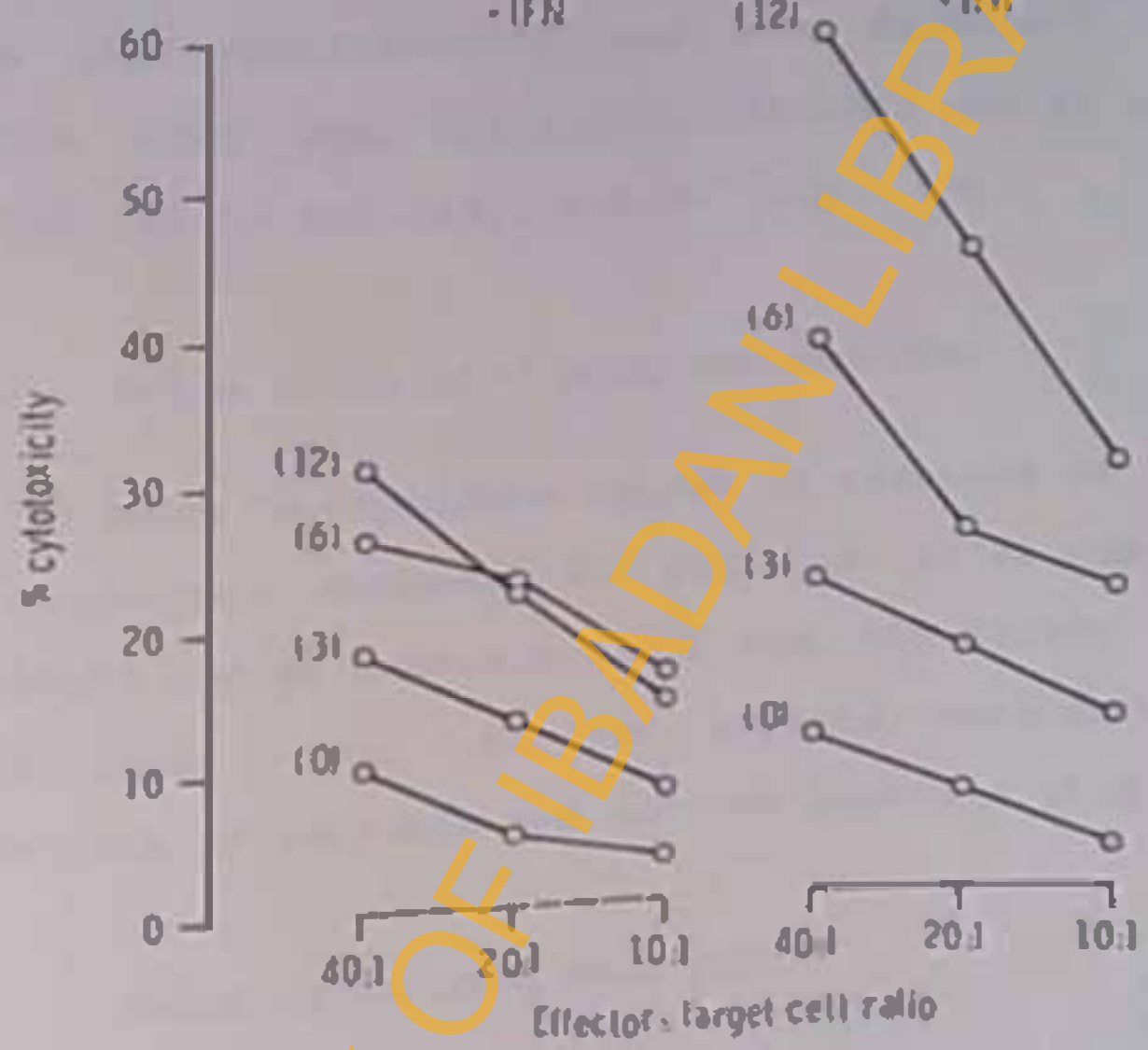

Fig 27: Effect of Interferon pretreatment on NK cell activity in one patient who had low pretreatment values, according to duration of treatment (months, in brackets).

limits 179-307), compared to 83 to 1073 cpm (mean 458 cpm, 95% limits 364-563) for 28 controls, P<0.001 (Fig. 28). At six months the proliferation was not different between 15 patients (380 cpm, 95% limits 230-608) and 15 controls (356 cpm, 95% limits 225-529), P=0.37 (Fig. 29).

b) Cells stimulated with SEA and TPA:

The mean ^{3}H-thymidine uptake in response to SEA + TPA was comparable between 29 patients (1338 cpm, 95% limits 1093-1607) and 28 controls (1277 cpm, 95% limits 1009-1576), P=0.53 (Fig. 28). Similar results were obtained at six months from 15 patients and matched controls (Fig. 29).

c) Cells stimulated with PPD:

In contrast, marked differences in in vitro reactivity to PPD were found between patients and controls in PCS. In response to 10 PPD, initial maximum ^{3}H-thymidine uptake ranged from 67 to 4100 cpm (mean 1518, 95% limits 1125-1969) among 29 patients and 481 to 8532 cpm (mean 2842, 95% limits 2097-3700) among 29 controls, P<0.001. Similarly, in response to 10U and 100U PPD the mean proliferative response for patients (1415 cpm, 95% limits 1049-1835, and 1078 cpm, 95% limits 773-1433 respectively) were significantly lower than control values (2465 cpm, 95% limits 1847-3173 and 1732 cpm, 95% limits 1360-2147, respectively), P<0.01 for each comparison (Fig. 28).

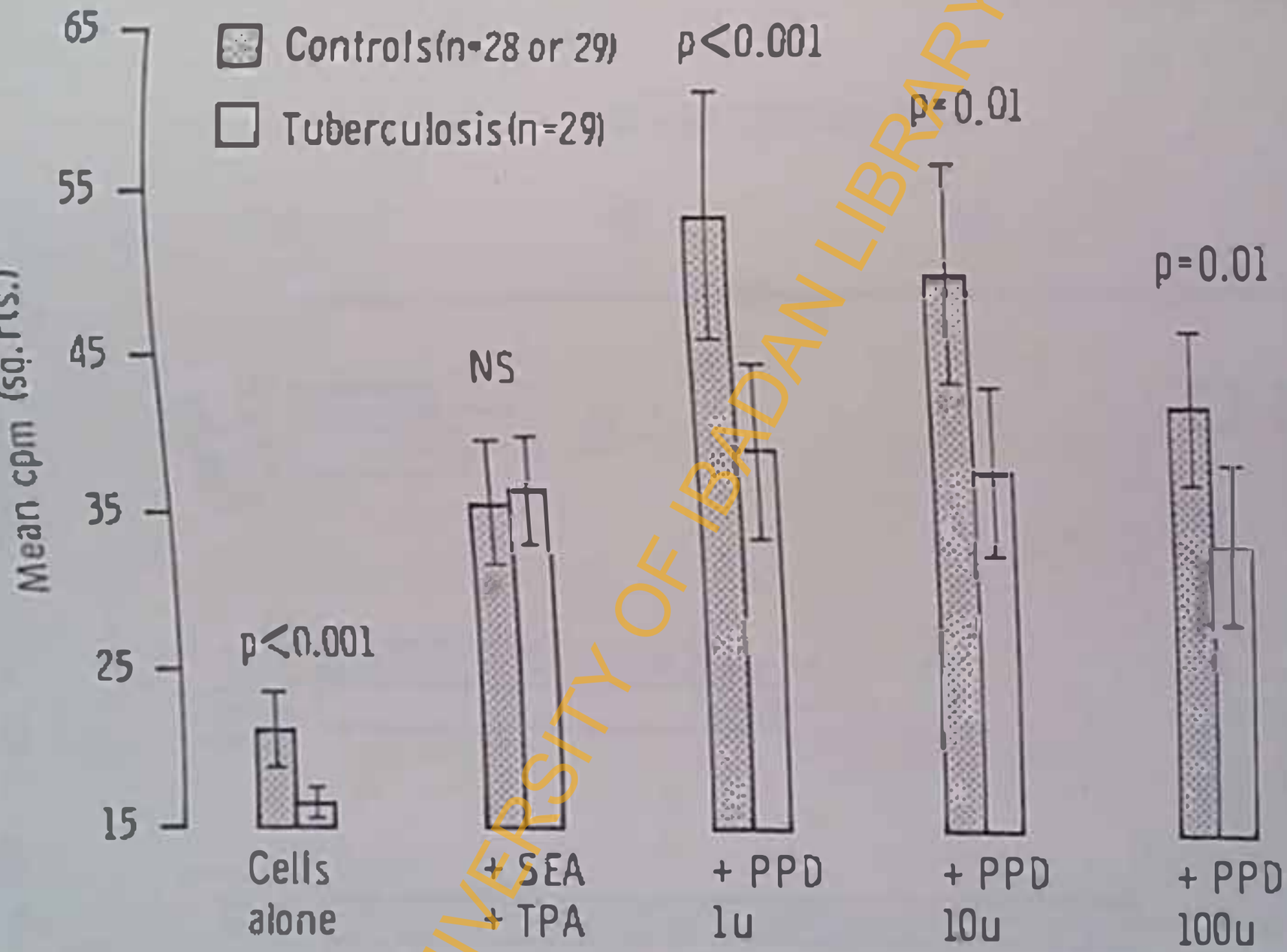

Fig 28: Maximum ^{3}H-thymidine uptake (mean cpm) into mononuclear cells from subjects before treatment. 95% confidence limits indicated.

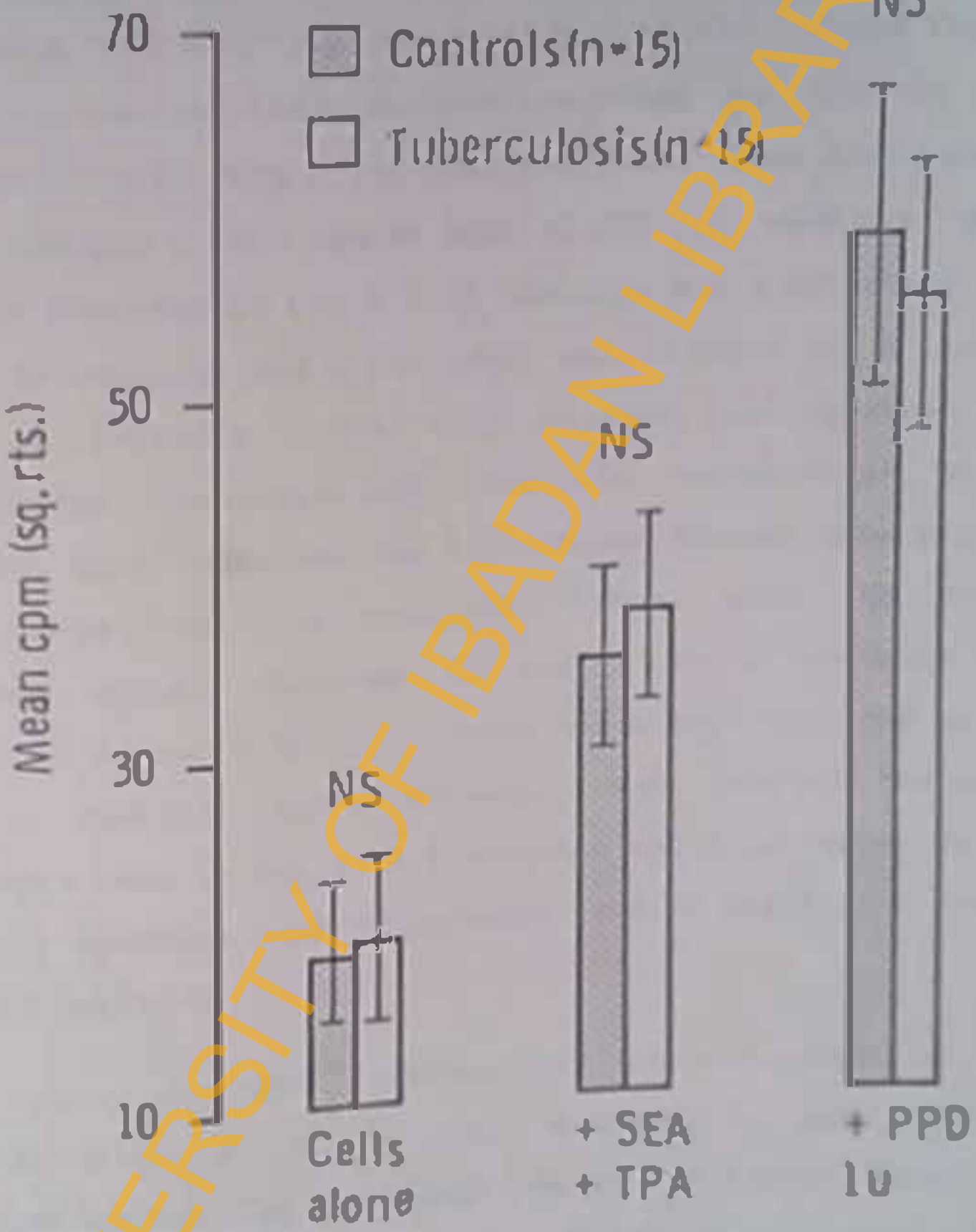

Fig 20: Maximum ^{3}H-thymidine uptake (mean cpm) into mononuclear cells of subjects after six months of chemotherapy. 95% confidence limits indicated

Furthermore, there were marked differences in the kinetics of the responses (Figs. 30-32). Cells from most controls reached their maximum response to PPD on Day 4 compared with Day 5 for most patients. This difference was most obvious at the lowest dose of PPD (1U) when the maximum uptake occurred on Day 3 in 3 controls and 3 patients, on Day 4 in 18 controls and 8 patients, and on Day 5 in 8 controls and 18 patients. The same tendency was observed at the higher PPD concentrations, but the reactivities in both groups were lower and the differences between them were less pronounced (Figs. 31 and 32). Thus, with 10U PPD the maximum uptake occurred on Day 3 in 2 controls and 6 patients, on Day 4 in 11 controls and 6 patients, and on Day 5 in 16 controls and 17 patients. With 100U PPD the maximum cpm were seen on Day 3 in 3 controls and 4 patients, on Day 4 in 18 controls and 12 patients, and on Day 5 in 8 controls and 13 patients.

Peak responses (irrespective of the time or cell concentration at which they occurred) for each individual plotted against the concentrations of PPD tested showed the maximum ^{3}H-thymidine uptake with 1U PPD in 20 controls and 12 patients, with 10U PPD in six controls and 12 patients, and with 100U PPD in three controls and five patients (Fig. 33).

After six months of chemotherapy proliferative responses to PPD were comparable between 15 patients and matched controls.

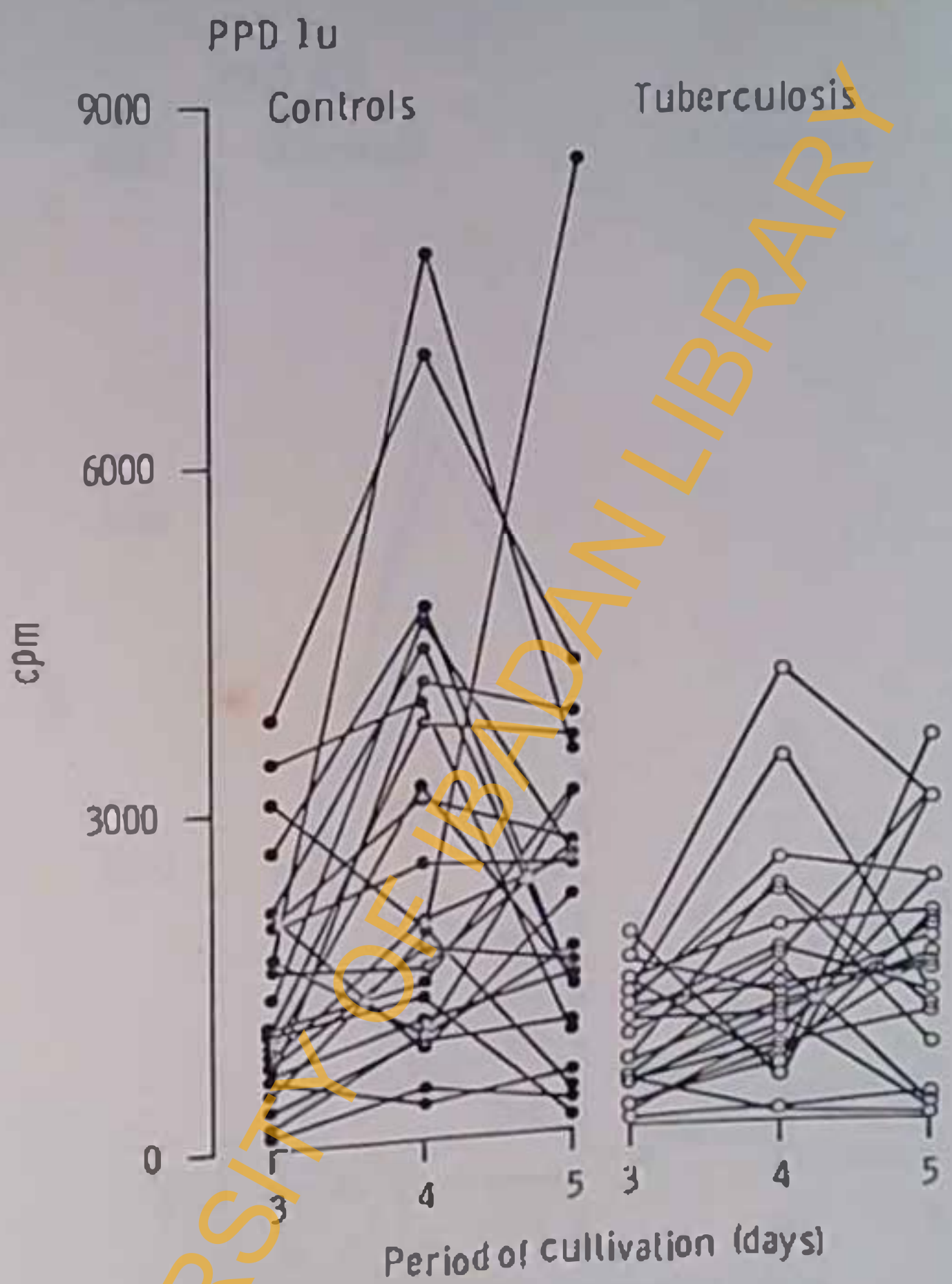

Fig 30: Kinetics of ^{3}H-thymidine uptake (cpm) in human mononuclear cells exposed to 1u PPD in culture for 3, 4 or 5 days. Each point represents the maximum uptake in triplicate cultures of 2, 4 or 8×10^4 cells per well. (The curves for five controls and six patients have been omitted for clarity, although their inclusion did not alter the shapes of the figures)

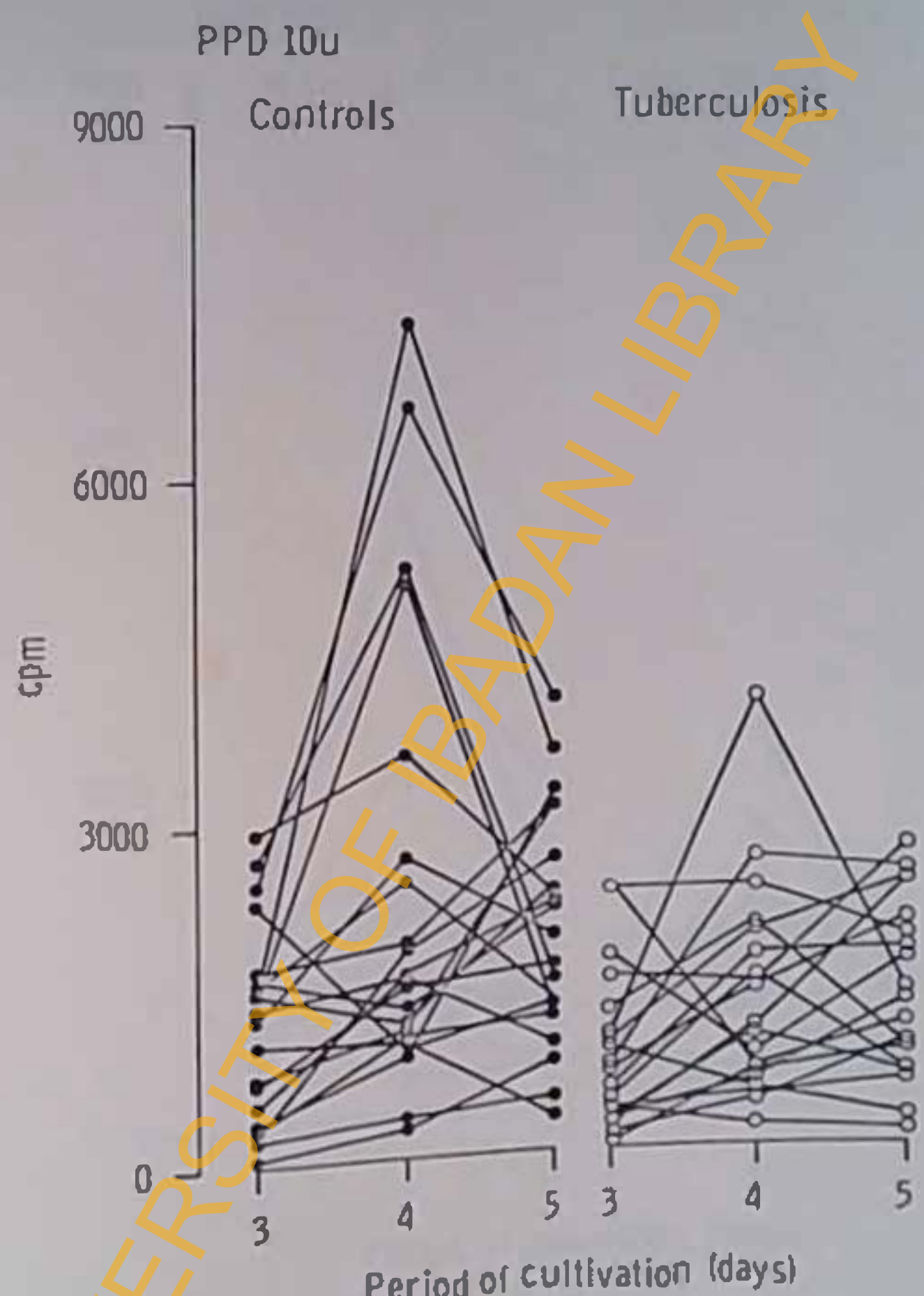

Fig 31: Kinetics of ^{3}H-thymidine uptake (cpm) in human mononuclear cells exposed to 10u PPD (For details see legend to Fig. 30).

Fig 32: Kinetics of ^{3}H-thymidine uptake (cpm) in human mononuclear cells exposed to 100u PPD (For details see legend to Fig. 30).

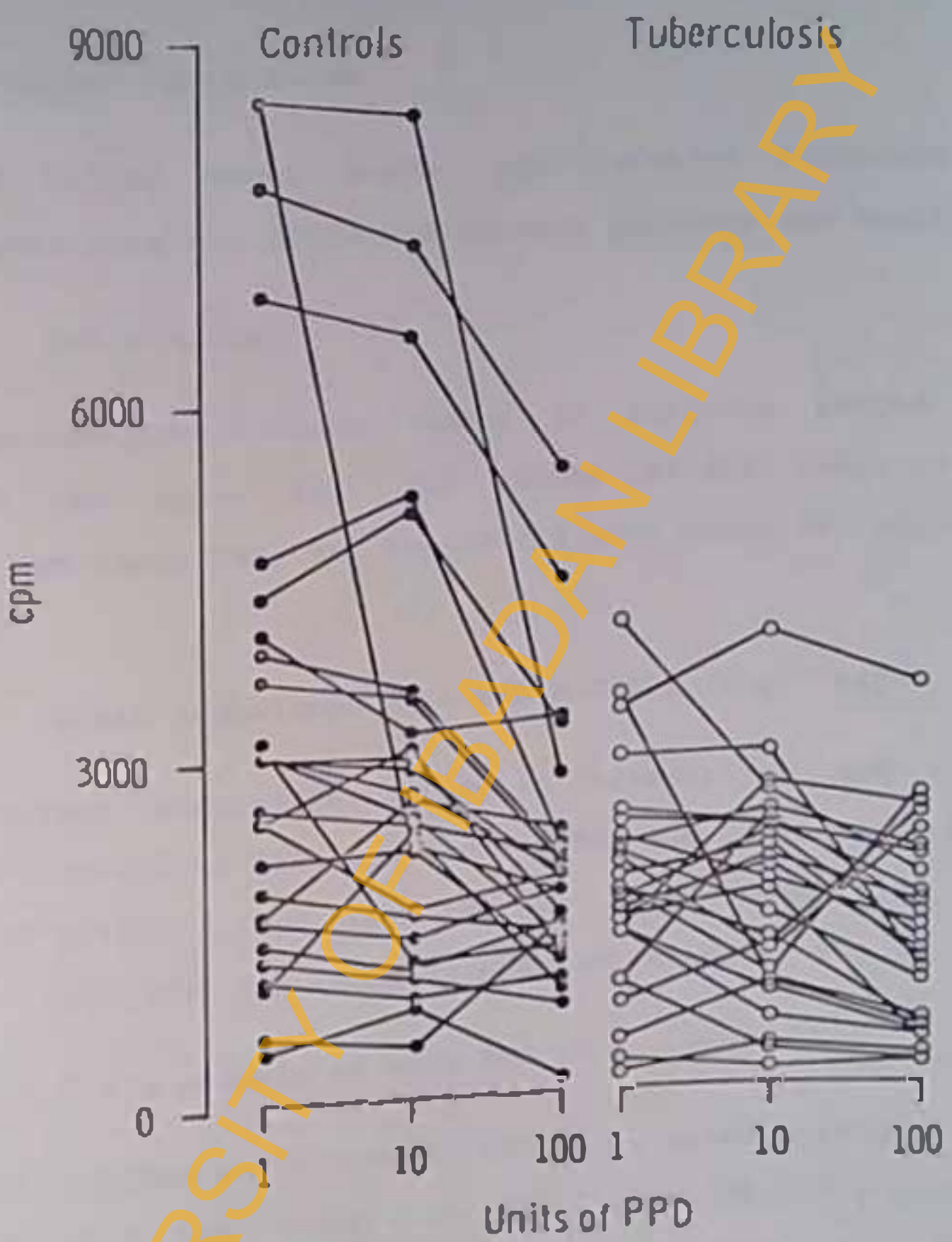

Fig 31: Dose-response curves of human mononuclear cells exposed to PPD. Peak responses (cpm), irrespective of time or cell concentration at which they occurred, were plotted for each subject against PPD concentration.

2. In normal human serum

In pooled human serum, proliferative responses to stimulants were not different between patients and controls.

a) Cells alone:

Maximum proliferation among 13 patients ranged from 118-974 cpm (mean 330, 95% limits 183-519) compared with 73-931 cpm (mean 393, 95% limits 262-549) among 13 controls, P=0.54

b) Cells stimulated with SEA and TPA (Fig. 34):

Maximum ^{3}H-thymidine uptake in response to SEA + TPA ranged from 243 to 3163 cpm (mean 1588, 95% limits 1101-2165) among 13 patients, and from 566 to 2425 (mean 1286, 95% limits, 925-1705) cpm among 13 matched controls, P=0.31.

c) Cells stimulated with PPD (Fig. 35):

In response to 10 PPD, ^{3}H-thymidine uptake ranged from 133 to 2657 cpm (mean 1116, 95% limits 596-1797) among 13 patients, and from 246 to 4374 cpm (mean 1325, 95% limits 674-2194) among 13 controls, P=0.54. Similarly, no differences were found between maximum responses to 100 PPD among 13 patients (range - 87 to 4447 cpm, mean 1333, 95% limits 728-2119) and 13 controls (292 to 5287 cpm, mean 1398, 95% limits 680-2371), P=0.89, or between maximum responses to 1000 PPD among 13 patients (range 67-3330 cpm, mean 1055, 95% limits 488-1837) and 13 controls (332-3560 cpm, mean 995, 95% limits 622-1456), P=0.87.

