SOME IMMUNOLOGICAL PARAMETERS AS INDICES OF AGEING IN NIGERIANS

BY

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DEDICATION

Unto Him that commanded light out of darkness,

the great I AM,

I lift my voice in praise.

A B S T R A C T

This study involved 541 participants. It assessed the state of body defence mechanisms during ageing in 217 healthy Nigerians aged 6-95 years. A different group of 274 healthy subjects of ages 6 to 60 years were studied for mantoux test responses. In vitro cell-mediated immune response was analyzed by the Leucocyte migration inhibitory factor (L-MIF) test using concanavalin A, candida antigen, measles and BCG vaccines. Numerical estimates of B-lymphocytes, T-lymphocytes, helper T-cells and suppressor T-cells were determined by immunofluorescence assay. Percentage null cell was derived by difference between 100 and the sum of pan T cell and B cell. Also examined were specific humoral immune factors including the immunoglobulins(IgG, IgA, IgM, IgD) and the determination of isohaemagglutinins. Antibody response to meningococcal polysaccharide vaccination was examined in another group of 50 subjects aged 18-55 years.

Non-specific cellular immune capacity was studied by the nitroblue tetrazolium (NBT) test and total and differential leucocyte counting. Non-specific humoral immune factors studied include complement components (C3c, C4, factor B) and acute phase proteins (transferrin, albumin, C-reactive protein, alpha 2-

macroglobulin). Miscellaneous immunologic indices were also analyzed. These include the levels of circulating immune complexes (CIC), the prevalence of antinuclear antibodies (ANA) and rheumatoid factors (RF).

Mean L-MIF activity decreased with rising age. The mean tuberculin reaction diameter increased progressively between the ages of 6 and 40 years, and began to decrease in subjects whose ages were above 50 years.

The numbers of B-cells, T-cells, null cells, helper T-cells, and suppressor T-cells were the same in the different age groups. T-cell subpopulation did not correlate ($P > 0.20$) with migration inhibition. Of the humoral factors, only C4, CIC and the prevalence of ANA and RF show significant alteration (increasing values) with age.

The results indicate a progressive decline in cellular immune function with age. The possibility that CIC and auto-antibodies may be useful in indexing ageing, especially in community studies, exists.

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CERTIFICATION

I certify that this work was done by Mr. G.O. Oyeyinka in the Department of Chemical Pathology, University of Ibadan.

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ABBREVIATIONS

IL-2	Interleukin-2
IFN-Y	Interferon gamma
IL2-R	Interleukin 2- receptor
mRNA	Messenger ribonucleic acid
PHA	Phytohacmagglutinin
PG	Prostaglandin
NBT	Nitroblue tetrazolium
SRID	Single radial immunodiffusion
PEG	Polyethylene glycol
RF	Rheumatoid factor
DNA	Deoxyribonucleic acid
tRNA	Transfer ribonucleic acid
ADH	Anti-diuretic hormone
GH	Growth hormone
ATPase	Adenosine triphosphatase
IL-1	Interleukin-1
NZB	New zealand black
LPS	Lipopolysaccharide
Ca^{++}	Calcium ion
Con. A	Concanavalin A
IGF-I	Insulin-like growth factor I
cAMP	Cyclic adenosine monophosphate

EDTA	Ethylene diamine tetra-acetic acid
SES	Socio-economic status
RPMI	Rosewell Park Memorial Institute medium
FCS	Foetal calf serum
PBS	Phosphate buffered saline
L-MIF	Leucocyte migration inhibitory factor
BCG	Bacille Calmette Guerin
PPD	Purified protein derivative of tuberculin
TRD	Tuberculin reaction diameter
Bf	Factor B
C.v.	Coefficient of variation
IUIS	International Union of Immunological Societies
CRP	C-reactive protein
FITC	Fluorescein isothiocyanate
Ig.	Immunoglobulin
RIA	Radioimmunoassay
ELISA	Enzyme-linked immunosorbent assay
CIC	Circulating immune complexes
O.D.	Optical density
SRBC	Sheep red blood cell
ANA	Antinuclear antibody
Ag.	Antigen
LMIT	Leucocyte migration inhibition test

INTRODUCTION AND AIM

It was first demonstrated in mice that the ability to mount an antibody response declines progressively with age after maturity. The decline was found to be due primarily to changes in tho tissues of the immune system and secondarily to changes in the systemic milieu (Makinodan and Peterson, 1962). Since then, there has been considerable evidence indicating that alterations in immune functions with age may be characteristic of all mammals (Makinodan, 1980). Old people are more susceptible to certain infections than the general population, and various intrinsic and extrinsic factors contribute to their increased susceptibility (Makinodan et al., 1984).

Immune dysfunction in the aged represents an intrinsic factor which plays a prominent role in the impaired resistance against infection. Epidemiologic studies have revealed that ageing human populations are deficient in their ability to generate adequate amounts of neutralizing antibodies in response to viral vaccination (Howells et al., 1975; Phair et al., 1978). Also, they possess significantly lower levels of anti-

bacterial antibodies in their blood (Batory and Redei, 1978).

Various immunologic indices have been measured to assess both humoral and cell-mediated immune responses. They revealed that with age certain activities decrease. Others show no change, or increase. Among those indices showing a decrease are cell-mediated immunity against viruses (Pazmino and Yunas, 1973), protozoa (Gardner and Remington, 1978) and intracellular bacteria (Patel, 1981). Others include primary antibody response (Makinodan and Peterson, 1962), production of high affinity antibodies (Goidl et al., 1976) and ability to respond to limiting doses of antigen (Price and Makinodan, 1972a). Indices showing an increase include the production of auto-antibodies (Cammarata et al., 1967), resistance to tolerance induction (Fujiwara and Cinader, 1974) and susceptibility to benign monoclonal gammopathy (Axelsson et al., 1966).

There is age-associated drop in the functional capability to produce interleukin 2 (IL 2) by peripheral blood lymphocytes from elderly humans. Interferon gamma (IFN-Y) production by these cells was found unaltered and no significant correlation between the level of IL 2 and

IFN-Y could be demonstrated (Shu-lin et al., 1986). This implies the existence of an alternative way of IFN-Y production which does not require the induction by IL 2. Exogenous IL 2 is known to be incapable of fully restoring the low proliferative response of cells from aged animals or elderly humans. There may be a defect in membrane IL 2R expression as there is evidence of decreased IL 2 and IL 2R MRNA expression in phytohaemagglutinin (PHA)-stimulated cells from old individuals (Nagel et al., 1988). Kinetic studies of PHA-induced proliferation of human lymphocytes indicate that after a delayed PHA-induced activation, the lymphocytes from aged donors have a near-normal ability to proliferate in vitro (Bonomo et al., 1986). Shedding of prostaglandin (PG) receptors by pre-incubation prevents PG-mediated suppression of mitogen induced lymphocyte response (Batory and Onody, 1986). It is possible that PG-dependent suppression is the main cause of reduced mitogen responses in advanced age.

The polymorphic effects of ageing on the immune system underscores the complexity of the mechanisms responsible for age-induced changes, and the magnitude of the task of relating individual immunologic indices to

susceptibility to infection. Most studies so far have focused attention on a few or related indices. This study has been designed to bring several immunologic indices, representing the major arms and sub-units of the immune system, to play in the assessment of altered parameters during ageing. It is envisaged that susceptibility to infection can be reduced in old individuals by modulating their immunologic responsiveness. The information on the state of immunologic parameters in the aged African is scarce. This study assesses the effector functional arm of cell-mediated immunity.

Ageing has been found to involve different patterns of change in immunoregulatory T-cell subsets in different strains of mice, depending on their genetic constitution (Clark et al., 1985). This study also aims at ascertaining the possibility of using the level of immune responsiveness as an index of the ageing process. Information gathered from this study will be important for public education on what period of life individuals should minimise activities that bring stress (i.e. the age that immunological depression sets in).

It is the aim of this study to assess immunological

parameters in individuals aged 6-95 years. Generally, the study consists of analyses of delayed hypersensitivity skin test responses and blood samples from Nigerians in each of the following age groups: 6-25 years, 26-45 years, 46-65 years and > 65 years.

Immunological parameters to be assessed include the following:

A. Non-specific cellular immune responses

(i) Total and differential white blood cell counts indicating neutrophil number and proportion

(ii) Assay of polymorphonuclear neutrophil function using Nitroblue tetrazolium (NBT) dye reduction test.

B. Specific cellular immune responses

(i) T-lymphocyte count by immunofluorescence using monoclonal antibodies.

(ii) Helper T-cell and suppressor T-cell enumeration by immunofluorescent staining with monoclonal antibodies.

(iii) Leucocyte migration inhibitory factor assay.

(iv) Passive cutaneous delayed hypersensitivity test.

C. Non-specific humoral immune responses

(i) Transferrin measurement by single

radial immunodiffusion (SRID).

(ii) Assay of complement components including C3, C4 and Factor B.

(iii) Analysis of two of the acute phase reactants: C-reactive protein and alpha 2-macroglobulin.

D. Specific humoral immune responses

(i) B-lymphocyte count by immunofluorescence.

(ii) Quantitation of the immunoglobulins IgG, IgA, IgM and IgD by single radial immunodiffusion.

(iii) Determination of iso-haemagglutinins.

(iv) Specific antibody responses to meningococcal polysaccharide vaccination.

E. Miscellaneous immunological indices

(i) Serum total protein and albumin by colourimetric assay.

(ii) Assay of circulating immune complexes by polyethylene glycol (PEG) precipitation.

(iii) Assessment of the incidence of rheumatoid factor (RF) positivity in sera by the Latex-agglutination method, and also by the Rose-Waaler test.

(iv) Assessment of the prevalence of antinuclear antibodies.

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(iii) Hepatitis-B surface antigen detection in serum by latex agglutination.

CHAPTER ONE

LITERATURE REVIEW

Immunity is the ability of an organism to resist disease. Ageing can be defined as a time-dependent process whereby one's body can no longer cope with environmental stress and change as easily as it once could. Hence, loss of physiological adaptability is one of the hallmarks of ageing. The immuno system fails, while the incidence of autoimmune diseases increases, with age (Butler, 1977). The increase in the incidence of autoimmune diseases may play a part in a wide range of chronic diseases from rheumatoid arthritis to senile dementia associated with old age. If the immune and other protective systems of the body were able to maintain themselves over the course of time with no age related degradation, the everyday loss of energy and vitality that occurs in the lives of older people as a consequence of viruses, arthritis and other debilitating circumstances would be greatly lessened. The goals of

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biomedical gerontology are to extend the productive years of life at the expense of the unproductive years of life, and to enable one to age graciously with a minimum of mental and physical disabilities (Makinodan, 1977).

It is well known that each species has a finite and unique life span: e.g a mayfly has a life span of about a day, a mouse about 3 years, a dog about 20 years, a horse about 40 years, and a human about 110 years. The average life span is generally significantly shorter than the maximum life span, and variation between individuals within a species is large (Makinodan, 1977). Those observations indicate that life span is genetically regulated and the difference between the maximum and average life span in a species reflects the influence of environmental factors to a great extent. Findings from studies of monozygotic and dizygotic progenies of parents with long and those with short life spans support genetic regulation. Kallman (1961) found the intrapair life span difference smaller in monozygotic than dizygotic twins and that the life expectancy of progenies

of parents with a longer life span is more prolonged than that of progenies of parents with a short life span. The cause of death was about twice as similar in monozygotic as in dizygotic pairs.

Studies of expectations of life span in the United States (Golenpaul, 1973) showed that the survival curve is becoming more rectangular (or box-like) in shape (Figure 1). This implies that the average life span is increasing significantly but the maximum life span is not. This has been attributed to the control of deleterious environmental factors through effective dietary, hygienic and vaccination programmes and through the use of antibiotics since the late 1940s. If the box-like trend continues, it is possible that in the near future most United States residents will have life spans approaching the genetic limit for man.

A United States Bureau of census report (Siegel and O'Leary, 1973) reveals that the maximum life span cannot be extended significantly by controlling environmental factors. According to this report, if the cause of death through malignant neoplasms is eliminated today, a child born tomorrow will have an increase in life expectancy of only 2.3 years and an adult 65 years old tomorrow will

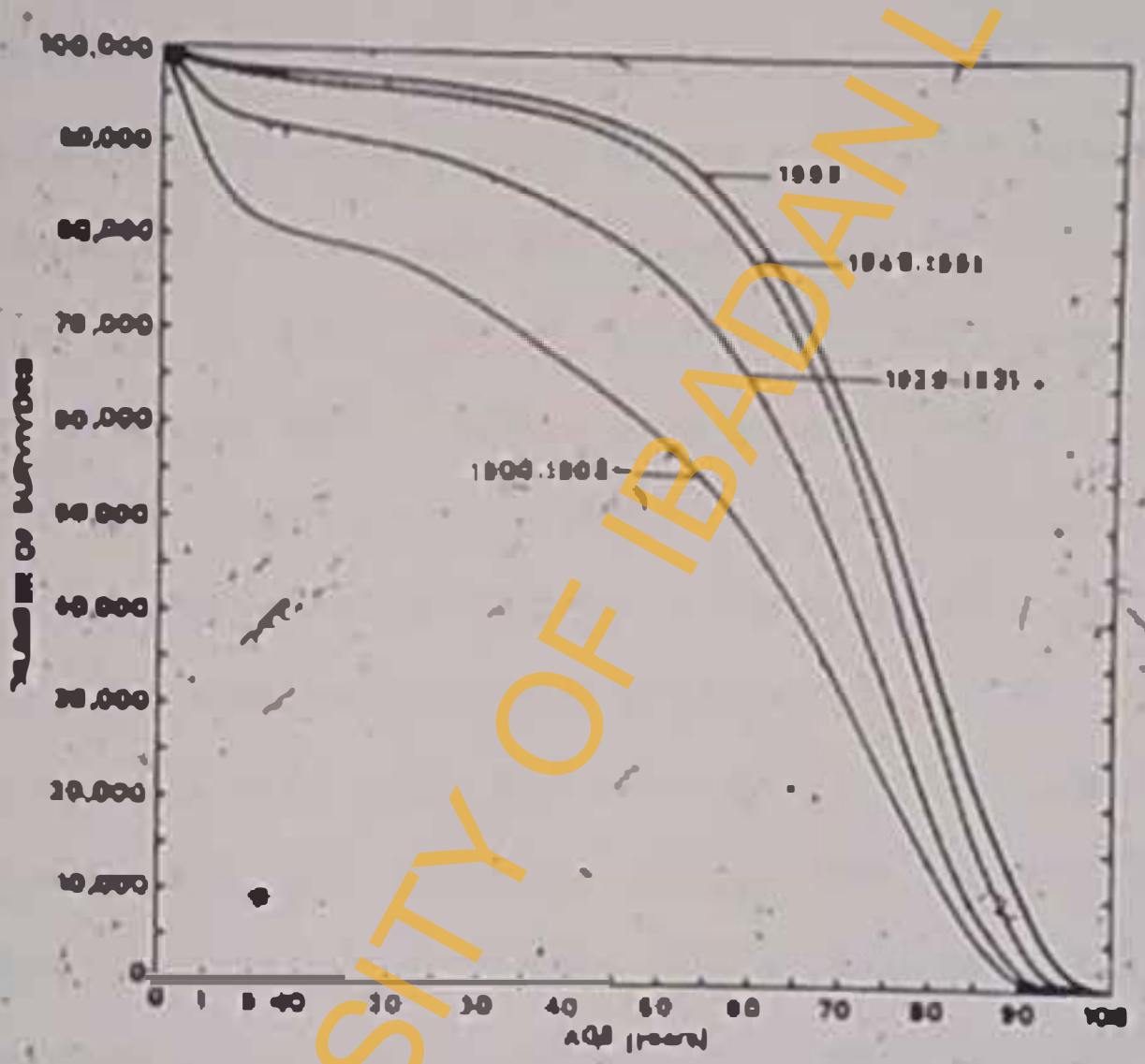

Figure 1. Expectation of life span in the United States, 1900-1980 (Golenpaul, 1973).

have an increase of only 1.2 years. Moreover, if all four major causes of death of the aged (i.e cardiovascular-renal diseases, heart diseases, vascular diseases affecting the central nervous system and malignant neoplasms) can be eliminated today, there will be only a 20-year gain in life expectancy of babies born tomorrow.

Physiological functions in general decline with age in a linear fashion (Makinodan, 1977). A fundamental question that has been asked over the years is whether the decline in various functions is initiated by the decline in function of only a few cell types or tissues or whether each tissue senesces independently of other tissues. Actuarial data suggest that ageing of individuals is caused by a senescence time clock built into a few cell types (Makinodan, 1977). Krohn (1962) found in mice that the skin had a life span longer than the mouse from which it originated. He had transplanted the skin of old mice into young, healthy mice in a serial manner. This means that skin ages in-situ because of factors extrinsic to it. Comparable results have been reported subsequently with several other tissues including bone and prostate tissues (Franks, 1970).

However, a limited in-vivo life span of certain

tissues (Siminovitch et al., 1964; Cudkowicz et al., 1964; Daniels et al., 1975) suggests that some tissues age because of changes intrinsic to them. Harrison's findings (1975) with haematopoietic stem cells of young and old mice, indicate that in-vivo transfer life span of a tissue is due to the number of traumatic experiences a tissue undergoes during its transfer handling rather than to the in-situ age of the tissue. In an approach to resolve this issue Hayflick (1965) assessed senescence of a homogeneous cell population in a defined in-vitro culture condition. He found that the cultures of human fibroblasts undergo about 50 doubling passages before they die (i.e on the average, one fibroblast can generate 10^{13} fibroblasts or 1 metric ton of fibroblasts).

To find out if the death of passaged fibroblast is due to a time clock built into them, fibroblasts that had previously undergone x number of passages were mixed with marker fibroblasts that had previously undergone y number of passages. The number of passages each type was still capable of undergoing was determined and found to be (50 - x) and (50 - y) more passages respectively. Thus, the in-vitro proliferative life span of human fibroblasts may be governed by a time clock built into

them. The question that arises is whether the time clock is in the nucleus or the cytoplasm and whether activation of the clock is genetic or stochastic. Recently, Harley et al. (1990) have shown that the amount and length of DNA in human fibroblast telomeres (termini of DNA helix) decrease as a function of serial passage during ageing in-vitro. It is not known whether this loss of DNA has a causal role in senescence.

Many physiologic systems have been the focus of biomedical gerontologic research. Of all the systems being examined, the immune system is perhaps the most attractive from both biologic and clinical points of view (Makinodan, 1977). The reasons are compelling:

a) The immune system, which is intimately involved in adaptation of the body to environmental stress and change, declines in its efficiency in performing certain functions with age.

b) Associated with the decline is the rise in susceptibility to viral and fungal infections, cancer, autoimmune and immune-complex diseases which can interfere with many physiologic functions of the body.

c) Probably more is known about differentiation,

ontogenetic and phylogenetic processes of the immune system at the cellular, genetic and molecular levels than any other system.

d) The immune system is amenable to precise cellular and molecular analysis and therefore offers great promise for successful manipulation.

e) There is a reasonable chance that a delay , reversal or decrease in the rate of decline in normal immune function may delay the onset and lessen the severity of diseases of ageing.

1.1 THEORIES ON AGEING

There are many theories of ageing, some conflicting and some mutually supporting. These theories appear to deal with different aspects of the same general event and fall into two general groups. One view is termed "fundamentalist", "intrinsic" or "controlled theory". This holds that ageing is an orderly genetically programmed event which is the consequence of differentiation, growth and maturation (Kanungo, 1976). The other general view is termed "epiphenomenalist", "extrinsic" or "random" theory. According to this theory, ageing

results from accumulation of random errors (Orgel, 1963). Some investigators place an intrinsic ageing chronometer in every cell, and others place it in a single centre - usually some-where in the brain (Moment, 1978). Efforts to elucidate the extrinsic theory have shown that once an error is made anywhere in the DNA-protein synthesizing apparatus by any agent, it could be magnified, producing faulty templates (Moment 1978). This, in turn, would serve as faulty models for the production of faulty enzymes, resulting in more faulty templates - a process leading to a gradually accelerating cumulative increase of errors until the final "error catastrophe" (death) occurs. Orgel (1970) has pointed out that "error catastrophe" is not inevitable with time but depends on a number of contingencies which may or may not occur.

The genetic explanation is generally favoured although definitive evidence for or against either type of theory is lacking (Makinodan, 1977). Thus, ageing can initiate at the transcriptional level , where it can be manifested as a mutation, DNA

deletion or macromolecular cross-linking of DNA. It can also initiate at the translational level where it can be manifested by altered RNA polymerase, altered tRNA and altered tRNA synthetase. Also, it can initiate at the post-translational level, where it can be manifested by stochastic alteration of certain vital, slowly-turning-over macromolecules such as enzymes essential for protein synthesis and DNA repair. Genetic heterogeneity combined with long life spans greatly limits the possibilities to test for genetic effects on patterns of human ageing. However, certain special kinds of premature human ageing (progeria), such as Werner's and Cockayne's syndromes, are generally accepted as having genetic causes. Individuals with these diseases fail to grow properly, become bald and wrinkled in their early teens, and almost invariably die before the age of 20, with extensive circulatory difficulties associated with lipid deposits in the blood vessels (Russell, 1978).

The many theories of ageing that have been put forward, include the following:

i. The free radical theory, which suggests the occurrence of age-associated interference with oxidative processes of proteins, which leads to molecular malfunction, misinformation or mutations (Harman, 1968; Sohal and Orr. 1992)

ii. The somatic mutation theory, which postulates that mutations in somatic cell DNA result in physiologic dysfunctions and deficiencies, ultimately leading to death (Curtis, 1971)

iii. The "accumulation of errors" theory of ageing, which states that age-associated progressive accumulation of errors of transcription and or translation results in accumulation of abnormalities of cellular information and deterioration of nuclear cytoplasmic functional controls (Price and Makinodan, 1973).

iv. The cross-linkage theory of ageing, which postulates that ageing is caused by the progressive cross-linkage of informational molecules and other molecules essential to life such as nucleic acids, nucleoproteins, enzymes, and structural proteins e.g. collagen

(Alexander, 1967).

v. The chalone theory of aging, which proposes that ageing is related to the appearance of specific molecules or chalones that inhibit essential processes of the central nervous system (CNS) and other organs (Bullough, 1971).

vi. The CNS - hormonal theory of ageing, which states that age-related changes are central nervous system-based, hormonally controlled and programmed, and result in progressive decline of mechanisms governing the rate of vital processes and corrective adjustments following cellular and environmental perturbation (Bellamy, 1967; Makinodan, 1973).

In addition to the foregoing list, other theories of ageing have been proposed. For instance, Walford (1974) proposed that conditions which prolong life must be based on those which influence either endocrine function or the immune system. He demonstrated that the life span of fish whose body temperature had been lowered to 15°C was markedly prolonged as compared to that of controls kept at 20°C. He argued that this finding may be a function

of the well-known temperature depressing influence on immune functions seen in the poikilotherms (Hildemann and Cooper, 1963). Even though the prolongation of life by temperature depression was accompanied by measurable changes in collagen reflecting a deceleration of the ageing process, Walford (1974) argues that the predominant influence on life span could be pre-eminently an immunological one.

In a similar fashion, Walford et al (1974) showed that calorie restriction, which decreases both cellular and humoral immune functions early in life, also results in preservation of immunological function late in life. By contrast, well-fed mice which showed vigorous immunological function early in life were characterised by early decline of immunologic functions and earlier death late in life. It is of interest that women, on the average, live about 8 years longer than men, who have a basal metabolic rate about 6 percent higher than women and more red blood cells per millilitre of blood (Moment, 1978).

1:2 DEVELOPMENTAL ASPECTS AND LIFE SPAN

Ageing and death of individual cells occur at all stages of development. Also, organs involute according

to a specific timetable. For example, the placenta involutes during the gestational period, the thymus during childhood and the ovary after five decades in normal females. A major distinction between the processes of development and ageing is that during ageing, no proteins, matrices, or organs with essentially new structures or functions arise, and there is a progressive decline of function (Goldstein, 1971).

Mammalian cells and tissues normally follow a defined schedule of growth. During embryo-genesis, all organs increase in size by cell division. By the time of adolescence, the growth of virtually all organs ceases as the somatic proportions of adulthood are attained. Thereafter, in general, much less mitotic activity is required to maintain the steady state. Thus, nervous tissue and muscle cells become virtually incapable of all mitotic activity during early adulthood. Fibroblasts, hepatic cells, renal tubular cells, and bone cells turn over slowly under normal conditions and regenerate less rapidly in older animals. Gastrointestinal and haematopoietic cells divide at a constant rate through adult life except for a tendency to decline under senescence (Post and Hoffman, 1968; Goldstein, 1971).

Burnet (1973) has suggested that the thymus may act as a biological clock which is genetically programmed to operate at a rate consistent with the optimal life time of the species.

Based on actuarial statistics in Europe and North America, it is known that about 2 percent of the population die before 1 year of age, 5 percent before 40 years of age, 15 percent before 60 years of age, 65 percent before 80 years of age, 90 percent before 90 years of age, and very few individuals survive the 100-year mark (Makinodan, 1977). At least 40 percent of all infant mortality is believed to result from genetic factors, while congenital malformations are the second leading cause of death of children under 1 year of age (Childs, 1975). Although it is difficult to assign specific genetic defects as a cause of death in the other age categories, about 20 percent of the general population have genetic defects which are known to shorten life span (Scriver et al., 1973; Goldstein, 1973; Ostrander et al., 1974). This defect may contribute significantly to the death of individuals below 60-70 years of age (Yunis et al., 1977). For example diabetes, which is one of the most common diseases in the general

adult population , may be considered. Its prevalence reaches 2 percent among men below 40 years of age, 9 percent before 60 years of age, and 11 percent before 70 years of age, (Ostrander et al., 1974). The significance of diabetes in ageing studies comes from its high frequency, the fact that it shortens life span significantly (Garcia et al., 1974), and that it is one of the most common and significant denominators of the final decline and death of long-lived individuals (Yunis et al, 1977). The familial nature of diabetes has long been known.

1.3 HORMONES AND THE AGEING LYMPHOID SYSTEM

The inevitability of hormone mediated senescence in the female reproductive system long before the death of the organism has long focused attention on the possibility that hormones mediate the ageing process. That ageing might reflect, at least in part, deficiency of hormone action raised the attractive prospect of the postponement of ageing with hormone replacement therapy. This prospect in large measure explains the inordinate amount of attention which hormonal factors have periodically received in attempts to account for senescence. It also explains the blemishes on nineteenth

and early twentieth century medical history of attempts to graft into old men (including some scientists) the gonads of goats and other animals to effect "rejuvenation" (Davies, 1978). Davies (1978) has listed conclusions which may be made from the many observations which attempt to relate function of the endocrine glands to ageing as follows:

i. there is no satisfactory evidence which substantiates involvement of endocrine gland in the process of ageing.

ii. ageing is characterized by normal circulating levels of thyroid hormone and cortisol; and decreased secretion of these substances in response to decreased degradation of hormones in peripheral tissues.

iii. endocrine tissue responsiveness to trophic hormones and stress is intact in the cases of the adrenal cortex and thyroid gland.

iv. the menopause is a hormone-mediated event which chronicles but does not regulate ageing, and appears to be initiated in the ovary.

v. the ovary is the only endocrine gland whose functional capacity predictably declines with

normal ageing. Androgen production by the testis tends to fall with age but wide inter-individual variations does not allow a description of decreased testosterone secretion as an inevitable consequence of ageing.

vi. for certain polypeptide hormones such as parathyroid hormone and prolactin, no impact of ageing has been consistently described. Anti-diurotic hormone (ADH) secretion is intact in older individuals, but tubular response to ADH may be impaired.

vii. available data regarding neuroregulation of the endocrine axes imply that such input may be decreased with age.

1.3.1 Growth hormone and the lymphoid system: The relevance of growth hormone (GH) for the ontogenetic development of the lymphoid system and primarily of thymus functions has been demonstrated from different experimental models. Thymus-dependent immunodeficiencies have been found in mice treated with anti-pituitary antisera (Pierpaoli and Sorkin, 1969) as well as in congenitally hypopituitary Snell dwarf mice (Fabris et al., 1970; Fabris et al., 1971a). The dwarf mice

immunodeficiency develops after weaning and is characterized by hypoplasia of the thymus-dependent areas, by impaired transplantation immunity and by slightly reduced humoral immune responses to thymus dependent antigens (Fabris et al., 1971a). Serum immunoglobulin levels are within normal range (Wilkinson et al., 1970). These findings have been confirmed in hypopituitary dwarf mice of the Ames (df) strain (Duquesnoy and Good, 1971; Duquesnoy, 1975).

The immunological deficiency of dwarf mice may be corrected by treating them daily for 30 days with bovine growth hormone, provided the thymus was not previously removed (Fabris et al., 1971b; Fabris et al., 1972). Growth hormone may influence DNA synthesis of thymocytes (Pandian and Talwar, 1971) especially as membrane receptors for GH are present on thymocytes (Arrembrecht, 1974). The GH requirements of the thymus dependent system are not limited to developmental stages, but last during the whole life of the animal, as shown by the deficient immunological recovery from x- irradiation in hypophysectomized adult rats (Duquesnoy et al., 1969). Also, there is increased graft versus host reaction mounted by adult spleen cells when injected into GH-

eated hybrid F_1 recipients (Pierpaoli et al., 1970).

.3.2 Insulin and the lymphoid system: Insulin receptors re present on the membrane of human circulating ymphocytes (Archer et al., 1973) and of human lymphoid ines (Gavin et al., 1974). The concentration of insulin eceptors on peripheral lymphocytes increases during lastic transformation (Krug et al., 1972). The binding f insulin on lymphocytes may have a biological ignificance since an insulin-dependent stimulation of membrane adenosino triphosphatase (ATPase) activity and glucose uptake has been observed (Hadden et al., 1972). The immunological deficiencies observed in insulin-deprived animals are similar to those shown by hypopituitary animals (Fabris, 1977). Since insulin and GH are physiologically linked by different direct and indirect relationships, the observed similarity may not be causal and may reflect a common underlying mechanism.

Advancing age is characterized by decreased insulin release in response to oral glucose challenge. Basal insulin secretion, turnover and tissue responsiveness are not influenced by normal ageing (Davies, 1978).

1.3.3. Thyroxine and the lymphoid system: The removal of the thyroid gland induces hypotrophy of the lymphoid system (Lundin, 1958). Administration of exogenous thyroxine to otherwise normal animals results in enlargemont of both central and peripheral lymphoid organs; and in increased outflow of lymphocytes from the

thymus (Ernstrom and Larsson, 1966). Functionally, thyroxine deprived animals show a generalized immunodepression (Pierpaoli et al, 1970; Fabris, 1973). Both antibody synthesis and cell-mediated immunity are strongly decreased in mice and rats with hypothyroidism. Such deficiency is fully restored by daily injection with exogenous thyroxine (Fabris, 1973). Neonatal or adult thyroidectomy in rats show that thyroxine is needed during the whole life of the animal in order to maintain the efficiency of the immune system, although the requirement seems to be higher during the ontogenetic development (Fabris, 1973).

1.3.4 Corticosteroids and the lymphoid system:

Corticosteroids, and particularly those of the cortisol type can suppress antibody response (Eliott and Sinclair, 1968) as well as cell-mediated immunity (Gunn et al., 1970). Excessive proliferation of lymphatic tissues, including the thymus, after adrenalectomy occurs in mice and rats (Dougherty, 1952; Ambrose, 1964; Gunn et al., 1970). These observations would imply that even at physiological levels, adrenal cortical hormones suppress the immunological responses or at least antagonize the proliferative stimuli exerted by other hormones.

1.3.5 Sex hormones and the lymphoid system: The greater capacity of the female compared to the male in terms of immune responsiveness has been documented in various mammalian species including man (Rowley and Mackay, 1969; Terres et al., 1968). Due to the complexity of the hormonal feedback mechanisms related to sex, it is difficult to define which one among the sex hormones is directly involved in immunopotentiation in the female. Both pituitary gonadotrophins and target gland hormones may well mediate this effect.

Other hormones such as vasopressin, parathyroid hormone (Whitfield, 1970) and epinephrine (Fabris, 1977) induce proliferation of thymocytes.

The observations that the wasting disease following thymectomy is accompanied by a number of non-immunological consequences indicate that the lymphoid system may affect some ageing processes. Such consequences include the degranulation of CH-producing cells in the adenohypophysis (Bianchi et al., 1970), sexual retardation (Nishizuka and Sakakura, 1969); impairment of liver regeneration and general body growth defects (Fabris, 1977). Moreover, athymic Nu/Nu mice

show alterations of (1) hormonal levels (Pantalouris, 1973; Pierpaoli and Sorkin, 1972), (2) liver enzyme activity and the ratio of soluble/insoluble collagen (Panta-louris, 1973), and (3) sexual maturation (Besedowski and Sorkin, 1974). These observations suggest that the lymphoid system and particularly the thymus may be linked to the general hormonal homeostatic mechanisms and directly, or through them, may control the function of other body tissues. The relevance of such a relationship for some age-related processes has been suggested by the observation that both hypopituitary dwarf and nude mice (Pantalouris, 1973) are affected by early ageing syndrome.

Both hormonal and immunological recoveries are required in order to prevent the early appearance of age-related symptoms. The achievement and the maintenance of the optimal ratio among functionally distinct subpopulations of lymphocytes, as well as their efficiency throughout life, might well depend on the adequacy of both the immune and the endocrine systems.

1.4 MOLECULAR BIOLOGY OF AGEING

Transplantation of young nuclei into old cells rejuvenates the old cells, whereas cells with old nuclei

and young cytoplasm behave like old cells (Brash and Hart, 1978). Thus, determination of senescence lies in the nucleus rather than the cytoplasm. This fact militates against cytoplasmic proteins, lysosomes, ageing pigments, and the cell's outer membrane as determinants of senescence, and points in favour of DNA. If strand-break or excision repair after damage is complete, the integrity of the DNA is restored. However, DNA damage accumulates with age in post-mitotic tissue indicating that repair is not always complete. This accumulation of DNA damage does not seem to be due to a decline in the function of repair systems with age as a decline occurs in these repair systems only in very old cultures (Brash and Hart, 1978).

Immunological methods suggest that proteins present only in foetal life begin to reappear with age, indicating erroneous gene derepression. Derepression of genes which control cell division may be the cause of the age-related proliferative diseases, cancer and arteriosclerosis, when they occur in post-mitotic tissues (Brash and Hart, 1978). Derepression of histocompatibility antigens could result in autoimmune disease.

Elastin, an extracelluar protein responsible for the elasticity of tissues such as blood vessels and lung tissues, forms cross-links between its own molecules with age and becomes less flexible. It may thus contribute to the increasing rigidity of these tissues and to wrinkling of skin. Calcium precipitates onto old elastin, a phenomenon reminiscent of arteriosclerosis.

Very few collagen molecules turn over. Therefore, damaged collagen molecules will not be replaced. Collagen becomes stiffer with age as a result of cross-links of several types. Stiffness of collagen containing tissues may restrict movable tissues such as lung, and may also interfere with tissue perfusion. While these phenomena do occur with age, the role of collagen in causing them is not clear. Cross-links in collagen are believed to arise not from mutation but from direct post synthetic modification by reactive molecules such as free radicals and aldehydes (Brash and Hart, 1978). Thus, collagen ageing and elastin ageing, and the pathologies that may result, may be a significant senescence pathway which is separate from the DNA-damage pathway.

The principal senescence pathways at the molecular

level seem likely to be:

i. DNA damage, with two branches - somatic mutation and gene derepression or repression;

ii. Collagen and elastin cross-linking; and perhaps

iii. Membrane lipid damage.

Evidence for the relative significance of these pathways is scanty. The collagen hypothesis rests on plausibility arguments as feeding of membrane stabilizers has been noted to result in extended life span (Brash and Hart, 1978).

1.5 THE THYMUS, OTHER LYMPHOID TISSUES AND AGEING

The thymus was an organ of mystery until when Miller (1961), Good et al. (1962) and Jankovic et al. (1962), through their neonatal thymectomy studies of experimental animals, revealed that it plays a major role in the development of the immune system. Osoba and Miller (1961) were then the first to present convincing evidence that a humoral thymic factor is responsible for T-cell maturation.

Age-related changes that occur in the thymus, which is the first organ to develop lymphoid characteristics, are particularly striking. The thymus reaches its

maximum weight early in life and thereafter, there is a rapid exponential regression (Schofield and Davies, 1978). This regression (Figure 2) affects mostly the thymic lymphatic mass, primarily as a result of cortical atrophy in both humans and animals beginning at the time of sexual maturity (Boyd, 1932; Santisteban, 1960; Good et al; 1964). Neonatal thymectomy causes severe impairment of immunological function and may cause "wasting disease" whose main features bear some similarity to normal ageing (Walford, 1969). In micro-anatomical terms thymic involution is characterized by a gross increase in both connective tissue and fats. Many diseases, especially if associated with starvation, cause exaggerated thymic involution (Schofield and Davies, 1978). The extent to which T-cells can mature is related to the degree of thymic involution. With advancing age, thymic tissues lose the ability to influence the following T-cell functions:

i. the influence on T-cells to home into T-cell dependent areas of lymph nodes (Hirokawa, 1977).

ii. the influence on splenic T-cells to respond mitogenically to T-cell-specific mitogens such as PHA, and allogeneic lymphocytes (Hirokawa, 1977).

