HOST ALLERGIC RESPONSE VARIATION IN CHILDREN WITH MEASLES INFECTION

by

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Submitted in partial fulfilment of the requirements for the degree of Doctor of Medicine in the Department of Paediatrics and Child Health, University of Natal.

1977, DURBAN

DECLARATION

This thesis is the candidates own original work.

Selected results from this thesis have been published in scientific journals. Research workers who were closely associated in these studies are co-authors in these publications.

1. Coovadia, H.M; Wesley, A.; Brain, Peter,; Henderson, L.G.; Hallett, A.F. and Vos, G.H. Immunoparesis and outcome in measles. Lancet (1977) 1, 619.

2. Coovadia, H.M.; Wesley, A.; Henderson, L.G.; Brain, Peter,; Vos, G.H. and Hallett, A.F. Alterations in immune responsiveness in acute measles and chronic post-measles chest disease. International Archives of Allergy and Applied Immunology. Accepted for publication, February 1977.

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ETHICAL CONSIDERATIONS

All the children studied were incapable of consenting on their own behalf to any non-therapeutic procedures performed. Therefore parental consent was obtained when indicated after they had been informed that the procedures undertaken were of no immediate benefit nor in any way significantly detrimental to the child's interests. Such consent was not obtained in studies which involved simple blood taking.

"And he will manage the cure best who has foreseen what is to happen from the present state of matters. For it is impossible to make all the sick well... but since men die, some even before calling the Physician, from the violence of the disease and some die immediately after calling him... before the Physican could bring his art to counteract the disease; it therefore becomes necessary to know the nature of such affections... and to learn a foreknowledge of this also."

HIPPOCRATES (460 - 370 BC)

From the book of Prognostics

C O N T E N T S

C O N T E N T S continued..... Page

SUMMARY

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4. SUMMARY

In many infections some patients recover while others die or are permanently disabled. These extremes in clinical outcome may be determined as much by the capacity of the host to eliminate the infecting agent as by the antigenic load on the individual. Children with measles who do not recover may succumb to acute complications (mainly respiratory) or chronic disease (respiratory and neurological) may develop. Analysis of immunological function antedating any of these final events would assist in understanding their pathogenesis and possibly aid in management.

In order to achieve this, immunological responsiveness was at first studied in 24 children with acute measles and compared with that in 20 children with established chronic post measles chest disease investigated 6 - 16 weeks after appearance of the rash. The immunosuppressive effects of acute measles were extensive. Total white cells were reduced and this reduction was accounted for entirely by lymphopenia which was equally expressed among the major lymphocyte sub-populations studied; the function of T cells, assessed by radioisotope incorporation into phytohaemagglutinin-transformed lymphocytes and by delayed skin hypersensitivity to dinitrochlorobenzene, was depressed. Serum IgA was reduced in acute measles patients. In contrast there was a relative sparing of the measured indices of immunity in patients with chronic post measles chest disease, with the major defect being an impaired delayed hypersensitivity reaction to dinitrochlorobenzene. There were minor alterations in complement components in both groups of patients with the evidence suggesting minimal utilisation of the alternative pathway in acute measles and classical pathway in chronic patients. High levels of heterophile antibodies to sheep red blood cells were detected in patients with chronic chest disease.

The results suggested that the conditions for chronicity of pulmonary disease in measles were unlikely to be determined by persistent abnormalities in the immunopathological factors enumerated, most of which were normal in chronic patients. It was not possible to interpret the findings of defective delayed hypersensitivity and complement components in patients with chronic chest disease as being either the cause or the effect of chronicity. The latter findings would have to be compared with results in children who had recovered from measles studied six weeks after onset of rash. An attempt was made to resolve this problem.

Twenty-two children with measles were studied in the acute stage of the rash and six weeks later and results compared with matched controls. The above findings in acute measles were confirmed: the total lymphocyte count and major lymphocyte sub-populations were significantly below control values. At six weeks the B cell and Null cell counts were still significantly diminished. The function of T cells assessed by radio-isotope uptake by phytohaemagglutinin-stimulated lymphocytes and by delayed skin hypersensitivity reaction to dinitrochlorobenzene was impaired during the acute stage and this persisted for six weeks. No important abnormalities were detected in serum immunoglobulins and complement components.

Partial reversal of immunological suppression caused by measles was therefore demonstrated at 6 weeks after the appearance of rash. Demonstration of a persistently defective delayed hypersensitivity in those who recovered made it unlikely that this anergy was important in the development of chronicity. Complement abnormalities were similarly unrelated to progression to chronic lung damage.

Children who recovered, when studied at six weeks, appeared to be worse off immunologically than those with established chronic chest disease following measles. Children with chronic chest disease were studied 6 - 16 weeks after onset of rash, by which time the partial reversal of immune deficiency, noted at 6 weeks, would be complete.

Among the group of children studied during the acute rash of measles there were five who subsequently died and one who progressed to chronic chest disease. Results in these six children were compared with those in six age-matched children who recovered from measles within a week.

In the children who subsequently died or developed chronic pneumonia, immunosuppression was more pronounced during the acute rash (i.e., 3 - 20 days before death) than in the children who recovered. The absolute total lymphocyte count (T and B cells) was significantly lower in those who died or developed chronicity. Mean serum C_3 was also lower in this group. There were no significant differences between the two groups for total white cells, neutrophils, Null cells, cells with both T and B cell markers, other complement factors, serum immunoglobulins and phytohaemagglutinin stimulation of lymphocytes.

The total lymphocyte count in a further nineteen patients with measles who had died, studied retrospectively, was significantly lower than that in twenty-seven patients with measles who recovered.

Children whose outcome was poor generally had absolute lymphocyte counts below 2000 cells/mm^3 whereas those who recovered had values above this level.

Therefore children who will die or develop chronic chest disease can be often distinguished, within two days of the appearance of the rash, from those who will recover.

In order to test the validity of this conclusion based on results obtained from a small sample the study was extended so as to increase the number of patients with measles who had severe lymphopenia (< 2000 cells/mm^3).

Seventy seven per cent of 30 children who had severe lymphopenia within 2 days of appearance of rash failed to recover: 30% died from pulmonary complications within a few days to two months of the onset of the exanthem while 47% developed chronic lung damage. This was significantly worse than the outcome in 30 children with lymphocyte counts above 2000 cells/mm^3, of whom 67% recovered, 33% developed chronic chest disease and none died. Persistence of severe lymphopenia (which was due to reduction in both T and B cells) in those with initial counts below 2000 cells/mm^3, for at least fifteen days after onset of rash, remained a good predictive index of morbidity and mortality. Reversal of immunoparesis in those with initial severe lymphopenia was slower and less complete 42 days from the appearance of the rash in children who subsequently died or progressed to chronicity than in those who recovered. All patients who died failed to produce an adequate or sustained antibody response to measles.

The results of these studies suggest that long term pulmonary and possibly neurological sequelae of measles are probably due to a transient widespread immunoparesis during early measles with persistent defects in specific immunity to measles and probably other viruses, whereas recovery is due to less severe effects of shorter duration.

In order to answer the question why some children do badly and others well after measles, studies on the HLA frequencies and measles antigen load have been undertaken in children with severe lymphopenia. Results of viral load are inconclusive and those of HLA suggest a trend towards histocompatibility linked genetic susceptibility to the development of severe lymphopenia in measles associated with HLA AW32.

The therapeutic implication of these studies is that children with measles who are at risk for death and chronic disease can be identified early in the disease and intervention at this stage may reverse the severe immunosuppression which leads to rapid demise or modify the immunopathological changes progressing relentlessly in some cases to permanent lung and brain damage and occasionally to death.

INTRODUCTION

1. INTRODUCTION

(i) HISTORICAL REVIEW

Rich and poor nations differ today in their patterns of disease; epidemics which contributed so much to human misery and death during the past few centuries are no longer a serious problem in the developed countries. Among less privileged peoples, however, these same widespread diseases account for a considerable morbidity and mortality. Deaths from measles among unvaccinated children in Britain are negligible[1] whereas in parts of Africa the combination of measles and malnutrition can result in a mortality of 50%[2]. Measles has been and remains an illness attended by grave complications.

No accurate records are available of Measles in antiquity. The widespread skin eruptions and epidemic nature of smallpox and measles may have accounted for some confusion between the two. Rhazes (860-932 AD) the great Persian clinician, is credited with the first authentic account in the literature of measles and also smallpox[3]. He draws an imperfect distinction between the two. In addition he delineates physical characteristics in the host which predispose to infection with either one or the other and which also account for severity of smallpox. With unerring clinical accuracy he observed of measles that "the diseases which are of a deep red colour are of a bad and fatal kind" - a fact which remains as true a thousand years later as it was then. Isaac the Israelite, a fellow physician of Rhazes, had a more fanciful explanation according to Davies.[4] He believed measles to be due to "the viciousness of menstrual blood". These noxious elements of blood passed from mother to foetus and the newly born baby was only purged of this when it broke out in a measles rash.[4]

Thomas Sydenham, the seventeenth century English physician, gave a first hand account of the clinical picture of measles,[5] thereby laying the basis for an unequivocal distinction from smallpox. He made special note of the nature of the rash and the development of pneumonia after measles. From his description of the extent of the rash, it would appear that measles was of the severe variety. The excellence of this account established Sydenham's clinical reputation. In 1675 he also clearly differentiated scarlet fever from measles.

An English pastor, Dr. Thomas Thacher, who had emigrated to the New England Colony, utilised the new facilities of a printing press to popularise ideas on measles and smallpox. In 1677 he distributed a one-page pamphlet entitled "A brief note to guide the common people of New England how to order themselves and theirs, in the small pocks or measles."[6]

Despite Sydenham's accurate description of measles, Michael Underwood, an English physician, still treated measles and smallpox in the same discussion in his book "Treatise in the diseases of Children" (1799).[6] During the 17th century the exanthemata remained inadequately recognised so that measles and rubella were not separated as clinical entities. In 1898 Henry Koplik described the spots on the buccal mucosa which are pathognomonic of measles and to this day bear his name.[7]

Measles henceforth could be completely identified by clincial examination. The viral aetiology of measles was suggested by experiments carried out between 1905 and 1939.[8] These involved transmission of the disease to human volunteers and animals by infected blood, serum and throat washings. For example, Ludvig Hektoen, an American pathologist, worked on experimental measles transmission by injections of blood (1905-1919).[9]

Munson emphasized that measles was passed on by the air borne route in the Texan epidemic of 1917.[10]

In 1949 Enders and his colleagues developed tissue culture methods which made possible the propagation of measles virus.[8] It was only in 1954 that techniques were found which allowed consistent propagation of measles virus in human and simian renal cells by Enders and Peebles in Harvard. The propagation of measles virus led to isolation of the Edmonston strain of measles which was successfully attenuated through serial passage and assured the development of the measles vaccine. The fine structure of the measles virion was described in 1961[11] and its electron microscopic, chemical and antigenic properties have been recently reviewed by Waterson.[12]

The word "measles", Davies notes, was first used in England by John Gaddesden and referred in general terms to the inmates of medieval leper houses.[4] Mathew Paris (1250) called these inmates "miselli". Leper homes were called "meselcotes".[13]

(ii) EPIDEMIOLOGY

Although the evidence is circumstantial, it would appear that measles in severe and epidemic form occurred during the time of Rhazes. By the 17th century, Sydenham had characterised the disease sufficiently accurately for precise recognition. The toll on human life was great.

A devastating epidemic of measles among Massachusetts Indians reduced their number from 30 000 to 3 000 in the early 17th century.[6] During the 18th century, when Edward Jenner was vaccinating people against

smallpox, epidemics of measles were noted to have increased in severity. Robert Watt of Glasgow[4] suggested that this effect was due to the protection of the worst off children against smallpox and their susceptibility to measles. Prior to vaccination, these worst off children would have been exposed to smallpox and many would have died from it. There was little reduction in death rate during the 19th century; during the seige of Paris (1871) half the *garde mobile* died of measles[4] and during the American civil war the confederates lost 2 000 of 40 000 troops with measles.[4] Measles also shaped military history during the war between Brazil and Paraguay in 1865.[4] The Paraguayans were defeated when they lost 20% of their army from measles. 25% of the entire Fijian population was wiped out in the catastrophic epidemic of 1875.[14] During an epidemic the attack rate is higher than for any other infectious disease. Among virgin populations (i.e., with no previous experience of measles) - Eskimos, Indians in Canada,[15] in Cape York, Australia[14] and in Greenland[16] it was shown to be more than 99%. This almost total attack rate is lower in endemic areas;[17] it was between 80 and 90% in the USA and Europe.

It is not clear why this should be so. However, recent evidence suggests that subclinical infection with measles is not very rare.[1] It is also possible, but less likely, that in endemic areas the lower attack rate is due to more susceptible individuals having been eliminated in previous epidemics. Mortality and the incidence of complications have been shown to be higher in first epidemics among virgin populations than in subsequent ones.[18] Subclinical infection probably also accounts for failure of susceptible children to develop measles in an epidemic. These subjects may respond with measles specific antibody[1] or cellular immunity.[19]

In the first half of this century the incidence of measles in the USA[20] and USSR[21] remained unchanged though the mortality was significantly reduced. In the U.K., the MRC trial on measles vaccine[1] has shown that with the use of live and killed vaccines the incidence of measles has dropped considerably.

Epidemics are said to occur[17] when the susceptible child population reaches 40% and to continue until this susceptible population is reduced to half. The incidence of the disease is highest between 2 to 4 years in developed countries[20] but in the Third World it occurs most frequently among even younger children.[22] Mortality is universally highest among the youngest[20,21,23,24] (< 2 years). Spread of infection is from case to case. Although there are no carriers, malnourished children with measles can excrete the antigen (not necessarily the virus) for up to 13 days.[2]

Immunity appears to be life-long. Good evidence for this is the resistance to measles among survivors of an epidemic of 1781 in the Faroe Islands during the next epidemic in 1846.[25] Similarly in Cape York, Australia,[26] those who contracted measles in 1944 were immune during the epidemic in 1953. Measles antibody persists for more than 10 years[27] after infection and cellular immunity to measles can be demonstrated in adults who had measles as children.[28]

(iii) PATHOGENESIS

There is at present an imperfect understanding of the events which occur in the human host from entry to elimination of measles virus. The processes during the incubation period, when the disease escapes clinical recognition, are probably least understood. Such gaps in our knowledge as threaten the continuity of interactions between host

and virus have been filled in with detail obtained from animal experiments. Foremost among these, and forming the classic experimental model of pathogenesis of virus disease, is ectromelia infection in the mouse as described by Fenner.[29] Evidence obtained from monkeys[30,31] infected with measles has also been extrapolated to the human situation (see Figures 1 and 2).

Infection occurs when droplets of nasopharyngeal secretions from patients with measles are transmitted to an uninfected person. The respiratory epithelium of the new host is the site of infection. Transmission of the disease by throat washings has already been alluded to under 'Historical Review'. Further it has been shown that patients with measles excrete measles virus in their nasopharyngeal secretion from 3 days before to 6 days after the onset of the rash.[32] Papp has proposed that the conjunctiva may be the primary site of infection.[33] Connection between the conjunctival sac and nasopharynx, however, would render infection of one as effective as infection of the other. Generally no symptoms occur at this stage though Goodall[34] has described transient respiratory illness. The period from exposure to first symptom (incubation period) is usually 11 days and that to the rash (incubation and prodrome) 14 days. In man leucocytes have been shown to transmit the disease during the incubation period.[35] Data from monkeys,[30,31] suggest that after lodging in the respiratory epithelium the virus infection extends to the regional lymph nodes on day 1. Multiplication in the nodes leads to a primary viraemia on day 2 with infection to lymphoid tissues and respiratory epithelium on days 3 to 5, with the formation of giant cells.

Rapid growth of virus particles leads to a secondary viraemia on day 5 with establishment of infection of the skin and possibly in the lung,

lymphoid tissues, blood, nasopharyngeal secretions and kidney. The brain may be infected also at this stage. This dissemination occurs on about day 7.

The prodromal phase, with clinically recognisable disease, begins on approximately the 11th day of illness. Virus has been shown to be present in man during this period[36] in blood, nasopharyngeal secretions, lung, cervical lymph nodes, spleen and kidney. In addition the characteristic giant cells seen in measles have been identified in nasopharyngeal secretions, lungs and various lymphoreticular tissues (appendix, tonsils, lymphnodes, thymus, Peyers patches and spleen)[36-43] Giant cells most probably represent virus infected cells[32] and may be of 3 types:[44]

1. Epithelial - in the respiratory epithelium and occasionally in the bladder mucosa.[36] The former may become detached and lie free in the lumina of air passages.

2. Reticulo-endothelial in lymphoid tissues (Warthin-Finkledey cells)

3. Thymus giant cells.

It is also well recognised that the measles virus forms giant cells in tissue culture.

It has been shown in monkeys[30] that the concentration of virus in both blood and tissues increases during this period.

Which cells can the measles virus infect? It has been pointed out that epithelial, reticulo-endothelial and thymic cells are infected. In addition it has been shown that in man, T and B lymphocytes and

monocytes from peripheral blood, but not polymorphonuclear leucocytes, can be productively infected by measles virus.[45] It is not known whether these same cells are also infected in lymph nodes and spleen. In monkeys lymphoid cells are not the site of infection; this has been shown to be primarily the reticular cells in germinal centres.[46]

Small quantities of antibody (haemagglutination-inhibition and complement fixing) may be present during the prodromal phase.[32] The rash erupts on day 14 and virus may be detected in blood and lungs for up to 32 hours[47] and in nasopharyngeal secretions for up to 6 days[32] after the appearance of the rash. Giant cells degenerate soon after the appearance of rash and then disappear. By day 15 there is increasing amount of antibody, viraemia ceases and tissue viral content diminishes. On day 17 the rash fades. Epithelial cells containing the viral antigen are excreted in the urine from 2 days before to 6 days after the rash.[48] In the skin, virus-like particles have been identified by electron microscopy in epithelial cells and endothielium of blood vessels.[49,50] Virus antigen can be detected in the epithelium, corium, hair follicles and sebaceous glands up to 5 days after the rash.[51] The immunopathological mechanisms which account for the rash are not clearly understood. Certainly virus antigen is found in blood vessels of the skin, but this is present in areas of skin with and without the exanthem.[51] It is thus an insufficient cause but may be a necessary one. The rash occurs during a period of minimal antibody[32] and probably maximum antigen concentration, conditions ideal for the development of soluble immune complexes. However, children with agammaglobulinaemia develop a measles rash[52] while those with cellular immune defects do not.[53,56] It is possible that the former do have undetectable antibody. Functional integrity of T and B cells, with co-operation at some level, is therefore probably the likeliest

basic requirement for development of the exanthem. Burnet's hypothesis for the development of the rash is illustrated in Fig. 2.[57]

In malnourished children[2] and those with severe measles[58] there is evidence for prolonged virus excretion; giant cells can be detected in the latter for up to 28 days after the onset of the rash. In uncomplicated cases giant cells with measles antigen are excreted 5 days before and up to 8 days after the appearance of the rash.[32]

Children debilitated by serious disease may develop a fatal measles giant cell pneumonia from which the virus can be isolated for up to a month after the onset.[59] Virus pneumonia following measles can be progressive and destructive and is usually associated with measles virus in the early post measles period (12 days) and thereafter with adenovirus and herpesvirus.[60] Bacterial infection supervening on a measles pneumonia is a not infrequent complication. Most of the adenovirus pneumonias seen locally follow on an attack of measles among Black children in Durban.[61] The end result of persistent lung disease after measles can be permanent lung damage.[62]

Subacute sclerosing panencephalitis is the prototype of a latent viral infection in man and may be due to altered host and measles virus interaction.[63] It is a rare condition among Black children in Durban. The other diseases associated with measles - multiple sclerosis[64] and partial lipodystrophy[65] (usually associated with hypocomplementaemic mesangiocapillary glomerulonephritis) - have not been reported as complications of measles in the Durban area.

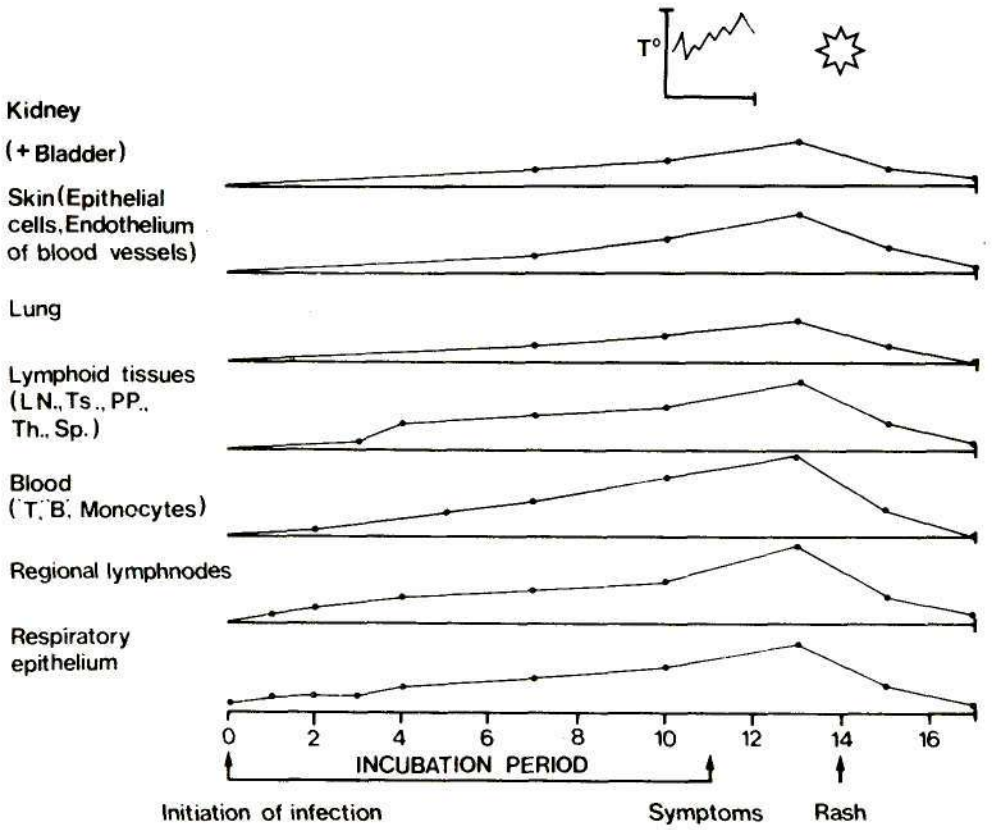

Fig. 1 PATHOGENESIS OF MEASLES

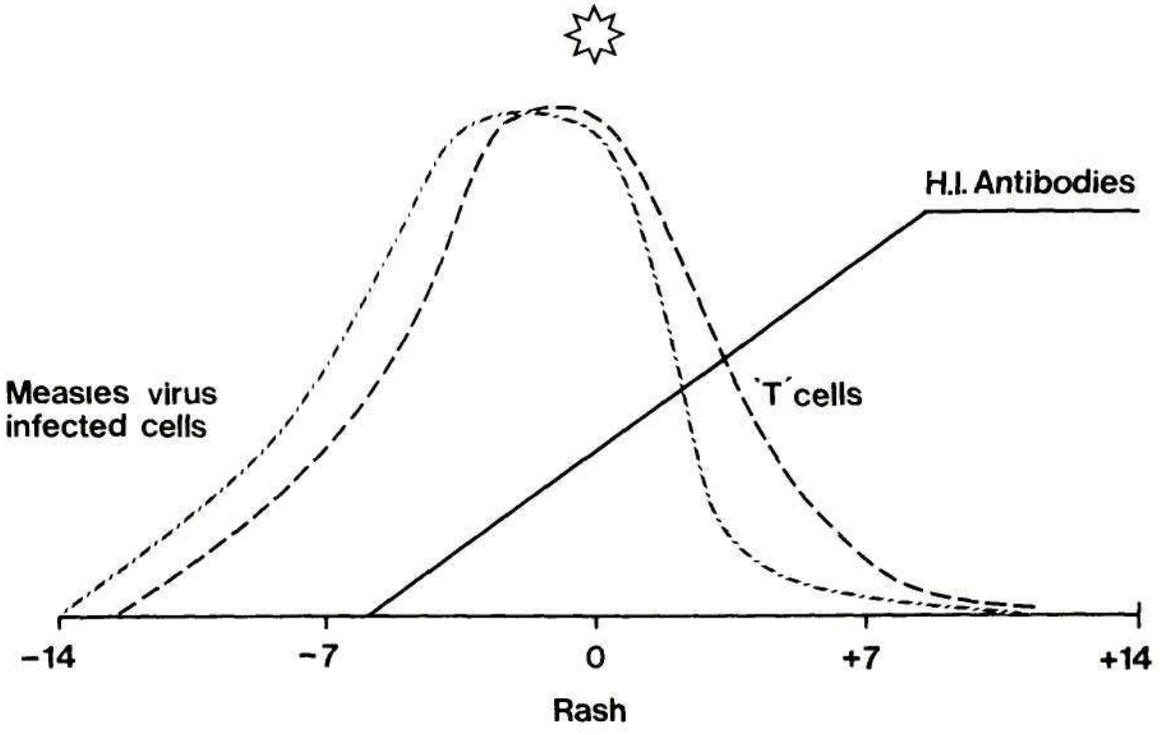

Fig. 2 IMMUNE RESPONSE TO MEASLES

(iv) THE NORMAL IMMUNE RESPONSE TO MEASLES

The complexity of interaction between host and parasite which is characteristic of many infections is at least restricted by the monotypic quality of the measles virus. Although there are four distinguishable antigenic determinants on the surface envelope of the virus,[12] there is no evidence that any one is more damaging than another and therefore all four can be considered as a single unit, the measles antigen. Evidence for antigenic uniformity[66] of measles virus has been:

1. Measles is a clearly defined clinical entity with symptoms constant, apart from variation in severity due to host and environment.

2. Life-long immunity.

3. Antibodies from different parts of the world are effective in neutralisation and complement fixation tests on a single type of virus.

Although non-specific mechanisms of host resistance, such as the pH and ciliary action of mucous surfaces, are important in defence against viruses,[67] their role in protection against measles has not been elucidated. In cell cultures infected with measles virus, an inhibitor of virus activity appears which has the characteristics of interferon.[68] Therefore it is likely that in vivo measles induces the production of interferon which in turn would restrict its dissemination in the early stages of the disease. Neutrophil numbers are not increased nor are these cells infected by measles virus.[45] Although important in bacterial infection, polymorphonuclear leucocytes probably play an

insignificant role in control of measles infection. Macrophages, which have an essential function in both the afferent and the efferent limbs of the immune response,[69] are certainly productively infected with measles.[45] It is uncertain whether they phagocytose and kill the virus or merely serve as a reservoir of replicating organisms.

A widespread dissemination of measles ensures a large quantity of cell associated antigen. Cells thus affected are mainly epithelial and lymphoreticular cells which assemble virions and express measles antigen on their surface.[70] This physical presentation of measles antigen would be expected to stimulate antigen sensitive T cells to proliferate in preference to B cells,[57] and a population of cytotoxic T cells would gradually be formed. Co-operation between T and B cells, or possibly direct stimulation of B cells, would generate activated B cells which go on to form plasma cells secreting specific measles antibody. Such antibody is a constant feature of clinical measles and T cells cytotoxic for fibroblasts infected with measles have been demonstrated in vitro.[71] Persons who have had measles in the past can produce a leucocyte migration inhibition factor when their cells are tested with measles antigen.[72] Further, lymphocytes of measles patients can liberate a macrophage migration inhibition factor and a lymphotoxin when stimulated with measles or measles-like (SSPE) viruses.[73] These findings suggest that measles virus stimulates the production of lymphokines from sensitised lymphocytes. However, the proliferation of sensitised lymphocytes on stimulation with measles virus has not been successfully or consistently demonstrated.[74,75,76] Measles complement fixing antigen may on the other hand, transform sensitised lymphocytes.[19]

Cells infected with measles during the incubation period could be effectively destroyed or modulated by a number of effective mechanisms: cytotoxic T cells, neutralising antibody, antibody with complement and complement alone. Antibody can combine with cell surface antigen, cause these antigens to aggregate into one polar end of the cell (i.e., "capping") and be shed into the circulation as complexes.[70] Antibody and complement have been shown to damage measles infected cells.[70] Measles virus activates the alternative pathway of complement both in vivo[77] and in vitro,[70] which would aid in elimination of infected cells.

Reaction between the population of infected measles cells and these many-pronged attacks by the immune response results in destruction of the former and an eruptive release of antigen, antigen-antibody complexes, virus and antibody, and the products of stimulated T cells, lymphokines. Spill over into the circulation and spread result in the clinical syndrome of measles. Elimination of infected cells completes recovery.

Antibody persists for more than 10 years[1] and correlates with protection while sensitised lymphocytes also last for long periods.[28] Neutralising antibodies appear early, peak on day 28 and remain for prolonged periods. Haemagglutination-inhibition antibody parallels neutralising antibody and persists for longer than 10 years. Complement fixing antibody appears on the first days of the rash and declines after 3 to 4 years.[73] Measles virus specific cytolytic antibodies have been demonstrated in Rhesus monkeys.[79] Injections of gammaglobulin given during the incubation period abort measles and passively acquired maternal antibodies protect babies against the disease. Clearly then, antibody prevents measles. However, children with agammaglobulinaemia handle measles virus normally and are immune to second attacks while those

with defects of cellular immunity develop a fatal disease.[52-56] Cell mediated immunity is probably important for recovery from existing infection while agammaglobulinaemics may have some undetectable antibody. Cell co-operation, however, may prove to be the most important element in immunity to measles infection.

(v) IMMUNOPATHOLOGICAL DISEASES LINKED WITH MEASLES

A. DISEASES IN WHICH MEASLES IS PROPOSED AS A CAUSATIVE FACTOR

1. CHRONIC NEUROLOGICAL DISEASE:

(a) Subacute sclerosing panecephalitis (SSPE)

SSPE is now recognised as the prototype of latent viral infection in man. The evidence associating this syndrome with measles is almost irrefutable. It is a disease of late childhood and early adolescence, following many years after an attack of measles, with an onset marked by behavioural and intellectual changes, leading on to increasing dementia, myoclonus and extrapyramidal signs and terminating in decortication and death in 6 to 12 months.[80,81]

These patients have high titres of measles antibodies in the serum[82] and CSF.[83] Some of the antibodies in the latter are produced within the brain itself and may have an oligoclonal character, i.e., they are of restricted heterogeneity. Measles virus has been identified in the brains of these patients by serological, immunofluorescence, and electron microscopic methods.[84] The virus has also been grown from infected brain tissues by methods of co-cultivation, i.e., using sensitive indicator cells for infection.[85]

Injection of SSPE virus into animals does not always lead to the disease; in some cases an acute encephalitis can be produced.[86]

The SSPE virus appears very closely related to measles virus but may not be identical. Subtle differences between these viruses suggest that SSPE virus could be measles virus in a defective form.[63] It is believed today that many chronic viral diseases are due to defective viruses.[87] Host factors may, however, also play an important role in determining outcome to an ordinary attack of measles. SSPE-like illnesses have been described in rare patients with impaired immunity[88] and the attacks of measles antedating SSPE have been shown to occur in a younger age group.[89] The complexity of virus-host interactions and the role of environmental factors in the aetiology of SSPE is suggested by the detection of this disease in Cape Town but not in Durban.

(b) Multiple sclerosis

The evidence linking multiple sclerosis and measles is less secure. It is based on the finding of high titres of measles antibodies in serum[90] and CSF[91] in these patients. Some of this antibody is present in an oligoclonal IgG band as in SSPE.[64] There is no convincing evidence of measles virus in the tissues of these patients.

2. PARTIAL LIPODYSTROPHY:

There has been a reported association between an attack of measles and the development of partial lipodystrophy;[65] a rare disorder of subcutaneous tissue which is related to a severe kidney lesion, hypocomplementaemic mesangiocapillary glomerulonephritis. This appears to involve particularly those areas of skin affected by the rash.

3. CHRONIC "AUTOIMMUNE" DISEASES:

In systemic lupus erythematosus[92] and chronic active hepatitis[93] antibodies to measles virus have been shown to be elevated. This, however, is more probably the effect rather than the cause of the disease.