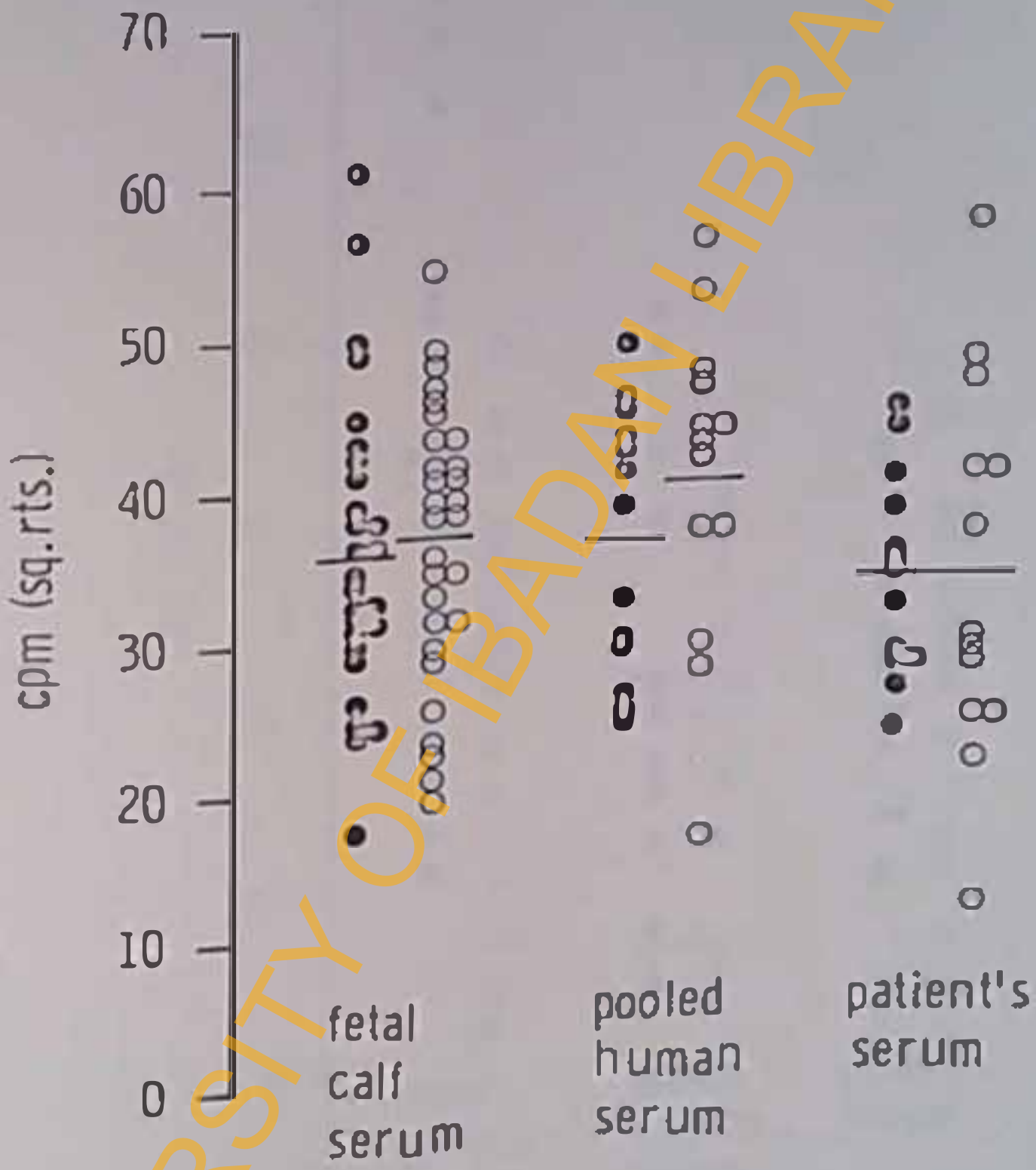

Fig 34: Comparison of maximum ^{3}H-thymidine uptake (cpm) in mononuclear cells from matched controls (●) and patients (○), cultured with SEA + TPA in three different sera.

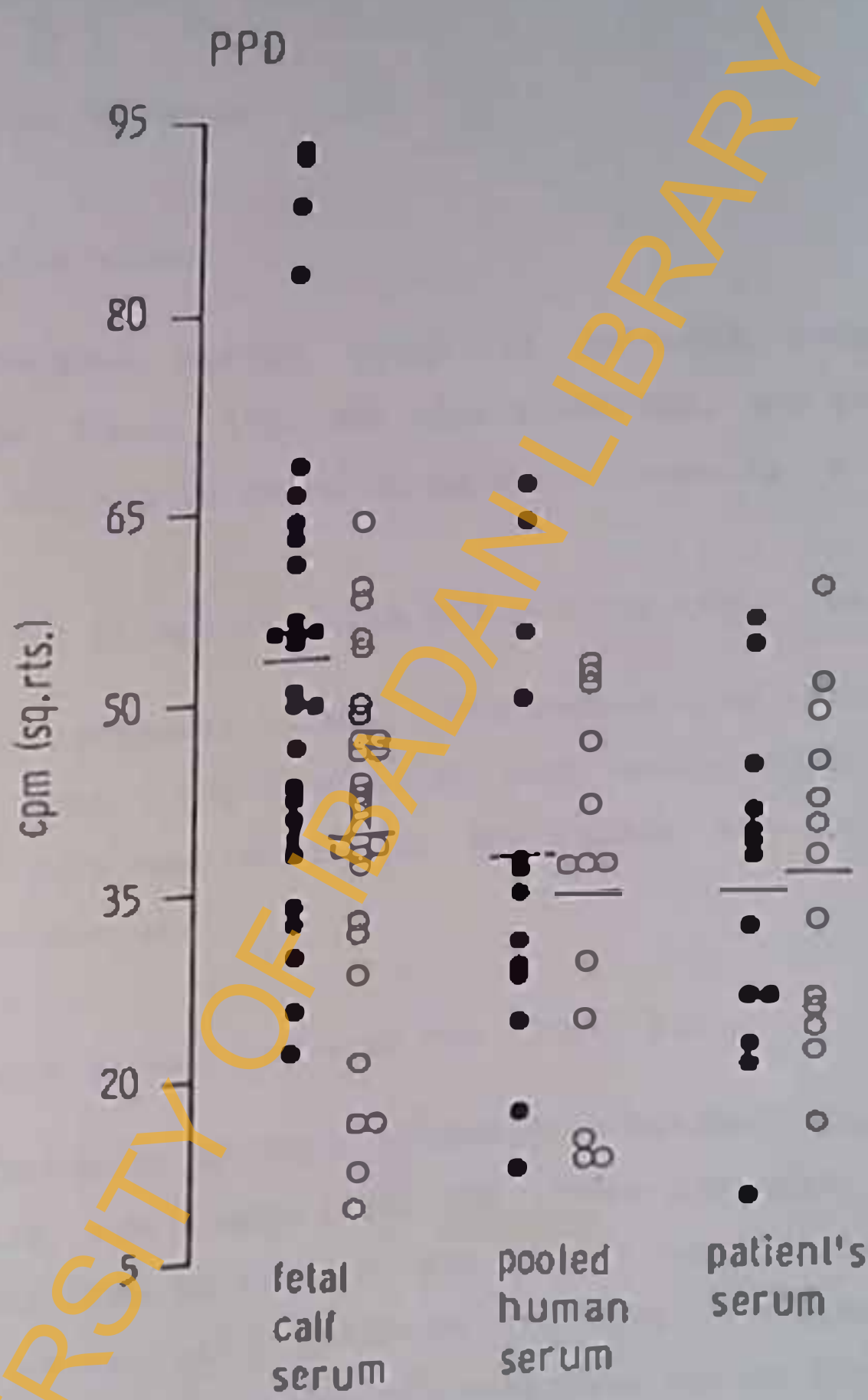

Fig 35: Comparison of maximum ^3H-thymidine uptake (cpm) in mononuclear cells from matched controls (●) and patients (○), cultured with 1u PPD in three different sera.

3. In patient's serum

a) Cells alone:

^{3}H-thymidine uptake among 13 patients ranged from 119-839 cpm (mean 375, 95% limits 266-502) and 154-742 cpm (mean 331, 95% limits 215-472) among 13 controls, P=0.59.

b) Cells stimulated with SEA and TPA (Fig. 34):

Maximum responses to SEA + TPA ranged from 112 to 3259 cpm (mean 1092, 95% limits 637-1668) among 13 patients and from 493 to 1979 cpm (mean 1088, 95% limits, 829-1382) among 13 controls, P=0.98.

c) Cells stimulated with PPD (Fig. 35):

In response to 1U PPD, ^{3}H-thymidine uptake ranged from 203 to 3320 cpm (mean 1198, 95% limits 710-1813) among 13 patients, and from 64 to 3061 cpm (mean 1095, 95% limits 619-1707) among 13 controls, P=0.75. Similarly, no differences were seen in maximum responses to 10U PPD (range 178-5204 cpm, mean 1542, 95% limits 770-2580 and range 349-2496 cpm, mean 1265, 95% limits 864-1743 respectively, P=0.54), and in maximum responses to 100U PPD (range 221-3722 cpm, mean 1091, 95% limits 624-1688 and range 460-2453 cpm, mean 980, 95% limits 663-1360 respectively, P=0.70).

G. Monocyte migration

Compared with matched controls, patients with tuberculosis had impaired monocyte migration towards the chemoattractant, casein.

Values for monocyte random locomotion (RL) and chemotaxis (CTX) were found to follow a normal distribution. RL values among 15 patients ranged from 1 to 11 μ (mean 4.5, 1 SD 3.5) compared with 1 to 14 μ (mean 6.8, 1 SD 3.9) among 17 controls, P=0.038. Values for chemotaxis among 15 Patients ranged from 8 to 27 μ (mean 20.7, 1 SD 6.1) compared with 16 to 32 μ (mean 23.3, 1 SD 5.4) among 17 control subjects, P=0.025 (Fig. 36). Three patients (20%) but no controls had values for chemotaxis less than 15μ (P=0.09, Fisher's exact test).

H. IFN production

No significant differences were found between patients and matched controls in mean titres of IFN-α induced by Newcastle Disease virus, IFN-γ induced by SEA + TPA, and IFN-γ induced by PPD. However, a subset of nine tuberculosis patients produced low titres (<100 u/ml) of IFN-γ in response to PBMC stimulation with PPD. In comparison to other patients, this group was characterised by lower IFN-α and IFN-γ responses to virus and mitogens respectively, relative anergy to tuberculin skin testing, depressed in vitro PBMC proliferative responses to PPD, and neutrophil leucocytosis, all of which returned to normal during chemotherapy.

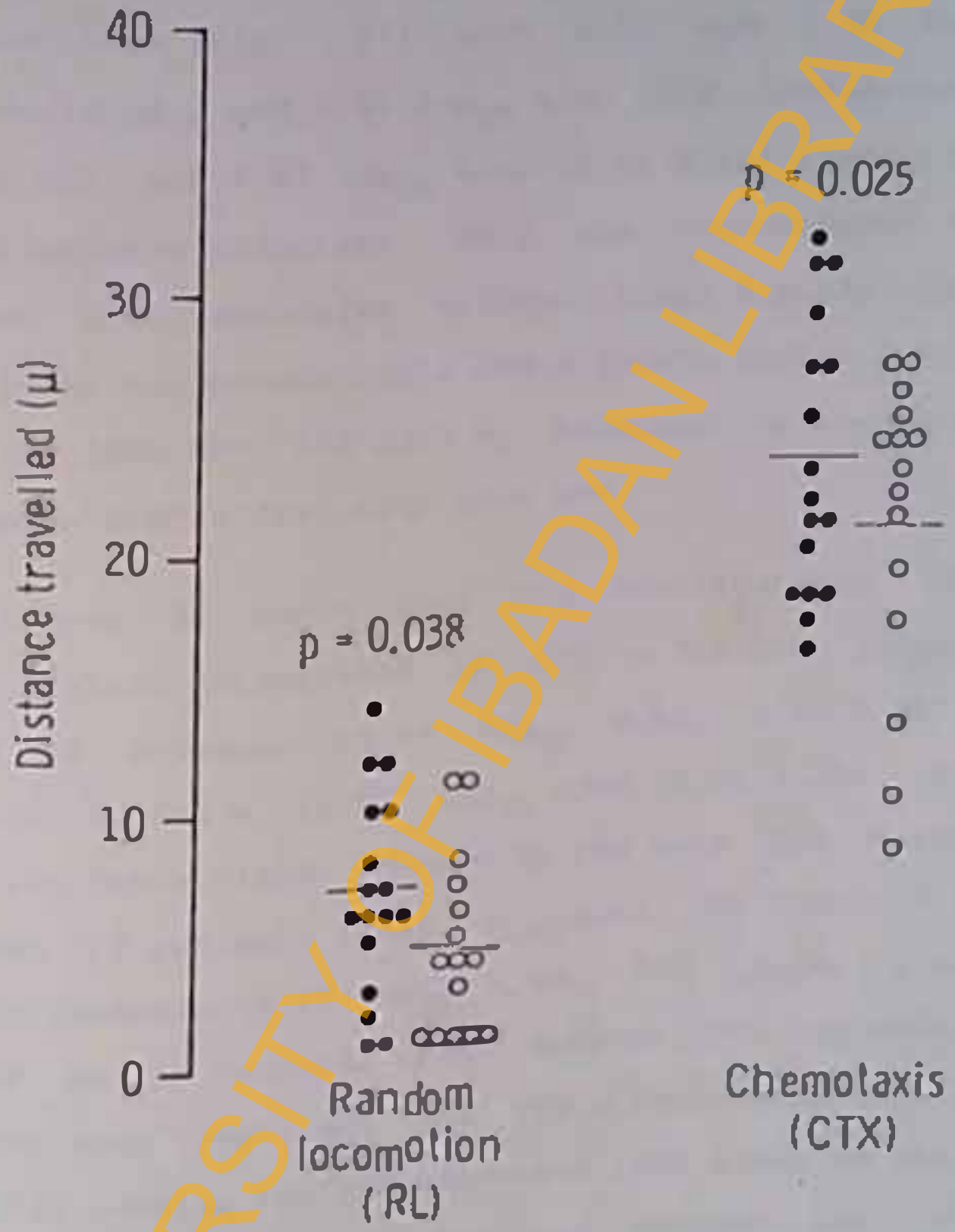

Fig 36: Comparison of random locomotion (in medium) and casein-stimulated chemotaxis of monocytes between 17 matched healthy controls (●) and 15 patients with tuberculosis (o).

The geometric mean IFN-α titres for 28 patients and 28 controls (Fig. 37) were 2.52 and 2.50 $\log_{10}$ u/ml (unstimulated), and 3.41 $\log_{10}$ u/ml (95% confidence limits 3.18-3.58) and 3.46 $\log_{10}$ u/ml (3.24-3.64) respectively for NDV-stimulated cultures. Only one malnourished alcoholic patient with extensive multibacillary disease and negative skin tests had undetectable IFN-α levels (below 2.0 $\log_{10}$ u/ml) by IRMA and <10 u/ml by bioassay on sensitive cells following PBMC stimulation with NDV.

Figure 38 shows that the distributions and mean IFN-γ titres stimulated by SEA + TPA were comparable among 14 patients (3.34 $\log_{10}$ u/ml, 3.04-3.56) and 14 matched controls (3.37 $\log_{10}$ u/ml, 3.16-3.55). Similarly, the mean IFN-γ titres induced by PPD were not different between 25 patients (2.56 $\log_{10}$ u/ml, 95% limits 2.23-2.88) and 25 controls (2.77 $\log_{10}$ u/ml, 95% limits 2.58-2.96), P>0.05 by the binomial test. However, the subjects could be divided into 'high' and 'low' IFN-γ responders by using the 20th centile for PPD responses (100 u/ml) as the cut-off. In response to SEA + TPA only one patient and no control subjects produced IFN-γ levels below 100 u/ml. In contrast, in response to PPD, nine patients (36%), including the low responder to SEA + TPA, but only one matched control (4%) produced IFN-γ titres below 100 u/ml (P<0.02, chi-squared test with Yates' correction).

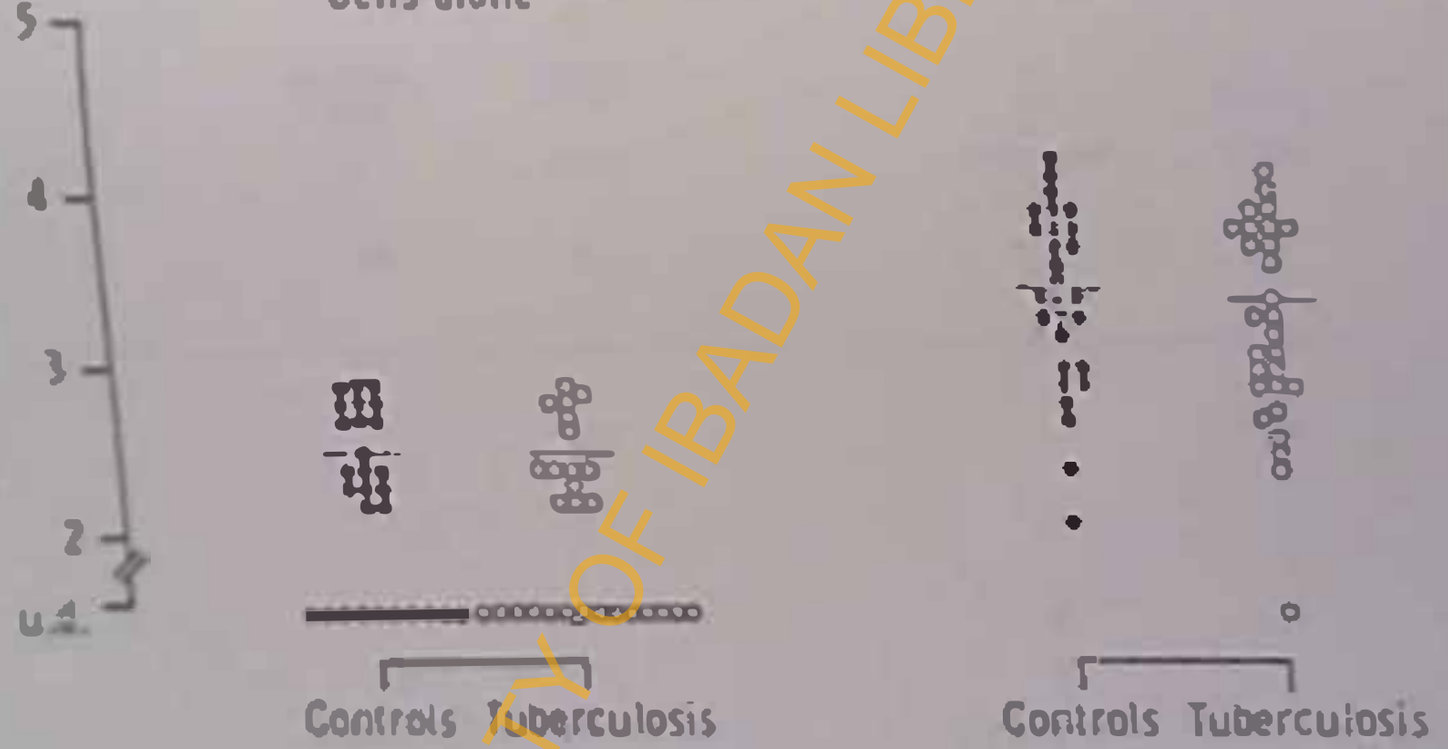

Fig 37: Interferon (IFN)-α responses of mononuclear cells from 30 matched healthy controls (•) and 26 tuberculosis patients (○) to stimulation with Newcastle Disease Virus (NDV).

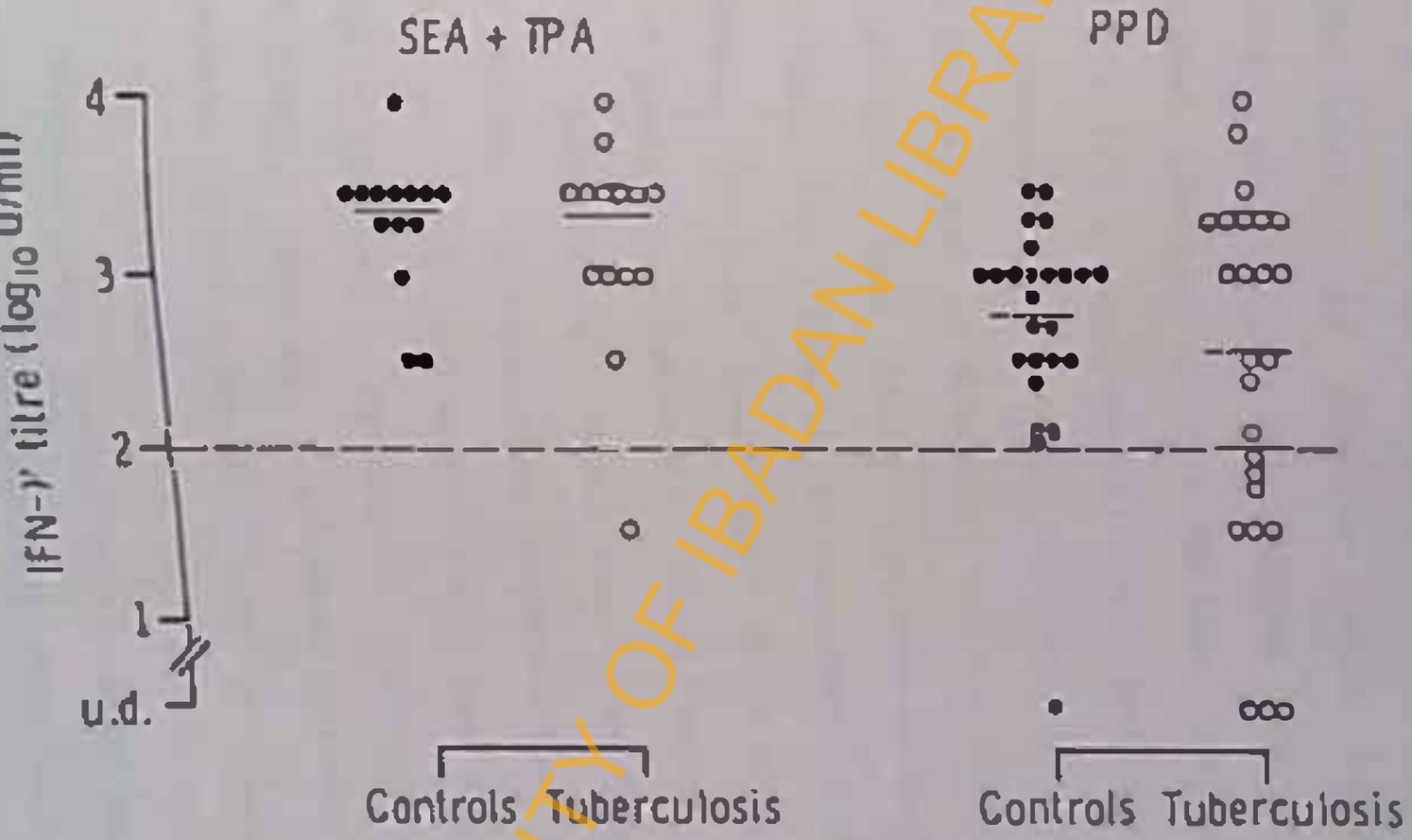

Fig 38: Interferon (IFN)-γ responses of mononuclear cells from matched healthy controls (●) and tuberculosis patients (o) to Staphylococcal Enterotoxin A plus Tetrahydrophorbyl Acetate, SEA + TPA (n = 14 per group) or Purified Protein Derivative, PPD (n = 25 per group).

Some clinical features of the high and low PPD-stimulated IFN-γ responder patients are shown in Table 5. The major differences were in the larger proportion of patients with negative skin tests to 1TU PPD, and the smaller mean reaction size among low IFN-γ responders. On repeat testing with 10 TU PPD, all but three patients (two were low IFN-γ responders) were positive. Both groups were comparable in age, sex and ethnic distributions, diet, site and extent of disease, and the presence or absence of cavitation. Low IFN-γ responders to PPD also produced lower mean IFN-γ titres in response to SEA + TPA, and to a lesser extent lower mean IFN-α titres in response to NDV stimulation (Table 6). They had a lower mean ESR and higher neutrophil counts than high responders. Also, their mean maximal ^{3}H-thymidine incorporation in response to PPD (915 cpm, 95% limits 285-1902) was significantly lower than among high IFN-γ producers (1954 cpm, 95% limits 1636-2300), P=0.01, although their mean proliferative responses to SEA + TPA (1186 cpm and 1501 cpm respectively) were not different, P=0.25 (Fig. 39). No differences were seen between these groups in terms of mean W/H^2, arm muscle circumference, skinfold thickness, serum sodium, serum total proteins, albumin, globulin, iron, TIBC, phosphate, calcium and immunoglobulins (Table 7). A healthy 35 year-old male control subject of BCG origin produced undetectable levels of IFN-γ in response to PPD. He was skin test-negative to 1 TU, but positive to 10 TU PPD, and had PBMC proliferative responses (cpm) to PPD that were below the lower 95%

TABLE 5

Clinical features of patients with high or low IFN-γ responses to PPD

	High IFN-γ responders to PPD (n = 16)	Low IFN-γ responders to PPD (n = 9)	P
Age (yrs)	33.9	31.9	0.37
M:F	9:7	7:2	0.25 (Fisher)
Ethnic origin:			
ISC	11	7	
Caucasian	5	1	
African	0	1	
Diet:			
vegetarian	10	4	0.33 (Fisher)
non-vegetarian	6	5	
Sites of disease:			
Pulmonary	10	8	
Miliary	2	0	
Extrapulmonary	4	1	
Extent of disease:			
<Area of RUL*	7	5	0.38 (Fisher)
>Area of RUL	9	4	0.20 (Fisher)
Cavitation: Yes:No	3:9	4:4	
Pleurisy with effusion	3	1	
Initial Mantoux test (1TU PPD)		[illegible]	
Negative	[illegible]	[illegible]	0.63 (Fisher)
Mean diam (SD) mm	10.1 (5.3)	13.2 (3.3)	0.06
Time of [illegible] (days)	60	54	0.21

* RUL = right upper lobe

TABLE 6

A comparison of anthropometric indices, interferon (IFN) production, peripheral blood mononuclear cell (PBMC) proliferation, monocyte migration and Natural Killer (NK) activity between patients with high or low IFN-γ responses to PPD (1SD or 95% confidence limits in brackets)

	High IFN-γ responders to PPD (n = 10)	Low IFN-γ responders to PPD (n = 9)	P
W/H^2	20.0 (1.9)	18.2 (4.3)	0.30
Arm muscle circumference (cm)	20.9 (2.7)	21.4 (2.3)	0.65
Skinfold thickness (mm)	8.9 (5.0-12.8)	6.0 (3.7-8.9)	0.19
IFN-α ($\log_{10}$ u/ml)	3.36 (3.21-3.61)	3.37 (3.21-3.52)	0.86
IFN-γ (EKA+TPA) ($\log_{10}$ u/ml)	1.9 ([illegible].20-3.[illegible])	2.70 ([illegible]-2.6[illegible])	0.0[illegible]
PBMC proliferation, cpm (EKA+TPA)	1341 (1174-1[illegible])	1180 (7[illegible]-5[illegible])	0.25
PBMC proliferation, cpm (PPD)	1916 (1036-3[illegible])	815 (203-1882)	0.0[illegible]
Monocyte			
Random locomotion (%)	4.8 (3)	4.3 (3)	0.88
Chemotaxis (%)	21.5 (6.[illegible])	16.8 (6.6)	0.4[illegible]
NK activity (% cytotoxicity)			
40:1	42 (11)	30 (13)	0.42
20:1	34 (13)	32 (15)	0.70
10:1	25 (12)	23 (13)	0.74

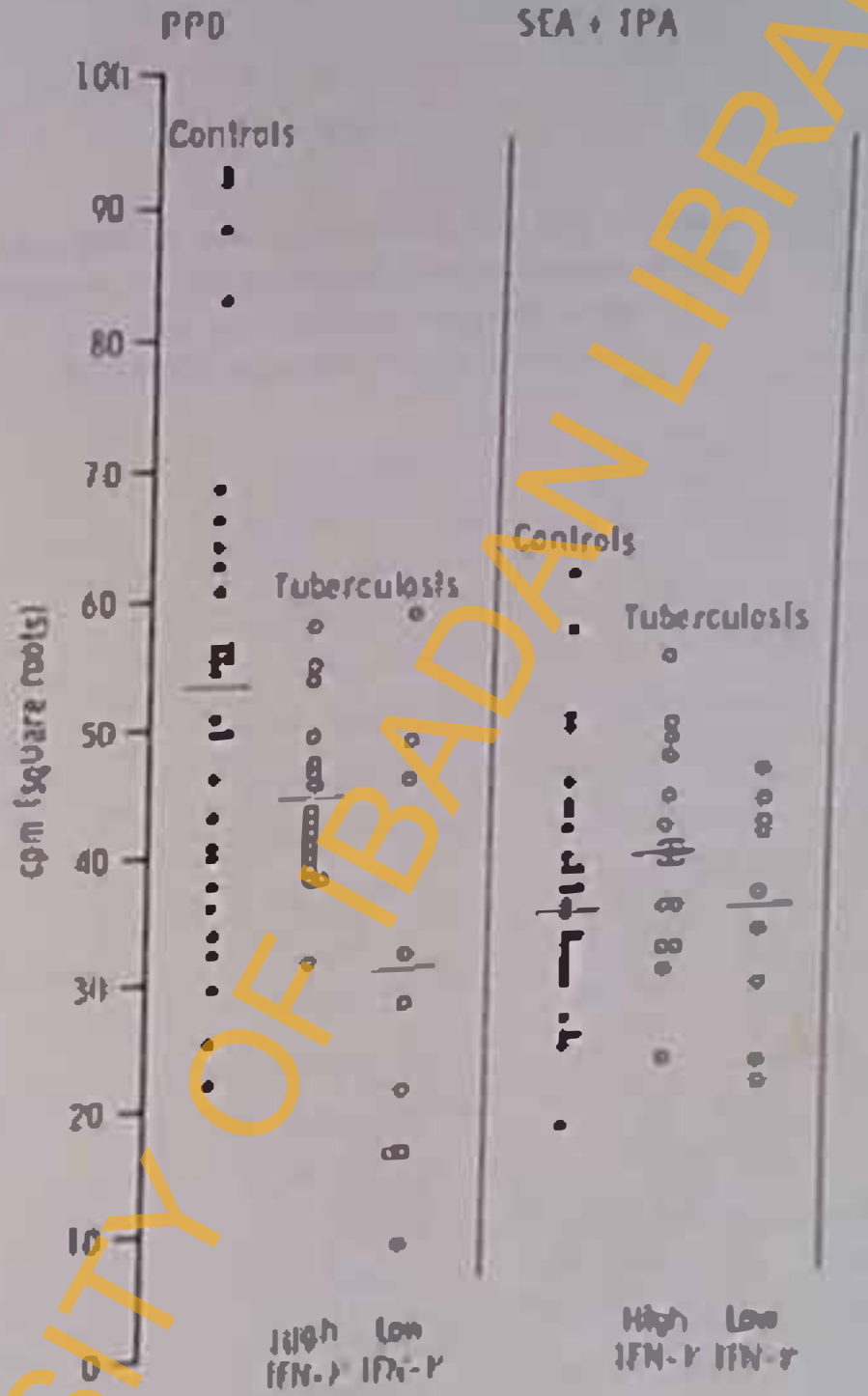

Fig 19: Maximum uptake of ^{3}H-thymidine (cpm) into cultures of mononuclear cells from matched healthy controls (•) and tuberculosis patients (o) classified as high or low (<100u/ml) IFN-γ responders to PPD.