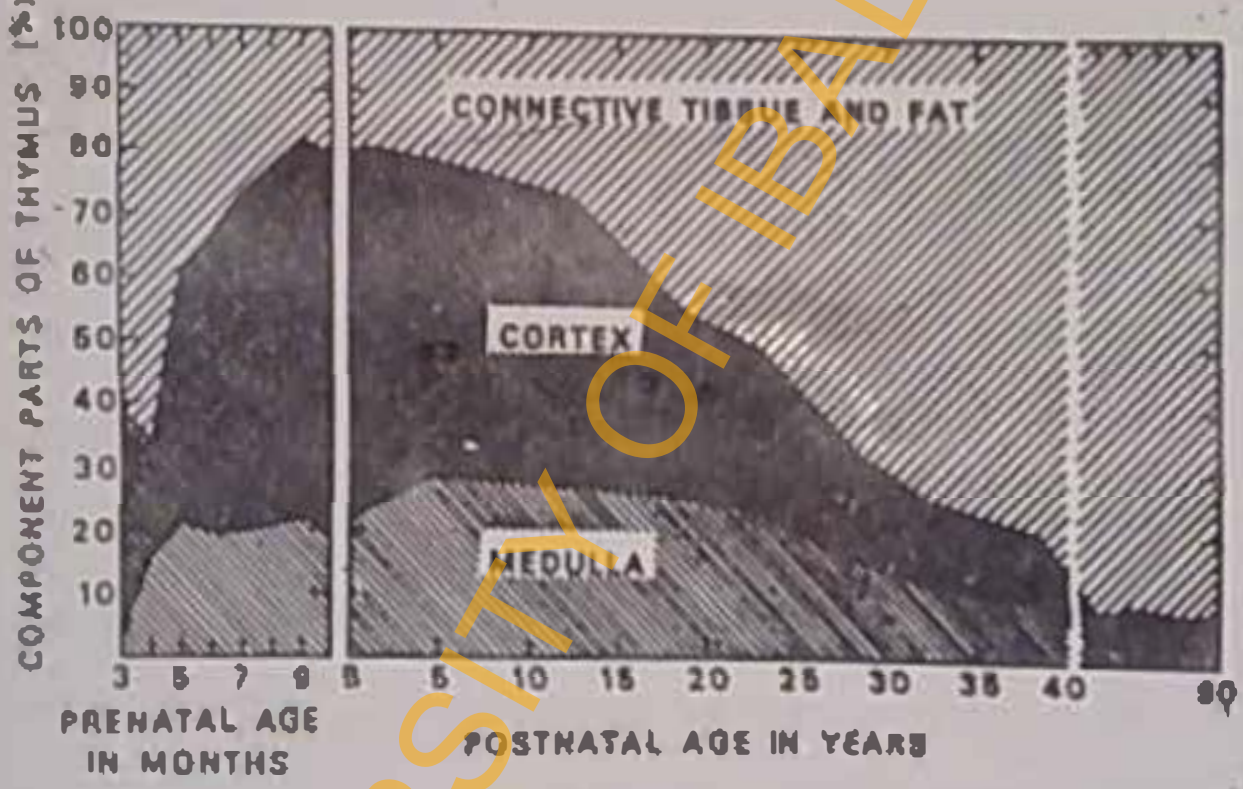

Figure 2. Age-related changes in the human thymus

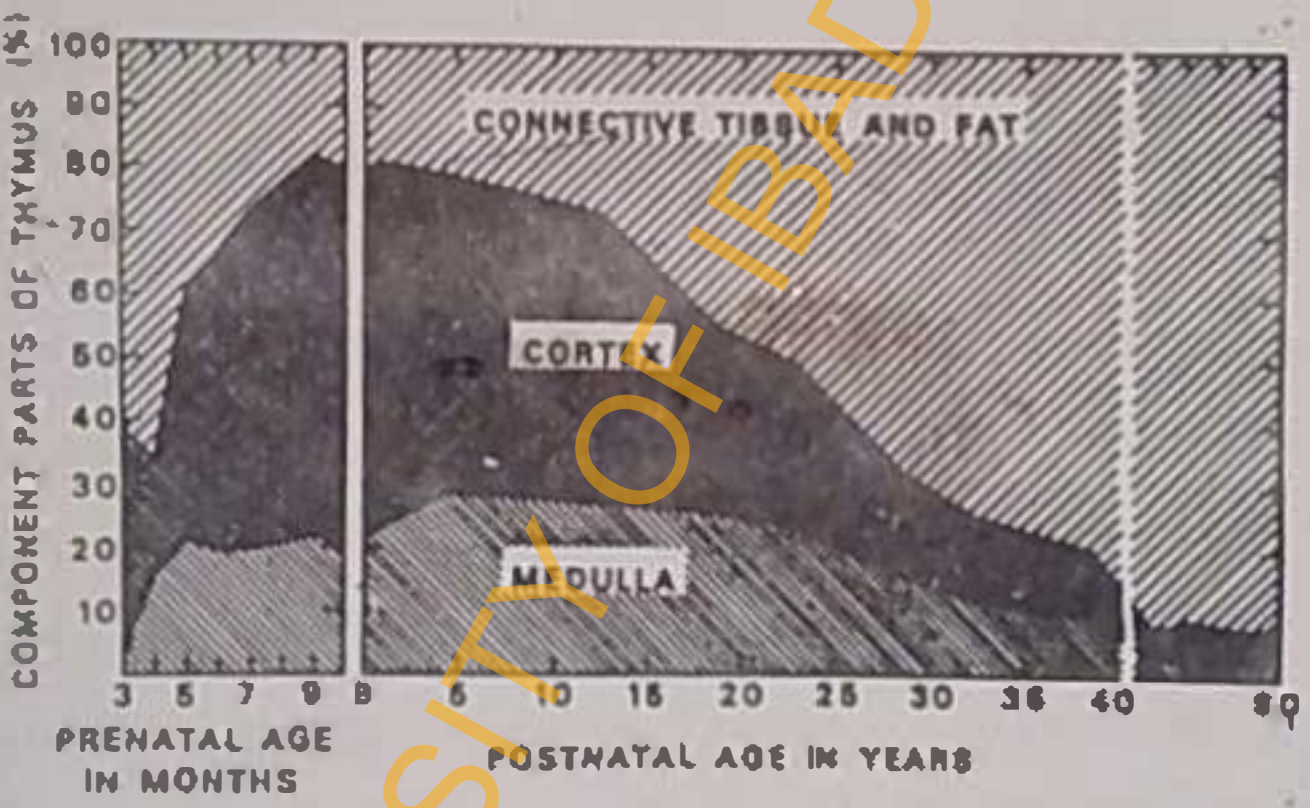

Figure 2. Age-related changes in the human thymus (Boyd, 1932).

iii. the influence on splenic T-cells to "help" B-cells in their response to sheep red blood cells (Hirokawa, 1977).

Whatever the mechanism in operation, a role of the thymus gland in the age-related immune defects is suggested by its morphological involution and the decline in serum thymic hormone levels which accompanies ageing (Goldstein et al., 1974).

Other tissues, which are part of the immune system, also show gross age-related changes. The maximum appendix weight is reached earlier in life in man than in other species and declines slowly throughout the remainder of the life span (Schofield and Davies, 1978). Tonsil weight also gradually declines throughout life (Schofield and Davies, 1978). The relative weights of lymph nodes decline slightly from a maximum in early life to young adulthood and then remain constant into old age (Schofield and Davies, 1978). Spleen weight gradually declines in man, with a slight presenile increase at approximately 40 to 50 years of age (Schofield and Davies, 1978). Although the size of lymph nodes and spleen remains the same or decreases slightly with age after adulthood in individuals without lymphatic neoplasia, the cellular composition of the tissues shift

so that there are diminished numbers of germinal centers, increasing numbers of plasma cells and macrophages, and an increase in the amount of connective tissue (Chino et al., 1971; Peter, 1973; Good and Yunis, 1974).

The first hint that various tissues of the immune system need not undergo changes with age at the same time comes from the observation that the thymus undergoes involution shortly after sexual maturity (Boyd, 1932), when the spleen and lymph nodes are approaching their plateau phase of growth (Makinodan et al., 1984). Subsequent studies revealed that age related changes in various humoral and cell-mediated immunologic activities of the spleen do not necessarily correlate with those of the peripheral lymph nodes (Stutman, 1974), which in turn do not correlate with those of gut-associated lymphoid tissues (Szewczuk and Campbell, 1980). The effects of ageing at the tissue levels is complex as the constitutive function of the thymus in synthesizing T-cell maturation factors undergoes drastic changes in a differential manner. Thus, in vivo studies (Hirokawa and Makinodan, 1975; Hirokawa and Sado, 1978; Hirokawa et al., 1982) revealed that the capacity to generate subpopulations of T-cells, which are needed to mount an allogeneic cytolytic response, decreases in an exponential manner with age. The decrease begins at

birth - long before the thymus reaches its maximum size (Hirokawa and Sado, 1978). On the other hand, the capacity to generate mitogen-responsive T-cells decreases only slightly with age, and the decrease begins at around mid-life, long after the involuting process commenced (Hirokawa and Makinodan, 1975).

1.6 CELLULAR FACTORS IN THE AGEING OF THE IMMUNE SYSTEM

The decline in normal immune functions with age may be due to changes in the cellular environment or to changes in the cells of the immune system or both. The results of cell transfer experiments (Albright and Makinodan, 1966; Price and Makinodan, 1972 a,b) reveal that changes both intrinsic and extrinsic to the cells affect the immune response. Only about 10 percent of the normal age-related decline can be attributed to changes in the cellular environment while 90 percent of the decline can be attributed to changes intrinsic to the old cells (Price and Makinodan, 1972 a, b). The responsible factor (s) in the cellular environment was shown to be systemic and non-cellular (Price and Makinodan, 1972 b; Goodman et al., 1972).

One of the characteristics of immunosenescence is the increase in variability of immune indices (Makinodan

et al., 1971). A decline in normal immune functions could be due to any of three types of cellular changes (Peter, 1971; Makinodan et al., 1976) viz:

i. an absolute decrease in cell number through death caused possibly by autoimmune cells.

ii. a relative decrease in cell number as a result of an increase in the number of "suppressor" cells.

iii. a decrease in functional efficiency possibly caused by somatic mutation.

1.6.1 Stem cells: In mouse bone marrow, which contains 90 percent of all stem cells, the total stem cell number remains constant with age (Coggle and Proukakis, 1970; Chen, 1971). Thus stem cells can replicate in situ throughout the natural life span of the mouse, unlike stem cells passaged in vivo whose ability to replicate is limited (Siminovitch et al., 1964; Lajtha and Schofield, 1971; Harrison, 1975). Furthermore stem cells do not lose their lympho-haematopoietic ability with age (Harrison and Doubleday, 1975). On the other hand, subtle changes have been detected with age. The rate of B cell formation seems to decline with age (Farrar et al., 1974) as does the ability to repair X-ray induced DNA damage (Chen, 1974). In addition, alterations in

certain kinetic parameters in spleen colony formation has been detected with age (Deitchman and Makinodan, 1975). There is also a decrease in the ability of bone marrow stem cells to home into the thymus (Tyan, 1977), and in the number of unipotent, pre-thymic precursor T-cells in the human and mouse bone marrow (Twomey et al., 1982). Associated with these decreases is an increase in the number of mitotically quiescent stem cells (Peterson et al., 1983).

1.6.2 Macrophages: It is reasonable to think that because macrophages confront antigens before the T and B cells, any defect in them could decrease immune functions without appreciable changes in the antigen-specific T and B cells. However, macrophages are not adversely affected by ageing in their handling of antigens during both the induction of immune responses and phagocytosis (Makinodan et al., 1977). For instance, the in vitro phagocytic activity of old mice was found equal to or better than that of young mice (Perkins and Makinodan, 1971). They also found the ability of antigen-laden macrophages of old mice to initiate primary and secondary antibody responses in vitro comparable to that of young mice. Heidrick and Makinodan (1973) reported that the capacity

of splenic macrophages and other adherent cells to cooperate with T-cells and B-cells in the initiation of antibody responses in vitro was unaffected by age. Finally, it has been observed that lysosomal enzyme activity increased rather than decrease with age in macrophages (Heidrick, 1972). However, the ability of murine thymic adherent cells to produce maturation factors declines sharply with age (Makinodan et al., 1984). The thymic adherent cells of newborn mice were found to synthesize both maturation factors and an inhibitory factor. Those of young adult mice synthesize only the maturation factors, and those of 20-month-old mice synthesize cnly inhibitory factors. Also, Chang et al. (1982) have shown that adherent cells have a role to play in the age related decline in murine interleukin 2 production. This would suggest that their capacity to synthesize IL-1, which is needed by T helper cells to produce IL-2, is affected. The capacity of macrophages of old mice to synthesize IL-1 has been reported to be reduced (Inamizu et al., 1983).

It has been suggested that the reason for reduced antigen processing is the failure of antigens to localize in the follicles of lymphoid tissues of antigen-stimulated old mice (Metcalf et al., 1966; Legge and Austin, 1968). Thus, the ability of individuals to

detect low doses of antigens, especially "weak" antigens (such as syngeneic tumour antigens) can decline with age. Indeed, there is age related poor immune surveillance against low doses of certain syngeneic tumour cells, in mice (Prehn, 1971). This could also explain why the resistance to allogeneic tumour cell challenge can decline more than a hundredfold with age in mice manifesting only a fourfold decline in T-cell mediated cytolytic activity against the same tumour cells (Goodman and Makinodan, 1975).

1.6.3 T cells: Present evidence suggest that the decline in immune functions which accompanies ageing is due primarily to changes in the T cell component of the immune system. T cell subpopulations shift with age but the data is conflicting. A decrease in helper T-cells and an increase in suppressor T-cells (Makinodan et al., 1984; Mascart-Lemone et al. 1982) have been reported. Others obtained an increase in helper T cells and a decrease in suppressor T-cells (Malet and Fournier, 1983). The percentage of circulating T-cells may be raised (Wallgren et al., 1978), depressed (Girard et al., 1977; Mascart-Lemone et al., 1982: Diaz-Jouanen et al., 1975a; Carosella et al., 1974; Alexopoulos and Babitis,

1976; Teasdale et al., 1976) or unchanged (Kishimoto et al., 1978; Duchateau et al., 1976; Gupta and Good, 1979; Weksler and Hutteroth, 1974; Hallgren et al., 1978) during ageing. Circulating lymphocyte number decreases progressively during or after middle age in humans to about 70 percent, of that of a young adult, by the sixth decade (Diaz-Jouanen et al., 1975a; Augener et al., 1974; Alexopoulos and Babitis, 1976). A proportional decrease in the number of T cells was observed while the number of B cells show little change. Other workers (Mascart-Lemone et al., 1982; Alder et al., 1982) found no difference in the absolute number of peripheral blood lymphocytes between young and old healthy individuals.

There is decreased colony formation in the elderly (Alder et al., 1982) and this defect may be due largely or completely to a defect in the number and/or proliferative ability of the colony-forming cells (Oyeyinka, 1984). The lymphocytes producing colonies are a subset of the lymphocytes responding to PHA (Stahn et al., 1978; Foa and Catovsky, 1979; Bernstein et al.,1980). A progressive decline in the magnitude of the splenic immune response to dinitrophenylated bovine gammaglobulin, a highly T cell-dependent antigen, also

occurs in C57BL/J mice with advancing age. Aged animals show a preferential loss of direct (IgG) and high avidity plaque-forming cells in both their primary and secondary responses (Goidl et al., 1976). Evidence for impaired helper and augmented suppressor cell activity in these animals was obtained from cell transfer experiments. As T cells are important in the generation of high affinity antibody and IgG antibody (Gershon and Paul, 1971; Anderson et al., 1974), the loss of these functions in old mice may be due to impaired helper T cell activity.

The delayed-type hypersensitivity response to ubiquitous antigens is depressed with advancing age (Toh et al., 1973; Mackay, 1972; Waldorf et al., 1968; Forbes, 1971; Roberts-Thomson et al., 1974). Ageing impairs the responsiveness of human lymphocytes to phytohaemagglutinin (Pisciotta et al., 1967; Hallgren et al., 1973; Weksler and Hutteroth, 1974; Roberts -Thomson et al., 1974). There is a decreased proliferative response of lymphocytes from elderly donors to antigens and T-cell mitogens in general (Hallgren et al., 1978; Czlonkowska and Korlak, 1979; Inkoles et al., 1977; Goodwin and Messner; 1979; Goodwin et al; 1982). Irrespective of the status of T

Cell number, the response of T cells of elderly donors to optimal mitogen stimulation is reduced due to decrease in the number of responding cells and to the decreased ability of these cells to undergo multiple divisions (Inkeles et al., 1977; Hefton et al., 1980). The decreased proliferation in old people was observed not due to a delayed response to PHA or to a prolonged cell cycle (Hefton et al., 1980). However, Tice et al. (1979), using a computer simulation of cell proliferation, found that aged lymphocytes enter the stimulated pool more slowly and that, although minimum cell cycle durations remained the same, mean and maximum cell cycle durations were significantly increased in aged lymphocytes. Ca^{++} uptake, which plays a fundamental role in cellular activation, is decreased in PHA - stimulated T cells of old humans (Kennes et al., 1981). Since the uptake of Ca^{++} is restricted to the first 24 hours after activation, these results suggest that the metabolic events occurring during the initial phase of T cell activation are altered in old individuals.

Evidence for a decrease in T helper cells function with age comes from the demonstration of a reduced interleukin (IL)-2 production (Chang et al., 1982; Gillis

et al., 1981; Miller and Stutman, 1981; Thoman and Weigle, 1981). At the surface membrane level, considerably fewer IL-2 receptors have been detected in antigen/mitogen-activated T cells of old than of young humans (Gillis et al., 1981) and mice (Chang et al., 1982), as judged by the ability of activated T-cells to absorb IL-2. Since stimulation by IL-2 is essential for activated T-cells to expand clonally, the reduction in the number of IL-2 receptors with age could contribute to the decline in the proliferative activity of T cells of old individuals, whose capacity to synthesize IL-2 is also compromised. In humans, a decrease in suppressor activity has been observed (Hallgren and Yunis, 1977). In contrast, Gupta and Good (1979) found an increase in the number of T gamma suppressor cells. Moreover, Antel and Arnason (1979) found that concanavalin A-activated suppressor cells from elderly individuals produce less suppressor factors but suppress the mitogenic response of autologous cells to a greater extent than do those of the young. In long-lived mice, there is an increase in T suppressor cells with age (Goidl et al.,1976; Makinodan et al., 1976; Segre and Segre, 1976) but a decrease in short-lived mice (Barthold

et al.,1974). Doria et al.(1982) demonstrated that old mice lose their sensitivity to suppression. This could account for the increase in autoantibody formation in old long lived mice (Makinodan et al., 1984).

Like the responses to the plant lectins PHA and concanavalin A (Con.A), there is an age-related decline in the ability of human peripheral blood lymphocytes to be activated by 12-O-tetradecanoyl phorbol-13-acetate (TPA) (Nagel et al., 1982). However, unlike the PHA or Con.A responses there is no augmentation of cellular ^{3}H-thymidine incorporation induced by the addition of the prostaglandin synthetase inhibitors such as indomethacin (Nagel et al., 1982). This is an indication that prostaglandin-secreting suppressor cells are not responsible for the diminished mitogenic response observed in the peripheral blood lymphocytes of elderly individuals activated by TPA.

1.7. HUMORAL FACTORS IN THE AGEING OF THE IMMUNE SYSTEM

1.7.1 B cells: The number of B cells in the spleen and lymph nodes does not seem to change appreciably with age in long-lived mice (Makinodan and Adler, 1975) whereas the number of plasma cells seems to increase in

autoimmune prone relatively short-lived mice (Good and Yunis, 1974). In humans, studies have been limited primarily to circulating B cells, and they indicate that the number of B cells also remains relatively constant (Diaz-Jouanen et al., 1975b). However, it is not known whether the number of circulating B cells corresponds to that in the spleen and lymph nodes. Lymphocytes from old donor NZB mice were found to be distributed in abnormally large numbers in the liver and in abnormally small numbers in the spleen and lymph node, of recipient NZB mice, as compared to the distribution found for cells of young donors (Fernandes et al., 1977).

In contrast to the constancy with age in the total B-cell population, its subpopulations may fluctuate. The number of B cells responsive to a T cell - independent antigen decreases slightly with age in long-lived mice (Price and Makinodan, 1972 a). Also, the immune response to lipopolysaccharide (LPS), a T-independent mitogen decreases with age (Cerbase-Delima et al., 1974). Indeed the level of serum IgG and IgA tends to increase with age while that of serum IgM tends to decrease (Haferkamp et al., 1966; Lyngbye and Kroll, 1971; Buckley et al., 1974). However, others have found the proliferative

capacity of mitogen sensitive B-cells unaltered with age (Hung et al., 1975a, 1975b; Makinodan and Adler, 1975). The responsiveness of B-cells to stimulation with certain T-cell dependent antigens decreases strikingly with age (Makinodan and Peterson, 1962; Makinodan et al., 1971).

1.7.2 Immunoglobulins: Nordby and Dodge (1974) reported on a large community based study of immunoglobulins of 3,213 persons aged 3 to 94 years. In keeping with earlier data on age related changes in total gamma-globulin (Mackay et al., 1977), the concentration of IgG and IgA increased with age whereas IgM did not change. Other reports described a fall, with age, in the levels of IgD (Leslie et al., 1975) and of IgE (Grundbacher, 1974; Orren and Davdle, 1975).

1.7.3 Natural antibody: Natural antibody is the antibody that is detectable in serum prior to immunization with the corresponding antigen and arising presumably from immunization with cross-reactive determinants. Somers and Kuhns (1972) investigated 197 people aged 15 - 50 years, 91 individuals aged 51 - 69 years, and 90 others from 70 to 98 years of age. Average titres of anti - A and anti- B iso - agglutinins decreased progressively from 20 years of age to very low

levels in senescence. This trend can be attributed to "refractoriness to immune stimuli" (Mackay et al., 1977).

1.7.4 Response to vaccines: Humoral immune responses to influenza vaccine were found to be maintained in the aged (Feery et al., 1976). Also, immune responses to tetanus toxoid vaccination was not altered in ageing individuals (Solomonova and Vizev, 1973).

1.7.5 Sex differences in the humoral immune response to ageing: There is a progressive decrease of total serum IgE and of the in vivo specific IgE antibody synthesis in ageing women,, but not in ageing men (Delespesse et al., 1977; Hanneuse et al., 1978). The age-associated increase in the prevalence of autoantibodies is much more pronounced in females than in males (Wittingham et al., 1971), whereas the frequency of circulating immune complexes is higher in the latter (Delespesse et al., 1980). Epidemiologic data (World Health Organisation,1982) show that males have a higher general mortality rate than females in most nations studied. However, it has been shown that germ-free male mice live as long or longer than germ-free female mice (Gordon et al., 1966). It is possible that conventionally raised females live longer than males because of the females' greater resistance to infection (Oyeyinka, 1984). The

same argument may apply to man.

1.8 THE PATHOGENIC ROLE OF AGE-RELATED IMMUNE DYSFUNCTION

Age-dependent immune dysfunctions can be classified into three groups: immune deficiency, autoimmunity and idiopathic paraproteinaemia. They are not always found as none of these dysfunctions may be detectable in some very old individuals, though they occur in combinations in others (Hijmans and Hollander, 1977). There is considerable data on the decline in the functions of the immune system during ageing (Pazmino and Yuhas, 1973; Makinodan, 1980; Patel, 1981). A major characteristic of the relationship between immunity and ageing is the increased occurrence of autoantobodies. Clinical observation reveals that autoantibodies can be found in no less than 50 percent of aged individuals without overt disease, although titres are usually low. These reduced titres are more frequent in females than in males (Hijmans and Hollander, 1977). Weakly positive tests for rheumatoid factor, low - level antinuclear antibodies, or antithyroglobulin antibodies may, therefore, have little medical significance in aged women. However, they can be considered to indicate a disturbance in the homeostatic mechanism.

The heterogeneity of the immunoglobulins is the hallmark of humoral immunity. A restricted heterogeneity is observed in certain situations, multiple myeloma being the extreme case. This is a malignant proliferation of plasma cells that can result in a greatly increased level of circulating monoclonal immunoglobulins - the M component or paraprotein - with a concomitant decrease in the other immunoglobulins. Idiopathic or benign paraproteinaemia is a far more frequent finding, and differs from myeloma in that it is not progressive, the level of the paraprotein is lower, and the decrease in the residual immunoglobulins is not as pronounced (Hijmans and Hollander, 1977). Idiopathic paraproteinaemia is of considerable gerontological interest because of its increase in frequency on ageing. These changes have been found at a frequency of one out of five in very old individuals (Englisova et al., 1968; Radl et al., 1975).

Among others, age-related pathology due to immune dysfunctions include infection, autoimmune diseases, malignancy and amyloidosis.

1.8.1 Infection: The lack of difference in PHA or skin test responses between healthy and chronically ill

elderly subjects obtained by Goodwin et al. (1982) suggests that the major determinant of depressed cellular immunity in the elderly is age and not age-associated diseases. Age-related immune dysfunction contributes to the vulnerability of old individuals to infection (Haddy, 1988). Animal model studies demonstrate the association between age-related decline in T-cell-dependent immunologic responses and the decline in resistance against viral, bacterial, and parasitic infections (Makinodan et al., 1984). Ageing human populations are deficient in their ability to generate adequate amounts of neutralizing antibodies in response to viral vaccination (Howells et al., 1975; phair et al., 1978). Also, they possess significantly lower levels of antibacterial antibodies in their blood (Batory and Rodei, 1978).

Common infections in the aged include those of the respiratory tract especially pneumonia. They are at a high risk for influenza. Infections of the urogenital tract are often incapacitating and involvement of the ki ney, as seen in pyelonephritis, may shorten the life expectancy (Hijmans and Hollander, 1977).

1.8.2. Autoimmune diseases: There is increased incidence of a number of autoimmune diseases, such as rheumatoid

arthritis and autoimmune thyroiditis, with ageing (Hijmans and Hollander, 1977). Other autoimmune disorders, however, are more evenly distributed throughout life (Hijmans and Hollander, 1977). Signs of a focal thyroiditis can be found in about 25 percent of post-mortem examinations in females over fifty years of age (Williams and Doniach, 1962). The aetiology of this group of diseases is unknown.

1.8.3 Malignancy: In the naturally occurring immune deficiency diseases in man a tumour incidence, which is several hundred times higher than that of the control groups, has been observed. However, the majority of these are lymphoreticular malignancies (Good, 1974). The Denver tumour registry contains data (Penn, 1974) showing that the iatrogenic immune deficiency states are associated with an increased incidence of malignancy of restricted spectrum. Two-thirds of the cases had epithelial tumours and the rest mesenchymal tumours. The immune surveillance as a natural defence mechanism against malignancy seems a reality only in relation to tumour associated antigen systems, such as viral antigens (Klein, 1975). This conclusion agrees with the increased susceptibility of the congenitally thymus-deprived nude mice to virus oncogenesis (Allison et al., 1974). Those

animals are known to develop more tumours spontaneously than control animals, in spite of being immunologically impaired.

1.8.4 Amyloidosis: Amyloid is a protein substance with a fibrillar structure deposited in extracellular tissues in certain disease states and in ageing. Amyloid fibrils consist of two major proteins that exist either singly or in combination. One is known as AL and consists of fragments of immunoglobulin light chains, and the other, AA, consists of a protein that is unrelated to immunoglobulin (Franklin, 1975). Amyloid deposits increase in number with age as senile plaques in many tissues and are said to be the best single indicator of the ageing process (Wright et al., 1969). The AA protein, present in the amyloid tissue from patients with secondary amyloidosis, was shown to be present in the serum of 60 percent of aged people. A younger group of adult blood donors had only a 3 percent frequency (Benson et al., 1975). Amyloid A protein level increases upon ageing (Rosenthal and Franklin, 1975). Amyloidosis can be observed in more than half the male population over 70 years of age. It can be considered as the most characteristic immune disorder of ageing (Hijmans and Hollander, 1977).

1.9 PROSPECTS FOR CORRECTION OF AGE-RELATED IMMUNE DYSFUNCTION

The multiplicity of senescence pathways means that there is no single cause of ageing. As it is impossible to halt all processes that cause damage, and thereby achieve immortality, it has been suggested (Brash and Hart, 1978) that the goal of research on ageing should be to search not for the cause of ageing, but for control points. Two broad classes of immune dysfunction are associated with ageing. These consists of immunodeficiency and increased incidence of autoimmune manifestations. Which of these are primary or whether both are coequal is difficult to prove. Corrective measures should focus on the restoration of the immune response capacity and/or prevention of the autoimmune aspects of ageing.

1.9.1 internal body temperature adjustment: Observations by naturalists, that lizards enjoy a longer life span in New England than in Florida (Walford et al., 1977), stimulated controlled laboratory investigations of the effect of body temperature on life span in poikilothermic vertebrates. The temperature phenomenon is paradoxical in terms of the relation between immunology and ageing. Lowering body temperature greatly suppresses both humoral and cellular immunity (Trump and Hildemann, 1970; Cone and Marchalonis, 1972). This would seem likely to accentuate the immunodeficiency of ageing; nevertheless, life span is greatly prolonged (Walford et al., 1977). The life span prolonging effects of caloric undernutrition and of lowering body temperature are maximally effective at the opposite extremes of life span: nutritional manipulation during the first half and temperature lowering during the last half. One of these regimes delays the

immunodeficiency (Walford et al., 1977), and the other probably ameliorates the autoimmunity (Liu and Walford, 1975) of normal ageing respectively. However, attempts to achieve prolonged low-grade hypothermia in homeothermic animals by drug administration have been disappointing (Liu and Walford, 1972).

1.9.3 Use of therapeutic agents: Patients with T-cell deficiencies demonstrate low serum thymic hormone levels. Wara et al. (1975) reported partial restoration of cell mediated immunity in a patient with thymic hypoplasia after daily injections of thymosin. Other investigators have reported that exposure to thymic factors in vitro may profoundly affect the lymphoid cells from patients with certain primary immunodeficiency diseases (Ajuti et al., 1975; Touraine et al., 1975a,b). It appears that thymus hormonal factors might favourably influence the immunodeficiency of ageing.

In addition to promoting linear growth in pre-pubertal children, growth hormone has anabolic, lipolytic, and diabetogenic properties (Vance, 1990). Growth hormone acts on the liver and other tissues to stimulate the production of insulin-like growth factor I (IGF-I) also known as somatomedin C. This substance is responsible for the growth-promoting effects of growth hormone and serves as an indicator of overall growth hormone secretion. Integrated 24-hour growth hormone

concentrations decline with increasing age. They are approximately one-third lower in healthy people more than 55 years old compared to people aged 18 to 33 years (Vance, 1990). IGF-I concentrations are similarly reduced (Ho et al., 1987). As indicated by serum IGF-I measurements however, the decline in growth hormone secretion with age is not universal. The prevalence of serum IGF-I concentrations below the range found in 20-29 year-old men and women was found to be 11 percent in the fourth decade of life, 20 percent in the fifth, 22 percent in the sixth, 42 percent in the seventh, and 55 percent in the eighth and ninth. At all ages, the serum level of IGF-I was inversely correlated with adiposity (Rudman et al., 1981).

Decline in growth hormone, IGF-I production; a decrease in muscle mass and increase in adiposity occur in healthy elderly subjects (Vance, 1990) and presumably also in adults with growth hormone deficiency. These observations led to attempts to determine whether the administration of growth hormone is beneficial in such people. Rudman et al. (1990) have reported the effects of the administration of growth hormone three times a week for six months on body composition in healthy 61 to 81 year-old men who had serum IGF-I concentrations below those of healthy younger men. In this study and in similar

studies of men and women with growth hormone deficiency (Salomon et al., 1989; Jorgensen et al., 1989), the administration of growth hormone increased the serum IGF-I concentrations to within the range found in young (less than 30 years old) healthy adults. There were also increases in lean body mass of 8.8-10 percent and decreases in the mass of adipose tissue of 14.4-16 percent, respectively, after six months of growth hormone administration (Rudman et al., 1990; Salomon et al., 1989). Our understanding of the potential benefits of growth hormone administration has been extended by these studies. However, studies of short-term administration of growth hormone have revealed that growth hormone can affect carbohydrate metabolism adversely, producing hyperinsulinaemia, glucose intolerance, and diabetes mellitus. It affects musculoskeletal system (producing hypertension, oedema, and congestive heart failure), as exemplified by acromegaly (Vance, 1990). It is not clear whether long-term administration of growth hormone produces similar effects, or whether the improved metabolism of nutrients observed in animals given growth hormone (Etherton et al., 1987) also occurs in humans. Therefore, further studies are required before the use of growth hormone in healthy older adults can be contemplated. It's general use in the elderly, and in adults with growth hormone deficiency, now or in the

immediate future is however not justified (Vance, 1990).

The age associated decline in the functional capability to produce interleukin 2 would suggest the need for IL-2 immunotherapy in the aged. However, a high frequency of non-opportunistic bacterial infections occur in patients receiving IL-2 (Murphy et al., 1988; Klempner et al., 1990). Klempner et al. (1990) have shown that these patients acquire an acute, profound and reversible defect in neutrophil chemotaxis that may contribute to the high morbidity resulting from bacterial infections. However, the concomitant administration of dexamethasone markedly reduces the side effects (e.g bacterial infections) of IL-2 therapy (Klempner et al., 1990). The mechanism is possibly through the inhibition of secondary cytokines such as tumour necrosis factor which is known to be capable of producing defective neutrophil chemotaxis in-vitro.

Although largely unexplored in the realm of ageing, synthetic polynucleotides may have potential for immunologic restoration. Studies have shown that naturally occurring DNA and RNA could restore the immune response in X-irradiated animals (Simic and Kanazir, 1968). Braun and Nakano (1967) had earlier reported that commercially available poly AU could enhance the anti-sheep red blood cell response in a manner resembling that of oligonucleotides obtained from digestion of calf thymus DNA.

Synthetic polynucleotides may exert their immunologic effect by raising the intracellular levels of cAMP. Spleen cells exposed in-vitro to poly AU, and other oligonucleotides show a rise in adenyl cyclase activity (Winchurch et al., 1971). In this respect the action of polynucleotides may be similar to that of other agents which raise intracellular cAMP levels, such as catecholamines and thymic humoral factors. Also, the stimulatory effect of poly AU on antibody response can be magnified by simultaneous administration of theophylline (Ishizuka et al., 1970) which is a phosphodiesterase inhibitor and stabilizer of cAMP levels. In as much as synthetic polynucleotides are believed to stimulate proliferation and/or differentiation of B - and/or T - cells, they may be useful in treating the immunodeficiency of ageing, perhaps by replacing the lost thymic functions.

1.10 INTERACTION OF NUTRITION WITH IMMUNOCOMPETENCE DURING AGEING

Changes occuring in the immune system during the ageing process have many similarities with the effects of some types of protein-calorie malnutrition (Chandra, 1993). However, in the absence of essential nutrient deprivation, energy intake restriction may actually retard ageing (Walford et al., 1987). Thus, nutritional status is a critical determinant of

immunocompotence (Chandra, 1991; Mc Murray, 1984). Thia has been demonstrated in atudies on young children with protein-energy malnutrition (PEM), young adults and the elderly (Chandra, 1993).

A calorically restricted but nutritionally aupplemontod diet (i.e. under-nutrition without malnutrition) markedly prolongs the life span (Ross, 1969; Walford ot al., 1974). Deficiencios of essential amino acids may influence the immune system in different ways (Jose and Good, 1973). For example, a deficit in valino alters humoral more than cellular immunity, whereas a doficit in lysine decreases both responses. Tho apparent deceleration of ageing obtained with a tryptophan-deficient diet (Segall and Timiras, 1975) might reflect altered immune function as well as alterations of brain monoamines (Timiras, 1975).

CHAPTER TWO

DESCRIPTION OF THE STUDY POPULATION SUBJECTS AND METHODS

2.1 AGE AND SEX DISTRIBUTION

Two hundred and seventeen Nigerian males and females aged 6 - 85 years have been studied. They include 122 males and 95 females. The age and sex distribution of the subjects are displayed in Table 1a. This study was conducted at the University College Hospital (UCH), Ibadan, Nigeria between August 1990 and December 1991. The individuals sampled were from the western, eastern and northern parts of Nigeria and include:

(1) 53 male - and 5 female - blood donors from the blood bank, U.C.H., Ibadan.

(2) 31 female nurses and health workers taking part in a family planning training programme at the Department of Obstetrics and Gynaecology, UCH, Ibadan.

(3) 18 female patients seeking consultation, at the

TABLE 11

Age-and-sex distribution of subjects.

a. Samples for Laboratory analysis

Age group (yrs)	Males	Females	Total
6-25	39	21	60
26-45	36	34	70
46-65	27	20	47
> 65	20	20	40
Total	122	95	217

b. Samples for Mantoux test

Age group (Yrs)	Males	Females	Total
6-25	89	47	136
26-45	71	27	98
46-65	26	14	40
Total	186	88	274

c. Samples for Meningococcal vaccination study

Age group (Yrs)	Males	Females	Total
6-25	5	2	7
26-45	25	2	27
46-65	16	0	16
Total	46	4	50

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Department of Obstetrics and Gynaecology, UCH, Ibadan, for infertility of unexplained origin who were not known to be infected in any way;

(4) apparently healthy 40 male and 31 female patients seeking consultation for eye problems such as cataract and glaucoma at the eye clinic, Department of Ophthalmology, UCH, Ibadan;

(5) 16 male and 11 female staff and students of the University of Ibadan;

(6) 13 male and 11 female elementary school students at Badeku primary school.

Another group of 274 healthy individuals (186 males, 88 females; age: 6 - 60 years) were studied for their in-vivo cell-mediated immune response by the Mantoux delayed hypersensitivity skin test.(Table 1 This was done at the University of Ibadan Health Services (Jaja) Clinic between the months of January and May 1992.

Specific antibody response to meningococcal polysaccharide vaccine was examined in 50 healthy Nigerian individuals in March and April of 1992. These individuals were aged 18 - 55 years and included 46 males and 4 females from Afon, Asa local government area of Kwara State, Nigeria.(Table 1c).

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2.2 COLLECTION OF BLOOD SAMPLES

Blood (20 ml) was collected from each of the subjects by venepuncture. Exactly 1 ml of blood was immediately transferred to a siliconized collection vial containing 20 units of heparin (catalog No. 840-20, Sigma chemical Co. Ltd, Dorset, England) for Nitroblue tetrazolium (NBT) dye reduction test. The blood was gently but well mixed by tilting the vial slightly and rolling it for approximately 30 seconds. The remaining blood was placed in a plastic universal container with heparin at 10 units per ml, and gently but well mixed. 8 ml of the sample was diluted for T lymphocyte and B lymphocyte enumeration. 8 ml was used for leucocyte migration inhibition assay. 1.5 ml of blood was mixed with ethylene diamine tetra-acetic acid (EDTA) anti-coagulant and centrifuged at 800 x G for 5 minutes. The remaining 1.5 ml of blood was also centrifuged. Both EDTA-plasma and heparin plasma samples were stored at - 20°C until analyzed.

Participants in the meningococcal polysaccharide vaccination study each provided about 3 ml of blood before being vaccinated and 3ml of blood about a month after vaccination.

2.3 SOCIO-ECONOMIC STATUS

The socio-economic status (SES) of the subjects was determined by the method described by Williams (1985) which is based on the level of formal education, occupation and income. Five socio-economic classes were defined as follows:

SES 1: highly educated professionals, academics, senior public officers and business executives.

SES 2: post-secondary school educated individuals and middle-level public officers.

SES 3: post-primary school educated individuals, lower level public officers and institutional staff and skilled workers.

SES 4: primary school-educated individuals and unskilled workers.

SES 5: illiterate poor peasant farmers and petty-traders.

Teenagers have been ascribed the SES of their parents or guardians.

Table 2 compares the SES of the individuals in the different age groups studied. The variables are comparable in the subject-groups (X^2 = 19.116; P>0.05).

TABLE 2:

Socio-economic status (SES) distribution among the subjects

Age group (years)	S E S				
	1	2	3	4	5
6-25	0	3	20	17	12
26-45	3	12	17	18	10
46-65	3	7	8	12	12
>65	1	3	7	7	14

Socio-economic classes 1 to 5 were defined based on the level of formal education, occupation and income.

$X^2 = 19.116$; $P>0.05$.

2.4 HEALTH STATUS

All subjects were fully ambulatory and living independently. The health status of these individuals was subjected to exclusion criteria, based on clinical information, laboratory data and on rules for the limitation of pharmacological interference, of the SENIEUR protocol applied for immunological studies (Ligthart et al., 1984). Those excluded are: infection, inflammation, malignancy, and diabetes mellitus.

