SERO-EPIDEMIOLOGICAL SURVEY OF THE LEVEL AND SIGNIFICANCE OF MEASLES ANTIBODY TITER IN PRIMARY SCHOOL CHILDREN IN KADUNA STATE

BY

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THESIS APPROVAL

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D E D I C A T I O N

This study and the future it brings is whole heartedly

dedicated to my mother

JULIANA IBIDUN OBAFEMI

who

out of almost nothing had struggled

to ensure my basic education on which

I am building.

When Things Go Wrong, as they Sometimes will,
When the road you're trudging seems all up hill,
When the funds are low and the debts are high,
And you want to smile, but you have to sigh,
When care is pressing you down abit,
Rest, if you must - but don't you quit.

Success is failure turned inside out -
The silver tint of the clouds of doubt
And you never can tell how close you are,
It may be near when it seems afar,
So stick to the fight when you are hardest hit
It's when things seem worst that you must not quit.

Sir Speedy

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SUMMARY

It has been observed that any detectable level of measles hemagglutination inhibition antibody is indicative of immunity to measles infection (Krugman, 1977). However, there have been many reports on re-infection with measles virus in individuals previously vaccinated and or after primary infection (McCormick et. al. 1977; Shopp et. al. 1976 and Wyll and White 1971). Thus it became very important for us to take a critical look at what level of antibody could be considered too low for adequate protection against infection and or re-infection.

In an effort to make our contribution towards the solution of some of the above problems facing the scientists of this country which need to be tackled with more vigour; primary school children between the ages of 6 years and 15 years were studied for the presence and serum level of measles hemagglutination inhibiting antibodies in relation to the constant measles epidemics in Kaduna State and other parts of Nigeria, as well as the neutralization potential of such antibodies when exposed to Edmonston B strain live measles virus and cultured in vero cell line.

The question of vaccine efficacy has been partially tackled (Eghafona 1978) and the present studies tried to deal partly with the aspect of the level of measles hemagglutinatin inhibiting antibody in the school children who appear to be the most vulnerable group to measles infection, it also looked into the neutralizing effect of different levels of measles hemagglutination inhibition antibodies on measles virus in vitro. Present study compared the presence and level of measles hemagglutination inhibition antibodies in areas where vaccination campaigns have been carried out in recent years and in areas where no such campaign is known to have been carried out so as to measure the advantages if any, of vaccination campaigns against measles.

The results suggest that a greater percentage of pupils in the unvaccinated areas and in particular in age group 6 years have considerably low levels of circulating measles hemagglutination inhibition antibodies (Table III and IV).

It was observed that in the age group of 6 years Ikara, Saminaka and Jama'a Federation Local Government Areas recorded the highest percentage of pupils with low levels of circulating hemagglutination inhibition antibody (Table III and IV).

The lowest antibody levels in all age groups studied are recorded in Jama'a Federation, Kachia and Saminaka Local Government areas. These Local Government areas are amongst those areas that have no record of immunization campaign.

The significant difference between the vaccinated and unvaccinated areas outlined in the statistical analysis suggests the possibility of attaining high level of vaccine efficacy in our community if the vaccines are used when potent. The neutralization test shows that during measles infection, replication of the virus could be inhibited for 3 days by antibody level of 1:16, a period of time which may possibly surfice for individuals with such level of circulating antibodies to mount anamnestic immunological response in a situation where measles infection may occur, a state of immune response which could boost the antibody level leading to complete neutralization of the virus and therefore prevent the virus from establishing an infection.

I N T R O D U C T I O N

Although in the developed countries clinical measles infection has greatly reduced during the past decade due to successful vaccination research on measles virus has recently received increased attention. One of the horizon of interest is the relative ease with which the virus can establish persistent infection both in vitro and in vivo (Gould et al. 1975; Norrby, 1967 and Rustigian, 1966) and to the fact that measles infections often involve the central nervous system (Miller et al. 1956; Tyler, 1957 and Katz, 1972). Results on recent studies have also shed some light on the variety of clinical conditions that may be due to persistence or latent infections with measles virus and other paramyxoviruses (Finkel et al 1973; Sullivan et al. 1975 and Zweiman et al. 1971). The regular epidemics with its complications which measles infections present in all parts of Nigeria are in many circumstances worse than malaria (Morley, 1961; Morley et al. 1963, 1964).

Several investigators have studied the incidence and problems associated with measles infection in Nigerian children,

Gans (1961) reported that measles bronchopneumonia accounted for a mortality rate of 34.7% of 896 deaths out of 2,355 children ranging from the age of newborn infants to about 6 years who were admitted into a hospital ward of the General Hospital in Lagos. Morley, Woodland and Martin (1963) reported that in children under 5 years of age in Imesi near Ilesha, Nigeria, of the acute infective fever, measles, with its complications was outstanding as a cause of death.