TABLE 7

A comparison of some blood counts, ESR, serum proteins, immunoglobulins and biochemical indices between patients with high and low IFN-γ responses to PPD (95% or 99% confidence limits in brackets)

	High IFN-γ responders to PPD (n = 16)	Low IFN-γ responders to PPD (n = 9)	P
Hb (g/100 ml)	13.0(1.8)	12.2(2.5)	0.38
Total WBC ($x10^3$/µl)	7.3(6.6-8.3)	8.0(6.6-9.9)	0.44
Neutrophils (/µl)	4878) 4284-5782)	6434(5057-8186)	0.05
Lymphocytes (/µl)	1646 (868)	1331 (755)	0.43
Monocytes (/µl)	346 (218-504)	447 (133-846)	0.64
ESR (mm/hr)	43 (32-62)	24 (1-54)	0.08
Total protein (g/l)	71 (8)	70 (10)	
Albumin (g/l)	37 (7)	37 (9)	
Globulin (g/l)	34 (6)	34 (6)	
IgG (g/l)	13.2(11.3-16.2)	13.0(11.1-15.3)	0.87
IgA (g/l)	2.6(2.3-3.1)	2.6(1.7-3.9)	0.86
IgM (g/l)	1.6(1.1-1.8)	1.1(0.6-2.2)	0.38
Alk. Phos (iu/l)	233 (170-334)	259 (159-421)	0.70
Asp. Trans (iu/l)	32 (15-33)	26 (13-48)	0.83
Iron (mmol/l)	7 (6-10)	5 (4-8)	0.11
TIBC (umol/l)	34 (16)	27 (20)	0.89
Sodium (mmol/l)	139 (3)	135 (7)	0.28
Calcium (mmol/l)	2.32 (0.17)	2.19 (0.25)	0.76
Phosphate (mmol/l)	1.19 (0.18)	1.07 (0.21)	0.36

confidence limit for the control group mean. The significance of low IFN-γ responses to PPD in such an apparently healthy tuberculin reactor is not known.

In all the patients followed up, chemotherapy restored skin test responses, PBMC proliferation, neutrophil counts and IFN-α responses to NDV. In the one low IFN-γ responder patient re-tested at six months, PBMC stimulation with PPD yielded an IFN-γ response within the normal range.

I. Reactions during chemotherapy

Only a few side-effects of chemotherapy were encountered, including further (transient) elevation of serum transaminase (22 patients), pellagra (2), peripheral neuropathy (1) and persistent fever (1). Two patients, both Caucasians, developed sudden unexpected respiratory distress, fatal in one case, a few weeks after the initiation of chemotherapy.

Case 12: A 54 year-old malnourished alcoholic with widespread tuberculous bronchopneumonia (Fig. 40), shedding "scanty" acid-fast bacilli (AFB) in sputum. He had a negative Mantoux skin test to 10 TU PPD, anaemia (Hb 12.8 g/dl), low serum sodium (127 mmol/l), albumin (24 g/l), iron (4 μmol/l), TIBC (33 μmol/l), lymphocytopenia (774/μl), and a profound depression of all anthropometric indices. PBMC stimulation with virus, mitogens and antigen yielded very low levels (<2.0 log 10 u/ml) of IFN-α and IFN-γ. His

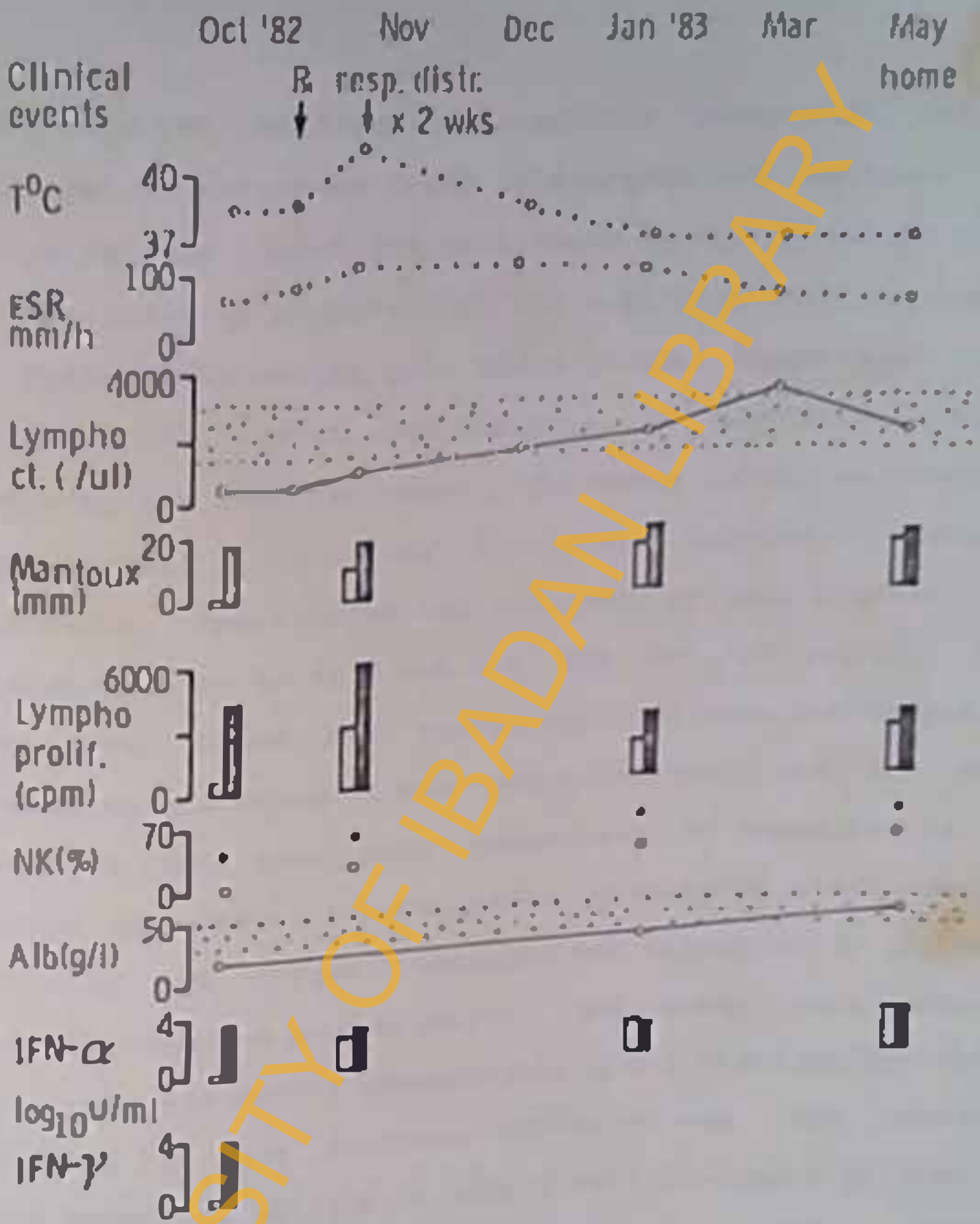

Fig 40: Serial clinical, laboratory and immunological data from one patient (Case 1) who survived a reaction during treatment. (Open bars/circles = values for patient; closed bars/circles = values for matched healthy controls; shaded areas = normal laboratory ranges).

spontaneous NK activity was grossly subnormal, and PBMC proliferative responses (cpm) to mitogens and antigens were far below the lower 95% confidence limit for all patients. The initiation of chemotherapy and ward diet with supplements was followed by weight gain and a gradual improvement in his clinical state, serum biochemistry, NK activity, Mantoux reactivity and PBMC responses. Two weeks later, he developed sudden severe respiratory distress, hypoxia, cyanosis, tachycardia, hypotension and worsening of lung shadows. His temperature rose to 41°C and his ESR to 110 mm/hr. Lung scans, ECG, blood and sputum cultures were not helpful in elucidating the cause of his deterioration, and the chest X-ray did not show lung congestion or pneumothorax. He remained very unwell for two weeks in spite of broad-spectrum antibiotic and oxygen treatment, but subsequently improved. His first negative sputum culture and smear were obtained four and six months respectively after starting treatment. In view of his slow response to treatment, and realistic doubts about his ability to comply with treatment at home, he was kept in hospital for seven months. He was readmitted unwell two weeks after discharge with a recurrence of symptoms and, remarkably, the sputum smear showed numerous AFB. His treatment was resumed, but he subsequently absconded from the Hospital.

Case 29: A 38 year-old barman admitted with a five month history of productive cough, severe weight loss (4 stone), fever and drenching night sweats, and found to have extensive bilateral lung consolidation with numerous AFB in sputum. He

had anaemia (Hb 10.5 g/dl), lymphocytopenia (332/µl), low serum sodium (129 mmol/l), total protein (44 g/l), albumin (18 g/l), iron (5 µmol/l) and TIBC (15 µmol/l) and gave a small positive (6 mm) skin induration to 1TU PPD. In contrast to Case 12, his NK activity, ^{3}H-thymidine uptake and IFN-α and IFN-γ production in response to mitogens, antigen and virus were within normal limits for healthy controls (Fig. 41). Five days after the initiation of chemotherapy he developed severe dyspnoea, pleuritic pain, increasing neutrophil leucocytosis and respiratory failure. He did not respond to methylprednisolone, heparin and positive pressure ventilation, and died in cardiac arrest on day 14. Autopsy revealed ante-mortem thrombi in the Pulmonary vessels and extensive lung infarction.

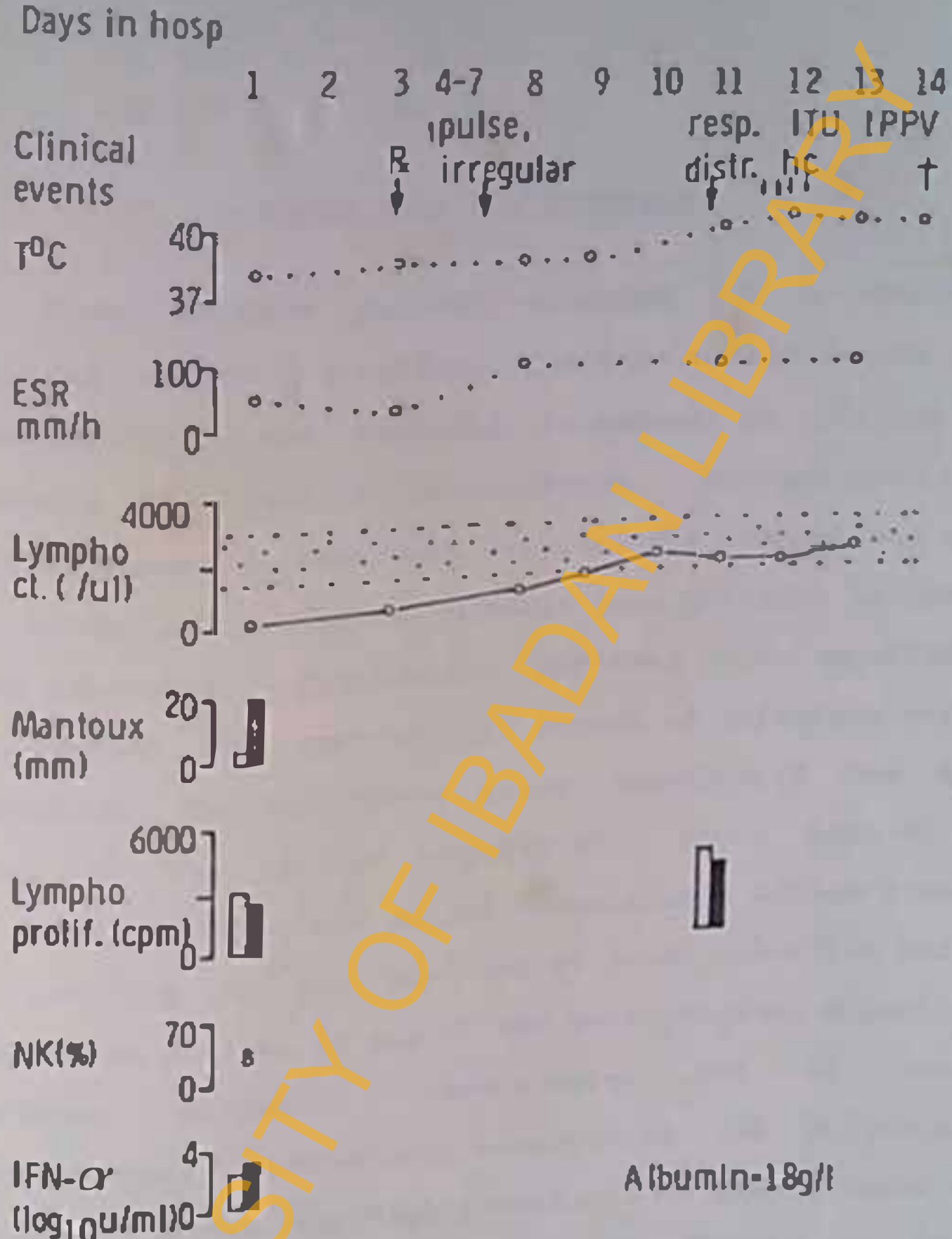

Fig 41: Clinical, laboratory and immunological data from another patient (Case29) who had a fatal reaction during treatment (ITU = intensive therapy unit; hc = hydrocortisone; IPPV = intermittent positive pressure ventilation; † = death. For other details see legend to Fig. 40).

CHAPTER FOUR - DISCUSSION

These studies provide evidence for a spectrum of clinical forms, nutritional, metabolic and immune response abnormalities, and clinical responses to therapy among patients with active tuberculosis. Whereas most patients were symptomatic, four were surprisingly without any symptoms in spite of extensive disease (one patient) or cavitation (two patients). Symptomless patients are important when considering the control of spread of infection within the community. The ISC ethnic group constituted the majority (70%) of the patient population. About half of all ISC patients had developed active tuberculosis within five years of settling in the UK, but at least one-fifth had active disease on arrival in the UK and were admitted directly from Heathrow Airport. Furthermore, 88% of cases of extrapulmonary tuberculosis occurred in ISC patients. The reasons for the very high prevalence of tuberculosis and the higher frequency of extrapulmonary disease in the ISC population of the UK are not known. There is evidence that the strains of M. tuberculosis acquired by ISC immigrants are prevalent in their countries of origin and are responsible for their subsequent disease (Grange et al, 1977). These strains tend to belong to phage type 1, are more susceptible to hydrogen peroxide and more sensitive to thiophen-2-carbonic acid hydrazide, suggesting that they are

less virulent than those isolated from British or African patients (Subbaiah et al, 1960; Mitchison et al, 1963; Bates and Mitchison, 1969; Grange et al, 1977). Thus, the greater frequency of reactivation (or re-infection) tuberculosis seen in this group is probably not related to the virulence of the organism, but to some characteristics of the host. Grange and colleagues (1977) suggested that ISC patients may have a natural susceptibility, or that immigration and the attendant physical and sociocultural problems may alter their resistance in some unexplained manner.

A. Nutrition

Compared to matched controls, patients with tuberculosis were significantly less heavy (measured as W/H^2), less fat (measured as skinfold thickness) and less muscular (measured as arm muscle circumference) and had significantly lower serum sodium, albumin, iron and TIBC. Hyponatraemia could have been caused by inappropriate ADH secretion (Edingham, 1983), subclinical Addison's disease or dietary deficiency.

Initiation of anti-tuberculous chemotherapy and ward diet with supplements was followed by a gradual repletion of body weight, fat, serum sodium, iron and TIBC, all of which were fully reconstituted by six months. However, mean arm muscle circumference and serum albumin levels remained persistently low for as long as 12 months after initiation of treatment, indicating that total body protein stores were not fully reconstituted during treatment. The initial rapid improvement in these indices became less marked, as may have occurred because of the ward diet during early

hospitalisation. However, in the present study, comparison with a non-hospitalised treatment group was not made.

Whether deficient protein reserves are a predisposing cause or an effect of tuberculous infection, or both, is not clear. Undernutrition favours reactivation of latent tuberculosis (Edwards et al, 1971) since deficiencies of calories, protein, various vitamins and trace elements consistently impair lymphocyte functions (Chandra, 1983). If undernutrition is an effect of tuberculous infection - and this is probable as weight loss is a cardinal symptom - it could be that protein reserves take longer than 12 months to replete, particularly among sedentary vegetarians who constituted half of our patient population. It is notable that mean anthropometric indices for the 30 healthy controls (70% of whom were of ISC origin) were slightly less than the norms compiled by Jelliffe (1966) for use in developing countries; even Jelliffe's standards underestimate the average measurements for Western countries (Blackburn and Biotrian, 1977). Tuberculosis patients are therefore profoundly malnourished at diagnosis, and protein malnutrition persists at least as long as the present standard nine-month chemotherapy regimen.

Initial anthropometric assessment should identify patients in need of special nutritional support. The institution of a high-protein diet might aid chemotherapy and facilitate recovery from the disease. However, it is not known what causes weight loss in tuberculosis, and whether

dietary protein supplementation would increase albumin stores. Loss of body mass is caused by an imbalance in which energy expenditure exceeds energy (food) intake (Horton, 1983). This may explain the cachexia of tuberculosis. Many patients are anorectic, and probably have a reduced food intake; most have fever and night sweats which would waste energy. Further studies are needed to measure precisely food intake, metabolic rate and intermediary metabolism in these patients.

B. Blood counts, ESR, lymphocyte subpopulations

At diagnosis, patients with tuberculosis had marked anaemia, neutrophil leucocytosis, lymphocytopenia and elevation of ESR, serum alkaline phosphatase, monocyte and eosinophil counts. These measurements had returned to normal after six months of treatment, confirming earlier work by others (Humber et al, 1980). Analysis of lymphocyte subpopulations showed that patients had significant reductions in absolute counts of total circulating T-cells, helper/inducer T-cells (T_H) and B cells. Their mean total suppressor/cytotoxic T-cell (T_S) counts and T_H/T_S ratios were not statistically different. However, two patients had decreased T_H/T_S ration which increased gradually during treatment, but were still subnormal at seven and 14 weeks respectively, when their total lymphocyte counts had returned to normal. One healthy control with decreased T_H/T_S ratio and increased lymphocyte count (i.e. increased T_S cells) had just recovered from chickenpox six weeks previously. An

increase in T_S cells (which include cytotoxic cells), described as T_S "augmentation" (Fahey, Detels and Gottlieb, 1983), is probably a normal response to common viral infections. This pattern of abnormality has been observed after primary infections of normal subjects with cytomegalovirus or Epstein-Barr virus (Reinherz, O'Brien, Rosenthal and Schlossman, 1980; Crawford, Brickell, Tidman, McConnell, Hoffbrand and Janossy, 1981; Carney, Rubin, Hoffman, Hansen, Healey and Hirsch, 1981), after herpesvirus infections of renal transplant recipients (Schooley et al, 1983), and in symptom-free promiscuous homosexuals (Pinching, Jeffries, Donaghy, Murday, McManus, Moshtael, Parkin and Harris, 1983). Our subject denied being homosexual.

Lymphocytopenia has been found previously in tuberculosis (Zwolinski et al, 1976; Shima et al, 1976; Al-Tawil and Thewaini, 1978; Katz et al, 1979; Humber et al, 1980), though not by all workers (Heish and Chong, 1981). The restoration of consistently normal counts within two weeks of treatment suggests a perturbation of lymphocyte traffic rather than a failure of production in the bone marrow. Such perturbation may be caused by selective lymphocyte sequestration into infected organ-systems which is thought to occur in active tuberculosis (Rook, Carswell and Stanford, 1976; Flouffe, Silva, Fekety and Baird, 1979; Kinman, Fryden, Eriksson, Moller and Link, 1981; Fujiwara, Okuda, Fukukawa and Tsuyuguchi, 1982). The mechanism of such lymphocyte trapping or its reversal by chemotherapy is not known. It is possible that the rapid killing of tubercle

bacilli by effective modern drugs allows the release and rapid return of the trapped lymphocytes into the circulation. While there were absolute reductions in total numbers of both T-cells and B-cells in this series, various authors have reported a predominantly T-lymphocytopenia (Al-Tawil and Thewaini, 1978; Skvor and Trnka, 1979; Katz et al, 1979; Humber et al, 1980). In agreement with Katz and colleagues (1979), the T-lymphocytopenia observed in this series was due mainly to a reduction in T_H cells. Absolute T_H-lymphocytopenia in the presence of a normal T_H/T_S ratio in this series implies some reduction in T_S cell count also, even though absolute T_S cell numbers were not significantly reduced. This finding contrasts with the data of Katz and colleagues who reported a proportional increase in T_S cells in tuberculosis patients. However, T_H-lymphocytopenia with normal or slightly reduced T_S counts could indicate a relative physiological excess of T_S activity. But there is no good evidence that numeracy of cell counts correlates directly with physiological activity.

The finding of B-lymphocytopenia in tuberculosis patients is surprising, and does not appear to correlate with in vivo over-production of IgG and IgA (but not IgM) observed before treatment (Fig. 15). Tuberculoproteins such as PPD are polyclonal B-cell mitogens (Sultzer et al, 1972). It is probable that the in vivo antibody response of the fewer B cells available is greatly potentiated by the powerful adjuvant activity of freely available mycobacterial antigens. However, Katz and colleagues (1979), using a haemolytic

plaque-forming assay to measure antibody production to sheep red blood cells, showed that B lymphocytes from tuberculosis subjects were hyporesponsive in vitro to pokeweed mitogen due to inhibition by adherent cells. Therefore the coexistence of B-lymphocytopenia and hypergammaglobulinaemia in this series may not be fully explained by the potent adjuvant and mitogenic effects of tuberculoproteins on B-cells.

The findings from this study contrast with previous reports by some investigators of normal (Williams et al, 1973; Gatner and Anderson, 1980; Hsieh and Cheng, 1981) and increased (Kventy, 1977) T-cell counts, and normal (Williams et al, 1973; Bhatnagar et al, 1977; Al-Tawil and Thewaini, 1978; Gatner and Anderson, 1980;) and increased (Hsieh and Cheng, 1981) B-cell counts in patients with tuberculosis. This may be due to differences in case selection, disease severity between study populations, or in enumeration techniques. Whereas most other workers used rosetting techniques, an arguably more accurate and specific technique using fluorescein-labelled monoclonal antibodies was employed in this study.

One alternative explanation for these findings is that there may be a reduction, not in absolute cell counts, but in the density of antigenic molecules on the cell surfaces recognised, for example, by antibody reagents. It is recognised that cell surface glycoproteins may alter in protein energy malnutrition (Chandra, 1983). However, one might expect such reductions to affect all lymphocyte subsets, which was not the case.

C. Tuberculin skin reactions

Early skin reactions (6-12 hrs) to low-dose (1 TU) tuberculin were significantly more frequent in patients than matched controls, although the proportions reacting by 72 hours were similar in both groups. Thus, at 6 hrs, 58% of 24 patients and none of 24 controls gave a reaction to 1 TU PPD; at 12 hrs, 71% of patients and only 8% of controls had reactions (P<0.001).

Clonchur, Fosslock and Silverman (1965) elicited such an early reaction to an ultrasonicate of M. tuberculosis in 91% of 106 patients and none of 61 controls. Similarly, Wilhelm and Romer (1977) described a peptide isolated from a ribonucleoprotein fraction of M. tuberculosis which elicited a skin-response maximum at 24 hours apparently only in those with active tuberculosis. More recently, Kardjito and colleagues (1982) have observed this early reaction using PPD in 72% of 107 patients but also in 3.5% of 143 controls. Since PPD is an impure mixture of mycobacterial products (Seibert and Munday, 1933), it would appear from these results that the discriminatory value of the accelerated skin reaction increases with the specificity of the antigen used. The cause of this early dermal response is not known, but Kardjito and Grange (1980) have suggested that it may represent an antibody-mediated Arthus reaction, and thus would be influenced by circulating antibodies to the antigen

used and less by the state of delayed hypersensitivity. The use of pure and specific antigens for this test, if they are made available by monoclonal antibody technology, may improve its diagnostic specificity and sensitivity. However, no correlations were found in this study between total IgG and IgA antibody titres and the timing or sizes of skin-test reactions.

Tuberculosis patients had a larger mean maximum induration to PPD than controls, but this would not have been of any value in making the diagnosis. Ten per cent of patients and 19% of healthy controls failed to give a positive skin reaction to 10 TU PPD. Among 10 patients, the proportions of early (6-8 hr) and delayed (48-72 hr) reactions were significantly increased, the latter to 100%, after six months of chemotherapy (Fig. 22). In contrast, repeat testing of 18 controls at six months showed an increase only in the proportion of early reactions. Furthermore, patients and controls gave significantly larger early and delayed reactions, patients at 12 months and controls at six months. These observations confirm previous reports (Steele and Willis, 1934; Magnus and Edwards, 1955; Narain, Nair, Rao, Chandrasekhar and Lal, 1966; Thompson, Glasgroth, Snider and Farer, 1979) that sequential tuberculin testing has a booster effect on tuberculin sensitivity in healthy humans.