The laboratory tests leading to exclusion include a haematological screening (leucocyte count with differentiation; see chapter 3.1) and serological screening of samples for amoebiasis, hepatitis-B virus infection, and salmonellosis (see Appendix; pp 292-296). The salmonella serotypes whose antibody levels were screened include Salmonella typhi O, S. typhi H, S. paratyphi O (groups A,B and C somatic antigens) and S. paratyphi H (flagellar antigens a,b and c). Malaria antibodies were not screened because the study was carried out in a malaria-endemic area where healthy individuals carry these antibodies for protection.

Haemoglobin genotyping was done on the samples by electrophoretic separation on cellulose acetate at pH 8.5 as described by Dacie and Lewis (1991). Subjects with haemoglobin genotype AA and those with sickle cell traits AS and AC were evenly distributed among the age groups (X^2 = 3.450; P>0.10). Those with genotype Hb.AA represent 73% of the total sample while those with Hb.AS and Hb.AC were 22% and 5% respectively. None of the sampled population was of genotype Hb.SS, Hb.SC or Hb.CC (see Appendix; pp 297-299).

2.5 NUTRITIONAL STATUS

It is believed that biochemical and immunological changes occur long before any anthropometric abnormality can be detected in the assessment of nutritional status. Olusi and Jessop (1977) have recommended the measurement of transferrin level as the most sensitive and most practical index of nutritional state. The nutritional status of the subjects in this study was assessed by measuring total plasma protein, albumin, transferrin and complement C3c concentrations. All four plasma components showed no significant difference in mean values among the age groups (see Appendix; pg. 300; sections 5.1 and 5.2).

2.6 STATISTICAL METHODS

Methods employed in the analysis of data collected in the course of this study include: Students' t-test, Chi-square analysis, Spearman's coefficient of correlation, Regression analysis and coefficient of variation (cv).

CHAPTER THREE

NON-SPECIFIC CELLULAR IMMUNE RESPONSES DURING AGEING IN NIGERIANS

3.1 TOTAL AND DIFFERENTIAL WHITE BLOOD CELL COUNTS

3.1.1 INTRODUCTION: The neutrophil and cells of the monocyte-macrophage series are the major effectors of non-specific cellular immunity. Although Bender et al. (1986) stated that changes in the absolute number of lymphocytes is not an age-associated finding, alteration in the numbers of the white blood cell subpopulations is expected to affect the immune response. Total and differential white blood cell enumeration was therefore done in this study to supply baseline data and to allow the assessment of the effect of age on these parameters in Nigerians.

3.1.2 SUBJECTS AND METHODS: Blood samples from 197 individuals were examined for total leucocyte count. These included 55, 67, 43 and 32 people aged 6-25, 26-45, 46-65 and >65 years respectively. 167 samples were stained for differential leucocyte counts and included samples from 47, 50, 38 and 32 people in the respective age groups. The methods

employed for total and differential white blood cell counts are as follows:

Total white blood cell count: 0.1 cm^3 whole blood was delivered into 0.9cm^3 of Tuerk fluid (1% glacial acetic acid tinted with gentian violet) and mixed. Using a capillary tube, a modified Fuchs Rosenthal counting chamber (0.2mm depth) was filled with the diluted blood. The cells in two diagonal square millimetres were counted using a 40 mm objective and x 10 eye piece. Calculation of cell number was done as follows:

Cell number = $n/2 \times 5 \times 10 \times 10^6$ cells/L

where n is the number of cells counted in two diagonal squares.

Differential white blood cell count: Differential white cell count was done on Leishman's stained thin blood films. Blood films were made from fresh drops of blood, dried in air for about 10 minutes and fixed in undiluted Leishman's stain for 2 minutes. Staining was for 10 minutes in Leishman's stain diluted 1 in 3 with Leishman buffer pH 6.8. Slides were examined microscopically using the oil immersion objective

and x 10 eye piece. The Longitudinal counting method was employed to record the proportions of neutrophil, lymphocyte, monocyte, eosinophil and basophil present. At least 200 white blood cells were counted and the proportions were expressed as percentages of the total white cells counted.

3.1.3 RESULTS: The results obtained are as follows:

Total white blood cell count

Total white blood cell counts obtained for the various age groups are displayed in Table 3. A mean count of 5.41± 2.32 x 10^9 leucocytes/L for the 6-25 year age group did not change significantly into old age (>65 years) as no age-related changes in the counts could be demonstrated ($r= -0.068$; $P > 0.20$). However, there was significant depression in leucocyte number in the 26-45 year age group compared to the 6-25 years age group ($t=2.940$; $P < 0.01$).

Differential white blood cell count

The numbers of neutrophils and monocytes obtained in the various age groups studied are presented in Tables 4 and 5 respectively.

TABLE 3.

Total white blood cell counts in Nigerians aged 6 to 95 years

Age group (in years)	n	Mean (x10⁹/L)	± s.d.
6-25	55	5.41	2.32
26-45	67	4.37	1.34
46-65	43	5.16	2.80
>65	32	4.92	1.21

$r = -0.068$; $P > 0.20$

Table 4 shows that neutrophil number was not altered significantly with age (r= -0.075; P >0.20). However, mean neutrophil number (x 10^9/L) decreased (t = 3.071; P<0.01) between 6-25 year age group (2.81±1.17) and 26-45 year age group (2.16 ± 0.86).

Results of monocyte count (x 10^9/L) are presented in counts
Table 5. Mean monocyte / (± Is.d) were 0.35± 0.24 for the 6-25 year age group, 0.29 ± 0.21 for the 26-45 year age group, 0.34 ± 0.34 for the 46-65 year age group and 0.35 ± 0.20 for the >65 year age group. All comparisons of mean values by students' t-test among the age groups showed no significant difference (P>0.10 in all cases). Furthermore, monocyte number is not correlated to age (r = -0.020; P>0.20).

Differences among mean values for the different age groups in basophil count were not significant (Students' t-test: P>0.05 in all cases). Mean values obtained ranged between 0.4 ± 0.74% and 0.8 ± 1.25%. Mean eosinophil count ranged between 3 ± 4.5% and 5 ± 5.0%. Comparisons of means among the age groups show no significant difference (Students' t-test: P>0.10 in all cases). No significant changes with age in lymphocyte figures (r = -0.086; P>0.20) were observed.

TABLE 1.

Neutrophil count in each of the age groups studied

Age group (in years)	n	Mean ($x10^9/L$)	1 s.d.
6-25	47	2.81	1.17
26-45	50	2.16	0.86
46-65	38	2.69	1.97
>65	32	2.34	0.94

$r = -0.075$; $P>0.20$.

TABLE 3.

Monocyte count in each of the age groups studied.

Age group (in years)	n	Mean ($x10^9/L$)	1 s.d.
6-25	47	0.35	0.24
26-45	50	0.29	0.21
46-65	38	0.34	0.34
>65	32	0.35	0.20

$r = -0.020$; $P>0.20$

3.2 NITROBLUE TETRAZOLIUM (NBT) DYE REDUCTION TEST

3.2.1. INTRODUCTION: It is well known that humoral and cellular immunological reactivity increases between birth and maturity and declines in old age (Weksler, 1986). However, data on alterations in phagocyte functions are scarce. Until the report of Rabatic et al. (1988) there was not a single report on age-related changes of phagocytes functions that covers all age groups in man. Their report was restricted to the investigation of antibody-dependent cell-mediated cytotoxicity and two stages of phagocytosis viz ingestion and digestion. The present study investigates neutrophil metabolic activity across the age groups by the NBT dye reduction test.

3.2.2 SUBJECTS AND METHODS: Both unstimulated and stimulated NBT tests were done on blood samples from 104 subjects. These were people aged 6-25 years (20), 26-45 years (35), 46-65 years (26), and over 65 years (23).

Nitroblue tetrazolium (NBT) dye reduction test

The Sigma Diagnostics (Sigma Chemicals Co. Ltd., Dorset, England) NBT test kit (procedure No.840) was employed in this study. Sigma's semi-quantitative NBT procedure is based on a modification of the method of Feigin et al. (1971), and conforms with the procedure of Park et al. (1968). Whole blood was tested within 2 hours of collection or stored in refrigerator at 4°C. A plastic syringe was used for venipuncture and 1cm³ blood was delivered into a siliconized collection vial containing 20 units of heparin (Catalog No. 840-20, Sigma Chemical Co. Ltd.). The vial was tilted slightly and rolled for about 10 seconds to mix.

For the unstimulated test, 50 ul of NBT solution was transferred to a vial (Catalog No. 840-50, Sigma Chemical). 50 ul of well-mixed heparinized blood was added and mixed gently but well. Incubation followed at 37°C for 10 minutes and at room temperature for an additional 10 minutes. The vial content was mixed again and a moderately thick smear was prepared. Smear was air-dried for about 10 minutes and treated with Wright stain (Catalog No. WS 10) as follows:

Dried smear was flooded with 1cm³ of undiluted stain for 15 seconds. To the flooded smear, 1cm³ of distilled water was added, and the mixture was allowed to stand for 30 seconds. Smear was then rinsed with water, allowed to drain and air-dried.

For the stimulated NBT procedure, 50 ul of NBT solution was transferred to a vial (Catalog No. 840-50). 25ul heparinized blood and 25ul of stimulant solution (non-viable bacterial extracts - Sigma Chemical) were added. Mixing was gentle, but adequate. Incubation was for 10 minutes at 37°C followed by a further 10 minutes at room temperature. Microscopical examination and counting was done as for the unstimulated procedure.

Stained smear was scanned using oil immersion objective. 100 or more neutrophils were counted. Those neutrophils showing formazan deposits were recorded as positive. Absolute number of positive neutrophils was calculated from total white blood cells and differential leucocyte counts.

3.2.3 RESULTS: The percentages of neutrophils showing intracytoplasmic deposits of formazan following NBT dye reduction are shown in Figure 3 for unstimulated assay and in Figure 4 for bacterial extract stimulated

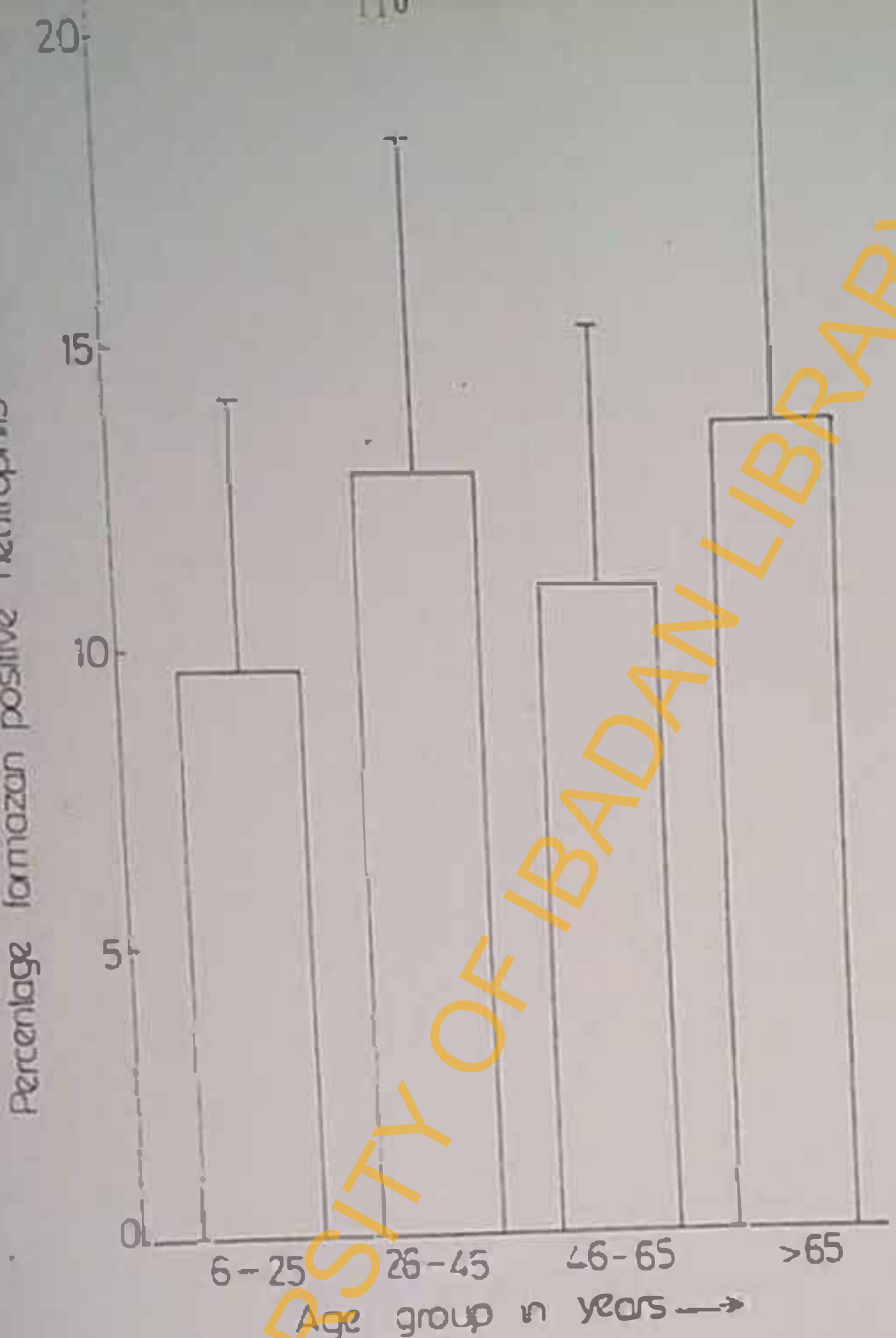

Figure 3. Mean (± 1 s.d) percentage formazan positive neutrophils in the age groups using unstinulated NBT test. $\chi^2 = 0.940$; $P > 0.10$.

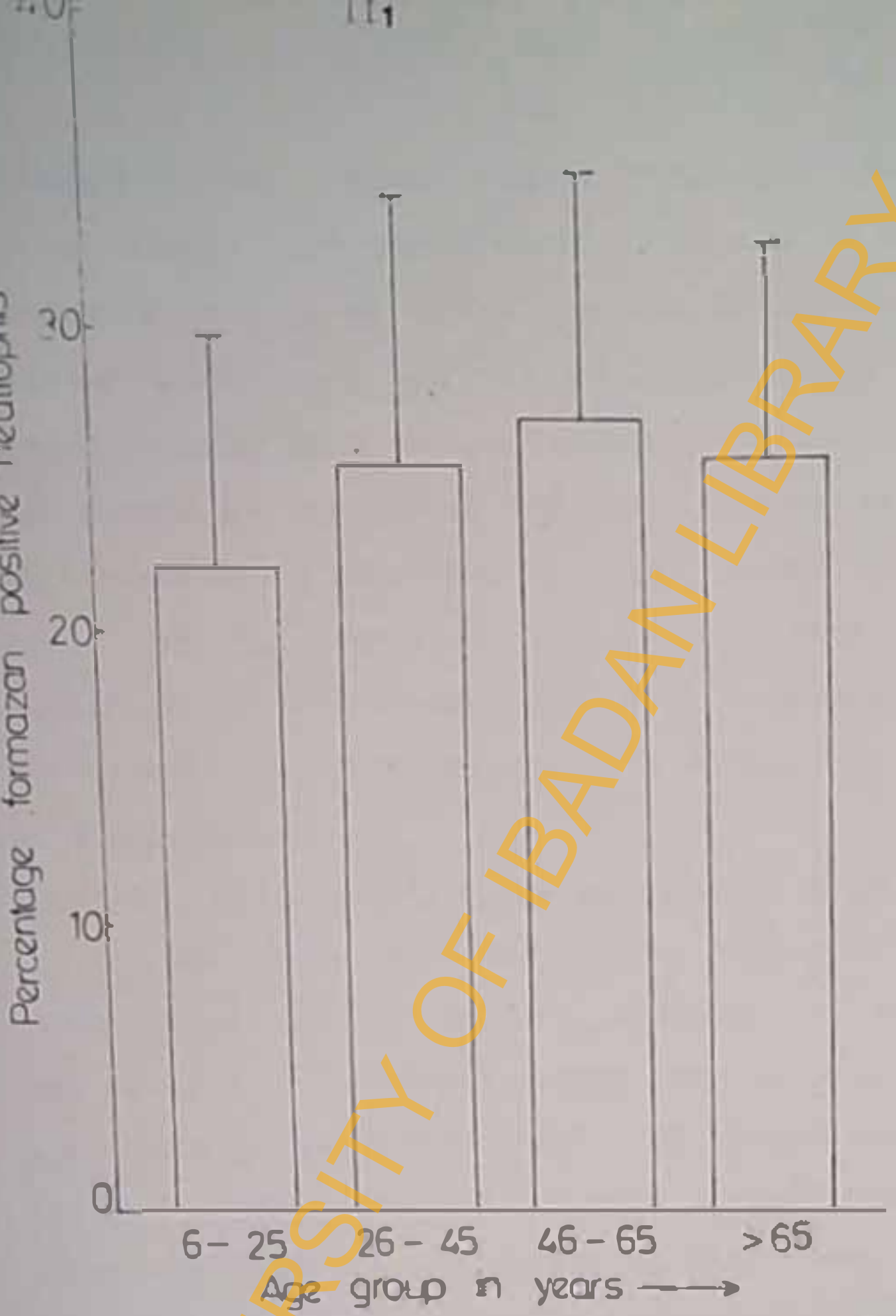

Figure 4. Mean (± 1 s.d) percentage formazan positive neutrophils in the age groups using stimulated NBT test. $X^2 = 0.790$; $P > 0.10$

determinations. Mean values displayed in Figure 3 reveal significant differences (Students' t-test) only between 6-25 year and 26-45 year age groups ($P<0.05$) and between 6-25 year and >65 year age groups ($P<0.05$). Comparisons of mean values (Students' t-test) among the age groups in stimulated NBT test (Figure 4) show no difference among them ($P>0.05$ in all cases). The changes with age in formazan positivity rate was not statistically significant for both unstimulated (X^2 = 0.940; $P>0.10$) and stimulated (X^2 = 0.790; $P>0.10$) tests.

3. 3 DISCUSSION:

Total and differential white blood cell counts

It has been recommended that leucocyte counts be reported in absolute numbers (Orfanakis et al., 1970). The results of leucocyte counts have been presented in absolute figures in this study. The counts obtained here for total leucocytes and for each type of leucocyte are generally lower than figures reported from studies in caucasian and negro subjects (Orfanakis et al., 1970). It is well established that total leucocyte counts are lower in tropic dwellers compared to values in caucasians (Greenwood and Whittle, 1981). Orfanakis et al. (1970) used electronic cell counting method while haemocytometer counting technique was employed here. The possibility

that the differences in absolute leucocyte counts between the two studies is due to the use of different methods is not supported by their own observation. They found that both electronic counting and the haemocytometer method gave the same normal blood concentration ranges for the several leucocyte types. However, this was only possible if appropriate attention was paid to methodological details.

No significant correlation was observed between total leucocyte counts and age in this study. This indicates that ageing per se does not alter leucocyte production. A mean total white blood cell count of 4.31± 1.34 x 10^9/l reported here for subjects aged 26-45 years agrees with that observed previously in normal control Nigerians aged 28-42 years (4.68 ± 1.71 X 10^9/l) by Onyemelukwe et al.(1990). However, the total white blood cell counts are generally lower than figures reported in five-year old Ghanian children (Abdalla, 1988). The Age of the Ghanian subjects may be responsible for the difference as Ghanians aged one year had considerably higher counts than those five years old in the same study (Abdalla, 1988).

Age related changes could not be demonstrated in neutrophil counts in this study. However, neutrophil functions may be

affected by ageing. Similar numbers of monocytes among the age groups suggest that antigen presenting cells are adequately maintained numerically during ageing. Basophil numbers also did not change with age. The same true for eosinophils.

Nitroblue tetrazolium dye reduction test

Although humoral and cellular immunological reactivity increase between birth and maturity and declines in old age, studies of age related changes of phagocyte functions that covers all age groups in man are scarce (Rabatic et al., 1988). Measurement of neutrophil chemiluminescence appears presently to be the most sensitive method for assessing neutrophil metabolic function. However, the NBT test which was used in this study is the most sensitive of the other methods and avoids the use of costly and unavailable liquid scintillation spectrometers.

Neutrophil metabolic activity assessed by the NBT dye reduction test did not demonstrate age related changes. This agrees with the findings of Corberand et al. (1986) who observed that human polymorphonuclear functions - chemotaxis, phagocytosis and intracellular killing of bacteria - do not change with age. However,

Fullop et al. (1985) showed an increase of phagocytosis and a decrease of intracellular killing with ageing. Bongrand et al. (1984) reported a decrease of Fc receptor-mediated phagocytosis in aged people.

Unlike in the present report, both ingestive and digestive ability of neutrophils have been reported to be significantly reduced in the aged (Rabatic et al, 1988). Reduced ingestive capacity of neutrophils in the aged may be due to decreased Fc gamma function with age. However, the reduced digestive capacity of neutrophils obtained by Rabatic et al. (1988) may not be real but may be due to decreased number of phagocytosed materials presented for digestion.

A lack of age related change in NBT dye reduction capacity observed in this study suggests that the digestive ability of neutrophils is not affected by ageing in Nigerians. Impairment of polymorphonuclear neutrophil function secondary to systemic diseases such as malnutrition and diabetes are well known (Greenwood and Whittle, 1981). The absence of such impairment in the older age groups studied here may be due to the exclusion of subjects with such conditions.

CHAPTER FOUR

SPECIFIC CELLULAR IMMUNE RESPONSES DURING AGEING IN NIGERIANS

4.1 TOTAL LYMPHOCYTES OBTAINED FROM DIFFERENTIAL LEUCOCYTE COUNTS

4.1.1 INTRODUCTION: The lymphocyte is of central importance in the development of adaptive immune responses. Although a longitudinal study in Baltimore indicates that total lymphocyte numbers do not change significantly with age (Bender et al., 1986), they may be altered in Nigerians due to genetic differences. Total lymphocytes have been obtained from differential leucocyte counts in this study to supply baseline data and to allow the assessment of age-related effects on this parameter in Nigerians aged 6-95 years.

4.1.2 SUBJECTS AND METHODS Blood samples from 167 subjects were examined for total lymphocyte count. These included 47, 50, 38 and 32 individuals in the age groups 6-25, 26-45, 46-65 and > 65 years respectively. The methods employed for total and differential white blood cell counts

have been described in chapter 3.1.2.

4.1.3 RESULTS: The lymphocyte numbers obtained in the various age groups studied are presented in Table 6. They show a gradual age-related slight increase in values from the 26-45 years age group. Correlation analysis revealed no significance in these changes with age ($r = -0.086$; $p > 0.20$). The mean absolute lymphocyte count of $2.17 \pm 1.34 \times 10^9/L$ obtained for the 6-25 year age group showed no statistical difference from mean values obtained for the other age groups except in comparison with the 26-45 years age group.

4.2 ENUMERATION OF T CELLS AND SUBSETS

4.2.1 INTRODUCTION: Prior studies of the changes of regulatory T lymphocyte subsets in ageing reported conflicting data. Decreases (Mascart-Lemone et al., 1982; Van De Griend et al, 1982) and a lack of alteration (Bender et al., 1986; Gupta and Good, 1981) in total number of T cells with ageing have been reported. Helper T cells were found to increase (Van DeGriend et al., 1982; Nalet and Fournier, 1983) or decrease (Mascart-Lemone et al., 1982; Gupta and Good, 1981). The frequency of the suppressor T cell population was also

TABLE 6.

Total Lymphocyte count in each of the age groups studied

Age group (in years)	n	Mean ($x10^9/L$)	1 s.d.
6-25	47	2.17	1.34
26-45	50	1.66	0.52
46-65	38	1.96	0.95
>65	32	2.00	0.56

$r = -0.086$; $P>0.20$.

reported to increase (Mascart-Lemone et al., 1982; Gupta and Good, 1981) or decrease (Nalet and Fournier, 1983; Ligthart et al., 1985) with ageing.

The characteristics of the subjects in previous studies may account for these contrasting observations regarding T cell subpopulations. Some of the elderly individuals may not have been "normal" subjects since they were nursing-home patients. In this study, monoclonal antibodies have been used to delineate the T cell subsets during ageing in healthy Nigerians.

4.2.2 SUBJECTS AND METHODS:T cell subsetting was done in 90 individuals. 12 of them were 6-25 years old and 28 were 26-45 years old. 26 subjects were 46-65 years old while people >65 years numbered 24.

A T cell monoclonal panel kit (code no. MCA 328) from Serotec, Oxford, England was used in the indirect immunofluorescence staining of cell surfaces in this study. The detailed procedure employed is as follows:

Preparation of cells from human peripheral blood: 8 cm^3 of heparinised blood was delivered into a universal plastic tube. 6.4 cm^3 of medium (RPMI 1640 + heparin - 5 i.u./cm^3 medium) was added to the blood which was then mixed. The diluted blood was carefully layered over 4.0

cm^3 of Ficoll Hypaque (9% aqueous solution of Ficoll and 33.9% Isopaque in water in the ratio 24:10; stirred to mix and millipore filtered) in two plastic centrifuge tubes. This was centrifuged at room temperature at 850 x G for 20 minutes. The mononuclear cells formed a visible, clean interface between the plasma and Ficoll Hypaque. Plasma was then aspirated and the mononuclear cell layer was carefully removed and placed in a clean plastic centrifuge tube. The tube was filled with RPMI 1640 + heparin, mixed and centrifuged at 300 x G for 5 minutes. Supernatant was aspirated and discarded. Pellet of cells was resuspended and RPMI 1640 + 5% FCS was added, mixed and spun at 300 x G for 5 minutes. Again, supernatant was aspirated and discarded. Pellet was resuspended and 5 cm^3 of RPMI 1640 + 5% FCS was added, gently mixed and cell number determined using a haemocytometer counting chamber. Cell viability was also determined at this stage, mixing 1 drop of 0.5% trypan blue with 4 drops of cell suspension. This was allowed to stand at room temperature for about 3 minutes. The number of cells excluding the stain was expressed as a percentage of the total number of cells present on microscopy. Cell suspensions showing a viability of at least 90% were processed further.

Indirect immunofluorescence staining of pan T-, helper T-, and suppressor T- cell surfaces: 1 X 10^6 mononuclear cells were added to each of 4 plastic precipitin tubes. One tube stood for each of pan T-, helper T- and suppressor T- cell assays. The fourth tube served as the reagent control. The cells were washed with washing medium (cold RPMI 1640 + 2% FCS +0.1% sodium azide), centrifuged at 300 X G for 5 minutes, supernatant aspirated and cell pellet resuspended. 50 ul of 1 in 20 diluted (in PBS buffer pH 8.0) monoclonal antibody to pan T cell (anti-T2, isotype IgG 2b), to helper/ inducer T- cell (anti-T4; isotype IgM) and to suppressor/cytotoxic T cell (anti-T8; isotype IgM) were added to corresponding tubes. Nothing was added to the reagent control tube. The cells were then incubated for 60 minutes on ice, agitating once during incubation, and then centrifuged at 300 X G for 10 minutes at 4°C. The supernatant was carefully aspirated and the cell pellet was resuspended and washed twice with cold washing medium. Centrifugation after each washing was at 300 X G for 10 minutes 4°C. Supernatant was aspirated from cell pellet and 50ul fluorescein-labelled anti-mouse immunoglobulin (1 in 50 dilution in PBS pH 8.0) was added to each of the tubes.

The 4 tubes were incubated for 30 minutes on ice and then centrifuged at 300 x G for 10 minutes at 4°C. The supernatants were carefully aspirated and the cell pellets were washed thrice with cold washing medium. Centrifugation after each washing was at 300 x G for 10 minutes. The cells were resuspended after aspirating the supernatant. For fluorescent microscopy, a drop of mounting medium (70% glycerol + 30% PBS at pH 8.6) was added to the cells and mixed well. A drop of this cell suspension was placed on a microscope slide and covered with a coverslip. The slides were read immediately using a fluorescent microscope, expressing the number of fluorescent cells as a percentage of the total lymphocytes counted. Absolute number of fluorescent lymphocytes was calculated from total white blood cell and differential leucocyte counts.

Null cell proportion and number: The proportion of null cells in the samples (expressed as percentage) was calculated by subtracting the sum of pan T cell and B cell proportions (6.2 below) from 100. Thus:

Null cell = 100 - (% pan T cells + % B cells)

The number of null cells was then obtained from the number of lymphocytes recorded for each sample.

6.2.3 RESULTS: The following results were obtained:

Enumeration of T-cells

The result of T lymphocyte enumeration is displayed in ible 7. Mean T cell counts ranged from 0.86 ± 0.29 x 10^9/L the 6-25 year age group to 1.09 ± 0.60 x 10^9/L in the 46-65 ar age group. Statistical analysis shows no correlation tween T cell number and age (r = 0.177; P > 0.05). Mean T cells were ±6.5% 54±6.7% 52±9.5% and 53±9.8% of total lymphocytes for the spective age groups.

Helper T cell count

Table 8 presents the number of helper T cells obtained for e different age groups. Mean absolute helper T cell counts 10^9/L) obtained in the 6-25 year, 26-45 year, 46-65 year and 65 year age groups were 0.54 ± 0.20, 0.55 ± 0.22, 0.59 ± 0.40 d 0.62 ± 0.23 respectively. No statistically significant trend uld be demonstrated between helper T cell counts and age (r = 0.114; P > 0.20). Mean helper T cells were 63±9.9%, 60±7.8%, ±13.8% and 60±14.9% of total T lymphocytes for the respective e groups.

Suppressor T cell count

Suppressor T cell numbers are shown in Table 9. Mean absolute unts ($x10^9$/L) of 0.35 ± 0.14, 0.37 ± 0.14, 0.51± 0.34 and 0.44± 22 cells were obtained in the respective age groups. There was significant correlation between suppressor T-cell number and e (r=0.176; P > 0.05). Mean suppressor T cells were 41±10.6%, ±7. %, 47±15.3% and 41±15.1% of total T lymphocytes for the spective age groups.

TABLE 7.

Mean T lymphocytes in the different age groups studied

Age group (in years)	n	Mean ($x10^9/L$)	1 s.d.
6-25	12	0.86	0.29
26-45	28	0.91	0.32
46-65	26	1.09	0.60
>65	24	1.04	0.33

$F = 0.177$; $P>0.05$.

TABLE 8.

Mean Helper T cells in the different age groups studied

Age group (in years)	n	Mean ($\times 10^9$/L)	1 s.d.
6-25	12	0.54	0.20
26-45	28	0.55	0.22
46-65	26	0.59	0.40
>65	24	0.62	0.23

$r = 0.114$; $P > 0.20$.

TABLE 9.

Mean Suppressor T cells in the different age groups studied

Age group (in years)	n	Mean ($x10^9/L$)	1 s.d.
6-25	12	0.35	0.14
26-45	28	0.37	0.14
46-65	26	0.51	0.34
>65	24	0.44	0.22

$F = 0.176$; $P > 0.05$

T4 : T8 Ratio

The mean T4 : T8 ratios obtained for the different age groups (6-25, 26-45, 46-65, > 65 years) in this study were 1.74 ± 0.81, 1.55 ± 0.54, 1.4±0.87 and 1.8 ±1.09 respectively. There were no significant differences among them (Students' t-test; p > 0.10 in all cases).

Null cells in the different age groups

Estimation of null cells yielded results shown in Table 10. The absolute counts ($x10^9/L$) obtained for the respective age groups were 0.21 ± 0.17, 0.23 ± 0.12, 0.29 ± 0.25 and 0.26 ± 0.17. There was no statistical difference in these mean values (r = 0.165; P>0.10). Mean null cells relative to total lymphocytes in the respective age groups were 13 ± 8.8%, 14 ± 7.0%, 15 ± 10.7% and 14 ± 9.7%

4.3 LEUCOCYTE MIGRATION INHIBITORY FACTOR ASSAY

4.3.1 INTRODUCTION: It is well known that T cell function as measured by delayed type hypersensitivity skin tests and proliferative responsiveness of lymphocytes to mitogenic stimulation decrease with increasing age in humans (Grossman et al., 1975; Muraskо et al., 1987). The

TABLE 10.

Mean Null-cells in the different age groups studied

Age group (in years)	n	Mean ($x10^9/L$)	1 s.d.
6-25	12	0.21	0.17
26-45	28	0.23	0.12
46-65	26	0.29	0.25
>65	24	0.26	0.17

$r = 0.165$; $P>0.10$

production of antigen-induced leucocyte migration inhibitory factor (L-MIF) in vitro correlates with the in vivo state of cell mediated immunity of the lymphocyte donor (Rocklin et al., 1970). Cellular immunologic capacity has been studied during ageing in Nigerians by carrying out L-MIF activity determinations.

4.3.2 SUBJECTS AND METHODS: In all, 150 subjects were studied. 35 of them are in the 6-25 years age group and 54 are 26-45 years old. Others include 33 subjects in the age group 46-65 years and 28 in the > 65 years age group.

Lymphocytes were activated with three antigens and one mitogen that stimulates T cell proliferation. They include measles virus, mycobacterial antigen, candida and concanavalin A.

The antigens: Live attenuated measles virus vaccine (lot EO382, Institut Merieux, Lyon, Franco) was used as antigen at a working dilution of 1 in 100 in RPMI (Rosewell Park Memorial Insitute) 1640 + 15% foetal calf serum (FCS). Bacille Calmotte Guerin (BCG) Pasteur vaccine (Lot R 5524, Institut Pasteur, France) served as the source of mycobacterial antigen used at a working dilution of 1 in 100. Concanavalin A (Pharmacia Fine Chemicals, Sweden) was used at a concentration of 20 ug/ml.

A heavy culture of candida species, in peptone water,

was obtained from Medical Microbiology Department of the University College Hospital, Ibadan, Nigeria. The culture was washed thrice in PBS pH 7.2 and killed by boiling at 100°C for 30 minutes. The final suspension gave an absorbance of 0.43 in a 10mm light path at 640nm using a SP6-200 spectro-photometer (PYE UNICAM Cambridge, England). This final suspension was diluted 1 in 1000 with RPMI 1640-FCS for use in leucocyte migration inhibition assay.

Dose-response experiment for the antigens: The working concentrations of the antigens stated above were determined, after dose-response trials. Measles and B.C.G vaccines were tested at 1 in 10, 1 in 100 and 1 in 1000 dilutions. Candida antigen suspension was tested at similar dilutions. Con. A was first tested at 100 ug/cm^3, 10 ug/cm^3 and 1 ug/cm^3; and then at 50 ug/cm^3 and 20 ug/cm^3 in a second preliminary experiment.

Procedure for the assay: The method described by Hudson and Hay (1976) was followed. 8cm^3 of heparinised blood was mixed with equal volume of 3% dextran solution (Grade A, molecular weight 200,000-275,000; BDH Chemicals Limited, Poole, England). This was allowed to stand at 37°C for 60 minutes. The leucocyte-rich supernatant plasma was spun in a bench centrifuge at 300 x G for 10

minutes at room temperature. Sedimented cells were washed three times in warm RPMI 1640. Centrifugation was at 300 x G for 10 minutes at room temperature. The cells were resuspended in 10 cm^3 of RPMI 1640 containing 15% foetal calf serum. They were counted in a haemocytometer and adjusted to 100 x 10^9 cells/L. Capillary tubes (haematocrit) were filled two-third full with the cell suspension, sealed with plasticine at one end, and packed by centrifugation at 850 x G for 10 minutes at room temperature. The capillary tubes were then cut at the packed cell-medium interface. A small drop of silicone grease (M494; Imperial Chemical Industries Ltd., U.K.) was put in one corner of each well of an improvised migration chamber. The cut capillaries were placed in the chamber and held in place by anchoring the sealed ends in silicone grease (Figure 5). The wells were immediately topped up with medium or antigen in medium solutions in duplicates. Both medium and antigen solutions contained 15% foetal calf serum. The chambers were covered with sterile culture plate sealing tapes avoiding air bubbles, and incubated for 24 hours at 37°C.

The image of the migration field was projected on the screen of an immunodiffusion plate reader (Osram 64 425; Behring Institut) and traced on a piece of graphic

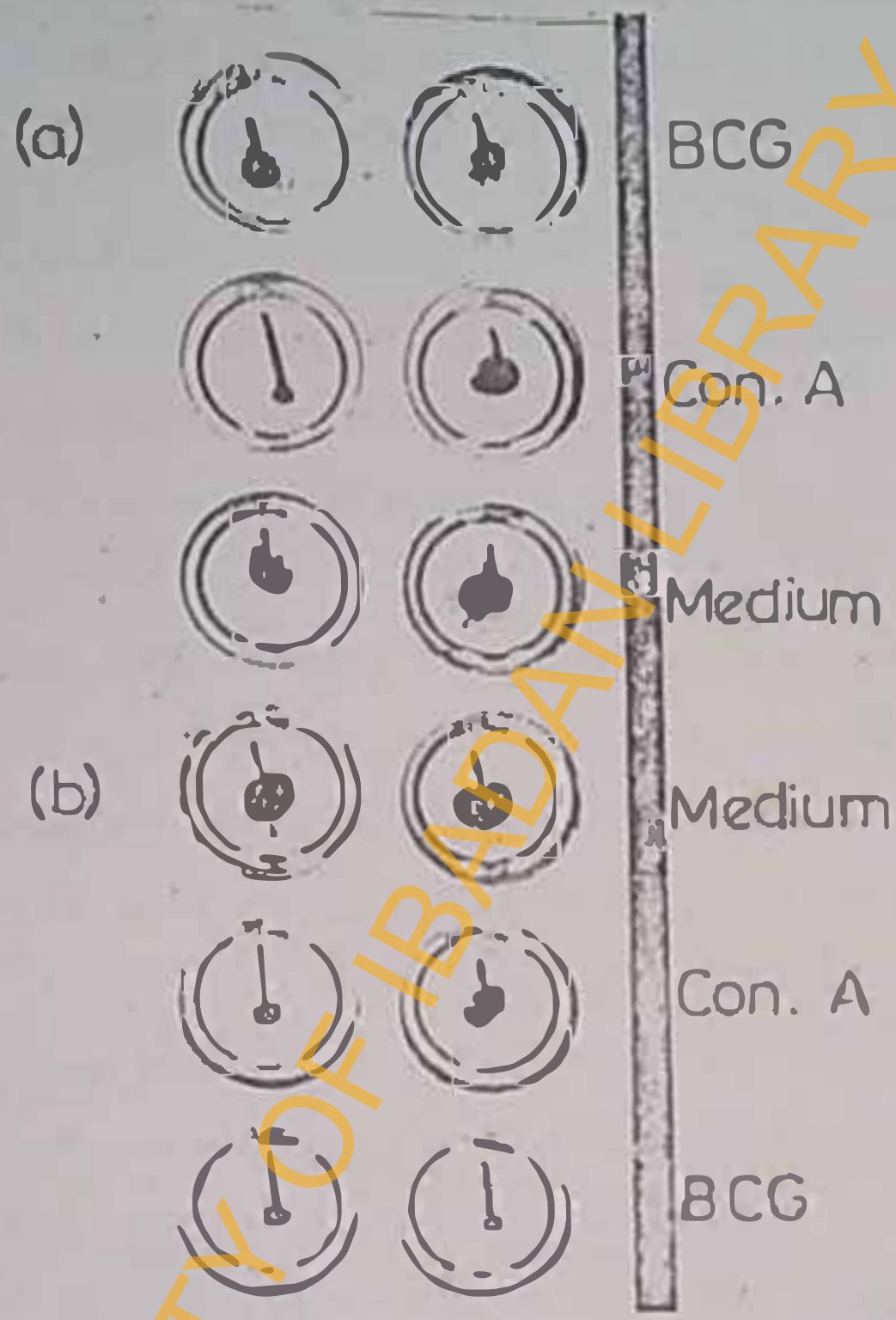

Figure 5. Leucocyte migration inhibitory factor test showi
leucocyte migration after 24 hours incubation in
medium, Con. A and BCG. The samples are from m
aged 72 years (a) and 33 years (b).