B. DISEASES WHICH ARE AMELIORATED BY MEASLES INFECTION

Children with the minimal change nephrotic syndrome in relapse have been noted to go into remission after measles.[94] As these patients nearly always respond to steroids it is possible that measles is effective by its powerful immunosuppressive mechanisms. We have observed this effect in minimal change nephrosis not only after measles, but also following on an attack of varicella. It is also possible that increased levels of adrenocortical hormones during these infections produce a response in nephrosis. There is anecdotal evidence that measles rarely aggravates an attack of allergic asthma.[95]

(vi) INFECTIONS FOLLOWING MEASLES

If the undoubted immunosuppressive effects of measles have clinical relevance, then an increased incidence of infections should be expected to follow on rubeola. In the previous chapter mention has been made of prolonged or delayed effects of measles infection on the nervous system, as in SSPE. This demonstrates an inability, under certain circumstances, to deal specifically with measles virus.

Ever since von Pirquet noted that the tuberculin reaction became negative during measles infection[96] there have been numerous attempts

to link causally the development of tuberculosis and the depression of skin reactivity to PPD. Implicit in this is the belief that delayed hypersensitivity and immunity are one and the same thing in tuberculosis. Although closely related, it would appear that these two facets of the allergic response to the tubercle bacillus are not identical.[97] This together with the varying degrees of severity of measles may account for some of the contradictory evidence in this regard.

Klein (1925),[98] Goebel (1929),[99] Constantino (1932),[100] Kohn (1932)[101] and Liesegang (1932)[102] reported studies of large groups of children in whom an attack of measles preceded the development of tuberculosis. These studies suggested that measles both activated and aggravated tuberculosis. Two large measles epidemics in the virgin populations of southern and western Greenland in 1951 and 1962 respectively added further support to the association between measles and TB. During April 1951, Christensen and co-workers[103] surveyed the townspeople of Narssak for pulmonary tuberculosis using fluoroscopy. It was found in 16%. The measles epidemic occurred in May and almost the entire population of the town contracted the disease. In September of that year the incidence of tuberculosis was found to be 19%. Flick[104] states that this difference is not statistically significant and that in any case there had been no control population (i.e., without measles). However, in the 1962 epidemic in Greenland a control group without measles was also studied.[105] Of 34 000 persons, 10 722 contracted measles whereas the remainder, being immune from the previous epidemics of 1954 and 1955, did not. Lange[106] found that in adults (> 20 years) in this population the incidence of tuberculosis was significantly higher in those who had suffered from measles than in those who had not. This evidence suggested that persons who had

measles were predisposed to development of tuberculosis. Starr and Berkovich 1965[107] found that measles had a detrimental effect on patients already on treatment for tuberculosis. Flick, in a review of the literature,[104] quotes a number of studies from which he draws the opposite conclusion, i.e., that measles may have a beneficial effect on existing tuberculosis. Our own experience is that, except in the few examples quoted in the previous chapter, measles is generally damaging to children and in particular predisposes them to tuberculosis. The strong impression is that, at least in Black children, a severe attack of measles not infrequently leads to pulmonary tuberculosis. This would be in accord with the recognition of polar forms of tuberculosis patients with anergy and disseminated tuberculosis at one end and at the other those with vigorous cell mediated immunity and a restricted lesion.[108]

Primary infection with herpes simplex virus is a common and innocuous disease of infancy and childhood. In measles, however, it can be devastating. Armengaud[109] found that of 225 cases of herpetic gingivostomatitis, 83% occurred in children recovering from measles; of these 25 died. Kipps *et al.*[110] in Cape Town found that of 93 cases of fatal disseminated herpes infection, 16 had been preceded by measles. Herpes simplex is thus not only common in measles but can be extremely dangerous. It has in fact been suggested that children with measles should be isolated from contact with those who develop herpes. The incidence of herpes and measles is maximal at the age of 1 year[110] and in Durban it has been reported that the maximum number of cases of disseminated herpes occurred during October-November when the incidence of measles was greatest. Disseminated herpes is a problem of the neonate, the malnourished and the child with measles. The common denominator among these three conditions is a variable

degree of immaturity or unresponsiveness of the immune mechanisms.

Moniliasis,[111] cancrum-oris[112] and pyogenic infections[23] also often complicate measles. This inability of the measles-infected host to handle effectively infections with viruses, fungi and pyogenic organisms hints at widespread immunodeficiency. These defects in immunity are considered in detail in the following chapters.

(vii) NON SPECIFIC IMMUNITY

It is always difficult and often impossible to induce measles infection in experimental animals. This means that most animals possess an inherent or natural immunity to the virus. The intratracheal inoculation of nasopharyngeal washings from measles patients can produce a measles-like disease without the respiratory complications in Rhesus Macacus monkeys.[113,114] However, even in these primates only freshly captured animals are susceptible; captive monkeys lose this susceptibility and develop resistance. Adapted strains of measles virus can infect newborn mice and hamsters.[115]

Man appears to have no such natural immunity. In virgin populations, i.e., with no previous experience of measles, the attack rate approximates 100%.[14,15,16]

However, in endemic areas this rate may be between 80 to 90%.[17] It has been suggested[17] that this may imply a natural immunity among a minority of the population in endemic regions. Recent evidence[1] suggests that this might in fact be due to inapparent infection.

The exact mechanisms of innate immunity are not at all clear. The demonstration of measles virus receptors on human lymphocytes[116] would

at least make it unlikely, in the absence of any other evidence, that in man any natural protection exists.

It has been pointed out[67] that reactions at mucous surfaces which are important in defence against viruses are not clearly defined in the case of measles. As the initial site of lodgement is the respiratory epithelium it is quite likely that any damage to the mucosa of the upper respiratory tract would influence the course and possibly severity of measles infection.

The numbers[117] and function[118] of white cells in the peripheral blood are transiently diminished during infection with measles.

Benjamin and Ward[117] showed that leucopenia did not occur during the incubation period of measles, was most pronounced during the rash, persisted to the period of convalescence and then recovered. This diminution in white cells was due to decrease in the numbers of neutrophils, lymphocytes, monocytes and eosinophils; lymphocytes and eosinophils were the earliest and most affected. Mean lymphocyte counts did not fall below 2000 cells/mm^3 though individual counts did drop below this level from the onset of rash to convalescence.

These changes are paralleled by leucocytic responses after live measles vaccine which results in leucopenia due to neutropenia, lymphopenia, eosinopenia and monocytopenia.[119] These effects are most intense on the day of onset of rash. In one study[119] of the effects of vaccine not a single patient had lymphocyte counts of less than 2000 cells/mm^3. This difference in the degree of lymphopenia induced by wild and attenuated measles viruses implies an inverse relationship between virulence and lymphocyte numbers.

However, within infections produced by attenuated measles virus there was no correlation between severity of vaccine disease and extent of lymphopenia.[119] Similarly lymphopenia was not of prognostic value with wild virus infection though during convalescence after infection with wild virus, one patient developed progressive lymphopenia (to 1000 cells/mm^3) and died from streptococcal septicaemia.[117]

The reasons forwarded for the decrease in leucocyte numbers in these studies have been either a sequestration or destruction of cells. Lymph nodes in children dying from measles reveal hyperplasia and cellular engorgement.[117] This finding together with the productive infection of white cells with measles virus[45] make it likely that both mechanisms are operative in rubeola.

Macrophages are critical in both the afferent and efferent limbs of the immune response. As measles virus infects these cells[45] it is probable that their function is altered. Monocyte derived atypical cells in viral infections[120] may also have abnormal functions. Therefore measles would be expected to affect immune responses at all levels. Anderson and co-workers[118] have shown that in white children with uncomplicated measles the following abnormalities of leucocyte function are present: there is gross impairment of both random migration and response to chemotactic stimuli of neutrophils; this defect in an in vitro test is reinforced by the demonstration of decreased neutrophil accumulation in an in vivo Rebuck skin window technique. Recovery from this chemotactic defect was complete 11 days after the onset of rash. Further, it was shown that this was due to an intrinsic cellular defect as measles serum generated normal chemotactic activity and bore no leucotactic inhibitors. These authors postulate that this immuno-deficiency may be due to elevated plasma cortisol, excessive consumption

of antigen - antibody complexes by neutrophils or direct infection of polymorphonuclear leucocytes by measles virus. There is no evidence to support the last mentioned mechanism. This defect of neutrophils during measles would be one explanation for the increased susceptibility to bacterial superinfection in these patients.

Evidence of pathological complement activation has been demonstrated in 35 to 40% of children with measles.[121,77] The function of the complement cascade as assessed by total haemolytic complement was shown to be reduced by Ecker *et al* in 1946.[121] Recently Charlesworth and his associates[77] have shown that both the classical and alternative pathways of complement are activated in measles. Reduced serum concentrations of $C1_q$, C_4 and C_3, with the presence of C_3d, suggest the former, while diminution of C_3, C_5 and properdin with detectable C_3d in the presence of normal quantities of $C1_q$ and C_4, point to utilisation of the alternative pathway. A few patients had an isolated lowering of $C1_q$. $C1_s$, C_1 esterase inhibitor, factor B and C_7 were normal. Determinations of cobra venom convertase gave inconclusive results, C_3 splitting activity was negative and no immune complexes were detected. Therefore these authors have shown activation of complement which would most likely be due to immune complexes; that these were not detected may be ascribed to the insensitivity of the assay systems used for their measurement. Bacterial superinfection may also be responsible for these effects though the data presented do not allow such an interpretation to be made.

These in vivo studies have their parallel in the experiments of Joseph, Cooper and Oldstone.[122] They showed that complement consumption was via the alternative pathway when antibody was directed against virus-infected cells while it was mediated through the classical pathway when antibody combined with free virus.

(viii) SPECIFIC MECHANISMS OF IMMUNITY IN MEASLES

Historically the first evidence of the immunosuppressive effects of measles was the observation of von Pirquet that the tuberculin reaction became negative during the exanthem.[96] This has been confirmed subsequently by a number of different investigators.[123-129,95] The only point of difference has been the duration of anergy. The tuberculin response becomes negative during the incubation period and this generally persists for about 6 weeks. However, in a study by Kipps *et al*,[129] recovery from anergy in some patients took as long as a year after the attack of measles. It is likely that in this report the immunosuppressive effects of measles were compounded by those of malnutrition which is in itself damaging to immunity.

In addition to PPD, candida antigen, streptococcal antigen and dinitrochlorobenzene (DNCB) have been utilised in demonstration of impairment of the delayed hypersensitivity response during measles infection.[112,130] In one report DNCB sensitisation gave a positive result.[112] It is possible that the powerfully sensitising properties of DNCB were able to overcome the suppressive effects of measles which had rendered the patients anergic to candida and streptococcal antigens. However, by and large tests of delayed skin hypersensitivity are negative in the incubation period and for variable periods during and after the clinical disease.

An extension of these observations has been the investigation of in vitro correlates of delayed hypersensitivity, i.e., lymphocyte transformation to mitogens and antigens. Most investigators have utilised the antigen of the tubercle bacillus (PPD) and the lectin Phytohaemagglutinin (PHA). The former measures the response of antigen sensitive cells whereas the latter tests the function of all thymus derived cells.

In tuberculin positive patients the transformation of lymphocytes by PPD was defective.[131] This has been confirmed by the finding of impaired transformation of sensitised lymphocytes (i.e., from patients with tuberculosis) when PPD and measles virus were added to the culture.[132]

Results of PHA transformation of lymphocytes have been contradictory. Some workers have obtained normal,[112] others impaired[130] transformation. This may be due to varying severity of the disease studied by different workers or to inherent difficulties in the technique. Finkel and Dent[133] reported normal responsiveness at optimum concentrations of PHA but decreased transformation at suboptimal concentrations. They postulated that as the response to suboptimal PHA is macrophage dependent,[134] the cells primarily affected by measles virus infection are macrophages. This is a reasonable explanation as the addition of measles virus to cultures of lymphocytes with added PHA gave conflicting results.[132,135] It is likely that in these in vitro systems the numbers of macrophages differed rather than there was an intrinsic lymphocyte variability to PHA stimulation.

The effect of measles virus on the co-operation of T and B cells has been studied in a murine model by measurement of the anti-hapten antibody response to DNP.[136] Measles virus was found to reduce the antihapten antibody response. This effect was due to impairment of helper T cell function.

Measles vaccine has also been reported to suppress delayed hypersensitivity responses in the skin to a variety of provoking antigens.[137]

The numbers of T cells were reduced during the exanthem and convalescence and took up to 3 months to return to normal.[138] There is inadequate data[130] on numbers of B cells and other lympocyte subpopulations.

The function of B cells in measles, however, was compromised although there was no change in pre-existing antibody titres to poliomyelitis, diphtheria and tetanus in recipients of live or killed vaccines.[137] During the early exanthem, levels of serum immunoglobulins were not markedly affected though IgG, IgA and IgM were low or normal.[112,139,77] IgA has been reported to be increased in severe measles.[139] The amount of heterophile antibody was unaffected.[112]

Production of antibodies, especially H, to TAB vaccine[112,140] has been shown to be defective in measles. This is of interest as the H antigen of *Salmonella typhi* appears to be a thymus independent antigen and therefore measures B cell function directly.[141] Antibodies to tetanus vaccine were diminished but this diminution escaped significance.[112]

(ix) PATHOLOGY IN MEASLES

An appreciation of histological changes taking place in the lymphoreticular and respiratory tissues is important for a clearer interpretation of immuno-pathological events occurring during measles. This is particularly relevant as immune function is generally tested by assessment of the quantity and function of cellular and soluble substances in the peripheral blood and not in other tissues.

Measles virus belongs to the group of larger myxoviruses (Newcastle disease, mumps, dog distemper and rinderpest) which have a common property

of causing formation of syncytia or giant cells in tissue culture.[142-146] This is an outstanding histopathological feature of measles infection in different tissues of man.

LYMPHORETICULAR TISSUES

Giant cells appearing during the prodromal phase of measles had been described by Warthin in 1931.[147] These cells were present in germinal centres and subepithelial tissues of the tonsils and pharyngeal mucosa. These observations were based on studies of tissue removed during tonsillectomy in 4 children who developed measles one to four days after the operation.

Another type of giant cell, which occurs after the prodrome and is mainly derived from lymphocytes in the thymus, has been described by White and Boyd.[44] These authors studied children who died at various intervals after developing clinical measles. They reported the following: Thymuses of children who died on the 4th day after the onset of rash showed relative preservation of normal architecture with local areas of atrophy in the cortex. There was giant cell formation of cortical thymocytes with up to 100 cells forming a syncytium. Nuclei were discrete or fused in these cells. All stages of fusion and nuclear degeneration were seen from single thymocytes with karyorrhexis of nuclei to large multinucleated giant cells. No inclusion bodies were detected in these cells: this serves to distinguish them from giant cells found in the lungs of measles patients. Similar changes, but to a lesser extent, affecting thymocytes and reticular cells, occurred in the medulla. Hassall's corpuscles were decreased in size but not in number and showed nuclear degeneration.

Thymic tissue obtained after the 4th day of onset of rash demonstrated more severe changes. There was loss of cortico-medullary differentiation, depletion of cortical and medullary thymocytes and a predominance of Hassall's bodies and reticular cells.

Atrophy of the thymus persisted for up to 64 days after onset of rash. Incomplete recovery from these changes was present in thymuses of patients obtained 3 to 4 months after the onset of exanthem. Aggregations of thymocytes were still present at 4 months.

Atrophy of the thymus was not specific to measles and occurred in children dying from a variety of illnesses, e.g., Fallot's tetralogy, atrial septal defect, hypoxia, gastro-enteritis, bronchopneumonia and trisomy 21. Giant cells, however, were not seen in any of these conditions.

Denton,[148] who examined the thymus of a patient dying 3 hours after developing a measles rash, found no significant changes. In children who died more than 2 days after the onset of the measles rash, Kipps and Kaschula,[60] reported severe depletion of cortical lymphocytes.

It would appear, therefore, that atrophy of the thymus occurs at about the 2nd day of the appearance of the measles rash and lasts for approximately 3 to 4 months.

The effect of measles on the other lymphoreticular tissues is less marked. White and Boyd[44] found minor abnormalities in the spleen, mesenteric lymph nodes and Peyers patches of the small intestine. Degen,[149] in an autopsy study of 100 cases, reported inflammation of the tonsils, follicular, lymphoid and reticulo-endothelial hyperplasia of the spleen and congestion with some diminution of lymphoid cells in

cervical, peribronchial and mesenteric lymphnodes. A cervical lymphnode biopsy taken on the first day of the rash from a patient who had a severe lymphopenia (900 cells/mm^3) has been studied by Benjamin and Ward.[117] They found markedly hyperplastic changes with follicles and medullary cords bulging with lymphocytes and numerous mitotic mononuclear cells. They concluded that lymphocytes proliferated in lymphnodes but failed to reach the circulation. Denton reported follicular hyperplasia of the spleen and inflammatory changes in lymphnodes.[148] Kipps and Kaschula,[60] however, found lymphatic atrophy in the lymphnodes, spleen and tonsils. As 66% of their post measles patients were underweight for age, it is possible that these changes were due to malnutrition rather than measles. Details other than weight of their well-nourished patients are not given.

RESPIRATORY SYSTEM

The reaction to measles virus of mucous surfaces and submucous tissues throughout the respiratory tract is similar though there may be variation in severity. Three types of lesions have been described in the mouth and pharynx:[148] inflammatory changes with focal necrosis of buccal epithelium, suppurative lesions in and about submucous glands and ducts, and inflammation of submucous lymph follicles. Kopliks spots were considered by Denton[148] to be inflammation of submucous glands. These changes are similar to those in the skin where sub-epithelial round cell infiltration with focal necrosis has been documented by Ewing.[150]

The histological descriptions of respiratory disease in measles offered by Denton[148] and by Degen[149] are similar and are described below:

There was oedema and congestion of the mucous membrane of the larynx and

trachea with some of the superficial and deeper cells having become necrotic. Occasionally ulceration of the mucous membrane was noted. Blood vessels in the submucous tissues were dilated with cuffs of inflammatory cells.

Bronchi and bronchioles showed similar changes to the above but with more extensive destruction of epithelium and a film of mucus and pus on the mucosal surfaces. Walls of bronchi and bronchioles were thickened by dilated blood vessels with inflammatory cells.

Inflammatory changes occurred in the peribronchial tissues and the alveoli contained large mononuclear cells with sheets of respiratory epithelium. Giant cells, pus cells and red cells were also found in the alveoli. Denton suggests that much of the alveolar content had been aspirated material from the bronchioles and that primary damage to the lung was minimal.

In brief the patients had laryngotracheitis, bronchitis, bronchiolitis and peri-bronchitis.

Kipps and Kaschula[60] reported more severe lesions in Cape African and Coloured children. They found necrotising bronchiolitis with interstitial pneumonia together with the presence of giant cells with intracytoplasmic and intranuclear inclusions in alveoli and bronchioles. These giant cells were typical of those described by Hecht[151] and which are said to occur in patients whose immunological responsiveness has been impaired.[53] Enders *et al*[53] were the first to culture measles virus from the lungs of patients with leukaemia who developed Hecht's giant cell pneumonia and died.

The sequel of an acute measles infection of the lungs may be follicular bronchiectasis.[62] This type of lesion has been described by Whitwell[62] as a bronchiectasis in which "the most prominent microscopic feature is an excessive formation of lymphoid tissue, occurring as follicles and nodes which are situated both in the walls of diseased bronchi and bronchioles and among the surrounding alveoli." Whitwell proposed the following evolution of this condition: Early changes were thickening of bronchiolar walls due to oedema and lymph follicle formation with destruction of elastic tissues near the follicles. These affected bronchioles were surrounded by a rim of interstitial inflammation. Further increase in lymph follicles led to widespread destruction of elastic tissue and muscle in bronchiolar walls. Robbed of their supporting elements bronchioles collapsed. Interstitial inflammation spread and fibrosis occurred around branches of the pulmonary artery.

In follicular bronchiectasis the other essential lesions were a destructive bronchitis, bronchiolitis and interstitial pneumonitis. These changes therefore resemble the early lesions of measles described above.

In summary the initial measles infection in most cases causes a mild reversible catarrhal inflammation of the respiratory tract which occasionally progresses to a destructive bronchiolitis with interstitial pneumonia. This pneumonia with Hecht's giant cells can be seen most frequently in immunologically compromised hosts. Rarely, inflamed bronchioles and bronchi become inadequately drained of secretions and are permanently damaged.

The organisms which have been associated with these pulmonary changes have been viruses and bacteria. Measles virus was responsible for lung

damage early in the disease.[60] Later, herpes virus and adenovirus have been cultured from post measles lung tissues.[60] Beta haemolytic streptococcus has been the most commonly isolated organism in one series.[149] In this study bacteria isolated from the lungs and blood of patients dying from measles were pneumococci, streptococcus viridans, staphylococcus aureus and albus. In Nigeria the commonest cause of death in measles was staphylococcal pneumonia.[152] Autopsy studies of measles patients in Uganda showed that 65% of deaths were due to viral rather than bacterial pneumonia.[153]

Wesley *et al* (1971)[154] have shown, by examination of antemortem and postmortem lung puncture aspirates, that the aetiology of pneumonia in Black children with measles in Durban was mainly bacterial. Sixty-eight per cent of antemortem cultures were positive for bacteria. The superinfecting organisms were commonly staphylococcus pyogenes, but from a third of patients gram negative bacteria were cultured. Mortality was highest in those with gram negative infections and malnutrition. Schonland,[155] on the other hand, in a post mortem study of measles patients drawn from the same source, found that the majority (> 70%) had died from viral pneumonia.

Antibiotic administration has probably reduced the frequency of measles deaths due to bacterial pneumonias.[60]

PURPOSE OF STUDY

2. PURPOSE OF STUDY

Measles, as has been discussed, is without doubt widely immunosuppressive. There is atrophy of the thymus with relative preservation of peripheral lymphoid tissues in children dying from measles. There is compelling evidence linking measles with at least one immunopathological disease. Histopathological changes in pulmonary tissues of children who die from chronic post measles chest disease reveal a heightened lymphocytic response to sites of an earlier measles infection. Events during the acute exanthem, which antedate these long term complications or death, are, however, not clearly understood. The purpose of this study is to detect critical alterations in immune responsiveness during the acute phase of measles which will influence clinical outcome.

The most common clinical presentations of measles in local Black children admitted to hospital are:

Acute: The vast majority get an acute infection, develop the classical picture of a morbilliform rash, upper respiratory tract infection and a mild chest infection, all of which resolve in about 7-10 days. All that remains as witness of rubeola is a brown-black staining of the skin which gradually fades.

Death: Within the above group of acute measles patients, a small but significant number develop overwhelming lung disease and succumb. The mortality is about 12 to 14%.

Chronic: A small yet significant number of patients have an acute episode, clinically indistinguishable from that just described, but go on to develop a chronic chest disease which persists from months to years, and in many cases leads to permanent lung destruction. These

children recover partially from the acute infection but have persistent lung changes as seen on chest radiographs with frequent exacerbations manifested by fever and respiratory distress. Observations in this unit have suggested that those children who still have significant radiological evidence of pneumonia six weeks after appearance of the rash, will go on to chronicity.

What then determines that a particular child with measles will develop a mild acute illness from which he presumably recovers fully, an acute fatal illness or a chronic debilitating and sometimes fatal disease? It is the purpose of this thesis to try to provide an answer to this interesting question and, in the broader view, to supply some understanding of the fundamental problem of the factors influencing acute elimination or chronic persistence of infectious agents in human hosts.

Measles as seen in children in Durban raises questions of a rather more general nature. They relate to the heterogeneity in the observable manifestations of infections, tissue damage and clinical expression, caused by the same agent in different hosts. It is generally recognised, though little understood, that within individuals of the same race and generally exposed to the same infective agent, e.g., poliovirus, streptococcus and Australia antigen, there are a number of possible outcomes. These range from a mild sub-clinical infection with complete recovery to a severe, rapidly evolving fatal illness or a chronic and persistent disease resulting always in morbidity and occasionally in death.

It is proposed to study the range of immunity functions in children with measles and to follow up with repeated investigations those with chronic chest problems. In this way it may be possible, by comparison of the

measurable parameters of immune responsiveness, to single out those factors which are critical for rapid elimination of antigen and which, when compromised, lead to chronic lung damage or death.

Clearly an understanding of the immune responses in infection is important not only for sound appreciation of the pathogenesis and clinical picture of disease states, but also for a rational application of modern therapeutic agents once these mechanisms have been elucidated.

If the factors leading to chronicity or death are identified, a careful programme of treatment with the appropriate therapeutic agents can be suggested. A major consideration is the possible reduction in cost of prolonged management of chronic chest disease following measles.

At our present stage of understanding it is difficult to separate the genetic from environmental causes of difference in clinical expression of disease. The increasing number of associations between HL A and human disease on the one hand, and the varying spectrum of chronic infections seen in a single host on the other, suggest that both these factors play a part in determining allergic responsiveness to infectious disease. Therefore the host and viral determinants which may influence outcome in measles will also be investigated.

METHODS

3. METHODS

GENERAL

Blood for investigation was always taken between 0800-0930 hours. This decreased possible errors introduced by diurnal variation in results of the tests performed.

1. ENUMERATION OF LYMPHOCYTE SUBPOPULATIONS

The method used has been developed and standardised by Professor Peter Brain and his co-workers at the Natal Institute of Immunology.[156]

Sheep blood was collected weekly from the same animal and stored at $4^{o}C$ in Alsever's solution. The 1% saline suspension used in the tests was prepared daily from cells washed three times.

Ten millilitres of venous blood was taken from the human subjects. A white cell count was done with a Coulter counter, and a smear stained with Giemsa for differential count. The blood was defibrinated by rotating with glass beads in a conical flask. The liquid part was diluted in the proportion of two parts of blood to three of saline and layered over a preparation of Ficoll-Hypaque, or of the commercial product Lymphoprep, with a specific gravity of 1.077,[157] in a large centrifuge tube. The tubes were spun for 45 min. at 450 g at a radius of 10 cm, after which the cells at the interface were pipetted off and washed three times in saline, each wash lasting 5 min at 250 g. The supernatant was finally removed and the cells resuspended in four drops of saline. One drop of fluorescein-labelled anti-human globulin serum (Burroughs Wellcome MF01) was added, the mixtures incubated for 30 min at room temperature, and the cells washed twice in saline. The super

natant was then removed and the button resuspended with two drops of saline. To two drops of this suspension in a 7.5 x 1 cm plastic round-bottom tube, two drops of sheep cell suspension were added and the tubes centrifuged for 5 min at 400 rev/min. They were then left undisturbed at $4^{o}C$ for 2 hr. Half the supernatant was finally pipetted off and the cells resuspended in the remainder by very gentle shaking. One drop of the suspension was examined immediately on a clean slide under a coverslip, using a Leitz Orthoplan microscope equipped so that incident illumination from a mercury vapour lamp could be quickly interchanged with weak dark-ground illumination via a glycerol-immersed substage condenser, using ordinary tungsten light.[158] In each preparation, the first hundred objects seen were classified either as rosettes with at least three sheep cells (the central cell was usually visible owing to the spreading out of the cells, and could be examined for fluorescence), as non-rosetting lymphocytes showing fluorescence, as non-fluorescing and non-rosetting lymphocytes and as rosetting lymphocytes showing fluorescence. It had been previously shown[158,159] that in this system only lymphocytes make sheep cell rosettes, and that cells other than lymphocytes which may contaminate the preparation are readily detected under tungsten dark-ground illumination; such cells were not counted. Every object seen was first identified individually by dark-ground illumination, and the illumination then switched to ultra-violet to note whether it was fluorescent. Proportions were thus obtained of T cells (forming rosettes), of B cells which are non-rosetting but fluorescent, of Null cells bearing neither of these markers and FT cells bearing both markers. From the lymphocyte count, the absolute numbers of T, B, Null and FT cells were determined by proportion.

Reproducibility of Results

Normal adults have been tested serially and the counts and T: B cell proportions were consistent from day to day. This method of identifying lymphocyte subpopulations, when discussed at an INSERM workshop on lymphocyte separation and identification, Montpellier, 1976, gave results which agreed with those laboratories getting higher figures for B cells.

2. TRANSFORMATION OF LYMPHOCYTES

A sterile technique was employed:
Blood was collected in sterile flasks and defibrinated by glass beads; this was then diluted with an equal volume (5 mls) of medium. The blood medium mixture was carefully layered on top of Ficoll-Hypaque (5 mls) in 3033 tubes. This was centrifuged at 400 g for 30 minutes at room temperature. The interface was removed with a sterile Pasteur pipette and the cells washed three times with medium in Falcon 3033 tubes; centrifugation at 400 g was carried out for 10 minutes with each wash. The cell pellets were resuspended after each wash with a sterile Pasteur pipette. After the third wash the pellets were resuspended in 1 ml of medium and the cells counted by microscopy.

Cell concentrations were adjusted to 1.33×10^6 cells/ml. 0.1 ml of antibiotics (penicillin and streptomycin in a 1/20 dilution) were added per 10 mls of cells. These cell concentrations were dispensed into the wells of round bottomed micro-titre plates as follows:

A. PHYTOHAEMAGGLUTININ (PHA) STIMULATION

PHA	0.4 ug/25 ul
CELLS (199,500)	150 ul
SERUM	25 ul

B. MEASLES VIRUS

Measles virus in various concentrations, ranging from 10^{0} to 10^{-10}	25 ul
CELLS (199,500)	150 ul
SERUM	25 ul

Plates were covered with a lid and wrapped in jiffy wrap. These were incubated at 37°C for the following times:

PHA	48 hours
Measles virus	120 hours

^{14}C Thymidine (0.075 uCi/10 ul) was then added to the cell cultures which were incubated for a further 24 hours.

The cultures were harvested on a multiple automatic sample harvester using Skatron glass fibre filter paper and water.

The filter papers were dried in an oven at 60°C for 1 hour after placing in glass counting vials.

2 mls of Instagel scintillation fluid was added to each vial and the amount of ^{14}C-Thymidine incorporated was counted on a Packard Tri-Carb liquid scintillation spectrometer model 3380.

Mean values and the coefficient of variation for triplicate cultures were calculated.

Results are expressed as stimulation index (S.I.) which is the ratio of stimulated to unstimulated counts per minute and as disintegrations per minute.

Reproducibility of Results

The coefficient of variation was always less than 15 and on average 7.35.

N.B. The culture supernatant of measles infected HeLa cells dialysed against normal saline for 24 hours was used for virus transformation. The titre of virus before dialysis was $TCID_{50}\,10^{-3.5}$ per ml as titrated on the BGM line of monkey kidney cells.

The supernatant was kindly supplied by Professor O.W. Prozesky of The National Institute for Virology, Department of Health, Sandringham.

3. DELAYED HYPERSENSITIVITY REACTION TO 2:4 DINITROCHLOROBENZENE (DNCB)

The method was to induce hypersensitivity with 10% DNCB dissolved in acetone applied to the forearm: after 9 days a challenge dose of 0.1 ml of 0.5% DNCB was applied to the opposite arm. The reactions were read after 48 hours and graded: 0, no reaction; I, erythema; II, erythema, induration, and vesiculation.

4. SERUM IMMUNOGLOBULINS AND COMPLEMENT COMPONENTS

Serum levels of IgG, IgM and IgA were determined by radial immunodiffusion (Behringwerke). Haemolytic complement was assayed by the method of Mayer, 1961,[160] using sheep red blood cells stored at $4^{o}C$ and rabbit haemolytic serum.

Complement components C_3, C_4 and C_3PA were estimated in plasma by radial immunodiffusion.[161] Tests for the presence of electrophoretically distinct metabolic breakdown products of complement C_3 were done by immuno-electrophoresis and Laurell's two-dimensional electrophoresis in agarose containing antibody, using barbitone buffer pH 8.6 and mono-specific anti-C_3 (B_1C/B_1A) antiserum (rabbit). Positive and negative controls using aged and fresh serum respectively were included for each test.

Details of the test for C_3 conversion products are given below:

C_3 CONVERSION PRODUCTS

A two-dimensional immunoelectrophoresis test was performed according to Laurell[162] modified by Clarke and Freeman.[163]

MATERIALS: 1.5% agarose

Barbitol buffer pH 8.6

41.2 g Sodium barbitol (sodium 5,5 diethylbarbiturate)

8.0 g Barbitol (5-5-diethylbarbituric acid) made up to 10 litres with distilled H_2O

Specimens were taken in EDTA and stored at - 70°C.

METHOD:

First dimension:

5 ml of 1.5% agarose in barbitol buffer pH 8.6 was poured on a 9 x 11 cm glass plate. A 4 mm well was cut and filled with 10 µl of sample. Serum was separated for 60 min. with a rectifier output-voltage of approximately 280V, a field strength of 8 - 10 V/cm, current per chamber approx 45 mA with water cooling.

Second dimension:

10 mls of melted agar + 30 µl of β1C β1A antiserum (Behring) were poured next to the previously poured strip containing the separated serum. The agarose was allowed to set and the plate then put back in the chamber. The current was run at right angles to its direction in the first run. The rectifier output voltage was approximately 80 V, Field strength 2 - 3 V/cm, current per chamber approximately 10 MA with an electrophoresis time of 18 hours.