Repeat testing of healthy controls in this series amplified cellular (T-cell) responses - but only in initial reactors - causing larger delayed reactions. If early reactions are indeed antibody-mediated, then repeat testing would also have enhanced antibody production - in both initial reactors and non-reactors - increasing the sizes of early reactions and the proportions of those reacting. However, repeat tuberculin testing did not induce delayed tuberculin hypersensitivity in initial healthy non-reactors. Therefore, the increase with time in the proportion of patients giving delayed reactions (to 100%) must have an alternative basis: probably a result of improved T-cell responsiveness associated with effective therapy (Goldstein et al, 1976). Additional evidence for this was seen in the parallel improvement observed with treatment in other lymphocyte responses - ^{3}H-thymidine uptake and interferon production.

D. Cytotoxicity

These data confirm other reports of wide variation in NK cytotoxicity among normal people (Forbes, Niblack, Fuchs, Richie, Johnson and Oldham, 1981; Antonelli, Stewart and Dupont, 1981). However, results from repeat tests in normals (Table 8) confirm that the level of endogenous NK activity in healthy donors is surprisingly stable (Pross and Baines, 1982) but that it is possible to categorize normal donors as "high" or "low" responders who remain high or low on repeated testing.

TABLE 8

Serial MK cytotoxicity (%) for mononuclear cells of healthy donors against K562 targets at an E:T cell ratio of 40:1

	Month						
Donor	1	2	3	7	9	11	14
J.O.	21	23	30	26		22	28
	24	28	38				
		28					
G.S.	42	37			54		46
A.J.			71		51		75
M.O.			"	55	66		68

Yoneda, Kasai, Ishibashi, Nishikawa, Tokunaga and Mikami (1982, 1983) reported that patients with pulmonary tuberculosis have enhanced NK activity, and that further augmentation occurred during chemotherapy. Their conclusion was supported in a separate study by Ina and colleagues (1982). The findings from the present study, however, do not agree with these reports. Levels of endogenous and IFN-augmented NK cytotoxicity were comparable in our group of 27 tuberculosis patients and 27 matched controls. Furthermore, no change was observed in mean spontaneous NK activity after six months of chemotherapy. Only one patient had subnormal NK activity which was unresponsive to IFN. He was an alcoholic with extensive disease, negative skin tests, diminished in vitro IFN-α response to virus and IFN-γ and proliferative responses of lymphocytes to PPD, and gross asthenia evidenced by severely reduced anthropometric indices, haemoglobin, serum albumin, iron and total iron-binding capacity. Alcohol consumption is associated with a depression of T-cell functions (Lundy, Raff, Deakins, Wanebo, Jacobs and Lee, 1975) and, paradoxically, elevation of NK activity (Saxena, Mezey and Adler, 1980a). Malnutrition, however, is a well-recognised cause of impaired cell-mediated immunity (Chandra, 1983). Also, Saxena, Saxena and Adler (1980b) showed that food deprivation in mice caused marked loss of NK activity.

The mean cytotoxicity (17.4% at 20:1 ratio) reported by Yoneda and colleagues for a healthy population is much lower than was found in this study (32.5%) and in most series

(Forbes et al, 1981; Antonelli et al, 1981; Penechow and Mackay, 1980; Fernandes and Gupta, 1981; Nagel, Collins and Adler, 1981). Low values in this population could not be attributed to the high proportion of elderly people (24 out of 54 were over 70 years) in their series, since NK activity does not decline with age (Fernandes and Gupta, 1981 ; Nagel et al, 1981). It is possible that these differences are inherent in the population studied since NK activity is genetically determined (Roder and Prose, 1982). However, Yoneda and colleagues did not state the level of background lysis in their assays (an indicator of assay quality), and also performed assays at only one E:T cell ratio. One advantage of assays at multiple ratios is that the investigator can confirm a linear increase in NK activity with E:T cell ratio, an important indicator of validity.

The present data show clearly that NK activity is usually normal in active tuberculosis and is not affected by standard chemotherapy (rifampicin, isoniazid and ethambutol) nor by the progressive enhancement of immune reactivity which occurs during treatment. These results suggest that the NK system probably does not play a primary role in host defence against tuberculosis, although it may become impaired as part of widespread anergy of advanced disease.

E. Mononuclear cell proliferation

In the ^{3}H-thymidine incorporation assays performed in fetal calf serum (FCS) the maximum responses of mononuclear cells from 29 patients cultured alone or with PPD were depressed significantly when compared with controls. The maximum difference was seen at 1u PPD. In contrast, no differences were seen in cultures stimulated with the T-cell mitogens SEA and TPA. When mononuclear cells from 13 patients and controls were cultured in 10% pooled normal human serum or 10% patient's serum, no differences were observed between the groups in ^{3}H-thymidine uptake by unstimulated or stimulated cells. Although sera were always heat-inactivated and filtered before use, the uptake into unstimulated cultures sometimes exceeded 500 cpm. This high background may be attributable to the mitogenic activity of some batches of FCS, probably caused by contamination with bacterial endotoxin (Parrant and Newton, 1981). However, the design of this study made it unlikely that variations between batches of FCS (or other reagents) would affect inter-sample comparisons. Each patient and matched control were always tested together under identical conditions, using the same reagents; maximum counts were compared by paired t-tests without subtraction of the background.

Among healthy controls, the maximum PPD response frequently occurred on Day 4 and with 1u PPD, while among patients maximum responses tended to occur later (Day 5 or after) and with higher concentrations of PPD. In vitro hyporesponsiveness to PPD and other antigens with or without relative preservation of mitogen reactivity has been reported

among tuberculosis patients (Heilman and McFarland, 1966; Ellner, 1978; Humber et al, 1980), but not consistently (Gatner and Andersen, 1980).

The findings from this study could be explained on the basis of differences in lymphocyte receptor affinities. According to the clonal selection theory (Burnet, 1969) subjects with tuberculosis or positive tuberculin skin reactions should have "PPD-specific" lymphocytes in their peripheral blood; this has been demonstrated (Smith and Reichmann, 1972; Ellner, 1978; Okubo, Kosama and Yano, 1982). However, it has been suggested that continuous exposure of tuberculosis patients to a large antigen load may lead to some degree of immunological tolerance and may induce lymphocyte populations with low-affinity receptors (Nilsson, 1972). Such low-affinity cells would presumably require larger doses of antigen and longer periods of cultivation for maximum activation in vitro, whereas healthy lymphocytes retain mainly high-affinity receptors which are promptly triggered by low doses of antigen. Anergy in miliary tuberculosis could be explained in this way. Also, it is of interest that the very first investigation on lymphocyte reactivity to PPD (Pearmain et al, 1963) showed that lymphocytes from tuberculosis patients did not proliferate when exposed to PPD in a low concentration, while cells from successfully treated cases did. That mononuclear cells from tuberculosis subjects in this study proliferated normally to the non-specific mitogens SEA + TPA, but not to the "specific" antigen PPD, would support this theory of low antigen-receptor affinity.

Contrary to expectations, PPD caused considerably more ^{3}H-thymidine uptake than SEA + TPA. Whereas mitogens will stimulate up to 80-90% of a particular lymphocyte class, mostly T-cells, PPD and other specific antigens stimulate only the small number of cells bearing specific receptors, usually less than 0.1% of the total (Wilkinson and Morris, 1983). However, there is evidence for non-specific recruitment of lymphocytes in the PPD-specific proliferative response (Tsuyuguchi, Shiratsuchi, Fujiwara and Teraoka, 1982). Also, in addition to its antigenic properties, PPD has non-specific mitogenic activity for B-lymphocytes which is probably mediated by mitogenic T-cell factors (Blomgren, 1975).

In conclusion, mononuclear cells (which consist predominantly of lymphocytes) from tuberculosis patients were relatively hyporesponsive to antigen but not to mitogens, although cells from a few patients with more extensive disease were unresponsive to both. In vitro lymphocyte anergy was probably induced by active infection, since normal reactivity was restored by treatment. Loss of reactivity is probably progressive, initially affecting antigen-sensitive lymphocytes but later involving other lymphocytes in advanced disease. One mechanism for lymphocyte hyporesponsiveness could be the induction of tolerance (Nilsson, 1972) by immunosuppressive cell wall substances of tubercle bacilli (Kleinhenz, Ellner, Spagnulo and Daniel, 1981). Evidence for a serum factor blocking lymphocyte proliferation (Heilman and Hofer land, 1966) was not found in this study.

F. Monocyte migration

Fifteen tuberculosis patients had relative impairment of mean random locomotion and casein-stimulated chemotaxis compared to matched controls. These findings confirm the reports of Campbell (1979) and Nielsen et al (1982), who attributed the defect to a circulating anti-chemotactic plasma factor. Whether this defect is a cause or effect of tuberculosis is not clear from this study, but Campbell found that monocyte migration returned to normal during treatment. Impaired monocyte migration to the site of a tuberculous infection, for example, could have serious implications for the granulomatous response which is essential for host resistance against M. tuberculosis (Youmans, 1979).

G. Interferon production

IFN-γ responses to tuberculin proteins appear to be relatively depressed in a proportion (36%) of tuberculosis patients in this study. This finding may be spurious, but it may be a cause or an effect of the tuberculous infection. IFN assays were set up blind for the patients and controls in parallel, and were consistent for the standard preparation tested on every plate, so it seems unlikely that the large differences observed arise because of inaccuracy in the assay.

It is not known whether low titres of IFN arise because cells of some tuberculosis patients are unable to respond as efficiently to PPD (and to a lesser extent T-cell mitogen or virus), or whether there is a smaller proportion of responding cells, or a deficiency of some accessory factor such as macrophage help or interleukin 2 in the preparation made.

Certain individuals may have a genetic predisposition to the development of inadequate IFN-γ responses following tuberculous infection, resulting in failure to activate macrophages, and progression of the infection to active disease. Evidence from studies of community groups (Large, 1964), twins (Kallman and Reisner, 1943) and HLA antigens (Al-Arif et al, 1979; Singh et al, 1983) strongly support a role for genetic factors in the control of the host immune response to tuberculosis. In the UK it has been suggested that the very high prevalence of tuberculosis among persons of ISC ethnic group may be attributable in part to a natural susceptibility (Grange et al, 1977). Whether this susceptibility resides in abnormal lymphokine (IFN-γ) production in response to infection deserves further investigation. Although both high and low responder patients were comparable for ethnic backgrounds, the single low-responder control subject in this study was of ISC origin.

Alternatively, reduced IFN-γ production may primarily reflect a quantitative deficiency in specific responder T-cells, since Leu 3a + helper cells, which were strikingly decreased in this series of tuberculosis patients appear to be the principal T-cell subset responsible for antigen-induced production of IFN-γ (Cunningham and Morigan, 1984).

H. Reasons for anergy

Suppression of antigen-induced cutaneous, proliferative and IFN-γ responses of mononuclear cells from some patients with tuberculosis may have occurred for a variety of other reasons. Firstly, anergy is known to occur in untreated Progressive tuberculosis (Chaparas, 1982) and correlations have been reported between anergy and extent of infection (Bhatnagar et al, 1977). Three patients who were anergic to tuberculin skin testing had radiologically extensive disease, decreased IFN-γ production and decreased ^{3}H-thymidine uptake by mononuclear cells in response to PPD. However, no strong overall correlations were observed between radiological extent of disease and these other parameters of CMI, although the extent of infection could not be compared in five patients with exclusively extra-pulmonary tuberculosis (two bone, two glandular and one meningeal). Also, normal proliferative (^{3}H-thymidine) responses to powerful T-cell mitogens exhibited by mononuclear cells from two of the three patients with skin-test anergy would argue against the concept of generalised anergy, or cell death in culture, as an explanation.

Secondly, selective sequestration and peripheral blood depletion of antigen-reactive cells may lead to the loss of antigen-induced cellular responses. Such selective compartmentalisation has been demonstrated in experimental systems (Schlossman, Levin, Rocklin and David, 1971), infected lymph nodes of patients with tuberculous adenitis (Rook et al, 1976), tuberculous pleural fluid (Fujiwara et al, 1982) and cerebrospinal fluid in tuberculous meningitis (Plouffe et al, 1979; Kinnman et al, 1981).

Anergy to tuberculin skin testing, sometimes seen in up to one third of patients with tuberculous pleurisy (Berger and Mejia, 1973), has been attributed to preferential sequestration of antigen-reactive lymphocytes in the pleural space (Stead and Bates, 1971). Selective sequestration would certainly account for the occurrence of peripheral blood lymphocytopenia and its rapid reversal within 14 days of starting treatment. However, all four patients with pleural effusions in this study were skin test positive, had normal mononuclear cell proliferative responses, and all but one produced normal levels of IFN-γ in response to PPD! Also, no overall correlation was found between circulating lymphocyte counts and the extent of infection, skin reaction sizes, or PPD-induced mononuclear cell proliferative or IFN-γ responses.

Thirdly, circulating suppressor cells may suppress the reactivity of antigen-sensitive cells in tuberculosis. Although IFN-γ production in responses to mitogens and antigens requires both T-lymphocytes and macrophages (Epstein et al, 1971; Arbeit, Leary and Levin, 1982), certain T-cells (Johnson, 1981) and macrophages in some situations (Arbeit et al, 1982) may actually inhibit IFN-γ production. In normal individuals, at least three pathways regulating lymphocyte proliferation have been described (Rice, Laughter and Twomey, 1979), and the suppression of responses to antigens or mitogens has been variously attributed to T-lymphocytes (Shou, Schwartz and Good, 1976), to B-lymphocytes (Bona, Audibert and Juy, 1976) and to cells of the monocyte-macrophage series (Laughter and Twomey, 1977).

Similarly, among patients with active tuberculosis, circulating suppressor monocytes (Ellner, 1978) and T-cells (Katz et al, 1979) have been reported, and it has been suggested that suppressor lymphocytes are induced by the infection (Tsuyuguchi, Shiratsuchi, Terasoko and Hirano, 1980; Wadee, Sher and Rabson, 1980). The group of low IFN-γ responders in this study share some similarities with a subset of anergic patients defined by Ellner (1978) except that the former had normal monocyte and higher neutrophil counts than the other (high-responder) patients.

Fourthly, circulating serum factors depressing lymphocyte reactivity have also been demonstrated in active tuberculosis (Hellman and McFarland, 1965), but not confirmed

(Hinz et al, 1970). There is evidence that such blocking factors may consist of potent immunosuppressive cell-wall substances of tubercle bacilli (Kleinhenz et al, 1981) which have been shown to suppress antigen-induced ^{3}H-thymidine uptake and lymphokine (MIF) production by human PMBC (Ellner and Daniel, 1979). There was no evidence for such blocking factors among 13 patients tested in this study. This finding must, however, remain inconclusive, as the number of patients tested was small. Also, inhibitory serum factors may be present only in certain (?anergic) patients and at such low titres that some means of concentrating the serum may be necessary to demonstrate their presence.

Finally, all the defects observed could be secondary to malnutrition associated with tuberculosis. Clinical studies have demonstrated that T-lymphocytopenia, decreased cutaneous delayed hypersensitivity, lymphocyte transformation and lymphokine (MIF) production in response to PPD may result from a deficiency of calories, protein (Chandra, 1983) or iron (Joyneon, Walker, Jacobs and Dolby, 1972), all of which were profoundly depressed in this group of tuberculosis patients.

Whether they are causes or effects of tuberculous disease, inadequate antigen-induced lymphocyte proliferation and production of IFN-γ and other lymphokines could result in failure to generate effector cells, failure of macrophage activation, and disease progression. Recent studies have demonstrated that human IFN-γ is the active lymphokine that

induces macrophages to exert enhanced antimicrobial activity against intracellular pathogens such as Chlamydia psittaci (Rothermel, Rubin and Murray, 1983), Toxoplasma gondii (Nathan, Murray, Wiebe and Rubin, 1983) and Leishmania donovani (Murray, Rubin and Rothermel, 1983). It may well be the premier lymphokine required to limit tuberculous infection.

t.

Reactions during chemotherapy

The earliest events observed following initiation of chemotherapy were a rapid rise of blood lymphocyte counts and a sharp increase in ESR to a peak within the first two weeks (Figs. 12 and 13). It was at this time when chemotherapy might be expected to be most effective that severe reactions occurred in two patients, one of whom died. Both were alcoholic, self-neglected, malnourished, and presented with radiologically widespread pulmonary disease and lymphocytopenia. While the older man had profound depression of tuberculin skin reactivity, lymphocyte proliferation, IFN-production and NK activity (Fig. 40), the younger who died had normal values for these markers of cell-mediated immunity (Fig. 41)

Ellis and Webb (1983) have drawn attention to an important group of patients who died suddenly, unexpectedly and without obvious cause within a few weeks of starting treatment for pulmonary tuberculosis. In their series, 14 out of 80 patients certified dead from tuberculosis came into

this category. They were young, had localised disease, and shared certain characteristics with the two seriously ill cases in this study: recent initiation of chemotherapy (a few weeks), absence of treatment with steroids, and a predominance of males. This pattern of response has been noted by other clinicians (Clague, El-Ansary and Hopkins, 1983; Barss, 1983; Smith and Onwubalili, 1983). Within a few weeks of starting antituberculous chemotherapy, some patients deteriorate clinically and may even die. Although this may result from intercurrent pulmonary embolism, myocardial infarction or septicaemia, there are often no findings to support such complications. It may be that this is the natural course of such advanced disease. However, it has often seemed that the initiation of therapy has hastened the death of these patients.

Acute adrenal insufficiency precipitated by treatment with rifampicin has been reported (El-Ansary and Zaria, 1983), and the timing of acute reactions often coincides with the time of peak hepatocellular enzyme induction by this drug: two weeks (Buffington, Dominguez, Piering, Herbert, Kauffman and Lemann, 1976). However, respiratory failure such as is frequently seen in these cases is difficult to attribute to rifampicin, ethambutol or isoniazid. More likely, it results from delayed hypersensitivity reactions precipitated by two immunological consequences of treatment.

First, data from this study have shown that rapid bacterial killing is usually followed within a few days, weeks or months by an increase in the numbers and antigen reactivity of circulating lymphocytes (Figs. 12 and 29).

Secondly, a huge dose of such antigenic material suddenly becomes available, and may be released into the circulation either directly or via macrophages. When tuberculoprotein is injected directly into the circulation of an immune animal, systemic reactions such as fever, malaise and shock ensue (Youmans, 1979); this was soon discovered by Koch in his initial unsuccessful attempts to treat tuberculosis patients with injections of old tuberculin (Koch, 1890; Bignall, 1982). Furthermore, killed mycobacteria are potent immunological adjuvants (Freund, 1956), especially in large numbers (Brown and Brown, 1982). A massive load of antigen presented suddenly to large numbers of "resuscitated" immunocompetent lymphocytes would precipitate severe systemic hypersensitivity. Where pulmonary reserve is already small, such as in extensive pulmonary tuberculosis, further inflammation in the lungs will result in increased alveolar capillary permeability and the clinical picture of the adult respiratory distress syndrome observed in some cases.

Other patients with less severe disease may have mild and self-limiting reactions in the lungs, lymph nodes, brain or other foci of infection. Such reactions may be entirely ~~subclinical~~ and asymptomatic in the majority, manifesting

only as a sharp rise in ESR. Occasionally they may result in persistent fever, gland swelling, gland tenderness or the appearance of new lesions (e.g. brain tubercles). It is probable that even in patients with limited lung disease who do not have clinical reactions following treatment, some changes occur in alveolar-capillary permeability.

Further studies are needed to establish the pathogenesis of treatment reactions in order to identify high risk patients. Nevertheless, it would seem prudent to treat some tuberculosis patients with steroids ab initio; but which patients? In a recent survey of deaths from pulmonary tuberculosis in England and Wales, Humphries, Byfield, Darbyshire, Davies, Nunn, Citron and Fox (1984) found that 163 of 1312 patients (12%) died before they had completed chemotherapy, most of the deaths (70%) occurring in the first month of treatment. Of the 163 patients who died, 96% were of white ethnic origin. Factors predisposing to death in this group included (in order of significance) radiographic extent of disease, age, extent of cavitation and a positive sputum smear result. These data suggest that older white patients with extensive cavitating smear-positive disease run the highest risk for death during treatment.

CHAPTER FIVE - CONCLUSION

A spectrum of immunity in tuberculosis

Those studies have shown that patients with active tuberculosis exhibit varying degrees of a wide range of metabolic and immunological derangements. These include elevation of ESR, protein-calorie malnutrition, anaemia, neutrophil leucocytosis, monocytosis, helper-T-lymphocytopenia, hyponatraemia, overproduction of immunoglobuline, accelerated cutaneous tuberculin hypersensitivity, defects in antigen-induced mononuclear cell proliferative and lymphokine (IFN-γ) responses, and impaired monocyte migration.

It is possible to categorise the patients into groups according to their reactivity in representative tests of cell-mediated immunity (Table 9). Group 1 consists of well-nourished patients with positive dermal responses (early and delayed) to tuberculin-PPD, normal in vitro mononuclear cell proliferative and lymphokine (IFN-γ) responses to PPD stimulation. In contrast, Group 5 consists of grossly malnourished patients with negative skin tests and reduced or absent in vitro mononuclear cell proliferative and lymphokine (IFN-γ) responses to PPD. Between these extremes is a graduated and continuous downward gradation (Groups 2,3 and 4) in cellular responses. One patient in Group 5 (number 12) had the longest time (four months) to bacteriologic sputum conversion, while four patients in Group 1 (numbers 1,2,4 and

TABLE 9

The spectrum of immunity in tuberculosis

	Mantoux test (PPD)		PBMC transformation (PPD)	IFN-γ production (PPD)	Nutritional status	Patients' case no
	6h	48h				
Healthy controls	-	+	+	+	+	
Patients						
Group 1	+	+	+	+	+	1,4,14,15,23, 24,38,39
				+	+	12,7,11,16,18, 21,27,225,37
Group 2	+	+	+		+	25,8,9,29
				+		
Group 3	-	+	+		+	3,10,13,17,20
				+		
Group 4	-	-	+		+	6,12,19,35
				+		
Group 5	-	-	+			

Key: - = negative
+ = positive
+ = normal (within control mean ± SD)
+ = low (below control mean - SD)

15) had the shortest time (less than seven days). Such an analysis indicates that there is a spectrum of immunological responses to tuberculosis ranging from a state of hyper-sensitivity (Group 1) to one of anergy (Group 5). The patterns defined are not merely chronological steps in a single sequence. Although patients with the most extensive disease were profoundly malnourished and generally hyporesponsive to PPD in vitro and in vivo, there was no strong overall correlation among radiological extent or duration of disease, nutritional state and the level of CMI. For instance, patients in Groups 3 and 4 had equally advanced disease by chest x-ray criteria; however, Group 3 patients, although undernourished, had normal cellular responses to PPD, while Group 4 patients had normal nutrition but poor cellular responses. Also, no correlation was observed between immunological patterns and age, sex, ethnic group, neutrophilia, lymphocytopenia, monocytosis, raised ESR, alkaline phosphatase or hyponatraemia, suggesting that these derangements are non-specific events. Similarly, serum immunoglobulin levels did not follow the pattern of cellular responses, and patients were seen in every group who had either high or normal IgG levels.

Skinsnes (1968) was the first to suggest that tuberculosis, like leprosy, is a disease characterised by an immune spectrum. The data of Bhatnagar and colleagues (1977) provided strong support for this concept, and Lenzini and associates (1977) proposed a four-group classification, from reactive (RR) to unreactive (UU), based on clinical,

radiological and histopathological findings which they correlated with the results of immunological tests. Thus at the reactive end were patients with positive skin tests, low antibody titres and localised paucibacillary disease which responded quickly to treatment. At the unreactive end were those with negative skin tests, high antibody titres and disseminated multibacillary disease which responded slowly. Agnihotri, Chaturvedi and Pande (1978) proposed a similar three-group immunological classification. Further support for the existence of an immune spectrum in tuberculosis has recently come from Daniel, Oxtoby, Pinto and Moreno (1981), and Ridley (1983).

The findings in this study agree with those earlier reports, except that serum antibody (immunoglobulin) levels did not correlate with cellular responses as reported by other authors (Bhatnagar et al, 1977; Lenzini et al, 1977). However, they measured specific anti-PPD antibodies. The immunological spectrum defined in this series is less florid than that found in leprosy (Ridley and Joplin, 1966). This is not surprising. Tuberculosis differs from leprosy essentially in that the mycobacterium is more virulent, more immunogenic, and would not permit a chronic 'lepromatous' form of tuberculosis to exist. Consequently, at the multibacillary anergic end of the tuberculosis spectrum there are only a few surviving cases at any one time because the infection is progressive and fatal in this group. Thus, the spectrum of tuberculosis is probably equivalent only to the upper half of the leprosy spectrum (Ridley, 1983).

Two hypotheses suggested for the genesis of the leprosy spectrum are equally applicable to tuberculosis. The 'delay' hypothesis (Godal, Myrvang, Stanford and Samuel, 1974) suggests that the onset of CMI is delayed, indefinitely in anergic patients; the 'suppression' hypothesis (Scott, Russell, Boughton and Vincin, 1976) proposes that early disease is accompanied by CMI which either increases or is suppressed. The latter agrees far better with the clinical experience of tuberculosis, and parallels the events in the host-parasite interaction that lead to anergy (Fig. 42). Thus, following primary infection in healthy well-nourished subjects, the majority of lesions (95%) heal and remain healed throughout life. Tuberculin reactivity (afferent) develops during healing of primary tuberculosis or BCG vaccination. However, in susceptible individuals (Fig. 42) active disease develops from reactivation (in about 5% of infected persons, Daniel, 1982), or directly follows primary infection without intervening healing. The variable clinical picture is modulated by the variable state of the host's immunity. Delayed-type hypersensitivity (efferent), specific T-cell immunity and antibodies are acquired early on. Hypersensitivity is of value in the early stage when it ensures a rapid detection of the organism and build-up of an effective immune response (Dannenberg, 1968). Monocyte proliferation and monocytosis result from increased consumption in high turnover granulomas formed in response to cell-wall antigens and lymphokines. Monocytes or their products suppress CMI either directly (Elner, 1978), or

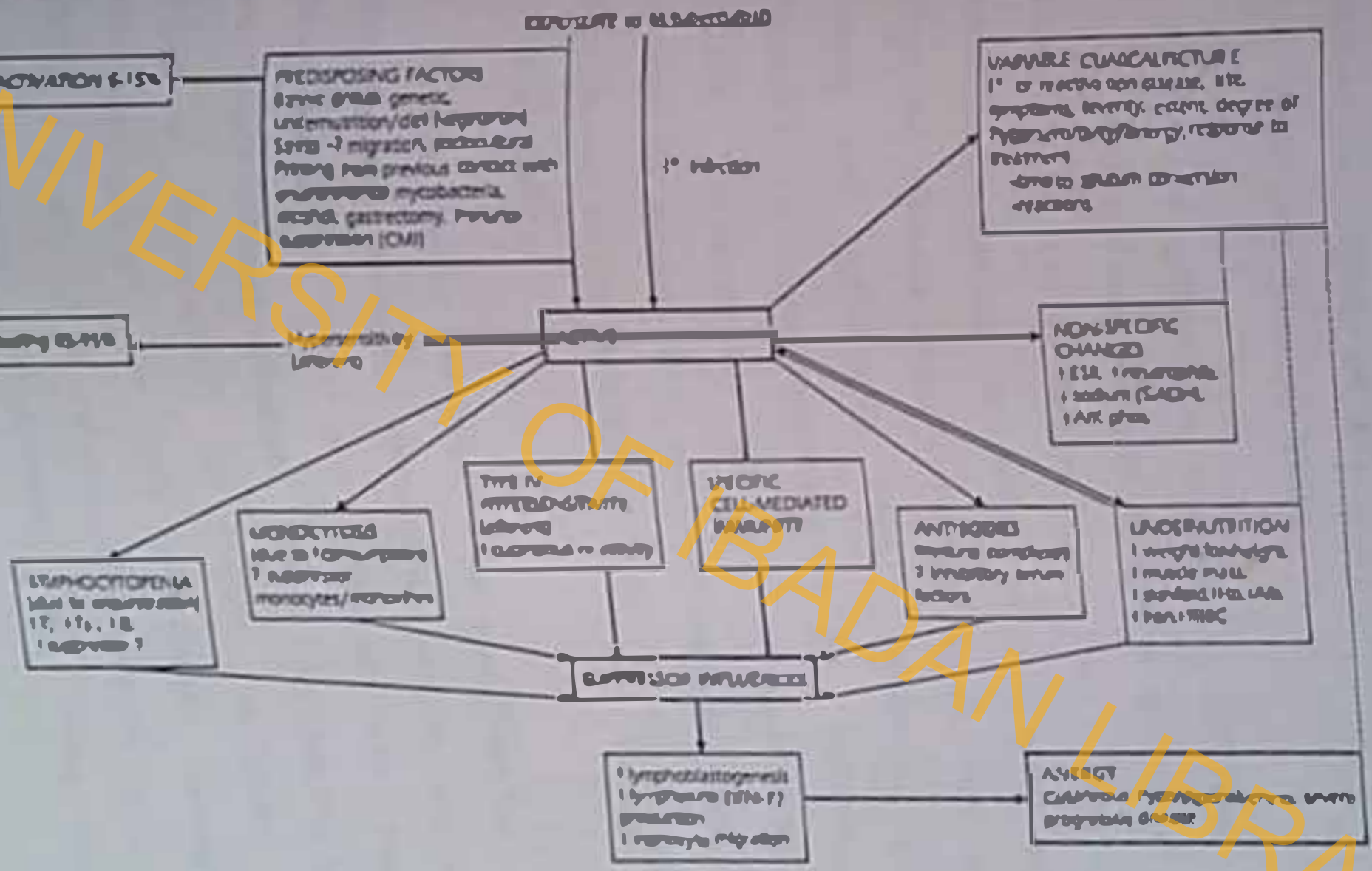

Fig 42: The natural history of infection with *M. tuberculosis*.

indirectly by inducing proliferation of suppressor T-lymphocytes (Katz et al, 1979) via the agency of monokines (Wadee, Sher and Rabson, 1980).