Other reports on the situation of measles in Nigeria are available, each report pointing to the severity of the infection and its complications (Morley, 1962; Morley et al. 1961, 1963 and Katz et al. 1962). Some have dealt with children between the age of 6 months - 5 years and their predisposition to measles infection. It appears that inspite of increased public health measles vaccination campaign in all parts of Nigeria, measles epidemics still occur in many parts of Nigeria every

year and in many instances among primary school children.

The frequency of measles epidemics in our society makes one wonder whether or not the measles immunization campaigns being carried out almost yearly in several parts of the country really has any advantage.

Based on the above fact, several questions tend to agitate our inquiring minds, firstly, could we really prove that measles vaccines on which the Nigerian government spends large sums of naira importing every year are eventually used in a potent form? Secondly if they are used when they are still potent, then what could be the efficacy level of the vaccines as applied to Nigerian community? Thirdly if the vaccine efficacy is as high as that obtained in many parts of the world, then does it mean that only a few selected areas are covered by the campaigns?

And forthly, if that is the case what could we do to ensure that the campaigns in Nigeria cover wider areas of the country than is presently the case.

LITERATURE REVIEW

Measles virus is a member of the family paramyxoviridiae (Fenner 1975). Two other viruses, Canine distemper and rinderpest, share a close antigenic relationship with measles virus (Gillespie et al. 1960; Imagawa, 1968; Orvell et al. 1974; Waterson et al. 1963 and Yahanouchi et al. 1970). These three viruses form a distinct group, the genus Morbillivirus (Fenner, 1975), which differ from other paramyxoviruses in several respects. These differences arise from the fact that these viruses do not possess any virion-associated neuraminidase activity nor do they adsorb to neuramic acid-containing cellular receptors (Choppin et al. 1975; Howe et al. 1970; Norrby. 1962; Perie's et al. 1962 and Waterson. 1965). More significantly measles virus is the only member of the genus that exhibits hemagglutination (Choppin et al. 1975; Howe et al. 1970; Norrby. 1962; Perie's et al. 1962 and Waterson. 1965).

Structure of Measles Virions.

Physical Structure

The physical structure of measles virus has been extensively reviewed (Nakai et al. 1969; Waterson 1965; and Waterson et al. 1973; Norrby et al. 1964; and Numazakai et al. 1966) with regards to its similarity to those of other paramyxoviruses (Choppin et al. 1975; Compans et al. 1971; and Kingsbury, 1972), in terms of its nucleic acid content (Hall et al. 1973; Waters et al. 1972 and 1974) as well as the nature of the nucleocapsids (Norrby et al. 1965 and 1972; Waters et al. 1974; Waterson, 1965; Waterson et al. 1961; and Nakai et al. 1969).

Structural Components

The presence of RNA within measles virus particles was first reported in 1964 (Norrby et al. 1964; and St. Geme, 1964). The single stranded RNA was shown to have an approximate molecular weight of 6.2×10^6 and a sedimentation coefficient of 52S in sucrose density gradients (Hall et al. 1973; Hammarskjold et al. 1974; Nakai et al. 1969; and Schluederberg, 1974). Thus measles RNA appears to be a little larger than the 50S RNA isolated from other paramyxoviruses (Choppin et al. 1975; Kingsbury, 1973).

Although measles RNA has a sedimentation coefficient of 52S, it has been documented that it does migrate with other paramyxovirus RNA's in polyacrylamide gels (Hammarkjold et al. 1974) and it also co-sediment with New Castle Disease Virus (NDV) 50S RNA in sucrose density gradients (Winston et al. 1973).

Structural Protein

In polyacrylamide gel electrophoresis studies, it has been found that measles virions are composed of six polypeptides, whose approximate molecular weights range from 38,000 - 79,000 units. Generally in the gel patterns measles virions were found to closely resemble those of canine distemper and rinderpest and also exhibited certain similarities to the other paramyxoviruses (Bussell et al. 1974; Hall et al. 1973; Underwood et al. 1974; and Waters et al. 1973).

The smallest polypeptide molecular weight of approximately 38,000 may be analogous to the membrane (M) protein which is beneath the lipid bilayer of the virus envelope and probably function in maintaining the integrity of the virus particle (Choppin et al. 1974). The second largest polypeptide, molecular weight approximately 70,000 is associated with the nucleocapsid and it had been thought to be part of the transcriptase complex (Mountcastle et al. 1977).

The major structural protein, the nucleocapsid polypeptides can be cleaved by proteolytic enzyme into two small units of polypeptide with molecular weights of 24,000 and 38,000 respectively. This cleavage characteristic has been correlated with its changes in appearance as observed by electron microscopy (Waters et al. 1974).