The agarose was pressed, dried and stained with Coomassii Brilliant Blue.

Reproducibility of Results:

Random examination of triplicate tests on single samples for serum immunoglobulins and complement have shown coefficients of variation of 0.7 for IgG, 0.9 for IgA and 0.0 for IgM and 2.4 for C_3, 0.0 for C_4, 0.0 for CH_{50} and 0.9 for C_3P A

5. MEASLES COMPLEMENT FIXING ANTIBODIES

Antigen was titrated in buffer.

Complement and haemolysin titrations were performed in complement fixation buffer using 4% sheep cells. The smallest amount of haemolysin and complement giving 50% haemolysis was used.

The test serum was diluted in buffer and doubling dilutions were used. Antigen and complement were added and allowed to react overnight at $4^{o}C$. The following morning dilutions were incubated at $37^{o}C$ for 30 minutes. Indicator system (2% sensitised cells) was added and incubated at $37^{o}C$ for 60 minutes. Haemolysis was measured.

6. RHEUMATOID AND ANTI-COMPLEMENTARY FACTORS AND OTHER ANTIBODIES

Rheumatoid factors were detected using the Hyland slide test. Collection, storage (-20^{o}C) and handling of blood samples used for determination of anti-complementary factors were carried out under aseptic conditions. The anti-complementary test utilises the binding of haemolytic complement to circulating complexes in serum. Heat-inactivated sera (0.1 ml) to which 2.0 and 2.5 CH_{50} units of guineapig complement had been added, were mixed with optimally sensitised washed sheep red cells (0.1 ml of a 2% suspension) in an icebath for 60 minutes and the release of haemoglobin was measured by spectrophotometer after further incubation at 37^{o} for 30 minutes. Complement consumption was measured by the van Krogh formula in the manner suggested by Mayer *et al.*[164]

Anti-F(ab) and Anti-whole IgG Antibodies

Papain digestion of anti-D IgG was carried out as described by Porter[165] and specificity of the fragments was ascertained by precipitin tests in gel with anti-F(ab) and anti-Fc antisera which indicated that the Fc part of the IgG molecule had been destroyed whereas the F(ab) remained intact. Coating of group O Rhesus positive red cells (R_1R_2) with the papain-digested anti-D was carried out by adding to 0.2 ml of washed packed cells 0.1 ml of digested anti-D and incubation at 37^{o}C for one hour. Agglutination tests were performed on glass tiles by adding to one drop of washed sensitised or unsensitised control cells (5% solutions) an equal volume of serial two-fold dilutions in saline of the serum to be tested. The tiles were then placed in a moist chamber at room temperature for one hour. The presence or absence of agglutination was determined with a x5 magnification head-fitting eyepiece. All titres were expressed as reciprocals of the final serum dilution showing definite agglutination.

In some cases cells sensitised with F(ab) anti-D can be agglutinated by antibodies directed against the hereditary Inv, Gm(f) and Gm(z) determinants present on the light and heavy chain of the F(ab) fragment. To differentiate this variety of antibody from F(ab) antibodies it is important that the test system includes cells sensitised with undigested anti-D immunoglobulin.

Sera reacting with undigested anti-D were re-examined for the presence of F(ab) antibodies after adding normal pooled sera lacking anti-F(ab) activity. This procedure discriminates true F(ab) antibodies from others.

Anti-Sheep Red Cell Antibodies

Heat inactivated sera were mixed with a 5% saline suspension of sheep red cells on translucent glass tiles and allowed to stand at room temperature ($22^{o}C$) for 60 minutes in a moist chamber. Results were macroscopically read and all titres given as reciprocals of the final serum dilution showing definite agglutination.

7. HL A ANTIGENS

HL A antigens were detected by M.G. Hammond and D. Appadoo at the Natal Institute of Immunology, using a technique described below:

Lymphocytes were isolated by the method of Boyum[157] using a Ficoll-Hypaque mixture, and the cytotoxicity test was performed in Falcon microtest trays using the 2-stage procedure recommended by the National Institutes of Health,[166] as follows:

1 µl of antiserum and 1 µl of cell suspension were added to each well under paraffin. After 30 min. at room temperature, 5 µl of unabsorbed

rabbit complement was added and, after a further 60 min. at room temperature, 5 µl of freshly prepared 0.6% trypan blue in saline was added. After 15 min. at room temperature, excess dye was flicked off and the wells were examined with an inverted microscope.

8. DETECTION OF MEASLES ANTIGEN ON NASOPHARYNGEAL SECRETIONS

Using a syringe and rubber catheter, 5 mls of sterile saline was injected into the right nostril and rapidly aspirated.[167] If testing was not done immediately 5 ml Hanks transport medium was added to the nasal washings. This was centrifuged at 1000 g for 10 minutes. After discarding the supernatant, 1 ml 2% dimercaptoethanol was added and mixed well using wide and fine bore needles alternately. The mixture was washed immediately with phosphate buffered saline three times. One drop of the deposit was placed on a slide, allowed to dry, fixed in acetone at $4^{o}C$ for 10 minutes and then examined by indirect immunofluorescence.

Measles specific antiserum was supplied by Professors P.S. Gardner, Royal Victoria Infirmary, Newcastle-Upon-Tyne and Pekka Halonen, Department of Virology, University of Turku, Finland.

9. PATIENTS AND CONTROLS

Patients:

Measles was diagnosed on the characteristic clinical presentation by at least two independent observers. The features noted were fever, cough, morbilliform rash, Koplik's spots and conjunctivitis.

None of these patients was critically ill on admission, when they were investigated. All had mild bronchopneumonia and diarrhoea.

Details of patients and controls are included for each study.

All patients were above the 10th Boston centile for weight and none had clinical evidence of Kwashiorkor or marasmus (by far the commonest forms of malnutrition seen here).

At the time of study measles vaccine had not been routinely available for either patients or controls.

Chronic post measles chest disease patients are those who had significant radiological signs of bronchopneumonia 6 weeks after the onset of the measles rash. Observations in this unit, which admits about 1 600 cases of complicated measles a year, have shown that mild to moderate bronchopneumonia can be expected to clear radiologically within 2 to 3 weeks of rash. In our experience, children who have bronchopneumonia for longer than 6 weeks usually run a fluctuant course over months to years, with persistent lung changes.

Controls

Healthy children were usually siblings of patients with minor illness attending the outpatients' clinic, or were attending a follow-up clinic after recovery from a preceding minor illness. None was ill at the time of investigation and informed consent was obtained prior to blood being taken.

TOTAL NUMBER OF PATIENTS AND CONTROLS

1.	Acute Measles (Studies I, II, III, IV)	:	89
2.	Chronic Post Measles Chest Disease: (Study I)	:	20
3.	Healthy Controls: (All Studies)	:	75
4.	Mild Measles (seen and treated in Paediatric Out Patients' Department): (Study V)	:	55
5.	Non-Measles: (Study V)	:	120
6.	Retrospective Study of Measles Patients who died: (Study II)	:	19

DETAILS OF IMMUNOLOGICAL AND OTHER INVESTIGATIONS UNDERTAKEN IN EACH GROUP ARE GIVEN IN STUDIES I TO V.

10. STATISTICAL METHODS

Statistical analysis of results was carried out by either parametric or non-parametric tests. The latter were utilised when no assumptions could be made on the symmetrical distribution of sample results about the mean and when data were discrete.

The parametric test used was the Student's 't' test and the non-parametric tests employed were either the Wilcoxon Signed Rank Sum test or the Chi-square. Yates' correction for continuity was applied to Chi-Square when any of the expected frequencies was less than 10.

Probability values less than 0.05 were taken as significant. Methods used for a particular study are given in it.

STUDY I

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4. S T U D Y I

(i) DESIGN AND PURPOSE OF STUDY

It was decided that a practical approach to the problem of detecting immunological factors during acute disease which might determine poor outcome would be in the first instance to compare two groups of patients:

1. Children with acute measles.
2. Children with established chronic post measles chest disease.

Although it was recognised that there would be difficulties in the interpretation of any results obtained from such a study, it was undertaken because of the following two reasons:

1. Comparison of data between acute or chronic patients might reveal some abnormality in the latter which was absent in the former or a persistent immune defect in chronic patients which was reversed in acute patients on 6 week follow-up. Clearly, if any defect was detected, it would not be possible to be certain that it was the cause and not the effect of chronicity.

2. With a known mortality of about 15% in patients hospitalised with measles, and an unknown incidence of chronic chest disease, it was reasonably assumed that there was at least a 20% chance of investigating patients during the acute phase who would subsequently do badly (die or develop chronic chest disease). Comparison of results in this group against those who did well (recovered) might provide the first lead to the detection of predictive factors during acute measles.

For the purposes of this study, there was no information available as to whether a particular immunological test would be more important than another. A wide range of immunity functions were therefore tested.

(ii) PATIENTS AND CONTROLS

Studies were carried out on 44 well-nourished African children (> 10th Boston centile for weight), 24 of whom had an acute measles infection (AM), investigated within 48 hours of appearance of the rash, and 20 with chronic pulmonary symptoms (CPMC) who had significant radiological signs of bronchopneumonia at least 6 weeks after the onset of the measles rash. Measles was diagnosed clinically by two independent observers. None of the AM or CPMC patients were critically ill at the time of study although all the former had a complication of measles (i.e., diarrhoea, mild bronchopneumonia or otitis media). Controls, except those for DNCB sensitisation, were healthy children, individually matched with patients for age and race. Results of DHR to DNCB in 19 race-matched children previously reported[111] have been used as controls. Neither controls nor patients had been immunised against measles.

The ages of the AM patients ranged from 7 to 48 months, with 17 females and 7 males. CPMC patients were aged from 6 to 30 months, with 10 females and 10 males. There were four deaths in the AM group and one among the CPMC group during the period of study.

Except where indicated, results of patients are compared with age and race-matched controls. All investigations were not performed on every patient; the actual number of patients studied for each investigation is indicated in the tables and figures. Statistical analysis was performed using Student's t, Chi-square or Wilcoxon Signed Rank sum tests. The last mentioned test was used to compare SI results.

(iii) RESULTS

The total white cell count, absolute numbers of lymphocytes and lymphocyte sub-populations in AM and CPMC patients and their respective controls are enumerated in table I and drawn in Fig. 3. There was significant diminution of total white cells, absolute lymphocytes and T, B and Null cells in AM; FT cells were normal in numbers. CPMC patients had normal numbers of all these cells.

PHA transformation of lymphocytes in 22 AM and 17 CPMC patients and controls expressed as the SI was (fig. 4): AM mean 131 $\pm$ 92 (SD), controls 343 $\pm$ 242; CPMC mean 264 $\pm$ 263, controls 292 $\pm$ 131. The SI was significantly depressed ($p < 0.01$) in AM patients; in CPMC patients it did not differ significantly from the normal value.

Assessment of DHR to DNCB (fig. 5) revealed a grade 0 reaction in 21 patients and grade I in a single patient in the AM group. There were 8 patients with grade 0 response and 2 each with grades I and II in CPMC children. Compared to controls (previously reported)[111] 68% (13/19) of whom responded with a grade II reaction and 32% (6/19) with a grade I, both AM and CPMC had decreased DHR to DNCB ($p < 0.001$, $p < 0.02$, respectively).

Results of IgG, IgA and IgM estimations are given in table II and Fig. 6. IgG was normal, IgA lower and IgM higher in acute measles as compared to controls; in CPMC, IgG and IgM levels were significantly increased, with IgA being normal.

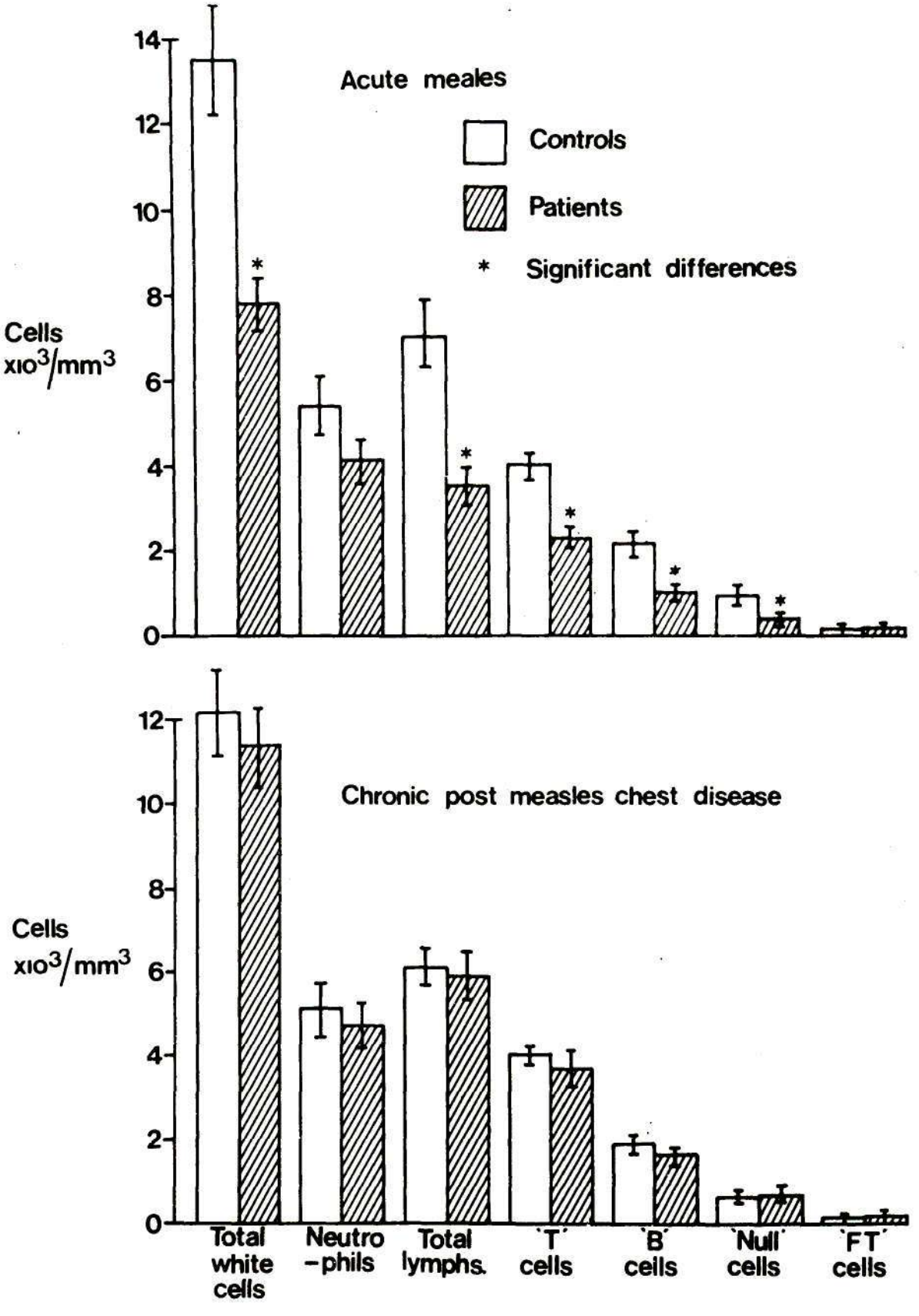
Measurements of complement components (Table III, Fig. 7) revealed that in 23 AM patients the mean C_3 (0.90 g/l $\pm$ 0.18) and C_4 (0.41 g/l $\pm$ 0.21) were not significantly different from values in 41 healthy children

TABLE I : THE TOTAL WHITE CELL, POLYMORPHONUCLEAR LEUCOCYTE AND LYMPHOCYTE COUNTS AND LYMPHOCYTE SUB-POPULATIONS IN 22 CHILDREN WITH ACUTE MEASLES AND 20 WITH CHRONIC POST-MEASLES CHEST DISEASE COMPARED WITH THEIR RESPECTIVE CONTROLS

	TOTAL WHITE CELLS	POLYMORPHO-NUCLEAR LEUCOCYTES	TOTAL LYMPHOCYTES	LYMPHOCYTE SUB-POPULATIONS			
				'T'	'B'	'NULL'	"FT'
ACUTE MEASLES							
CONTROLS	13,446[+] ± 1436[*]	5,406 ± 776	7,064 ± 809	3,991 ± 397 (57)	2,145 ± 276 (30)	927 ± 198 (13)	49 ± 14 (<1)
PATIENTS	7,800 ± 613	4,055 ± 473	3,566 ± 446	2,212 ± 250 (62)	981 ± 164 (28)	373 ± 50 (9)	47 ± 14 (1)
	<.0005[@]	0.10	<.0005	<.0005	<.0005	.005	0.47
CHRONIC PATIENTS							
CONTROLS	12,130 ± 1008	5,094 ± 591	6,127 ± 434	3,929 ± 251 (58)	1,825 ± 165 (30)	674 ± 78 (11)	116 ± 43 (1)
PATIENTS	11,370 ± 951	4,692 ± 632	5,832 ± 611	3,607 ± 404 (61)	1,540 ± 159 (25)	679 ± 136 (12)	118 ± 24 (2)
	0.30	0.35	0.35	0.48	0.10	0.48	0.47

\+ Absolute numbers of cells per cu.mm
* Standard error
@ P value of difference between patients and controls
() Percentage of total lymphocytes

Fig. 3



MEAN ABSOLUTE WHITE CELL COUNTS IN 22 ACUTE MEASLES AND 20 CHRONIC POST MEASLES CHEST DISEASE WITH RESPECTIVE MATCHED CONTROLS

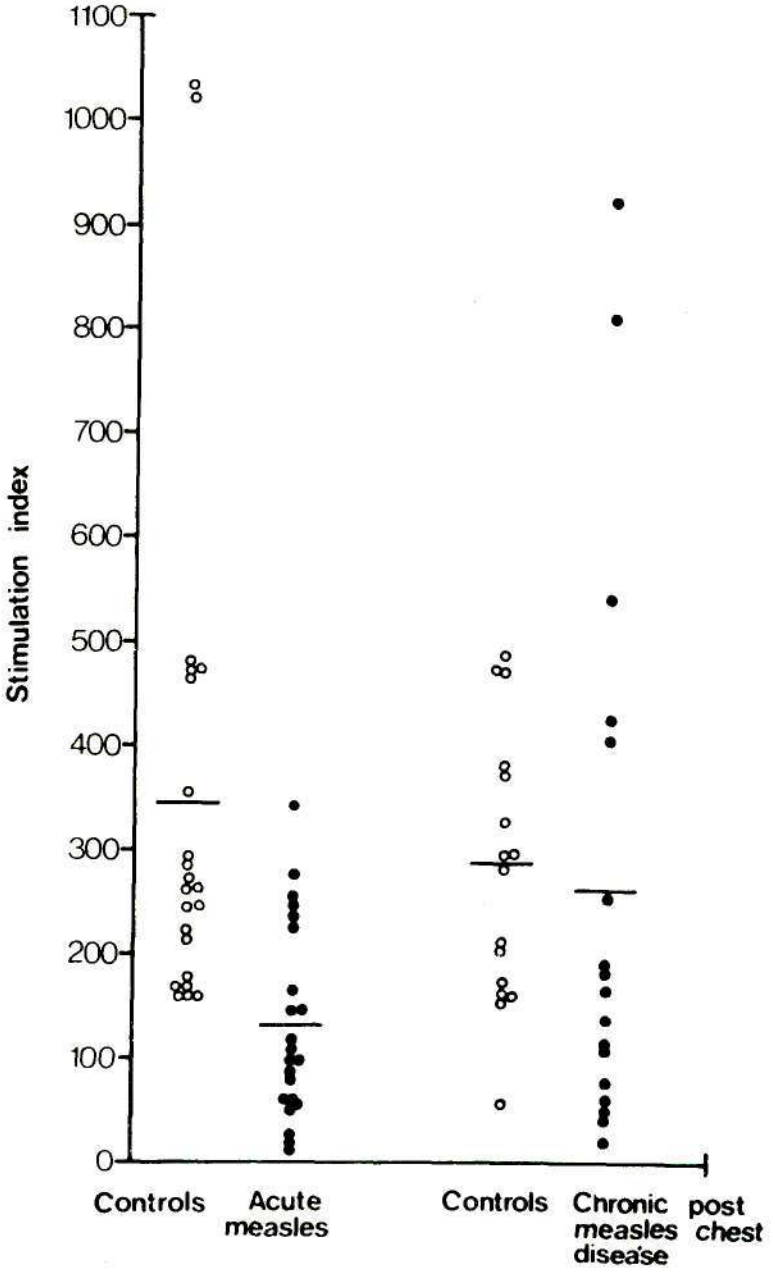


Fig. 4 PHA transformation of lymphocytes assessed by ^{14}C thymidine uptake in 22 patients with acute measles, 17 with chronic post-measles chest disease and matched controls.
Horizontal bars represent mean values.
Stimulation Index = ratio of stimulated to un-stimulated counts/min.

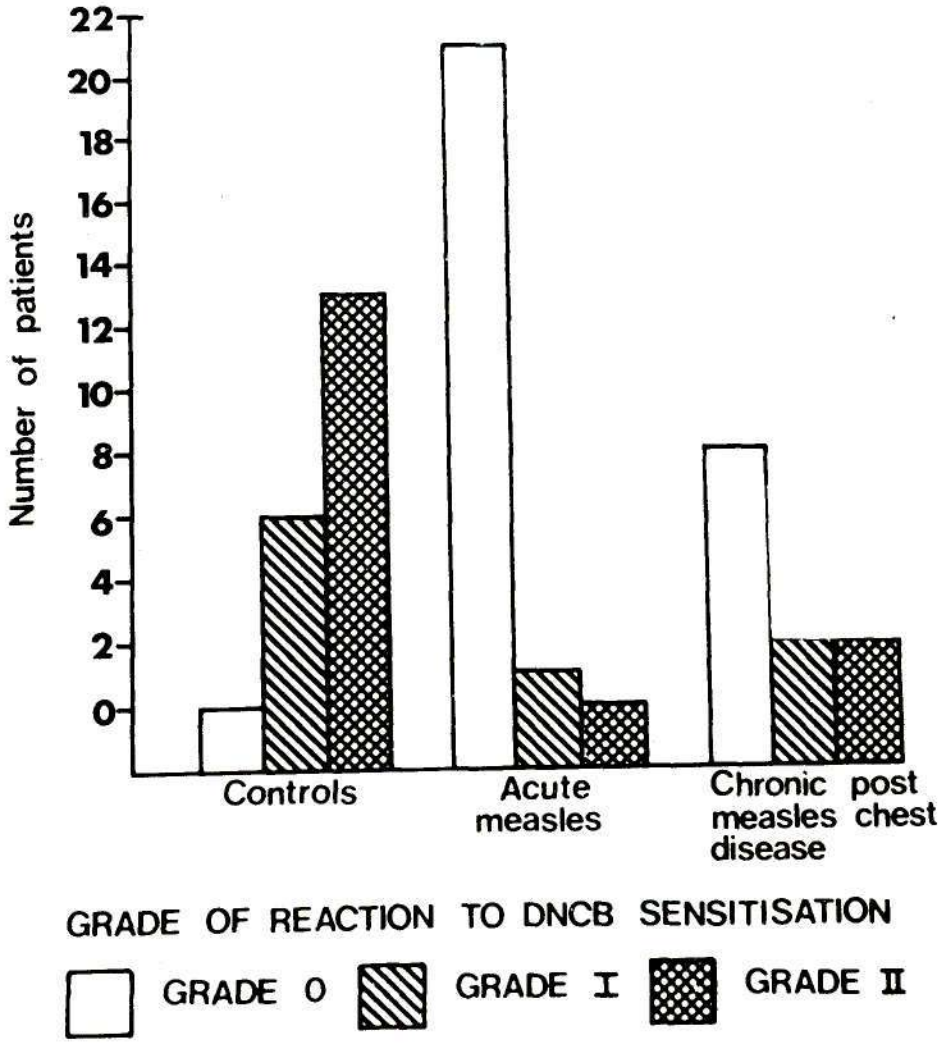


Fig. 5 Delayed skin hypersensitivity to dinitrochlorobenzene in 22 patients with acute measles, 12 with chronic post-measles chest disease and 19 controls.

TABLE II : SERUM IgG, IgA AND IgM LEVELS IN 24 CHILDREN WITH ACUTE MEASLES AND 18 WITH CHRONIC POST-MEASLES CHEST DISEASE COMPARED WITH AGE AND RACE MATCHED CONTROLS

SERUM IMMUNO-GLOBULINS	CONTROLS	AM PATIENTS	CONTROLS	CPMC PATIENTS
IgG	15.84 ±1.15	14.48= ± .81*	15.61 ±1.81	21.94 ±2.05
	0.20@		0.025	
IgA	.96 ± .11	.72 ± .05	.98 ± .24	1.30 ±.24
	0.025		0.20	
IgM	1.68 ± .13	2.30 ± .20	1.91 ± .16	3.29 ±.40
	0.01		0.0025	

= mean, in GMS/litre

* Standard error

@ P value of difference between patients and controls.

TABLE III

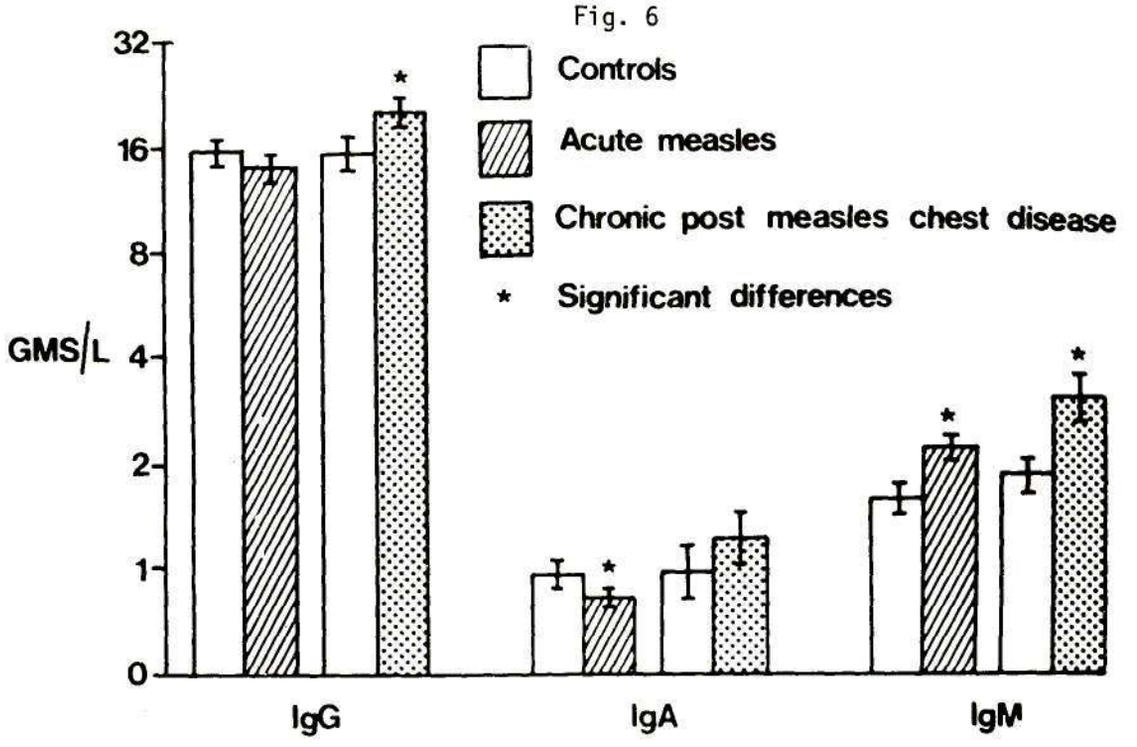
COMPLEMENT COMPONENTS IN 23 ACUTE MEASLES AND 18 CHRONIC POST MEASLES CHEST DISEASE

	AM	CONTROLS	CPMC
C_3	.90 ± .18	.96 ± .18	1.08 ± .18 *
C_4	.41 ± .21	.47 ± .15	.33 ± .18 *
C_3PA	.27 ± .10 *	.35 ± .16	.29 ± .14
CH_{50}	54 ± 10 *	66 ± 11	63 ± 12

All figures in gms/L.

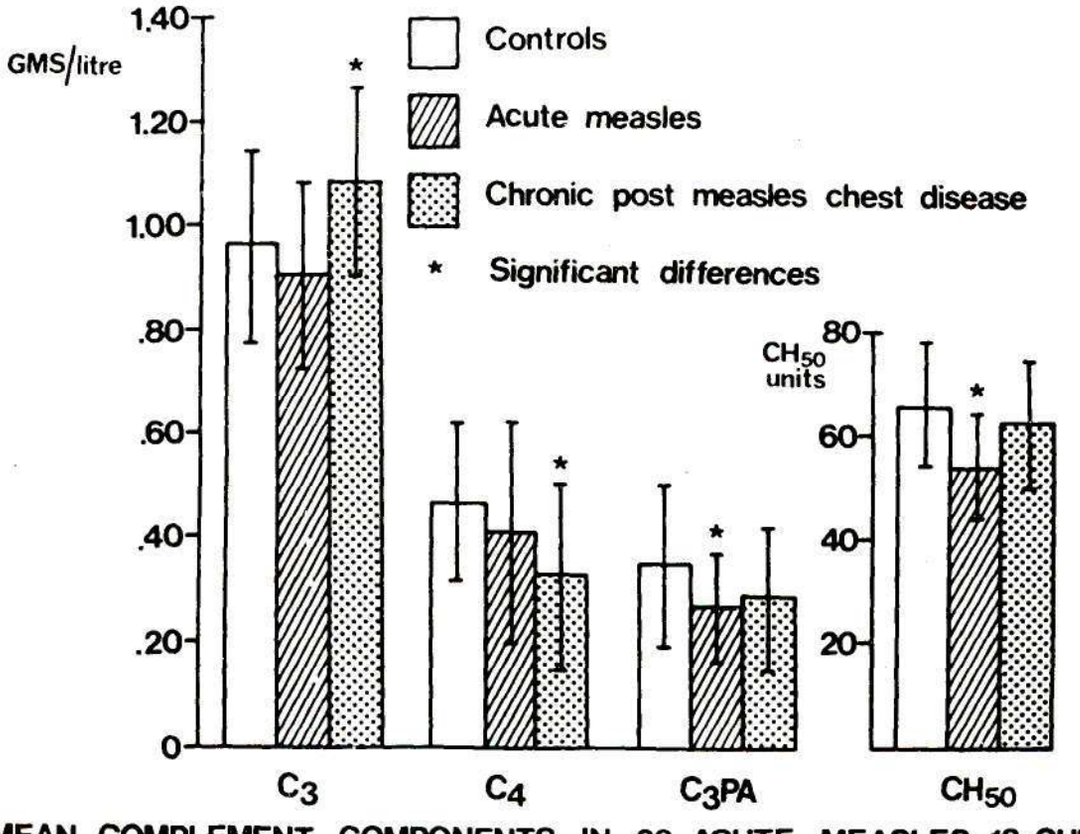
* = Significant difference

Fig. 6



MEAN SERUM IMMUNOGLOBULINS IN 24 ACUTE MEASLES & 18 CHRONIC POST MEASLES CHEST DISEASE WITH MATCHED CONTROLS

Fig. 7



MEAN COMPLEMENT COMPONENTS IN 23 ACUTE MEASLES, 18 CHRONIC POST MEASLES CHEST DISEASE & 41 CONTROLS

TABLE IV

ANTIBODIES AND ANTICOMPLEMENTARY FACTORS IN 22 ACUTE MEASLES, 17 CHRONIC CHEST DISEASE AND 34 CONTROLS

	RHEUMATOID FACTOR	ANTI		ANTI-COMPLEM-ENTARY FACTORS	ANTI-SRBC			
		IgG	F(ab)'		1/5	1/10	1/20	1/40
CONTROLS	1	0	0	0	27	4	0	3
AM	2	0	3	0	20	1	1	0
CPMC	1	2	2	0	2	1	4	10

Numbers of Patients/Controls with +ve tests.

serving as controls for both AM and CPMC patients (0.96 g/l ± 0.18, and 0.47 g/l ± 0.15, respectively). Mean C_3PA (0.27 g/l ± 0.10) and CH50 (54 ± 10) were significantly lower ($p < 0.05$) than controls (0.35 g/l ± 0.16 and 66 ± 11, respectively). In 18 CPMC patients mean C_3 (1.08 g/l ± 0.18) was significantly increased ($p < 0.05$) and mean C_4 (0.33 g/l ± 0.18) significantly lower ($p < 0.01$) than controls. C_3PA (0.29 g/l ± 0.14) and CH50 (63 ± 12) in this group were not different from controls. C_3 conversion products were not detected in any patient.