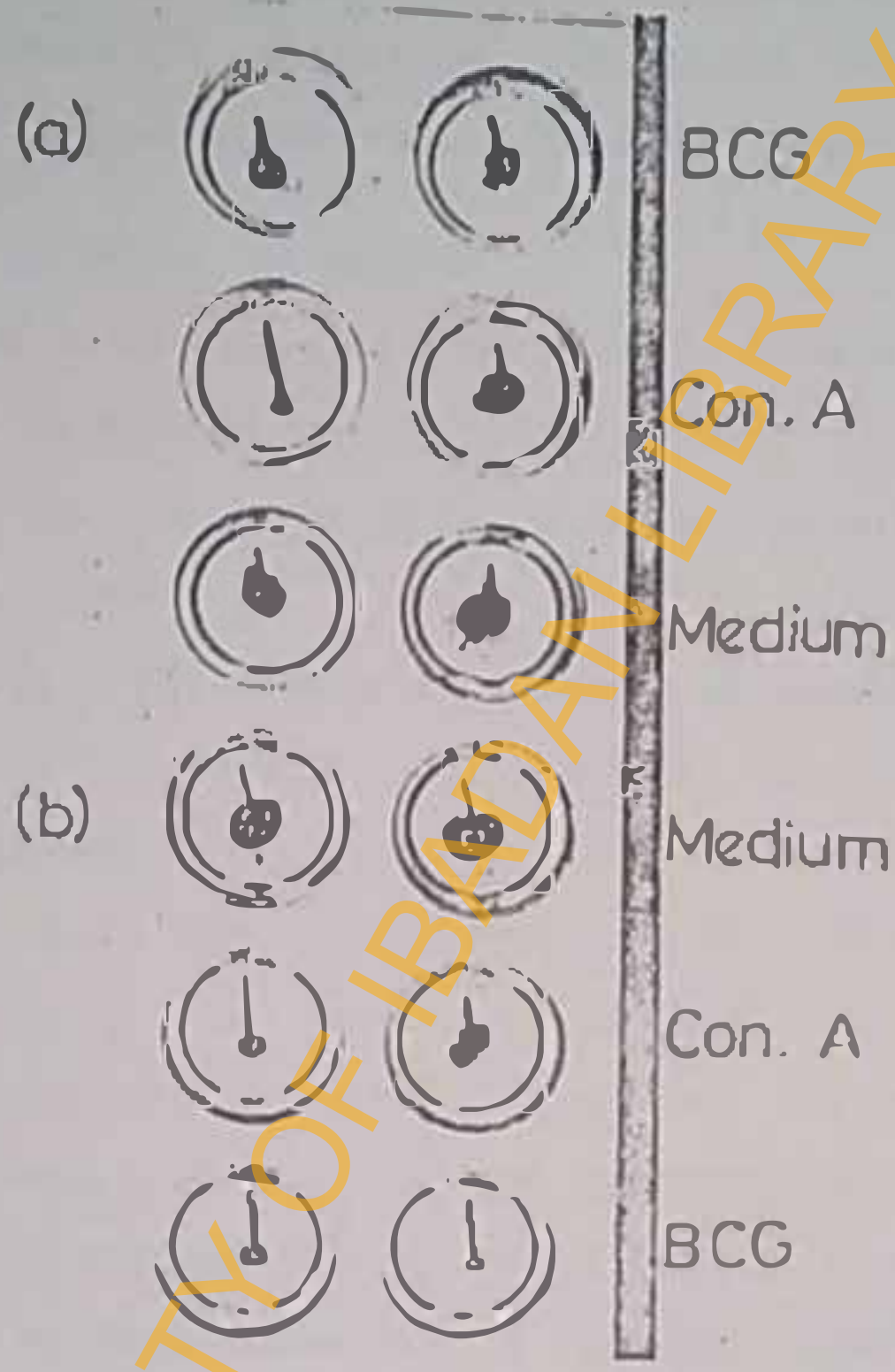

Figure 5. Leucocyte migration inhibitory factor test showing leucocyte migration after 24 hours incubation in medium, Con. A and BCG. The samples are from mal aged 72 years (a) and 33 years (b).

paper. The area of the traced portion was assessed by counting the number of small squares enclosed (Figure 6). The percentages of migration inhibition due to the presence of the antigens were calculated thus:

$$\% \text{ migration inhibition} = \frac{C - T}{C} \times 100$$

where C is the area of migration in medium and T is the area of migration in antigen solution.

A migration inhibition value of 20 percent or greater was taken as positive.

4.3.3 RESULTS: Preliminary experiments to determine the working concentrations of concanavalin A, Candida species antigen, measles virus and BCG vaccines in the L-MIF assays were done. Results obtained for Con. A are shown in Table 11. As much as it was practicable, concentrations giving results close to 50% migration inhibition were selected for the determinations. This was to ensure that quantitative variations in migration inhibition could be assessed. A second dose-response experiment was done with Con. A because none of the concentrations (i.e 100 ug/ml, 10ug/ml and 1ug/ml) used in the first experiment gave migration inhibition value close to 50%. For concanavalin A the working concentration chosen was 20 ug/ml. It was 1 in 1000 dilution for the locally prepared Candida species antigen suspension. Measles virus and BCG vaccines were used at 1 in 100 dilution. These different chosen concentrations of Con. A, Candida, Measles virus and BCG vaccines gave mean migration inhibition

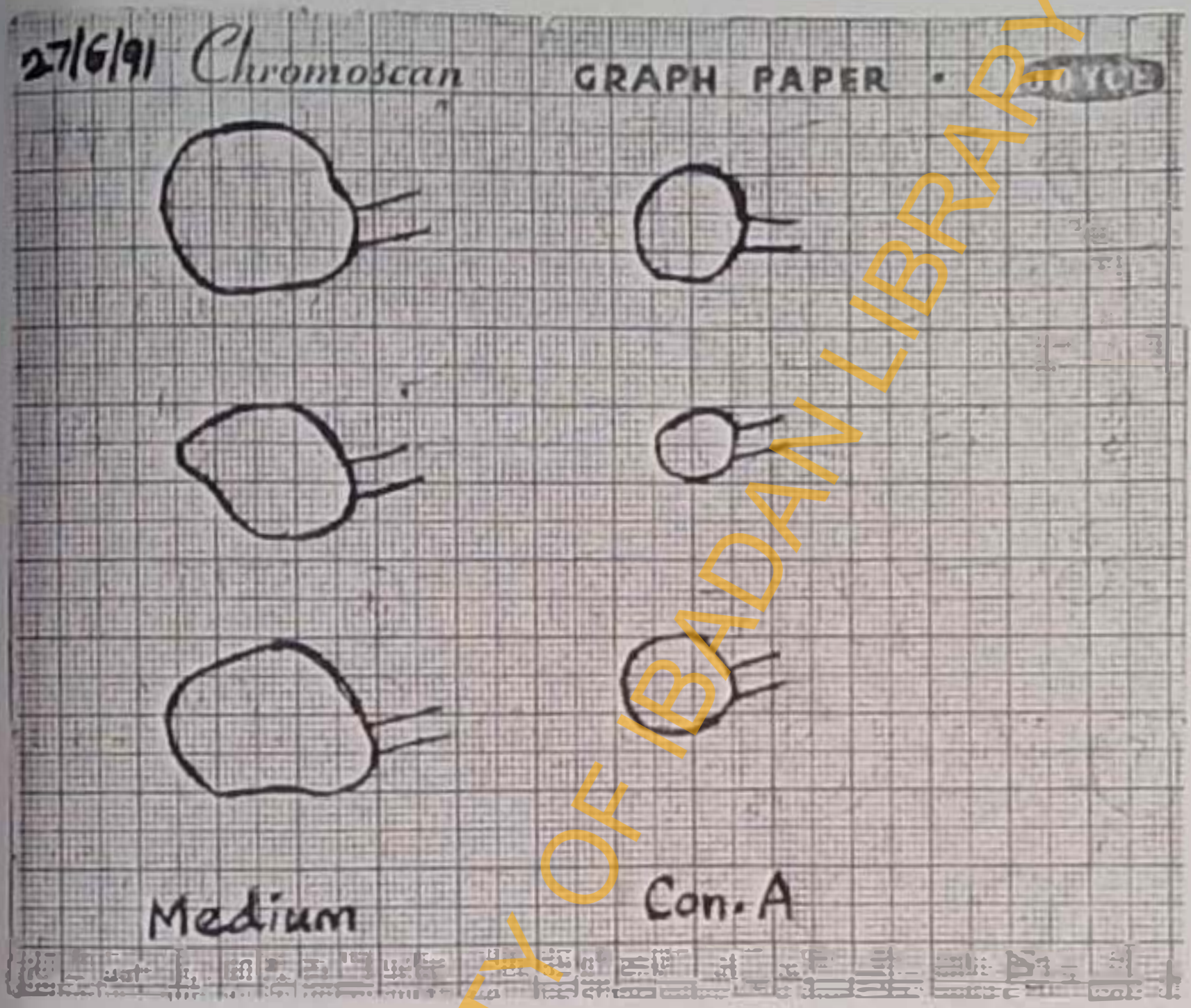

Figure 6. Graphic paper showing examples of migration areas traced.

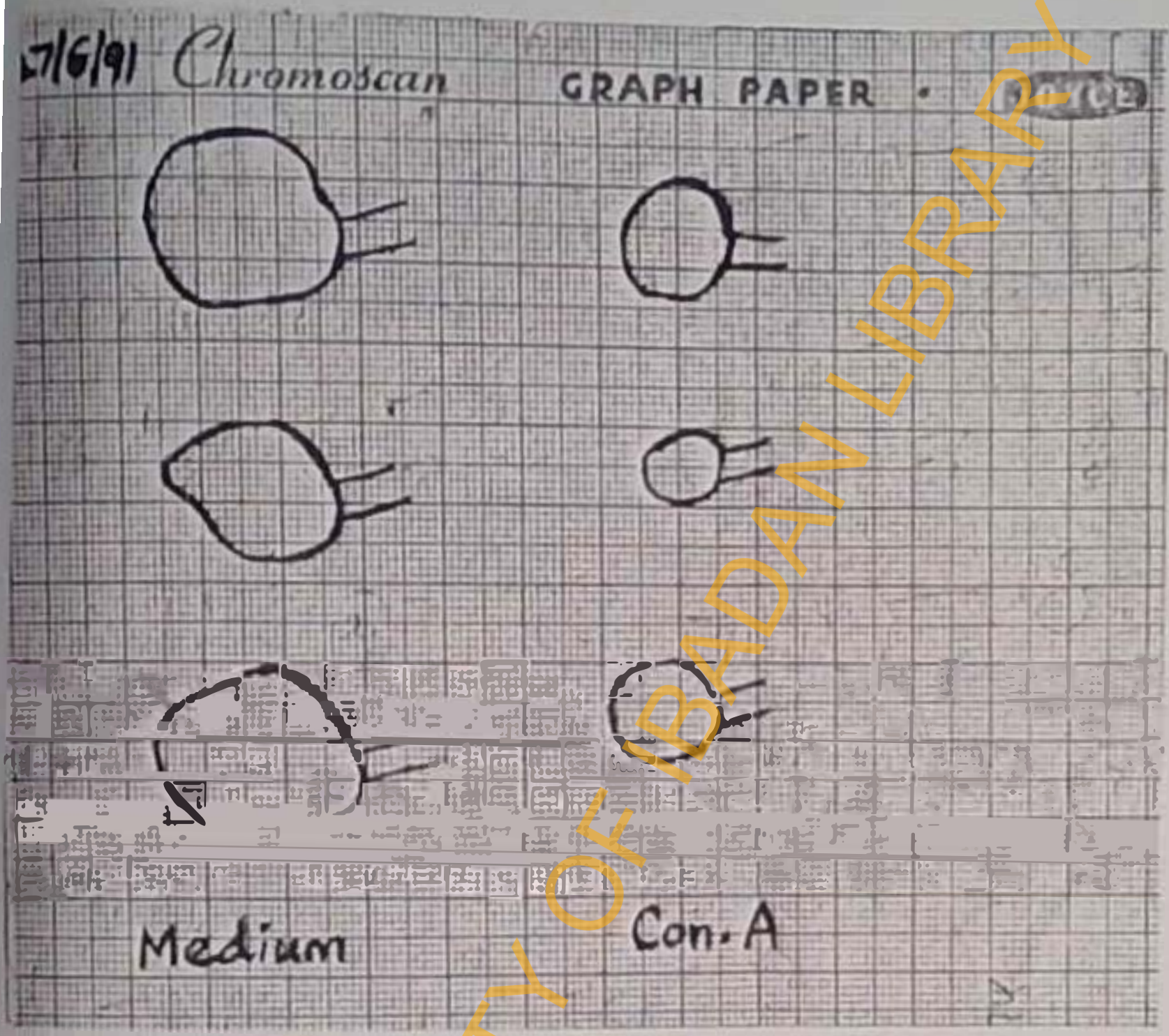

Figure 6. Graphic paper showing examples of migration areas traced.

TABLE 11.

Dose-response trials for Con. A in leucocyte migration inhibitory factor assay.

	MITOGEN CONCENTRATION (ug/ml)		
Subjects	100	10	1
1.	58.5	0	0
2.	72.3	45.4	55.4
3.	86.1	40.3	44.4
4.	90.9	10.9	0
5.	94.8	29.3	36.2
6.	96.2	11.2	22.6
$\bar{x}$	83.1	23.2	26.4
s.d	14.8	18.0	23.1

	MITOGEN CONCENTRATION (ug/ml)	
Subjects	50	20
1.	92.9	14.3
2.	97.3	62.2
3.	96.6	75.9
4.	96.4	75.0
$\bar{x}$	95.8	56.9
s.d	2.0	29.0

Values are the Migration Inhibition in percentages.

values of 56.9±29.0%, 33.0±22.9%, 31.6±21.9% and 30.5± 32.3% respectively in the preliminary experiments. The level of migration inhibition (of leucocytes from the subjects studied) obtained with each of the lymphocyte activators are as follows:

Concanavalin A: Mean percentage migration inhibition values obtained with Con. A in the different age groups are shown in Table 12. A maximum of 41.8% migration inhibition was recorded in the 6-25 year age group.This value declined progressively with age down to 14.3% migration inhibition in the > 65 year age group. This is only 34% of the migration inhibition obtained for the 6-25 year age group. The depression with age in the magnitude of migration inhibition obtained showed statistical significance in general ($r = -0.425$; $P < 0.01$; Figure 7).

Candida antigen: Table 13 shows the mean migration inhibition obtained with Candida antigen in the different age groups. The 6-25 year age group gave the maximum migration inhibition of 42.4%. This value decreased progressively with age reaching 16.3% migration inhibition in the > 65 year age group. This represents 38% of the migration inhibition value for the 6-25 year age group. A significant inverse correlation ($r = -0.197$; $P < 0.01$) was obtained between migration inhibition and age (Figure 8).

Measles virus vaccine antigen: Percentage migration

TABLE 12.

Mean Percentage migration inhibition with Con. A in the different age groups

Age group (yrs):	6 - 25	26 - 45	46 - 65	> 65
n	35	54	27	28
mean	41.8	28.1	23.3	14.3
s.d	27.3	21.6	24.7	18.7

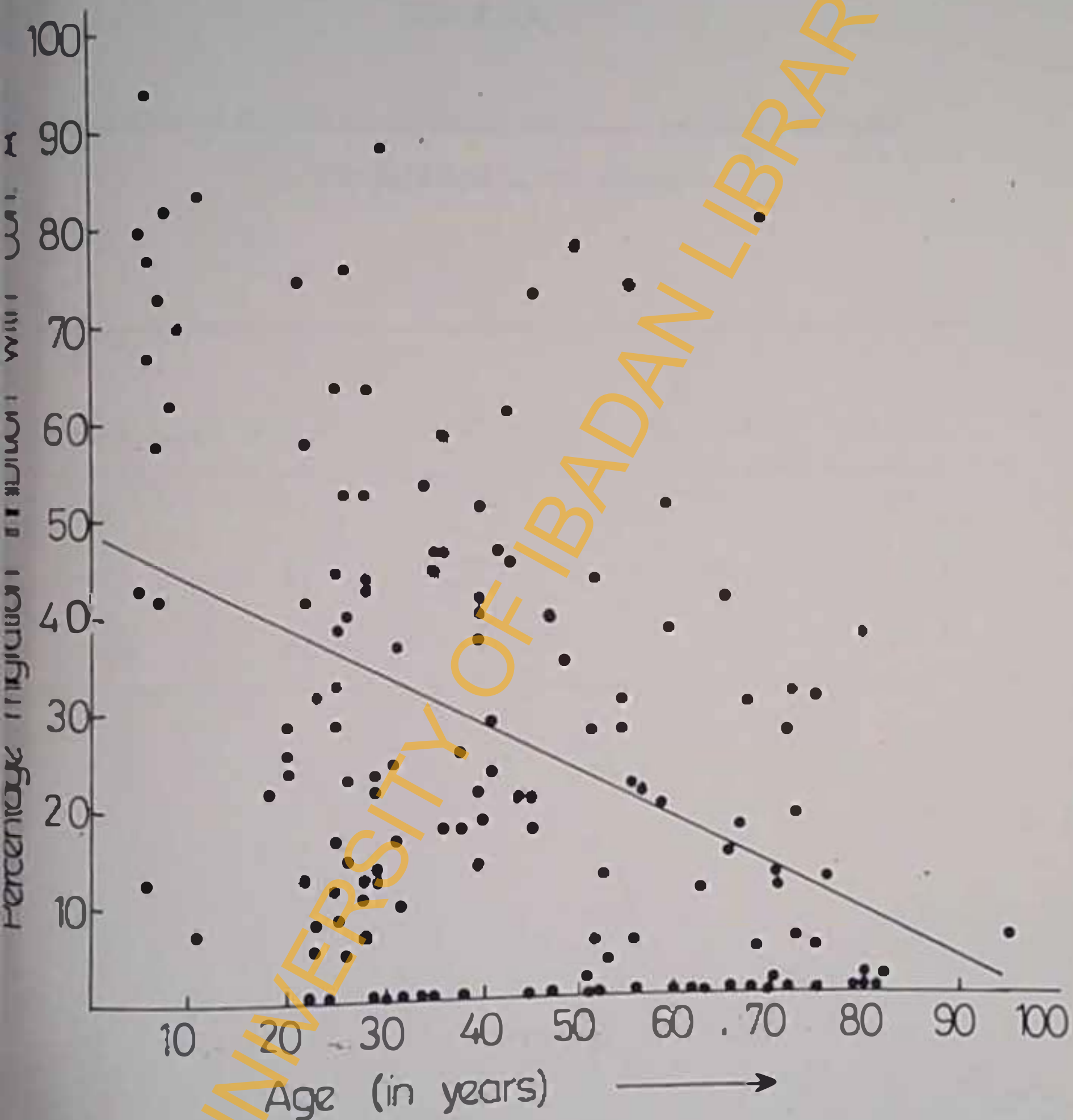

Figure 7. Correlation of leucocyte migration inhibition using Con. A with age. Y=48.47 - 0.498X. r = -0.425; P < 0.01.

TABLE 11.

Mean Percentage migration inhibition with Candida antigen in the different age groups.

Age group (yrs):	6 - 25	26 - 45	46 - 65	> 65
n	31	54	33	28
mean	42.4	23.9	19.1	16.3
s.d	27.5	16.8	20.9	17.7

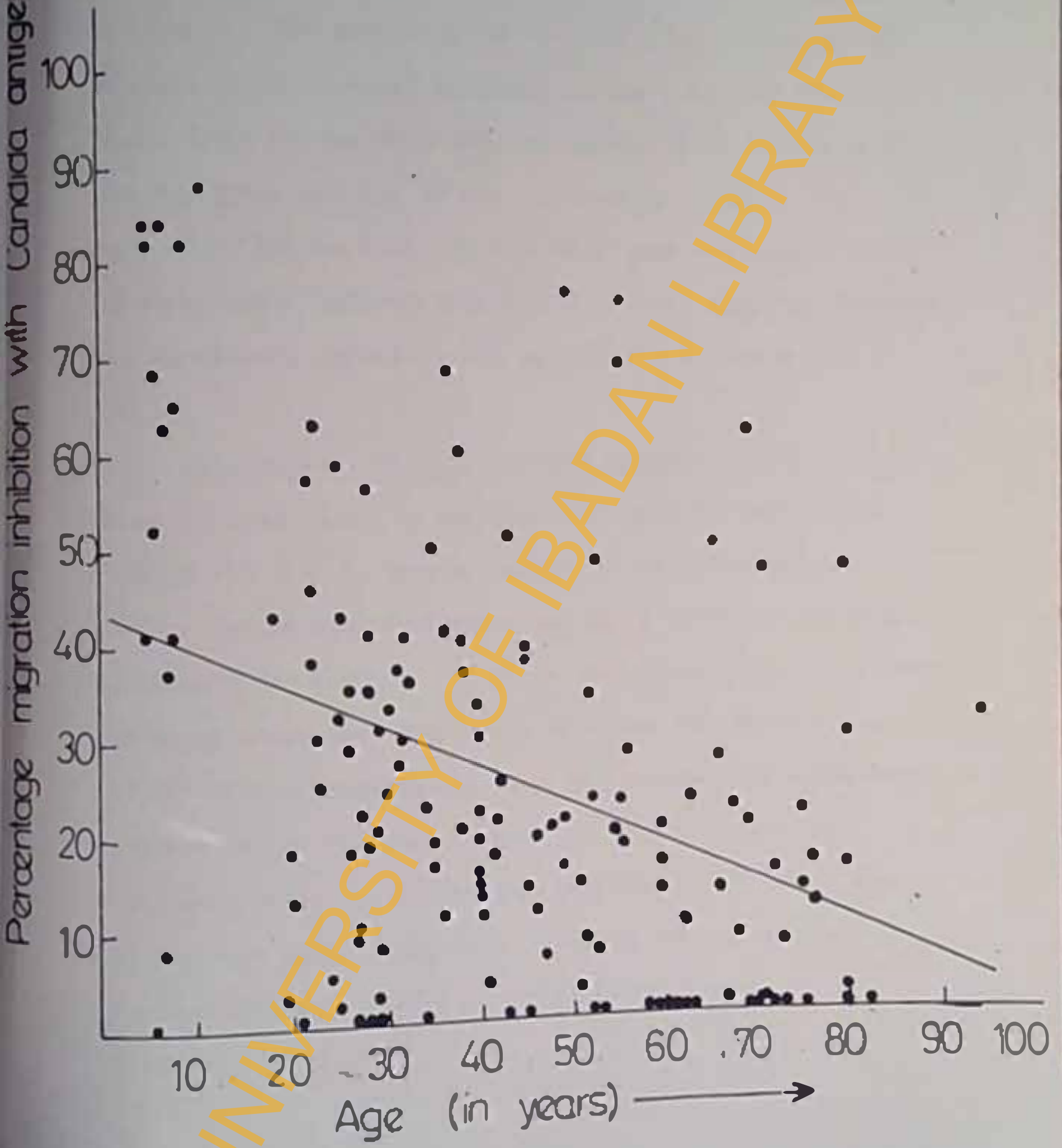

Figure 8. Correlation of leucocytu migration inhibition using Candida antigen, with age Y=43.28 - 0.423X. r=-0.397; P<0.01.

inhibition figures obtained with measles antigen are shown in Table 14. The general trend was that of decreasing values with age: 37.6% migration inhibition for the 6-25 year age group, 33.8% for the 26-45 year ago group; 29.3% for the 46-65 year age group and 21% for the > 65 year age group. The mean values for the 6-25 year and 28-45 year age groups were not significantly different ($t=0.624$, $P > 0.20$). Migration inhibition was significantly correlated with age (Figure 9: $r = -0.235$; $p < 0.05$)

Mycobacterial (B.C.G. Vaccine) antigen: Table 15 shows the mean values of migration inhibition (in percentages) obtained with B.C.G. vaccine antigen in the different age groups. People aged 6-25 years and 26-45 years did not differ significantly ($t = 0.867$; $p > 0.20$) in their mean values. However significant progressive depression in values (Students' t-test; $p < 0.05$) were obtained in the older age groups. Mean migration inhibition of 6.8% for the > 65 year age group was only 27% of the mean value (24.8% migration inhibition) obtained in the 6-25 year age group. Migration inhibition was significantly correlated with age (Figure 10: $r=-0.372$; $p < 0.01$).

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TABLE 14.

an percentage migration inhibition with measles antigen in the different age groups

Age group (yrs):	6 - 25	26 - 45	46 - 65	> 65
n	22	41	21	20
mean	37.8	33.8	29.3	21.0
s.d	21.9	26.5	23.2	23.6

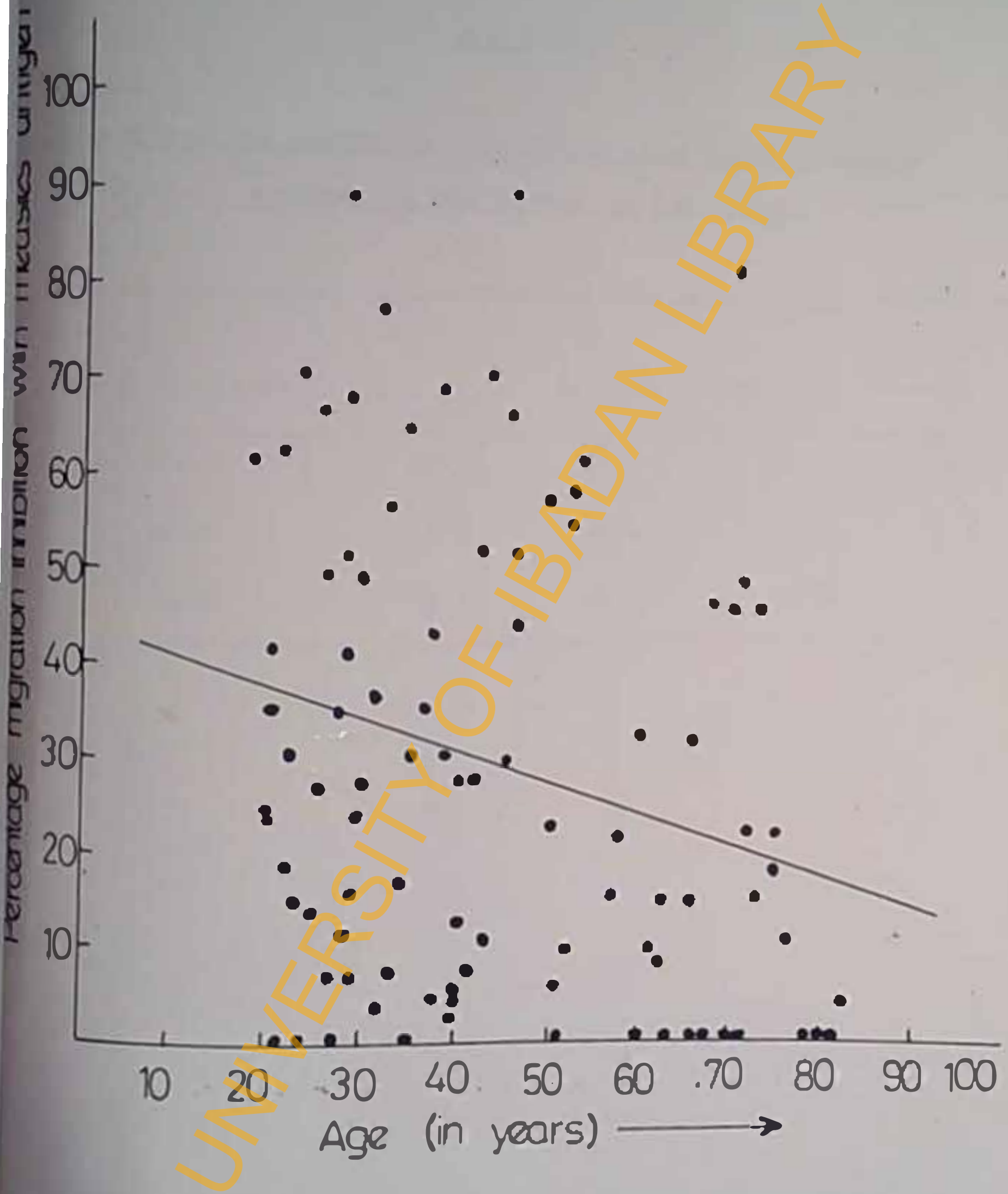

Figure 9. Correlation of leucocyte migration inhibition using measles antigen with age $Y=43.75-0.318X$.
$r=-0.235$; $P<0.05$.

TABLE E.

Mean Percentage Migration Inhibition with B.C.G. vaccine antigen in the different age groups

Age group (yrs):	6 - 25	26 - 45	46 - 65	> 65
n	23	47	21	20
mean	24.8	29.6	18.1	6.8
s.d	19.9	22.6	19.5	7.5

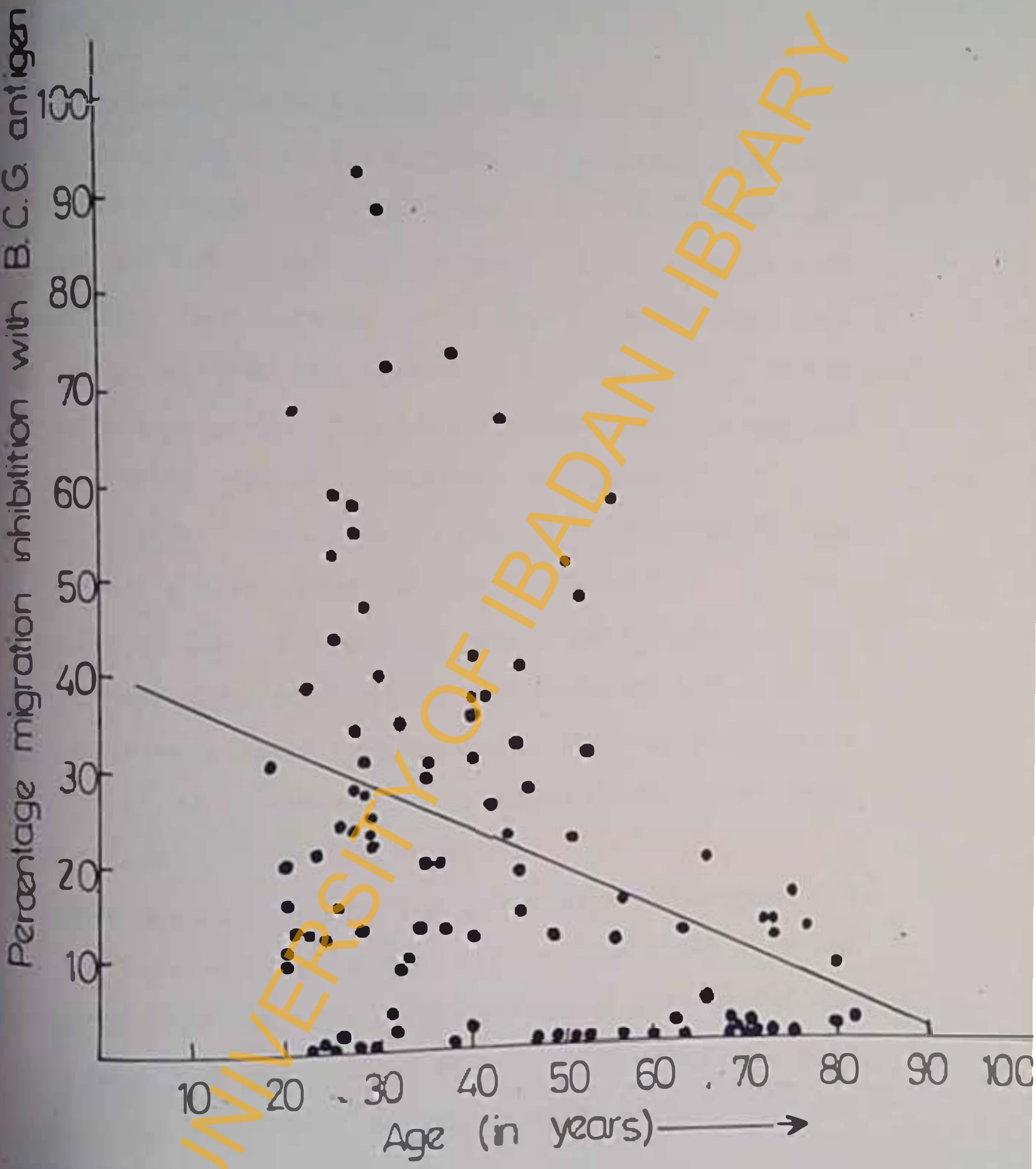

Figure 10. Correlation of leucocyte migration inhibition using B.C.G. with age Y=40.90-0.448X. r=-0.372; P<0.01.

Positivity rates in migration inhibition: Exhibited in Figure 11 are positivity rates in leucocyte migration inhibitory factor assay with con. A, Candida antigen, measles and B.C.G. vaccine antigens. The prevalence rate of positive results with con. A of 74% in the 6-25 year age group declined progressively with age to 25% in the >65 year age group. Positivity rates were 57% and 52% for the 26-45 year and 46-65 year age groups respectively. Statistical analysis by Chi-square test show clear significance (X^2 = 49.63; P<0.005).

74% of people aged 6-25 years were positive with Candida antigen. Other age groups recorded 56%, 36% and 36% positivity rates respectively, showing progressive reduction in values. Chi-square analysis show significance (X^2 = 40.13; P<0.005).

For measles antigen the positivity rates were 67% for the 6-25 year age group, 61% for the 26-45 year age group, 50% for the 46-65 years age group and 40% for the >65 year age group. Statistical analysis show significant depression in values with age (X^2 = 17.31; P<0.005).

The positivity rates with B.C.G. vaccine antigen were 44%, 68%, 33% and 6% respectively for the different

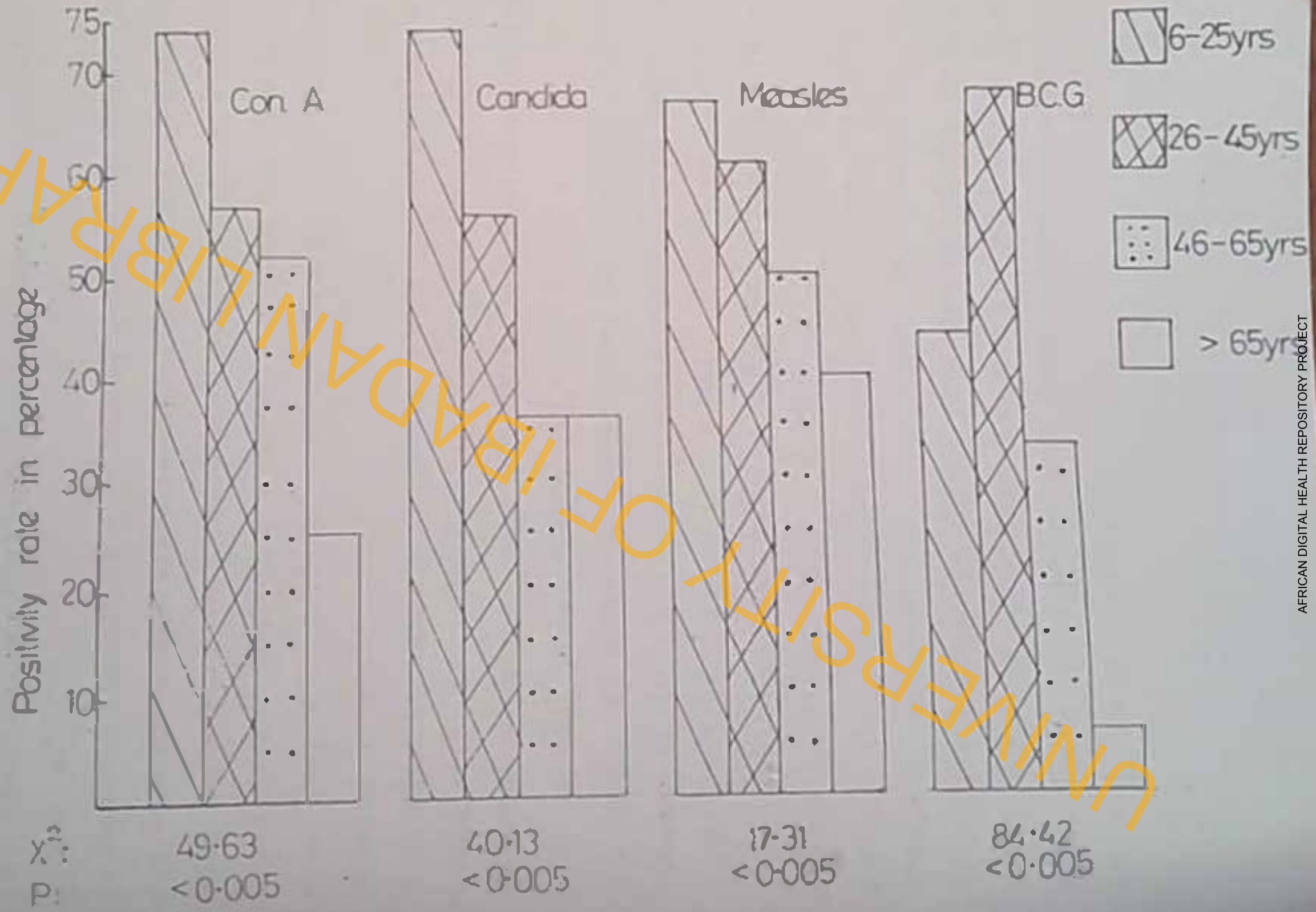

Figure11. Positivity rates leucocyte migration inhibitory fa
assay in the age groups studied using four different

age groups. The differences in mean values were significant (X^2 = 84.42; P<0.005).

Correlation analysis between leucocyte migration inhibition and:

Plasma transferrin levels: Plasma transferrin levels (see 5.2) did not correlate with migration inhibition obtained with any of the four lymphocyte activators (r= - 0.104, P > 0.20 for Con. A; r = 0.030, P > 0.20 for Candida; r = -0.144, P > 0.20 for BCG; r= -0.246, P > 0.05 for measles antigen).