The surface of the measles virus envelope is involved in the hemagglutinating (HA), Hemolytic (HL) and cell fusion properties of the virion (Waterson, 1965) whereas in other paramyxoviruses, these activities as well as neuraminidase activity are associated with two surface glycoproteins that form the spikes (Choppin et al. 1975). It has been possible to separate particles that contain primarily HA activity in Caesium Chloride from infectious virions that contain both hemagglutinating and hemolysing activities (HA and HL) (Norrby 1964; Norrby et al. 1964; 1972). The HL component was found to be more sensitive than HA to thermal inactivation, as well as to treatment with formalin - ether or Tween 80 - ether (Norrby et al. 1965, 1975), and both hemagglutinin and hemolysin (HA and HL) are known to be antigenically distinct (Ehrnst, 1975; Norrby et al. 1972,

1975a, 1975b,. However so far, out of several strains of measles virus, researchers have been able to identify only one glycoprotein that corresponds to the largest structural polypeptide with a molecular weight of 79,000 (Breschkin et al. 1977; Bussell et al. 1974; Hardwick et al. 1976; Mountcastle et al. 1977; Waters et al. 1973).

On the other hand Hall and Martin, (1973) identified in their measles preparations two glycopolypeptides, with molecular weights of 69,000 and 53,000 respectively, that form the spikes and can also be removed by proteolytic enzyme digestion. When these proteins were further purified and characterized HA activity was found to be associated with the larger glycoprotein whereas HI and cell fusion activities required both glycoproteins and membrane - lipids (Hall et al. 1974). However Mountcastle and Choppin (1977), working on the same strain of measles virus were unable to confirm the presence of glycoprotein with molecular weight of 53,000 as reported by earlier workers, the reasons for this discrepancies still remain unresolved.

VIRUS CELL INTERACTIONS
CPE (Cytopathic Effects).

The first in vitro isolation of measles virus was done in 1954 by Enders and Peebles by inoculating primary human and Simian Kidney cells with clinical specimens.

Shortly after, it was reported that the virus could also replicate in established cell lines and chick embryos (Fankel et al. 1958; and Milovanovic et al. 1957). Many investigators have subsequently shown that a variety of primary and continous cell line could support the growth of measles virus but that the best of the cell cultures are those of human and simian origin (Matumoto, 1966).

Enders and Peebles (1954) also described a very distinctive cytopathic effect produced by measles virus infections. These cytopathic effects are characterized by the formation of multinucleated giant cells (Syncytia). The chromatin assumes a marginal position and is replaced by intranuclear inclusions; eosinophilic inclusions develop in the cytoplasm. In addition, chromosomal aberrations and breakage have been observed in measles virus - infected cells (Csonk et al. 1975; Fjelde et al. 1962; Levenshtam et al. 1969; and Nichols et al. 1965).

Measles virus has been found to produce two types of syncytia formation, such as fusion from within and fusion from without types of syncytia (Cascardo et al. 1965).

The formation of syncytia (fusion from within) is virus-specific protein dependent. The protein "fusion factor" is present on the surface of an infected cell (Cascardo et al. 1965; and Choppin et al. 1975). Some data also support the fact that this "fusion factor" may be identical to the Hemolysin (HL) obtained from temperature-sensitive mutants of measles virus.

Also syncytia (fusion from without) can occur within 5 hrs. when cells are infected at high multiplicities (Cascardo et al. 1965). Fusion from without can occur even in the absence of virus replication since it had been found that noninfectious measles virus particles, such as ultraviolet-irradiated virions and even the small Hemagglutinating (HA) particles did produce syncytia (Norrby 1964; Schluederberg, 1962).

'Strand forming' is another type of CPE produced by measles virus infection. This is characterized by the appearance of spindle shaped or stellate cells that have long and irregular cytoplasm processes (Oddo et al. 1961). Strand forming cytopathic effect (CPE) always occur when cells are infected with undiluted passaged inoculum and infectious virus yields are often greatly reduced (Oddo et al. 1961; Parfanovich et al. 1971).

Interactions with Erythrocytes

Measles virions are capable of several interactions with erythrocytes thus the development of HA (Norrby, 1962; Perie's et al. 1960; Rosen, 1961) HA-inhibition (Norrby, 1962; and Rosen, 1961) and HL (Norrby et al. 1964; and Perie's et al. 1960) assays have greatly enhanced the ease with which virus replication could be monitored and quantified. Perie's and Chany (1960) first reported that measles virus would agglutinate only monkey or baboon erythrocytes. The most important characteristics which make measles virus unique amongst the paramyxoviruses are that measles virus HA assay is maximal at 37°C and that because of the lack of neuraminidase, there is no spontaneous elution of the virions. In addition, HA is not influenced by P^H changes between 4.8 and 10.1, and there is a minimal requirement for the presence of mono - or divalent ions (Choppin et al. 1975; Norrby, 1962; and Waterson 1965).