There was no significant detection of anti-complementary factors, or an increase in rheumatoid factors in either group of patients (Table IV). Anti-whole IgG was present in 2 of 17 CPMC patients and not detected in any of 22 AM patients and 34 controls. Anti-F (ab) antibodies were found in 2 of 17 CPMC and 3 of 22 AM patients and in none of 34 controls. These changes did not reach significance.

Anti-SRBC antibodies in a titre of more than 10 were detected in 3 of 34 controls, 1 of 22 AM patients and 14 of 17 CPMC patients. Compared to controls, their incidence is significantly increased ($p < 0.001$) in CPMC patients.

(iv) DISCUSSION

These studies have defined the extensive damaging effects of acute measles infection at different levels of the immune response. The findings reported here extend and clarify the recognised immunodeficiency in measles with the early documentation by von Pirquet[96] of a negative tuberculin reaction in a previously positive patient. In contrast, there was a relative sparing of the measured indices of immunity in patients with chronic chest disease following on measles.

Reduction of T, B and NULL lymphocyte sub-populations has been shown in acute measles and was reflected in an absolute lymphopenia. Functional assessment of T cells by lymphocyte transformation to PHA and *in vivo* DHR to DNCB revealed that both were impaired in these patients. We have shown in a previous study[130] that the function of B cells as determined by antibody response to typhoid, paratyphoid A and paratyphoid B vaccine was impaired in children with measles. Serum IgA was low, the haemolytic function of complement (CH50) and C_3PA, a factor utilised in activation of the alternative pathway, were decreased. 3 of 23 AM patients had detectable antibodies to the F (ab) fraction of IgG.

Patients with chronic chest disease following measles had impaired DNCB skin reactivity and low serum C_4; antibodies to whole IgG and F(ab) were detected in a minority of these children and heterophile antibodies to SRBCs in the majority.

Dissociation of *in vivo* and *in vitro* correlates of cellular immunity occurred in some CPMC patients. The DHR was defective (i.e., grade 0 reaction) although T cell numbers in 3 patients and PHA transformation of lymphocytes in 5 were within range of control values (mean - 1 SD). There were no clinical differences between these patients and those in whom all these three parameters of cellular immunity correlated. These findings are paralleled in SSPE.[71] It is possible that these functions are effected by different sub-sets of T lymphocytes[168,169] and therefore unequally compromised by infection.

It is interesting that polymorphonuclear leucocytes were not increased in both groups of patients, suggesting that either secondary infection with pyogenic organisms did not occur, or that, if it did, the neutrophil response was inhibited by measles infection. Chemotaxis of leucocytes

has been shown to be impaired in children with measles.[118] The inference that superadded bacterial infection did not make a significant contribution to the clinical problems of these children was supported by the small number of positive blood cultures obtained - 2 each from AM and CPMC patients. However, the possibility that viruses posed the greatest threat to these patients was suggested by the presence of moderate to severe infection of the oropharynx by *herpes simplex* in 7 of these children (5 acute and 2 chronic patients). Further, *herpes simplex* and *adenovirus* have been cultured in addition to measles virus from lung tissue[60] and there is frequently evidence of *adenovirus* on histology of the lungs of children dying from post-measles bronchopneumonia.[170,171] The reduced efficiency of thymus-dependent immune responses with only minor alterations in complement proteins in acute measles would preferentially diminish protection against viral rather than bacterial infections.[172] The identified immuno-deficiency in AM may allow entry of and damage by other viruses, but of itself is an insufficient cause for chronicity, as measurements of immunity were relatively normal in CPMC patients.

The unexpected finding of low serum IgA in AM patients may be accounted for by either of the following possibilities: as patients admitted to hospital usually had complications of measles (with respiratory disease and eye or ear infections) they may have been selected for susceptibility to the relatively severe form of the disease by a pre-existing IgA deficiency;[173] measles, on the other hand, may have suppressed the formation or release of IgA. In the latter instance IgA deficiency, as is well recognised,[174] could be secondary to poor thymic function. Of particular importance is the reported association of SSPE, in which there is subtle alteration of T dependent functions, with low serum IgA.[75] IgA deficiency can under other circumstances cause wide-ranging

immunopathological effects[173] but appears, in this instance, unrelated to chronic post-measles tissue damage.

Pathological activation of the classical and alternate pathways of the complement system in measles has been demonstrated both in patients[77] and *in vitro*.[122] Complement abnormalities were not striking in the present study with the evidence suggesting minimal utilisation of alternate pathway in AM (low C_3PA) and classical pathway in CPMC (depressed C_4). These discrepancies are probably not due to patient differences since those studied by Charlesworth *et al*[77] were apparently less severely affected than the children reported here. It is more likely that in the present series the serum measurements (as has been suggested by Williams *et al*[175]) did not accurately reflect deviations in synthesis and catabolism of complement. However, serious and prolonged alterations in complement metabolism would be expected to lower serum complement concentration. The hypothesis (arising from the detection of hypocomplementaemia in partial lipodystrophy and mesangiocapillary glomerulonephritis[65,176]) that prolonged complement depletion may lead to subsequent tissue injury, is unsubstantiated by findings in this study: CPMC patients had minor complement abnormalities with normal C_3.

Previous studies have shown that the incidence of F(ab) antibodies is always significantly greater in hospital patients than in healthy blood donors.[177] It has been suggested that F(ab) antibodies are probably formed through the release of lysosomal enzymes by granulocytes or macrophages when antibodies are complexed with bacterial antigens.[178,179] These findings of anti-F(ab) activity in some AM and CPMC patients, but not in the controls, indicated the formation of immune complexes and the ability of the patient's immune apparatus to catabolise them. This is confirmed by the absence of anti-complementary factors, C_3 conversion

products and minimal changes in the complement system in these children.

Patients in whom neither anti-F(ab) antibodies nor anticomplementary factors were detected may have been free of soluble immune complexes. The inability to detect immune complexes in measles patients has been documented[77] and presumably reflects insensitivity of the assay systems used. The possibility remains that in these patients circulating immune complexes remain undetected and could be damaging to tissue if poorly catabolised. None of the 5 patients who died during the study had anti-F(ab) antibodies. Anti-whole IgG antibodies may represent antibodies to allospecific markers on heavy or light chains of IgG.[180]

Human antibodies directed against the red cells of foreign species of animals are generally described as heterophile antibodies, and the explanation most frequently offered for the formation of such antibodies in man is that the causative agents, particularly bacteria, share antigenic determinants with the animal red cells used for detecting the antibodies.

In the present study the finding of raised antibody titres to sheep red cells in CPMC patients, as opposed to AM patients and controls, is of interest. The results suggest that infection by the measles virus alone need not lead to raised antibody activity to sheep cells but that other factors, possibly bacterial or other viral infections, associated with post-measles chest disease are linked to the formation of heterophile antibody. The high levels of heterophile antibody activity observed in measles patients susceptible to chronic pneumonia also reveals that chronicity may not be related to restrictions in humoral antibody activation.

The results in these patients suggest that the conditions for chronicity of pulmonary disease in acute measles are unlikely to be determined by persistent abnormalities in the immunopathological factors enumerated, most of which were normal in CPMC, but by as yet unidentified processes which may have their roots in variable antigen load at onset of infection, genetic predisposition to persistent disease, or defective measles specific immunity. It is not possible to interpret the findings in CPMC patients in this report as being either the cause or the effect of chronicity. The critical study providing answers to this problem will be a comparison of the results in those AM patients who die or develop chronic chest disease with those who recover. This has been has been done in study 3.

The investigations have, however, emphasised the profound non-specific immunodeficiency in acute measles in contrast to the relative integrity of immune responses in chronic post-measles chest disease. Patients with late neurological sequelae of measles (SSPE) similarly retain to a large extent an intact non-antigen-specific defence mechanism though they may have subtle alterations of specific immunity.[71]

(v) SUMMARY

Immune responses in 24 children with acute measles were compared with those in 20 children who had chronic pulmonary complications following measles. The immunosuppressive effects of acute measles were extensive: total white cells were reduced and this reduction was accounted for entirely by lymphopenia which was equally expressed among the major lymphocyte sub-populations studied; the function of T cells, assessed by radioisotope incorporation into phytohaemagglutin transformed lymphocytes and delayed skin hypersensitivity to dinitrochlorobenzene,

was depressed. Serum IgA was reduced in AM patients. In contrast there was a relative sparing of the measured indices of immunity in patients with chronic post-measles chest disease, with the major defect being an impaired DHR to DNCB. There were minor alterations of complement components in both groups of patients.

(vi) CONCLUSIONS

There are two important conclusions to be drawn from the results of this study:

1. Children with chronic post-measles chest disease have a defect in DHR to DNCB and alterations in complement components (C_4 and C_3). In order to test whether these are causally related to the chronic lung disease, it will be necessary to assess acute measles patients who have recovered at 6 weeks after onset of rash. This has been done in study III.

2. Of the 24 acute measles patients studied within 48 hours of the rash, 4 died at varying periods thereafter and 1 developed chronic chest disease. Results in these children are compared with those who recovered, in Study II.

Therefore the objectives of this study outlined in (i) have been achieved.

STUDY II

(i) DESIGN AND PURPOSE OF STUDY

This report is a comparison of immunological tests during acute measles between patients who subsequently died or developed chronic chest disease and those who recovered.

(ii) PATIENTS AND CONTROLS

Group AB - Five children who died from measles and one who had persistent bronchopneumonia 6 weeks after the onset form the basis of this report. Five are part of Study I. One further patient, similarly studied and who died, has been added. Their mean age was 16 months (range 7-48 months) and three were females. All were well-nourished (weight > 10th Boston centile). The five deaths occurred 3, 6, 12, 19 and 20 days after the appearance of the rash, and after a clinical course characterised by severe progressive pneumonia. In addition, laryngo-tracheobronchitis developed in one of the patients who died and hepatic failure with pneumococcal septicaemia developed in another.

Group AR - Six age-matched children who recovered from measles within 7 days were selected from Study I.

Normal Controls - Six race and age matched healthy children were investigated.

Retrospective Group - Total lymphocyte counts were reviewed in nineteen children with measles who died during the year preceding this study. Their mean age was 14 months (range 5-26 months) and ten were male. Blood counts had been obtained within 14 days of the onset of the rash and 1-4 days before death. These nineteen were compared with twenty-seven who recovered satisfactorily from measles. Twenty of these

twenty seven children form part of the group of acute measles patients in Study I. Statistical analysis was performed by using the Wilcoxon-signed-rank sum test.

(iii) RESULTS (See Table V, VI, Fig. 8)

The mean absolute lymphocyte count in group AB (2096 ± 635 S.E.M. cells/mm^3) was significantly less than that in group AR (4113 ± 606 cells/mm^3) ($p = 0.05$). Five of the six group-AB patients had absolute lymphocyte counts below 2000 cells/mm^3 and all of group AR patients had counts of more than 2000 cells/mm^3. The mean T cell (1328 ± 375 cells/mm^3) and B-cell counts (571 ± 208 cells/mm^3) in group AB were significantly less ($p = 0.05$ for both) than those (2578 ± 305 T cells and 1107 ± 276 B cells/mm^3) in group AR. The proportions of these cells, however, were similar in both groups. The mean T cell count in group AR was significantly less ($p = 0.05$) than that in the healthy controls (3989 ± 417 cells/mm^3). In group AB white-cell and null cell counts were less than in group AR and the FT cell count was higher than in group AR. However, none of these differences between groups AB and AR was statistically significant. Neutrophil counts were similar in groups AB and AR and in controls.

The mean S.I. of P.H.A.-transformed lymphocytes in group AB (102 ± 34) resembled that in group AR (132 ± 32) although both were significantly less ($p = 0.05$) than that in controls (459 ± 143). The S.I. was lowest (13 and 50) in two patients who died. There were no significant differences in serum concentrations of IgG, IgA, IgM, C3, C4, C3 pro-activator, and total haemolytic complement units in groups AB and AR and controls. However, mean serum-C3 was considerably lower in group AB (0.75 ± 0.08 g/l) than in group AR (0.92 ± 0.03 g/l) ($p = 0.10$).

TABLE V

TOTAL WHITE CELLS, NEUTROPHILS, ABSOLUTE NUMBERS AND SUB-POPULATIONS OF LYMPHOCYTES AND LYMPHOCYTE TRANSFORMATION TO PHA IN MEASLES PATIENTS AND CONTROLS

	TWC	N	AL	'T'	'B'	'NULL'	'FT'	'SI'
CONTROLS	12033*	4107	6617	3989	1743	888	97	459
	(1829)+	(966)	(864)	(417)	(374)	(183)	(40)	(143)
AR[x]	8900	4457	4113	2578	1107	429	44	132
	(887)	(1060)	(606)	(305)	(276)	(101)	(16)	(32)
AB[o]	6950	4693	2096	1328	571	197	106	102
	(1054)	(802)	(635)	(375)	(208)	(67)	(44)	(34)
'P' VALUE								
CONTROL/AR	>.05	>.05	.1	.05	>.05	.1	>.05	.05
AR/AB	>.05	>.05	.05	.05	.05	>.05	>.05	>05

* Absolute count, in cells/cu.mm (mean values)
\+ Standard error
x Measles patients who recovered
o Measles patients who either died or became chronic

TABLE VI

SERUM IMMUNOGLOBULINS AND COMPLEMENT COMPONENTS IN MEASLES PATIENTS AND CONTROLS

	IgG	IgA	IgM	C_3	C_4	C3PA	CH50
CONTROLS	11.37*	0.57	1.86	1.05	0.63	0.35	65
	(0.70)+	(0.15)	(0.35)	(0.04)	(0.06)	(0.04)	(4)
AR[x]	15.37	0.70	3.07	0.92	0.32	0.34	57
	(2.34)	(0.07)	(0.61)	(0.03)	(0.08)	(0.06)	(2)
AB[o]	13.63	0.70	1.94	0.75	0.42	0.31	58
	(2.04)	(0.12)	(0.27)	(0.08)	(0.07)	(0.05)	(5)

* Mean in Gms/Litre (Except CH50)
\+ Standard error
x Measles patients who recovered
o Measles patients who either died or became chronic

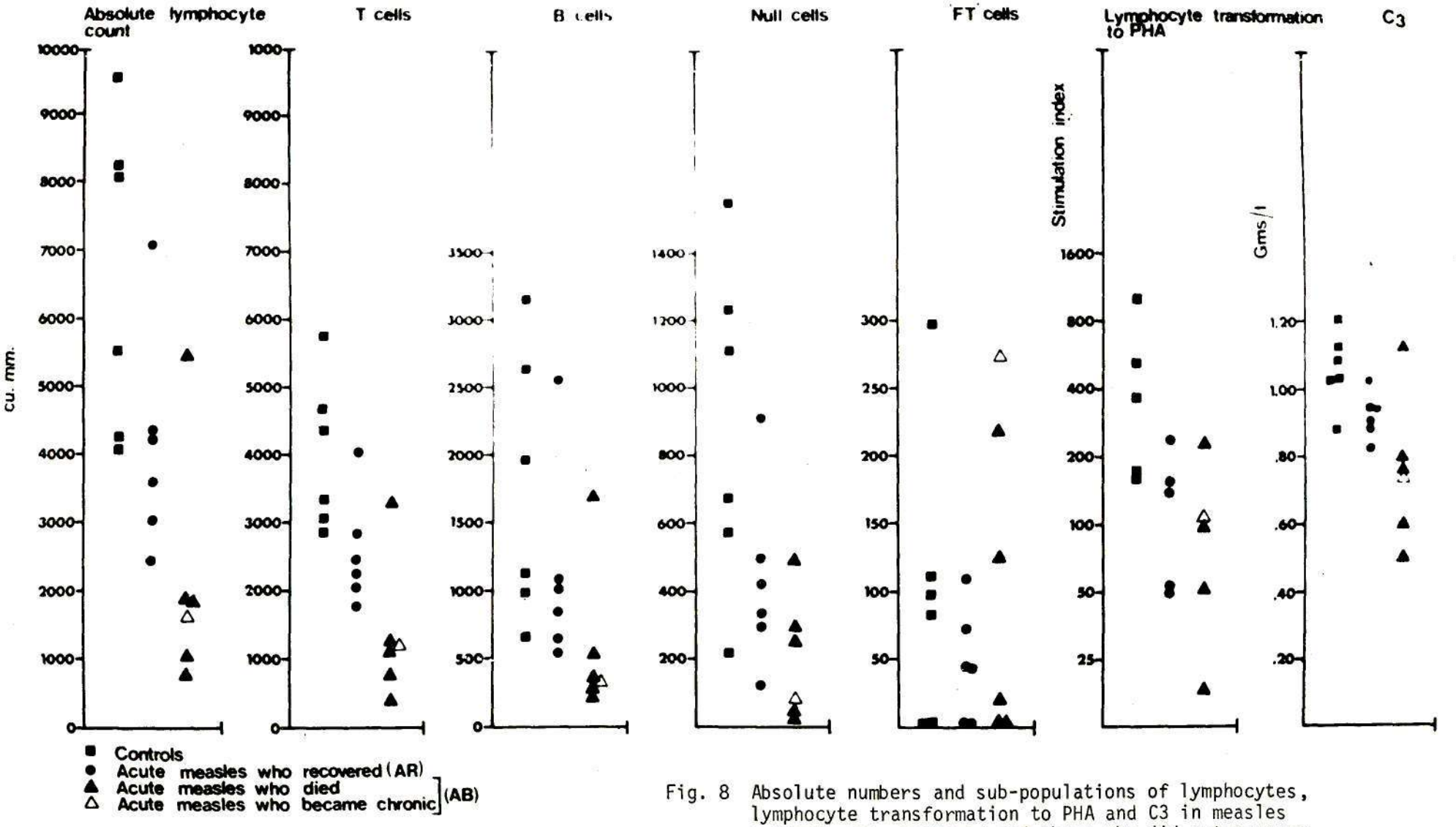


Fig. 8 Absolute numbers and sub-populations of lymphocytes, lymphocyte transformation to PHA and C3 in measles patients who recovered and those who did not recover and controls.

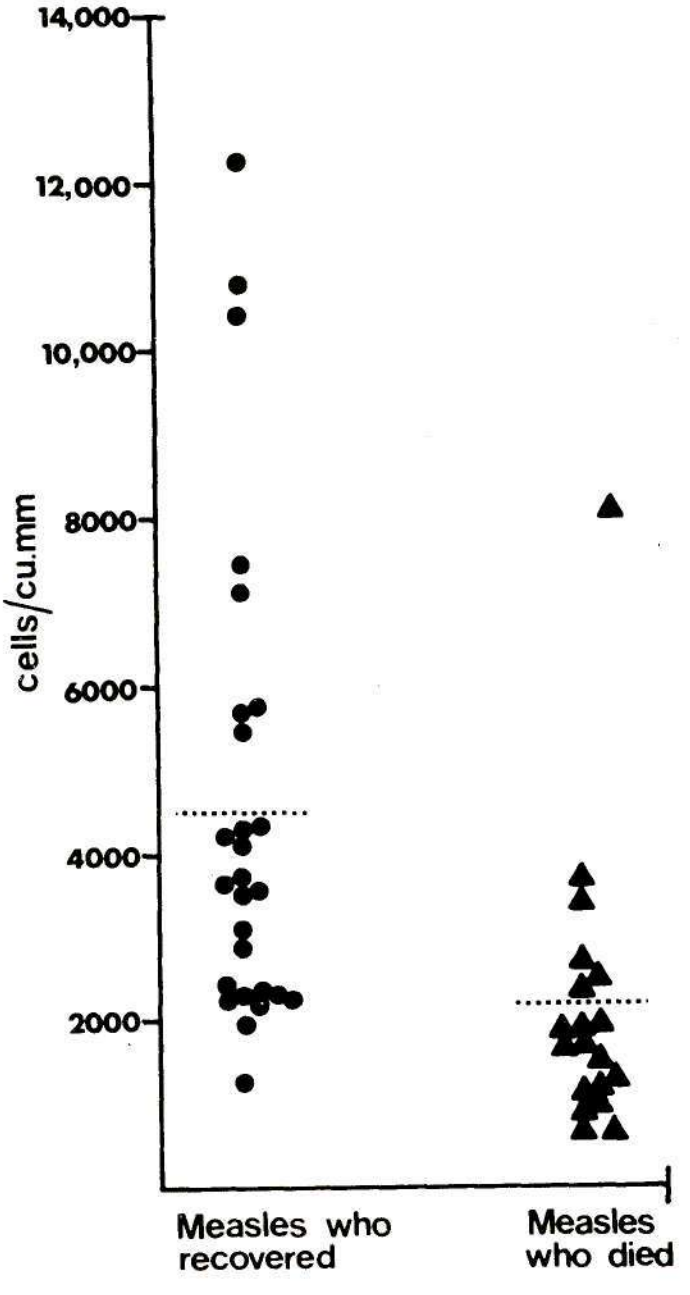


Fig. 9 Absolute lymphocyte counts in 19 measles patients who died (retrospectively studied) and 27 who recovered.

In the retrospective study the mean lymphocyte count in nineteen children who died was 2117 ± 375 cells/mm^3 (Fig. 9). This was significantly lower ($p < 0.01$) than the mean value (4487 ± 540 cells/mm^3) in twenty seven children with measles who recovered. Of these twenty seven children only two had total lymphocyte counts of less than 2000 cells/mm^3, with low counts of T (684 and 1448 cells/mm^3) and B (317 and 496 cells/mm^3) cells.

(iv) DISCUSSION

In this study of the acute stage of measles infection a critical breakdown of defence mechanisms was demonstrated which can be linked to the severity of outcome. In those who subsequently died or whose disease became chronic the immunosuppressive effect of measles during the acute rash was intensified. This could have allowed progression to chronic disease or death. These damaging effects were evident in the significant reduction in the absolute lymphocyte count (five of six patients had counts of less than 2000 cells/mm^3). This decrease in total lymphocytes was caused by a reduction in both T and B cells. These results in a small number of patients were supported by those in a retrospective analysis of another nineteen measles patients who died, most of whom also had severe lymphopenia (< 2000 cells/mm^3). Smythe *et al*[111] demonstrated lymphopenia in 26.8% of children with protein-calorie malnutrition who died and in 1.59% of those who survived. Infection was likely to have been the immediate cause of death in these malnourished children.

Although not all the patients with measles who died had lymphopenia, there could have been a drop in lymphocyte concentrations in these patients immediately before death. More important, of twenty seven patients with acute measles who recovered, two had lymphocyte counts

of less than 2000 cells/mm^3 with low T and B cell counts. The demonstration of intact thymolymphatic tissues in patients with sudden infant death syndrome,[181] which may in some cases be caused by infection, suggests that factors other than severe lymphopenia may be important in determining morbidity and mortality. Irreversible damage to the individual's immune mechanisms may be one of these factors. In this investigation a reduction in total lymphocyte count was detected early on in the disease process.

The fact that counts of Null and FT cells (which may be precursors of T and B cells or as yet unidentified lymphocyte subpopulations[182,183]) were little altered indicates that these cell types may be less important than the T and B cell types in determining outcome in measles infection.

The lack of increase in neutrophil count in group AB makes it likely that death or chronic chest disease was the result of virus dissemination rather than bacterial superinfection. The correlation of impaired T cell function (assessed by P.H.A. transformation) with poor outcome in measles was less secure, although the lowest indices in this test occurred in two patients who died. Serum C3 was significantly lower in group AB than in group AR. Other complement components and functions and serum immunoglobulins in group AB were not significantly different from group AR. Serum immunoglobulins were similarly unaffected in malnourished children who died.[111] Sparing of immunoglobulins around the time of death probably reflects their persistence in serum and is a function of their half-lives.

Subacute sclerosing panencephalitis (S.S.P.E.) may be the result of an altered measles virus or modulation of host immune responses by infection.[63] In this chronic illness there are minor defects in non-antigen-specific

immunity and pronounced abnormalities of measles-specific immune responses.[71] It is possible that pronounced immunoparesis at the onset of measles, which may be transient, allows persistence of antigen and leads to the development of S.S.P.E.

It has been demonstrated that profound immunosuppression in measles chiefly affects the T and B cell subpopulations, has less severe effects on T cell function and C3 concentrations, and in most cases distinguishes between children who will die or develop chronic chest disease and those who will recover.

(v) SUMMARY

In five children with measles who subsequently died and in one with measles in whom chronic bronchopneumonia developed (group AB) immunosuppression was more pronounced during the acute rash (i.e., 3-20 days before death) than in six children with measles who recovered (group AR). The absolute total lymphocyte count (T and B cells) was significantly lower in group AB. Mean serum C3 was also lower in group AB than in group AR. There were no significant differences between the two groups for other complement factors or for serum immunoglobulins. The mean phytohaemagglutinin stimulation index (S.I.) for lymphocytes from patients in group AB resembled that in group AR although the S.I. in both groups was significantly lower than that in healthy controls. S.I.s. were lowest in two patients who died. Counts of total white cells, neutrophils, Null cells, and those with both B and T cell markers were not significantly different in groups AB and AR. The total lymphocyte count (mean 2117 $\pm$ S.E.M. 375 cells/mm^3) in a further nineteen patients with measles who had died, studied retrospectively, was significantly lower than that (4487 $\pm$ 540 cells/mm^3) in

twenty seven patients with measles who recovered.

(vi) CONCLUSION

Children who will die or develop chronic chest disease can be distinguished within two days of appearance of the measles rash from those who will recover.

S T U D Y III

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6. S T U D Y I I I

(i) DESIGN AND PURPOSE OF STUDY

Defects in the DHR and complement components C_4 and C_3PA were documented in children with established chronic post measles chest disease in Study I. Study II was undertaken in order to determine whether these immunodeficiencies occurred only in chronic chest disease and not in measles patients who recovered clinically. Children with measles were studied during the acute rash and six weeks later.

(ii) PATIENTS AND CONTROLS

Twenty-two African children, of whom 11 were males, aged 6 months to 6 years (median 12 months) were studied. Their nutrition was satisfactory in that their weights were greater than the 10th Boston percentile and their mean haemoglobin was 10.2 $\pm$ 1.06 gms/dl.

Tests were done on these children during the measles rash and repeated six weeks later when all had made a complete clinical recovery, except one who had persistent bronchopneumonia. Twenty two age, sex and race matched children of comparable nutrition were studied as controls. Consent for procedures was obtained from the parents of the children.

The statistical method used was the Students' 't' test.

(iii) RESULTS

1. Total lymphocyte counts, Lymphocyte Subpopulations and Lymphocyte Transformation (Table VII)

During the acute phase of measles there was a significant decrease below control levels of the absolute count of lymphocytes ($p < 0.01$) and of T cells ($p < 0.01$). Both counts reached control values by 6 weeks.

The mean of the B and Null cell counts, which were significantly depressed initially ($p < 0.001$) failed to reach normal values after 6 weeks : B cells $p < 0.01$ and Null cells $p < 0.001$. The FT cell count in the acute phase and 6 weeks later was not significantly different from that of controls, although the rise over this period was significant ($p < 0.05$).

The transformation of lymphocytes by PHA was reduced significantly in the acute phase ($p < 0.001$) and remained low at 6 weeks ($p < 0.001$).

2. Serum Immunoglobulins (Table VIII)

(a) Ten of the 22 patients had their serum immunoglobulins measured. The mean level of IgG of patients rose significantly ($p < 0.02$) in the 6 weeks after measles. Neither level, however, was significantly different from that in 10 controls.

(b) While the rise in IgM was not significant, the mean level at 6 weeks was higher than controls ($p < 0.025$).

(c) IgA was shown not to differ from control levels, nor alter over the period of study.

TABLE VII

LYMPHOCYTE COUNTS AND TRANSFORMATION BY PHA IN 22 CHILDREN WITH MEASLES AND IN 22 CONTROLS

GROUP	ABSOLUTE* LYMPHOCYTES	T-CELLS	B-CELLS	NULL-CELLS	FT-CELLS	LYMPHOCYTE Θ TRANSFORMATION
ACUTE	4210 ± 654Δ	2989 ±455	987 ± 166	234 ± 63	81 ± 22	9100 ± 1784
SIX WEEKS	5918 ± 431	4364 ±302	1270 ± 151	285 ± 47	162 ± 31	7970 ± 1751
CONTROLS	7603 ± 742	4746 ±453	2051 ± 247	805 ±128	140 ± 40	31745 ± 5249
ACUTE/ CONTROL	<.005 ∇	<.01	<.001	<.001	NS	<.001
SIX WEEKS / CONTROL	NS	NS	<.01	<.001	NS	<.001

* Cells/mm^3

Δ Mean ± standard error

Θ Disintegrations per minute

∇ Probability Value

NS Not significant

TABLE VIII

SERUM IMMUNOGLOBULINS AND COMPLEMENT FACTORS (GMS/L) IN 10 CHILDREN WITH MEASLES AND 6 WEEKS LATER, AND IN 10 HEALTHY CONTROLS: MEANS AND STANDARD ERRORS

G R O U P	IgG	IgA	IgM	C_3	C_4	C_3PA	CH_{50}
ACUTE	13.86 ±1.47	1.03 ±.25	1.87 ±.20	.88 ±.05	.42 ±.05	.46 ±.03	65 ±4
6 WEEKS	22.19 ±2.77	1.65 ±.24	2.24 ±.26	1.04 ±.04	.42 ±.04	.44 ±.04	78 ±3
CONTROLS	17.97 ±1.74	1.35 ±.25	1.62 ±.13	1.02 ±.08	.48 ±.06	.40 ±.04	64 ±3
Acute/Control	$p < 0.05$	NS	NS	NS	NS	NS	NS
6 Weeks/Control	NS	NS	$p < 0.025$	NS	NS	NS	$p < 0.0025$
Acute/6 Weeks	$p < 0.02$	NS	NS	$p < 0.05$	NS	NS	$p < 0.02$

3. Serum Complement Components (Table VIII)

Ten of the 22 patients had serum complement components measured. C_3 rose significantly over 6 weeks ($p < 0.05$) although the initial reduced level in early measles was not significantly different from the value in 10 controls.

CH_{50} also rose significantly in 6 weeks ($p < 0.02$), the final level being significantly above controls ($p < 0.0025$). C_4 and C_3PA remained within normal limits.

4. DHR to DNCB

During the measles rash, DNCB sensitisation failed in 8 of the 10 children tested, was Grade I in one and Grade II in another. Six weeks later 7 patients still failed to react, while 3 produced a Grade II reaction.

(iv) DISCUSSION

Incomplete reversal of the immunodeficiency of acute measles in children has been shown to occur within 6 weeks of infection. A decrease in the total lymphocyte count of up to 50% has been shown following measles infection or attentuated measles vaccine.[137,117,119] Yata *et al*, 1974,[138] have shown the diminution in lymphocyte numbers in measles to be mainly due to T cells.

The total lymphocyte count and lymphocyte subpopulations, with the exception of FT cells, were significantly reduced in the acute stage of measles. Recovery 6 weeks later was noted only in the total and T cell counts and had not occurred in B cells and Null cells.

The role of Null cells in immunity has not been clearly established but they may be precursors of T and B cells.[182] A fall in Null cells may be the primary event in measles and acount for the reduced T and B populations. Alternatively, their reduction may reflect a faster maturing to T and B cells in response to the observed significant reduction in the initial total lymphocyte count. Persistent depletion of Null cells at 6 weeks suggests a continuation of the latter mechanism with the recovery of T cell, and to a lesser extent B cell numbers, at the cost of the precursor. In malnutrition, also a disease of depressed cellular immunity, the number of Null cells was increased, and showed in vivo cytoxicity.[185]

In vitro T cell function, assessed by PHA transformation of lymphocytes, was depressed during acute measles and was still subnormal by 6 weeks. Also, in vivo estimation of the T cell function by DHR to DNCB showed a continued defective response in the majority 6 weeks after infection.

The present evidence confirms the prolonged functional depression of cellular immunity following measles, with recovery of competence being delayed for longer than 6 weeks in many patients. In Study I we have shown that children with established post measles chronic chest disease studied six to sixteen weeks (Mean : 9 weeks) after onset of the rash, have normal PHA transformation and subpopulations of lymphocytes.

The return to normal of B cell numbers in this study was not accomplished by the sixth week after the onset of the disease. Nevertheless at no stage was serum immunoglobulin production overtly affected. On the contrary, the level of serum IgG and IgM showed a small but significant rise over the six weeks.

The ability to produce antibody to an antigen stimulus can be reduced in measles resulting not only from immunoparesis of helper T cells but also from direct depression of B cell function.[136,140] Therefore it is of interest, in view of depression of both B cell number and function in measles, that in this study the IgG and IgM did show a significant change. Immunoglobulin levels in serum are to some extent a function of their half-lives and thus may fail to reflect, in the short term, changes in immune activity.