Selective lymphocyte trapping in infected organs (Rook et al, 1976) causes lymphocytopenia affecting predominantly T-helper cells, depleting the circulation of lymphokine-producing and other antigen-reactive lymphocytes. Antibodies, immune complexes and ?inhibitory serum factors (Hellman and McFarland, 1966; Campbell, 1979) may also contribute to immunosuppression by antagonizing T-cell function. Progressive disease is associated with deranged intermediary metabolism, reduced food intake and worsening malnutrition which profoundly impairs cell-mediated responses (Chandra, 1983).

If the viable bacterial load continues to increase and dissemination occurs, immunosuppressive cell-wall products (Kleinhenz et al, 1981) are released into the circulation from "stuffed" macrophages. These suppressive influences act singly or in concert to induce variable degrees of unresponsiveness. On this sequential spectrum there is usually a point of no return, above which there is a tendency to immunological enhancement, and below which there is a progression to anergy.

As in leprosy, treatment reactions probably occur as a result of upgrading of patients in the middle and lower zones of the spectrum. Such reversal reactions may result from two consequences of sudden massive bacterial killing: first, a

reduction in viable bacterial load causes rapid reversal of the lymphocyte trap and a sudden increase in the number and activity of T-cells; secondly, presentation of this massive antigen load to a reconstituted and 'resuscitated' lymphocyte population results in a severe inflammatory (delayed-type hypersensitivity) reaction in the lesion. This analysis would explain the timing of such reactions, mostly occurring 10 to 30 days after initiation of treatment - the time that it takes for lymphocytes to recover full immune competence and for the bactericidal drugs to produce the maximum antigen load. It would also explain why patients in the middle of the spectrum - who retain a relatively high level of hypersensitivity pre-treatment - probably have the most severe treatment reactions (the one fatality of two patients with reactions in this series belonged to Group 3).

This investigation suggests that there are several profitable areas for research in immunity in tuberculosis. First, the important antigens of *M. tuberculosis* need to be isolated to improve the specificity, sensitivity and diagnostic utility of tuberculins. Such pure antigens could be applied in diagnostic skin testing and in the development of an in vitro test that correlates precisely with immunity. Secondly, lymphokines appear to be important in limiting the infection. It would be exciting to investigate further the role of interferons, especially as purified natural IFN-α and recombinant IFN-γ are now available for clinical trials. It would also be worth investigating the therapeutic potential of IFN-γ in patients whose production is deficient.

Furthermore, the possible use of other immunoenhancing agents (levamisole, thymic extracts, transfer factor, IL-2 and monoclonal antibodies that ablate specific suppressor T-cell clones) to hasten recovery in anergic patients at the lower end of the spectrum must be considered. Thirdly, the possible role of dietary protein supplementation to facilitate recovery in malnourished patients needs to be investigated in a properly controlled clinical trial. Fourthly, the changes that regularly occur in lung function following the institution of chemotherapy, and fifthly, the definition of patients who need corticosteroids and the role of those drugs in the prevention of acute treatment reactions deserve further study. Finally, the possible role of serum factors, antibodies and immune complexes in immunosuppression and in the evolution of the spectrum of tuberculosis should be a profitable area for future research.

REFERENCES

Abstracts of the International Society for Immunopharmacology, July 1982. Second International Conference on Immunopharmacology, Washington DC. Int J Immunopharmacol 1982;4,pt 4.

Addison IE and Babbage JW. A raft technique for chemotaxis: a versatile method suitable for clinical studies. J Immunol Methods 1976;10:385-388.

Agnihotri MS, Chaturvedi UC and Pande SK. Immunological classification of pulmonary tuberculosis. Indian J Tuberc 1978;25:65-76.

Al-Arif LI, Goldstein RA, Affronti LF and Janicki BW. HLA-BW 15 and tuberculosis in a North American black population. Am Rev Respir Dis 1979;120:1275-1278.

Al-Tawil NG and Thowaini AJ. Study of the immunological status of patients with pulmonary tuberculosis. Scand J Immunol 1978;8:333-338.

Ali SK, Puri S, Chandra S and Chandra RK. 1983, Miscellaneous disorders, in Primary and Secondary Immunodeficiency Disorders, ed by Chandra RK, New York, Churchill-Livingstone, p. 284.

Antonelli P, Stewart IW and Dupont B. Distribution of natural killer cell activity in peripheral blood, thymus, lymph nodes and spleen, and the effect of in vitro treatment with interferon preparation. Clin Immunol Immunopathol 1981; 19:161-169.

Arbeit RD, Leary PL and Levin MJ. Gamma interferon production by combinations of human peripheral blood lymphocytes, monocytes and cultured macrophages. Infect Imun 1982;35: 383-390.

Arloing S and Courmant P. Sur la recherche et la valeur clinique de l'agglutination du bacille de Koch par le sorum sanguin de l'homme. C R Acad Sci 1898;127:425-428.

Armitage P. Statistical Methods in Medical Research, Oxford, Blackwell, p. 127-312, 1971.

Armstrong JA and Hart PD. Response of cultured macrophages to Mycobacterium tuberculosis, with observations on fusion of lysosomes with phagosomes. J Exp Med 1971;134:713-740.

Armstrong JA and Hart PD. Phagosome-lysosome interactions in cultured macrophages infected with virulent tubercle bacilli: reversal of the unusual non-fusion pattern and observations on bacterial survival. J Exp Med 1975;142:1-16.

Aspergren N and Roroman H. Mitosis-stimulating effect of tuberculin on lymphocytes from peripheral blood. J Allergy 1964;35:433-439.

Astor SH, Spitler LE, Frick OL and Fudenberg HH. Human leukocyte migration inhibition in agarose using four antigens: correlation with skin reactivity. J Immunol 1973;110: 1174-1179

Babior BM. Oxygen-dependent microbicidal killing by phagocytes. N Engl J Med 1978;298:659-721.

Baldwin ER, Kinghorn IM and Allen AM. Studies on immunity in tuberculosis: the properties of the serum of immunized rabbits. Med News 1905;87:636-638.

Bates P. Unexpected deaths in pulmonary tuberculosis. Lancet 1983;1:1437.

Bates JH and Mitchison DA. Geographic distribution of bacteriophage types of M. tuberculosis. Am Rev Respir Dis 1969;100: 189-193.

Beatman JE, Kleinman H, Glyer WW and Lacroix CM. A study of variability in tuberculin test reading. Am Rev Respir Dis 1964;90:913-919.

Beteaovskii BA, Kogan BI and Mostovoy YM. Study on some genetic characteristics in patients with pulmonary tuberculosis. Probl Tuberk 1981;10:48-50.

Berger HW and Mejia E. Tuberculous pleurisy. Chest 1973; 63:88-92.

Bhatnagar R, Malaviya AN, Narayanan S, Rajgopalan P, Kumar R and Bharadwaj OP. Spectrum of immune response abnormalities in different clinical forms of tuberculosis. Am Rev Respir Dis 1977;115:207-212.

Bignall JR. A century of treating tuberculosis. Tubercle 1982; 63:19-22.

Blackburn CL and Meltzer BR. 1977. Nutritional support resources in hospital practice, in Nutritional support of Medical Practice, ed by Schneider HA, Anderson CE and Coursin DB. Hagerstown, Md. Harper and Row.

Block H. Studies on the virulence of tubercle bacilli: isolation and biological properties of a constituent of virulent organisms. J Exp Med 1950;91:197-217.

Blomgren H. Role of B cells in the expression of the PPD response of human lymphocytes in vitro. Scand J Immunol 1975; 4:499-510.

Bona CP, Audibert F and Juy D. Cell suppression in PPD-induced blast specific response of human peripheral blood lymphocytes. Clin Exp Immunol 1976;26:258-266.

Boucot KR, Dillon ES and Cooper DA. Tuberculosis among diabetics: The Philadelphia Survey. Am Rev Tuberc 1952;65(pt.2):1-50.

Boyum A. Isolation of leukocytes from human blood. Methyl cellulose, dextran, and Ficoll as erhtyrocyte-aggregating agents. Scand J Clin Lab Invest (suppl) 1968;21:31-50.

British Thoracic and Tuberculosis Association (BTTA) Report. Short-course chemotherapy in pulmonary tuberculosis. Lancet 1976;ii:1102-1104.

Browder A and Griffon A. Tuberculin tine tests on medical wards. Am Rev Respir Dis 1972;105:299-301.

Brown CA and Brown IN. Mycobacterium bovis, BCG, modulation of murine antibody responses: influence of dose and degree of aggregation of live or dead organisms. Br J Exp Pathol 1982;63:133-143.

Buckley CE and Dorsey FC. A comparison of serum immuno-globulin concentrations in sarcoidosis and tuberculosis. Ann Intern Med 1970;72:37-42.

Buckley CE, White DH and Seigler HF. HLA-B7 associated tuberculin hyporesponsiveness in BCG-treated melanoma patients. Monogr Allergy 1977;11:97-105.

Buffington CA, Dominguez JH, Piering WF, Herbert LA, Kauffman HM and Lemann J. Interaction of rifampicin and glucocorticoids. JAMA 1976;236:1958-1960.

Burnet FM. The clonal selection theory of acquired immunity. Cambridge University Press, London and Cambridge, 1959.

Campbell PA. Immunocompetent cells in resistance to bacterial infections. Bacteriol Rev 1976;40:284-313.

Campbell PB. Defective leukotaxis in monocytes from patients with pulmonary tuberculosis. J Infect Dis 1979;139:409-417.

Carney WP, Rubin RH, Hoffman RA, Hansen WP, Healy K and Hirsch MS. Analysis of T-lymphocyte subsets in cytomegalovirus mononucleosis. J Immunol 1981;126:2114-2116.

Carr RI, Chakraborty AK, Brunda MJ, Davidson PT, Damle PS, Hardtke MA, Gilbride KJ and Minden P. Immune complexes and antibodies to BCG in sera from patients with mycobacterial infections. Clin Exp Immunol 1980;39:562-569.

Carrol MEW, Jackett PS, Aber VR and Lowrie DB. Phagolysosome formation, cyclic adenosine 3':5'-monophosphate and the fate of Salmonella typhimurium within mouse peritoneal macrophages. J Gen Microbiol 1979;110:421-429.

Chan SH, Lee SK and Simons MJ. Levamisole augmentation of lymphocyte hyporesponsiveness to phytohaemagglutinin in patients with pulmonary tuberculosis. Proc Soc Exp Biol Med 1976;151;716-719.

Chandler PJ, Allison MJ, Margolis C and Goretton E. The effects of intermittent hyperbaric oxygen therapy on the development of tuberculosis in the rabbit. Am Rev Respir Dis 1965;91:858-880

Chandra RK. Nutrition, immunity and infection: present knowledge and future directions. Lancet 1983;1:688-691.

Chaparas SD. Immunity in tuberculosis. Bull WHO 1982; 60:447-462.

Chapman JS and Dyerly MD. Social and other factors in intra-familial transmission of tuberculosis. Am Rev Respir Dis 1964;90:48-60.

Ching C and Lopez C. Natural killing of Herpes simplex virus type 1-infected target cells: normal human responses and influence of antiviral antibody. Infect Immun 1979;26:49-56.

Chukanova VP. Role of hereditary factors in tuberculosis. Probl Tuberk 1981;11:46-50.

Clague HW, El-Ansary EH and Hopkins CA. Unexpected deaths in pulmonary tuberculosis. Lancet 1983;i:1437.

Committee on Nomenclature. Interferon Nomenclature. Nature 1980;286:110.

Comstock GW. Community research in tuberculosis. Muscogee County, Georgia. Public Health Rep 1964;79:1045-1056.

Comstock GW, Foreboe SH and Hammes LM. A controlled trial of community-wide isoniazid prophylaxis in Alaska. Am Rev Respir Dis 1967;95:935-943.

Comstock GW, Livesay VT and Woolpert SF. The prognosis of a positive tuberculin reaction in childhood and adolescence. Am J Epidemiol 1974;99:131-138.

Comstock GW. Proof revisited: The modern epidemiology of tuberculosis. Am J Epidemiol 1975;101:363-382.

Comstock GW and Edwards PQ. The competing risks of tuberculosis and hepatitis for adult tuberculin reactors. Am Rev Respir Dis 1975;111:573-577.

Comstock GW. Tuberculosis in twins: a re-analysis of the Prophit Survey. Am Rev Respir Dis 1978;117:621-624.

Comstock GW. Advances towards the conquest of tuberculosis. Public Health Rep 1980;95:444-450.

Cox RA, Arnold DR, Cook D and Lundberg D. HLA phenotypes in Mexican Americans with tuberculosis. Am Rev Respir Dis 1982; 126:653-655.

Crawford DH, Brickell P, Tidman N, McConnell I, Hoffbrand AV and Janossy G. Increased number of cells with suppressor T-cell phenotype in the peripheral blood of patients with infectious mononucleosis. Clin Exp Immunol 1981;43:291-297.

Cruchaud A, Girard JP and Hitoglou S. The functions of human monocytes in normal subjects and in disorders associated with immune deficiency. Int Arch Allergy Appl Immunol 1977; 54:529-537.

Cunningham RS, Sabin SR, Sugiyma S and Kindwall JA. The role of the monocyte in tuberculosis. Bull J Hopk Hosp 1925;37:231-234.

Cunningham AL and Morigan TC. Leu 3+ T-cells produce γ-interferon in patients with recurrent Herpes labialis. J Immunol 1984; 132:197-202

Daniel TM and Janicki BW. Mycobacterial antigens: A review of their isolation, chemistry and immunological properties. Microbiol Rev 1978;42:84-92.

Daniel TM, Ortoby MJ, Pinto E and Moreno E. The immune spectrum in patients with pulmonary tuberculosis. Am Rev Respir Dis 1981;123:556-559.

Daniel TM. Selective primary health care: Strategies for control of disease in the developing world. II. Tuberculosis. Rev Infect Dis 1982;4:1254-1265.

Dannenberg AM. Cellular hypersensitivity and cellular immunity in the pathogenesis of tuberculosis: Specificity, systemic and local nature, and associated macrophage enzymes. Bacteriol Rev 1968;32:85-102.

Dannenberg AM, Ando M and Shima K. Macrophage accumulation, division, maturation, and digestive and microbicidal capacities in tuberculous lesions. III. The turnover of macrophages and its relation to their activation and antimicrobial immunity in primary BCG lesions and those of reinfection. J Immunol 1972; 109:1109-1121.

Dannenberg AM, Ando M, Shima K and Tsuda T. 1975 Macrophage turnover and activation in tuberculous granulomas. in Mononuclear Phagocytes in Immunity, Infection and Pathology, ed by van furth R, Oxford, Blackwell Scientific Publications, p. 959

DeVries RRP, Nijenhuis LE, Lui A, Pat RFM and vanrood JJ. HLA-linked genetic control of host response to Mycobacterium leprae. Lancet 1976;1:1328.

DeVries RRP, Kreeftenberg HG, Laggen HG and Vanrood JJ. In vitro immune responsiveness to vaccinia virus and HLA. N Engl J Med 1977;297:692-696.

Doan CA and Wiseman BK. The monocyte, monocytosis, and monocytic leukosis: a clinical and pathological study. Ann Intern Med 1934;8:383-414.

Draper P. Bacteriology of Mycobacterium leprae: state of the art paper. Ann Microbiol (Paris) 1982;133:13-14.

Durnin JVCA and Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness:measurements on 481 men and women aged from 16 to 72 years. Br J Nutr 1974; 32:77-97.

Eagle H. Amino acid metabolism in mammalian cell cultures. Science 1959;130:432-437.

Edwards LB, Livesay VT, Acquaviva FA and Palmer CE. Height, weight, tuberculous infection and tuberculous disease. Arch Environ Health 1971;22:106-112.

Edwards PQ. Tuberculin negative. N Engl J Med 1972;286:373.

Einhorn S, Blomgren H and Strander H. Interferon and spontaneous cytotoxicity in man. Acta Med Scand 1978;20:477-483.

El-Ansary EH and Earis JE. Rifampicin and adrenal crisis. Br Med J 1983;286:1861-1862.

Ellis ME and Webb AK. Cause of death in patients admitted to hospital for pulmonary tuberculosis. Lancet 1983;1:665-667.

Ellner JJ. suppressor adherent cells in human tuberculosis. J Immunol 1978;121:2573-2579.

Ellner JJ and Daniel TM. Immunosuppression by mycobacterial arabinomannan. Clin Exp Immunol 1979;35:250-257.

Epstein LB, Cline MJ and Merigan TC. PPD-stimulated interferon: in vitro macrophage-lymphocyte interaction in the production of a mediator of cellular immunity. Cell Immunol 1971;2:602-613.

Epstein LB, Cline MJ and Merigan TC, 1972 The in vitro interaction of immune macrophages and lymphocytes in the antigen (PPD)- induced production of interferon, in Proceedings of the 6th Annual Leucocyte Culture Conference, ed by Schwartz HA London and New York, Academic Press, P 203-207.

Fahey JL, Detels R and Gottlieb MS. Immune cell augmentation (with altered T-subset ratio) is common in healthy homosexual men. N Engl J Med 1983;308:842-843.

Farrant J and Knight SC. Help and suppression by lymphoid cells as a function of cellular concentration. Proc Natl Acad Sci, USA 1979;76:3507-3510.

Farrant J and Newton C. Relative ability to provide help: an explanation for Con A-induced suppression. Clin Exp Immunol 1981;45:504-513.

Faulkner JB, Carpenter RL and Patnode RA. Serum protein and immunoglobulin levels in tuberculosis. Am J Clin Pathol 1967; 48:556-560.

Fennet P, Martin SP and Pierce SP. The enumeration of viable tubercle bacilli in cultures and infected tissues. Ann NY Acad Sci 1949;52:751-758.

Fernandes G and Gupta S. Natural killing and antibody-dependent cytotoxicity by lymphocyte subpopulations in young and aging humans. Clin Immunol 1981;1:141-148.

Forbes JT, Niblack GC, Fuche R, Richie RE, Johnson HK and Oldham RK. Human natural cell-mediated cytotoxicity. I. Levels in peripheral blood, cord blood and thoracic duct lymphocytes. Cancer Immunol Immunother 1981;11:139-145.

Fox W. Whither short-course chemotherapy? Br J Dis Chest 1981; 75:331-357.

Fox W. Compliance of patients and physicians: experience and lessons from tuberculosis - I. Br Med J 1983;287:33-35.

Fahey JL, Detels R and Gottlieb MS. Immune cell augmentation (with altered T-subset ratio) is common in healthy homosexual men. N Engl J Med 1983;308:842-843.

Farrant J and Knight SC. Help and suppression by lymphoid cells as a function of cellular concentration. Proc Natl Acad Sci, USA 1979;76:3507-3510.

Farrant J and Newton C. Relative ability to provide help: an explanation for Con A-induced suppression. Clin Exp Immunol 1981;45:504-513.

Faulkner JB, Carpenter RL and Patnode RA. Serum protein and immunoglobulin levels in tuberculosis. Am J Clin Pathol 1967; 48:556-560.

Fenner P, Martin SP and Pierce SP. The enumeration of viable tubercle bacilli in cultures and infected tissues. Ann NY Acad Sci 1949;52:751-758.

Fernandes G and Gupta S. Natural killing and antibody-dependent cytotoxicity by lymphocyte subpopulations in young and aging humans. Clin Immunol 1981;1:141-148.

Forbes JT, Niblack CC, Fuchs R, Richie RE, Johnson HK and Oldham RK. Human natural cell-mediated cytotoxicity. 1. Levels in peripheral blood, cord blood and thoracic duct lymphocytes. Cancer Immunol Immunother 1981;11:139-145.

Fox W. Whither short-course chemotherapy? Br J Dis Chest 1981; 75:331-357.

Fox W. Compliance of patients and physicians: experience and lessons from tuberculosis - I. Br Med J 1983;287:33-35.

Freund J. The mode of action of immunologic adjuvants. Adv Tuberc Res 1956;7:130-148.

Frost WH. Age selection of mortality from tuberculosis in successive decades. Am J Hyg 1939;30:91-96.

Frost P and Lance EM, 1973 The relation of lymphocyte trapping to the mode of action of adjuvants, in Immunopotentiation. Ciba Foundation Symposium 18, Amsterdam. Elsevier, Excerpta Medica, p 29-38.

Frucht H, Kunkol P and Spiro HM. Pulmonary tuberculosis following gastric resection. Ann Intern Med 1957;46:696-705.

Fujiwara H, Okuda Y, Fukukawa T and Teuyuguchi I. In vitro tuberculin reactivity of lymphocytes from patients with tuberculous pleurisy. Infect Immun 1982;35:402-409.

Furcolow ML, Howell B and Nelson WE. Quantitative studies of the tuberculin reaction. Tuberculin sensitivity in relation to active tuberculosis. Am Rev Tuberc 1942;25:504-510.

Garland J. Deleterious effects of ACTH and cortisone on tuberculosis. N Engl J Med 1951;245:662-664.

Gatner EMS and Anderson R. An in vitro assessment of cellular and humoral immune function in pulmonary tuberculosis: correction of defective neutrophil motility by ascorbate, levamisole, metoprolol and propranolol. Clin Exp Immunol 1980;40:327-336.

Gelb AF, Loffler C, Brower A, Mascatello V and Lyons HA. Miliary tuberculosis. Am Rev Respir Dis 1973;108:1327-1333.

Germain RN, Williams RM and Benacerraf B. Specific and non-specific antitumour immunity. II. Macrophage-mediated nonspecific effector activity induced by BCG and similar agents. J Natl Cancer Inst 1975;54:709-720.

Girard JP, Paychere J, Cuevas J and Fernandes B. Cell-mediated immunity in an aging population. Clin Exp Immunol 1977; 27:85-91.

Glasgow LA. Leukocytes and interferon in the host response to viral infections. II. Enhanced interferon response of leukocytes from immune animals. J Bacteriol 1966;91:2185-2191.

Glasser RM, Walker RI and Herion JC. The significance of haematologic abnormalities in patients with tuberculosis. Arch Intern Med 1970;125:691-695.

Glenchur H, Fosslock BE and Silverman M. An immediate skin test for the diagnosis of active pulmonary tuberculosis. Am Rev Respir Dis 1965;92:741-748.

Godal T, Myrvang B, Stanford JL and Samuel DR. Recent advances in the immunology of leprosy with special reference to new approache in immunoprophylaxis. Bull Inst Pasteur 1974;72:273-278.

Goldstein RA, Ang UH, Foellmer JW and Janicki BW. Rifampicin and cell-mediated immune responses in tuberculosis. Am Rev Respir Dis 1976;113:197-202.

Gordon AH, Hart PD and Young MR. Ammonia inhibits phagosome-lysosome fusion in macrophages. Nature 1980;286:79-80.

Goren MB, Brokl O and Schaefer WB., Lipids of putative relevance to virulence in Mycobacterium tuberculosis : correlation of virulence with elaboration of sulphatides and strongly acidic lipids. Infect Immun 1974;9:142-149.

Goren MB, Hart PD, Young MR and Armstrong JA. Prevention of phagosome-lysosome fusion in cultured macrophages by sulfatides of Mycobacterium tuberculosis. Proc Natl Acad Sci USA 1976; 73:2510-2514.

Goren MB and Brennan PJ, 1979 Mycobacterial lipids: chemistry and biological activities, in Tuberculosis, ed by Youmans GP, Philadelphia, WB Saunders Co, p 63-193.

Goraki AJ, Dupont B, Hansen JA and Good RA. Leukocyte migration inhibitory factor (IMIF) induced by concanavalin A : standardiaed microassay for production in vitro. Proc Natl Acad Sci US. 1975;72:3197-3200.

Grange JM, Aber VR, Allen BW, Mitchison DA, Mikhail JR, McSwiggan DA and Collins CH. Comparison of strains of Mycobacterium tuberculosis from British, Ugandan and Asian immigrant patients : a study in bacteriophage typing, susceptibility to hydrogen peroxide and sensitivity to thiopen-2-carbon acid hydrazide. Tubercle 1977;58:207-215.

Grange JM, Aber VR, Allen BW, Mitchison DA and Goren MB. The correlation of bacteriophage types of Mycobacterium tuberculosis with guinea-pig virulence and in vitro indicators of virulence. J Gen Microbiol 1978;108:1-7.

Grange JM, Gibson J and Naseau B. Enzyme-linked immunosorbent assay (ELISA): A study of antibodies to Mycobacterium tuberculosis in the IgG, IgA and IgM classes in tuberculosis, sarcoidosis and Crohn's disease. Tubercle 1980;61:145-152.

Green JA, Cooperband SR and Kibrick S. Immune-specific induction interferon production in cultures of human blood lymphocytes. Science 1969;164:1415-1417.

Greenberg LJ, Gray JED and Yunis EJ. Association of HLA-5 and im
responsiveness to streptococcal antigens. J Exp Med 1975;
141:935-943.

Grosset I, Maury C, Krese C, Blangy D and Mancury M. Role of
interferon in the Pathogenesis of virus diseases in mice as
demonstrated by the use of anti-interferon serum. VI. Polyoma
virus infection. Int J Cancer 1979;24:178-183.

Grieco MH and Chmel H. Acute disseminated tuberculosis as a
diagnostic problem. A clinical study based on twenty-eight
cases. Am Rev Respir Dis 1974;109:554-560.

Gump DW, Pekoty R Jr, Urbanetti J and Nosenzo C. Studies of
human leukocyte culture as an in vitro test of delayed hyper-
sensitivity. Am Rev Respir Dis 1967;95:470-476.

Halber W and Hirzfeld L. Studien uber die Konstitution-
serologie. Z Immunitaetsforsch 1926;48:34-68.

Harrison BDW, Tugwell P and Fawcett IW. Tuberculin reactions in
adult Nigerians with sputum-positive pulmonary tuberculosis.
Lancet 1975;1:421.

Hart PD. Statement of the questions. Ann NY Acad Sci 1960,154:1-7

Hart PD, Armstrong JA, Brown CA and Draper P. Ultrastructural
study of the behaviour of macrophages toward parasitic myco-
bacteria. Infect Immun 1972;5:803-807.

Heberman RB, Ortaldo JR and Bonnard GD. Augmentation by interfero
of human natural and antibody dependent cell-mediated
cytotoxicity. Nature 1979;277:221-223.

Heilman DH and McFarland W. Inhibition of tuberculin-induced mitogenesis in culture of lymphocytes from tuberculous donors. Int Arch Allergy 1966;30:58-66.