HA titers can however be enhanced by carrying the assay out in buffers containing 0.8M ammonium sulfate especially for the salt dependent HA (Gould et al. 1976; Schluederberg et al. 1971; and Shirodaria et al. 1976). Some strains of measles virus will agglutinate only in the presence of high salt concentration (Breschkin et al. 1977; Gould et al. 1976). Infected cells can hemadsorb erythrocytes, especially during late infection after the HA protein has been inserted into cellular membrane (Baker et al. 1965; Bancio, 1962; and Kohn, 1962). Although HA and HL activities can be differentiated,

the presence of HL appears to be a necessary pre-requisite for HL activity (Breschkin et al. 1977; and Norrby et al. 1964). HL activity is optimal at P^H 8.0 and it is greatly inhibited by Ca^{++} ions (Norrby et al. 1964).

REPLICATION

The seve ity of measles virus infection, the length of time required for the appearance of progeny virions, and the amount of virus ultimately produced are significantly influenced by such variables as the virus strain, the host cell and the multiplicity of infection (Dejong et al. 1970; Nakai et al. 1969; Portner et al. 1973; and Rapp, 1964). The replication cycle can be divided into three major events.

Adsorption, Penetration and Uncoating

Measles virions attach to cellular receptors by means of the spikes on the virus envelope. One such receptor for measles virus according to Duffy and Howe, (1976), is a glycoprotein that contains only low levels of sialic acid. Studies with other paramyxoviruses had suggested that the virions enter the cell either by 'viropexis' - (phagocytosis) (Compans et al. 1966) or by fusion of the viral membrane with the cellular membrane (Morgan et al. 1968). Either process is ultimately followed by the release of nucleocapsids into the cyloplasm (uncoating) and it is likely that both occur during infection (Choppin et al. 1975).

Synthesis of Virus-Specific Macromolecules

Data by Choppin et al. (1975); and Kingsbury, (1972, 1974) suggested that replication of measles RNA is similar, if not identical, to that of other paramyxoviruses.

The synthesis of virus RNA is initiated shortly after infection (Portner et al. 1973) and this is then followed by the synthesis of virus proteins and genomic RNA which is encapsidated into progeny nucleocapsids. These events appear to take place in the cytoplasm of infected cells.

It has also been reported that measles RNA can be isolated from the nuclear fraction of cell extracts (Carter et al. 1973; and Schluederberg et al. 1974) and that treatment with actinomycin D could lead to a reduction in genomic RNA and protein synthesis (Hall et al. 1977; and Schluederberg et al. 1972) and also in virus yield (Schluederberg et al. 1972).

According to Follett et al. (1976) unnucleated cells could support the growth of measles virus though the yield of infectious virus was greatly reduced compared with the results obtained in nucleated cells. However, virus-specific polypeptides were synthesized in the unnucleated cells.

The measles virus genome needs the synthesis of smaller complementary RNAs (plus strands) to function as a message. Although enzymatic activities which is associated with nucleocapsid has not been reported in measles virus (Huang et al. 1971; Robinson, 1971;

and Stone et al. 1971), but virus-specific RNA transcripts which have approximately sedimentation values of 18S, 22S, and 35S have been detected in measles virus-infected cells (Carter et al. 1973; Kiley et al. 1974; Winston et al. 1973; and Yeh, 1973). These RNAS are complementary to genomic RNA (Hall et al. 1976; and Yeh, 1973) and they have been isolated in association with five different size classes of polyribosomes (Kiley et al. 1974). At a later stage in the replication cycle, the virus must transcribe complete 52S positive - (+) strands to serve as templates for the formation of 52S negative - (-) strand genomes (Kingsbury. 1974).

Unlike the Sendai virus (Portner et al. 1972; and Robinson, 1971) and mumps virus (East et al. 1971) 50S RNA whose synthesis has been found to be inhibited by cycloheximide (suggesting that genomic RNA replication requires continued protein synthesis), the synthesis of measles 52S RNA has not been proved to be inhibited by cycloheximide (Hall et al. 1977; and Kiley et al. 1974). Kingsbury's data (1974), showed that the progeny molecules were rapidly encapsidated since free paramyxovirus RNA has not been seen in infected cells.