The utilisation of complement can occur during immune cytolysis.[187] Activation of both the classical and alternative pathways has been demonstrated in measles.[122,77] In this study the activation of complement in the acute phase was suggested by the significant rise in C_3 and CH_{50} during convalescence.

The partial reversal within 6 weeks of immunological suppression by measles infection has been demonstrated, with major effects on lymphocyte sub-populations and T cell function and minor variation in serum immunoglobulins and complement components.

(v) SUMMARY

Twenty two children with measles were studied in the stage of the rash and 6 weeks later and results compared with matched controls.

The total lymphocyte count and lymphocyte sub-populations with T and B cell markers and those with absence of both markers (Null cells) were significantly below control levels in the acute phase. At 6 weeks the B cell and Null cell counts were still significantly diminished.

The function of T cells assessed by ^{14}C uptake of phytohaemagglutinin stimulated lymphocytes and delayed skin hypersensitivity reaction to dinitrochlorobenzene was impaired during the acute stage and this persisted for 6 weeks.

Over the 6 weeks of study there was a small but significant rise in serum IgG, IgM and complement factors.

(vi) CONCLUSIONS

This report has answered a few questions posed by findings in Study I but raised a few unanswered ones itself.

Children who recovered from acute measles had persistently defective DHR to DNCB. Therefore the demonstration of this anergy in those with chronic chest disease cannot be important in the development of chronicity.

Complement studies obscured rather than clarified issues. Diminution in C_4 which was shown in chronic chest disease was not detected in the acute phase of measles. Therefore the predictive value of this component could not be assessed. Reduction in C_3PA during acute measles (Study I) was not confirmed by these results.

B cells, Null cells and the transformation of lymphocytes by PHA which were normal in chronic chest disease were reduced in children who recovered from measles. It is extremely unlikely that this difference is causally related to the development of chronicity. If anything, the children who recovered appeared to be worse off immunologically than those who had a serious complication of measles. The explanation

probably lies in the fact that children with chronic measles chest disease were studied later than 6 weeks after onset of rash (range 6 - 16 weeks). Reversal of immune deficiency, which was partial at 6 weeks, would probably be complete soon after this period.

STUDY IV

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7. STUDY IV

(i) DESIGN AND PURPOSE OF STUDY

The conclusions drawn from Study II have provided an important index to immunological events in acute measles which can serve as a guide to subsequent outcome. However, the number of patients on which these observations have been based is not large. Therefore Study IV was undertaken to enlarge and test this evidence.

Lymphocytes which are necessary for mediating specific immune responses[189] are easily and frequently measured in the peripheral blood and infrequently reduced in common illnesses.[190] There appears to be an important link between lymphocyte numbers and the immediate and late complications of certain diseases: Pre-treatment lymphocyte counts in breast cancer correlate with severity and serve as a predictive index of metastases occurring within 5 years;[191] a marked diminution of B cells precedes death in smallpox;[192] profound lymphopenia (<2000 cells/mm^3) during the exanthem in measles, in most cases distinguishes between children who will die or develop chronic chest disease from those who will recover.

There is extensive immunosuppression during acute measles and elements of this persist for periods varying from 6[95] to 52 weeks.[129] In children with the late pulmonary and neurological (subacute sclerosing panencephalitis (SSPE)[71]) sequelae of measles there is a relative sparing of most measured indices of immunity with some subtle immunologic abnormalities. The sequence of immunological events occurring between paresis in acute severe disease and rehabilitation during chronicity is not understood. Elucidation of these changes would

assist in understanding the pathogenesis of chronic disease and may be helpful in its prevention.

An attempt has been made to clarify these points and consolidate earlier findings by studying 30 children with measles who had an initial severe lymphopenia (< 2000 cells/mm^3) sequentially, from acute rash to recovery, chronic chest disease or death. The outcome in this group has been compared to that in 30 children with measles with a moderate initial lymphopenia (> 2000 cells/mm^3).

(ii) PATIENTS AND CONTROLS

The nutritional state of all patients investigated was satisfactory. None had any of the clinical features of protein-calorie-malnutrition and all were between the 10th and 75th Harvard centiles for weight with serum albumens of over 30 gms/L.

Group A. Thirty African children with absolute lymphocyte counts below 2000 cells/mm^3 at 48 hours after the onset of the rash were studied sequentially. Blood for immunological studies was taken within the first 15 days and on the 21st and 42nd day after the appearance of the rash. Chest radiographs were done on admission and on 42nd day. Their mean age was 19 months (range 6 - 72 months) and 14 were females.

Group B Thirty African children with absolute lymphocyte counts above 2000 cells/mm^3 within 48 hours of the appearance of the rash, had their clinical course noted and chest radiographs done on admission and on day 42. Their mean age was 13 months (range 5 - 60 months) and 17 were female.

Normal Controls. Thirty healthy children, race and age matched with those in group A, had immunological studies done except for measles antibodies.

CHRONIC CHEST DISEASE

Observations in this unit suggest that children who have radiological evidence of pneumonia 6 weeks after the appearance of the measles rash, usually run a fluctuating course over months to years with persistent lung changes. For purposes of this study, chest radiographs taken on the 6th week were examined independently by a consultant radiologist, Dr. F. Rooknoodeen, and by Dr. Anne Wesley without foreknowledge of the patients' clinical condition or group status.

Bronchopulmonary changes were graded out of a total of eleven in the following manner: Points to a maximum of 4 were awarded for areas of patchy consolidation, and one point each for atelectasis, involvement of peripheral lung fields either by consolidation or emphysema or for effusion. Increased vascular markings, increased interstitial pattern and hilar shadow each were given a point. Since these last three features are common in viral infection and not of great pathophysiological significance, an abnormal score was 4 or more. Children with a score of 4 or more on the 6 week chest radiograph were classified as 'chronic chest disease'.

METHODS

Statistical analysis of results was carried out by either the Students' 't' or Chi-square test.

(iii) RESULTS

1. INITIAL LYMPHOCYTE COUNTS AND OUTCOME (GROUPS A & B)

The mean initial absolute lymphocyte counts in group A (1318 ± S.E.M. 89 cells/mm^3) and group B. (4232 ± 314 cells/mm^3) were significantly lower ($p < .0005$ for both) than that in healthy controls (6833 ± 553 cells/mm^3). In group A there was clinical recovery in 7 children, chronic chest disease in 14 and death in 9. However, in group B, 20 patients recovered, 10 progressed to chronic chest disease and none died. The differences between these two groups of patients for failure to recover (i.e., chronicity or death) and for progression to chronic chest disease alone, are significant ($p < 0.01$, $p < 0.05$ respectively). In either group, there was no significant difference in mean absolute lymphocyte counts in those whose course was unfavourable compared with those with a good outcome. The mean absolute lymphocyte count of 10 group B children who developed chronicity was 4330 ± 424 cells/mm^3. There was no significant difference in the initial (< 48 hours of rash) absolute lymphocyte counts between those children who subsequently recovered (1320 ± 143 cells/mm^3) and those who died or developed chronic chest disease in group A (1317 ± 110 cells/mm^3).

The mean age, in group A children who recovered was 20 months (range 9 - 36 months) in those who developed chronicity was 22 months (range 6 - 72 months) and in those who died was 13 months (range 6 - 24 months). These differences are not significant ($p > 0.05$). The 9 deaths occurred between 4 and 65 days after appearance of the rash and following a clinical course characterised by severe progressive pneumonia.

2. SEQUENTIAL LYMPHOCYTE COUNTS AND LYMPHOCYTE SUBPOPULATIONS (Group A) (Table IX and Figs. 10-12)

There was a rapid rise in absolute lymphocytes within 15 days of appearance of the rash. However, during this period, of 14 patients whose lymphocyte counts did not rise above 2000 cells/mm^3 (i.e., 30% of normal control value), 12 failed to recover (6 died, 6 chronic chest disease). Similarly, of 10 patients whose T cells persisted below 1268 cells/mm^3 (i.e., 30% of controls), 9 did not recover (4 died, 5 chronic chest disease), and of 14 children whose B cells remained less than 556 cells/mm^3 (i.e., 30% of controls) 12 did badly (6 died, 6 chronic chest disease). During this period Null cells were highest in 6 patients who did not recover (2 died, 4 chronic chest disease) and of 14 children who had an absence of FT cells 12 did not recover (4 died, 8 chronic chest disease).

The distinguishing pattern in absolute lymphocyte counts, T, B, Null and FT cells during the first fifteen days after onset of rash, between measles patients who recovered and those who did not, was less clearly detectable at 21 and 42 days. Lymphocyte subpopulations, excepting Null cells, had reached the level of normal controls at the third week after onset of rash in those who recovered. At the third week of the rash lymphocyte subpopulations, excepting FT cells, were still significantly below normal in children who did not recover. At the 6th week only the T cells in addition had reached normal in these children whereas the absolute lymphocytes, B cells and Null cells were still significantly depressed.

TABLE IX
SEQUENTIAL LYMPHOCYTE SUB-POPULATIONS IN GROUP A PATIENTS.

PATIENTS WHO RECOVERED

Patient Number	Day after Onset of Rash	Absolute Lymphocytes	Lymphocyte Sub-populations T	B	N	FT	C X R Grades on 42nd day.
1	2	1650	-	-	-	-	3
2	3	3741	2132	1459	150	37	3
3	5	4680	3931	468	281	187	2
4	5	4290	3218	815	257	0	1
5	5	3150	2520	599	32	32	2
6	7	1200	924	228	48	0	2
7	8	4840	3775	1016	48	48	3
All cases◆	21	6684±1833*	5022± 1159	1513± 674	150± 66	108 ± 45	
		>.05▲	>.05	>.05	<.001	>.05	
All cases◆	42	7248±1258	5603± 901	1459± 342	187± 92	129± 83	
		>.05	>.05	>.05	<.001	>.05	

PATIENTS WHO DEVELOPED CHRONIC CHEST DISEASE OR DIED

8	2	2337	1355	935	47	47	9
9	2	780	390	359	31	125	Died
10	2	1482	-	-	-	-	9
11	2	1530	-	-	-	-	Died
12	3	1836	1246	294	294	0	Died
13	3	1032	753	237	41	21	Died
14	3	4464	3660	625	179	0	4
15	4	936	-	-	-	-	Died
16	5	1870	1103	524	243	0	Died
17	5	530	201	329	0	0	4
18	6	5002	3902	950	150	200	4
19	7	2790	2009	502	279	112	Died
20	7	1287	978	309	0	103	4
21	7	910	783	109	18	0	6
22	8	10564	6972	1268	2324	0	7
23	8	1836	1175	294	367	73	8
24	9	720	583	108	29	0	6
25	9	6000	4680	1320	0	0	9
26	10	9300	6789	1953	558	186	5
27	11	3472	2535	451	486	0	7
28	12	4300	3526	473	301	0	Died
29	14	4620	3326	1016	277	0	6, Died
30	15	5200	4056	1144	0	0	4
All cases●	21	4531±500	3280± 326	836± 93	215±58	88± 27	
		<.005	<.05	<.001	<.001	>.05	
All cases■	42	5075±633	3881± 447	1078±195	126± 36	194± 43	
		<.05	>.05	<.005	<.001	>.20	

HEALTHY CONTROLS

6833±553* 4228± 365 1854± 169 756±96 121±38

KEY:

* Mean ± standard error

▲ p value of difference between patients and controls.

◆ Results of 5 patients

● Results of 15 patients

■ Results of 10 patients.

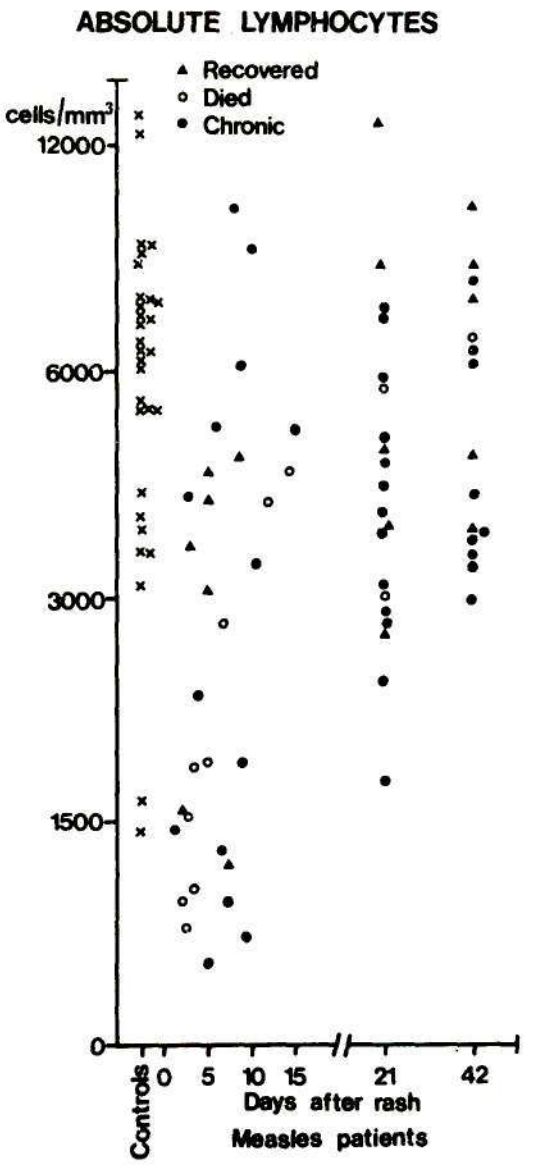

Fig. 10 SEQUENTIAL ABSOLUTE LYMPHOCYTE COUNTS IN GROUP A PATIENTS WHO RECOVERED, DIED OR DEVELOPED CHRONIC CHEST DISEASE AND IN CONTROLS.

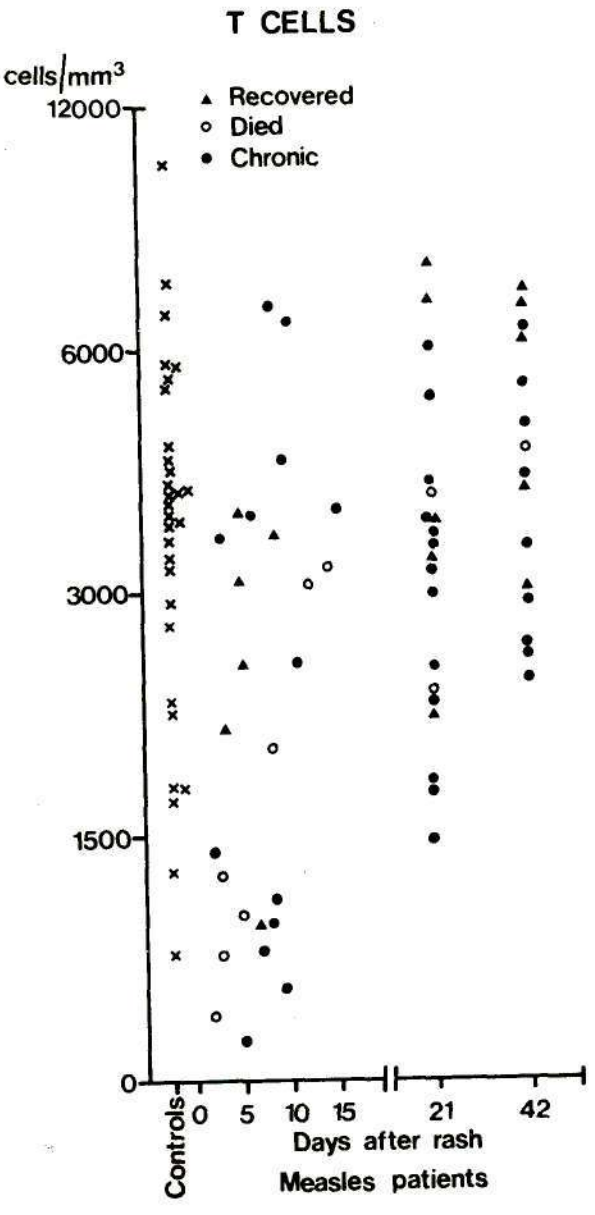

Fig. 11 SEQUENTIAL T CELLS IN GROUP A PATIENTS WHO RECOVERED, DIED OR DEVELOPED CHRONIC CHEST DISEASE AND IN CONTROLS.

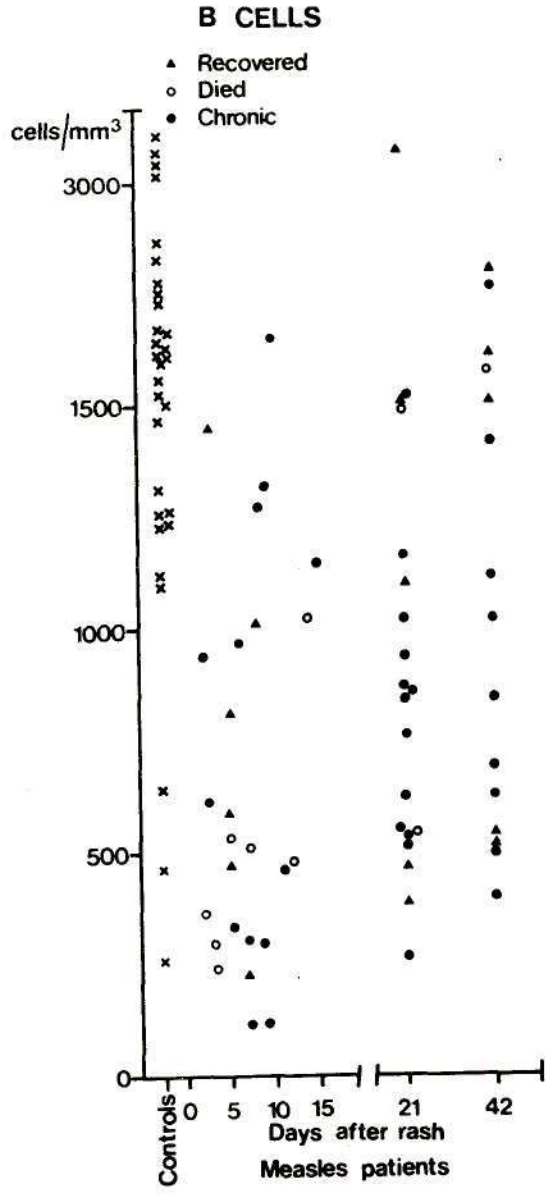

Fig. 12 SEQUENTIAL B CELLS IN GROUP A PATIENTS WHO RECOVERED, DIED OR DEVELOPED CHRONIC CHEST DISEASE AND IN CONTROLS.

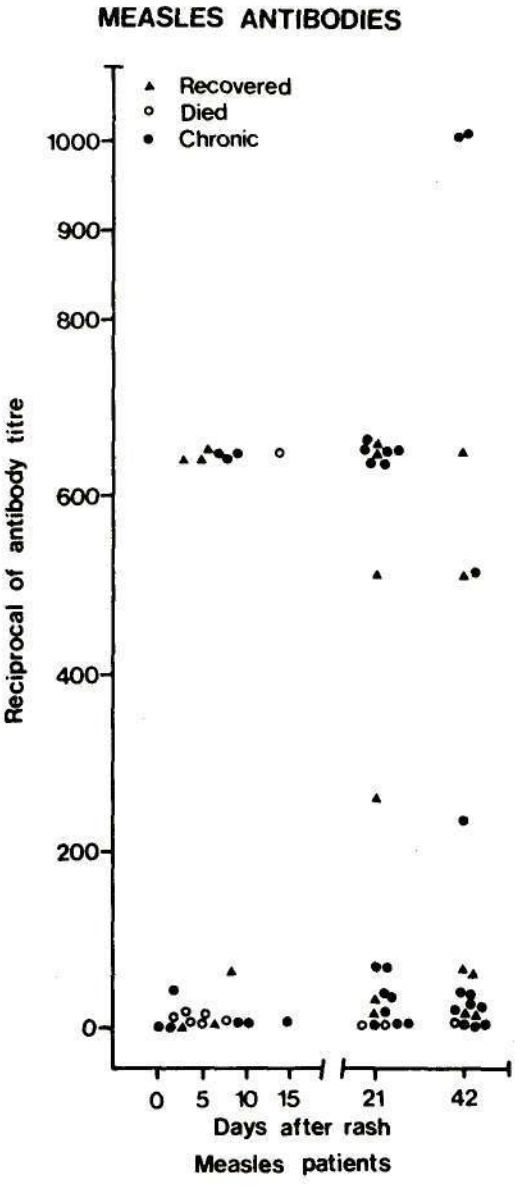

Fig. 13 SEQUENTIAL MEASLES COMPLEMENT-FIXING ANTIBODY TITRES IN GROUP A PATIENTS WHO RECOVERED, DIED OR DEVELOPED CHRONIC CHEST DISEASE.

3. MEASLES ANTIBODIES

(Group A) (Fig. 13)

Measles antibodies, during the 15 days after onset of rash, were below a titre of 1/10 in 6 of 7 children who subsequently died and below 1/32 in 6 of 9 who developed chronic chest disease. In 6 patients who recovered, there were equal numbers who had low (< 1/64) and high (1/640) titres. One patient who died had a titre of 1/640 on the 14th day after onset of rash and this rapidly fell to undetectable levels (<1/2) on days 21 and 42. A further patient who died had no detectable antibody on day 21. There was no distinguishable pattern in antibody levels on day 21 separating out those who developed chronicity from those who recovered. By day 42 most surviving patients had antibody titres of < 1/64.

4. C_3

(Group A)

There were no differences in C_3 between children who did well and those who did not, at any stage of the disease.

(iv) DISCUSSION

Children with measles at risk for mortality and serious morbidity have been identified by the severity of immunoparesis during the first two days of the rash. Estimation of lymphocyte numbers in the peripheral blood has been shown to be a reliable index of outcome in measles. More than 75% of children with a lymphopenia of less than 2000 cells/mm^3 during the early rash failed to recover: 30% died from pulmonary

complications within a few days to two months of onset of the exanthem, while 47% developed chronic lung damage. When depression of immunity was less severe (lymphocyte counts above 2000 cells/mm^3) recovery was more frequent and mortality insignificant. However, these patients had a high incidence (33%) of chronic lung damage. This probably reflects a more critical examination of chest radiographs and strict criteria for admission to hospital, with entry being restricted to the moderately and severely ill children with measles.

The profound immunosuppression during the first few days of the rash in measles which can determine prognosis has been shown (Study II) to affect chiefly the T and B cell sub-populations with less severe effects on C_3 and T cell function assessed by Phytohaemagglutinin transformation of lymphocytes. This quantitative defect in lymphocyte sub-populations was transient in the majority of children with severe lymphopenia. However, when this effect persisted for at least 15 days after appearance of the rash, it was nearly always associated with a poor outcome. Eighty six to ninety per cent of these children with prolonged immunoparesis died or developed progressive pulmonary damage. Estimation of lymphocytes after 48 hours of the rash in those children with rapid though incomplete reversal of lymphopenia was not an accurate index of eventual recovery, continuing disease or death. Some of these children recovered but many did not.

The predictive value of measles effects on Null and FT cells is less secure. In Study II, Null cells were lower and FT higher in patients with lymphocyte counts below 2000 cells/mm^3. This study, however, showed that in group A FT cells were undetectable and Null cells higher for at least 15 days after the rash in those who did not recover

when compared to those who did. Null cells have also been reported to be elevated in patients dying from smallpox.[192] These changes cannot be meaningfully interpreted until the function of these cells is more clearly understood.

The transformation of lymphocytes by phytohaemagglutinin and the dialysed culture supernatant of measles-infected HeLa cells in 12 group A patients studied sequentially was both inconsistent and unhelpful in distinguishing between those who did well and those who did not.

The mechanisms of lymphopenia in Group A may be due to any or all of the following: an elevated cortisol level which is a frequent homeostatic response during stress; the 'trapping' of lymphocytes in peripheral lymphoid tissues; virus-induced destruction of cells.

Increase of cortisol has been shown to be suppressive at various levels of the immune response:[193] the numbers and function of T and B cells are diminished, monocyte function is impaired, the inflammatory response is inhibited; complement components of the classical pathway are affected and there is thymolymphatic atrophy. Therefore it is entirely possible that many of the immunosuppressive effects caused by measles are due to elevated plasma cortisol. These effects would be expected to persist for the duration of the acute infection only. It would be difficult to explain prolonged immunological abnormalities on this basis alone.

Antigen-reactive cells have been demonstrated in peripheral lymphoid tissues when they have been undetectable in the blood in patients with infection who are anergic to skin testing with specific antigen.[194] The detection of severe lymphopenia in measles in the presence of exuberant lymphocytic formation in lymphnodes would make 'trapping' a likely event.[117]

Replication of measles virus has been shown to occur in T and B cells and monocytes.[45] Virus antigen is expressed on the surfaces of these cells which can be cytolysed by a number of effector mechanisms including antibody, complement and cytotoxic cells.[70] The interplay of such immunological activity may account for reduction in numbers of these cells during measles.

Atrophy of the thymus which is frequent in children dying from measles would reduce the supply of T cells to the periphery.[44]

The function of measles antigen sensitive cells is probably impaired during severe measles infection. The majority of children in Group A had a poor antibody response. Complement fixing measles antibodies are usually detectable within a day or two after onset of rash, attain peak levels soon after the first week and begin to decline only after the second month.[47] All the children who subsequently died failed to produce an adequate antibody response during the period of study.

One child who had responded early on in the disease rapidly lost all measurable antibody by the 3rd week after the appearance of the rash. It is difficult to explain this phenomenon. However, it has been shown that the maintenance of serum concentrations of antibody is dependent on the cyclical production of plaque forming cells.[195] In these experiments a few animals responded with only a single burst of plaque forming cells with a subsequent fall off in serum antibody titres.

It is possible that in this patient for some reason, either too much antigen or too incapable a lymphocyte, there was a similar restriction in antibody forming cells with a rapid diminution of antibody levels. Either or both of these mechanisms may have driven the lymphocyte to a catastrophic exhaustion of its immunological potential.

The link in this study between chronicity and antibody response was suggestive though it was tenuous when comparison was made with the response in those who recovered. Many children who developed chronic chest disease did not reach peak antibody titres by day 21 and the level had begun to fall by day 42.

The muted antibody response in those children who did badly was probably clinically important as patients with secondary immunodeficiency who die or develop fatal Hecht's giant cell pneumonia after measles also fail to produce adequate antibody.[2,59]

Factors which are intrinsic or extrinsic to the sensitive lymphocyte may determine the quantity of specific antibody produced. In malnourished children it has been demonstrated that when the nutritionally handicapped lymphocyte was confronted by a large quantity of measles antigen there was a feeble antibody response.[2] The children reported in this study were not malnourished. Diminution in antibodies in these patients may have been due to excessive measles antigen bombarding an intrinsically abnormal lymphocyte. Either of these factors may have accounted for decreased antibody in those who did not recover and possibly in Group A as a whole. A genetically determined inability of the lymphocyte to respond adequately to the virus may therefore be an additional and important reason for the recognised severity of measles in some African children.[196] Estimation of viral load and frequencies of HL A antigens in children with severe lymphopenia in measles may help in unravelling these problems.

Acute death in measles could be due to overwhelming and irreversible immunological paralysis, detectable by lymphopenia and inadequate specific antibody response. Chronicity could be explained by less

severe effects which would allow establishment of persistent virus infection. Viruses other than measles frequently complicate lung infection in measles,[60] and rarely brain infection in SSPE.[197] Measles infection leading to SSPE occurs most often in children under 2 years of age[89] when immunological responses are immature[198] and infection is associated with a high mortality.[196]

In this study those children who finally recovered had a more rapid reversal of immunoparesis than those who died or developed chronicity. There was incomplete immunological rehabilitation at six weeks in those who did not recover. However, when children with established chronic chest disease have been studied after six weeks, (Study I) immunological recovery is almost complete. Similarly in SSPE, immunological parameters are generally normal except for alterations in measles specific immunity.[71] These results suggest that long term pulmonary and neurological sequelae of measles are probably due to a transient widespread immunoparesis during early measles with persistent defects in specific immunity to measles and probably other viruses, whereas recovery is due to less severe effects of shorter duration.

The therapeutic implication of our studies is that children with measles who are at risk for death and chronic disease can be identified early in the disease and intervention at this stage may reverse the severe immunosuppression which leads to rapid demise or modify the immunopathological changes progressing relentlessly in some cases to permanent lung and brain damage and occasionally to death.

(v) SUMMARY

77% of 30 children with measles who had severe lymphopenia (< 2000 cells/mm^3) within 2 days of appearance of rash (Group A) subsequently died or progressed to chronic chest disease. This was significantly worse than the outcome in 30 children with measles who had lymphocyte counts above 2000 cells/mm^3 (Group B) of whom 67% recovered ($p < .01$). In group A children the persistence of severe lymphopenia (which was due to reduction in T and B cells) for at least fifteen days after onset of rash, remained a good predictive index of morbidity and mortality. Reversal of immunoparesis in group A was slower and incomplete 42 days from appearance of the rash in those who subsequently died or developed chronic chest disease than in those who recovered. All patients who died failed to produce adequate measles antibodies. The therapeutic implications and immunopathological significance of these findings for chronic complications following acute measles are discussed.

(vi) CONCLUSIONS

Children with measles who are at risk for death and chronic chest disease can be identified within 15 days of appearance of the rash by the severity of lymphopenia and failure of the specific antibody response.

The sequence of immunological events from acute disease to final outcome is clearly different in children who died or developed chronicity from those who recovered. Reversal of immunoparesis was slower and less complete six weeks after the rash in those whose course was unfavourable.

STUDY V

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8. S T U D Y V

DESIGN AND PURPOSE OF STUDY:

A. The immunological factors influencing outcome in measles have been defined in the preceding studies. A critical breakdown of defence mechanisms during acute measles had a high association with an increased morbidity and mortality. The fundamental cause of differences in clinical expression of a disease caused by a single type of virus,[199] however, are not understood. This study was therefore undertaken to analyse the role of viral-related and host-related factors determining outcome.

B. Lymphopenia was shown to be the single most important criterion of death or chronicity in measles. The incidence of lymphopenia in children with a number of different diseases was recorded.

A. 1. VIRAL LOAD AND OUTCOME IN MEASLES

(i) INTRODUCTION:

Exfoliated giant cells in nasopharyngeal secretions have been considered specific for measles infection[200,201] and prolonged excretion of these cells a feature of malnourished children.[2,58] However, this finding has not been confirmed by adequate laboratory virus identification.[2]

A fluorescent antibody technique, using nasopharyngeal secretions, has been shown to be a reliable method for measles virus identification by correlation of immuno fluorescence results with clinical diagnosis, tissue

culture and serology.[32] We have obtained measles-specific antiserum used in these studies from Professor P.S. Gardner and attempted to estimate viral load in nasopharyngeal secretions.

(ii) METHODS AND RESULTS:

Viral load in nasopharyngeal secretions was assessed by noting the number of fluorescing cells per high power field and the strength of immunofluorescence per cell taken.

(1) Total number of tests done = 28

(2) False positives (i.e., negative in test, positive in control) = 1

(3) True positives = 0

(iii) CONCLUSIONS:

This test has been unsuccessful in detecting measles antigen and therefore no conclusions can be made.

A. 2. HISTOCOMPATIBILITY LINKED GENETIC CONTROL OF SUSCEPTIBILITY TO SEVERE MEASLES

(i) INTRODUCTION:

Specific immune responses to a wide range of antigens is under genetic control.[202] This control, in the majority of instances, is linked to the major histocompatibility locus of a particular species.[203,204]

There is good evidence to show that resistance or susceptibility to specific viral infections in mice is linked to the histocompatibility locus H2.[205]

In man immune responsiveness to vaccinia[206] and influenza 'A'[207] viruses and streptococcal antigens[208,209] is associated with specific HL A types. Furthermore, susceptibility of individuals to development of paralytic poliomyelitis[210] and clinical variation in leprosy[211] is linked to HL A genes. Infection of human lymphoblastoid cell lines, however, suggest no significant interaction between measles virus and surface HL A antigens.[217]

The deviation in HL A frequencies in children with severe measles has been studied.

(ii) PATIENTS:

Measles is a universal disease with an almost 100% attack rate. Therefore nearly all susceptible persons exposed to the disease will develop infection. The frequency of HL A types among Blacks in Natal has been established.[218] Therefore HL A investigations were done only in children with severe measles (i.e., those with absolute lymphocyte counts of below 2000 cells/mm^3 soon after the onset of rash). Twenty four children with measles who had severe lymphopenia within 48 hours of onset of rash were studied. Seven of these children subsequently recovered whereas 12 did not (2 died, 10 chronic chest disease) and the outcome in 5 could not be ascertained.