Heppner GH. Blocking antibodies and enhancement. Ser Haematol 1972; 5:41-66.

Hewitt J, Coates ARM, Mitchison DA and Ivanyi J. The use of murine monoclonal antibodies without purification of antigen in the sero diagnosis of tuberculosis. J Immunol Methods 1982;55:205-211.

Hibbs JB Jr Macrophage nonimmunologic recognition: target cell factors related to contact inhibition. Science 1973;180:868-870.

Hillinger SM and Herzig GP. Impaired cell mediated immunity in Hodgkin's disease mediated by suppressor lymphocytes and monocytes. J Clin Invest 1978;61:1620-1627.

Hinz CF Jr, Daniel TM and Baum GC. Quantitative aspects of the stimulation of lymphocytes by tuberculin purified protein derivative. Int Arch Allergy Appl Immunol 1970;38:119-125.

Ho M and Enders JF. Further studies on an inhibitor of viral activity appearing in infected cell cultures and its role in chronic viral infections. Virology 1959;9:446-477.

Hoffman PM, Spitler LE, Hsu M and Fudenberg HH. Leukocyte migration inhibition in agarose. Cell Immunol 1975;18:21-30.

Hoffman PM, Spitler LE and Hsu M. Leukocyte-migration inhibition in guinea pigs. 1. Correlation with skin test reactivity and macrophage-migration inhibition. Cell Immunol 1976;21:358-363.

Holden M, Dublin MR and Diamond PH. Frequency of intermediate strength tuberculin sensitivity in patients with active tuberculosis. N Engl J Med 1971;285:1506-1509.

Hong Kong Chest Service/Tuberculosis Research Centre, Madras/ British Medical Research Council. A study of the characteristics and course of sputum smear-negative pulmonary tuberculosis. Tubercle 1981;62:155-167.

Horton ES. Introduction: an overview of the assessment and regulation of energy balance in humans. Am J Clin Nutr 1983; 38:972-977.

Kaieh K and Cheng R. Different responses of monocyte and active T lymPhocytes to in vitro challenge of purified protein derivative (PPD) in patients with active tuberculosis and healthy tuberculin reactors. Ann Allergy 1981;46:159-163.

Kumber DP, Nsanzumuhire H, Aluoch JA, Webster ADB, Aber VR, Mitchison DA, Girling DJ and Nunn AJ. Controlled double-blind study of the effect of rifampicin on humoral and cellular immune responses in patients with pulmonary tuberculosis and in tuberculosis contacts. Am Rev Respir Dis 1980;122:425-436.

Humphries MJ, Byfield SP, Darbyshire JC, Davies PDO, Nunn AJ, Citron KM and Fox W. Deaths occurring in newly notified patients with Pulmonary tuberculosis in England and Wales. Br J Dis Chest 1984;78:149-158.

Hyde L. Clinical significance of the tuberculin skin test. Am Rev Respir Dis 1972;105:453-454.

Ina Y, Takada K, Sugiura T, Moriohita M, Aoki M, Torii Y, Ichimura K, Suzuki M, Hachigami M and Yamamoto M. Killer and Natural Killer activities in patients with pulmonary tuberculosis. Kekkaku 1982;57:295-298.

Isaacs A and Lindenmann J. Virus interference. I. The interferon. Proc R Soc Lond 1957;147:258-267.

Isaacs D, Clarke K, Tyrrell DAJ, Webster ADB and Valman HB. Deficie production of leucocyte interferon (interferon-alpha) in vitro and in vivo in children with recurrent respiratory tract infections. Lancet 1981;11:950-952.

Jackett PS, Aber VR and Lowrie DB. Virulence of Mycobacterium tuberculosis and susceptibility to peroxidative killing systems. J Gen Microbiol 1978;107:273-278.

Jackett PS, Aber VR, Mitchison DA and Lowrie DB. The contribution of hydrogen peroxide resistance to virulence of Mycobacterium tuberculosis during the first six days after intravenous infection of normal and BCG-vaccinated guinea-pigs. Br J Exp Pathol 1981;62:34-40.

Jain RC. ABO blood groups and pulmonary tuberculosis. Tubercle 1970;51:322-323.

James WRL. The relationship of tuberculosis to the development of massive pneumoconiosis in coal workers. Br J Tuberc 1954;48:89-96.

Jelliffe DB, 1966. The assessment of the nutritional status of the community with special reference to field surveys in developing regions of the world. WHO Monograph 53, Geneva, World Health Organization.

Jiang ZF, An JB, Sun YF, Mittal KK and Lee TD. Association of HLA-BW35 with tuberculosis in the Chinese. Tissue Antigens 1983;22;86-88.

Joffe MI and Rabson AR. Suppression of LIF production but not blastogenesis in patients with tuberculous meningitis. Clin Immunol Immunopathol 1981;18:245-253.

Johnson HM. Cellular regulation of immune interferon production. Antiviral Res 1981;1:37-46.

Johnson JR and Davey WN. Cortisone, corticotropin, and antimicrobial therapy in tuberculosis in animals and man: a review Am Rev Tuberc 1954;70:623-636.

Johnson MW, Maibach HI and Salmon SE. Skin reactivity in patients with cancer. N Engl J Med 1971;284:1255-1257.

Johnson HM, Stanton GJ and Baron S. Relative ability of mitogens to stimulate production of interferon by lymphoid cells and to induce suppression of the in vitro immune response. Proc Soc Exp Biol Med 1977;154:138-141.

Johnson NM, McNicol MW, Burton-Lee EJ and Mowbray JF. Circulating immune complexes in tuberculosis. Thorax 1981;36:610-617.

Joynson DHM, Walker DM, Jacobs A and Dolby AE. Defect of cell mediated immunity in patients with iron-deficiency anaemia. Lancet 1972;11:1058-1059.

Kalish SB, Radin RC, Phair JP, Levitz D, Zeiss CR and Metzger E. Use of an enzyme-linked immunosorbent assay technique in the differential diagnosis of active pulmonary tuberculosis in humans. J Infect Dis 1983;147:523-530.

Kallmann FJ and Reisner D. Twin studies on the significance of genetic factors in tuberculosis. Am Rev Tuberc Pulm Dis 1943; 47:549-574.

Kamat SR, Dawson JJY, Devadatta S, Fox W, Janardhanan B, Radhakrishna S, Ramakrishnan CV, Somasundaram PR, Stott H and Velu S. A controlled study of the influence of segregation of tuberculous patients for one year on the attack rate of tuberculosis for a 5-year period in close family contacts in South India. Bull WHO 1966;34:517-532.

Kaplan MH and Chase MW. Antibodies to mycobacteria in human tuberculosis. I. Development of antibodies before and after antimicrobial therapy. J Infect Dis 1980;142:825-834.

Kardjito T and Grange JM. Immunological and clinical features of smear-positive pulmonary tuberculosis in East Java. Tubercle 1980;61:231-238.

Kardjito T, Handoyo I and Grange JM. Diagnosis of active tuberculosis by immunological methods. I. The effect of tuberculin reactivity and previous BCG vaccination on the antibody levels determined by ELISA. Tubercle 1982;63:269-274.

Kato M. Studies of the biochemical lesion in experimental tuberculosis in mice. VII. Effect of derivatives and chemical analogs of cord factor on structure and function of mouse liver mitochondria. Am Rev Respir Dis 1968;98:668-676.

Katz P, Goldstein RA and Fauci AS. Immunoregulation in infection caused by Mycobacterium tuberculosis: the presence of suppressor monocytes and the alteration of subpopulations of T lymphocytes. J Infect Dis 1979;140:12-21.

Kent DC and Schwartz R. Active pulmonary tuberculosis with negative tuberculin skin tests. Am Rev Respir Dis 1967;95: 411-418.

Kerby GR. Correlation of tuberculin skin reaction with in vitro lymphocyte transformation. Am Rev Respir Dis 1968; 97:904-908.

Khomenko AG. Distribution of HLA antigens in patients with tuberculosis. Ter Arkh 1981;53:135-138.

Kiessling R, Klein E and Wigzell H. "Natural" killer cells in the mouse. Eur J Immunol 1975;5:112-121.

King GW, Barn C and Lobuglio AF. The effect of tuberculosis and neoplasia on human monocyte staphylocidal activity. Cell Immunol 1975;16:389-395.

Kinnman J, Fryden A, Eriksson S, Moller E and Link H. Tuberculous meningitis: Immune reactions within the central nervous system. Scand J Immunol 1981;13:289-296.

Kitahara M, Eyre HJ and Hill HR. Monocyte functional and metabolic activity in malignant and inflammatory diseases. J Lab Clin Med 1979;93:472-479.

Klebanoff S, 1980. Cytocidal mechanisms of phagocytic cells. in Immunology, ed by Rougereau M and Dausset J, London, Academic Press.

Kleinhenz ME, Ellner JJ and Daniel TM. Immunosuppressive properties of tubercle bacillus arabinogalactan (Abstr.) Clin Res 1979;27:638A.

Kleinhenz ME, Ellner JJ, Spagnuolo PJ and Daniel TM. Suppression of lymphocyte responses by tuberculous plasma and mycobacterial arabinogalacten. J Clin Invest 1981;68:153-162.

Knight SC and Farrant J. Comparing stimulation of lymphocytes in different samples: separate effects of numbers of responding cells and their capacity to respond. J Immunol Methods 1978; 22:63-71.

Knight SC, Harding B, Burman S, O'Brien JA and Farrant J, 1979, Clinical applications of leucocyte culture: the importance of cellular concentration, in The Molecular Basis of Immune Cell Function, ed by Kaplan J, Amsterdam, Elsevier/North Holland, p. 181.

Koch R. Die Aetiologie der Tuberculose. Berlin klin Wochenschr 1882;15:221-230.

Koch R. Weitere Mittheilungen uber ein Heilmittel gegen Tuberculose. Dtsch Med Wochenschr 1890;16:1029.

Krieg RE and Meyers WM. Demonstration of Mycobacterium leprae in tissues from bacteriologically negative treated lepromatous leprosy patients. Int J Lepr 1979;47(suppl):367-374.

Kventy J. T-lymphocyte determination in tuberculosis. Scand J Respir Dis 1977;58:181-184.

Langford MP, Stanton GJ and Johnson HM. Biological effects of staphylococcal enterotoxin A on human peripheral lymphocytes. Infect Immun 1978;22:62-68.

Langford MP, Weigent DA, Georgiades J, Johnson H and Stanton GJ. Antibody to staphylococcal enterotoxin A induced human immune interferon (IFN-gamma). J Immunol 1981;126:1620-1623.

Large SE. Tuberculosis in the Gurkhas of Nepal. Tubercle 1964; 45:321-335.

Laughter AH and Twomey JJ. Suppression of lymphoproliferation by high concentrations of normal human mononuclear leukocytes. J Immunol 1977;119:173-179.

Ledingham JCG, 1983, Water and electrolyte disturbances, in Oxford Textbook of Medicine, ed by Weatherall DJ, Ledingham JCG and Warrell DA, Oxford, Oxford University Press, p. 18,26.

Lefford MJH, McGregor DD and Mackaness GB. Properties of lymphocytes which confer adoptive immunity to tuberculosis in rats. Immunology 1973;25:703-715.

Lenzini L, Rottoli P and Rottoli L. The spectrum of human tuberculosis. Clin Exp Immunol 1977;27:230-237.

Lester CP and Atwell RJ. The tuberculin skin reaction in active pulmonary tuberculosis. Am Rev Tuberc 1958;78:399-402.

Lichtenstein IH and Macgregor RR. Mycobacterial infections in renal transplant recipients: report of five cases and review of the literature. Rev Infect Dis 1983;5:216-226.

Littman BH and Ruddy S. Production of the second component of complement by human monocytes: stimulation by antigen-activated lymphocytes or lymphokines. J Exp Med 1977;145:1344-1352.

Loken MR and Herzenberg LA. Analysis of cell populations with a fluorescein-activated cell-sorter. Ann NY Acad Sci 1975;254: 163-171.

Loudon RG and Spohn SK. Cough frequency and infectivity in patients with pulmonary tuberculosis. Am Rev Respir Dis 1969; 99:109-111.

Lowrie DB, Aber VR and Jackett PS. Phagosome-lysosome fusion and cyclic adenosine 3':5'-monophosphate in macrophages infected with Mycobacterium microti, Mycobacterium bovis BCG or Mycobacterium lepraemurium. J Gen Mircobiol 1979; 110:431-441.

Lowrie DB, Jackett PS, Aber VR and Carrol MEW, 1980, Cyclic nucleotides and phagosome-lysosome fusion in mouse peritoneal macrophages, in Mononuclear Phagocytes: Functional Aspects, ed by van Furth R, The Hague, Boston and London, Martinus Nijhoff, p 1057-1075.

Lowrie DB. The macrophage and mycobacterial infections. Trans R Soc Trop Med Hyg 1983;77:646-655.

Luelmo F. BCG vaccination. Am Rev Respir Dis 1982;125.3 pt 2: 70-72.

Lundy J, Raff JH, Deakins J, Wanebo HJ, Jacobs FA and Lee TD. The acute and chronic effects of alcohol on human immune system. Surg Gynecol Obstet 1975;141:212-218.

Lurie MB. Studies on the mechanism of immunity in tuberculosis: the fate of tubercle bacilli ingested by mononuclear phagocytes derived from normal and immunized animals. J Exp Med 1942;75: 247-267.

Lurie MB, 1964, Resistance to tuberculosis: Experimental studies in native and acquired defensive mechanisms. Cambridge, Mass, Harvard University Press.

Mackaness GB. The growth of tubercle bacilli in monocytes from normal and vaccinated rabbits. Am Rev Tuberc 1954;69:495-504.

Mackaness GB. The immunological basis of acquired cellular resistance. J Exp Med 1964;120:105-120.

Mackaness GB. The relationship of delayed hypersensitivity to acquired cellular resistance. Br Med Bull 1967;23:52-54.

Mackler BF, Altman LC, Rosenstreich DL and Oppenheimer JJ. Induction of lymphokine production by EAC and of blastogenesis by soluble mitogens during human B-cell activation. Nature 1974; 249:834-837.

Magnus K and Edwards LB. The effect of repeated tuberculin testing on post-vaccination allergy. Lancet 1955;11:643.

Mandeville R and Rocheleau N. Natural cell mediated cytotoxicity in normal human peripheral blood lymphocytes and its in vitro boosting with BCG. Cancer Immunol Immunother 1983;15:17-22.

Meade CJ, Lachmann PJ and Bronner S. A sensitive assay for cellular hypersensitivity based on the uptake of radioactive colloidal gold. Immunology 1974;27:227-239.

Meltzer MS, Jones EE and Boetcher DA. Increased chemotactic responses of macrophages from BCG-infected mice. Cell Immunol 1975;17:268-276.

Middlebrook G and Dubos RJ. Specific serum agglutination of erythrocytes sensitized with extracts of tubercle bacilli. J Exp Med 1948;88:521-528.

Miller SD and Jones HE. Correlation of lymphocyte transformation with tuberculin skin-test sensitivity. Am Rev Respir Dis 1973; 107:530-538.

Minden P, McClatchy JK, Cooper R, Bardana EJ and Parr RS. Shared antigens between Mycobacterium bovis (BCG) and other bacterial species. Science 1971;176:57-58.

Mitchison DA. Treatment of tuberculosis. The Mitchell Lecture 1979. J R Coll Physicians Lond. 1980;149:91-99.

Mitchison DA, Selkon JB and Lloyd J. Virulence in the guinea-pig, susceptibility to hydrogen peroxide, and catalase activity of isoniazid-sensitive tubercle bacilli from South Indian and British patients. J Pathol Bacteriol 1963;86:177-186.

Mitchison DA, Aber VR, Ahmad FJ, Allen BW and Devi S. Evaluation of a serological test for tuberculosis. Br Med J 1977;1:1383-1387.

Moore CE, Corner RE and Franklin HA. Culture of normal human leukocytes. JAMA 1967;199:519-524.

Moore M, 1983, Interferon and the immune system, 2: Effect of IFN on the immune system, in Interferons: from Molecular Biology to Clinical Application, Symposium 35, ed by Burke DC and Morris AG, Cambridge University Press.

Morbidity Mortality Weekly Report (MMWR). Centers for Disease Control. Tuberculosis - United States, 1981. MMWR 1982;31: 443-446.

Moretta L, Webb SR, Grossi CE, Lydyard PM and Cooper MD. Functional analysis of two human T cell subpopulations: help and suppression of B-cell responses by T cells bearing receptors for IgM or IgG. J Exp Med 1977;146:184-200.

Moticka EJ. The nonspecific stimulation of immunoglobulin secretion following specific stimulation of the immune system. Immunology 1974;27:401-412.

MRC Report. The geographical distribution of tuberculosis notifications in a National Survey of England and Wales (1978-9). Report from the MRC Tuberculosis and Chest Diseases Unit. Tubercle 1982a;63:75-80.

MRC Report. Tuberculosis in children in a National Survey of notifications in England and Wales, 1978-9. Report from the MRC Tuberculosis and Chest Diseases Unit. Arch Dis Child 1982b; 57:734-741.

Murray HW, Rubin BY and Rothermel CD. Killing of intracellular Leishmania donovani by lymphokine-stimulated human mononuclear phagocytes: evidence that interferon-γ is the activating lymphokine. J Clin Invest 1983;72:1506-1510.

McMurray DN and Echeverri A. Cell-mediated immunity in anergic patients with pulmonary tuberculosis. Am Rev Respir Dis 1978; 118:827-834.

McMurray DN. Mechanisms of anergy in tuberculosis (Editorial). Chest 1980;77:4-5.

McNicol M. Trends in the epidemiology of tuberculosis - a Physician's view. J Clin Pathol 1983;36:1087-1090.

Nagel JE, Collins CD and Adler WH. Spontaneous or natural killer cytotoxicity of K562 erythroleukemic cells in normal patients. Cancer Res 1981;41:2284-2288.

Narain R, Nair SS, Rao CR, Chandrasekhar P and Lal P. Enhancing of tuberculin allergy by previous tuberculin testing. Bull WHO 1966;34:623-628.

Nash DR and Douglass JE. Anergy in active pulmonary tuberculosis. Chest 1980;77:32-37.

Nasseau E and Merrick AJ. The fluorescent antibody test in human tuberculosis: a pilot study. Tubercle 1970;51:430-436.

Nasseau E, Parsons ER and Johnson CD. Detection of antibodies to Mycobacterium tuberculosis by solid phase radioimmunoassay. J Immunol Methods 1975;6:261-271.

Nasseau E, Parsons ER and Johnson CD. The detection of antibodies to Mycobacterium tuberculosis by microplate enzyme-linked immunosorbent assay (ELISA). Tubercle 1976;57:67-70.

Nathan CF, Karnovsky ML and David JR. Alterations of macrophage functions by mediators from lymphocytes. J Exp Med 1971;133: 1356-1376.

Nathan CR, Murray HW, Wiebe ME and Rubin BY. Identification of interferon-γ as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. J Exp Med 1983;158:670-689.

Nathan CF. Mechanisms of macrophage antimicrobial activity. Trans R Soc Trop Med Hyg 1983;77:620-630.

Nicholls AC. A serodiagnostic test for tuberculosis. J Clin Pathol 1975;28:850-853.

Nicholls AC and Horefield R. Serological diagnosis of tuberculosis: a report of 12 months' clinical experience. Thorax 1976;31:289.

Nielsen H, Bennedsen J, Larsen SO, Rhodes JM and Viskum K. Defective monocyte chemotaxis in pulmonary tuberculosis. Eur J Respir Dis 1982;63:122-129.

Nilsson BS. The response of lymphocytes from tuberculin-positive or -negative humans to various doses of PPD-tuberculin in vitro. Cell Immunol 1972;3:493-500.

North RJ. Importance of thymus-derived lymphocytes in cell-mediated immunity to infection. Cell Immunol 1973;7:166-176.

North RJ. T-cell dependence of macrophage activation and mobilisation during infection with Mycobacterium tuberculosis. Infect Immun 1974;10:66-71.

O'Brien J, Knight B, Quick NA, Moore EH and Platt AS. A simple technique for harvesting lymphocytes cultured in Terasaki plates. J Immunol Methods 1979;27:219-223.

Okubo Y, Kusama S and Yano A. PPD-specific proliferative response in humans. I. Analysis of PPD-specific proliferative cells from tuberculous pleurisy patients and healthy controls with monoclonal antibodies specific for human T subsets. Microbiol Immunol 1982;26:511-521.

Osborne L, Georgiades J and Johnson H. Classification of interferons with antibody to immune interferon. Cell Immunol 1980;53:65-70.

Pantalone R and Page RC. Enzyme production and secretion by lymphokine-activated macrophages. J Reticuloendothel Soc 1977;21:343-357.

Para M, Sagone A, Balcerzak S and Tobuglio A. Metabolism of normal and activated monocytes (abstract). Clin Res 1972; 20:742A.

Parlett RC and Youmans GP. An evaluation of the specificity and sensitivity of a gel double-diffusion test for tuberculosis: a double-blind study. Am Rev Respir Dis 1959;80:153-166.

Patterson RJ and Youmans GP. Demonstration in tissue culture of lymphocyte-mediated immunity to tuberculosis. Infect Immun 1970;1:600-603.

Pearmain G, Lycette RR and Fitzgerald PH. Tuberculin-induced mitosis in peripheral blood leukocytes. Lancet 1963;1;637-638.

Penachow J and Mackay IR. NK and K cell activity of human blood: differences according to sex, age and disease. Ann Rheum Dis 1980;39;82-86.

Piessens WF, Churchill WH and David JR. Macrophages activated in vitro with lymphocyte mediators kill neoplastic but not normal cells. J Immunol 1975;114:293-299.

Pinching AJ, Jeffries DJ, Donaghy M, Munday PE, McManus TJ, Moshtael O, Parkin JM and Harris JRW. Studies of cellular immunity in male homosexuals in London. Lancet 1983;ii:126-130.

Plouffe JF, Silva J, Fekety R and Baird I. Cerebrospinal fluid lymphocyte transformations in meningitis. Arch Intern Med 1979;139:191-194.

Poplack DG, Sher NA, Chapara SD and Blaese RM. The effect of Mycobacterium bovis (bacillus Calmette-Guerin) on macrophage random migration, chemotaxis and pinocytosis. Cancer Res 1976; 36:1233-1237.

Prabhu T and Reddy VM. Active and total E-rosette forming T-lymphocytes in pulmonary tuberculosis. Indian J Med Res 1983;77:308-313.

Preble OT, Black RJ, Friedman RM, Klippel JH and Vilcek J. Systemic lupus erythematosus: presence in human serum of an unusual acid-labile leukocyte interferon. Science 1982;216: 429-431.

Proos HP and Baines MG. Studies of human natural killer cells. I. In vivo parameters affecting normal cytotoxic function. Int J Cancer 1982;29:383-390.

Radin RC, Zeiss RC and Phair JP. Antibodies to purified protein derivative in different immunoglobulin classes in the diagnosis of tuberculosis in man. Int Arch Allergy Appl Immunol 1983; 70:25-29.

Rees RJW, 1976, The macrophage in mycobacterial infections, in Infection and Immunology in the Rheumatic Diseases, ed by Dumonde DC, Oxford, Blackwell Scientific Publications, Chapter 5, p.31.

Reggiardo Z, Vazquez E and Schnaper L L. Kl.ISA tests for antibodies against mycobacterial glycolipids. J Immunol Methods 1980;34:55-60.

Reinherz EL, O'Brien C, Rosenthal P and Schlossman SF. The cellular basis for viral-induced immunodeficiency: Analysis by monoclonal antibodies. J Immunol 1980;125:1269-1274.

Resold-O'Donnell E and Resold HO. The enhancement of macrophage adenylate cyclase by products of activated lymphocytes. J Biol Chem 1974;249:3622-3627.

Rice L., Laughter AH and Twomey JJ. Three suppressor systems in human blood that modulate lymphoproliferation. J Immunol 1979;122:991-996.

Rich AR, 1951. The Pathogenesis of Tuberculosis, 2nd ed. Springfield Illinois, Thomas.

Ridley DS and Jopling WH. Classification of leprosy according to immunity. A five-group system. Int J Lepr 1966;34: 255-273.

Ridley DS, 1983. The histopathological spectrum of the Mycobacterioses. In The Biology of the Mycobacteria, Vol 2. ed by Ratledge C and Stanford J, London, Academic Press. P.129-171.

Rieger H, Trnka L and Steer J. Immunoprofile studies in Patients with pulmonary tuberculosis. IV. Tuberculin skin reaction and test of inhibition of blood leukocyte migration. Scand J Respir Dis 1978;59:313-317.

Ringdon O, Rynnel-Dagoo B, Kunori T, Smith CIE, Hammarstrom L, Froijd A and Moller E. Induction of antibody synthesis in human B lymphocytes by different polyclonal B cell activators: Evaluation by direct and indirect PFC assays. Immunol Rev 1979;45:195-218.

Rocklin RE, MacDermott RP, Chess L, Schlossman SF and David JR. Studies on mediator production by highly purified human T and B lymphocytes. J Exp Med 1974;140:1303-1316.

Rocklin RE, Bendtzen K and Greineder AD. Mediators of immunity: lymphokines and monokines. Adv Immunol 1980;29:55-136.

Roder JC and Proose HF. The biology of the human Natural Killer cell. J Clin Immunol 1982;2:249-263.

Rook CAW, Carswell JW and Stanford JL. Preliminary evidence for the trapping of antigen-specific lymphocytes in the lymphoid tissue of "anergic" tuberculosis. Clin Exp Immunol 1976;26:129-132.

Rooney JJ, Crocco JA and Krasor S. Further observations on tuberculin reactions in active tuberculosis. Am J Med 1976;60:617-622.

Rosenthal T, Scholtz S, Klimek R, Albert ED and Blaha M. HLA-A antigens and haplotypes in patients with tuberculosis. Z Immunitatsforsch 1973;144:424-432.

Rosenthal AS, Lisnky PE and Shevach EM. Macrophage-lymphocyte interaction and antigen recognition. Fed Proc 1975;34:1743-1748.

Rothermel CD, Rubin BY and Murray HW. γ-interferon is the factor in lymphokine that activates human macrophages to inhibit intracollular *Chlamydia psittaci* replication. J Immunol 1983;131:2542-2544.

Rouillon A, Perdrizet S and Parrot R. Transmission of tubercle bacilli: the effect of chemotherapy. Tubercle 1976;57:275-299.

Rubinstein M, Rubinstein S, Familletti PC, Miller RS, Waldman AA and Pestka S. Human leukocyte interferon: Production, purification to homogeneity, and initial characterization. Proc Natl Acad Sci USA 1979;76:640-644.

Ryan TA, Joiner BL and Ryan BF, 1981. Minitab Reference Manual. Pennsylvania State University

Saal JC, Riethmuller G, Eiber EP, Hadam M, Ehinger H and Schneider W. Regional BCG therapy of malignant melanoma: In vitro monitoring of spontaneous cytolytic activity of circulating lymphocytes. Cancer Immunol Immunother 1977;3: 27-33.

Saha N and Banerjee B. Incidence of ABO and Rh blood groups in Pulmonary tuberculosis in different ethnic groups. J Med Genet 1968;5:305-307.

Sahn SA and Neft TA. Miliary tuberculosis. Am J Med 1974; 56:495-505.

Salvin SB, Youngner JS and Leder WJ. Migration inhibitory factor and interferon in the circulation of mice with delayed hypersensitivty. Infect Immun 1973;7:68-75.

Sasazuki TY, Kohno Y, Iwamoto I, Tanimura M and Naito S. Association between an HLA haplotype and low responsiveness to tetanus toxoid in man. Nature 1978;272:359-361.

Rothermel CD, Rubin BY and Murray HW. γ-interferon is the factor in lymphokine that activates human macrophages to inhibit intracellular Chlamydia psittaci replication. J Immunol 1983;131:2542-2544.

Rouillon A, Perdrizet S and Parrot R. Transmission of tubercle bacilli: the effect of chemotherapy. Tubercle 1976;57:275-299.

Rubinstein M, Rubinstein S, Familletti PC, Miller RS, Waldman AA and Pestka S. Human leukocyte interferon: Production, purification to homogenity, and initial characterization. Proc Natl Acad Sci USA 1979;76:640-644.