T lymphocyte number: There was no significant correlation between migration inhibitory factor activity and the number of resting T cells and its subsets (see 4.2) in most cases (Table 16).

C4: Table 17 displays significant inverse correlation between C4 (see 5.1) and migration inhibition with Con. A and candida antigen (P < 0.05). Migration inhibition with measles and BCG vaccine antigens lacked significant correlation with C4 (P > 0.20).

Circulating Immune Complexes: Results of correlation analysis between CIC concentrations (see 7.1) and migration inhibitory factor activity are shown in Table 18. None of the four lymphocyte activators shows significant correlation of migration inhibition with CIC (P> 0.05 in all cases).

TABLE 16.

Correlation-analysis between migration inhibition (M.I.) and T lymphocyte number

Variables	r	t	P
Con. A - MI/pan T cell	0.164	1.703	> 0.05
Candida Ag. - MI /pan T cell	-0.094	0.562	> 0.20
Measles Ag.-MI /pan T cell	0.293	3.000	< 0.01
BCG Ag. - MI/pan T cell	-0.057	0.129	> 0.20
Con. A - MI/ helper T cell	-0.099	0.609	> 0.20
Candida Ag.-MI/helper T cell	-0.105	0.839	> 0.20
Measles Ag.-MI/helper T cell	0.274	1.610	> 0.10
BCG Ag.- MI/helper T cell	-0.064	0.163	> 0.20
Con. A -MI/suppressor T cell	-0.069	0.549	> 0.20
Candida Ag.-MI/suppressor T cell	-0.040	0.319	> 0.20
Measles Ag.-MI/suppressor T cell	0.163	0.029	> 0.20
BCG Ag.-Mg/suppressor T cell	-0.054	0.112	> 0.20

TABLE 17.

Correlation analysis between C4 and migration inhibition of leucocytes (MI).

Variables	r	t	P
C4 / Con. A - MI	- 0.203	2.286	< 0.05
C4 / Candida Ag.- MI	- 0.194	2.229	< 0.05
C4 / Measles Ag. - MI	- 0.087	0.782	> 0.20
C4 / BCG Ag. - MI	- 0.096	0.883	> 0.20

TABLE 18.

Correlation analysis between circulating immune complexes (CIC) and migration inhibition (MI) of leucocytes

Variables	r	t	P
CIC / Con. A - MI	- 0.160	1.925	> 0.05
CIC / Candida Ag.- MI	- 0.122	1.480	> 0.10
CIC / Measles Ag. - MI	- 0.090	0.868	> 0.20
CIC / BCG Ag. - MI	- 0.042	0.418	> 0.20

Rheumatoid factor (Rose- Waaler test) titres: No correlation was observed between the titres of rheumatoid factors (see 7.2) and leucocyte migration inhibition (Table 19; P > 0.10 in all cases).

4.4 MANTOUX DELAYED HYPERSENSITIVITY SKIN TEST

4.4.1 INTRODUCTION: It is generally agreed that there is a decrease in cellular immunity with increasing age (Murasko et al., 1986). However, the findings from different laboratories on mitogen responses and studies of delayed-type hypersensitivity are inconsistent (Murasko et al., 1986). Skin testing remains by far the most direct means for the clinical assessment of cellular responses in vivo (Spitler, 1976). The depression of delayed hypersensitivity skin responses to ubiquitous antigens (including mycobacteria) with advancing age is well known (Toh et al., 1973; Mackay, 1972; Waldorf et al., 1968). Correlation studies have shown that mortality in old people may be related to the failure of cell-mediated immunity as shown by skin test responses (Roberts-Thomson et al., 1974). This study examines Mantoux skin test responses during ageing in Nigerians.

4.4.2 SUBJECTS AND METHODS: The Mantoux test scores of 274 healthy students and staff at the University of Ibadan and their families were studied. Their ages ranged between 6 to 60 years. Mantoux test was carried out as part of general medical examination for students on admission and for the staff and their families on appointment. Those included in the study

TABLE 19.

Correlation analysis between the titres of rheumatoid factor (Rose-Waaler test) and migration inhibition (MI) of leucocytes

Variables	r	t	P
RF / Con. A - MI	- 0.014	0.142	> 0.20
RF / Candida Ag.- MI	- 0.057	0.605	> 0.20
RF / Measles Ag. - MI	0.190	1.547	> 0.10
RF / BCG Ag. - MI	0.095	0.802	> 0.20

RF is rheumatoid factor.

were found to be healthy. The test was therefore used to assess cell mediated immunity and not infection with mycobacteria.

Each of the subject received 0.1 ml (i.e. 2 tuberculin units - T.U.) of tuberculin purified protein derivative (PPD) intradermally on the forearm. The site of the injection with PPD (Batch RT 23; Staten Serum Institute, Copenhagen; Denmark) was marked with a circle about 5 cm in diameter using an indelible marking pencil. Results were read after 48 hours as the diameter of erythema and induration produced. Subjects with negative results were re-tested three months later. Some of these (8 of 12 people aged 6-25 years and 2 of 5 subjects aged 26-45 years) failed to report for re-testing and were thus excluded from the study. Excluded were 5.6%, 2.0% and 0% of people aged 6-25 years, 26-45 years and 46-65 years of age respectively. All those included in this report were Mantoux test positive. Differences in mean reaction diameters between males and females were analysed for the subjects.

4.4.3 RESULTS: Mean tuberculin PPD reaction diameters displayed in Table 20 show significant increase in the value for the 26-45 years age group in comparison with the mean for the 6-25 years age group. The responses began to decrease afterwards. No significant difference between the two sexes ($P > 0.20$) was obtained in tuberculin PPD reactivity in the subjects (Table 21). The mean values observed for females were slightly higher than those of males. The values for males increased between the 6-25 years and the 26-45 years age groups and then decreased. A similar pattern of change in mean values was observed for females. The stepwise changes in the mean values for males show statistical significance ($P < 0.05$ in both cases). In females, the mean value for the 26-45 years age group is significantly higher than the mean values for the 6-25 years ($P < 0.01$) and slightly higher than the 46-65 years ($P > 0.20$) age groups.

TABLE 20.

Mean tuberculin PPD reaction diameter (TRD) of healthy Nigerians during ageing.

Age group (yrs) :	6-25	26-45	46-65
n	136	98	40
mean (mm)	11.1	14.2	12.2
s.d	4.70	4.89	7.17
t-test	P<0.01	P>0.10	P>0.05

TABLE 21.

Sex comparison of tuberculin (PPD) reactivity in healthy Nigerians during ageing.

Age group (years)	Sex	n	mean (mm)	s.d	P (Student's t-te
6 - 25	M	89	10.9	4.33	
	F	47	11.6	5.36	>0.20
26 - 45	M	71	13.8	5.07	
	F	27	15.0	4.33	>0.20
46 - 65	M	26	11.9	6.91	
	F	14	12.6	7.87	>0.20

DISCUSSION

otal lymphocytes obtained from differential leucocyte counts:

The microscopic method of counting total lymphocytes is still of acceptable accuracy. A more accurate determination by fluorescence activated cell sorting is not yet possible as most available monoclonal antibody markers for lymphocytes (e.g CD37, D57) also recognise other tissue components. Enumeration of total peripheral blood lymphocytes in the study population show progressive but generally insignificant increase with age. Differential white blood cell counts show that absolute lymphocyte numbers did not differ significantly among the age groups except for a reduction in the 26-45 years age group which is unexplained. The mean count for this age group ($1.66 \pm 0.52 \times 10^9$/L) compares well with the value reported previously ($1.63 \pm 0.71 \times 10^9$/L) in twelve control Nigerians aged 28-42 years (Onyemelukwe et al., 1990). Weksler and Hutteroth (1974) also found that the absolute number of peripheral blood lymphocytes were the same in old and young persons. Lymphocyte depletion cannot be responsible for reduced cell mediated immunity in ageing.

Enumeration of T cells and subsets: An age-related decrease in T cell function as defined by skin tests (Grossman et al., 1975) and mitogen responsiveness (Murasko et al., 1986; Murasko et al., 1987) hasbeen described in humans. A possible hypothesis is that this decrease in T cell function is associated with changes in

4.5 DISCUSSION

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T lymphocyte subpopulations. The absolute numbers of T cells and null cells did not differ significantly between the groups in this study. These results agree with the findings of Weksler and Hutteroth (1974). It was found here that helper T-cell and suppressor T-cell numbers do not alter significantly with age. Matour et al. (1989) also could not find significant differences in the absolute numbers of T-lymphocytes and its subsets between old and young persons.

The relative T lymphocyte subpopulation of 57± 9% reported by Onyemelukwe et al. (1990) is similar to the figures of 52-54% observed presently. The absolute T lymphocyte count in both reports are also similar. Salimonu et al. (1982) obtained 45± 10% T lymphocytes in peripheral blood before measles virus immunization in children. The lower value obtained by Salimonu et al. (1982) compared with the other two reports may be because they employed sheep red blood cell rosetting technique. Cell surface staining by specific antibody was used by Onyemelukwe et al. (1990) and also in the present study. Also, Salimonu et al.(1982) studied young children while the subjects in the other two studies are adults.

The proportion and number of null cells reported here is a little lower than those documented by Onyemelukwe et al. (1990). This may be so because

monoclonal antibodies used in immunofluorescent assay of T and B cells in this study are more restricted in their cell surface antigen recognition than polyclonal antibodies used previously. In the present report, relative values of null cells and T cells are similar to values obtained by Greenwood et al. (1977) in children on recovery from malaria infection. However, their absolute figures are higher than in this report. Null cells are known to increase in acute malaria infection and lymphocytes from such patients show increased K cell activity against chicken red blood cells (Greenwood et al., 1977). It is likely that some of the increased number of null cells were K cells. Similar number of null cells obtained in this study compared to those in healthy children (Greenwood et al., 1977) and adults (Onyemelukwe et al., 1990) suggests that the subjects are free from infection.

There are no previous reports of helper T cell or suppressor T cell subpopulations in healthy Nigerians with which the present findings can be compared. However, the relative figures observed here are comparable with the value of 60% of T cells for helper T cells and higher than 30% of T cells for suppressor T

cells (Stites, 1982) previously reported in caucasians. Environmental or genetic reasons may be responsible for any difference.

Leucocyte migration inhibitory factor assay: It appears that age-related changes in the number of circulating T cells are minimal and the determination of changes in T cell function during ageing may prove more important. Most previous studies on the functional analysis of cell-mediated immunity during ageing have employed lectin-lymphocyte stimulation assays and delayed type hypersensitivity skin tests. This study used the Leucocyte Migration Inhibitory factor activity determinations to assess cellular immune function during ageing in Nigerians. Migration inhibitory factor is produced (along with other lymphokines) by sensitized lymphocytes when activated in-vitro by the sensitizing antigen. The production of antigen-induced migration inhibitory factor in vitro correlates with the in-vivo state of cellular hypersensitivity of the lymphocyte donor (Rocklin et al., 1970). The measurement of migration inhibition of leucocytes on activation by a mitogen (concanavalin A) and three antigens commonly encountered in the Nigerian environment (Candida, measles virus and B.C.G. vaccine antigens) in this study thus

offers an opportunity to study the responses of these cells in the laboratory at the effector functional level.

The percentage migration inhibition results obtained are generally lower than values previously reported (50 ±10%) for this environment in ten control Nigerians (Idoko et al., 1987) using Staphylococcus aureus to activate lymphocytes in vitro. Ade-Serrano et al., (1975) have also used the Leucocyte Migration Inhibition test to study B.C.G. immunoconversion in healthy young adult Nigerians. It is difficult to compare the present values with their findings as they obtained reversed results showing rising migration indices on immunoconversion.

A progressive age-related depression in cellular immune function was revealed by this study. Both mean migration inhibitory factor activity and the incidence of positive tests were found to decrease as Nigerians age. In general, individual data (which can be visualised from the range of values reported) indicate that though mean cellular immune responses are reduced in ageing people, it is not common to all of them. For instance, the migration inhibition generated by lymphocytes from some old people in this study was comparable to those obtained

in lymphocytes from young people. It will be interesting to know if immune status (especially cellular immunity) in ageing individuals correlates with eventual longevity.

Roberts-Thomson et al. (1974) found that the mortality rate of people over eighty years of age, who were hyporesponsive in tests for delayed hypersensitivity, was significantly greater over a two-year period than that of comparable people who were not hypo-responsive. Also, Murasko et al. (1986) obtained results that suggest that there may be a selection process in which the subjects who live to the age of 90 are those whose immune systems demonstrate the least decrease in activity. Correlation of mortality rate in old people with failure of cell-mediated immunity as shown by delayed-type hypersensitivity reactions has been obtained also in the case of cancer, where mortality rate was greater in those anergic to dinitrochlorobenzeno (Eilber and Morton, 1970; Wells et al., 1973). The increased mortality rate associated with impaired cell mediated immune responses, whether in old age or in cancer, is unexplained (Roberts - Thomson et al., 1974). One possibility is predisposition to more numerous and more frequently fatal infections, especially illnesses associated with seasonal epidemics due to new

types or strains of bacterial or viral pathogens.

The possibility that the depression with age in lymphokine (L-MIF) activity observed in this study is due to nutritional modulation is ruled out because those studied had comparable levels of components used to index nutritional state (see 2.5). This is confirmed by the lack of significant correlation between migration inhibition with any of the four lymphocyte activators and plasma transferrin levels. There was also no significant correlation between migration inhibition by any of the four activators and the number of resting T cells and its subsets. Thus, numerical alterations in these cells cannot be responsible for the results obtained. Alterations in the levels of complement proteins may be expected to affect immune responses as complement and immune response genes are associated at the major histocompatibility complex. Correlation between C4 and migration inhibitory factor activity with Con. A and Candida antigen were demonstrated in this study. However, there may be no direct relationship between complement and age-related changes in lymphokine activity as no correlation was made with measles and BCG antigens. Also, only C4 and not C3 and Bf levels showed significant alteration with age. Leucocyte migration inhibition and CIC concentrations did not correlate. This indicates that cause and effect relationships between immunity and soluble immun

complexes formation may not be established in ageing by measuring CIC levels. A lack of correlation between rheumatoid factor titres and migration inhibition suggests no role for helper T cells in the pathogenesis of age-associated autoimmunity.

The geriatric population is growing and the extent of a person's decline in immunocompetence with age is a key factor in the quality of life (Cearlock, 1988). While some general trends in the immune function of elderly individuals can be identified, the variability of immunocompetence from person to person increases progressively with age (Cearlock, 1988). Thus it is becoming more and more crucial to be able to perform and evaluate tests of immune function. Procedures currently performed predominantly in research laboratories will have to be increasingly adapted to the clinical laboratory so that the growing geriatric population may be better served. The traditional lectin-lymphocyte stimulation tests for the assessment of cellular immunity in ageing is costly, requires considerable technical expertise and is therefore getting out of reach for even research laboratories in the developing world. The leucocyte migration inhibition assay used to assess cellular

immunity in the present study endears itself for adaptation to clinical laboratories because it is relatively cheap and simple to perform.

Mantoux delayed hypersensitivity skin test: This study indicates a depression in delayed hypersensitivity skin test reactions after the age of forty-five years in Nigerians. Prior to this age, there was an increase in responses with age. Analysis of the results by sex indicates that the depression in skin test reactivity also begins after the age of forty-five years in both sexes. In an age-related study, Goodwin et al. (1982) also reported depressed skin hypersensitivity test responses in a healthy elderly population in New Mexico, U.S.A. These reports support the general view that there is a decrease in immune responsiveness as one ages (Murasko et al., 1986). They also agree with the postulate that a decrease in T-cell function is the primary immunological change with age (Kay, 1979; Meredith and Walford, 1979). The effector cells in delayed-type hypersensitivity skin reactions are T lymphocytes.

Increasing mean tuberculin PPD reaction diameters observed between age groups 6-25 years and 26-45 years in

this study may be due to an increased exposure to mycobacteria with rising age. Greenwood and Whittle (1981) have reported progressive increases with age in the prevalence of positive tuberculin test in the tropics. Repeated exposure to antigens would be expected to result in a more profound immune response. As it is possible that individuals who were Mantoux test negative had not been previously exposed (sensitized) to mycobacteria, they were retested after 3 months. This was to rule out false negative results.

All the healthy subjects reported in this study were Mantoux test positive. Goodwin et al. (1982) also observed positive responses in delayed hypersensitivity skin test in all of the healthy young subjects that they studied. However, they reported that 33% of their healthy elderly subjects (greater 65 years old) tested negative. Tuberculin positivity is maintained in the adult population by the presence of a latent infection or by repeated re-immunization (Greenwood and Whittle, 1981). However, cell-mediated immunity may be lost within a few years of BCG immunization, particularly in the tropics (Heyworth, 1977).

Although the increased mortality rate associated

with impaired delayed hypersensitivity responses in old age (Roberts-Thomson et al., 1974) and in cancer (Eilber and Morton, 1970; Wells et al., 1973) is unexplained, the observations suggest that the modulation of cellular immune responses may lead to a prolonged life span.

CHAPTER FIVE

NON-SPECIFIC HUMORAL IMMUNE RESPONSES DURING AGEING IN NIGERIANS

5.1 COMPLEMENT COMPONENTS C3c, C4 AND FACTOR B

5.1.1 INTRODUCTION: Available studies on complement levels during ageing are few. The increased susceptibility to infection in the aged may be associated with defects in the complement system as complement is a major effector arm of humoral immunity. Plasma levels of complement proteins C3c, C4 and factor B (Bf) have therefore been investigated during ageing in healthy Nigerians.

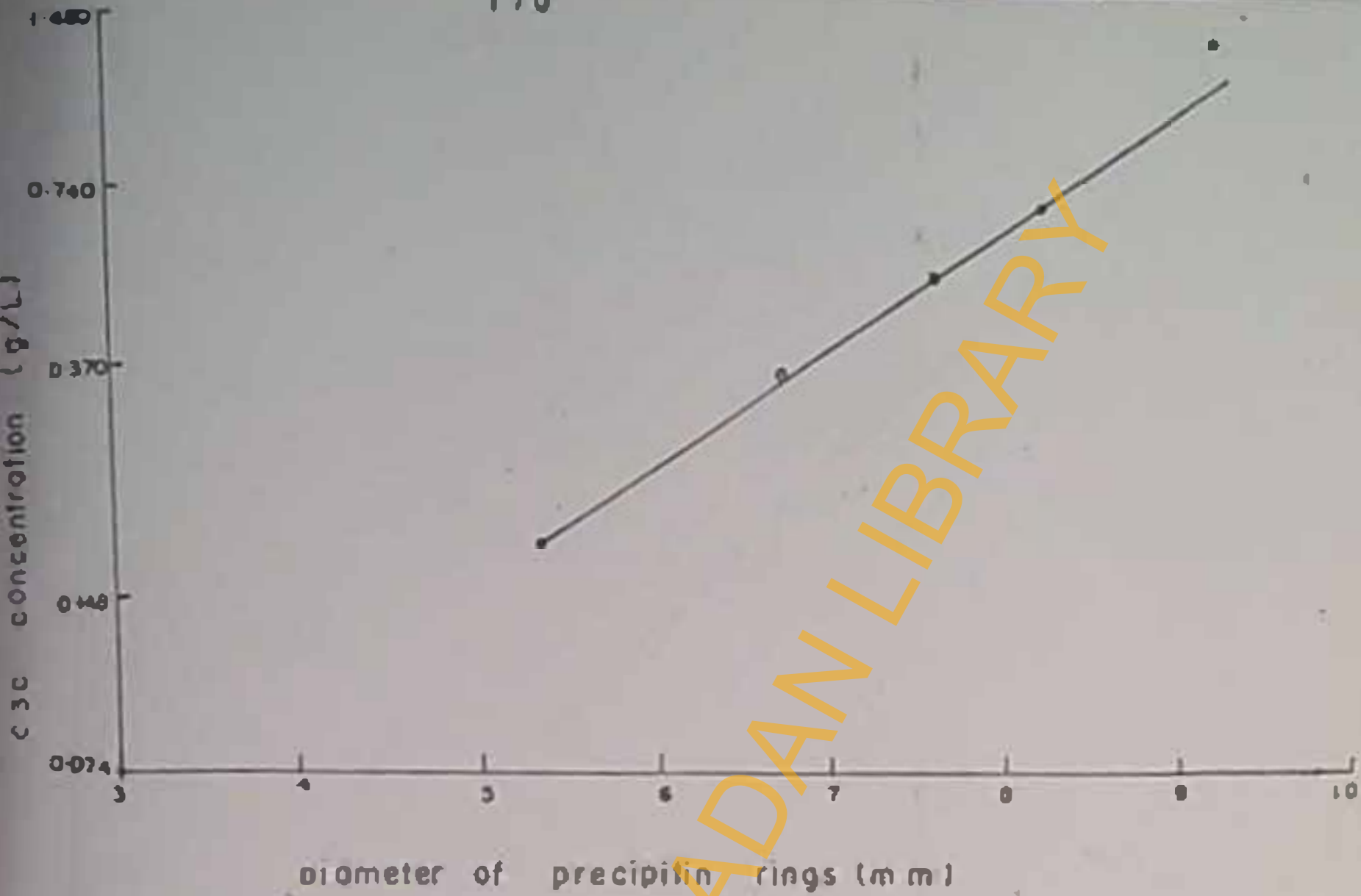
5.1.2 SUBJECTS AND METHODS: A total of 188 plasma samples were studied. 47 of the samples came from people aged 6-25 years and 54 from those 26-45 years old. The remaining samples include 47 from subjects 46-65 years old and 40 from those > 65 years of age.

Plasma C3c, C4 and Bf levels were measured by single

radial immunodiffusion technique (Fahey and McKelvey, 1965 as modified by Salimonu et al. 1978) using commercial monospecific antisera to human C3c, C4 and Bf respectively (Serotec, Oxford OX5 1JE, England). The levels of C3c and C4 were measured against a commercial serum standard (Standard Human Serum - lot No. 041025C; Behringwerke AG, Marburg, Germany). Bf levels were measured against a pooled plasma standard obtained from 10 adult healthy blood donors.

5.1.3 RESULTS: All the samples for C3c, C4 and Bf measurements were run in a single batch and the standard curves for these complement components are displayed in Figures 12 and 13. Intra-batch coefficient of variation (c.v) was 4.5% for C3, 8.6% for C4 and 11.5%for Bf.

Table 22 shows plasma concentrations for the complement components in the age groups. The mean values for C3c and Bf did not change significantly with age (P > 0.10 in both cases). However, C4 levels demonstrate significant increase with rising age ($r = 0.232$; $P < 0.01$). Sex differences in plasma C3c and C4 levels are shown in Tables 23 and 24 respectively. None of the age groups revealed significant difference in mean values between the two sexes ($P > 0.05$ in all cases). However, females aged 26-45 years had higher mean C3c level ($P < 0.01$) than males of the same age group. Both males and females showed no significant changes in mean C3c values



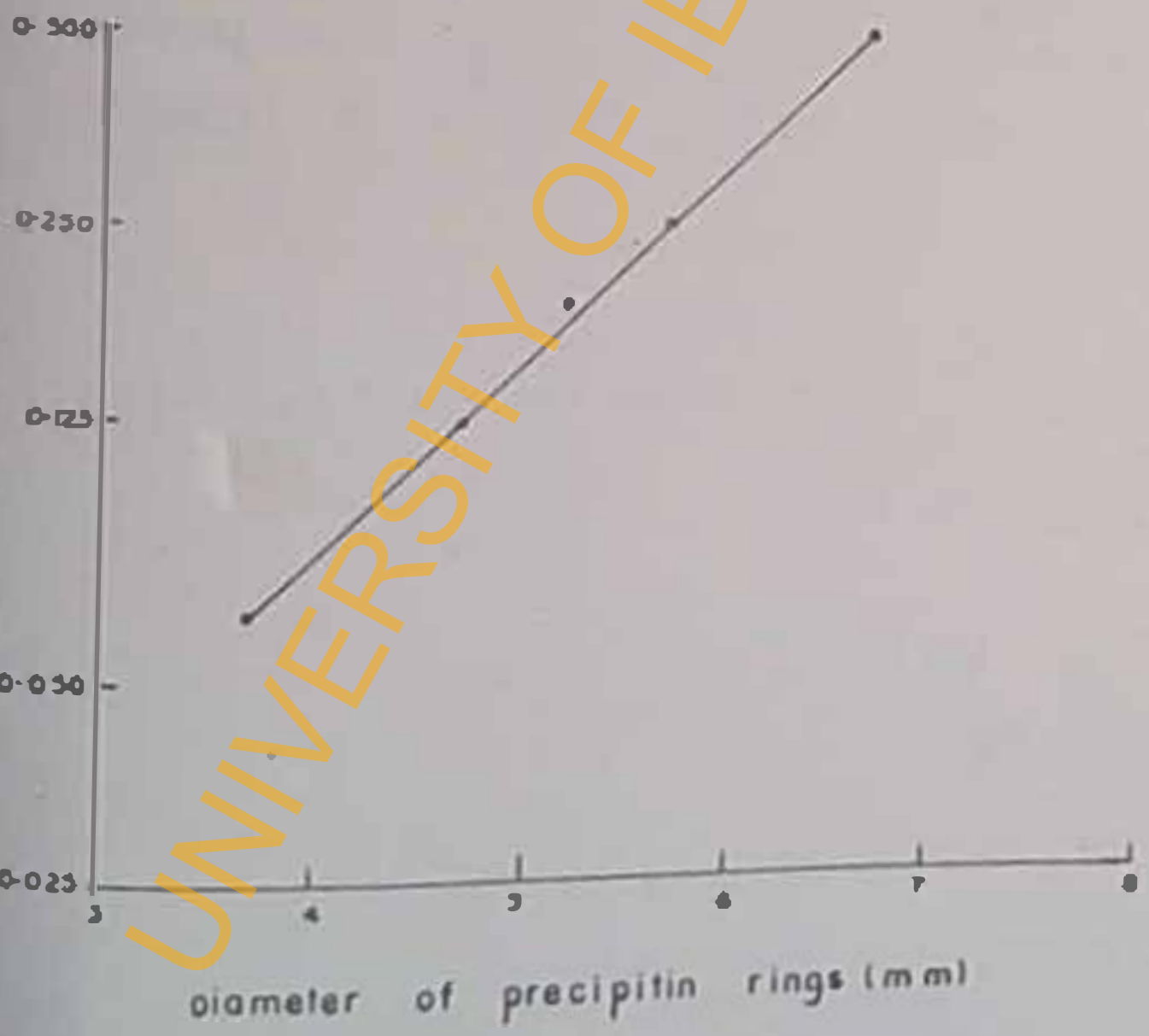


Figure 12. Standard curves for C3c and C4.

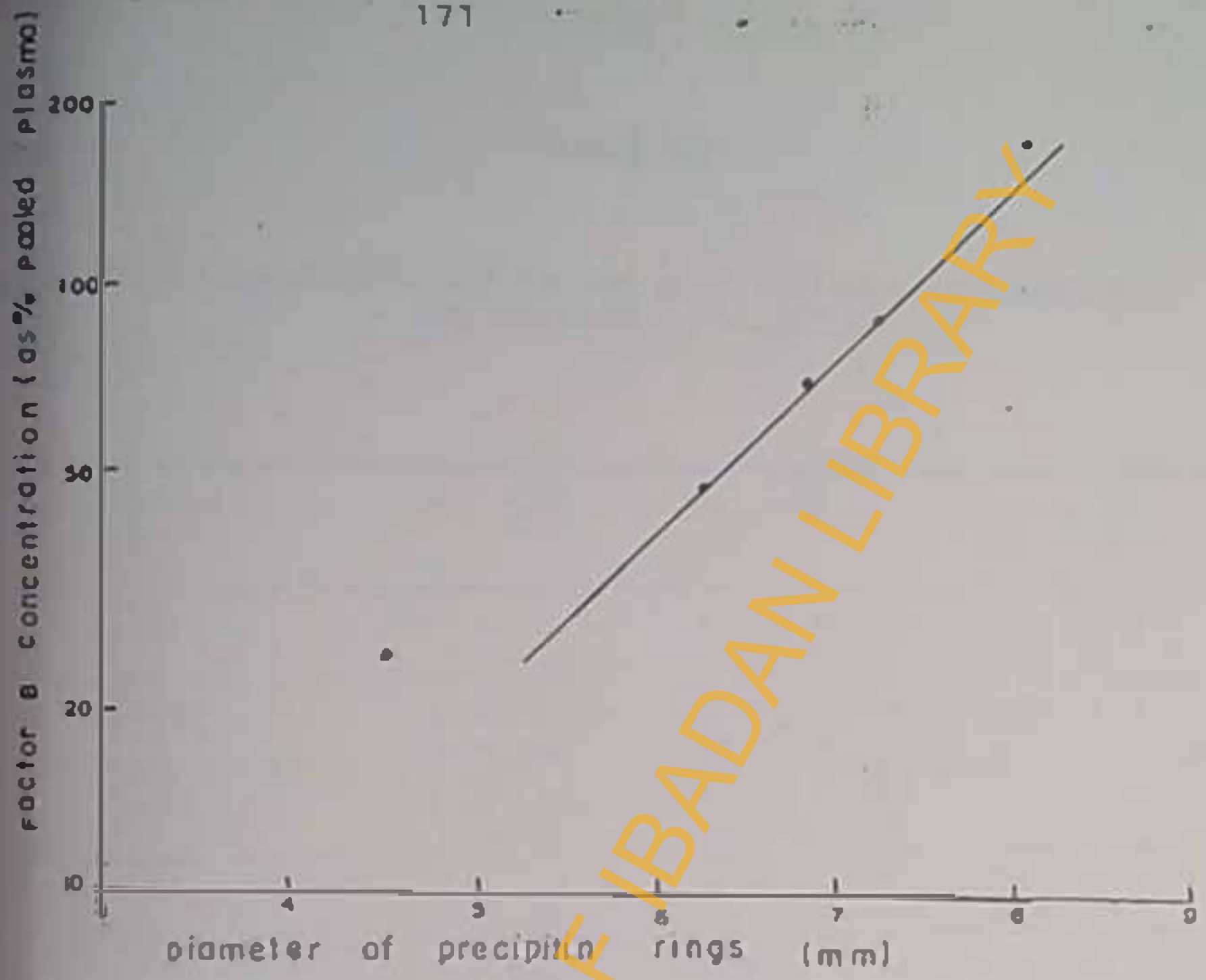


Figure 13. Stardard curve for factor B.

TABLE 22:

Mean (± ls.d) plasma concentrations of complement components in the different age groups.

Age group (yrs)	C3c (g/L)	C4 (g/L)	Factor B (%pooled serum)
6-25	0.64 ± 0.10 n= 47	0.25 ± 0.08 n = 47	105 ± 44.0 n = 20
26-45	0.64 ± 0.13 n = 48	0.32 ± 0.20 n = 54	108 ± 35.9 n = 42
46-65	0.60 ± 0.12 n = 43	0.36 ± 0.20 n = 47	106 ± 37.0 n = 26
> 65	0.61 ± 0.10 n = 32	0.33 ± 0.21 n = 40	102 ± 33.1 n = 27
r	- 0.118	0.232	- 0.065
p	> 0.10	< 0.01	> 0.20

TABLE 23:

Comparison of mean (± 1s.d) C3c levels (g/l) in plasma in males and females

Sex	Age group (in years)			
	6-25	26-45	46-65	> 65
Males	0.64 ± 0.08	0.56±0.09	0.58±0.13	0.58± 0.08
n	27	21	22	20
Females	0.62 ± 0.12	0.68 ± 0.13	0.62 ± 0.10	0.62 ± 0.11
n	20	27	21	12
t	0.645	3.765	1.132	1.312
P	> 0.20	< 0.01	> 0.20	> 0.10

TABLE 24

Comparison of mean (± l.s.d) C4 levels (g/L) in plasma in males and females

	Age group in years					
Sex	6-25	26-45	46-65	> 65	r	P
Males	0.27 ± 0.08	0.29 ± 0.18	0.35 ± 0.23	0.35 ± 0.25	0.202	>0.05
n =	27	22	26	20		
Females	0.23 ± 0.07	0.33 ± 0.21	0.37 ± 0.14	0.35 ± 0.12	0.304	<0.01
n =	20	32	21	20		
t	1.816	0.740	0.365	0.000		
P	>0.05	>0.20	>0.20	>0.20		

with changing age. The increase in C4 levels with age observed in males lacked statistical significance (P > 0.05), but female values were significant (P< 0.01). Sex analysis was not done for Bf because the number of samples that could be measured with available reagent were not large enough to split.

5.2 ACUTE PHASE PROTEINS: TRANSFERRIN, ALBUMIN, C-REACTIVE PROTEIN AND ALPHA-2 MACROGLOBULIN

5.2.1 INTRODUCTION: Acute phase proteins have been used to diagnose certain primary conditions and superimposed infections (Kushner and Mackiewicz, 1987). They have also been utilised to monitor disease activity thus serving as prognostic indices. Their greatest use appears to be in distinguishing those diseases with significant amount of inflammation or tissue damage from those in which these pathological processes are minimal or absent. A wide range of chronic diseases and other debilitating circumstances leading to everyday loss of energy and vitality are associated with old age. In this study, transferrin, albumin, C-reactive protein (CPR) and alpha-2 macroglobulin levels were measured to assess the possibility of using one or more of these proteins to index the ageing process.

5.2.2 SUBJECTS AND METHODS: A total of 152 subjects were sampled for the quantitation of transferrin, albumin,

alpha-2 macroglobulin and CRP in plasma. This consisted of 41 subjects aged 6-25 years and 40 of age 26-45 years. 39 individuals were 46-65 years old and those >65 years of age were 32.

Transferrin, alpha-2 macroglobulin and CRP were quantified by single radial immunodiffusion method of Fahey and Mckelvey (1965) as modified by Salimonu et al. (1978). Commercial monospecific antisera to human transferrin, alpha-2 macroglobulin and CRP (Serotec, Oxford OX5 IJE, England) were used. The levels of these acute phase proteins were measured against commercial serum standards (Behringwerke AG, Marburg, Germany). The detailed method has been described under 5.1.2.

Plasma albumin levels were determined by the brilliant cresol green method as described by Doumas and Biggs (1971). Briefly, 0.02cm³ of the test plasma was added to 4.0 cm³ of working solution of bromocresol green (BCG) dye. Also, 0.02 cm³ of albumin standard (3.1% solution) was added to 4.0 cm³ working BCG solution. After standing at room temperature for 10 minutes, the absorbance of the test and standard were read at 630 nm against a reagent blank. The concentration of albumin was determined as follows:

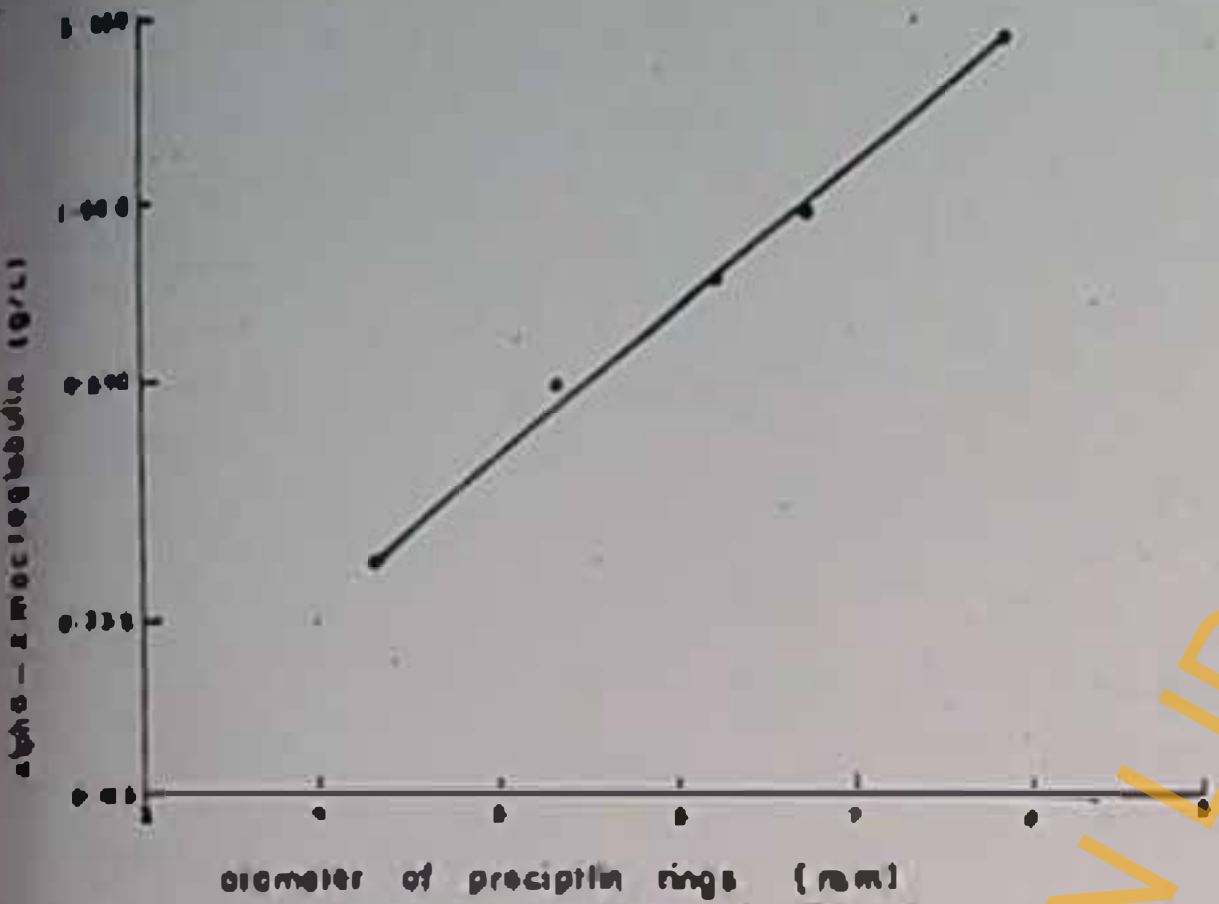
$$\text{Albumin (g/L)} = \frac{\text{O.D. of test}}{\text{O.D. of standard.}} \times \text{conc. of standard}$$

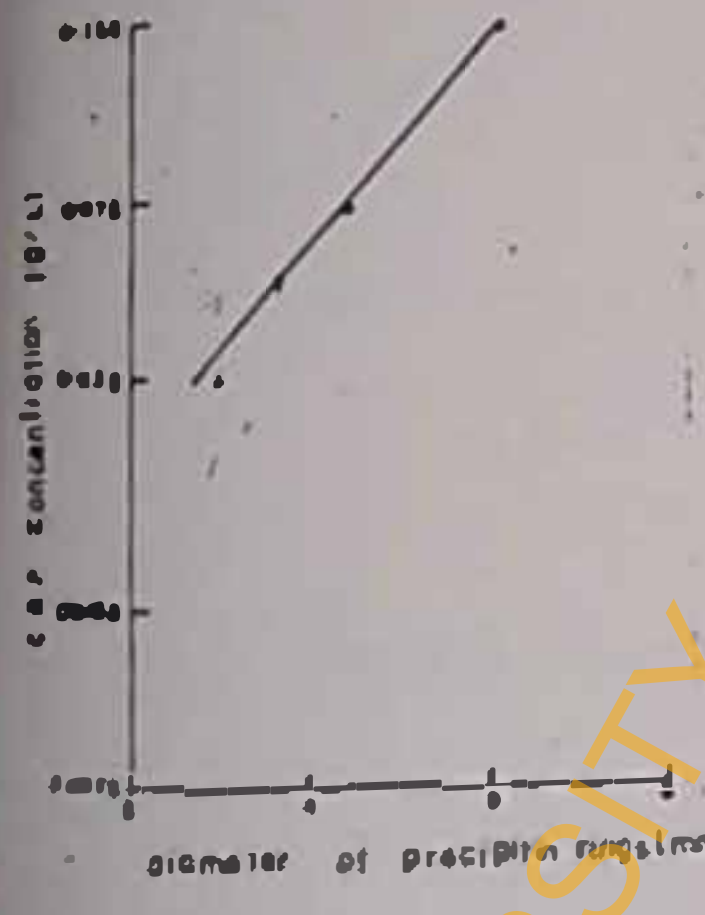
5.2.3 RESULTS: The samples were run in a single batch. The standard curves for transferrin, alpha-2 macroglobulin and CRP are shown in Figure 14; and the one for albumin in Figure 15. Intra-batch C.V. was 8.4% for transferrin, 7.4% for alpha-2 macroglobulin, 12.2% for CRP and 16.2% for albumin.