(iii) RESULTS:

The results are given in the table X

All comparisons made with controls. There was no difference between children who recovered and those who did not.

TABLE X

HLA FREQUENCIES IN CHILDREN WITH SEVERE LYMPHOPENIA (<2000 CELLS/MM^3)

	CONTROL N=731 %	ALL CASES WITH SEVERE LYMPHOPENIA N=24 %	RECOVERED N=7 %	DID NOT RECOVER N=12 %
HLA A 1	5.6	12.5	28.6	8.3
A 2	19.8	4.2	0	8.3
A 3	13.7	0	0	0
A 11	0.1	4.2	0	8.3
A 23	19.3	20.8	0	33.3
A 24	3.4	8.3	0	8.3
A 25	14.1	8.3	14.3	0
A 26	7.7	16.7	14.3	16.7
A 28	20.2	29.2	0	33.3
A 29	17.0	16.7	14.3	16.7
AW 30	39.3	20.8	28.6	16.7
AW 31	12.6	4.2	0	0
AW 32	1.6 {25.3} (<.02)*	16.7	28.6 {27.0} (<.02)*	16.7 {14.4}(<.02)*
AW 33	0	0	0	0
1 Antigen Blank	25.6	33.3	71.4	25.0
HLA CW 1	0	0	0	0
CW 2	13.0	25.0	14.3	33.3
CW 3	5.9	16.7	14.3	16.7
CW 4	15.8	8.3	14.3	0
CW 5	4.1	0	0	0
1 Antigen		41.7	42.9	50.0
Blank		54.2	57.1	50.0

{} Chi-square

* p value (multiplied by number of antigens tested i.e. 39)

continued./....

TABLE X continued.....

		CONTROL N = 731 %	ALL CASES WITH SEVERE LYMPHOPENIA N = 24 %	RECOVERED N = 7 %	DID NOT RECOVER N = 12 %
HL A	B 5	2.1	0	0	0
	B 7	15.9	12.5	14.3	8.3
	B 8	14.1	12.5	0	25.0
	B 13	4.9	0	0	0
	B 14	6.2	4.2	0	8.3
	B 15	6.0	0	0	0
	B 16	2.3	4.2	0	0
	B 17	38.2	54.2	28.6	75.0
	B 18	3.4	8.3	14.3	8.3
	BW 21	0.4	0	0	0
	BW 22	0	0	0	0
	BW 27	0.4	0	0	0
	BW 35	7.5	12.5	28.6	8.3
	BW 37	0.	0	0	0
	BW 40	1.5	0	0	0
	BW 41	1.4	0	0	0
	BW 42	27.9	12.5	0	8.3
	BW 44	16.1	20.8	14.3	16.7
	BW 45	6.2	4.2	14.3	0
	BW 53	3.4	0	0	0
1 Antigen		42.1	41.7	57.1	41.7
	Blank		4.2	14.3	0

The incidence of HL A AW_{32} was significantly increased ($p < .02$) over that of controls in the group of children with severe lymphopenia as a whole and separately for those who did or did not recover. The frequency of HL A A 11 was significantly increased ($p < .02$) in children with severe lymphopenia and in those who did not recover when compared to controls.

(v) COMMENTS:

These early results suggest a trend showing linkage between severe lymphopenia and HL A AW_{32}. This trend for an A antigen would be unusual in that most HLA-disease associations have been linked with B series antigens.[202]

It would appear from these results that there may be a histocompatibility linked genetic susceptibility to the development of severe lymphopenia in measles associated with HLA AW_{32}.

The mechanisms responsible for this association may be due to any of the following:

(1) HL A may be linked to an immune response gene[202] or immune suppression gene[212] which determine responsiveness to measles virus or measles virus infected cell surface products of infection. This would then account for an inability to handle measles infection adequately as shown by a poor antibody response and possibly other aspects of specific immunosuppression which would lead to severe lymphopenia.

(2) HL A products may provide favourable receptor sites for virus attachment on cells infected with measles virus, e.g., lymphocytes, monocytes, respiratory epithelial cells and neurons. This would facilitate damage to affected cells with consequent immunoparesis, lung and brain damage.

(3) One possibility is that measles virus carries antigenic determinants similar to HL A AW_{32} and A 11-"molecular mimicry".[213] Hence the response to this self-antigen would be expected to be diminished allowing for a more severe clinical outcome.

B. INCIDENCE OF LYMPHOPENIA

(i) INTRODUCTION:

Lymphopenia is infrequently present in common diseases[190] and when detected is often associated with serious illness.[191]

(ii) PATIENTS:

The absolute lymphocyte counts in 3 different groups of patients have been investigated.

(1) Acute mild measles: these were children with no major complication of measles, seen in paediatric out-patients and treated as out-patients.

(2) Moderate to severe measles: children with some complication of measles admitted to the fever unit for in-patient treatment.

(3) Non-Measles: the case notes of 120 consecutive in-patients to the Paediatric wards in King Edward VIII Hospital were studied. The major problem in 109 of these children was infection; in 11 it was not related to infection.

(iii) RESULTS:

Results are given in Table XI.

(iv) DISCUSSION:

These results have shown that severe lymphopenia (< 2000 cells/mm^3) is uncommon in mild measles but frequent in moderate to severe measles; when present it was associated with a high mortality. Severe lymphopenia was present in 9% of non measles patients and unrelated to mortality.

(v) CONCLUSIONS:

Absolute lymphocyte counts below 2000 cells/mm^3 appear to be an index of mortality primarily in children with measles and not in other common hospital infections.

TABLE XI

LYMPHOCYTE COUNTS AND MORTALITY IN CHILDREN WITH MEASLES AND NON-MEASLES

	NUMBER OF PATIENTS	NUMBER OF FEMALES	MEAN AGE IN MONTHS (RANGE)	MEAN ABSOLUTE LYMPHOCYTE COUNT (± S.E.M.)	NUMBER WITH ABSOLUTE LYMPHOCYTE COUNTS < 2000 CELLS/MM^3	MORTALITY	
						> 2000 LYMPHOCYTES/MM^3	< 2000 LYMPHOCYTES/MM^3
MILD MEASLES	55	33	19(6-72)	5526 ± 463	3 (5%)	0	0
MODERATE/ SEVERE MEASLES	33	22	16(6-48)	4036 ± 502	5 (15%)	1 (4%)	4 (80%)
NON-MEASLES	120	55	25(0.25-132)	5011 ± 298	11 (9%)	9 (8%)	1 (9%)

N.B. The 80% mortality in children with moderate to severe measles with lymphopenia below 2000 cells/mm^3 should be treated with reserve as Study IV has shown that in a larger number of these patients the mortality is 30%

A P P E N D I X

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9. APPENDIX

DETAILS OF CHILDREN WHO DIED DURING ACUTE MEASLES

Details of children who died during acute measles are given in Table XII.

COMMENTS

(1) All these children had pulmonary complications following measles which were probably the cause of death in the majority.

(2) Histopathological changes in the lungs were compatible with viral pneumonia. 'Smudge cells', which indicate adenovirus infection, were present in the lungs of 2 patients. Measles virus giant cells were not identified in any patient. Evidence for bacterial pneumonia was found in 2 patients.

(3) The thymus was atrophic whenever examined.

(4) The spleen and lymphnodes showed evidence of lymphocytic hyperplasia with germinal centres.

(5) Haemorrhage in the adrenals (1 case) may have been due to disseminated herpes.

(6) Septicaemia was present in 2 patients and hepatic failure in one.

CONCLUSIONS

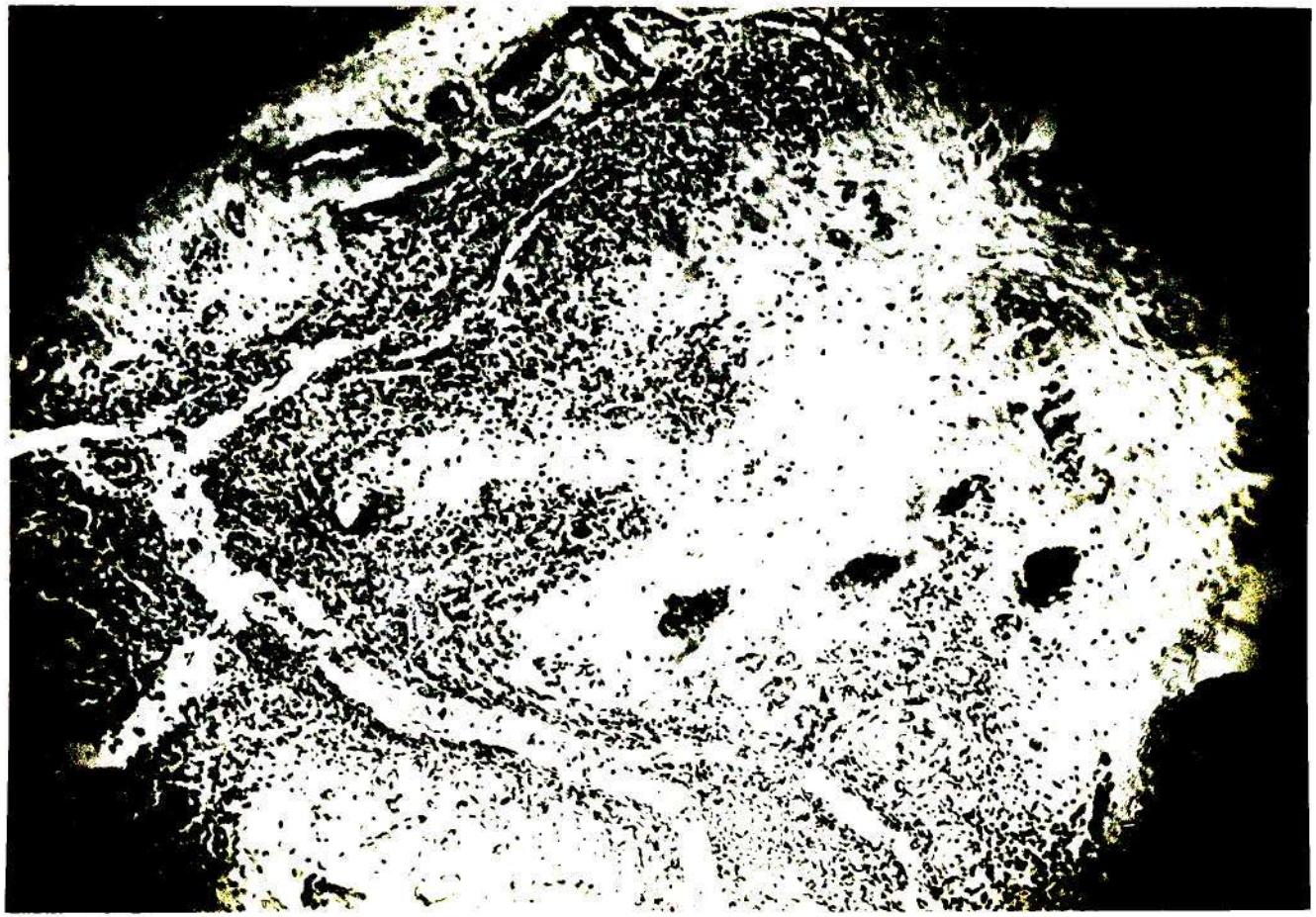
All the children with measles had pulmonary complications which were the cause of death in the majority. Pneumonia was nearly always of viral aetiology with bacterial infection being detected in 2 of 4 patients.

There was atrophy of the thymus with a lymphocytic response in the spleen and lymphnodes. This would support the concept of 'trapping' of lymphocytes in peripheral lymphoid tissues being associated with a profound lymphopenia.

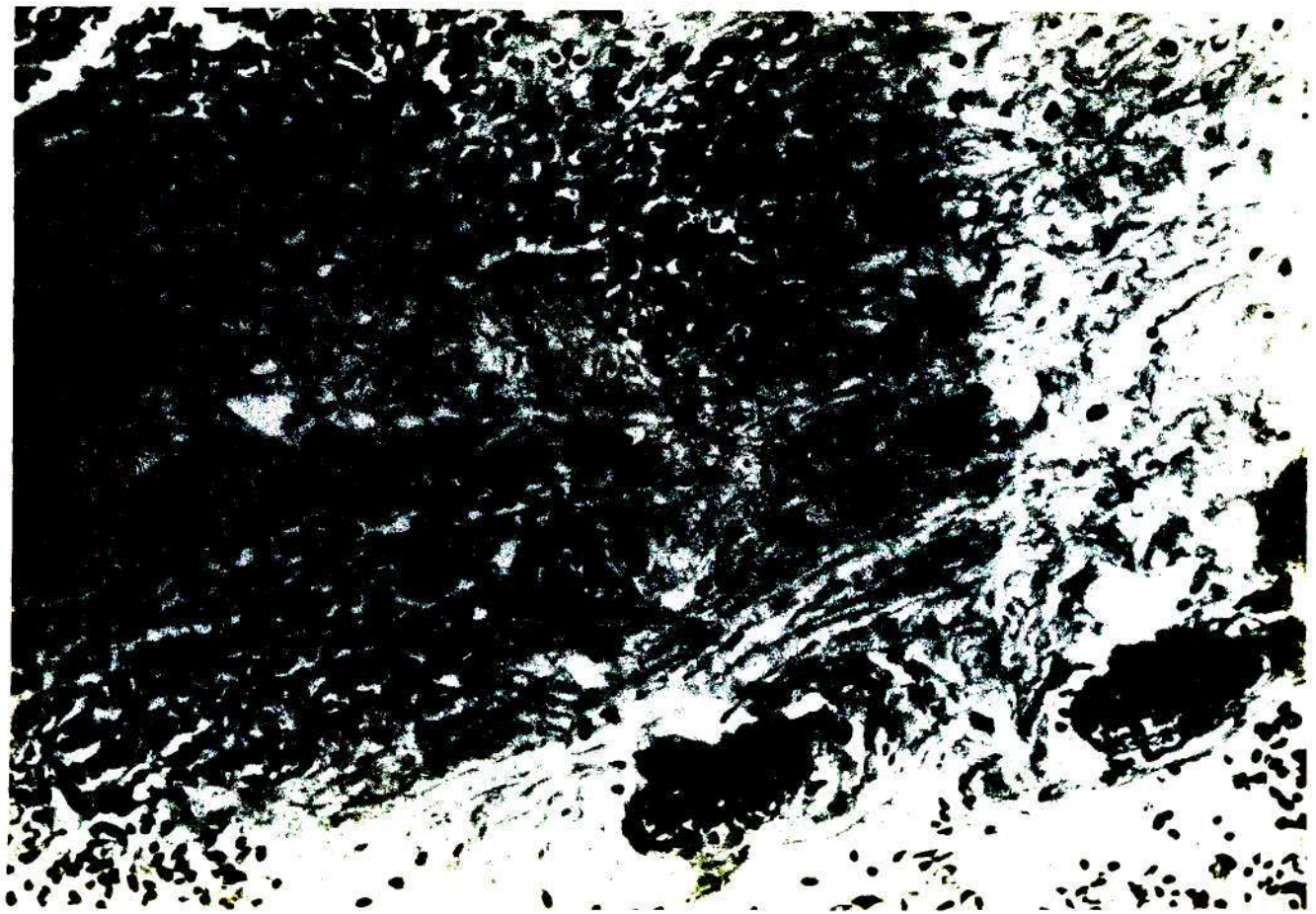
TABLE XII

DETAILS OF PATIENTS WITH ACUTE MEASLES WHO DIED

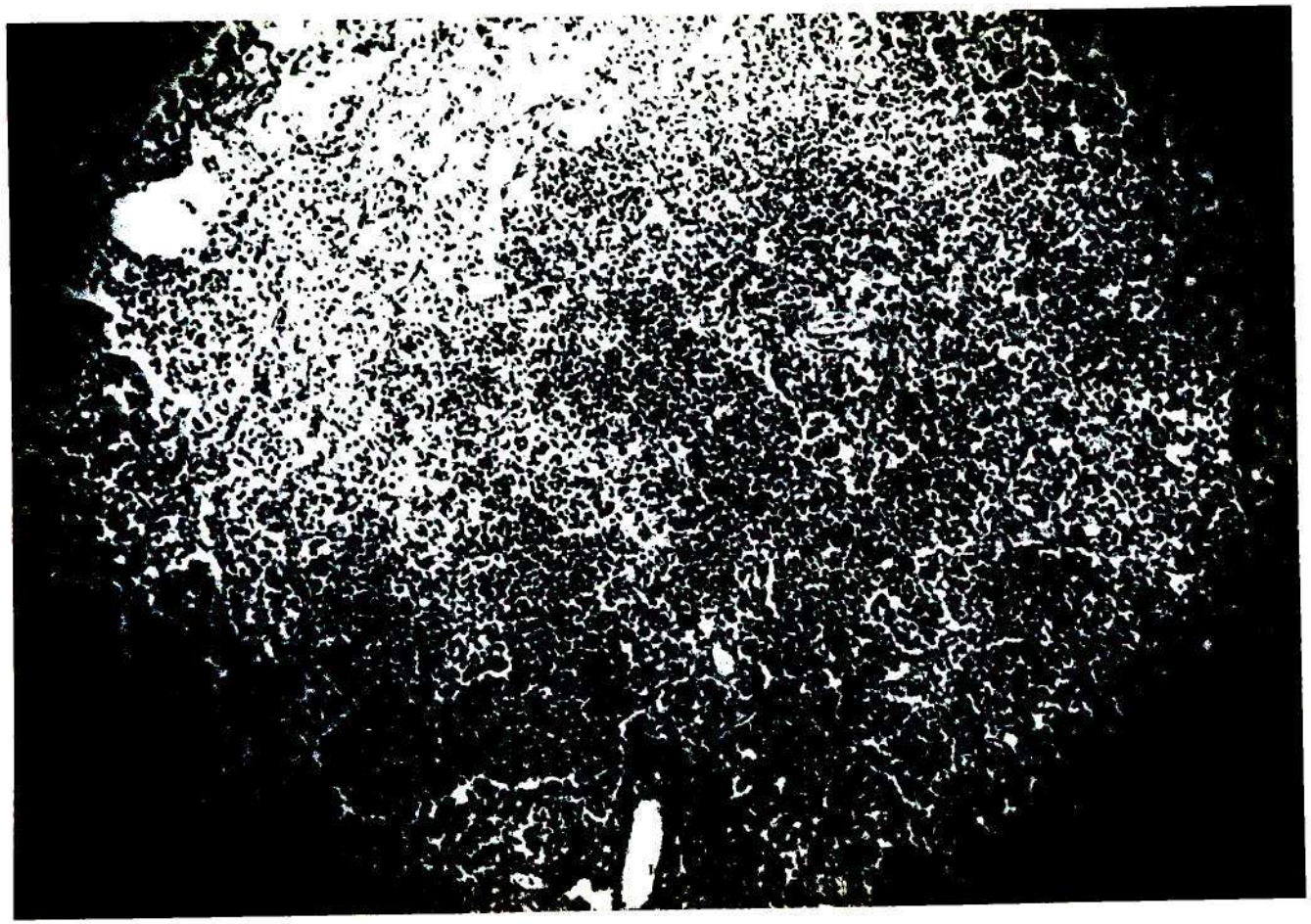
5	6	7	8	9
16	14	24	24	6
F	M	F	M	F
6	23	14	4	6
1. Bronchopneumonia 2. Laryngotracheobronchitis 3. Herpes stomatitis	1. Bronchopneumonia 2. Dysentery 3. Pneumococcal septicaemia 4. Hepatic failure	1. Bronchopneumonia 2. Herpes Stomatitis 3. Pleural Effusion	1. Bronchopneumonia 2. Otitis media	1. Bronchopneumonia
Penicillin, Cefzol, Gentamycin	Penicillin, Sulfas, Cefzol, Gentamycin, Neomycin, Vit.K	Penicillin, Streptomycin	Penicillin, Kanamycin Neomycin	Penicillin, Kanamycin Chloromycetin, Neomycin
1870	696	1032	780	936
Bilateral Bronchopneumonia	Bilateral Perihilar + Right Lower Lobe Bronchopneumonia	Perihilar Bronchopneumonia + Right pleural effusion	Extensive Bilateral Bronchopneumonia	Right Upper Lobe Consolidation
< 10	< 10	< 10	< 10	< 2
Not available	Not available	Not available	Not available	Not available