Ryan TA, Joiner BL and Ryan BF. 1981, Minitab Reference Manual, Pennsylvania State University

Saal JC, Riethmuller G, Elber EP, Hadam M, Ehinger H and Schneider W. Regional BCG therapy of malignant melanoma: In vitro monitoring of spontaneous cytolytic activity of circulating lymphocytes. Cancer Immunol Immunother 1977;3: 27-33.

Saha N and Banerjee B. Incidence of ABO and Rh blood groups in pulmonary tuberculosis in different ethnic groups. J Med Genet 1968;5:306-307.

Sahn SA and Neff TA. Miliary tuberculosis. Am J Med 1974; 56:495-505.

Salvin SB, Youngner JS and Leder WJ. Migration inhibitory factor and interferon in the circulation of mice with delayed hypersensitivty. Infect Immun 1973;7:68-75.

Sasazuki TY, Kohno Y, Iwamoto I, Tanimura M and Naito S. Association between an HLA haplotype and low responsiveness to tetanus toxoid in man. Nature 1978;272:359-361.

Saxona QB, Mozey E and Adler WH. Regulation of natural killer activity in vivo. II. The effect of alcohol consumption on human peripheral blood natural killer activity. Int J Cancer 1980a;26:413-417.

Saxona RK, Saxena QB and Adler WH. Regulation of natural killer activity in vivo. I. Loss of natural killer activity during starvation. Indian J Exp Biol 1980b;18:1383-1386.

Schacter EN. Tuberculin negative tuberculosis. Am Rev Respir Dis 1972;106:587-593.

Schlossman SF, Lovin HA, Rocklin RE and David JR. The compartmentalisation of antigen-reactive lymphocytes in desensitised guinea-pigs. J Exp Med 1971;134:741-750.

Schmidt ME and Douglas SD. Monocyte IgG receptor activity. dynamics, and modulation - normal individuals and patients with granulomatous disease. J Lab Clin Med 1977;89:332-340.

Schmidtke JR and Johnson AG. Regulation of the immune system by synthetic polynucleotides. I. Characteristics of adjuvant action on antibody synthesis. J Immunol 1971;106:1191-1200.

Schmitt E, Mouret G and Stiz L. Monocyte recruitment in tuberculosis and sarcoidosis. Br J Haematol 1977;35:11-17.

Schooley RT, Hirsch MS, Colvin RB, Cosimi AB, Tolkoff-Rubin NE, McCluskey RT, Burton RC, Russell PS, Herrin JT, Delmonico FL, Giorgi JV, Wenle W and Rubin RH. Association of herpesvirus infections with T-lymphocyte subset alterations, glomerulopathy and opportunistic infections after renal transplantation. N Engl J Med 1983;308:307-313.

Schrek R. Cell transformations and mitoses produced in vitro by tuberculin purified protein derivative in human blood cells.

Saxena QB, Mozey E and Adler WH. Regulation of natural killer activity in vivo. II. The effect of alcohol consumption on human peripheral blood natural killer activity. Int J Cancer 1980a;26:413-417.

Saxena RK, Saxena QB and Adler WH. Regulation of natural killer activity in vivo. I. Loss of natural killer activity during starvation. Indian J Exp Biol 1980b;18:1383-1386.

Schacter EN. Tuberculin negative tuberculosis. Am Rev Respir Dis 1972;106:587-593.

Schlossman SF, Levin HA, Rocklin RE and David JR. The compartmentalization of antigen-reactive lymphocytes in desensitized guinea-pigs. J Exp Med 1971;134:741-750.

Schmidt ME and Douglas SD. Monocyte IgG receptor activity, dynamics, and modulation - normal individuals and patients with granulomatous diseases. J Lab Clin Med 1977;89:332-340.

Schmidtke JR and Johnson AG. Regulation of the immune system by synthetic polynucleotides. I. Characteristics of adjuvant action on antibody synthesis. J Immunol 1971;106:1191-1200.

Schmitt E, Mouret G and Stix L. Monocyte recruitment in tuberculosis and sarcoidosis. Br J Haematol 1977;35:11-17.

Schooley RT, Hirsch MS, Colvin RB, Cosimi AB, Tolkoff-Rubin NE, McCluskey RT, Burton RC, Russell PS, Herrin JT, Delmonico FL, Giorgi JV, Henle W and Rubin RH. Association of herpesvirus infections with T-lymphocyte subset alterations, glomerulopathy and opportunistic infections after renal transplantation. N Engl J Med 1983;308:307-313.

Schrek R. Cell transformations and mitoses produced in vitro by tuberculin purified protein derivative in human blood cells. Am Rev Respir Dis 1963;87:734-738.

Scott GC, Russel DA, Boughton CR and Vincin DR. Untreated leprosy: probability for shifts in Ridley-Jopling classification. Int J Lepr 1976;44:110-122.

Secher DS. Immunoradiometric assay of human leucocyte interferon using monoclonal antibody. Nature 1981;290:501-503.

Seibert FB and Munday B. Chemical composition of active principle of tuberculin: comparison of nitrogen partition analyses of proteins from different acid-fast bacilli and relationship to biological activity. J Biol Chem 1933;101: 763-771.

Selby R, Barnard JM, Buehler SK, Crumley J, Larsen B and Marshall WH. Tuberculosis associated with HLA-B8 Bf6 in a Newfoundland community study. Tissue Antigens 1978;11:403-408.

Shapiro SS and Francia RS. An approximate analysis of variance test for normality. JASA 1972;67:215-216.

Shima K, Takanaka S, Ando M and Tokuoni H. Studies on T and B cell subpopulations in patients with pulmonary tuberculosis. Kekkaku 1976;51:363-374.

Shou L, Schwartz SA and Good RA. Suppressor cell activity after concanavalin A treatment of lymphocytes from normal donors. J Exp Med 1976;143:1100-1110.

Silwer H and Oscarsson PM. Incidence and coincidence of diabetes mellitus and pulmonary tuberculosis in a Swedish county. Acta Med Scand 1958;161(suppl 335):1-48.

Singh MM, Kumar P, Malaviya AN and Kumar R. Levamisole as an adjunct in the treatment of pulmonary tuberculosis. Am Rev Respir Dis 1981;123:277-279.

Singh SPN, Mehra NK, Dingley HB, Pande JN and Valdya MC. Human leukocyte antigen (HLA)-linked control of susceptibility to pulmonary tuberculosis and association with HLA-DR types. J Infect Dis 1983;148:676-681.

Skinsnes OK. Comparative pathogenesis of mycobacterioses. Ann NY Acad Sci 1968;154:19-31.

Skvor J and Trnka L. Immunoprofile studies in patients with pulmonary tuberculosis. I. Correlation of pretherapy cellular tests with characteristics of the disease. Scand J Respir Dis 1979;60:161-167.

Smith H and Onwubalili J. Unexplained deaths in pulmonary tuberculosis. Lancet 1983;i:984-985.

Smith JA and Reichmann LB. Lymphocyte transformation. An aid in the diagnosis of tuberculosis in patients with nonreactive skin tests. Am Rev Respir Dis 1972;106:194-201.

Snider DE. Jejunoileal bypass for obesity: A risk factor for tuberculosis (Editorial). Chest 1982;81:531.

Sokal JE. Measurement of delayed skin-test responses. N Engl J Med 1975;293:501-502.

Spitznagel JK and Allison AC. Mode of action of adjuvants: retinol and other lysosome-labilizing agents as adjuvants. J Immunol 1970;104:119-127.

Starr S and Berkovich S. Effect of measles, gamma globulin-modified measles and vaccine measles on the tuberculin test. N Engl J Med 1974;270:386-391.

Stead WW and Bates J, 1977. Tuberculosis, in Harrison's Principles of Internal Medicine, 8th ed., ed by Thorn GW, Adams RD, Braunwauld E, Isselbacher KJ and Petersdorf RG, New York, McGraw-Hill, p.900.

Steele AH and Willis HS, 1934, A study of the increase in sensitiveness in normal children produced by repeated injections of tuberculin, in Transactions of the 30th Annual Meeting of the National Tuberculosis Association, p.120-122.

Stinebring WR and Absher MP. production of interferon following an immune response. Ann NY Acad Sci 1970;173: 714-718.

Subbaiah TV, Mitchison DA and Selkon JB. The susceptibility to hydrogen peroxide of Indian and British isoniazid-sensitive and isoniazid-resistant tubercle bacilli. Tubercle 1960;41:323-333.

Sultan L, Myka W, Mills C, O'Grady P, Wells W and Riley RL. Tuberculosis disseminators. A study of the variability of aerial infectivity of tuberculous patients. Am Rev Respir Dis 1960;82:358-369.

Sultzer BM and Nilsson BS. PPD-tuberculin : a B-cell mitogen. Nature 1972;240:198-200.

Suter E. Multiplication of tubercle bacilli within mononuclear phagocytes in tissue cultures derived from normal animals and animals vaccinated with BCG. J Exp Med 1953;97:235-247.

Thomas JW, Clements D and Orzybowski S. in vitro lymphocyte responses and skin test reactivity following BCG vaccination. Clin Exp Immunol 1977;9,611-623.

Thompson NJ, Glassroth, JL, Snider DE Jr and Farer LS. The booster phenomenon in serial tuberculin skin testing. Am Rev Respir Dis 1979;119:587-597.

Thorn PH and Brookes VS. Peptic ulcer, partial gastrectomy and pulmonary tuberculosis. Br Med J 1956;1:603-608.

Timonen T, Saksela E, Virtanen I and Cantell K. Natural killer cells are responsible for the interferon production induced in human lymphocytes by tumour cell contact. Eur J Immunol 1980;10:422-427.

Tracey DE. The requirement for macrophages in the augmentation of natural killer cell activity by BCG. J Immunol 1979;123: 840-845.

Tsuyuguchi I, Shiratsuchi H, Teraoka O and Hirano T. Increase in T-cells bearing IgG Fc receptors in peripheral blood of Patients with tuberculosis by in vitro stimulation with Purified protein derivative. Am Rev Respir Dis 1980;121: 951-957.

Tsuyuguchi I, Shiratsuchi H, Fujiwara H and Teraoka O. Non-specific recruitment of lymphocytes in Purified Protein Derivative-induced lymphocyte proliferative response of Patients with tuberculosis. Infect Immun 1982;37:702-709.

Uberoi S, Malaviya AN, Chattopadhyay C, Kuman R and Shrivinas K. secondary immunodeficiency in miliary tuberculosis. Clin Exp Immunol 1975;22:404-408.

Urbanitz D, Gregoritz E, Fechner I and Gross R. Reduced phagocytosis of monocytes from patients with tuberculosis under treatment. Klin Wochenschr 1974;52:544-548.

Vilcek J, Sulea IT, Vovovitz P and Yip YK, 1980, Characterietics of interferons produced in cultures of human lymphocytes by stimulation with Corynebacterium parvum and phytohemagglutinin, in Biochemical Characterization of Lymphokines, ed by DeWock AL, Kristensen F and Landy M, London, Academic Press, p. 323-329.

Viljanen MK, Eskola J and Tala E. Enzyme-linked immunosorbent assay (ELISA) for antibodies to purified protein derivative of tuberculin (PPD): IgM-, IgA- and IgG-anti-PPD antibodies in active pulmonary tuberculosis. Eur J Respir Dis 1982; 63:257-262.

Wadee AS, Sher R and Rabson AR. Production of a suppressor factor by human adherent cells treated with mycobacteria. J Immunol 1980;125:1380-1386.

Wadee AA, Joffe MI, Lowitzer R and Rabson AR. Mononuclear cell function in Mycobacterium tuberculosis infected guinea pigs. Clin Immunol Immunopathol 1983;28:325-333.

Wahl SM, Wilton JM, Rosenstreich DL and Oppenheim JJ. The role of macrophages in the production of lymphokines by T and B lymphocytes. J Immunol 1975;114:1296-1301.

Wahl LM, Wahl SM, Mergenhagen SE and Martin GR. Collagenase production by lymphokine-activated macrophages. Science 1975;187:261-263.

Waldorf DS, Willkens RF and Decker JL. Impaired delayed hypersensitivity in an aging population. JAMA 1968;203:831-834.

Walker L and Lowrie DB. Killing of Mycobacterium microti by immunologically activated macrophages. Nature 1981;293:69-70.

Wallace R, Dicna BB, Greenborg L and Jessamino AF. A study of tuberculosis antibodies by bentonite flocculation. Can Med Assoc J 1966;94:947-950.

Wallace BA, Diena BB, Jossamine AG and Groonberg L. Circulating antibody response in BCG vaccination, tuberculous infection and sarcoidosis. Can Mod Assoc J 1967;96:585-588.

Weismann C, Goldstein I, Hoffstein S, Chauvet G and Robinoaux R. Yin/Yang modulation of lysosomal enzyme release from polymorphonuclear leukocytes by cyclic nucleotides. Ann NY Acad Sci 1975;256:222-231.

Wekaler ME and Huttoroth TH. Impaired lymphocyte function in aged humans. J Clin Invest 1974;53:99-104.

Wheelock EF. Virus replication and high titre interferon Production in human leucocyte cultures inoculated with Newcastle Disease virus. J Bacteriol 1966;92:1415-1421.

WHO Memorandum. Trial of BCG vaccines in south India for tuberculosis prevention: first report. Bull WHO 1979:57: 819-827.

WHO Memorandum. Immunological Research in Tuberculosis: Memorandum from a WHO Meeting. Bull WHO 1982;60:723-727.

Wilhelm C and Romer C. Untersuchung uber die Wertigkeit eines neu aufgefundenen aus Mycobacterium tuberculosis isolierten, kleinmolekularen peptids mit spezifischer Antigenstruktur fur die Tuberkulosediagnostik. Zentralbl Bakteriol (Orig A) 1977;239:379-396.

Wilkinson M and Morris AG, 1983. Interferon and the immune system, 1: Induction of interferon by stimulation of the immune system, in Interferons: from Molecular Biology to Clinical Application, Symposium 35, ed by Burke DC and Morris AG. The Society for General Microbiology, Cambridge University Press.

Wilkinson PC, 1982, Chemotaxis and Inflammation. 2nd ed. Edinburgh, Churchill Livingstone.

Williams RC, Dobord JR, Mellbye OJ, Messner RP and Lindstrom PP. Studies of T and B lymphocytes in patients with connective tissue diseases. J Clin Invest 1973;52: 283-295.

Winters WD and Cox RA. Serodiagnosis of tuberculosis by radioimmunoassay. Am Rev Respir Dis 1981;124:582-585.

Wolfe SA, Tracey DE and Henney CS. Induction of "natural killer" cells by BCG. Nature 1976;262:584-586.

Yassen NY, Thewaini AJ, Al-Tawil MO and Jazrawi FY. Trial of immunopotentiation by levamisole in patients with pulmonary tuberculosis. J Infect 1980;2:125-136.

Yip YK, Pang R, Urban C and Vilcek J. Partial purification and characterization of human gamma (immune) interferon. Proc Natl Acad Sci USA 1981a;78:1601-1605.

Yip YK, Pang R, Oppenheim J, Hadibar MS, Henriksen D, Zerobeckyj-Eckhardt I and Vilcek J. Stimulation of human gamma interferon production by diterpene esters. Infect Immun 1981b;34:131-139.

Yoneda T, Ishibashi J, Nishikawa K, Mikami R, Kasai M and Tokunaga T. NK cell activity in pulmonary tuberculosis. Kekkaku 1982;57:233-237.

Yoneda T, Kasai M Ishibashi J, Nishikawa K, Tokunaga T and Mikami R. NK cell activity in pulmonary tuberculosis. Br J Chest Dis 1983;77:185-188.

Yoshida T, Sonozaki H and Cohen S. The production of migration inhibition factor by B and T cells of the guinea pig. J Exp Med 1973;138:784-797.

Youmans GP, 1978. Tuberculosis, Philadelphia, W.B.Saunders.

Youmans GP. Mechanisms of immunity in tuberculosis. Pathobiol Annu 1979;9:137-162.

Zeitz SJ, Ostrow JH and Van Arsdel PP Jr. Humoral and cellular immunity in the anergic tuberculous patient. A proapective study. J Allergy Clin Immunol 1974;53:20-32.

Zwolinski J, Wieczorek Z and Skibinski G. T and B lymphocytes in patients with pulmonary tuberculosis. Arch Immun Ther Exp 1976;24:689-698.

APPENDIX I: RAW DATA. TABLE A1

Anthropometric indices and haemoglobin
(wt/ht², arm muscle circumf. or Hb less than control mean - 2 SD)

Case No	W/H²		Skinfold thickness (mm)		Arm muscle circumference (cm)		Haemoglobin (g/dl)	
	Controls	Patients	Controls	Patients	Controls	Patients	Controls	Patients
1	20.4	24.9	10	20	25	24	15.5	14.7
2	24.9	21.7	19	-	21	-	14.9	13.4
3	22.8	23.0	-	15	-	22	-	13.0
4	18.4	18.5	2	2	23	21	-	12.6
5	17.9	16.2(+)	13	12	22	17(+)	13.4	11.0(+)
6	19.9	13.7(+)	10	7	25	19(+)	16.4	9.8(+)
7	21.0	19.4	8	7	24	23	13.6	13.0
8	17.9	14.7(+)	6	8	20	17(+)	13.0	12.4
9	22.0	14.3(+)	10	-	25	-	14.5	10.1(+)
10	19.0	14.7(+)	15	6	20	18	13.2	7.9(+)
11	23.2	19.9	16	5	24	23	15.6	17.3
12	24.2	13.8(+)	11	2	26	17(+)	14.1	12.8
13	24.5	17.3	14	5	27	22	15.9	15.1
14	21.6	21.1	-	8	-	21	15.7	14.8
15	17.7	18	9	5	20	19	13.1	13.2
16	23.0	27.2	19	10	20	24	13.2.5	-
17	25.6	20.3	20	6	-	25	14.7	11.0(+)
18	25.3	21.6	20	5	23	22	17.1	17.3
19	27.2	15.8(+)	14	1	30	23	15.5	10.2(+)
20	22.0	25.6	10	6	24	27	15.4	14.1
21	20.2	17.5	25	-	19	-	14.0	14.2
22	21.4	21.3	18	21	23	19	13.5	10.8(+)
23	20.9	24.4	14	81	21	25	15.0	13.6
24	23.1	23.5	10	37	23	21	14.1	14.3
25	23.7	18.0	26	-	26	-	15.1	14.0
26	26.6	17.5	21	9	23	22	14.2	12.8
27	21.3	19.3	10	10	21	19	14.3	13.6
28	22.7	21.3	8	7	25	24	15.9	-
29	21.0	24.3(+)	8	3	28	17(+)	14.8	10.3(+)
30	24.0	18 (+)	8	9	24	17(+)	14.9	13.9

TABLE A2

White blood cell counts and erythrocyte sedimentation rates
(+= lymphocyte count less than control mean - 2 SD)

Case no.	Neutrophils (/µl)		Lymphocytes (/µl)		Monocytes (/µl)		ESR (mm/hr)	
	Controls	Patients	Controls	Patients	Controls	Patients	Controls	Patients
1	4884	4600	1624	2635	464	236	2	15
2	4416	5540	1725	1410	552	752	-	80
3	-	-	-	-	-	410	-	7
4	3340	7458	-	2200	253	700	-	46
5	3355	4312	1650	896(+)	440	352	2	110
6	3762	9900	2046	2430	320	1080	1	140
7	1874	-	2640	1526	69	-	3	60
8	2420	5568	1936	-	0	289	4	37
9	7760	4897	1261	2980	500	166	10	64
10	2862	5395	2160	1040	162	65	4	7
11	1932	5670	1090	1620	336	324	1	9
12	2784	7740	1776	774(+)	192	86	5	67
13	4408	5382	2050	1774	836	546	4	13
14	3300	3711	2107	700(+)	-	47	1	74
15	3240	6640	3080	960(+)	150	240	7	30
16	3234	5100	2214	1400	295	700	4	-
17	3010	4914	1520	567(+)	160	819	3	16
18	-	3700	1782	800(+)	105	300	2	26
19	2200	5504	3030	640(+)	167	256	2	53
20	4148	5540	1977	1520	432	-	1	37
21	4340	10375	2320	1635	140	250	3	28
22	4290	3249	1930	1824	195	513	5	104
23	3080	4224	2214	1729	230	330	6	73
24	4290	4821	1830	1563	375	252	3	19
25	1912	6984	1470	2106	210	1150	1	75
26	2750	6240	2163	1794	236	136	10	40
27	3524	5215	2610	2500	288	640	1	67
28	5403	4535	2500	1940	254	400	1	-
29	1554	7353	2224	332(+)	168	332	3	40
30	6592	7590	2070	2070	288	575	6	13

TABLE A3

Levels of serum proteins and immunoglobulins
(ie Albumin less than normal laboratory range)

Case No	Albumin (range 35-50g/l) Controls	Albumin Patients	Globulin (g/l) Controls	Globulin Patients	IgG (g/l) Controls	IgG Patients	IgA (g/l) Controls	IgA Patients	IgM (g/l) Controls	IgM Patients
1	40	43	29	29	12.0	10.0	2.1	2.5	1.2	3.8
2	45	30	28	30	7.7	6.7	2.0	1.0	1.1	0.7
3	-	-	-	-	-	-	-	-	-	-
4	46	43	-	-	-	17.0	-	7.7	-	2.4
5	46	33	31	49	2.9	12.0	2.4	4.7	1.3	2.4
6	45	31	27	34	11.7	10.3	1.5	2.1	1.8	1.1
7	-	-	33	33	10.1	8.9	2.7	2.1	1.6	0.9
8	46	30	31	33	12.9	11.1	2.2	2.8	2.0	0.6
9	43	20(a)	34	40	14.3	17.0	2.2	4.7	2.8	1.2
10	43	39	36	30	8.3	12.0	1.4	3.0	0.3	2.3
11	46 46	37	35	40	9.1	21.6	1.2	2.8	1.7	0.9
12	40	30(a)	23	36	8.1	12.9	0.7	6.2	1.8	2.6
13	47	43	28	37	13.5	17.0	2.0	1.5	0.9	2.5
14	40	34	27	31	11.3	9.7	1.7	3.7	1.2	1.1
15	40	41	20	33	14.3	12.0	2.3	2.7	1.4	3.3
16	42	30	29	35	13.4	13.1	2.2	7.9	1.3	1.1
17	51	43	27	28	9.0	10.3	2.4	1.8	1.1	0.6
18	45	34	36	30	8.0	15.0	2.5	2.1	1.0	2.4
19	46	30(a)	33	31	13.3	11.1	2.3	1.4	1.1	0.1
20	45	43	31	30	12.0	-	1.3	2.5	1.3	0.4
21	47	42	38	37 37	9.4	18.0	6.1	-	2.8	2.3
22	44	37	40	36	11.0	18.9	2.5	2.7	0.7	1.2
23	47	37	32	30	13.1	17.0	4.3	2.3	1.1	1.7
24	44	40	29	30	18.0	12.2	2.0	2.5	0.4	1.4
25	45	30	29	34	10.3	15.3	1.4	4.0	3.7	3.8
26	44	37	29	38	12.3	19.0	2.5	2.9	0.8	3.8
27	49	40	27	38	10.0	12.8	1.0	1.7	2.0	1.3
28	40	47	23	38	7.1	11.7	6.0	1.8	2. 1.0	-
29	45	18(a)	33	38	10.0	8.9	5.7	2.8	1.4	0.1
30	47	47	38	31	12.0	14.1	2.3	2.5	1.3	1.4

TABLE A4

Levels of serum sodium, calcium, iron, TIBC and alkaline phosphatase
(+ - TIBC less than control mean - 71)

Case No	Sodium (mmol/l) Controls	Sodium (mmol/l) Patients	Calcium (mmol/l) Controls	Calcium (mmol/l) Patients	Iron (µmol/l) Controls	Iron (µmol/l) Patients	TIBC (µmol/l) Controls	TIBC (µmol/l) Patients	Alkaline Phosphatase (IU/l) Controls	Alkaline Phosphatase (IU/l) Patients
1	142	139	2.40	-	4	11	81	69	-	150
2	142	-	2.37	-	10	-	73	-	315	200
3	-	142	-	-	-	-	-	-	-	-
4	140	138	2.31	2.30	-	-	-	-	-	317
5	141	139	2.26	2.02	4	8	90	57	-	268
6	-	-	2.35	2.12	27	3	50	-	153	316
7	136	135	2.37	2.36	24	7	75	64	130	999
8	139	136	2.37	2.25	23	4	84	45(+)	161	177
9	140	136	2.28	2.24	14	4	75	38(+)	50	-
10	139	123	2.34	2.09	15	4	54	64	161	173
11	143	139	2.43	2.25	-	-	-	54	143	308
12	144	137	2.23	1.78	23	4	54	33(+)	130	417
13	-	-	2. 2.30	2.53	24	11	60	50	231	220
14	142	141	2.63	2.21	24	7	62	38(+)	86	300
15	134	139	2. 2.40	2.29	10	7	81	37	117	161
16	138	137	2.31	2.23	10	16	66	64	167	299
17	138	139	2.43	2.30	20	7	41	43	167	180
18	139	134	[illegible]	2.14	20	8	64	50	202	320
19	140	135	2.20	1.80	16	4	63	37(+)	153	236
20	-	-	2.43	2.43	18	8	75	60	132	368
21	137	137	2.24	2.32	9	8	43	40	131 130	[illegible]
22	139	130	2.21	2.36	13	7	60	43(+)	213	230
23	138	[illegible]	[illegible]	2.37	15	9	60	43(+)	164	176
24	142	[illegible]	2.36	2.16	13	10	65	75	176	233
25	144	[illegible]	2.40	2.30	-	10	-	41(+)	134	233
26	111	134	2.30	2.71	14	4	71	64	181	[illegible]
27	142	139	2.37	2.57	16	5	[illegible]	68	221	300
28	141	141	2.42	2.37	20	16	60	60	202	160
29	134	129	2.30	1.83	10	8	55	33(+)	130	176
30	[illegible]	141	2.46	2.35	12	8	71	38(+)	207	170

TABLE A3

Absolute counts (/μl) of total lymphocytes, total T (Leu 1 or 4+), suppressor-T (Leu 2a+), helper-T (Leu 3a+) and B (Leu 12+) lymphocytes, and helper/suppressor T-lymphocyte (T_H/T_S) ratios

Case No.	Total lymphocytes		Leu 1/4+ (pan T)		Leu 2a+ (T_S)		Leu 3a+ (T_H)		Leu 12+ (B)		T_H/T_S ratio	
	Controls	Patients	Controls	Patients	Controls	Patients	Controls	Patients	Controls	Patients	Controls	Patients
Pneumonia (untreated)												
22	1950	1826	1385	1290	327	381	1073	1049	-	-	2.08	2.71
24	2143	3496	1907	3073	655	[illegible]	1332	1373	175	750	2.03	1.31
73	2580	3368	2099	2047	641	837	1280	2544	230	301	1.85	2.04
75	2278	870	2130	685	267	65	1670	421	89	44	6.25	0.66
76	2053	5063	1463	790	472	723	841	130	103	53	1.71	0.18
12	1572	1003	1441	173	563	481	1105	391	112	70	1.97	0.84
13	1570	475	3104	350	1607	93	1535	235	357	20	0.96	3.53
14	1918	810	3007	697	1040	200	1701	440	750	100	1.67	2.22
15	3301	1630	2718	1030	411	459	2103	753	237	131	3.44	1.64
16	1726	1215	1574	961	384	349	1314	571	213	109	0.62	1.60
17	1796	4130	1597	3432	632	1336	875	2308	126	165	1.21	[illegible]
30	2381	3461	1919	1364	667	536	1105	838	115	82	1.64	1.56
31	-	2979	-	2061	-	849	-	1352	-	94	-	1.57
Other lung (untreated)												
19 [illegible]	-	1306	-	610	-	808	-	157	-	56	-	0.20
20 [illegible]	-	3053	-	1926	-	1516	-	1107	-	395	-	0.74
21 [illegible]	-	1000	-	1117	-	902	-	647	-	59	-	0.72
23 [illegible]	-	1143	-	1082	-	608	-	501	-	74	-	0.98