Virus Antigens and Structural Proteins

Studies using metabolic inhibitors technique showed that the protein synthesis in measles virus can be detected within 3 hours after infection and reaches a peak long before the appearance of infectious virions (Portner et al. 1973). Immunofluorescent technique has shown that virus-specific antigen (nucleocapsid) could

be detected in the cytoplasm within few hours. The fluorescence first appears as fine dots or granules in the perinuclear region, and as the infection proceeds and the intensity of the staining increases, this fluorescence often completely encircles the nucleus (Llanes-Rodas et al. 1965; Norrby, 1972; and Rapp et al. 1960). Finally the nucleocapsid antigens become dispersed throughout the cytoplasm (Llanes-Rodas et al. 1965; Norrby, 1972; and Rapp et al. 1960); even the antigens are sometimes detected in the nuclei of some cells. Nuclear staining is most prevalent late in infection, and the antigens are associated with nuclear inclusion bodies (Enders et al. 1954; Llanes-Rodas et al. 1965; Norrby, 1972; Oyanagi et al. 1970; and Rapp et al. 1960).

The envelope antigens (HA and HL) first appear in the cytoplasm at one pole of the nucleus, and they produce a mesh work of fine threads of fluorescence (Norrby, 1972).

Later in the infection, the antigens become dispersed through out the cytoplasm and preferentially accumulate in the cytoplasmic membrane (Ehrnst et al. 1974; Norrby, 1972; and Rapp et al. 1960). Trypsin treated infected cells showed that HA first appears at the poles of cells and then spreads over the surface of the cellular membrane (Ehrnst et al. 1975). Dubors-Dalcq and Barbosa (1973) have shown that the budding of nucleocapsids to form infectious virions occurs at those sites on the membrane where virus-specific antigens are present.

Assembly and Release of Progeny Virions

Electron microscopy has helped a lot to give some information on measles virus maturation and assembly. Dubors-Dalcq et al. (1973); Nakai et al. (1969); and Oyanagi et al. (1971) in their studies, found that early in infection, intracytoplasmic inclusion bodies composed of nucleocapsid filaments are formed.

The inclusion bodies tend to accumulate in the perinuclear region of the cell and are most frequently observed in multinucleated giant cells. The cell membrane becomes altered in appearance to more closely resemble the virus envelope, the membrane becomes thicker and more electron dense, and spikes appear. The nucleocapsids assemble beneath these areas of the cellular membrane and align in parallel arrays. The membrane then buds to form progeny virions (Dubors-Dalcq et al. 1973; Naka et al. 1969; and Oyanagi et al. 1971). Late in infection, crystal-like nucleocapsids appear in the intranuclear inclusion bodies (Ba ker et al. 1960; and Nakai et al. 1971). But it has been observed that nucleocapsids are rarely seen in both nucleus and cytoplasm of the same cell, and there is no evidence that they migrate from one site to another. However, Nakai et al. (1969) found that when the nuclear nucleocapsids are predominant in an infected culture, little virus budding from the cells surface is observed and low yields of infectious virus are obtained.

Temperature-Sensitive Mutant.

Stability

Measles virus is a relatively thermolabile virus. Black (1959) noticed that the half-life of the infectivity at 37°C is about 2 hours at P^H 7.5. At 56°C after 30 minutes measles virus loses its infectivity. The virus is stable between P^H 5.0 and P^H 10.5 (Musser and Underwood, 1960). The storage of the virus in the cold is greatly enhanced by the presence of serum or other protein material. In the absence of protein the infectivity falls even at - 70°C. When lyophylized, there is a small initial drop in titer after which the infectivity remains relatively constant (Waterson, 1965).

Various research works and data have revealed that beta and gamma radiation as well as alpha particles and deuterons easily inactivate the virus (Girardi et al. 1958; Musser and Underwood 1960; and Benyesh et al. 1958). The virus can also be inactivated readily by treatment with formalin (Norrby et al. 1975). Several groups of investigators have isolated temperature-sensitive mutants of measles virus (Brergholz et al. 1975; Haspel et al. 1975; and Yamazi et al. 1972). Three complimentation groups of temperature-sensitive mutants have been isolated (Haspel et al. 1975; and Bergholz et al. 1975). Most of the mutants isolated by Haspel et al. (1975) are classified as HMK - negative because they do no synthesize

virion RNA or produce detectable virus antigens at the nonpermissive temperature (39°C). More than half of the measles virus strains isolated by Bergholz et al. (1975) were RNA negative.

The RNA positive mutants were defective in functions associated with the mutants isolated by Bergholz et al. (1975) they did not hemeadsorb or produce syncytia, but virus-specific antigens were detected in the cytoplasm and nucleus. The other group of RNA positive mutants synthesized virion RNA, nucleocapsids and HA, but did not form syncytia in infected cells; and no HL antigen could be detected by immunofluorescence tests (Haspel et al. 1975).