Table 25 showed no significant change with age in mean concentrations of all the four acute phase proteins (P>0.10 in all cases).

5.3 DISCUSSION

Complement components C3c, C4 and factor B: Information on the levels of complement proteins in healthy Nigerians is scanty. Abdu-rrahman et al. (1981) reported C3 levels only in the first year of life in healthy Nigerian children. Other reports have monitored changes in relative concentrations of complement components in various diseases (Greenwood and Brueton, 1974, Greenwood et al., 1976; Greenwood and Whittle, 1976; Olusi et al., 1976; Oyeyinka and Onyemelukwe, 1983; Oyeyinka et al., 1987). The quantitative concentrations of C3 and C4 obtained here in apparently healthy Nigerians can





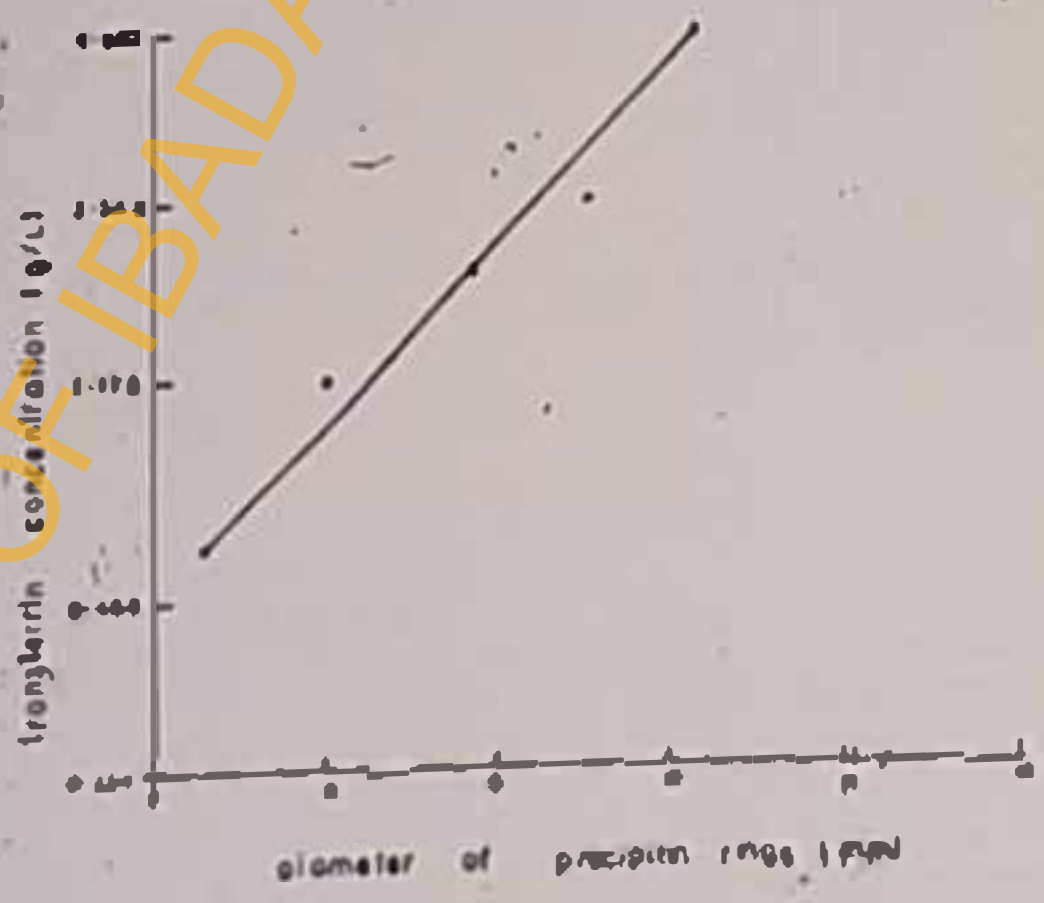


Figure 14. Standard curves for alpha - 2 macroglobulin, CRP and transferrin.

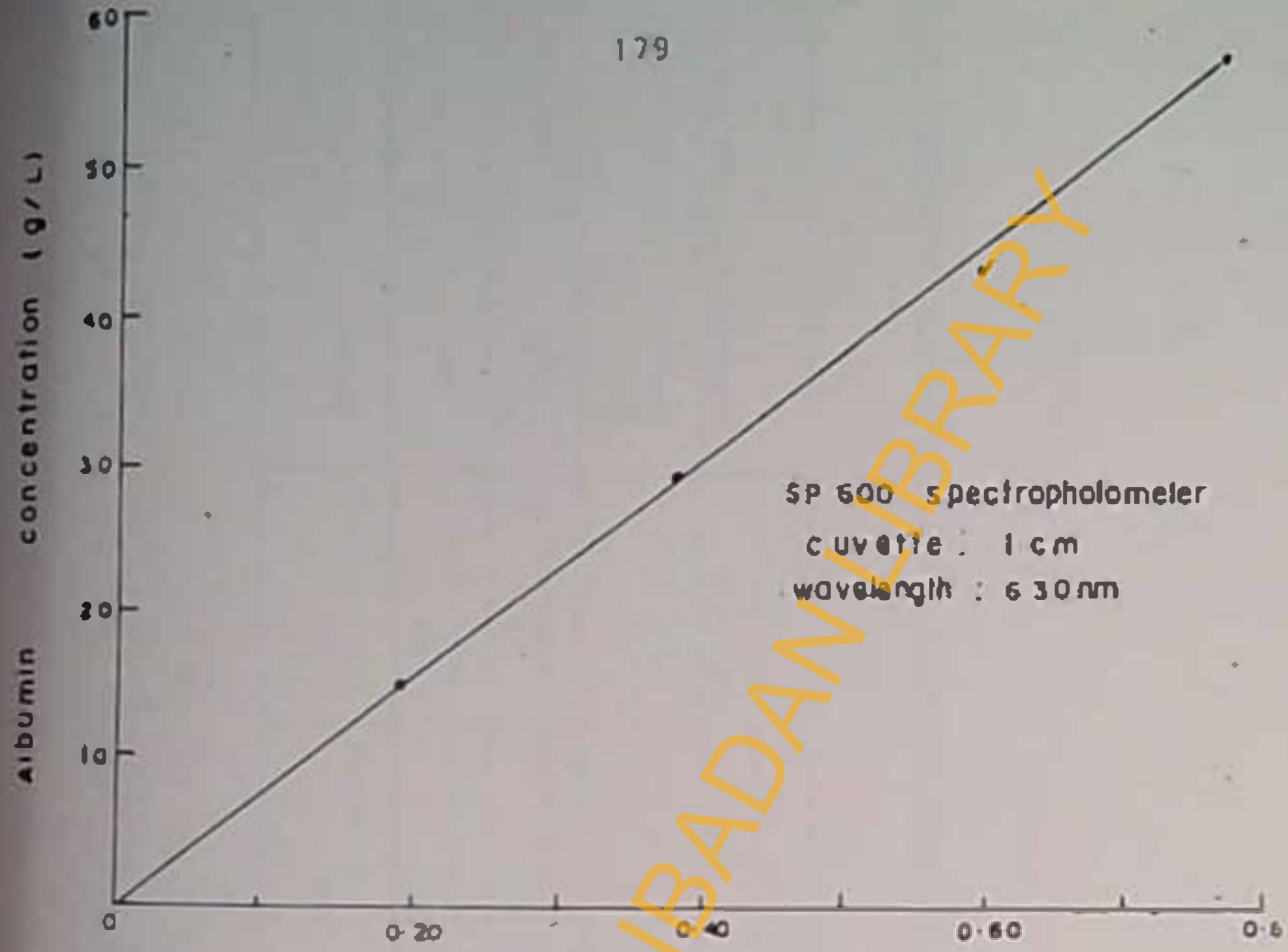


Figure 15. Standard curve for albumin.

TABLE 25

an (± 1s.d.) plasma concentrations of acute phase reactants in the different age groups

Acute Phase Reactants		Age group in years 6 - 25	26 - 45	46 - 65	> 65	r	P
Transferrin (g/L)	Mean n	2.30 ± 0.93 20	2.45 ± 1.22 34	2.32 ± 1.28 36	2.74 ± 1.32 32	0.134	> 0.10
Albumin (g/L)	Mean n	40.1 ± 10.4 41	43.6 ± 10.3 23	39.6 ± 9.8 28	38.8 ± 9.8 29	- 0.075	> 0.20
Alpha 2 Macro-globulin (g/L)	Mean n	3.62 ± 0.74 15	4.08 ± 0.83 16	3.09 ± 0.71 15	4.09 ± 0.84 16	- 0.048	> 0.20
C-Reactive Protein** (g/L)	Mean n	0.005 ± 0.01 39	0.001 ± 0 40	0.004 ± 0.01 39	0.012 ± 0.03 32	- 0.038	> 0.20

** Detectable in 13%, 0%, 8%, and 19% of subjects in the respective age groups.

therefore not be compared with most previous reports. However, the C3 figures in this report are lower than values found in normal healthy subjects in India (Ganguly et al., 1978). The discrepancy may be because anti-C3 was used in radial immunodiffusion technique to quantify the Indian samples. It has been recommended that only anti-C3c should be used in the immuno-chemical estimation of C3 levels (IUIS/WHO Working Group, 1981). Environmental factors may also be responsible for the difference.

Salimonu (1985) has reported a higher mean C3 level, which is lower than that found in India, in Nigerian children aged 1-4 years. The younger age of the children may be responsible for the higher level compared to this report as C3 levels are high in cord plasma of Nigerian neonates (Abdurrahman et al., 1981).

The mean plasma BF levels (expressed as percentage of a pooled local serum standard) observed in this study are similar to previously reported percentage concentrations of this complement component (Greenwood and Whittle, 1976; Greenwood et al., 1976; Oyeyinka and Onyemelukwe, 1983) in apparently healthy Nigerians.

A lack of significant difference among the age groups in C3c and Bf levels suggest that there are no age-related differences in the complement system between ages 6 and

95 years. It is therefore unlikely that the system is being activated in healthy subjects during ageing. However, in the absence of turnover studies, it cannot be concluded that the activation pathways of complement are not stimulated as serum levels reflect a balance between catabolism and rate of biosynthesis (Colten, 1976). Increased biosynthesis or reduced catabolism may be responsible for elevated C4 levels observed with increasing age. The few available studies in the literature show no gross deficiencies in complement levels and if anything show increases in the levels of complement components in the elderly (Schneider, 1983; Nagaki et al., 1980).

The reason for significant higher mean C3c level in females than in males aged 26-45 years is not obvious. Whether this has any relationship with the fact that IgG and IgM levels are usually higher in females than in males (Stoop et al, 1969) is also not clear. However, the results generally indicate no disparity based on sex in C3c and C4 levels.

Acute phase protein: A lack of significant alteration in transferrin, albumin, alpha-2 macroglobulin and CRP levels with age in this study shows that acute phase responses in aged individuals is not a consequence of old age per se. The may therefore not be useful in indexing the ageing process. CRP is an outstanding positive acute phase reactant in man, rising by up to a thousand-fold

its basal plasma level in response to inflammatory stimuli (Kushner, 1988). It has been found useful as an indicator of active bacterial infection (Sabel and Hanson, 1974; Peltola and Jaakkola, 1988) and may also serve as a helpful index of infection in the aged. This suggestion is supported by the observation of Peltola and Jaakkola (1988) that CRP values were elevated in at least 89% of bacteraemic or fungaemic episodes regardless of the age or immunologic status of the patient.

Despite being a non specific acute phase protein and thus by no means a substitute for specific microbiologic tests, the CRP is of diagnostic value in that it increases rapidly enough after the onset of infection (Peltola and Jaakkola, 1988). The normal levels of CRP obtained in the age groups in this study is an indication that our subjects are from a healthy population. This is further strengthened by normal levels of the two negative acute phase reactants quantified (albumin and transferrin) in all the age groups. Although Rapin and Lagier (1988) observed that serum albumin concentrations showed a tendency to decrease with age, auch tendency was non significant. As transferrin level is decreased in malnutrition (Razban et al., 1975), it should prove useful in monitoring nutritional changes during ageing.

The mean values obtained here for alpha-2-macroglobulin are higher than values reported by Salimonu

et al. (1986) in Nigerian babies. However, the present values compare well with those reported by Salimonu (1985) for alpha-2-macroglobulin and CRP in children aged 1-4 years. It would seem that adult levels of alpha2-macroglobulin are reached in infancy in Nigerian children. Although Razban et al.(1975) observed reduced levels of alpha-2-macroglobulin in malnutrition, Crockson et al. (1966) found this acute phase protein consistently unresponsive to surgical trauma. Alpha-2-macroglobulin may not be a sensitive stress monitor during ageing.

CHAPTER SIX

SPECIFIC HUMORAL IMMUNE RESPONSES DURING AGEING IN NIGERIANS

6.1 TOTAL LYMPHOCYTES OBTAINED FROM DIFFERENTIAL LEUCOCYTE COUNTS (see chapter 4.1)

6.2 ENUMERATION OF B-CELLS AND QUANTITATION OF IMMUNOGLOBULINS

6.2.1 INTRODUCTION: Adequate antibody responses in the aged have been observed for influenza (Feery et al., 1976) and tetanus toxoid (Solomonova and Vizev, 1973) vaccines. This suggests that antibody forming cells (B lymphocytes) are not impaired functionally during ageing. Diaz-Jouanen et al.(1975b) found that the number of circulating B cells remains relatively constant with age. However, the level of serum IgG and IgA tend to increase with age while that of serum IgM tends to decrease (Haferkamp et al., 1966; Buckley et al., 1974). In this study, B-cell enumeration and immunoglobulins G,A,M, and D quantitation has been carried out to reveal the pattern of change with age in these immunologic indices in Nigerians.

6.2.2 SUBJECTS AND METHODS: B-cells were counted in 98

blood samples obtained from 20 subjects aged 6 - 25 years, 28 subjects aged 26 - 45 years, 26 subjects aged 46 - 65 years and 24 subjects aged > 65 years. Immunoglobulins G, A, M and D estimation was done in plasma samples from a total of 172 subjects comprising 51 in the 6 - 25 year age group, 46 in the 26 - 45 year age group, 43 in the 46 - 65 year age group and 32 in those > 65 years of age.

B cell count was done by direct immunofluorescent staining of cell surfaces with flourescein isothiocyanate (FITC) conjugated anti-human IgG, A,M and L chains obtained from Serotec, Oxford, England (cat. no. AHP001F). The preparation of cells from human peripheral blood and the procedure for lymphocyte staining are as described in 4.2.2.

IgG, IgA, IgM and IgD levels were measured by single radial immunodiffusion technique (Salimonu et al., 1978) using commercial mono-specific antisera to human IgG, IgA, IgM and IgD (Serotec, Oxford OX5 IJE, England) respectively. The levels of IgG, IgA, and IgM were measured against commercial serum standards (Precinorm U: Boehringer Mannheim, Germany). IgD levels were quantified against the World Health Organisation standard

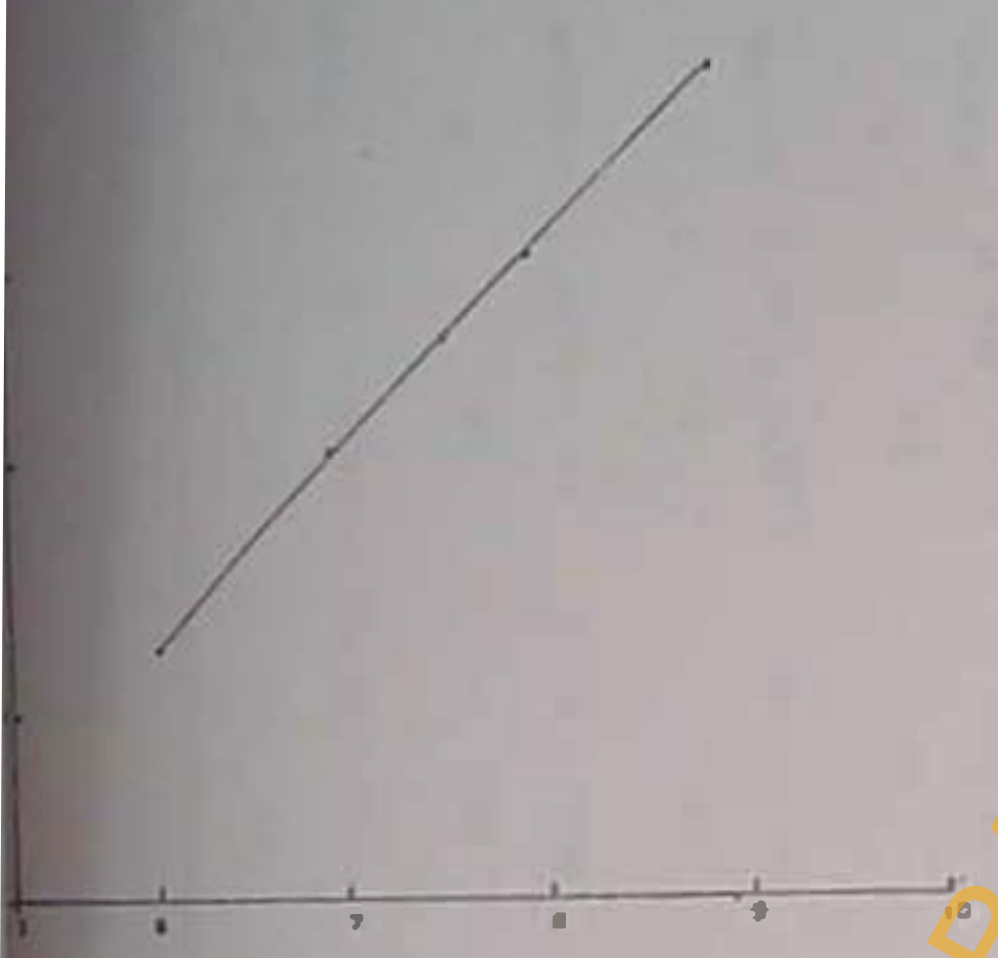
67/37 (Rowe et al., 1970).

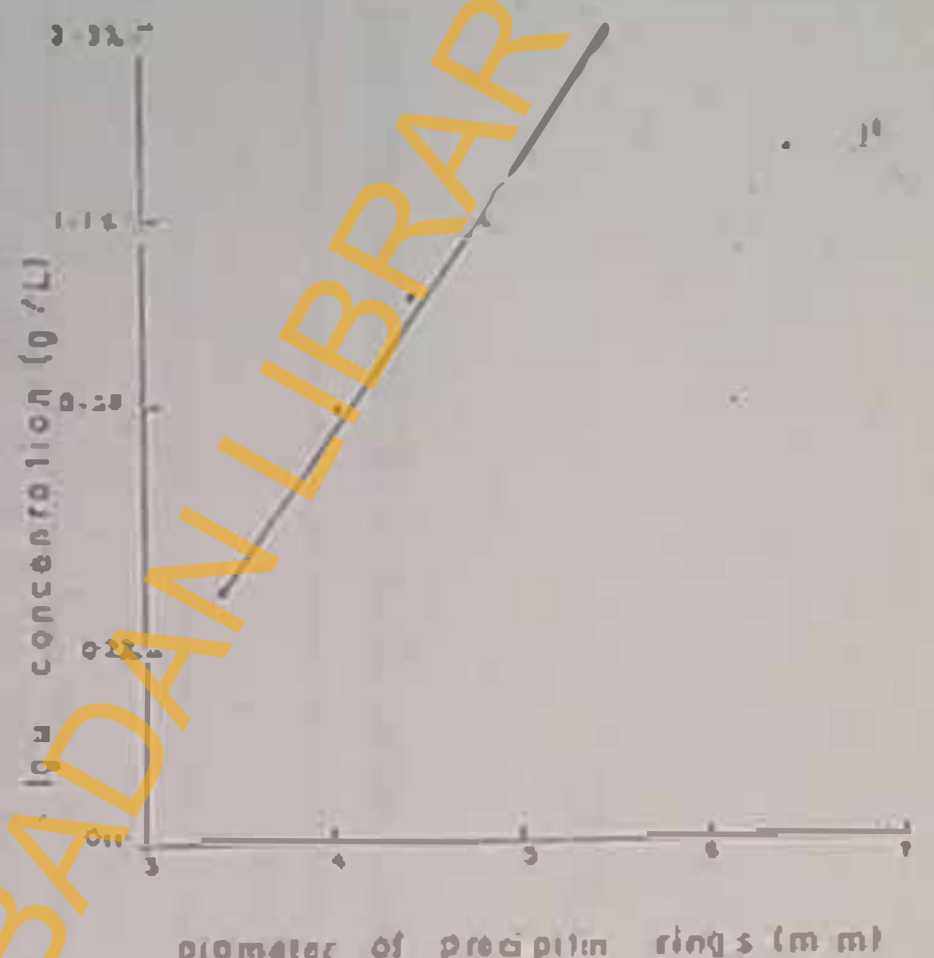
6.2.3 RESULTS: Estimation of the samples for immunoglobulins was done in a single batch. The standard curves for IgG, IgA, IgM and IgD are displayed in Figure 16. Intra-batch coefficient of variation was 10.8% for IgG, 6.8% for IgA, 14.4% for IgM and 8.9% for IgD. The mean plasma concentration of the immunoglobulins are shown in Table 26. No significant age-related changes in mean values could be demonstrated for any of the four immunoglobulins (P >0.05 in all cases).

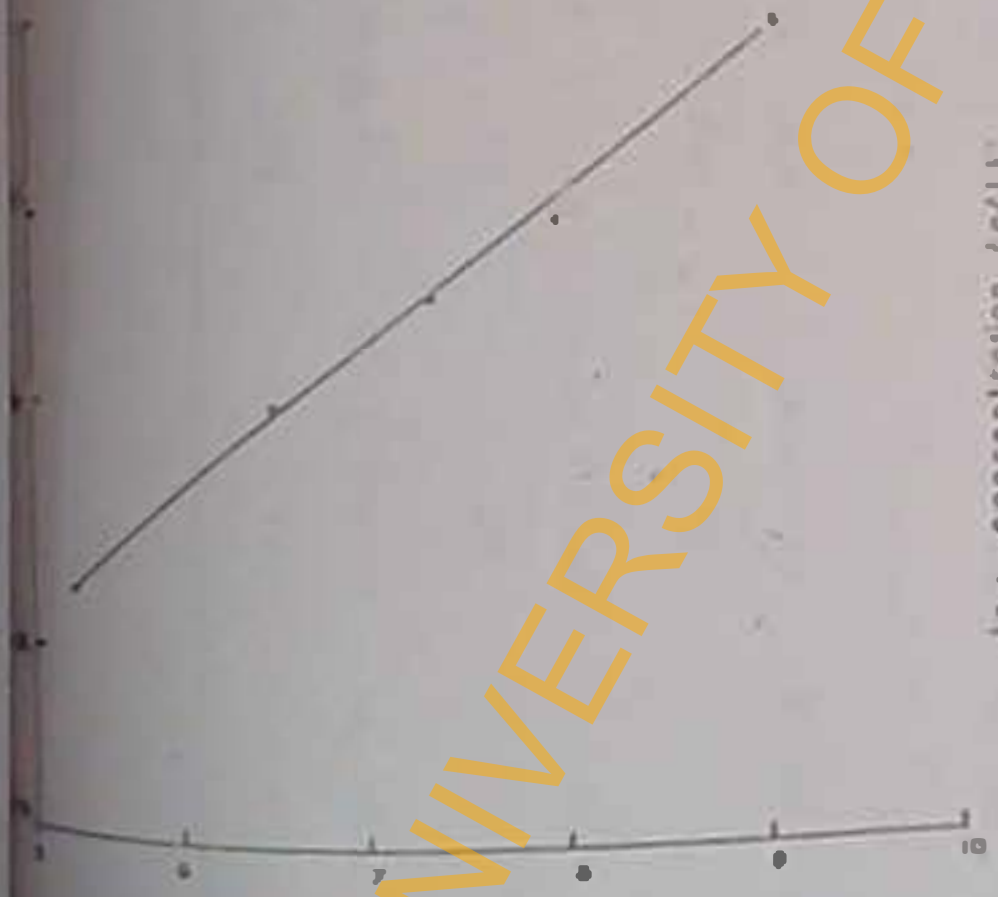
Generally, absolute B lymphocyte count did not change significantly with age (Table 27: r=0.178; P>0.05). B cells represented 35±7.6%, 32±6.3%, 33±9.7% and 33±7.1% of total lymphocytes counted in the respective age groups.

6.3 DETERMINATION OF ISOHAEMAGGLUTININS

6.3.1 INTRODUCTION: Isohaemagglutinins are usually IgM and are thought to arise through immunization against antigens of the gut flora (Roitt, 1988). These antigens are similar to the blood group substances so that the antibodies formed cross-react with the appropriate red cell type. For instance, an individual of blood group A is tolerant to antigens closely similar to A and will only form cross-reacting antibodies capable of agglutinating B red cells (Roitt, 1988). Somers and







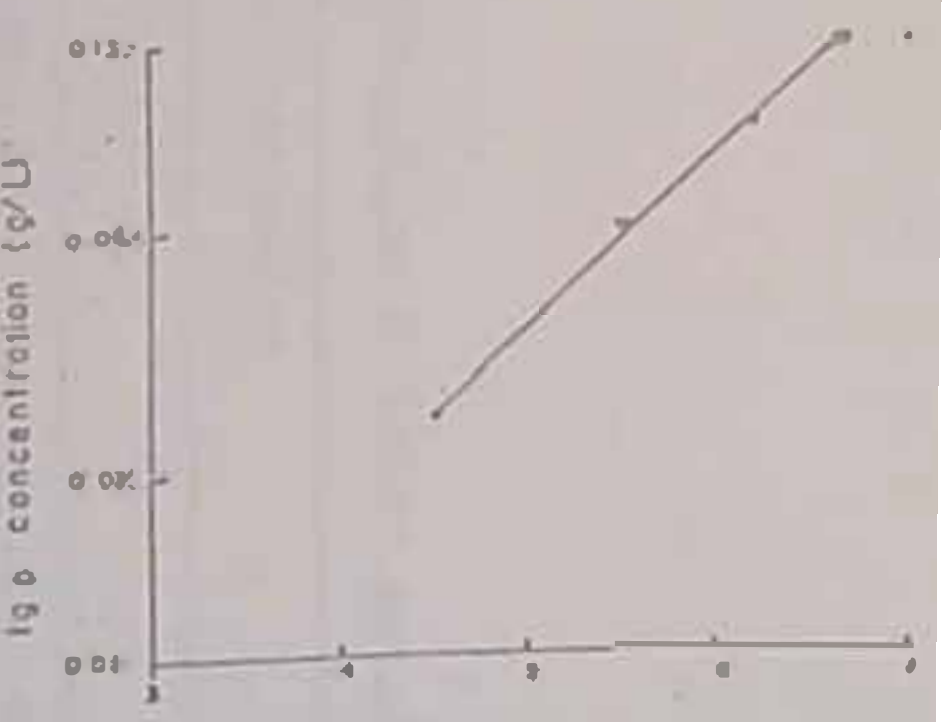


Figure 16. Standard curves for IgG, IgM, IgA and IgD.

TABLE 26

Mean (±1 s.d) plasma concentration of immunoglobulins in the different age groups

Immunoglobulin(g/L)		Age group (in years) 6 -25	26 - 45	46 -65	> 65	r	P
IgG	mean:	16.95 ± 3.76	14.58 ± 3.35	16.88 ± 5.25	16.16 ± 5.34	-0.005	>0.20
	n	51	46	43	32		
IgA	mean:	1.84 ± 0.93	1.80 ± 0.44	2.02 ± 0.97	2.23 ± 1.23	0.143	>0.05
	n	51	46	43	32		
IgM	mean:	2.89 ± 1.67	2.37 ± 2.00	2.07 ± 1.10	2.14 ± 1.33	-0.152	>0.05
	n	49	44	37	29		
IgD	mean:	0.06 ± 0.04	0.08 ± 0.14	0.02 ± 0.02	0.11 ± 0.22	0.167	>0.20
	n	13	16	13	16		

TABLE 27.

Mean B lymphocyte numbers in the different age groups

Age group (in years)	n	mean ($X 10^9/L$)	1 s.d
6 - 25	20	0.56	0.24
26 - 45	28	0.53	0.20
46 - 65	26	0.71	0.48
> 65	24	0.68	0.27

$r = 0.178$; $P > 0.05$

Kuhns (1972) have investigated people from teen age to old age and observed that average titres of anti-A and anti-B iso-agglutinins decreased progressively from 20 years of age to low levels in senescence. This study examines the concentrations of anti-A and anti-B isohaemagglutinins during ageing in Nigerians.

6.3.2 SUBJECTS AND METHODS: Anti-A antibody titre was estimated in a total of 124 subjects. These included 37 people in the 6-25 year age group; 35 people in the 26-45 year age group; 27 individuals aged 46-65 years and 25 subjects who were >65 years of age. Similarly anti-B antibody titres were determined in a total of 106 subjects. They included 35, 26, 23 and 20 people in the respective age groups.

The titration of anti-A and anti-B in plasma was performed by the method of Baker et al. (1966). Serial doubling dilutions of the sera were prepared. To this was added an equal volume (50 ul) of a well washed, freshly prepared, 1% suspension of appropriate red cells (A cells or B cells). Agglutination was read after 2 hours at room temperature. The titre of the serum recorded

was the highest dilution showing agglutination. A and B cells were obtained from blood of 10 donors each. They were thrice washed with phosphate buffered saline (PBS) pH 7.2. Control sera were titrated along with the samples. Control anti-A (human; Lot 0120358) and anti-B (human; Lot 0121128A) were obtained from Behringwerke AG, Marburg, Germany.

6.3.3 RESULTS: The mean isohaemagglutinins A and B titres obtained in the various age groups are displayed in figure 17. The titres show no significant correlation with age for both anti-A (r = 0.057; t=0.630; P>0.20) and anti-B (r= -0.096; t = 0.971; P>0.20).

6.4 ANTIBODY RESPONSE TO MENINGOCOCCAL POLYSACCHARIDE VACCINATION

6.4.1 INTRODUCTION: Antibody responses following immunization with various vaccines in man has been studied in Nigerians. For instance, Salimonu (1980) and Salimonu et al. (1982) have shown antibody levels in children immunized with tetanus toxoid or measles virus vaccine. Also, the duration of antibody response to meningococcal

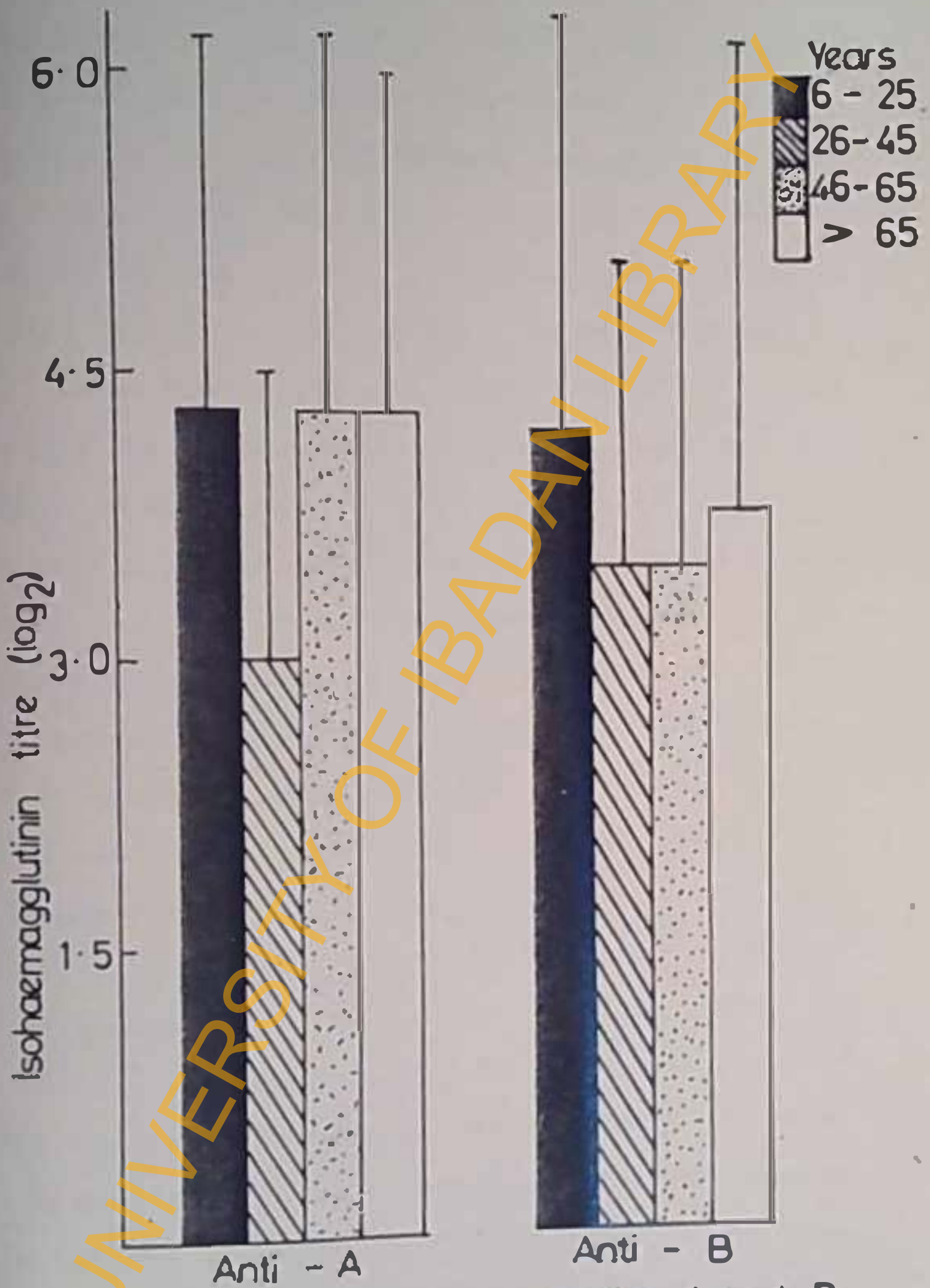


Figure 17: Mean ± 1s.d. isohaemagglutinins A and B titres in the different age groups.

polysaccharide vaccination has been followed (Greenwood et al., 1980; Mohammed et al., 1984). Maintenance of humoral immune responses in the aged has been reported for tetanus toxoid (Solomonova and Vizev, 1973) and for influenza vaccine (Feery et al., 1976). Roberts-Thomson et al. (1974) obtained the same levels of total antibody to monomeric flagellin in young and old people though the T-cell dependent late IgG antibody response was significantly lower in old people. This study assesses the pattern of antibody responses to meningococcal (A + C) polysaccharide vaccine during ageing in Nigerians.

6.4.2 SUBJECTS AND METHODS: Fifty healthy Nigerians were bled for a pre and post-meningococcal (A + C) polysaccharide vaccination samples. These were only 46% (50 out of 109) of those who donated a prevaccination blood sample. It was impossible to obtain informed consent from the rest and from other envisaged participants in the study. 2 cm^3 of blood was drawn on each occasion and serum samples were stored at -20°C until analysed. The post vaccination sample was collected 32 days after

vaccination. The subjects were aged 18-55 years and included 46 males and 4 females. They were all recruited from Area local government secretariat, Afon, Kwara State of Nigeria. Each received a subcutaneous injection of 0.5cm³ bivalent A + C vaccine (Lot D 0271, Institut Merieux, Lyon, France) containing 50 ug of A and C polysaccharide antigens.

Antibodies to the meningococcal vaccine in decomplemented sera (at 56°C for 30 minutes) were measured in microtitre plates by indirect haemagglutination technique (Mohammed et al., 1984). Human group O (rhesus D-negative) red blood cells coated with meningococcal (groups A and C combined) polysaccharide at a concentration of 25 ug/ml were used. Incubation was first at room temperature for 4 hours and then at 4°C for 16 hours. Results were read as $\log_2$ titres.