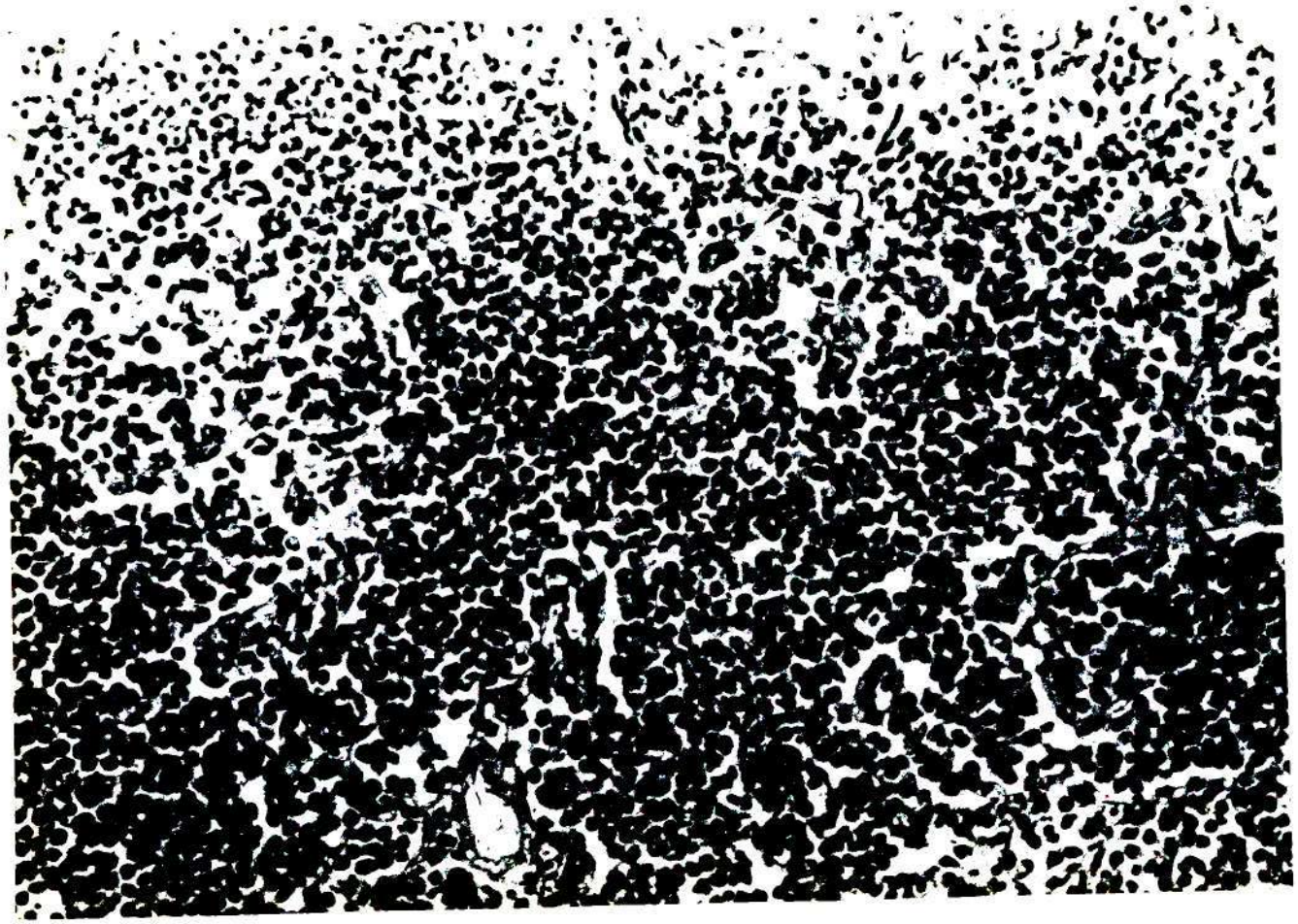
M1 ATROPHY OF THYMUS



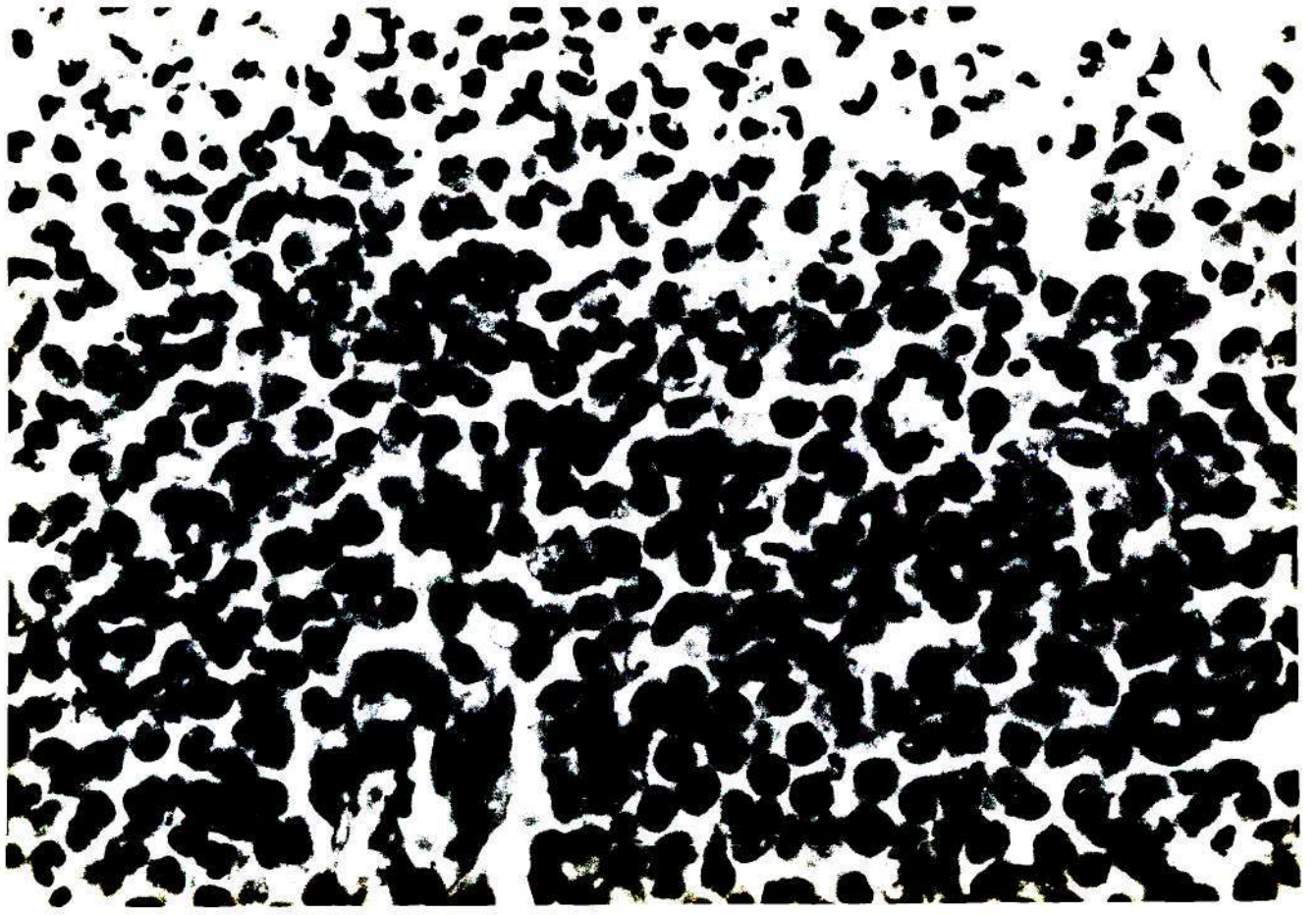
M1 ATROPHY OF THYMUS



M1 HISTOLOGY OF LUNG SHOWING NEUTROPHIL INFILTRATION SUGGESTIVE OF BACTERIAL PNEUMONIA

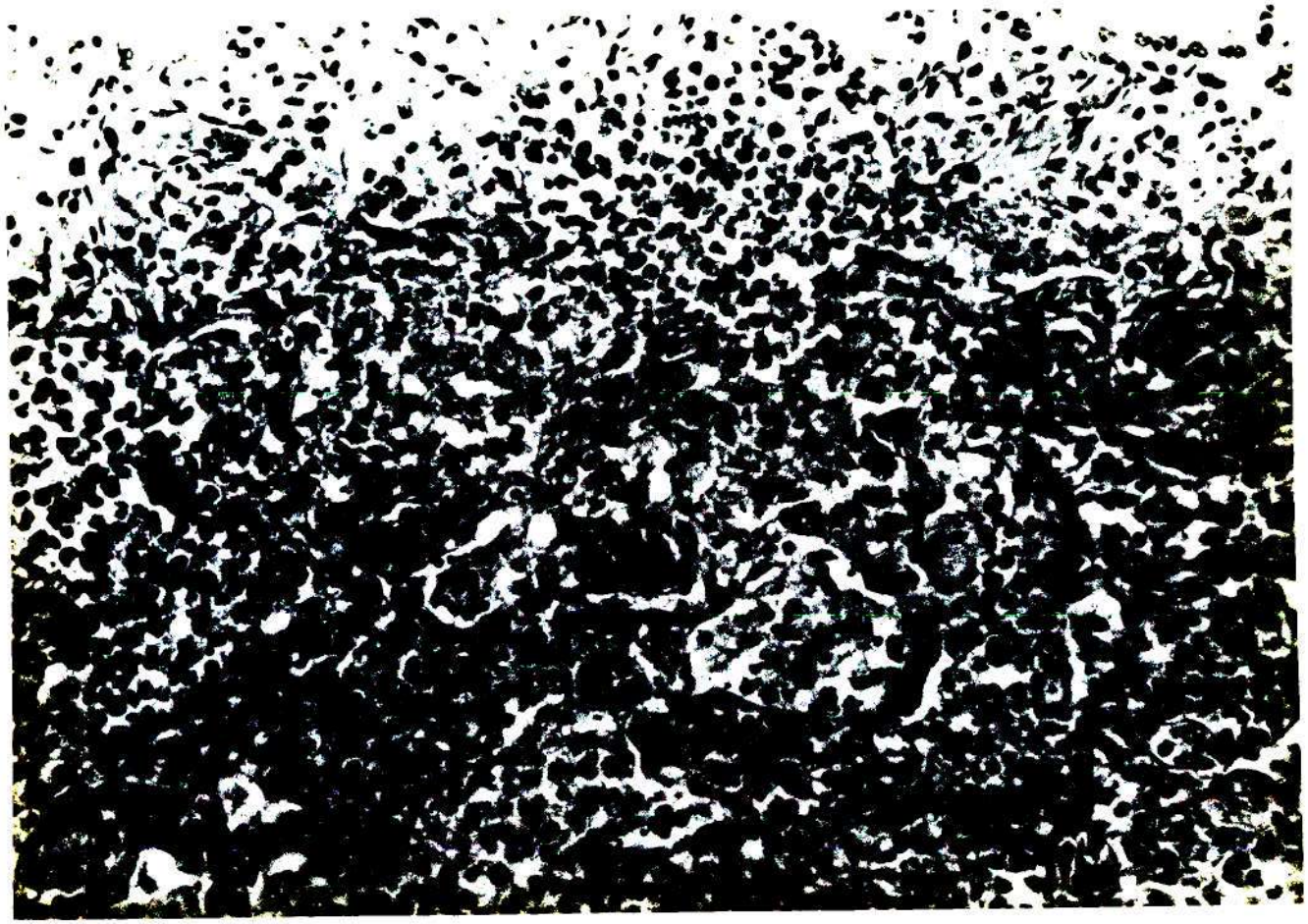


M1 BACTERIAL PNEUMONIA

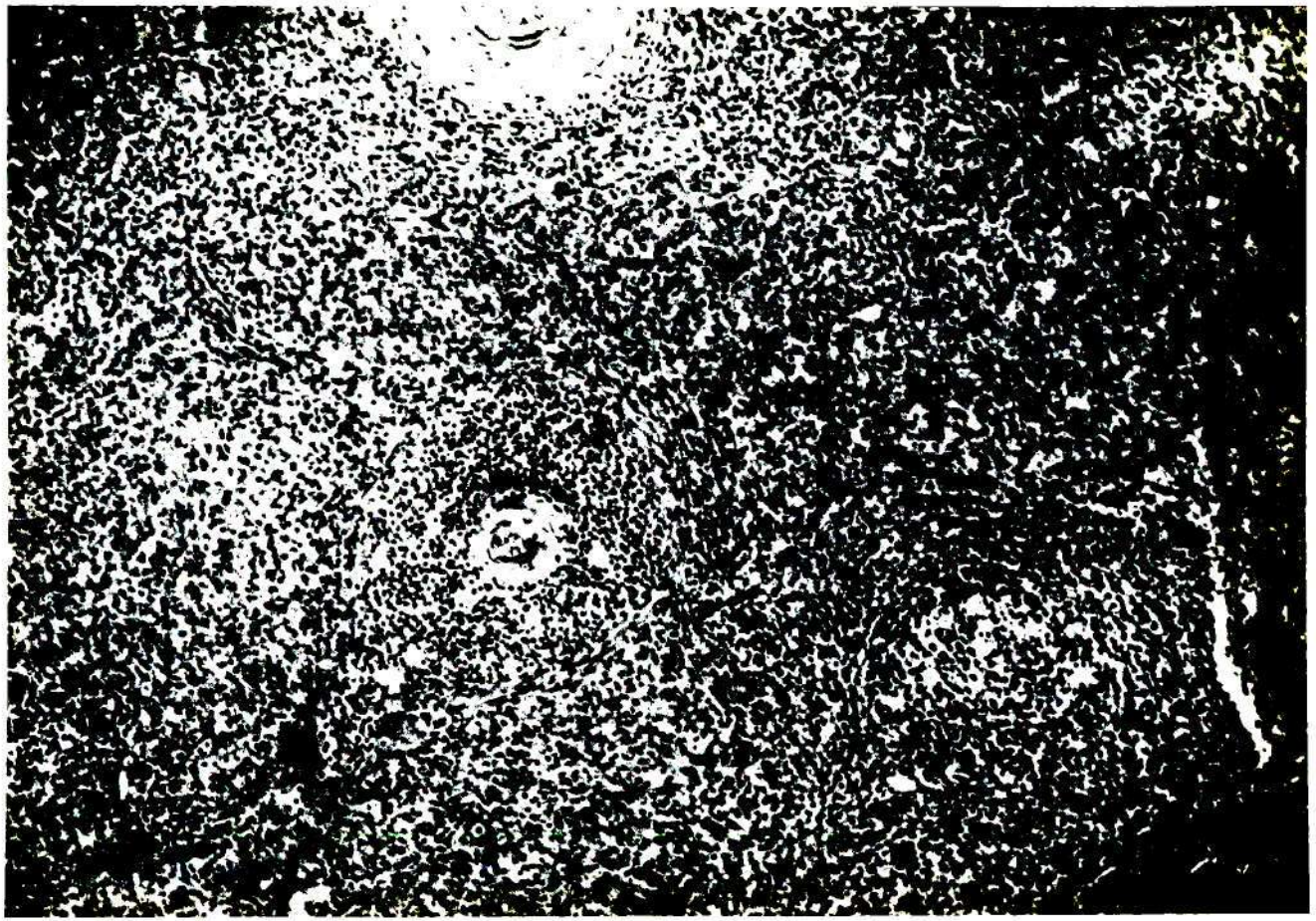


M1 BACTERIAL PNEUMONIA

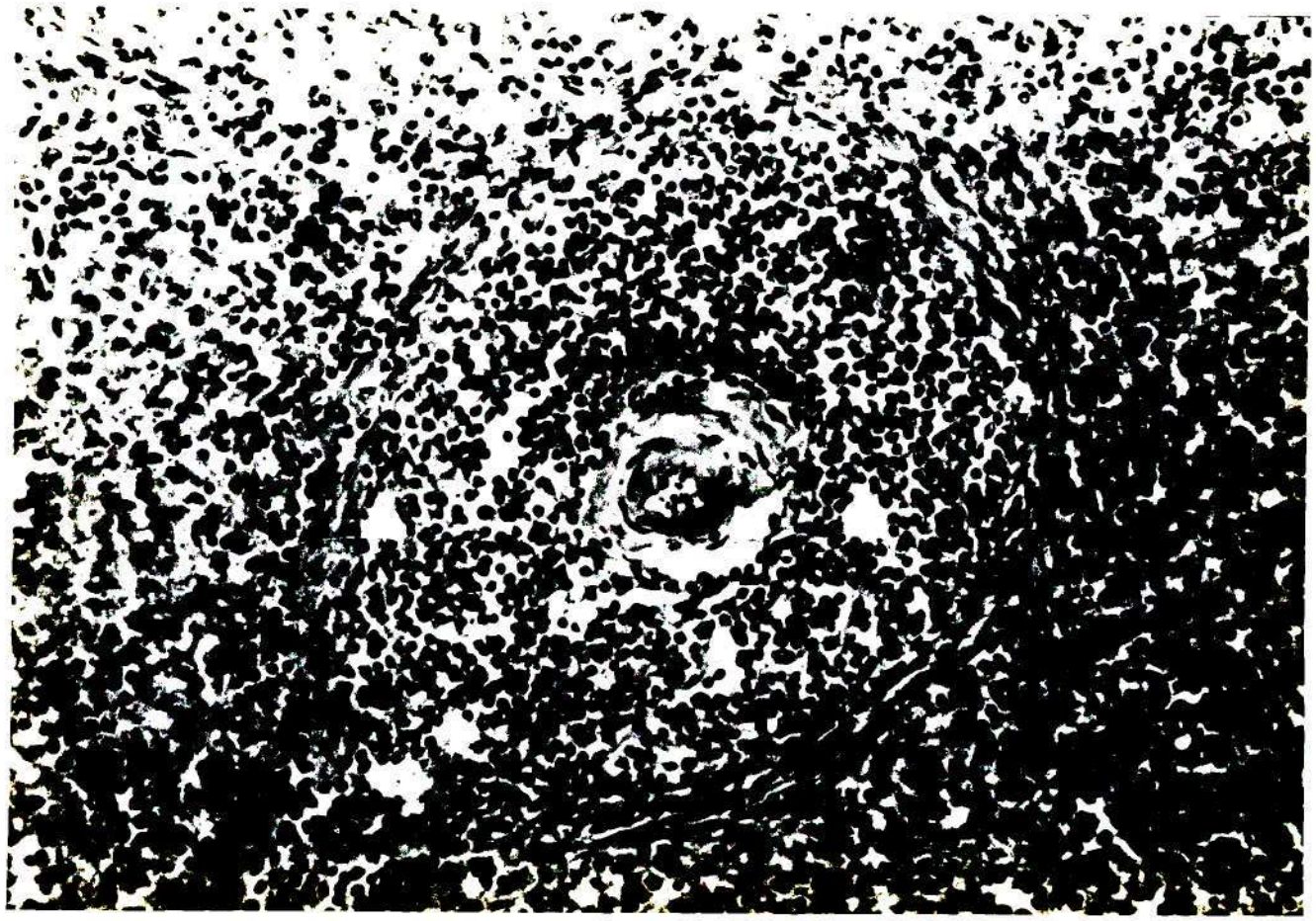
M3 HISTOLOGY OF LUNG SHOWING "SMUDGE CELLS" AND NEUTROPHILS



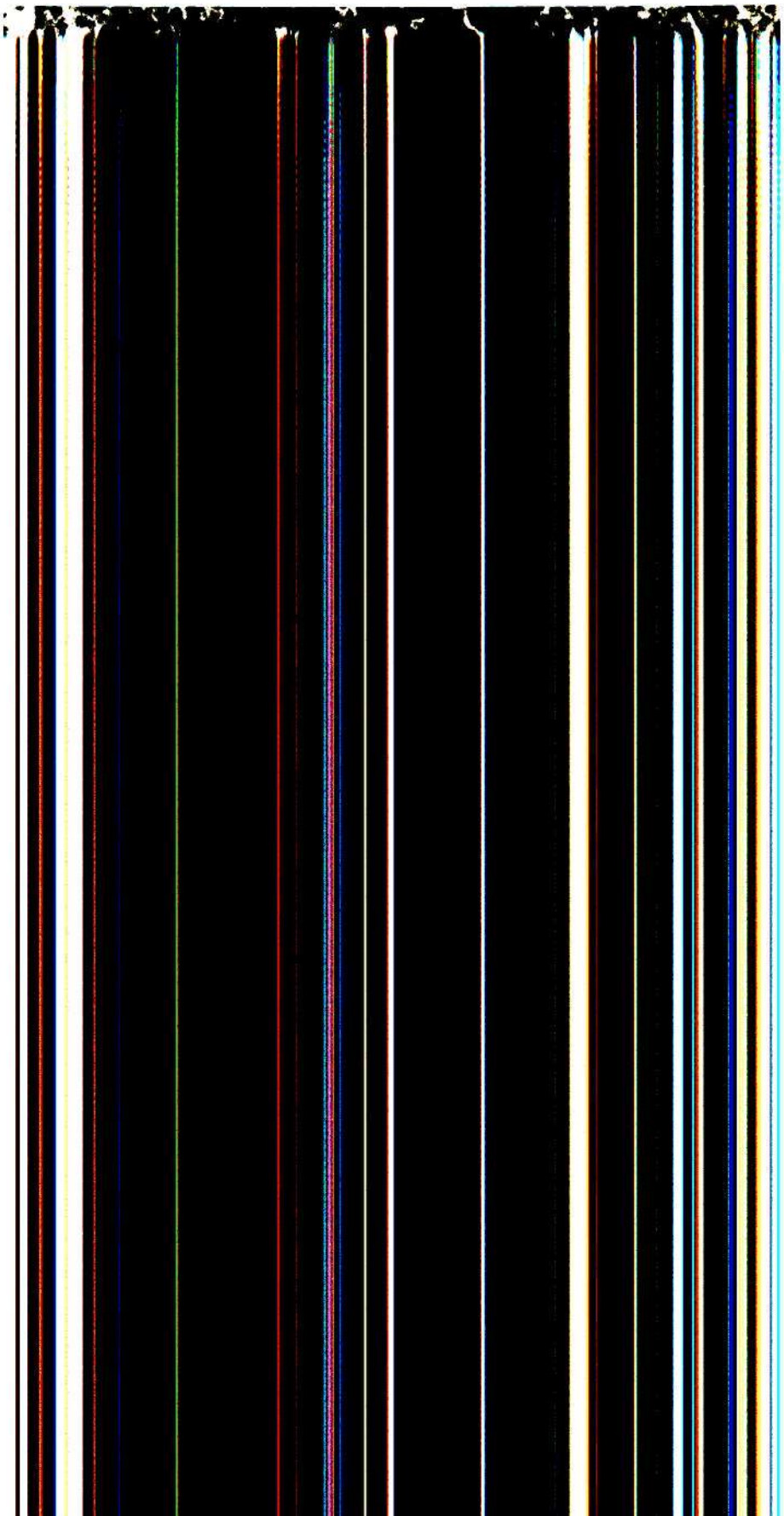
M3 LUNG TISSUE SHOWING "SMUDGE CELLS" AND NEUTROPHILS



M3 HISTOLOGY OF SPLEEN SHOWING PERI-ARTERIOLAR LYMPHOID SHEATH AND LYMPHOID FOLLICLE



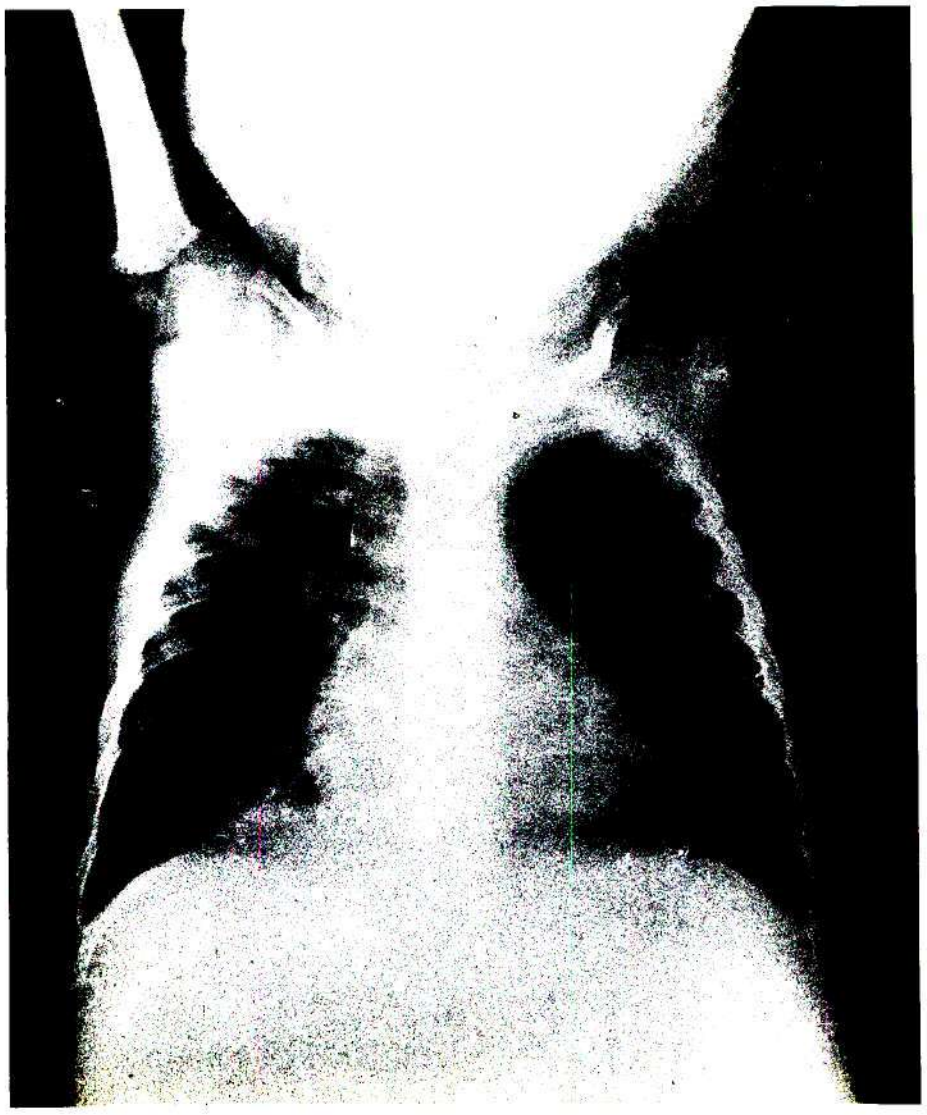
M3 PERIARTERIOLAR LYMPHOID SHEATH (SPLEEN)



SEQUENTIAL CHEST RADIOGRAPHS IN FOUR PATIENTS WHO DEVELOPED CHRONIC CHEST DISEASE

KEY:

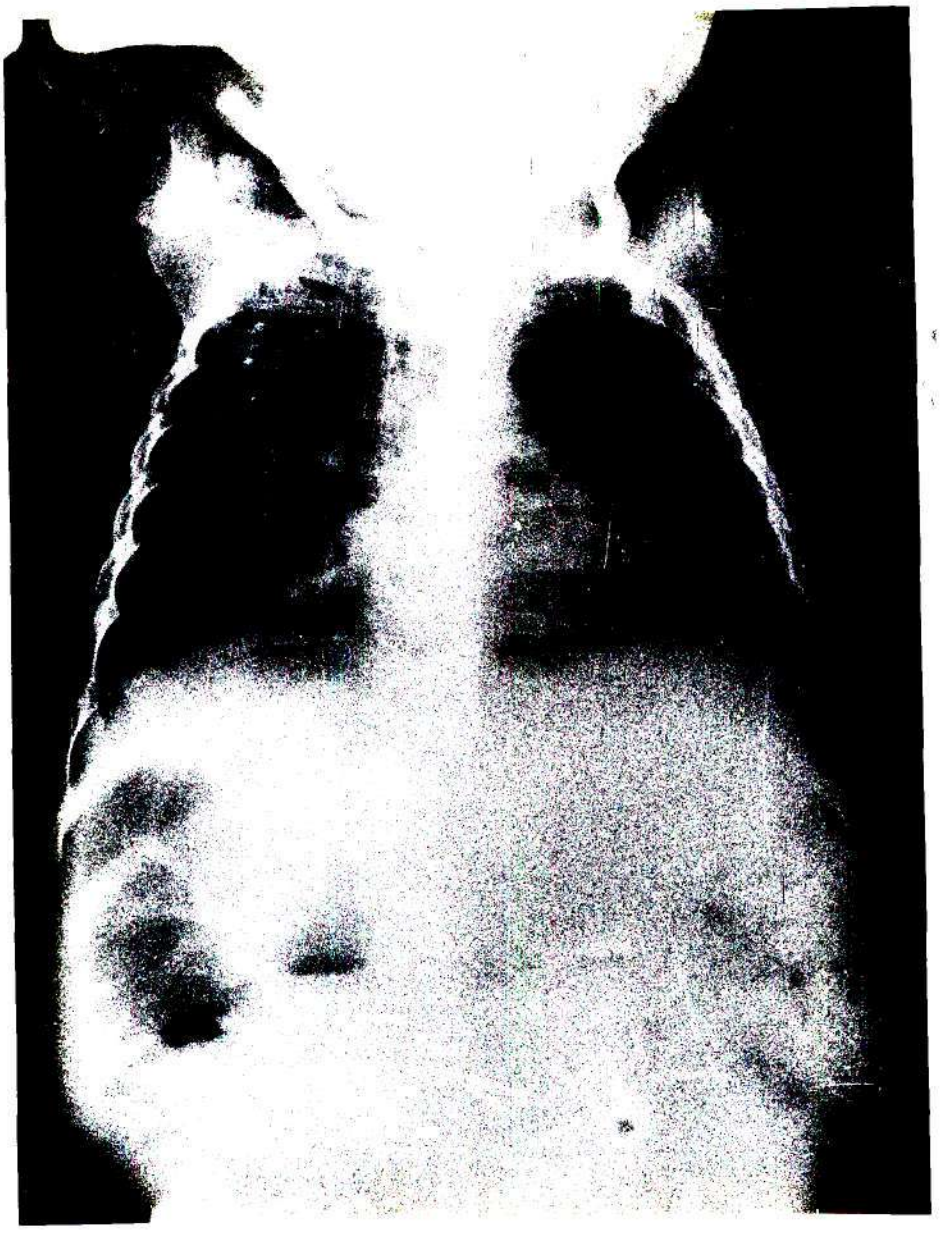
M	=	SERIES NUMBER
DAY	=	DAY AFTER APPEARANCE OF RASH
CXR GRADE	=	SEVERITY OF BRONCHOPULMONARY CHANGE (see page 71 for details)



M56

DAY 12

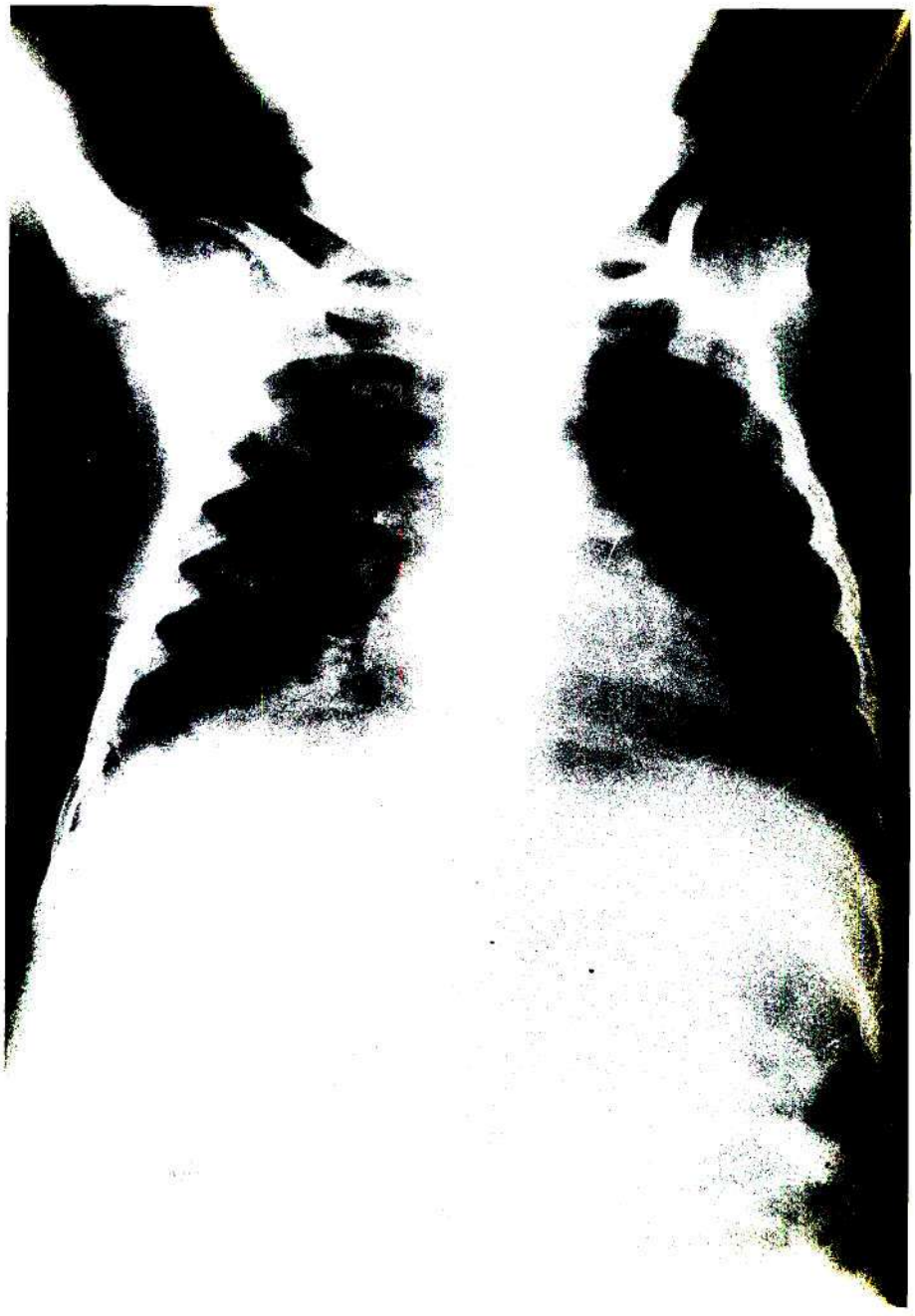
CXR GRADE 6



M56

DAY. 36

CXR GRADE 5



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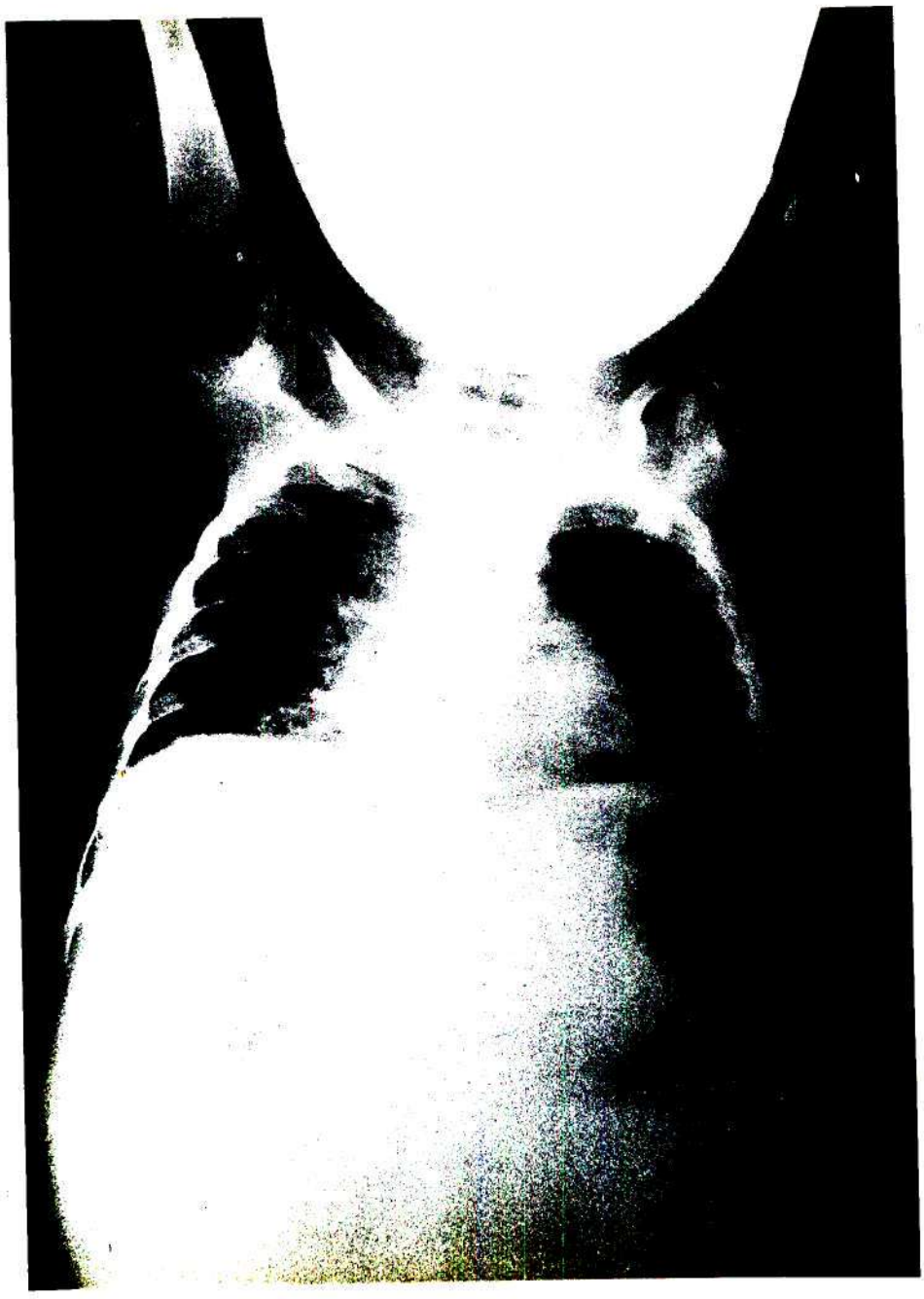

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DAY 74

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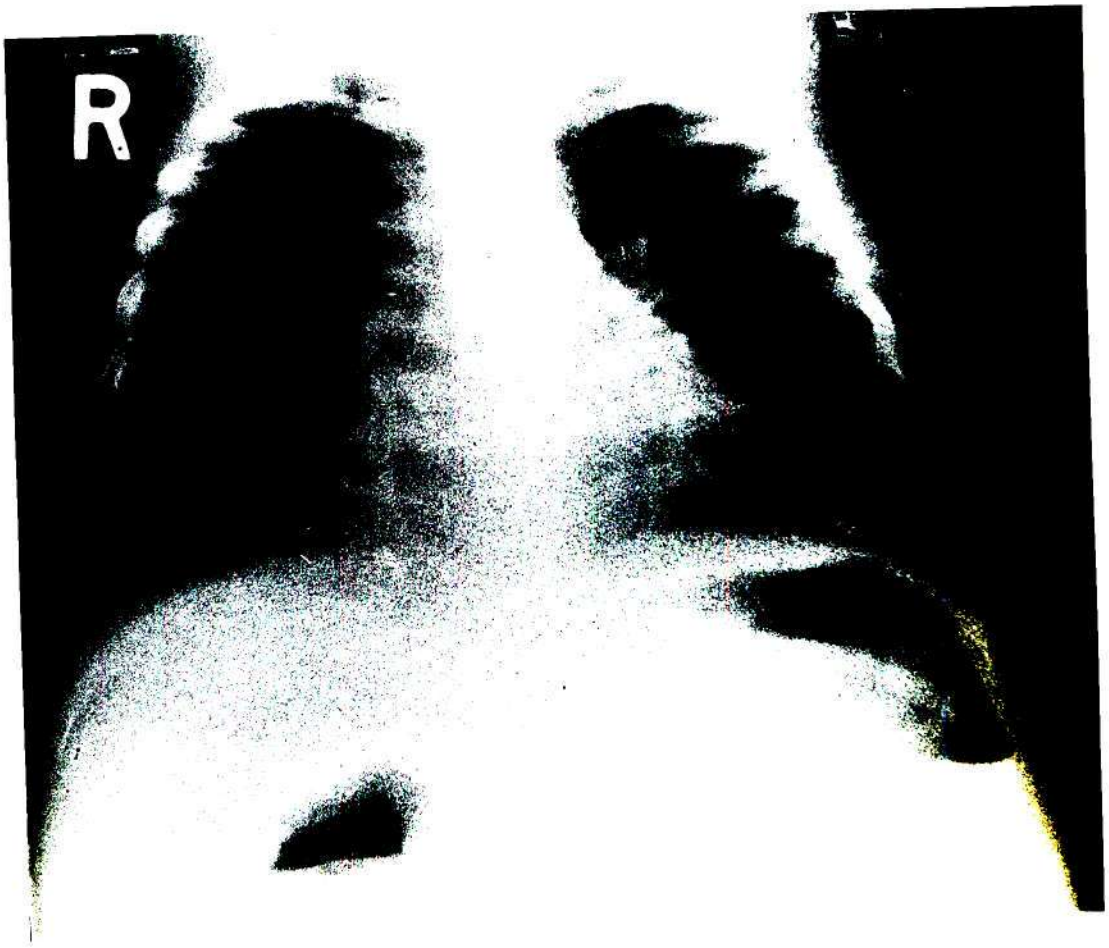

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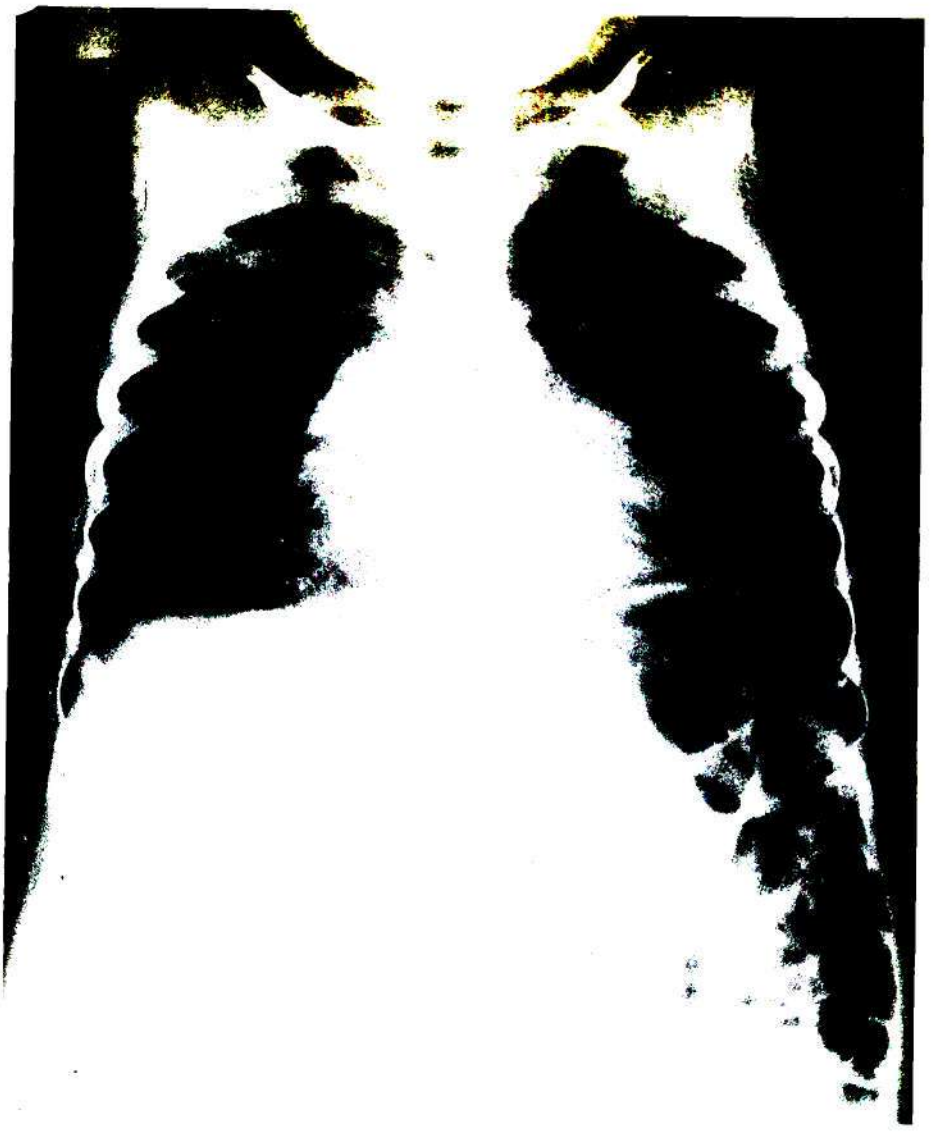