Interferon

Measles virus had been found to induce the synthesis of interferon during *in vitro* and *in vivo* replication (DeMaeyer et al. 1961, 1965; Ho et al. 1969; Mirchamsy et al. 1969; Pertralli et al. 1965).

The inhibition of the virus by interferon was also demonstrated in the works of Anderson et al. (1964); Mirchamsy et al. (1969) and Rapp, (1964), when they found that addition of low concentration of actinomycin D to infected cells inhibited interferon synthesis and enhanced both virus yields and plaque size.

The temperature-sensitive mutants of measles virus, grown at either permissive or nonpermissive temperature, had unlike reovirus (Lai et al. 1973) Sindbis virus (Atkins et al. 1976) and Semliki Forest virus (Lonniczi et al. 1970), been found not to induce interferon.

Further studies had suggested that the inability of those temperature sensitive mutants to induce interferon might be as a result of the selection of virus particles that were unable to induce interferon before mutagenesis. For example a wild type revertant of measles virus was also unable to induce interferon and stocks of wild type virus appeared to consist of a mixture of virus particles, some of which induced interferon and some of which did not (Breschin et al. 1976). Actually, the strains of measles virus from which most of the termperature sensitive mutants were derived (Haspel et al. 1975) consisted primarily of non-interferon-inducing particles (Mokimm et al. 1977). In fact, the ability or inability of measles virus to induce interferon may be partially under the genetic control of the virion, since plaque-purified clones of measles virus that induce interferon generally retain this characteristic upon subsequent passage. Nevertheless some non-inducing clones regain the ability to induce interferon, and others produce greater amounts of interferon after serial passage in Vero cells (Mokimm et al. 1977).

PATHOGENESIS

Spread of Measles Virus.

As far as it is known, there is no animal reservoir for measles virus. Transfer occurs from persons with the disease and carrier state is yet to be documented.

Parum (1846) suggested a respiratory mode of transmission. Entry into a new host probably occurs as a result of deposition of virus in the upper respiratory epithelium including perhaps the conjuctival sac.

Incubation.

When Ender and Co-workers (1959) introduced measles virus into monkey, they found that viremia occurred on the 5th to 7th day. During this 5 - 7 day interval, it was presumed that intense viral multiplication occurred in reticuloendothelial tissue possibly following an early viremia (1st - 2nd day) which transfer virus from regional respiratory lymph nodes to t e entire reticuloendothelia system and viscera.

Rapp (1937) and Katz et al. (1962) found that, if measles virus is administered to human by subcutaneous or intraveneous route, the incubation period is shortened by 3 to 4 days. This gives the indication that early multiplication in the respiratory epithelium and regional lymph nodes might have been by-passed and the antigen was more rapidly introduced to the reticuloendothelial system and viscera.

If measles neutralizing antibody is administered early in the incubation period subsequent disease can be prevented in the exposed susceptible subject (Nickhann, 1937). If less gamma globulin is used, modification of the illness results and in some cases the incubation period is prolonged.

In the Simian disease, consistent and marked leucopenia was observed during the secondary viremia (Sergiev et al. 1960). It has been shown that the suspension of white blood cell can support measles virus growth in vitro (Berge et al. 1961). Measles can also be transmitted to susceptible volunteers by transfer of washed leucocytes from the persons with the disease (Rapp, 1937), and measles virus can be recovered in tissue culture from washed leucocyte suspension (Cresser et al. 1963).

Very little is known concerning the morphologic alterations associated with measles virus development in the first few days following contact with the virus. Within 3 days following incubation in monkeys, however, some degree of generalized lymphoid hyperplasia and giant-cell formation has been noted (Sergiev et al. 1960). These changes appear to be continously progressive until the onset of prodromal symptoms. Symptoms are lacking during the early phase of the incubation period. Goodal (1925) and Partington et al. (1959) revealed their observation of typical corneal lesions

in one patient and transient respiratory symptoms in others shortly after exposure to measles infection, suggesting that closer observation for minor manifestations may reveal clinical effects of viral multiplication.

The clinical phase of recognizable measles infection is heralded by a 2-4 days period of fever and increasing respiratory symptoms. These symptoms are followed by the appearance of small irregular spots of a bright red color. In the centre of each spot as described by Koplik in 1896 is a minute bluish white speck. This sign in most instances precedes the characteristic rash.

Viremia does occur during the prodrome and the respiratory secretions yield large amounts of virus. The proliferation of measles virus appears to be wide spread. This is supported by the isolation of the virus from lungs, spleen, and cervical lymph nodes of a child dying during the prodromal period (Sherman et al. 1958). Warthin, (1931) and Finkeldeg et al. (1931) observed epithelial and lymphoid giant cells during the prodrome.