6.4.3 RESULTS: $\log_2$ antibody titres obtained in various age groups (6-25, 26-45 and 46-65 years) before vaccination with meningococcal (A+C) polysaccharide are shown in Figure 18. There was no significant difference in $\log_2$ titres among the age

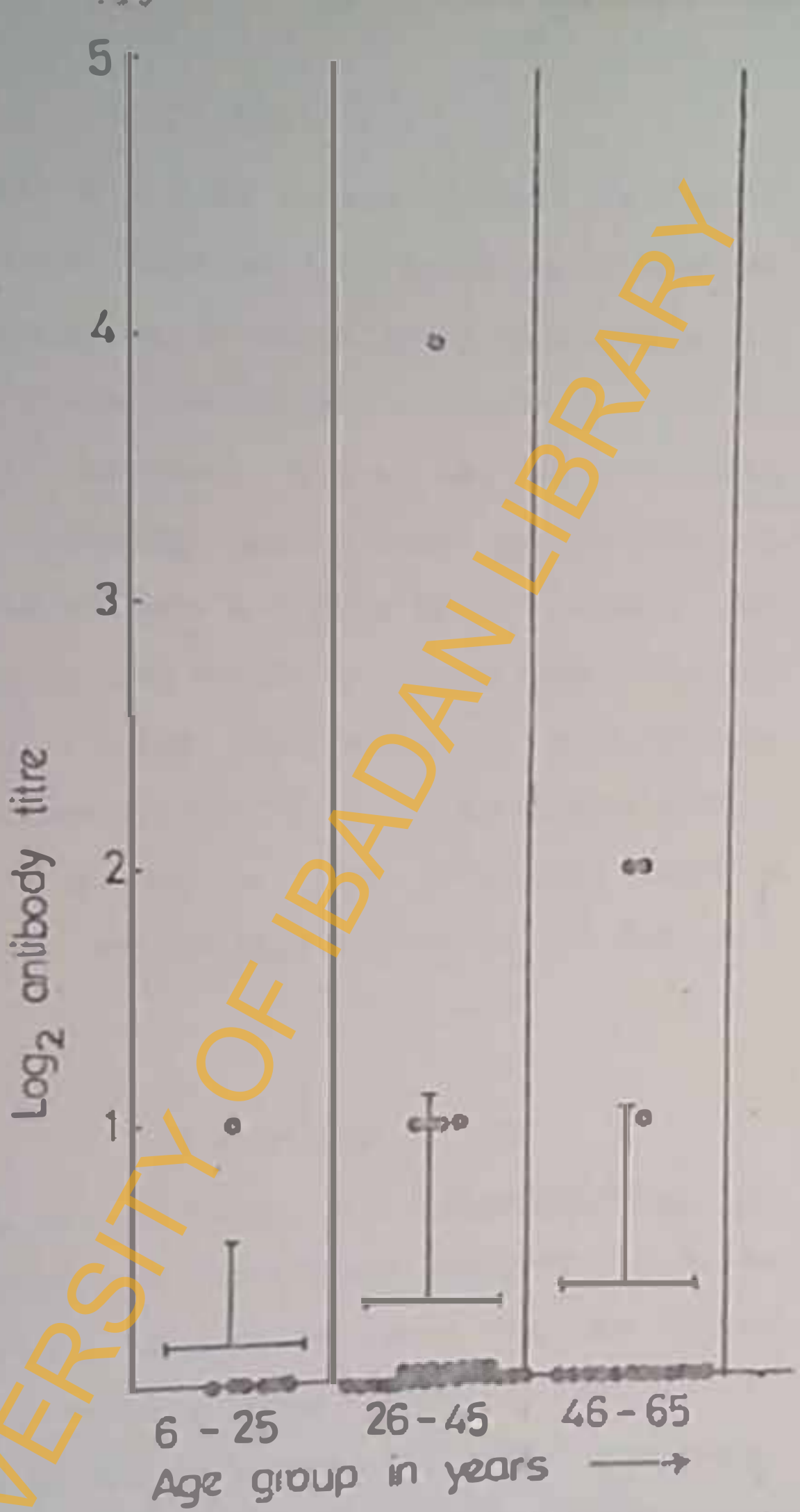


Figure 18: Log_2 antibody titres (±s.d) obtained in various groups before vaccination with meningococcal (A.C) polysaccharide.

groups (t-test: P > 0.20 in all cases). Figure 19 displays post-vaccination log_2 antibody titres in the same individuals. No significant difference was recorded among the age groups (t-test: P >0.20 in all cases). However, there was significant increase in antibody levels over prevaccination values (paired t-test: P < 0.01 in all cases). The mean increase in log_2 antibody titres over baseline levels 32 days after vaccination in various age groups are presented in Figure 20. No statistically significant correlation was obtained between antibody titres and increasing age (r = 0.032; P > 0.20).

6.5 DISCUSSION

Enumeration of B-cells and quantitation of Immunoglobulins: No significant differences in the absolute numbers of B-cells among the age groups were observed in this study. Diaz-Jouanen et al. (1975 b) also found the number of B-cells relatively constant with age. Over thirty percent of peripheral blood lymphocytes found to be B cells in this report is higher than figures previously

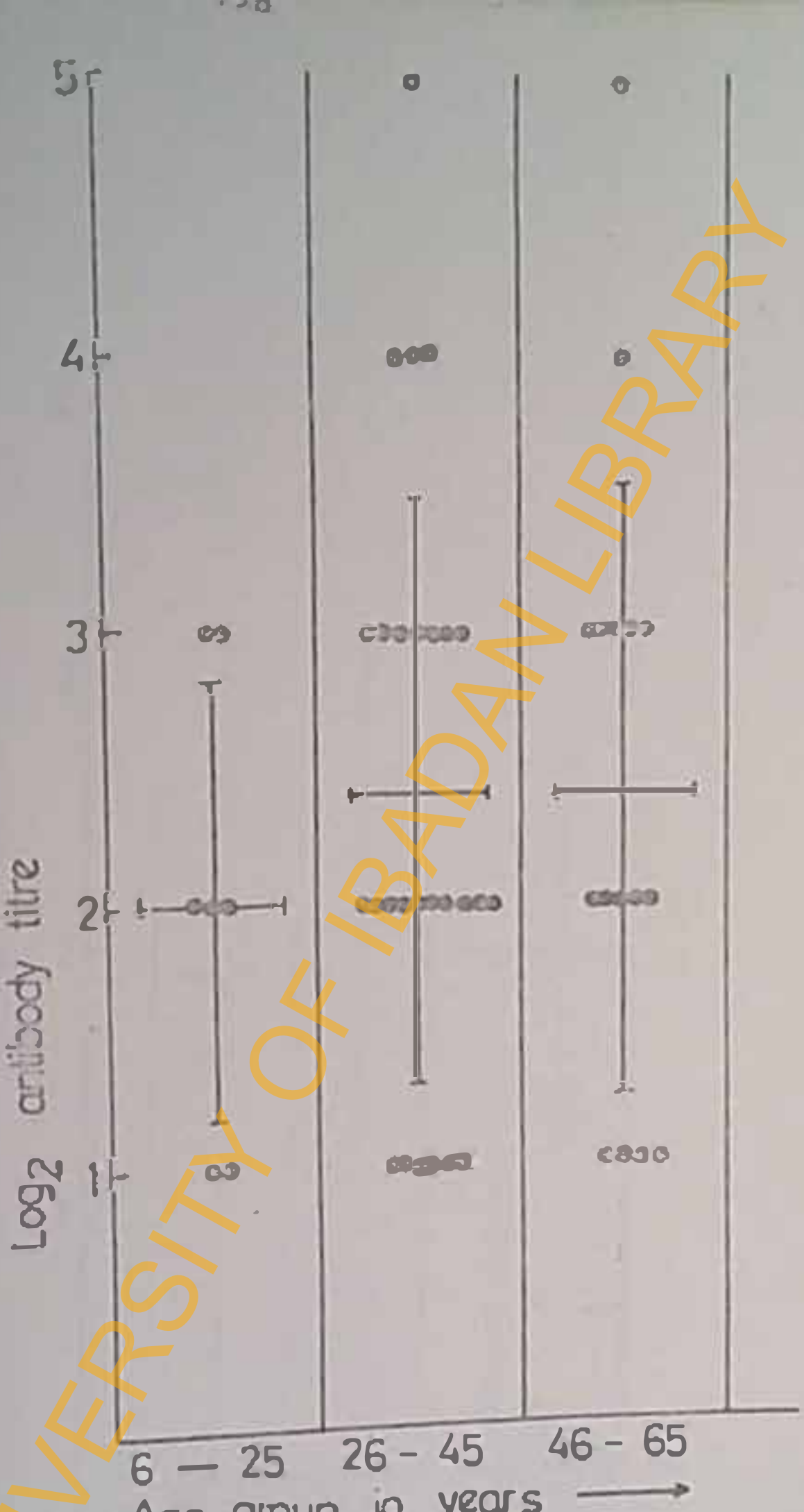


Figure 19: Log_2 antibody titres (±1s.d) obtained in various age groups 32 days after vaccination with meningococcal (A+C) polysaccharide

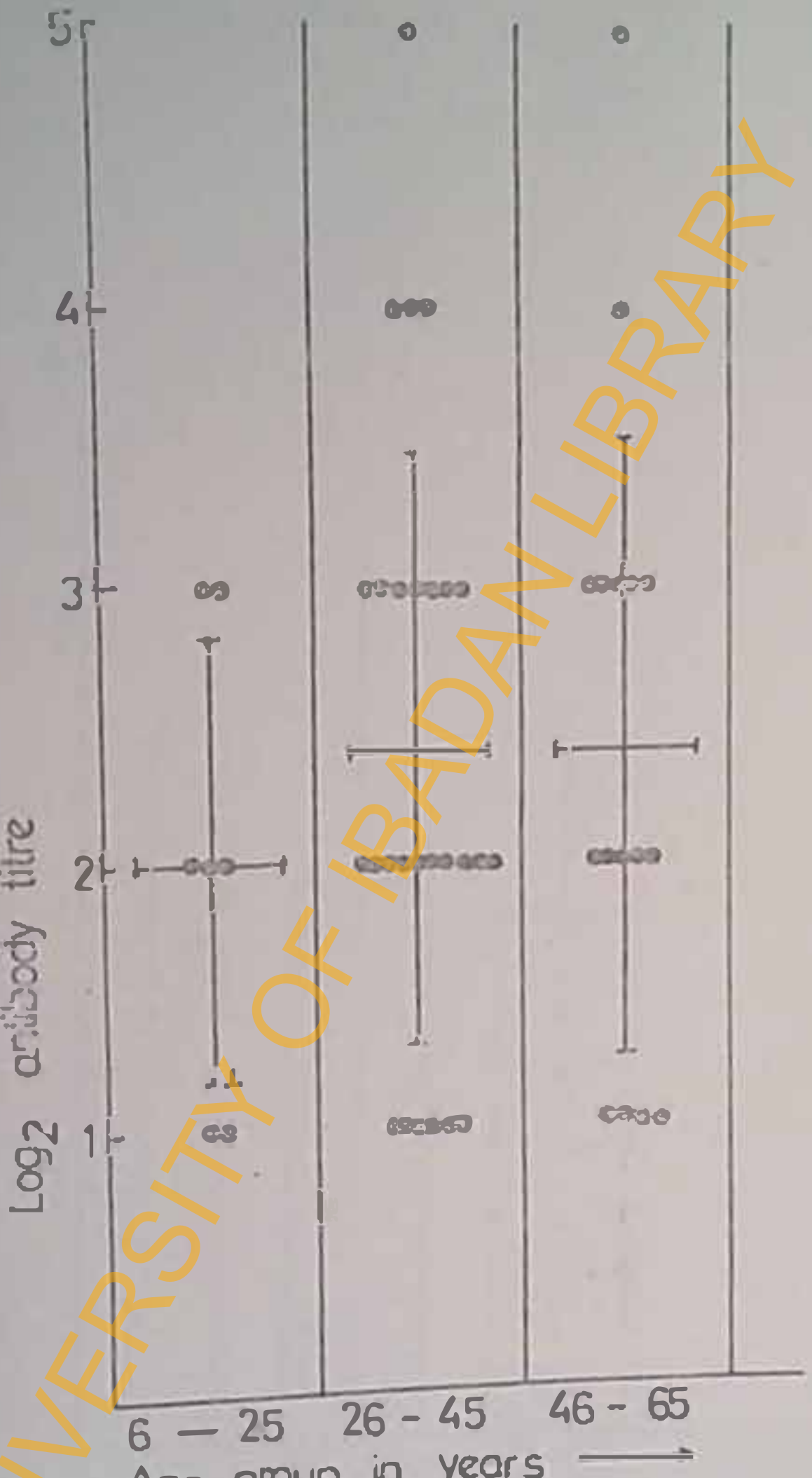


Figure 19: Log_2 antibody titres (±1s.d) obtained in various age groups 32 days after vaccination with meningococcal (A. C) polysaccharide.

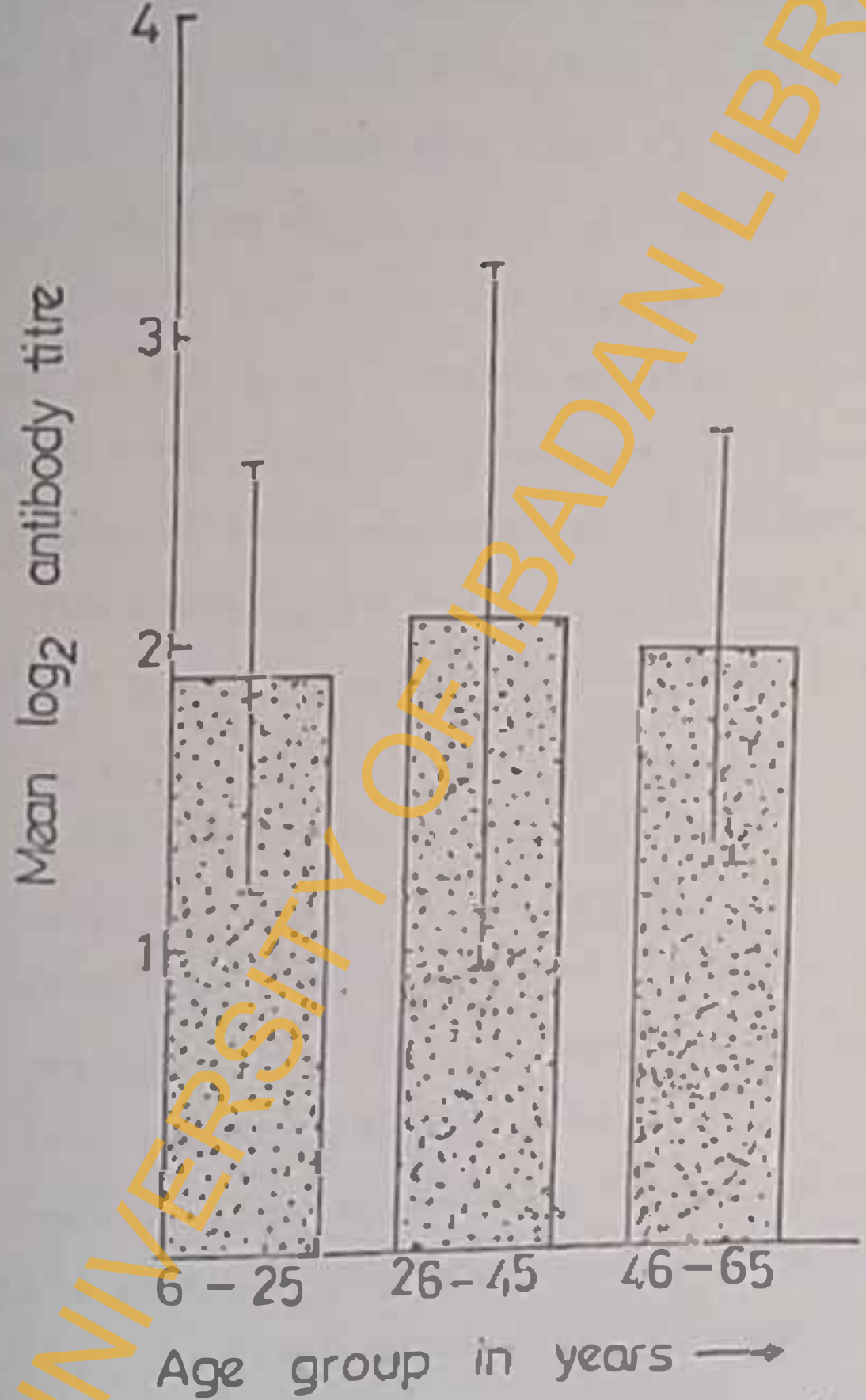


Figure 20. Mean increase (±1 s.d.) in meningococcal log_2 antibody titres over pre-vaccination levels in various age groups. $r = 0.032$; $P > 0.20$.

reported in some control Nigerians. Salimonu et al. (1982) using rosette test obtained 14 ± 5 percent B lymphocytes in 1 - 4 year - old children. Onyemelukwe et al. (1990) employing membrane-bound immunoglobulin found 17.4 ± 3.7 percent B lymphocytes in people aged 28 - 42 years. It is possible that the present result differs from that of Salimonu et al. (1982) because different methods were used and also because of the limited age of the group studied earlier. However, the reason for the difference between the present value and that reported by Onyemelukwe et al. (1990) is not obvious. The differences in the cut-off of the age groups (26-45 years as against 28-42 years) may be contributory. Greenwood et al. (1977) have reported similar relative values of B-cells to the one observed here but their absolute figures are higher than in this report.

The observation that IgA level tends to increase with age and IgM values tend to decrease agrees with some previous reports (Haferkamp et al., 1966 ; Buckley et al 1974). However, these tendencies did not reach statistical significance in the present study. Oyeyinka et al. (1984) also reported increasing IgA concentrations with

advancing age in Nigerians. Unlike the findings of Buckley and Dorsey (1970, 1971) which described a fall in mean concentrations of IgG after the age of 35 years in North Carolina, no significant alteration in IgG mean values with age was observed in this study ($r = -0.005$; $P>0.20$). The mean IgD figures presented here are similar to those previously reported in Nigerians (Oyeyinka et al., 1982). A general lack of age-related changes in the four immunoglobulins estimated suggests that they will not be important for the purpose of indexing the ageing process.

Determination of Isohaemagglutinins: No significant age-related change in isohaemagglutinins A and B titres could be demonstrated in this study. This observation differs from the report of Somers and Kuhns (1972) who reported age-associated decrease in anti-A and anti-B isoagglutinins. A decreased degradation of hormones in peripheral tissues of ageing people is well documented (Davies, 1978). The lack of statistical difference in the titres of the two isohaemagglutinins among the age groups noted in

the present study may also be due to a decreased degradation of these proteins in the aged and ageing populations.

None of the subjects studied here showed a complete lack of isohaemagglutinins in plasma. Though isohaemagglutinin levels tend to decrease during the course of leukaemia, a complete lack, or congenital absence of isoagglutinins A and B is rare (Ogata and Hasegawa,1977). In addition immunoglobulin levels in the subjects were normal in this study. This suggests that none of them had the relatively more common lack of isoagglutinins related to hypogammaglobulinaemia.

Antibody response to meningococcal polysaccharide vaccination:

The subjects in the various age groups in this study responded with antibody pro-duction when challenged with meningococcal poly-saccharide vaccine. Experience gained from the use of this antigen for vaccination for over twenty years now (Gotschlich et al., 1969; Goldschneider et al., 1972; Greenwood and Wali,1980) indicate that responses in adults are adequate (Goldschneider et al.,1969; Greenwood et al.,1980) but poor in infants and young children (Monto et al., 1973;

Peltola et al., 1977). The fact that all the subjects studied here were adults allowed the analysis of the effect of ageing on a homogenous population of responders. However, infants and young children were not studied because it was not possible to obtain informed consent from their parents.

The T-cell independence of polysaccharide antigens (Schneerson et al., 1982) makes them of low immunogenicity. This may be responsible for the lack of higher than 5 log$_2$ antibody titres in this study. Similar figures to those observed here were previously reported in Nigerians receiving the vaccine for the first time (Mohammed et al., 1984). Methods that have been described for measuring meningococcal antibody include radio-immunoassay (RIA), enzyme-linked immunosorbent assay (ELISA) and indirect haemagglutination (Mohammed et al., 1984). The indirect haemagglutination test is not as sensitive as RIA and ELISA although Artenstein et al. (1971) have found its serogroup specificity an advantage. Higher antibody titres might have been demonstrated in this study if RIA or ELISA had

been used.

No significant age-related change in the level of antibody production to meningococcal polysaccharide vaccine was obtained here. This suggests that the immunosuppression usually associated with ageing does not affect B-cell responses, especially as this vaccine is T-cell independent. Age-related changes in immune responses to tetanus toxoid and influenza vaccine could also not be demonstrated in previous reports (Solomonova and Vizev, 1973; Feery et al., 1976). Although Roberts-Thomson et al. (1974) found that the T-cell dependent late IgG antibod' response was significantly lower in old people, they obtained the same levels of total antibody to monomeric flagellin in young and old people.

CHAPTER SEVEN

MISCELLANEOUS IMMUNOLOGIC INDICES DURING AGEING IN NIGERIANS

7.1 CIRCULATING IMMUNE COMPLEXES

7.1.1 INTRODUCTION: Using the polyethylene glycol precipitation method, the levels of circulating immune complexes (CIC) have been documented in healthy Nigerians (Fakunle et al., 1978; Salimonu, 1985; Salimonu and Akinyemi, 1986; Okpala and Salimonu, 1990). These were obtained in control individuals in clinical studies. However, Onyewotu (1978) has measured immune complexes in healthy Nigerians by radiobioassay. Increased CIC have been reported in the healthy elderly compared to young controls in New Mexico, USA (Goodwin et al., 1982). Elevated immune complex levels may be expected in sera from old people either as a consequence of the vulnerability of old individuals to infection or the age associated increase in the prevalence of autoantibodies. In the present study, the concentrations of circulating immune complexes were measured in healthy Nigerians to see

the variations in values with age.

7.1.2 SUBJECTS AND METHODS: A total of 212 plasma samples were studied. 56 of the samples (males: 33, females: 23) came from people aged 6-25 years and 69 (males: 36, females: 33) from those 26-45 years old. The remaining samples included 47 (males: 27, females:20) from subjects 46-65 years old and 40 (males:20, females: 20) from those >65 years of age.

Quantitation of CIC was done by the PEG precipitation method described by Haskova et al. (1978). Freshly collected plasma stored at - 20°C and thawed only once were diluted 1:3 with borate buffer PH 8.4. 0.22 cm^3 of the diluted plasma was added to 2 cm^3 of 4.166% PEG 6000 solution and mixed thoroughly. For each diluted plasma, a blank was set up by mixing 2 cm^3 of borate buffer with 0.22 cm^3 of the diluted serum. Incubation was at room temperature for 1 hour. The optical densities were read at 450 nm in a spectrophotometer against the plasma blank. The concentration of CIC in each sample was read off a standard immune complex calibration curve (Figure 21) prepared as described

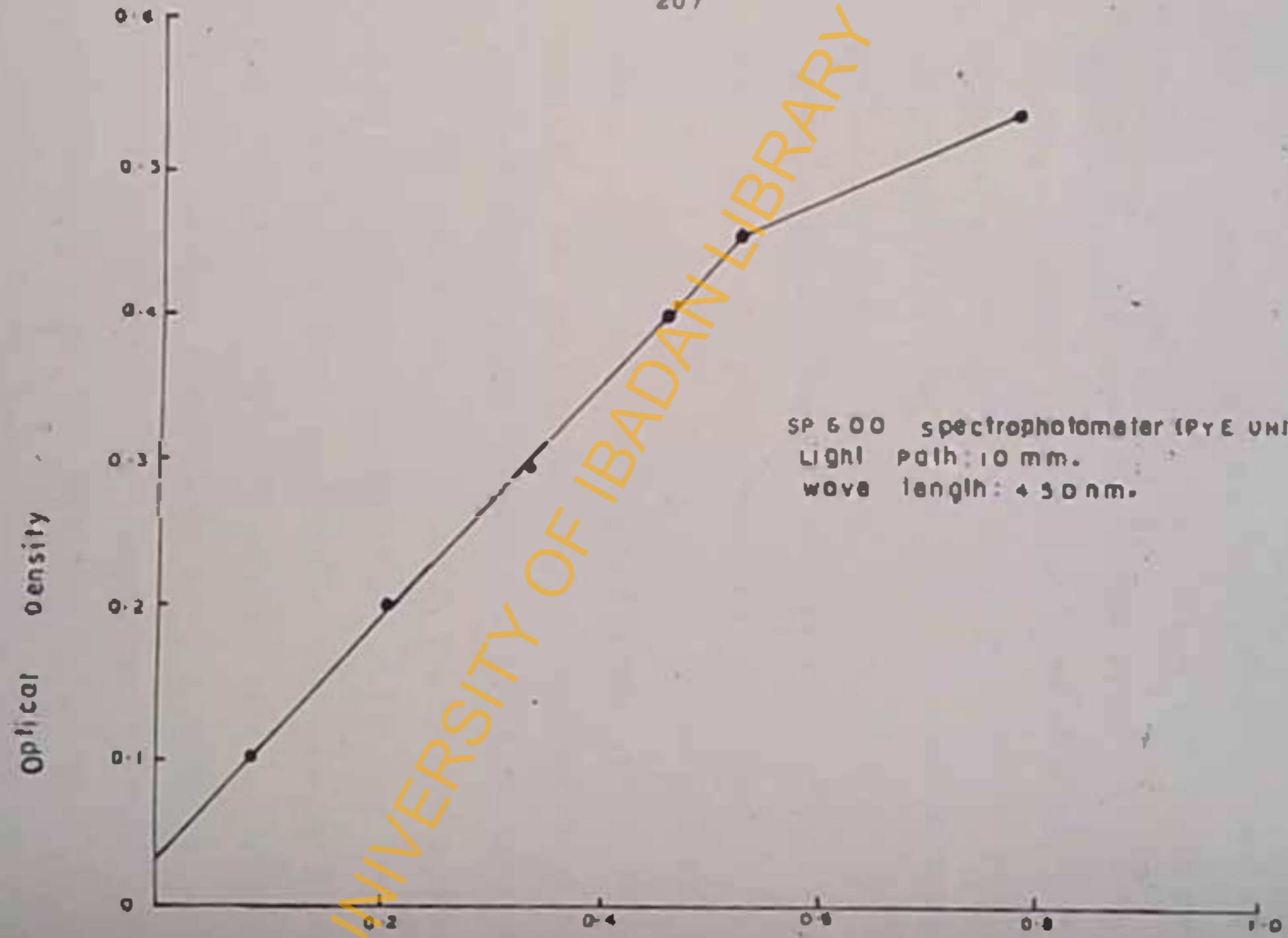


Figure 21. Standard curve for the quantitation of cic.

below:

Aggregated human globulin (AHG) was employed as an *in vitro* model of human immune complexes. AHG was prepared by heating 1.6g% of reference test serum (Sevac, Czechoslavakia), which is normal human globulin, in a water bath at 65°C for 30 minutes. The following concentrations were prepared from the aggregated human globulin: 80, 50, 40, 20, 10, 5 and 2.5mg/cm³. To 0.2cm³ of each concentration was added 0.2cm³ of borate buffer. Two sets of tubes were set up, one for the test and the other for the blank. To the test was added 5.4 cm³ of 4.166% PEG solution and to the "blank" 5.4cm³ of borate buffer.

To both sets of tubes were then added 0.2cm³ from the mixture of the prepared aggregated human globulin and borate buffer. The amount of turbidity was measured at 450nm after incubation for 60 minutes at room temperature. A standard curve (Figure 21) of optical densities against concentrations of aggregated human globulin (immune complexes) was plotted on arithmetic graph paper. This was employed in the determination of the

levels of immune complexes in the sera. The coefficient of variation for this assay was 6%.

7.1.3 RESULTS: The samples for immune complex quantitation were run in four batches. Inter-batch coefficient of variation was 12.5%. Figure 22 displays the levels of CIC in the different age groups. There was significant positive correlation ($r = 0.197$; $P<0.01$) between CIC concentration and age.

Comparison of the mean levels of CIC in males and females (Table 28) show no difference between the sexes (t-test, $P>0.20$ in all cases). No significant correlation between CIC and age was obtained in males ($r = 0.178$; $P> 0.05$) but female values showed significant correlation ($r = 0.212$; $P<0.05$). Respective male and female CIC incidence rates were 67% and 70% for the 6-25 year age group; 67% and 61% for the 26-45 year age group. The frequency of detectable CIC was 74% in males aged 46-65 years and 90% in those >65 years of age. The corresponding female figures were 55% and 85% respectively.

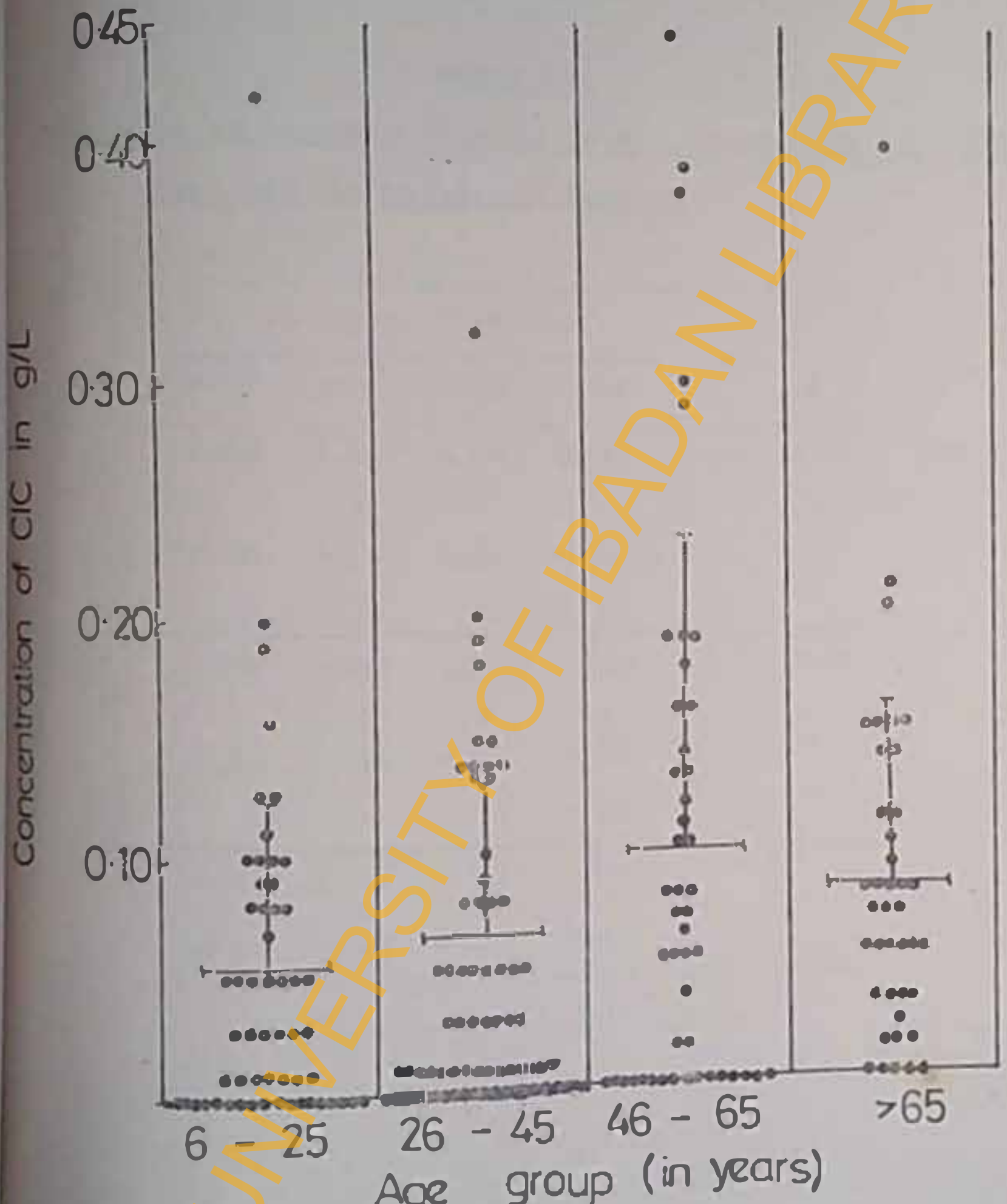


Figure 22 : Mean (±1s.d) levels of circulating immune complexes in the different age groups. r = 0.197; P < 0.01.

TABLE 28.

Comparison of the mean (±1s.d) levels of circulating immune complexes in males and females.

	Age group (in years)					
Sex	6-25	26-45	46-65	>65	r	p
Male: Mean (g/L)	0.055 ± 0.081	0.047 ± 0.071	0.104 ± 0.110	0.065 ± 0.061	0.178	>0.05
n	33	36	27	20		
Female: Mean (g/L)	0.048 ± 0.058	0.046 ± 0.060	0.093 ± 0.166	0.094 ± 0.089	0.212	<0.05
n	23	33	20	20		
t	0.376	0.063	0.257	1.198		
P	>0.20	>0.20	>0.20	>0.20		

7.2 ANTINUCLEAR ANTIBODY AND RHEUMATOID FACTOR DETERMINATIONS

7.2.1 INTRODUCTION: Many investigators have reported an increase in the prevalence of various autoantibodies with age (Hallgren et al., 1973; Cammarata et al., 1967; Mackay, 1972). However, Pandey et al (1979) found no association between antinuclear antibodies (ANA) and age in a large population of healthy caucasians. Rheumatoid factors (RF) are found in patients with a variety of acute and chronic infections and also frequently in the serum of healthy subjects living in the tropics (Greenwood and Whittle, 1981). They are often present at a high titre in serum (Houba and Allison, 1966; Greenwood et al., 1971). Greenwood et al (1971) have reported that the number of positive RF tests increases with age in Nigeria so that nearly half the population over the age of 50 years were found to have a high titre in the latex fixation test. This study takes a look at the frequency of ANA and RF in ageing Nigerians. It is hoped that more light will be shed on the rather high prevalence of RF as presently documented in the literature.

7.2.2 SUBJECTS AND METHODS: One hundred and eleven sera were examined for the presence of antinuclear antibodies. They included 25 each from those 6-25 years, 26-45 years and 46-65 years old. 36 of the subjects were > 65 years of age. Only 68 people were studied by the latex agglutination method for RF because of limited quantity of reagents. These subjects have been classified into those < 65 years (36 subjects) and those > 65 years of age (32 subjects). In addition, RF was also analysed by employing the Rose-Waaler technique to study 153 subjects: 40 each in the 6-25 year and 26-45 year age groups; 37 and 36 respectively in the 46-65 year and > 65 year age groups.

Antinuclear antibody: This was determined using impression smears of rat liver. Tissue smears were fan dried for about 1 hour. They were then covered with a 1 in 10 dilution of sera to be tested. A serum positive for ANA was used as positive control and a serum without ANA served as the negative control. Some smears were treated with PBS pH 7.2 as a control of nonspecific staining by the Ig.-FITC conjugate. Smears were then incubated for 30 minutes at room temperature

in a humid chamber. The smears were rinsed and washed with gentle stirring in PBS for 30 minutes. FITC- conjugated polyvalent antihuman immunoglobulin (anti-human IgG, A, M, and L chains; Serotec, Oxford, England) diluted 1 in 50 in PBS pH 7.2 was applied to the smears for 30 minutes. The slides were rinsed and washed with PBS for 30 minutes, and mounted in 70% glycerol in PBS pH 8.6. Examination of slides was under the ultraviolet microscope (Olympus, Japan).

Rheumatoid factor: Rapi Tex RF latex reagent kit (Behringwerke AG, Marburg, Germany) was used for the detection of RF in plasma samples in this study. In an immunochemical agglutination reaction, RF in plasma was expected to bind to latex particles coated with human gamma-globulin. The plasma samples and reagents were brought to room temperature. 40 ul of patient sample, 1 drop (approximately 40 ul) of positive and 1 drop of negative control sera supplied with the kit were applied on to separate fields of a test plate. 1 drop (approximately 40 ul) of Rapi Tex RF was placed next to each sample, mixed well with a stirring rod, and then rotated slowly. After 2

minutes, they were examined for agglutination. The presence of RF was indicated by distinct agglutination. Samples that did not react with Rapi TEX RF either contained no RF or contained concentrations below 20 i.u./cm^3 .

Semi-quantitative determination of RF was done by the Rose-Waaler sheep red blood cell (SRBC) agglutination test. Rabbit antibody to SRBC was prepared by injecting 1 cm^3 of a 10% suspension of SRBC into each of three rabbits. Injection was intra-venous and on days 0, 10 and 14. The animals were bled on day 22. The SRBC was stored in alsevers solution and washed in PBS (pH 7.2) before use. The blood was allowed to clot and retract. The serum was separated. This was tested in a 2-fold serial dilution against 1% washed SRBC. The anti-SRBC titre of the serum was found to be 1 in 256.

Test sera were decomplemented at 56°C for 30 minutes. To a 1 in 5 dilution in PBS of these sera was added 0.2 cm^3 of washed packed SRBC. The tube contents were mixed, left at room temperature for 60 minutes, and then at 4°C for 60 minutes. Tubes

were spun at 500 x G for 15 minutes and the supernatant was considered as a 1 in 5 dilution of test serum.

SRBCs were sensitized by mixing equal volumes of 1% cells and rabbit anti-SRBC at 1 in 1024 dulution - both in PBS pH 7.2. Incubation was at room temperature for 15 minutes.

Doubling dilutions in PBS of decomplemented test sera which were SRBC - absorbed, were set up. A positive and a negative control sera (Behringwerke AG, Marburg, France) were included. An equal volume of 0.5% unsensitized SRBC was added to the first well of each serum dilution, and an equal volume of 0.5% sensitized SRBC to all the other wells of each serum dilution. The microtitre plates were incubated at 4°C for 16 hours. The titre was taken as the initial dilution of serum in the well (before the addition of SRBC) showing agglutination.

7.2.3 RESULTS: Antinuclear antibodies were not detectable in any of subjects of age 6-25 years (0 out of 25) and 26-45 years (0 out of 25). They were detectable in 4% (1 out of 25) of subjects aged 46-65 years and 8.3% (3 out of 36) of subjects aged >65 years. Weakly reacting ANA were involved in

positive results as none of them remained positive on serum dilution. Statistical analysis indicate that there is age-associated increase in ANA prevalence (Figure 23; X^2 = 15.65; P<0.01).

Positivity rates in rheumatoid factors detected by latex agglutination in old subjects (>65 years of age) and others are shown in Figure 24. The results demonstrate a frequency of positive test of 2.7% in people <65 years of age and 9.4% in older subjects. The two frequencies differed significantly (X^2 = 3.948; P<0.05).

Analysis of sera for RF by the Rose-Waaler technique reveals results displayed in Figure 25. Incidence of positive results increasedwith age (X^2 = 198.08; P< 0.01). Subjects with age >65 years had 22% seropositivity rate (8 out of 35). The other age groups (46-65 years, 26-45 years, and 6-25 years) had 13.5% (5 out of 37), 7.5% (3 out of 40) and 2.5% (1 out of 40) positivity rates respectively.

Only 11% (4 out of 36) of subjects >65 years old were positive at serum dilutions higher than the mandatory 1 in 10 required for the test. The

Figure 23 Positivity rates in antinuclear antibody test in the different age groups. $X^2 = 15.650$; $P < 0.01$.