TABLE 4E

Skin reactions, cytotoxicity and monocyte migration

Case No.	Skin reactions(mm) to 1(of 10)TU PPD				Spontaneous cytotoxicity (% ^{51}Cr-released)		Monocyte migration (µl			
	Controls		Patients				Random locomotion		Chemotaxis	
	1h	48h	1h	48h	Controls	Patients	Controls	Patients	Controls	Patients
1	-	10	-	10	38	52	9	-	18	-
2	-	-	-	10	63	56	-	-	-	-
3	0	-	0	0(0)	34	53	-	-	-	-
4	0	30	0	17	26	29	-	-	-	-
5	0	17	0	0(0)	36	51	-	-	-	-
6	0	24	0	0(0)	52	27	-	-	-	-
7	0	0(10)	3	10	54	44	-	-	-	-
8	0	0(0)	0	0(0)	38	40	-	-	-	-
9	0	10	6	25	41	33	-	-	-	-
10	0	10	3	13	58	57	-	-	-	-
11	0	16	0	16	48	38	-	-	-	-
12	0	50	0	0(5)	46	19	12	-	27	-
13	0	25	0	10	35	18	-	-	-	-
14	0	0(0)	0	25	47	37	-	-	-	-
15	0	10	5	13	-	47	1	1	16	10
16	0	0(0)	0	21	38	33	3	1	17	0
17	-	10	-	10	36	45	10	1	21	13
18	0	0(0)	0	20	53	43	0	0	28	25
19	0	15	0	0(0)	32	42	-	-	-	-
20	-	0(0)	-	16	39	31	7	7	20	19
21	0	0(0)	0	12	-	-	12	11	32	28
22	0	0(0)	0	17	56	60	10	11	18	22
23	0	-	0	25	57	69	10	8	26	24
24	0	0(0)	5	30	56	32	6	6	25	21
25	-	15	-	-(0)	46	30	7	6	16	17
26	0	10	0	-(30)	59	53	6	7	22	26
27	-	0(0)	-	20	-	-	1	1	31	27
28	0	-	5	20	42	-	6	1	31	27
29	0	0(0)	0	0	46	40	6	6	27	28
30	0	10	0	0(0)	35	46	2	5	32	22

TABLE A7

Mononuclear cell proliferation with SCA+TPA (counts per minute)

Case no	In 10% fetal calf serum		In 10% patient's serum		In 10% normal human serum	
	Controls	Patients	Controls	Patients	Controls	Patients
1	2611	2305	-	-	-	-
2	1685	617	-	-	-	-
3	663	416	-	-	-	-
4	566	1447	-	-	-	-
5	-	-	-	-	-	-
6	1474	1247	-	-	-	-
7	3206	2429	-	-	-	-
8	1148	2978	-	-	-	-
9	1398	1174	-	-	-	-
10	1793	3080	-	-	-	-
11	3752	2026	-	-	-	-
12	2456	1664	-	-	-	-
13	2011	2068	-	-	-	-
14	1813	1886	772	822	1095	2228
15	1166	1555	665	557	566	3367
16	1313	1527	1210	425	502	743
17	503	685	734	543	1026	2773
18	1307	972	720	2284	1829	1012
19	1051	1708	603	182	2425	734
20	934	807	1857	3133	1850	1883
21	1048	1739	1171	1337	1442	1749
22	1002	1647	1305	1608	1774	1331
23	817	1139	1142	742	647	1347
24	578	980	1489	1508	833	823
25	232	384	-	-	-	-
26	657	860	-	-	-	-
27	896	1603	-	-	-	-
28	-	901	1979	1259	1978	2147
29	1500	2194	960	184	838	1879
30	970	1894				

TABLE A7

Mononuclear cell proliferation with SLA-TPA (counts per minute)

Case no	In 10% foetal calf serum		In 10% patient's serum		In 10% normal human serum	
	Controls	Patients	Controls	Patients	Controls	Patients
1	2411	2105	-	-	-	-
2	1685	632	-	-	-	-
3	663	416	-	-	-	-
4	566	1467	-	-	-	-
5	-	-	-	-	-	-
6	1424	1247	-	-	-	-
7	3206	2429	-	-	-	-
8	1168	2978	-	-	-	-
9	1398	1174	-	-	-	-
10	1793	1080	-	-	-	-
11	3753	3026	-	-	-	-
12	2456	1664	-	-	-	-
13	3011	2068	-	-	3099	2220
14	1813	1886	772	822	566	2163
15	1164	1555	665	557	583	243
16	1313	1527	1210	425	1026	2773
17	583	489	734	543	1039	1812
18	1307	972	730	2284	2425	734
19	1051	1700	493	112	1639	1883
20	934	807	1057	2133	1442	1749
21	1049	1725	1171	1327	1774	1331
22	1002	1447	1585	1606	607	1347
23	817	1159	1142	742	823	823
24	570	500	1109	1598	-	-
25	233	314	-	-	-	-
26	659	660	-	-	-	-
27	890	1685	-	-	1976	2147
28	-	961	1979	3339	838	1879
29	1500	2194	960	384		
30	970	1874				

TABLE 28

Mononuclear cell Proliferation with 1u PPD (counts per minute)
(+ = patient/control less than 70%)

Case no	In 10% foetal calf serum			In 10% patient's serum		In 10% normal human serum	
	Controls	Patients	Patient / Control	Controls	Patients	Controls	Patients
1	1036	1501	145	-	-	-	-
2	8527	1407	17(+)	-	-	-	-
3	625	424	68(+)	-	-	-	-
4	1288	973	76	-	-	-	-
5	-	-	-	-	-	-	-
6	4710	246	5(+)	-	-	-	-
7	4390	3096	48(+)	-	-	-	-
8	2460	1745	71	-	-	-	-
9	1652	2148	130	-	-	-	-
10	8532	772	9(+)	-	-	-	-
11	6927	4100	59(+)	-	-	-	-
12	3939	983	25(+)	-	-	-	-
13	7629	2378	30(+)	-	-	-	-
14	3178	3343	105	1061	1897	6017	2402
15	3091	2413	78	1448	936	1318	1660
16	3088	1755	57(+)	1576	565	246	136
17	1119	236	21(+)	933	1476	711	1288
18	4091	1450	35(+)	620	283	2016	1372
19	3088	1459	47(+)	64	482	122	133
20	870	67	7(+)	1387	3330	1285	2586
21	2502	1301	53(+)	601	2307	838	512
22	7455	1045	59(+)	2817	1482	4276	3094
23	1410	2161	152	698	2876	524	3637
24	1005	1570	85	361	416	771	772
25	401	134	28(+)	-	-	-	-
26	3620	1632	102	-	-	-	-
27	3716	1841	50(+)	-	-	-	-
28	-	2465	-	1803	603	3981	678
29	2131	3639	137	1793	1301	1151	1253
30	3017	2008	69(+)				

TABLE X5

Mononuclear cell proliferation with 1% PPD (counts per minute)
(* = patient/control less than 70%)

Case no	In 10% foetal calf serum			In 10% patient's serum		In 10% normal human serum	
	Controls	Patients	Patient % control	Controls	Patients	Controls	Patients
1	1036	1501	145	-	-	-	-
2	8527	1407	17(*)	-	-	-	-
3	635	424	68(*)	-	-	-	-
4	1288	973	76	-	-	-	-
5	-	-	-	-	-	-	-
6	4710	346	5(*)	-	-	-	-
7	4390	2094	48(*)	-	-	-	-
8	2460	1745	71	-	-	-	-
9	1652	3148	190	-	-	-	-
10	8532	372	4(*)	-	-	-	-
11	6937	4100	59(*)	-	-	-	-
12	3939	983	25(*)	-	-	-	-
13	7039	2378	34(*)	-	-	-	-
14	3178	2343	105	3061	1897	4017	2402
15	3091	2419	78	1660	936	1318	1460
16	3088	1758	57(*)	1578	965	206	136
17	1119	336	21(*)	933	1976	711	1388
18	4091	3458	35(*)	830	203	2016	1272
19	3088	1459	47(*)	64	482	122	133
20	870	67	7(*)	1367	3320	1781	2384
21	2592	1781	55(*)	606	2287	878	532
22	2685	1485	59(*)	2813	1482	4374	3084
23	2418	2161	152	638	2476	139	3657
24	1045	1570	65	361	816	772	772
25	481	124	38(*)	-	-	-	-
26	1630	1652	103	-	-	-	-
27	3711	1881	50(*)	-	-	-	-
28	-	2045	-	1885	603	2081	178
29	2131	2939	137	1293	1301	1351	1252
30	2017	2086	69(*)				

TABLE A8

Mononuclear cell proliferation with 1u PPD (counts per minute)
(‡ - patient/control less than 70%)

Case no	In 10% foetal calf serum			In 10% patient's serum		In 10% normal human serum	
	Controls	Patients	Patient/control %	Controls	Patients	Controls	Patients
1	1036	1501	145	-	-	-	-
2	8527	1407	17(‡)	-	-	-	-
3	625	424	68(‡)	-	-	-	-
4	1288	873	76	-	-	-	-
5	-	-	-	-	-	-	-
6	4710	246	5(‡)	-	-	-	-
7	4390	2096	48(‡)	-	-	-	-
8	2460	1745	71	-	-	-	-
9	1652	2148	130	-	-	-	-
10	8532	772	9(‡)	-	-	-	-
11	6937	4100	59(‡)	-	-	-	-
12	3530	983	25(‡)	-	-	-	-
13	7029	2378	39(‡)	-	-	-	-
14	3178	2343	105	3061	1897	4017	2602
15	3091	2013	70	1460	926	1318	1669
16	3088	1755	57(‡)	1576	969	246	136
17	1119	336	31(‡)	923	1876	711	1288
18	6091	3450	57(‡)	630	203	2016	1272
19	2068	1459	7(‡)	64	482	122	133
20	870	87	53(‡)	1267	3328	1703	3504
21	2502	1281	50(‡)	604	2397	870	512
22	2455	1445	152	2017	1482	6374	2896
23	2010	2161	85	630	2876	120	7457
24	1065	1570	28(‡)	361	416	772	772
25	481	134	103	-	-	-	-
26	1630	1652	98(‡)	-	-	-	-
27	2714	1881	-	-	-	-	-
28	-	2965	137	1885	603	2481	170
29	2131	2939	49(‡)	1293	1301	1151	1253
30	2017	2046					

TABLE A9

Titres of NDV-induced Interferon-α, SEA+TPA-induced Interferon-γ and PPD-induced Interferon-γ. (- indicates < 2.0 $\log_{10}$ u/ml)

Case no	IFN-α (NDV) $\log_{10}$ u/ml		IFN-γ (SEA+TPA) $\log_{10}$ u/ml		IFN-γ (PPD) $\log_{10}$ u/ml	
	Controls	Patients	Controls	Patients	Controls	Patients
1	3.8	3.8	3.48	3.78	3.0	3.95
2	-	-	-	-	-	-
3	3.9	3.9	3.48	3.0	3.0	<2.0(4)
4	-	-	3.30	3.48	3.0	3.30
5	3.1	2.9	-	-	-	-
6	3.4	2.8	2.52	2.52	2.05	<2.0(4)
7	3.7	3.8	3.30	1.95	3.3	3.78
8	3.8	3.4	-	3.48	-	3.52
9	3.8	3.3	3.30	3.48	3.0	3.30
10	3.7	3.8	3.95	-	3.48	4.95(4)
11	4.2	4.0	-	-	-	-
12	3.9	3.7	3.48	3.0	3.48	<1.6(4)
13	3.4	3.2	3.0	3.48	3.48	1.57(4)
14	2.9	3.1	3.48	3.48	3.52	3.0
15	3.3	3.7	-	-	2.30	3.3
16	3.0	2.4	3.48	3.0	2.52	2.52
17	3.3	3.0(4)	3.48	1.57(4)	2.52	1.57(4)
18	2.9	3.9	-	-	2.7	3.1
19	4.1	3.0	-	-	3.0	1.8(4)
20	3.8	3.3	2.52	3.0	<1.57(4)	1.57(4)
21	4.8	4.1	-	-	-	-
22	3.9	3.7	3.48	3.48	2.52	3.48
23	3.8	3.6	-	-	3.10	3.0
24	3.4	3.7	-	-	3.3	3.3
25	2.1	3.8	-	-	2.7	-
26	3.3	3.3	-	-	2.08	3.8
27	3.4	3.4	-	-	3.8	3.3
28	3.8	3.8	-	-	2.4	3.8
29	3.5	3.2	-	-	3.8	2.4
30	3.8		-	-	2.1	1.81(4)

TABLE A10

Pre-treatment skin reactions to ITT PPD in 28 patients with tuberculosis and 26 matched healthy controls

(P was derived by chi-squared test with Yates' correction unless otherwise stated)

		Tuberculosis	Controls	P
5 hrs	% with reactions	58 (14/24)	0 (0/26)	<0.001
	mean size (SD) mm	5.1 (3.4)	0	
12 hrs	% with reactions	71 (17/24)	8 (2/26)	<0.001
	mean size (SD) mm	8.2 (2.7)	0.9 (2.8)	
24 hrs	% with reactions	71 (20/28)	42 (10/24)	NS
	mean size (SD) mm	12.8 (3.2)	6.9 (3.4)	<0.05 (pooled t test)
48 hrs	% with reactions	75 (21/28)	62 (16/26)	NS
	mean size (95% limits) mm	17.3 (14.0-18.2)	12.8 (8.6-15.1)	<0.05 (pooled t test)
72 hrs	% with reactions	79 (22/28)	62 (16/26)	NS
	mean size (SD) mm	14.0 (4.2)	13.0 (4.7)	NS

TABLE A11

Skin reactions to 1TU PPD after six months of anti-tuberculosis chemotherapy in 10 patients and 10 matched healthy controls

(P was derived by chi-squared test with Yates' correction)

		Tuberculosis	Controls	P
6 hrs	% with reactions	70 (7/10)	22.2 (6/18)	<0.05
	mean size (1SD) mm	10.4 (4.6)	13.5 (6.6)	NS
12 hrs	% with reactions	80 (8/10)	38 (7/18)	NS
	mean size (1SD) mm	13.8 (3.8)	13.4 (9.6)	NS
24 hrs	% with reactions	100 (10/10)	50 (9/18)	<0.05
	mean size (1SD) mm	13.3 (4.3)	16.0 (8.3)	NS
48 hrs	% with reactions	100 (10/10)	55.6 (10/18)	<0.05
	mean size (1SD) mm	16.0 (4.4)	17.3 (3.8)	NS
72 hrs	% with reactions	100 (10/10)	55.6 (10/18)	<0.05
	mean size (1SD) mm	14.3 (3.8)	14.7 (3.3)	NS

TABLE A12

Skin reactions to 1TU PPD 12 months after the initiation of anti-tuberculosis chemotherapy in 10 patients and 10 matched healthy controls

(P was derived by chi-squared test with Yates' correction)

		Patients	Controls	P
6 hrs	% with reactions	90 (9/10)	20 (2/10)	<0.02
	Mean size (±SD) mm	11.7 (5.2)	11.0 (4.6)	NS
12 hrs	% with reactions	100 (10/10)	30 (3/10)	<0.02
	Mean size (±SD) mm	14.7 (6.7)	13.7 (5.8)	NS
24 hrs	% with reactions	100 (10/10)	50 (5/10)	<0.05
	Mean size (±SD) mm	19.6 (5.1)	16.8 (5.8)	NS
48 hrs	% with reactions	100 (10/10)	50 (5/10)	<0.05
	Mean size (±SD) mm	20.9 (5.7)	16.3 (5.1)	NS
72 hrs	% with reactions	100 (10/10)	50 (5/10)	<0.05
	Mean size (±SD) mm	17.9 (4.5)	15.4 (5.0)	NS

APPENDIX 2 : STUDY PROFORMA

THE SPECTRUM OF IMMUNITY IN TUBERCULOSIS

Date of test: 1.

Name: Hosp/Location Diet (delete)

Date of birth: Sex: Occupation: vegetarian/non-vegetarian

Address & Tel no:

Ethnic origin: Country of birth:

Migration history:

Date of migration to Britain:

Dates + duration of last 3 visits to country of ethnic origin:

Pregnant: Yes/No

Drug treatment - current/previous:

Treatment for previous TB + date:

BCG status + date:

Diagnosis: pulmonary / miliary / extrapulmonary (specify)

Sputum/CSF/pleural fluid/other : Microscopy -

Culture -

Severity of disease:

a. Systemic symptoms - wt loss, sweats, fever, haemoptysis, dyspnoea, other

b. Duration -

X-ray showing: Extent: 1. Nil 2. Less than 5 cm² 3. 5 cm² to less than 15 cm²

4. 15 cm² to [illegible] 5. Larger than [illegible]

Cavitation: Yes / No

Triceps skinfold:

Height:

Weight:

Mantoux : 0h [illegible] 24h 48h 72h 0h [illegible] 24h 48h 72h

1:10,000

1:1,000

Drug treatment + date commenced

Laboratory Tests:

Hb | Total WBC | Neutr | Lymphs | Mono
EOS | Baso | ESR | Sodium | Creat
Total prot | TIBC | Alk. Phos | ALT | Calcium | Phos

	Effector/Target ratio	Spontaneous	+IFN
NK assay: (^{51}Cr release)	40:1		
	20:1		
	10:1		

Lymphocyte proliferation assay (maximum ^{3}H-thymidine uptake, cpm):

Day	AB + TPA	PPD I	PPD II	PPD III	Cell conc.
					80,000/well
					40,000 "
3					20,000 "
					80,000 "
					40,000 "
6					20,000 "
					80,000 "
					40,000 "
					20,000 "
9					

Interferon Titres (log_{10} u/ml)	Unstimulated	Stimulated
IFN-α:		
IFN-γ (PHA, TPA):		
IFN-γ (PPD):		

Macrophage [illegible] (%): [illegible] -
[illegible] -

Lymphocyte Subpopulations (%): Total T T_H T_S T_H/T_S B

APPENDIX 3 : Details of laboratory methods

(a) Cell counting:

(i) Principle:

The standard Neubauer chamber is 0.1 mm deep and the area of each large corner square is 1.0 sq mm. With the coverslip properly positioned (i.e. showing Newton's rings) the volume enclosed by each large square is 0.1 x 0.1 - 0.1 cu mm. If average count per square is y, cell concentration - y/0.1 cu mm. Multiplied by dilution factor (z), cell concentration - zy/0.1 cu mm, or 10,000 yz/ml, since 1000 cu mm - 1 ml.

(ii) Method:

Cell suspension was mixed and 100 µl pipetted into a sterile Bijoux bottle containing 100 µl of 0.1% Trypan blue (x 2 dilution). The contents were thoroughly mixed. The mixture was dispensed with a Pasteur pipette into the Counting chamber of a Neubauer haemocytometer, and live cells (excluding dye) were counted in 25 large squares at three separate sites. The average count per 25 large squares was calculated. Cell concentration - dilution factor x cell count/25 large squares x 10,000/ml.

(b) Cell freezing:

The cell suspension was spun down and resuspended in 125 µl of RPMI 1640 plus 10% FCS in a freezer tube. 125 µl of 10% Dimethylsulphoxide, DMSO (BDH Chemicals, Poole) in RPMI 1640 plus 10% FCS were added, and within 5 mins, ampoules were immersed in glycerol at -30°C and left for 30 mins, then transferred straight into liquid nitrogen.

(c) Cell thawing:

20% FCS in RPMI 1640 was prepared. Ampoules (one at a time) were taken out of liquid nitrogen and waved intermittently in a water-bath at 37°C until the last ice crystal melted. 20% FCS was pipetted slowly dropwise, shaking between drops, up to 50 mls. The suspension was centrifuged at 1000 rpm for 10 mins, resuspended and washed twice more. This medium exchange removed DMSO, a cell poison that dehydrates cells.

(d) Thymidine dilution:

Tritiated thymidine from the Radioisotopes Division was supplied at a concentration of 48.4 µg/ml and specific activity of 1mCi/mmole, both of which were diluted before use. Using a 1 ml syringe, the bottle from Radioisotopes was emptied into a plastic universal bottle and rinsed out with 2 ml sterile normal saline. 0.07 ml cold thymidine was carefully added with a Hamilton dispenser which was then rinsed with 1 ml saline (total volume - 4 ml). A further 2.5 ml saline were added to bring the total volume to 6.5 ml. Using the Eppendorf pipette, 26 aliquots of 0.25 ml were dispensed and stored at 4°C.

(e) Monocyte migration assay:

Fixing and staining: Cellulose membranes (Sartorius) were washed with normal saline and immersed successively in:

Propan-2-ol (1) for 10 mins
Distilled water (to hydrate), until membranes sank
Harris haematoxylin for 20 mins
Distilled water in petri dish (shaken)
Propan-2-ol (2) for 10 mins
Propan-2-ol (3) for 10 mins, and
Cedar wood oil (kept dark) for 30 mins or longer, to clear.

Membranes were stored in cedar wood oil until examined.

(f) IFN-α assay:

Media: a) Blocking/diluting medium - phosphate buffered saline plus 0.5% bovine serum albumin and 0.05% sodium azide.

b) Washing medium - Blocking medium plus 0.05% Tween-20.

Prior to an assay the wells of each radioimmunoassay (RIA) tray were filled with blocking medium and left overnight at 4°C. The RIA trays were sucked out and 200 µl of each test sample were incubated in each well (in duplicate) with a polystyrene bead coated with sheep anti-IFN-α antibody (Celltech Ltd, Slough) overnight (18-20 hrs) at 4°C. Any IFN-α present in the test sample bound specifically to the bead, which was then washed three times

with washing medium. 200 µl of ^{125}I-monoclonal anti-IFN-α antibody (Celltech Ltd, Slough) were added to each well and further incubated with each bead overnight at 4°C. The radiolabelled monoclonal antibody bound to a second site on the IFN molecule. The IFN-α Standard (Celltech Ltd, Slough), calibrated against the NRC 69/19B International Reference Preparation (National Institute of Biological Standards and Control, London) was similarly detected and assayed in parallel. The beads were washed three times and counted in a Wallac CTL 300/1000 gamma counter. Readings from the standard were used to plot a dose-response curve of specific counts bound (= cpm minus non-specific binding) versus $\log_{10}$ IFN-α concentration. The titre of each test sample was determined by interpolation from this curve.

(g) IFN-γ assay:

Media: a) Growth medium - Eagle's Basal Medium (Eagle, 1959) buffered with sodium bicarbonate (13 mM) plus 15% newborn calf serum. Penicillin (100 u/ml), streptomycin (100 u/ml) and l-Glutamine (2 mM).

b) Maintenance medium - Eagle's Basal Medium buffered with sodium bicarbonate (13 mM) plus 2% newborn calf serum, penicillin (100 u/ml), streptomycin (100 u/ml) and l-Glutamine (2 mM).

HEP-2 cells were seeded onto plastic culture plates (Sterilin Ltd) at 4×10^4 in 0.2 ml growth medium per well, and incubated for two days at 37°C in a humidified 5% CO_2 atmosphere, until confluent monolayers formed. Three-fold dilutions of each test sample or the laboratory Reference IFN-γ Standard in maintenance medium were dispensed (150 µl) per well in duplicate. Wells designated 'virus' or 'cell' controls received medium only. The cultures were incubated overnight (18-20 hrs) at 37°C. Medium was decanted, and each well (except the 'cell' controls) was challenged with 200 plaque-forming units (pfu) of Semliki Forest Virus in 200 µl of maintenance medium. The plates were incubated for a further 48-72 hrs, by which time 'virus' control wells usually showed 90-100% cytopathic effect (cpe). Plates were fixed in 10% formol saline (15 mins) and stained with 0.1% crystal violet in 20% industrial methylated spirit (15-30 mins). IFN-γ titres were read by eye and microscopically as the reciprocal of the dilution at which approximately 50% of the cells were protected from viral cpe. The titres were adjusted to a Laboratory Reference Standard.

APPENDIX 4: Statistical methods

(a) Testing data for normal distribution (Shapiro and Francia, 1972):

A computer program, Minitab (supplied by the Statistics Department, 215 Pond Laboratory, The Pennsylvania State University, University Park, Pa 16802, USA) was used for all data analysis on the DEC 2060 mainframe computer at the Clinical Research Centre, Harrow. Data from various measurements were entered in columns against individual subjects in rows. To do a normal probability plot of the data in, say, column 5, normal scores of C5 were put in a new column C99, using the instruction NSCORES C5, PUT IN C99. C5 was then plotted against C99 using the command PLOT C5 vs C99. If the sample was from a normal population, the points in the plot would probably fall roughly in a straight line. If the sample was from a non-normal population, the plot would show a curvature. The "straightness" of the Probability plot was measured by the correlation coefficient of the points in the plot. The command CORRELATION OF C5 AND C99 measured straightness, and the powerful Shapiro-Francia test (1972) for normality was based on this correlation. A very high correlation was consistent with normality, whereas the hypothesis of normality was rejected if the correlation fell below the appropriate value in the table below (Ryan, Joiner and Ryan, 1981):

	alpha		
N	0.10	0.05	0.01
4	0.8951	0.8734	0.8318
5	0.9033	0.8804	0.8320
10	0.9347	0.9180	0.8804
15	0.9506	0.9383	0.9110
20	0.9600	0.9503	0.9290
25	0.9662	0.9582	0.9408
30	0.9707	0.9639	0.9490
40	0.9767	0.9715	0.9597
50	0.9807	0.9765	0.9664
		0.9799	0.9710
60	0.9835	0.9835	0.9757
75	0.9865		

(b) Transformations

The purpose of data transformation was to achieve approximate normality so that such data could be summarised with mean and standard deviation (SD), and parametric methods of statistical analysis (t-tests, regression, analysis of variance, etc) used. For every set of non-normal data in this study, base-ten logarithmic, square root, square and reciprocal transformations were tried in turn. The transformation that gave the highest correlation coefficient above the appropriate value in the table (above) was selected for use in analysis. For example, the following commands were given to determine the correlation coefficient for the log transformation:

LOGTEN C5, PUT IN C99

NSCORES C99, PUT IN C100

PLOT C99 vs C100

CORRELATE C99 AND C100

It was not possible to find a suitable transformation for serum sodium, which was therefore compared by the Wilcoxon's rank sum (non-parametric) test.

(c) Calculating confidence intervals

The variability of transformed data was expressed as a 95% confidence interval for the sample mean, defined as the interval which would include the true (but unknown) population mean in 95% of a long series of random samples from the population concerned. The 95% interval was calculated as mean $\pm t_{(n-1)(0.05)} \times SD/\sqrt{n}$, where $t_{(n-1)(0.05)}$ was read off the table of t-distribution for n-1 degrees of freedom and a probability of 0.05 for a two-tailed test.

APPENDIX 5: Publications and Scientific Meetings

Publications relating to work in this thesis:

Onwubalili JK and Scott CM. Natural killing and interferon-α production in tuberculosis. Antiviral Res (Abstr.), 1983;1(2):71.

Onwubalili JK and Scott CM. Natural killer cell activity in tuberculosis. Br J Dis Chest 1984 (in press).

Onwubalili JK, Scott CM and Robinson JA. Deficient immuno interferon production in tuberculosis. Clin Exp Immunol 1984 (in press).

Publications in other fields:

Onwubalili JK. Hysterical mutism: A case report. Niger Med J 1979;9:741-743.

Onwubalili JK. Overt diabetes mellitus without glycosuria in a patient with cutaneous ureterolleostomy. Br Med J 1982;284:1836-7.

Onwubalili JK. Sickle cell Anaemia: An explanation for the ancient myth of reincarnation in Nigeria. Lancet 1983;1:503-5.

Onwubalili JK. Oliguric ronal failure associated with Benoxaprofen treatment. Nephron 1983;35:279-80.

Onwubalili JK. Sickle Cell Disease and infection: A review. J Infect 1983;7:2-20.

Scott GM, Ward RJ, Wright DJ, Robinson JA, Onwubalili JK and Gauci CL. Effects of cloned interferon-α 2 in normal voluntoers: febrile reactions and changes in circulating corticosteroids and trace metals. Antimicrob Agents Chemother 1983;23:589-592.

Scientific Meetings:

The contents of this thesis have been presented, in part, by the author at the following scientific meetings:

1. Meeting of the Journal Club, Clinical Research Centre, Harrow, June 1982
2. Conference of the British Society for Immunology, London, November 1982.
3. The 2nd International TNO Meeting on the Biology of the Interferon System, Rotterdam, April 1983.
4. 'Tuberculosis Today': a postgraduate course for general practitioners, June 1983.