There is also conjuctival inflammation in both man and monkeys at this stage which also continues even after the appearance of the rash. Actually the prodrome corresponds to a period of intense viral spread and multiplication during which considerable cellular damage occurs which results in the symptomatology of the remaining period of the disease.

Although the disease is traditionally said to be apparent at onset of the rash, it is evident that intense viral activity has preceeded this visible evidence of measles virus infection. The rash progresses for about 3 days from head to foot though it is first detected about the hairline and face on the 14th day of post-infection. During the progression of the rash the systemic and respiratory symptoms reach a climax and recede rapidly after. Usually recovery is complete within 4-5 days. After the viral multiplication might have ceased the blood and respiratory secretions continue to yield virus for as long as 42 hours following the onset of the rash (Ruckle et al. 1959). At about the 1st - 4th day of rash, antibody appears, and the level increases and the virus becomes undetectable.

The measles rash are usuelly benign but seldomly it can be hemorrhagic and bleeding on the skin may be accompanied by visceral haemorrhage in severe, often fatal 'black' measles, although this type is hardly seen today (Krugman et al. 1960).

EPIDEMIOLOGY

The changing incidence of measles in a community is governed by the immune status of the population. There is no natural reservoir of infection but man. Attempts at transmission to lower animal other than monkeys were unsuccessful (Rake and Shaffer 1932; 1941). Except for a few isolated population, and inspite of vaccination against the disease, measles is endemic in man throughout the world. It's incidence rises to epidemic levels at regular intervals in a pattern that depends upon climate, season, size and density of population and especially the emergence of new generation of susceptible hosts (Bech, 1965).

Measles occur more constantly in large populations. It may disappear from isolated communities for long intervals but when it is re-introduced into the communities, epidemics of great intensity and abnormal severity are likely to occur (Bech, 1965). It is also important to note that when measles is introduced into a fully susceptible population, it spreads rapidly whatever the season, and the attack rates are almost 100% (Bech, 1965).

It was observed in Greenland in 1951 to 1954 during an epidemic that only 4 out of 4,262, less than 0.1% susceptible individuals

escaped infection and that about 50% developed complication and mortality rate was about eighteen times higher than that in a subsequent epidemic (Bech, 1965).

High correlation between the measles incidence and climate conditions had been observed and it was found that measles morbidity and the physical resistance of the virus are relatively correlated to a low relative humidity (de Jung and Winkler, 1964; de Jung, 1965).

In Imesi, Nigeria, it was observed that measles epidemics began in the middle of the dry season and declined with the onset of the wet season (Morley, 1962). Gans et al. (1962) also found that measles occurred in annual outbreak with greatest intensity in the months before the rains and that, pressure is highest in the large towns in the southwest of Nigeria and less in rural areas in the savannah.

Social habits of communities can also determine the extent of epidemics. Morley et al. (1963) observed that measles epidemics was usually at its peak during the dry season when the people of Imesi community celebrate various festivals. During these festivals which are usually spent in dancing and feasting children

are usually backed and this provides excellent opportunities for the spread of droplet infections (Morley et al, 1963).

About 20% of susceptible individuals in a population could maintain the virus at endemic incidence while 25% is sufficient to initiate a seasonal epidemic (Barbbot and Gordon, 1954). The peak age incidence depends upon local conditions, population density and the chance of exposure (Barbbot and Gordon, 1954).

MORTALITY

The manifestations of measles in Nigerian children have been described (Morley 1962; Morley Woodland and Martin 1963) and it was shown that measles in Nigerian children was outstanding among the acute infectious diseases causing death.

High mortality is often associated with lack of food, especially under protein starvation. In this circumstance, the disease is as severe as it was 150 years ago in Western European countries (Morley et al. 1963). The mortality rate as observed by Morley (1961) was about 25% among infants and young children. Generally the records showed a very high mortality rate in Africa as a whole compared to other parts of the world. McCregor (1964) reported a mortality rate of 14-15 percent and 22 per cent in Gambia. Savage (1967) in Zambia found that the mortality rate at that time was as high as 30% and that the peak age was between 6-18 months. Morley et al. (1967) reported a mortality rate of 5.7% for East Africa. Although the mortality percentage as a result of measles is on the decline, there are still some areas in Nigeria or Africa as a whole where children especially infants die from the disease due to social taboos carelessness and ignorance.

PERSISTENT INFECTION

Measles virus, like other paramyxoviruses, had been found in vitro to produce persistent infection (Choppin et al. 1975; Rapp et al. 1974). Measles infection usually results in widespread cellular destruction, but when surviving cells are selected and subcultured, they are often found to be persistently infected.

It is characteristic of most persistent infections that the cultures are usually refractory to superinfection with measles virus, but are readily infected with other unrelated viruses (Menna et al. 1975; Norrby 1972; and Rustigian 1966).