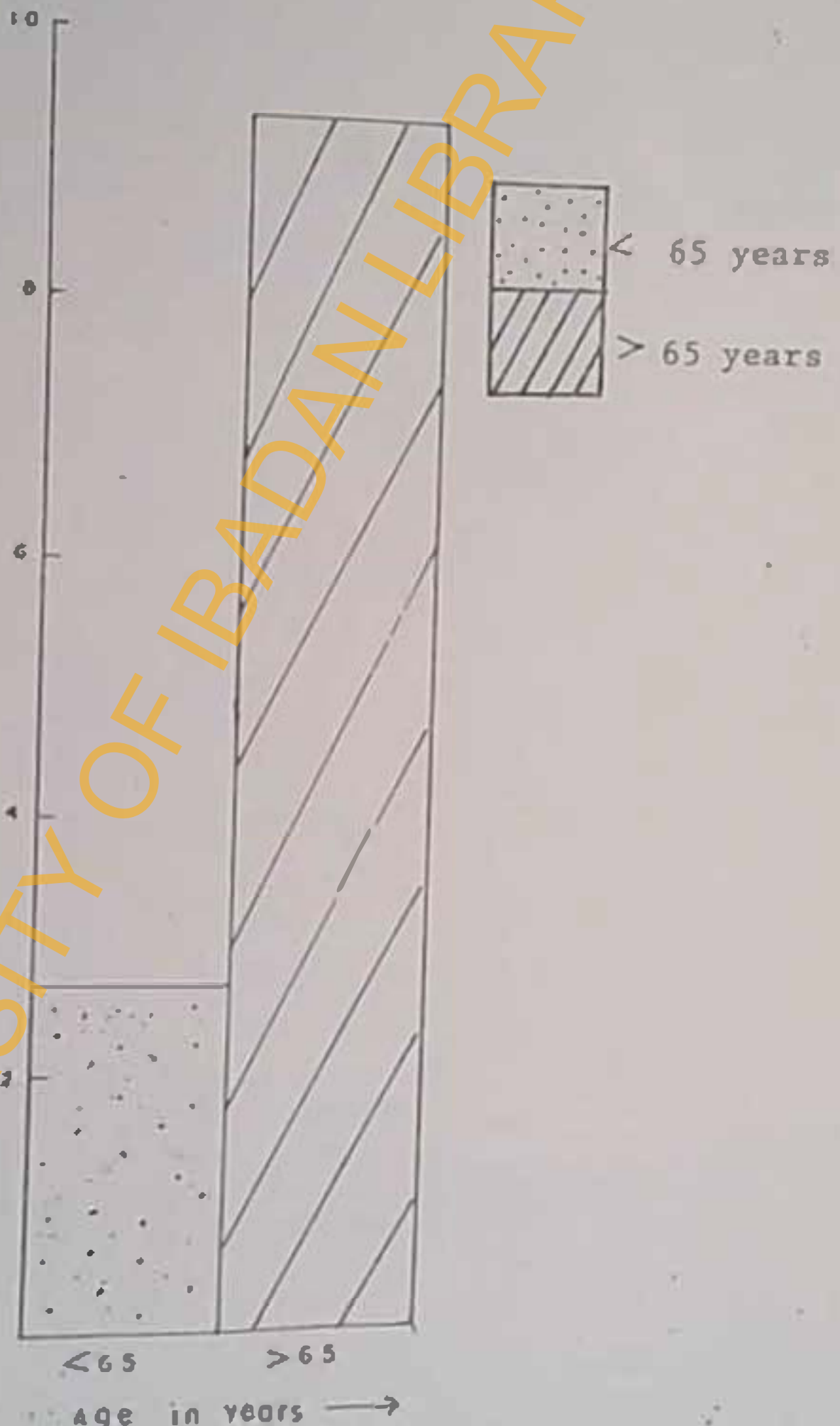


Figure 24. Positivity rates in rheumatoid factor latex agglutination test in old individuals and others.
$X^2 = 3.948$; $P < 0.05$.

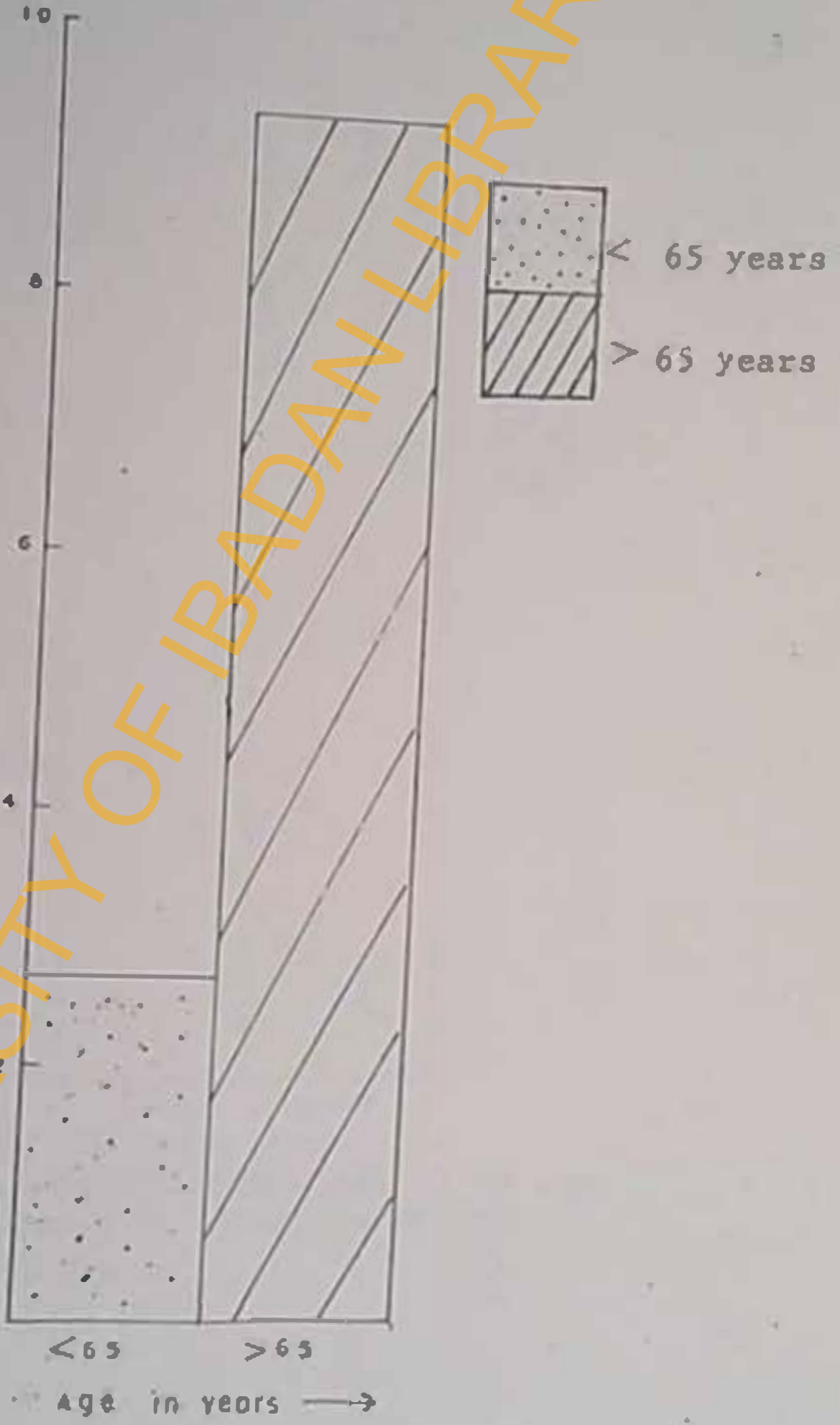


Figure24. Positivity rates in rheumatoid factor latex agglutination test in old individuals and others.
$\chi^2 = 3.948$; $P < 0.05$.

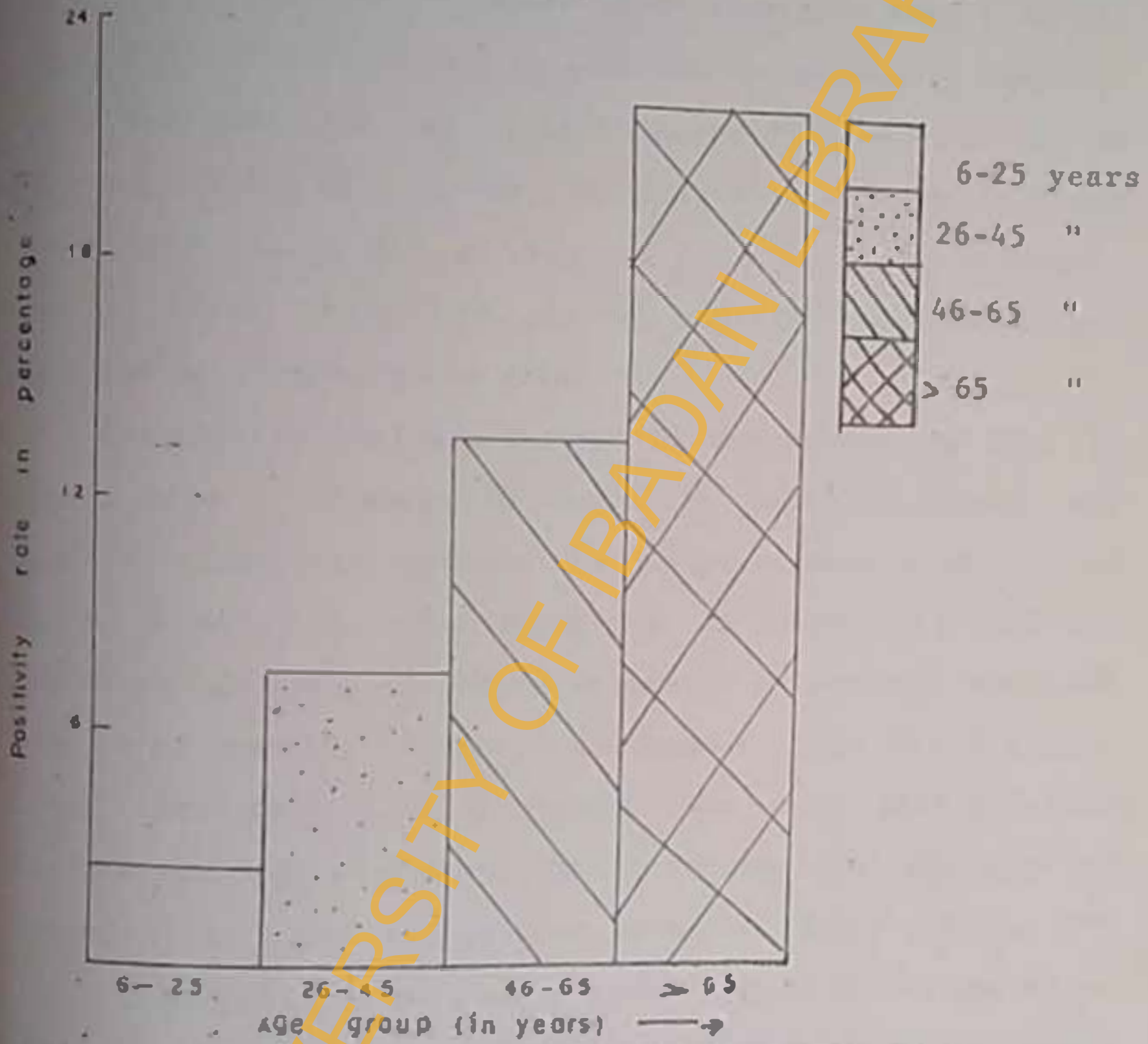


Figure 25 · Positivity rate in rheumatoid factor test, by the Rose-Wooler technique, in the different age groups.

$X^2 = 198.08$; $P < 0.01$.

highest titre was 1 in 40. For subjects aged 46-65 years the highest titre obtained was also 1 in 40 and only 5.4% (2 out of 37) were positive at higher serum dilutions than 1 in 10. All the 3 subjects that were positive in the 26-45 year age group were positive at >1 in 10 serum dilution and two of them had a titre of 1 in 320. The only 6-25 year old that had a positive result did so only at 1 in 10 serum dilution. Overall 47.1% (8 out of 17) of positive results became negative on further serum dilution.

Correlation analysis between RF concentrations and CIC levels (see 7.1) shown in Figure 26 indicate significant positive association between the two parameters ($r=0.195$; $t=2.355$; $P< 0.02$) in these Nigerians. In addition, there was increased prevalence of RF autoantibody in subjects with CIC (10 out of 100:10%) compared with those without (2 out of 43; 4.7%). However, this difference was not statistically significant ($X^2=2.08$; $P>0.10$). Of the immunoglobulins, only IgM showed significant correlation with RF titres (Table 29; $P<0.01$). RF positivity rates by latex agglutination was 6% (3 out of 50) for those with CIC and 5.6% (1 out of 18) for those without. This difference was also not significant ($X^2= 0.560$; $P>0.10$). For AHA, positive tests were obtained in 4% (3 out of 75) subjects with CIC and in 0% (0 out of 28) of those

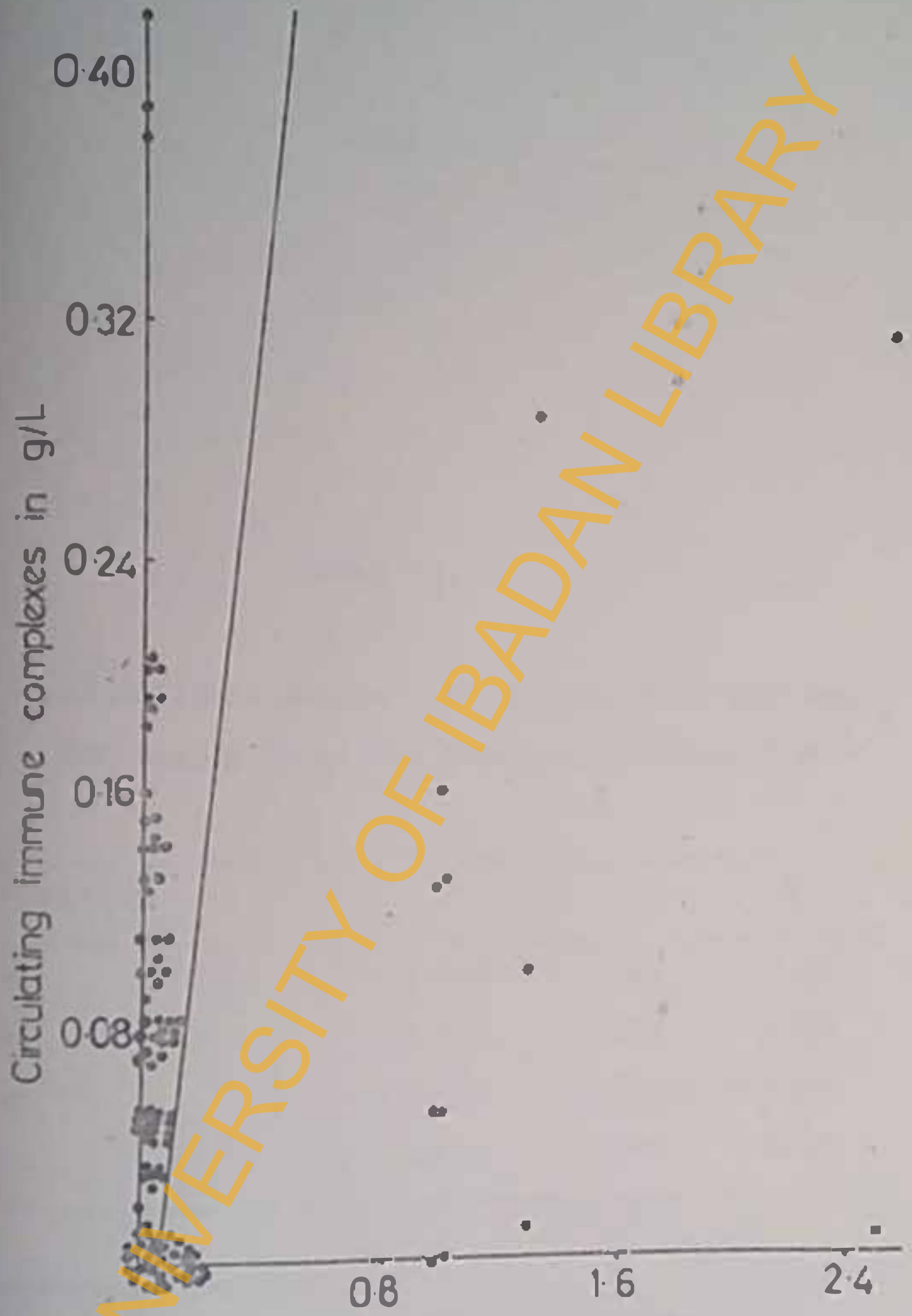


Figure 26: Scatter diagram relating cic levels to RF titres.
$y = 0{\cdot}050 + 0{\cdot}977r = 0{\cdot}195;\ p < 0{\cdot}02$

TABLE 29.

Correlation analysis between the titres of rheumatoid factors (Rose-Waaler test) and immunoglobulin levels

Variables	r	t	P
RF / IgG	- 0.154	1.716	> 0.05
RF / IgA	0.076	0.843	> 0.20
RF / IgM	0.292	3.236	< 0.01
RF / IgD	-0.049	0.319	> 0.20

RF is rheumatoid factor.

without. The difference was significant ($X^2 = 4.082$; $p < 0.05$).

7.3 DISCUSSION

Circulating immune complexes: The range of concentrations of CIC obtained in healthy Nigerians in this study is similar to those reported by Okpala and Salimonu (1990) in control individuals. Progressively increasing levels of CIC with age observed here is similar to the report of Goodwin et al. (1982). They found that CIC (determined by the ^{125}I-C1q solid phase assay) and autoantibodies were increased in the healthy elderly when compared with young controls. The expectation that CIC levels may be elevated in ageing people is supported by this report. However, this is not likely to be due to age-associated infections as healthy subjects were studied here. A possibility that increased prevalence of certain autoantibodies in ageing people may have contributed to these results cannot be ruled out.

The frequency of detectable levels of CIC increased in subjects > 65 years of age with a higher frequency in males than females. Delespesse et al. (1980) have also reported that circulating immune complexes are more frequent in males than in females. Higer frequency of CIC in males than in females may be because immune complexes are more likely to form in the presence of antibodies of low avidity. The immunologic capacity of the female as reflected by antibody formation, is known to be relatively

superior to that of the male (Kenny and Gray, 1971; Eidinger and Garrett, 1972). CIC levels may be a useful index of ageing.

Antinuclear antibodies and rheumatoid factor determinations: There is a clear increase in the percentage of sera from old subjects that were positive for antinuclear antibody compared to those from younger people in this study. However, a positivity rate of 8.3% observed here in subjects >65 years is lower than the prevalence of 18% reported by Goodwin et al. (1982) in New Mexicans. Positive sera were only weakly reactive here as they could not stand dilution. Greenwood and Whittle (1981) have also reported that ANA is usually present at only a low titre in healthy tropic dwellers.

In agreement with previous observations (Houba and Allison, 1966; Awoyinfa et al., 1971; Greenwood et al., 1971; Greenwood and Whittle, 1981), rheumatoid factors have been demonstrated in healthy Nigerians in this study. Also, old people have been found to have a significantly higher frequency of positive results compared to others. RF may therefore be useful in indexing the ageing process. This will be especially true if the high titres obtained in ageing individuals in latex fixation test (Greenwood et al., 1971) is confirmed. However, though the incidence of positive results increased with age in the present report, RF antibody titres in old subjects were not higher than in other age groups.

The observation of 9.4% frequency of RF positivity (by latex agglutination) in people >65 years of age in this study is lower than figures reported by Greenwood et al. (1971) in Africans over the age of 50 years. The high frequency of RF that they obtained in these subjects may not be real. For instance, highly lipaemic samples may cause non specific reactions (Rapi Tex RF, Behringwerke) probably through reduction in pH. Spontaneous agglutination of latex particles occurs at the isoelectric point of gammaglobulin, pH 6.6 (Singer and Plotz, 1956).

The Rapi Tex RF latex reagent used in the present study could not detect RF concentrations lower than 20 i.u./cm^3. More of the subjects studied may have RF at this lower range. This may explain the difference in prevalence rates reported here and those documented by Greenwood et al. (1971). It also explains the higher frequencies of RF positivity obtained by the rabbit antibody-coated SRBC agglutination test. About 47% of positive sera gave negative results on further dilution than the mandatory 1 in 10 required for the test. SeroPositivity rates of 22% for subjects >65 years of age and 13.5% for those 46-65 years old by the Rose-Waaler test can still not support near-50% positivity rate obtained by Greenwood et al. (1971). Goodwin et al. (1982) have reported a prevalence of 14% in old subjects in New Mexico by the latex agglutination method.

The observation that CIC and autoantibodies (ANA and RF) were increased in the healthy elderly compared to the younger groups in this study agrees with the findings of Goodwin et al.(19

there was increased prevalence of ANA and RF autoantibodies in subjects with CIC, compared with those without, but the difference was statistically significant for ANA only. The observation that CIC and RF are positively associated in these Nigerians does not agree with the findings of Goodwin et al. (1982). However, Delespesse et al. (1980) also found an association between the presence of CIC and the presence of one or more autoantibodies in healthy subjects. Increased prevalence of CIC in old people may result from persistent formation of autoantibody-antigen complexes. Immune complex disease is usually more likely to occur in chronic than in acute infections, and in those who produce poor affinity antibody (Greenwood and Whittle,1981). Autoimmunity is a chronic condition and elderly subjects produce antibodies with poor antigen avidity (Powers et al., 1987). The size of an immune complex also influences its fate; tissue damage being most likely to be produced by moderately large complexes (Greenwood and Whittle 1981). Very large complexes are taken up by phagocytes and destroyed; and very small complexes formed in antigen excess continue to circulate without being deposited in the tissues (Greenwood and Whittle,1981). Formation of small-sized immune complexes may be the one involved in the subjects in the present study as none of them had clinical signs of immune complex disease.

Correlation of IgM levels with rheumatoid factor titres suggests that this autoantibody is of IgM isotype. Rheumatoid factors are generally believed to be IgM autoantibodies specific for host IgG.

CHAPTER EIGHT

GENERAL DISCUSSION

It is generally agreed that there is a decrease in cellular immunity with increasing age (Pazmino and Yuhas, 1973; Gardner and Remington 1978; Patel, 1981) but the findings from different laboratories examining age-related changes in several immunologic indices are inconsistent (Goodwin et al. 1982; Muraako et al., 1986). The major problem with human gerontological research may be the separation of physiological changes of age from those that are secondary to diseases that frequently accompany ageing. Therefore only subjects that were considered to be in good health were admitted into this study. Also as nutrition plays a critical role in the modulation of immune responses (Chandra, 1993) the subjects studied were those with comparable levels of components used to index nutritional state (total plasma protein, albumin, transferrin and C3).

The results obtained in these Nigerians agree

with those from previous studies in other populations. Those showed decreased delayed hypersensitivity skin test responses (Roberts-Thomson et al, 1974), increased circulating immune complexes (Goodwin et al., 1982) and increased prevalence of rheumatoid factor (Greenwood et al., 1971) and anti-nuclear factor (Goodwin et al., 1982) autoantibodies with ageing. Lymphokine (migration inhibitory factor) activity was also found to decrease progressively with age in this study. L-MIF is being studied to assess cellular immunity during ageing in Nigerians for the first time. Complement component C4 levels had age-associated increase in agreement with the observation of Nagaki et al. (1980). Antibody formation in response to meningococcal polysaccharide vaccination remained unchanged with ageing. This is similar to the findings of Solomonova and Vizev (1973) with tetanus toxoid vaccine.

Matour et al. (1989) found no correlation between mitogen induced lymphoproliferation and T-cell subsets. The present study also revealed no significant correlation between migration

inhibitory factor activity and the number of T-cells and its subsets (see 4.3.3). This indicates that alterations in the numbers of resting T-cell subsets are not responsible for the depressed effector function observed during ageing in this study. Instead, there may be a defect in the ability of a subpopulation of cells to respond to antigenic stimuli. Matour et al. (1989) observed that the elderly demonstrate a decreased number of all T cell subsets after PHA stimulation compared to young subjects. The most pronounced decrease was in $CD8^+$ cells. Also, Grossmann et al. (1989) have obtained results in the elderly showing a 20-30% reduction in the proportion of $CD8^+$ cells proliferating in response to PHA and anti-CD3. They observed up to 40% reduction in the rate of cell-cycle progression of the responding cells.

Other factors might influence age-dependent depression in cellular immunity. One of them is a drop in the functional capability to produce IL-2 by peripheral blood lymphocytes (Shu-lin et al., 1986). However exogenous IL-2 is incapable of fully restoring the low proliferative response of

cells from elderly humans (Grossmann et al., 1989). The possibility that prostaglandin E2 (PG E2) produced by monocytes is a significant growth-retarding factor in lymphocytes from the aged is not supported by the report of Grossmann et al. (1989). The progressive loss of T cell function with age is probably not caused by one specific defect but rather by the accumulation of harmful changes with time (Cearlock, 1988).

Of the complement components studied, only C4 show significant alteration (increasing values) with age. Complement (C2, C4, Bf) and immune response genes are associated at the major histocompatibility complex. This would suggest that reduced cell-mediated immunity observed in ageing people in this study may also be associated with alterations in the level and function of these complement components. Correlation analysis (see 4.3.3.) show significant inverse correlation between C4 and migration inhibitory factor activity with Con.A and Candida antigen. Migration inhibition with measles and BCG vaccine antigens lacked significant correlation with C4. Although the levels

of C3c and Bf are not altered with age, this hypothesis may explain the age-associated elevation of C4 levels in this study. However, significant correlation of C4 levels with migration inhibitory factor activity was obtained with only two of the four lymphocyte activators used. This suggests that any association which may exist between complement and immune response genes may be partial.

Elevation of CIC levels in serum may be due to either increased production and/or decreased clearance of these complexes from the circulation. Clearance of CIC requires an effective phagocyte function. Evidence from this study (NBT dye reduction by neutrophils) shows that phagocyte function is not depressed during ageing. However, this does not rule out the presence of defective clearance of CIC. The finding of increased levels of CIC with age in this report, in the presence of apparently normal levels of C3 and elevated levels of C4, appears a paradox. This is because increased levels of CIC are often associated with the activation and consumption of classical pathway complement components. Procaccia et al. (1988) made an analogou

observation in patients suffering from multiple sclerosis. It is possible that high levels of CIC stimulate increased production of some complement componenta.

There was no significant correlation between CIC and migration inhibitory factor activity induced by any of the four lymphocyte activators used in this study (see 4.3.3). Although cause and effect relationships between immunity and soluble immune complexes formation may not be established in ageing by measuring CIC levels, immune complexes may serve as a valuable index of the ageing process. There was a profound overlap in CIC values among the age groups. Longitudinal studies may reveal that increases in CIC over individual baseline levels in the absence of infection or disease, may be more relevant in monitoring ageing.

Age-related increase in the prevalence of rheumatoid factors and anti-nuclear antibodies has been demonstrated in this study. It is ironic with ageing that autoimmunity appears to rise while cellular and certain humoral immune functions appear to decline. It has been postulated that a

decrease in T cell function is the primary immunological change with age (Kay, 1979; Meredith and Walford, 1979). Thus, the increase in autoantibodies is attributable to a loss of suppressor T cell function. No correlation was observed between the titres of rheumatoid factors and leucocyte migration inhibition in this study (see 4.3.3). This does not exclude the possibility that suppressor T cell function may be associated with the levels of RF. However, Goodwin et al. (1982) also reported no correlation between measures of cellular immunity (including PHA stimulation of lymphocytes) and those of autoimmunity.

Although the exact role of the autoantibodies is not known, they may cause slowly progressive tissue damage and in this manner may contribute to physical ageing (Weksler, 1981). Interestingly, some autoantibodies have been shown to interact with brain neurons in mice (Nandy, 1982). These may play a role in the aetiology of age-associated cognitive decline (Lal and Forster, 1988). Auto-antiidiotypic antibody may play a role in shutting

down the immune response. Old laboratory animals have been found to produce more of this antibody during the immune response than do young animals (Weksler, 1982).

Significant association between the titres of RF and immunoglobulin levels was found with IgM only (see 7.2). IgM was positively correlated with RF titres while IgG showed inverse correlation which missed statistical significance marginally. The results agree with the general view that rheumatoid factors are IgM autoantibodies directed against host IgG.

The decreased responsiveness of the immune system would be expected to contribute to increased susceptibility to infections in the elderly. Clinical studies have shown that immunization prevents some infectious diseases in the elderly (Haddy, 1988). Current recommendations require that patients aged 65 years or older should receive influenza vaccine annually, booster tetanus toxoid every ten years, and pneumococcal vaccine once (Powers et al., 1987).

C O N C L U S I O N

From the results of this study the following conclusions are reached:

1. Cellular immune responses at the effector functional

level, as assessed by the leucocyte migration inhibitory factor determinations, decrease progressively with age in Nigerians.

2. Tuberculin PPD skin test reactivity, a measure of cell-mediated immunity, is depressed after the age of forty-five years in Nigerians.

3. The capacity to form antibodies in response to vaccination with meningococcal polysaccharide is unaltered during ageing in Nigerians. Also, the levels of isohaemagglutinins are unchanged with age. These suggest that, unlike T-cells, B-cells are not affected by ageing.

4. Complement component C4 levels in plasma increases with age in Nigerians and may prove useful in indexing the ageing process.

5. Circulating immune complexes levels rise with increasing age in Nigerians, and may be a useful index of ageing in the tropics.

6. Old Nigerians have increased frequency of rheumatoid factors and anti-nuclear antibodies compared to younger ones.

7. Neutrophil metabolic function remains intact during ageing in Nigerians.

8. No numerical alterations in resting lymphocytes or their subsets are responsible for the depressed cellular immune capacity that occurs during ageing.

The polymorphic effects of ageing on the immune system make it difficult to relate individual immunologic indices to susceptibility to infection. Assessment of several immunologic parameters during ageing in this study reveals age-related decline in adaptive cellular immune responses. Humoral responses were maintained. This novel information on the immunologic profile in the aged African agrees substantially with reports from studies in other populations (Augener et al., 1974; Alexopoulos and Babitis, 1976; Muresko et al., 1986).

SUGGESTED FURTHER STUDIES

The pathogenesis of defective cellular immunity that occurs during ageing has not been fully elucidated in this study. Enumeration of resting T-cells and its subsets showed no significant alteration. A functional assay of purified T-lymphocyte subsets, rather than mere cell counts, may reveal changes that can explain depressed cellular immune responses, and increased prevalence of autoantibodies during ageing.

Elevated concentrations of circulating immune complexes found during ageing in this study suggests that immune complexes may have a role to play in the pathogenesis of depressed cellular immunity during ageing. Although the levels of circulating immune complexes

and a measure of cell mediated immunity (leucocyte migration inhibitory factor activity) did not correlate significantly in this study, a re-examination of this hypothesis may be rewarding.

Sex differences in the activity of leucocyte migration inhibitory factor during ageing was not analysed in this study because the number of participants studied was not large enough to split. Further studies should supply this information.

This study showed that antibody production in response to meningococcal polysaccharide vaccination did not differ significantly across the age groups. T-cell-dependent vaccines such as tetanus toxoid should be used in subsequent studies to assess antibody production during ageing as many antigens to which man is exposed are T-cell dependent.

Though neutrophil metabolic function was found unaltered during ageing in this study, it is important that future studies should analyse chemotactic function of these cells.

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APPENDIX I

MEASUREMENT OF AMOEBIC ANTIBODIES BY INDIRECT HAEMAGGLUTINATION

METHOD: Cellognost amoebiasis reagent kit (Behringwerke AG, Marburg, Germany) was used to detect amoebic antibodies by indirect haemagglutination. Positive control serum and test plasma were prediluted 1 in 8 with Tris buffer solution pH 8.0. A two-fold serial dilution was then prepared in a microtitration plate. 25ul of sensitized human group O erythrocyte suspension was added to 50 ul of each serial plasma dilution. Erythrocytes were sensitized with soluble purified E. histolytica (HK 9 strain) antigen. The plate was incubated at room temperature for 3 hours after which it was read. Agglutination of the cells was taken as positive while sedimentation of cells (ring or button formation) was recorded as negative.

RESULTS: Only 9 of 238 samples were positive and they were excluded from the study. The distribution of positive results among the age groups is shown in Table 30.-

TABLE 30

Distribution of positive tests in amoebic antibody assay in the study population

Age Group (Years)	No. of Samples that were negative	No. of Samples that were positive					Total	% total number tested
		Titre						
		32	64	128	256	512		
6-25	64	0	0	1	2	0	3/67	4.5
26-45	75	0	0	1	0	0	1/76	1.3
46-65	49	0	2	0	1	0	3/52	5.8
> 65	41	0	1	1	0	0	2/43	4.7

APPENDIX 2

SEROLOGICAL SCREENING FOR HEPATITIS-B VIRUS INFECTION

METHOD: Latex agglutination by the BIOMAN HEPAT-B latex test (Bio-Diagnostic Products Limited, Yaba-Lagos, Nigeria) was employed for hepatitis-B virus surface antigen (HBs.Ag.) detection in this study. Reagents were brought to room temperature and mixed gently before use, ensuring that the latex reagent is completely in suspension. 50 ul of undiluted test plasma, positive and negative control sera were placed on separate agglutination areas on a tile. 50 ul of latex reagent (coated with antibodies against HBs.Ag.) was added to each cell on the slide. They were mixed with separate applicator sticks and the fluid was spread over the entire area of the cell . The slide was tilted back and forth slowly for 5 minutes and was then examined for distinct agglutination.

RESULTS: 12 of 238 samples gave positive results. These were excluded from the study and consist of 4 subjects in the 6-25 years age group (6.0%), 5 subjects aged 26-45 years (6.6%), 2 individuals of age group 46-65 Years (3.8%) and 1 subject in the age group > 65 years (2.3%).

APPENDIX 3

DETERMINATION OF ANTIBODIES TO SALMONELLA SEROTYPES

METHOD: Antibodies to Salmonella antigens were semi-quantitated by microtitration uaing stained salmonella suspensions (TESTUS Diagnostics, Ikeja, Nigeria). A two-fold serial dilution of test samples, positive and negative controls was done in a microtitration plate using normal saline. Equal volume of 1 in 100 diluted salmonella suspensions was added to each appropriate well. The plate was rocked to mix antigen and serum and was incubated in a water bath at 48° C for 18 hours in the case of Salmonella 'O' antigens. Incubation was for only 2 hours at the same temperature for Salmonella 'H' antigens. The plates were then examined for agglutination.

RESULTS: The mean titres of salmonella antibodies observed in the study population are shown in Table 31. Significantly higher titres ($P < 0.05$) of antibodies to S. paratyphi O group A antigen were obtained in subjects aged 6-25 years compared to other age groups. The frequency distribution of the individual values reveals that the mean titre is not due to a few high values. Also, people >65 years old have significantly lower titres of antibodies ($P < 0.05$) to group B antigen of S. paratyphi O compared to other age groups.

TABLE 31

Mean (±s.d) titres of plasma antibodies to some Salmonella serotypes in the study population

Age gp. (yr.)	Anti-S. typhi 'O' (group D antigen)	anti-S. typhi 'H' (flagellar ag.d)	anti-S. paratyphi 'O'			anti-S. paratyphi 'H'		
			(gp. A ag.)	(gp. B ag.)	(gp. C ag.)	(ag. a)	(ag. b)	(ag.c)
6-25	16.9 ± 13.2 n = 56	13.0 ± 11.4 n = 56	28.7 ± 27.6 n=54	1290± 1387 n=55	6.9 ± 5.0 n=55	40.4 ± 31.4 n=54	27.4 ± 47.1 n=55	24.7± 17.3 n=55
26-45	15.6 ± 23.0 n = 62	13.3 ± 6.3 n = 63	16.3± 10.3 n=63	1666 ± .1544 n=63	7.6 ± 5.0 n=63	48.7 ± 59.4 n=63	22.1 ± 40.1 n=63	21.0 ± 22.2 n=63
46-65	19.1 ± 9.8 n = 35	15.3 ± 12.3 n = 35	18.4 ± 12.5 n=35	1211. ± .1637 n=35	8.2 ± 5.8 n=34	37.7 ± 39.9 n=35	37.1 ± 52.9 n=35	32.4± 40.6 n=34
>65	17.6 ± 10.5 n = 29	17.1 ± 9.4 n = 28	15.3± 9.9 n=29	686 ± 1471 n=26	7.9 ± 5.0 n=28	30.3 ± 25.4 n=29	33.7 ± 29.0 n=27	20.4± 16.7 n=28
6-95	17.0 ± 16.4 n = 182	14.2 ± 9.8 n = 182	20.2± 20.3 n=181	1319± 1541 n=179	7.6 ± 5.1 n=180	41.2 ± 44.2 n=181	28.4 ± 42.2 n=180	24.2 ± 25.0 n=180

ag. = antigen.

APPENDIX 4

SICKLE CELL TYPING BY CELLULOSE ACETATE ELECTROPHORESIS

METHOD: Sickle cell genotyping was done by haemoglobin electrophoresis on cellulose acetate as follows:

1. Haemolysates of the samples were prepared by lysing 1 volume of packed cells with 4 volumes of 50m mol./l EDTA.
2. The compartments of an electrophoresis tank were filled with Tris-EDTA-Borate (TEB) buffer pH 8.5. The wicks were soaked and positioned.
3. In a separate dish, the cellulose acetate membranes were soaked in TEB buffer for at least 5 minutes avoiding air bubbles.
4. The membranes were blotted between two pieces of absorbent paper and haemolysates were applied immediately.
5. 10 ul of each diluted sample was placed in a sample well into which an applicator is dipped in order to transfer the sample to the cellulose acetate membrane.
6. Haemolysates were applied to the cellulose acetate approximately 2 cm from one end of the strip.
7. The strip was placed upside down in the tank so that the wicks were in contact with the buffer and the

cellulose acetate, the application line being towards the cathode.

8. Power was then applied at 350 V for about 20 minutes after which it was switched off, the strip was removed and stained for 5 minutes with Ponceau S.

9. The strip was removed, drained and excess stain eluted with three consecutive 2 minute washes with 50 cm^3/l acetic acid.

10. Dehydration was done in absolute methanol for 5 minutes, clearing in 20% acetic acid in methanol for 8 minutes and the strips were dried in a 65°C oven for 6 minutes. They were then kept in a plastic envelope.

11. The pattern given by unknown samples were compared with that given by control haemolysates containing haemoglobins A, S and C in each electrophoretic run.

RESULTS: The distribution of haemoglobin genotype in the subjects studied is shown in Table 32. Nobody in the screened population was of genotype HbSS, HbSC or HbCC.

TABLE 32

Distribution of haemoglobin genotype in the subjects

Age group (years)	Haemoglobin Genotype				
	AA	AS	AC	SS	SC	CC
6-25	23	8	3	0	0	0
26-45	49	17	4	0	0	0
46-65	17	3	0	0	0	0
> 65	9	2	0	0	0	0
Total	98	30	7	0	0	0

APPENDIX 5

TOTAL PLASMA PROTEIN ASSAY

METHOD: Total protein was assayed colourimetrically by the Biuret method. 100ul of sample or standard was mixed with 2.9cm^3 of distilled water and 3.0 cm^3 of Biuret reagent. This was shaken and incubated at 37^{o}C for 10 minutes. 3.0m^3 of distilled water mixed with 3.0 cm^3 of Biuret reagent served as blank. Reading of results was done at 540 nm wavelength.

RESULTS: The mean ± 1 s.d. total plasma protein levels obtained for the various age groups are shown in figure 27. They did not differ significantly among the age groups.

Figure 27. Mean (± 1s.d.) total plasma protein levels in the subjects. $r = 0.146$; $P > 0.05$.