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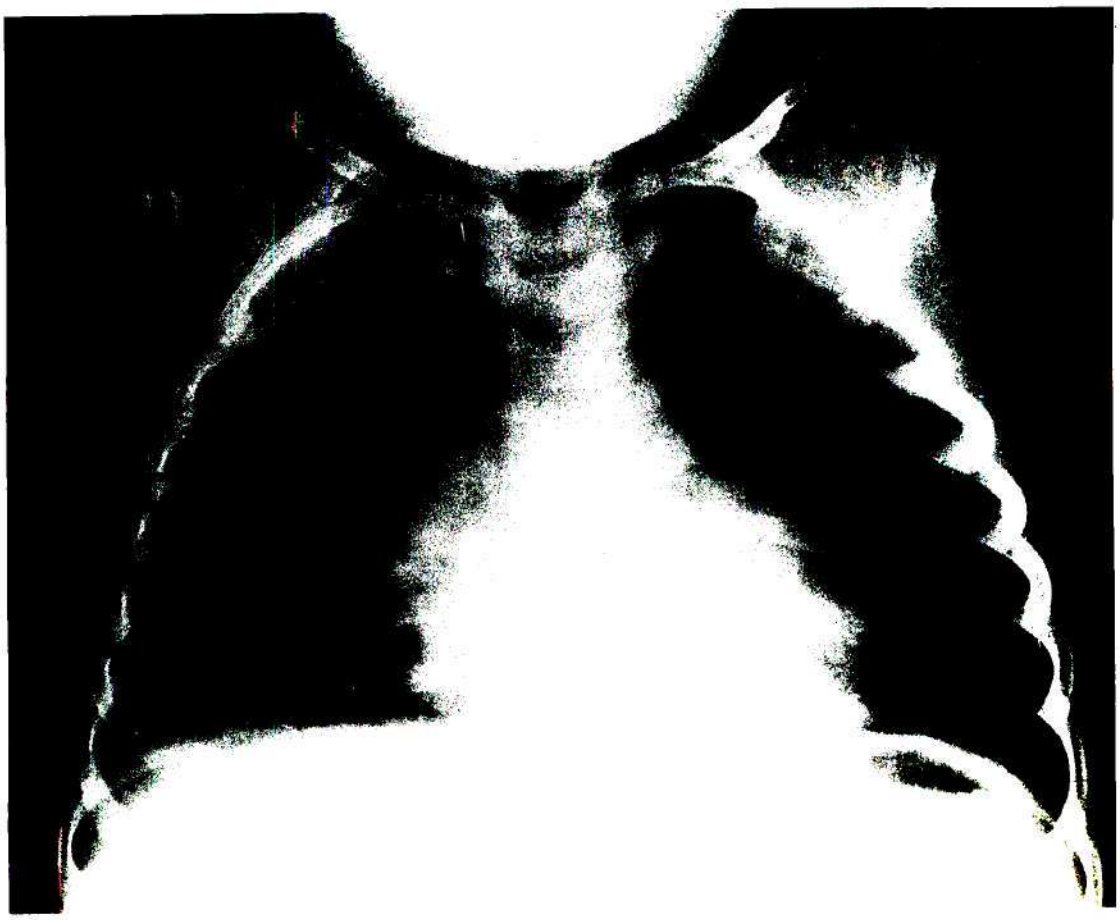

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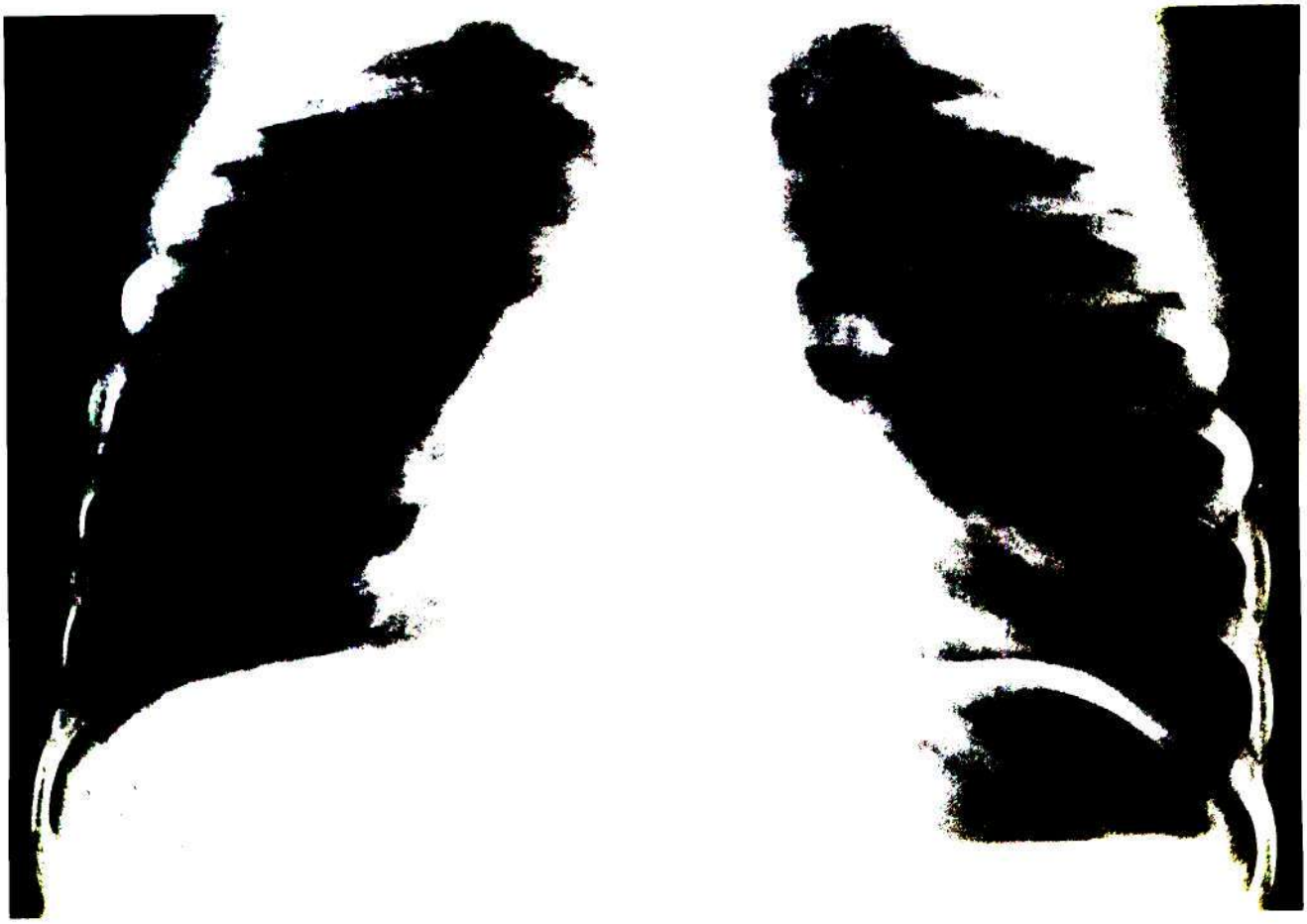

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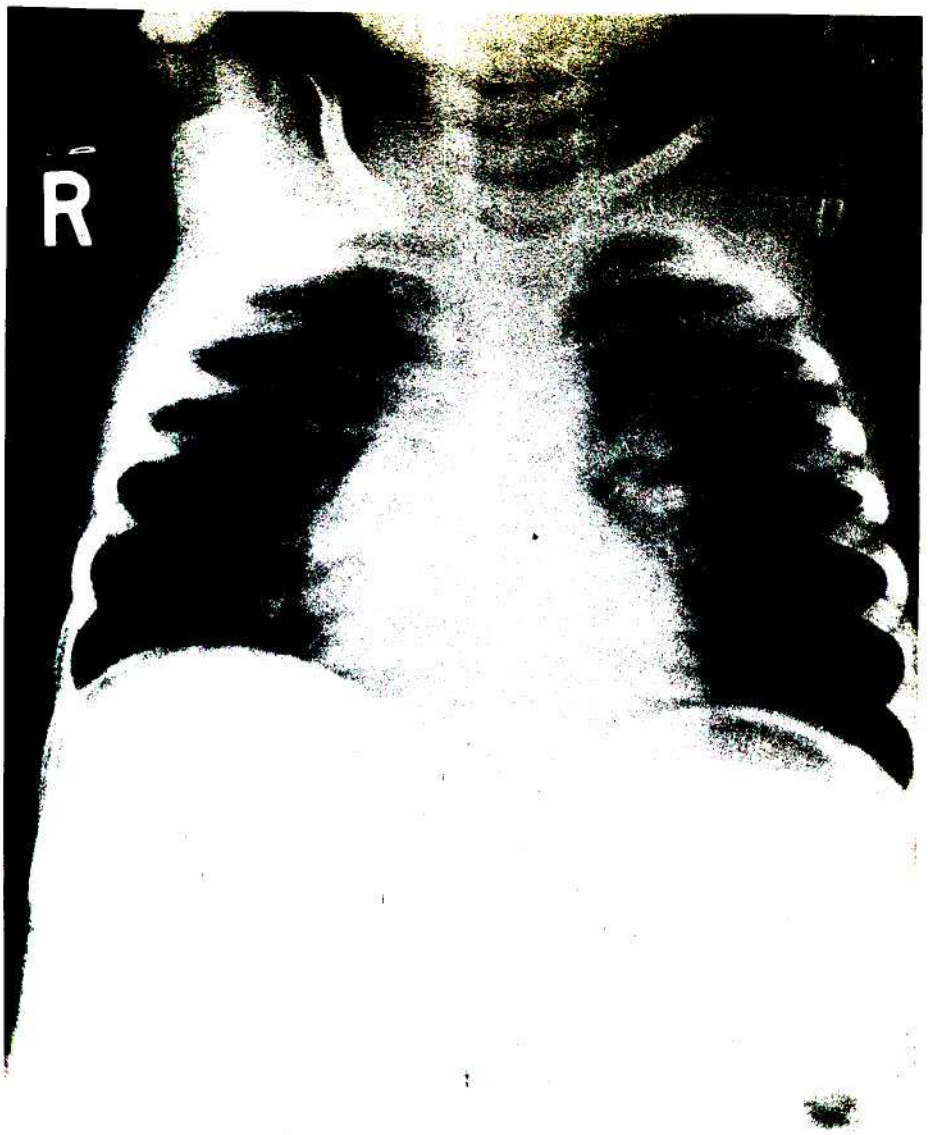

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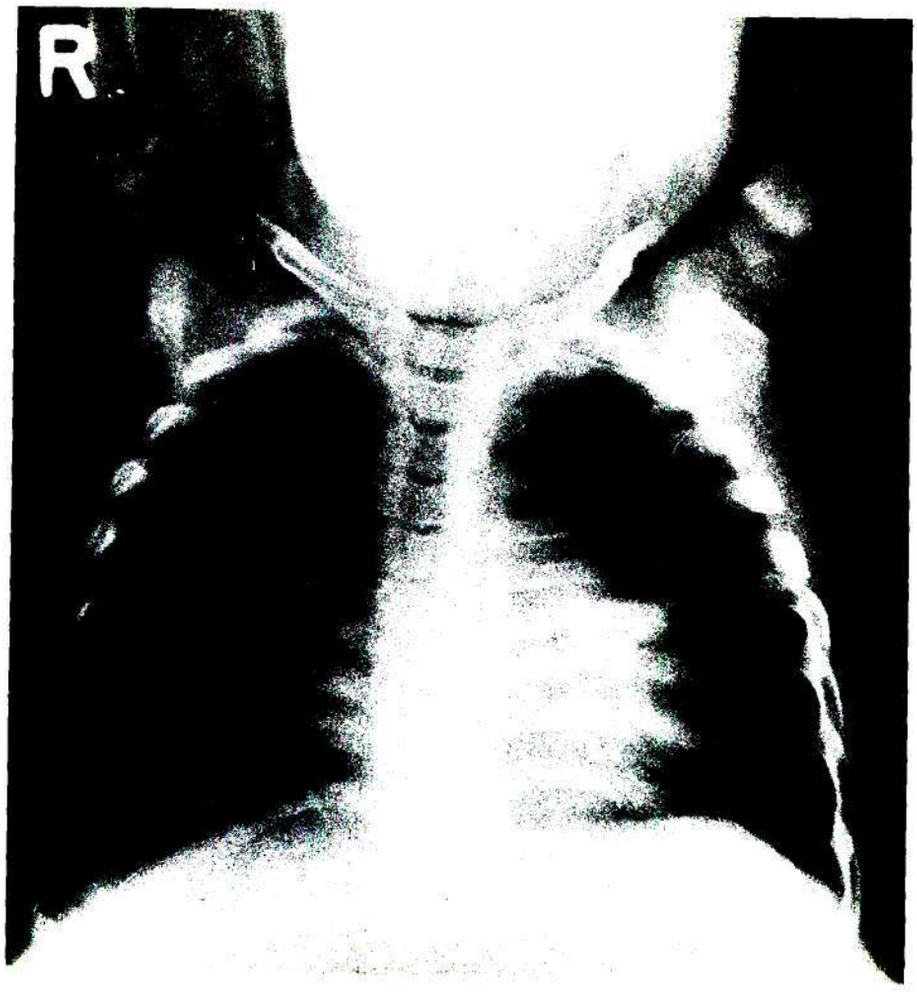

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DAY 5

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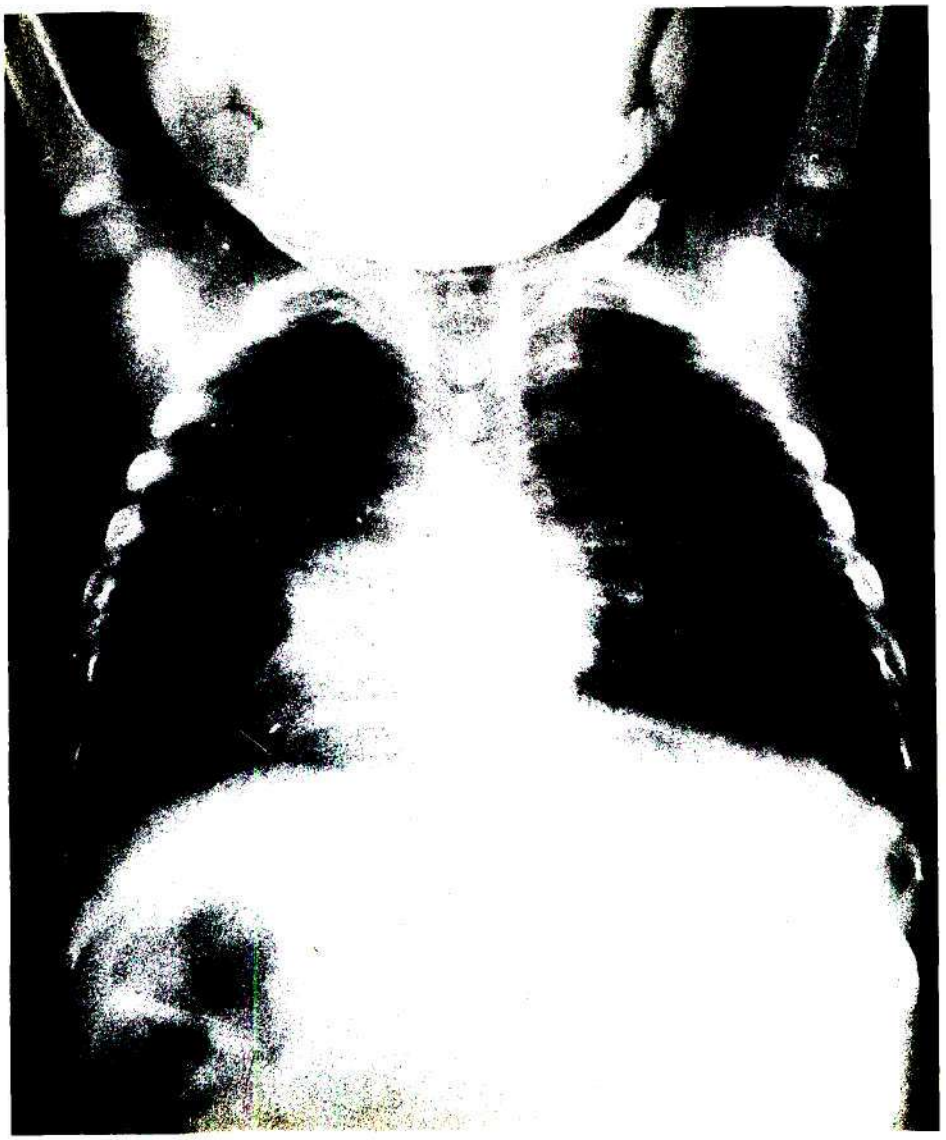

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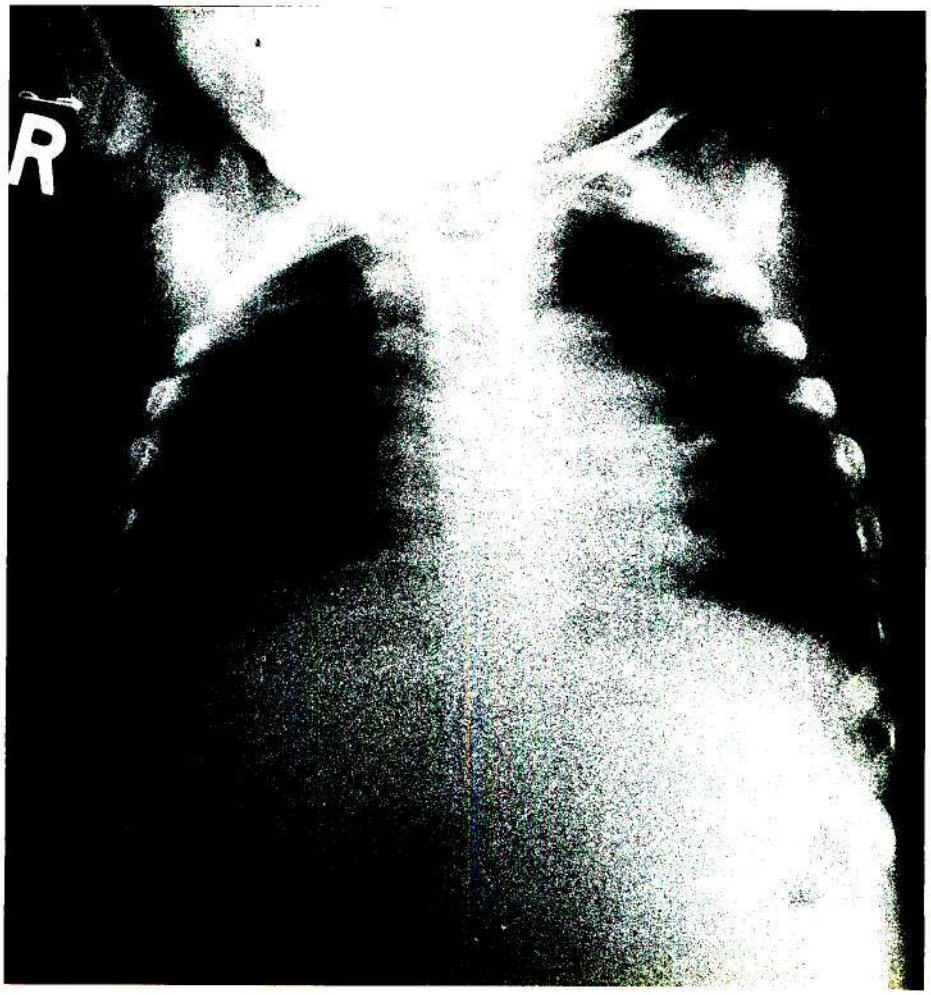

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DAY 47

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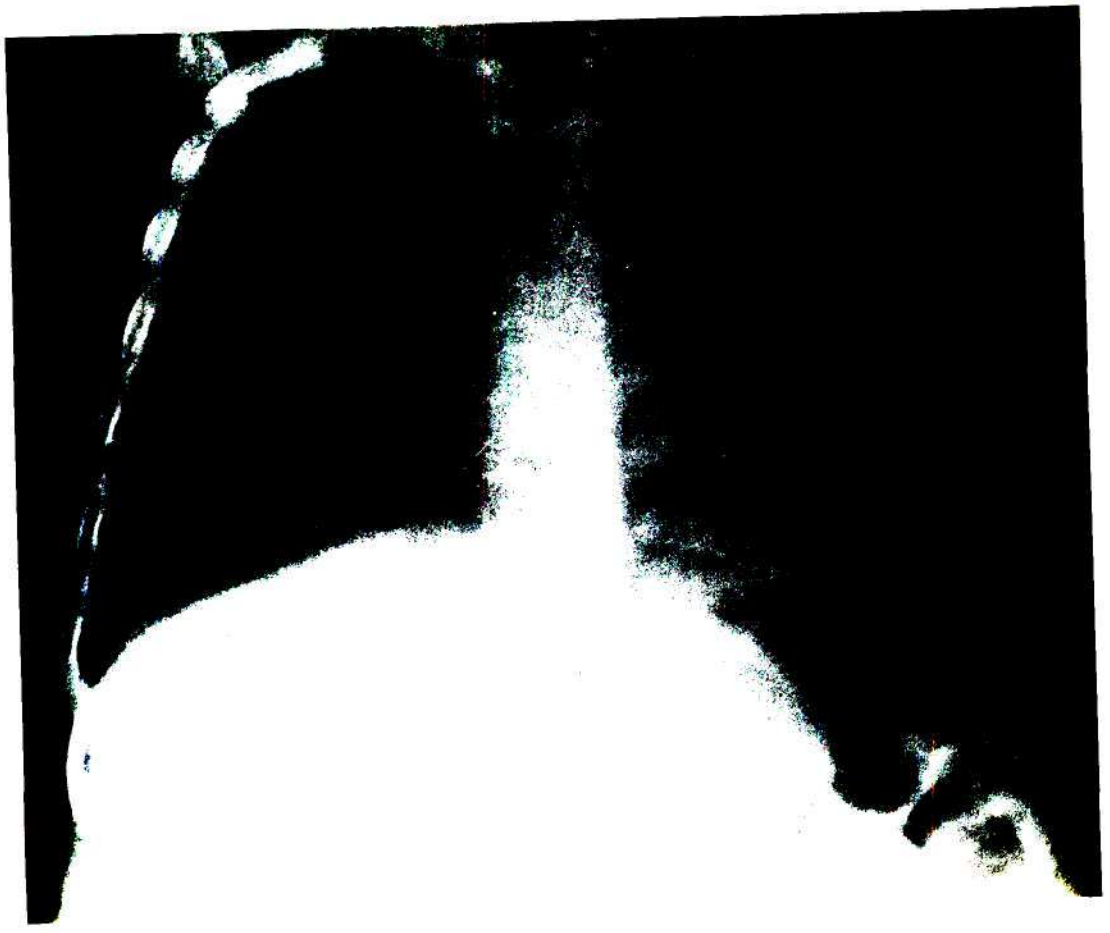
M75

6 months Pre Measles



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Day 3

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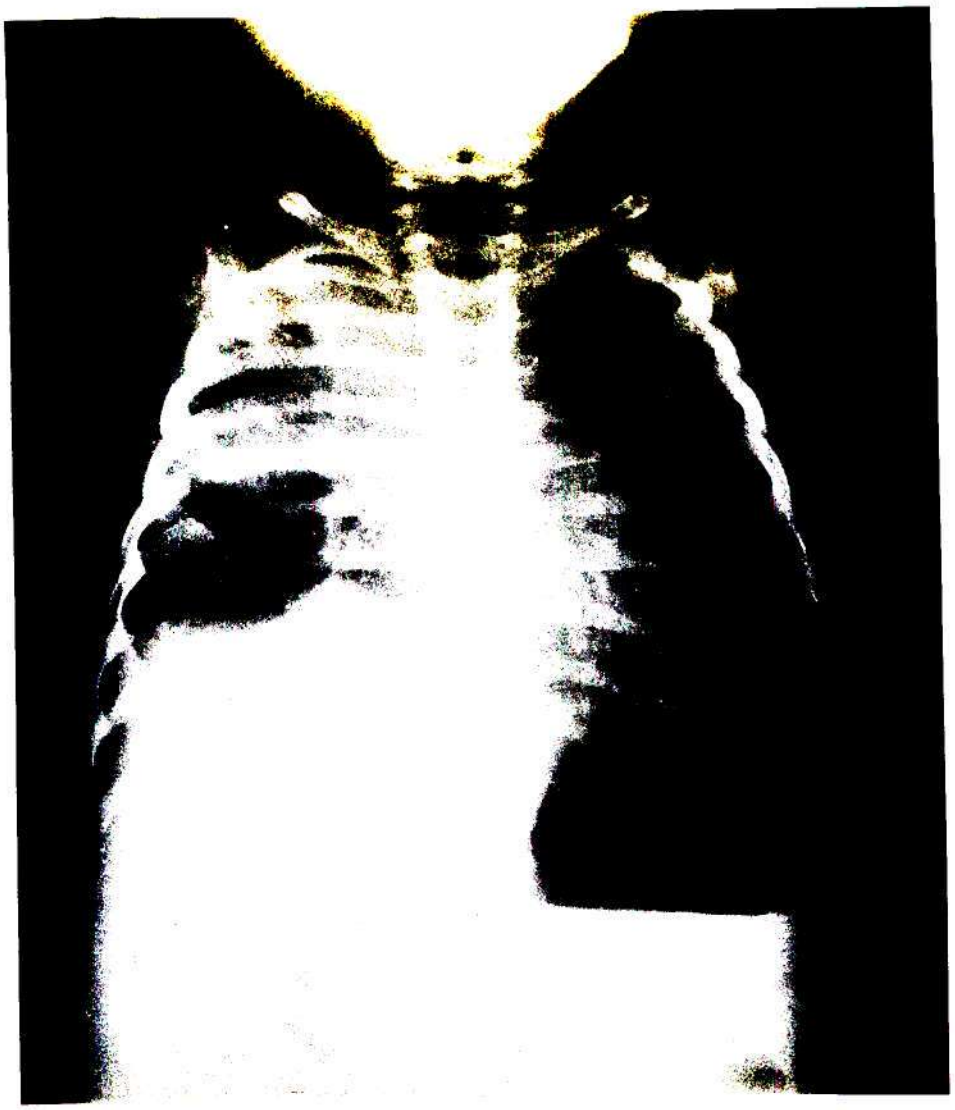

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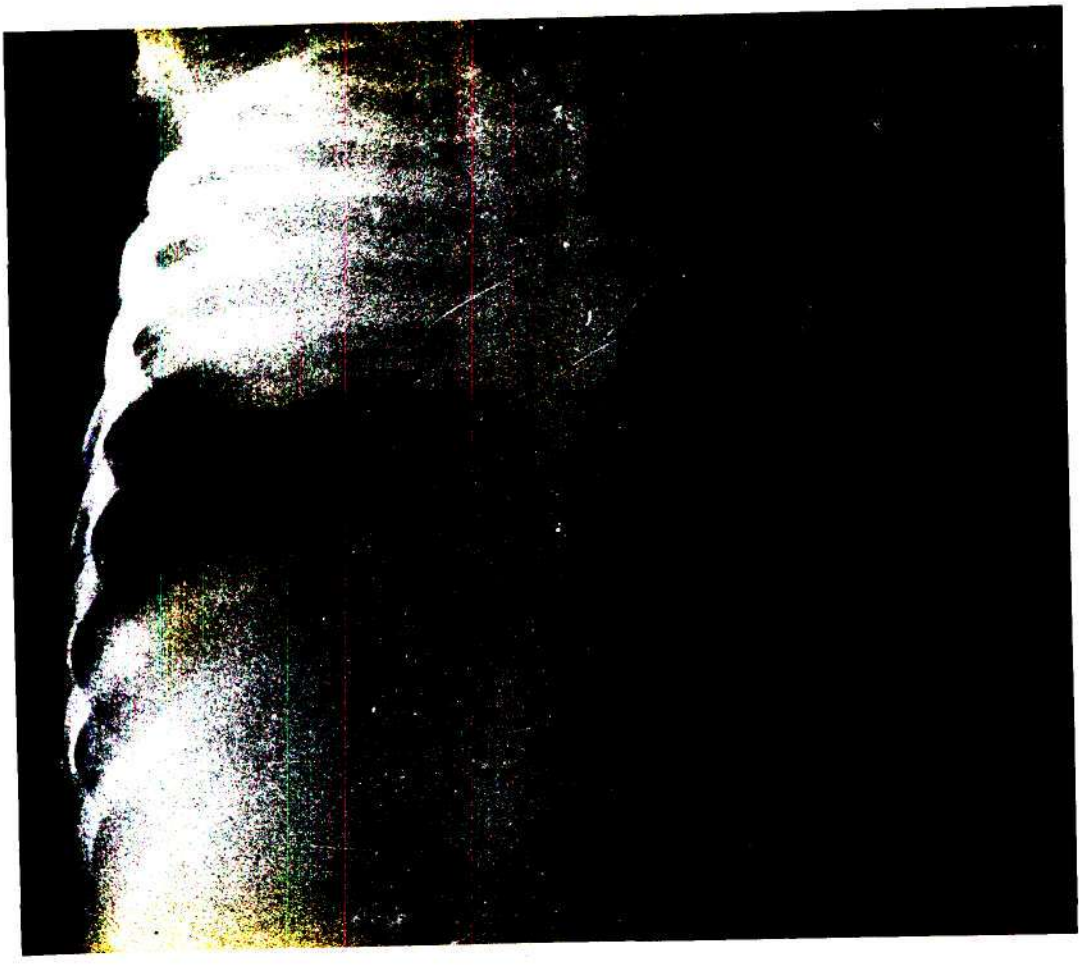

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DAY 50

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DISCUSSION

the clinical expression of disease is determined by both host and infecting organism. The immunopathological events which occur as a result of this interaction are not well documented.

Immunoparesis during acute disease and the persistence of immunological dysfunction appear to influence the development of chronicity in HB_sAg hepatitis.[216] There is some evidence to show that lymphocyte numbers are related to severity of disease in breast carcinoma[191] and in malnutrition.[111] At best this information is fragmentary and inconclusive.

The pattern of natural history of measles infection in Black children lends itself to an investigation of the problems discussed above. There is clinical variation in measles which can be objectively assessed. The majority of children recover and a minority do not. The latter either die or develop chronic pulmonary damage.

These studies which were carried out during the acute stage of measles infection have shown a critical breakdown of defence mechanisms which could be linked to severity of outcome. It has been demonstrated that profound immunosuppression in early measles which chiefly affected the T and B cell subpopulations and the specific antibody response to measles in most cases distinguished between children who subsequently died or developed chronic chest disease from those who recovered. Estimation of lymphocytes in the peripheral blood and complement fixing antibodies to measles were shown to be reliable indices of outcome. Seventy-seven per cent of children with a lymphopenia of less than 2000 cells/mm^3 during the early rash failed to recover; 30% died and 47% developed chronic chest disease. All the patients who died and many of those who progressed to chronicity failed to

A C K N O W L E D G E M E N T S

I wish to record my gratitude to Professor P.M. Smythe who has been a source of constant inspiration and guidance to me throughout this study.

I am also deeply grateful to Professor Peter Brain, Natal Institute of Immunology, whose wholehearted co-operation and helpful criticism at every stage, made this project possible. In particular he has placed at my disposal much of the laboratory facilities in his Institute.

My thanks are due to Dr. Anne Wesley who shared so much of the clinical burden and contributed meaningfully in interpretation of some of the results.

I would like to thank Dr. A.F. Hallett for estimations of immunoglobulins, complement and measles antibodies and Dr. G. Vos for lymphocyte transformation work and autoantibody measurements.

I am grateful to Mr. M.G. Hammond and Mr. D. Appadoo for HL A typing, Professor O.W. Prozesky for supplying measles virus, Professors P.S. Gardner and Pekka Halonen for their generous donation of measles antiserum and Dr. F. Rooknoodeen for assistance in grading of chest radiographs.

I acknowledge with gratitude the help of the Medical Research Council South Africa, whose grants supported in part these investigations.

I wish to thank the following for excellent technical assistance, R. Cooper, P. Kiepiela, J. Webster and R.E. Formo. My thanks are also due to Mrs. D. Bolt for keeping all my data in order and Mrs. J. Pretorius for typing many of the manuscripts.

My thanks are due to Mr. G.H. Haffajee, Medical School Library, for invaluable assistance with references used in the text.

I would like to express my appreciation to my wife who has borne a great deal of inconvenience because of the demands of these studies with equanimity.

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