However, there are various types of persistently infected cultures. There is the "carrier culture" persistent infection produced by measles virus. In this case, low levels of infectious virus are continually shed into the medium (Barry et al. 1976; Gibson et al. 1972; Gould et al. 1975; Haspel et al. 1973; MacIntyre et al. 1976; Norrby et al. 1977; Rustigian, 1962; 1966). Rustigian (1966) had confirmed that such a culture could be maintained for a long period.

Persistent measles infections can be established by growing infected cells in the presence of specific antiserum (Gould et al. 1975; Norrby 1967; Rustigian, 1966). It has also been found that interferon induction does not appear to be responsible for the low virus yields (Gibson et al. 1972; Norrby 1967; and Rapp et al. 1974).

It was reported by Maulen et al (1976) that cells persistently infected with canine distemper virus release into the medium a factor that selectively inhibits the replication of canine distemper and measles virus.

The second type of persistent infection reported is the "non-yielder". These non-yielder cultures were found to fail to produce infectious virus and the cultures also contain virus-specific intra-cellular antigens (Knight et al. 1972; Lenna et al. 1975; and Rustigian, 1966) and all size classes of virus-specific RNA (Winston et al. 1973).

These two types of persistent infection are some how indistinctable since Rustigian, (1966) found in his works that, by subculturing carrier cultures of Hela cells in the presence of antiserum, some clones that failed to release infectious virus were isolated. Conversely persistently infected hamster embryo fibroblasts that did not produce infectious virus (Knight et al. 1972) began to spontaneously release low levels of infectious virus when the cells were repeatedly passaged (Haspel et al. 1973).

Infectious virus has been recovered from cells persistently infected with measles virus by co-cultivating the cells with a different susceptible cell line (Knight et al. 1972), and increased virus yields have been obtained by lowering the incubation temperature

(Haspel et al. 1973; Norrby, 1967).

Treatment with such drugs like (iododeoxy-uridine, bromodeoxyuridine or mitomycin C) (Rapp et al. 1974) did not prevent virus yields. In another studies Flanagan et al (1976) and Henna et al. (1975) found that treatment with inhibitors produced an increase in number of cells that would haemadsorb, but infectious virus was not detected. It has been suggested that virus may be blocked at a latter stage in the replication cycle, since studies demonstrated that infectious virus could be recovered within 6 hours after co-cultivation, even in the presence of RNA and protein inhibitors (Haspel et al. 1973).

Role of Defective Particles in Persistent Infections.

Defective interfering particles often arise during serial, undiluted passage of virus stocks, and they are particles that contain only part of the virus genome. Huang et al. (1970) suggested that defective interfering particles may be associated with persistent infections in vitro and in vivo. Although defective particles require the presence of non-defective homologous virus for their replication, they concomitantly interfere with the replication of standard virus (Huang, 1973). Although it is still a speculation that defective interfering particles can arise in measles virus infections nevertheless such has been described for

several paramyxovirus systems (Kingsbury et al. 1970; Kolakofsky, 1976; and Roman et al. 1976).

However, Hall et al (1974) recently reported undiluted passaged measles virus stocks, which demonstrated many of the characteristics of defective interfering particles. The virus stocks consisting these particles were able to establish persistent infections in Vero cells.

Role of Temperature-sensitive Mutants in Persistent Infections.

It has been suggested that temperature-sensitive mutants may participate in the establishment, and or maintenance of persistent infections and that they may be selected for, during the course of a persistent infection (Preble et al. 1975).

There have been reports on the isolation of temperature-sensitive mutant from cells infected by some other viruses such as Senda virus (Nagata et al. 1972) and Newcastle Disease Virus (Preble et al. 1972 and 1973; Stanwick et al. 1976; Youngner et al. 1975). Even isolation of temperature-sensitive mutants of measles virus has also been reported (Haspel et al. 1973).

Knight et al (1972) established a "non-yielder" culture of hamster embryo cells which spontaneously began to produce small amounts of virus after 18 passages at 37°C. The same culture also was made to increase its virus yield when the incubation temperature was lowered to 33.5°C and the virus that was released was temperature-

sensitive (Haspel et al. 1973). Gould and Linton (1975) also isolated a temperature-sensitive mutant from Hep-2 cells persistently infected with measles. But when cultured at 39°C the culture appeared "cured" due to the loss of detectable intracellular antigens. However, the antigens reappeared when the cells were shifted back to 37°C and subcultured. Furthermore, it has been found that the temperature-sensitive mutant of measles virus was lost from persistently infected hamster cells during further subculture and was replaced with a virus that replicated like the wild-type virus (Haspel et al. 1973; and Rapp et al. 1974).

COMPLICATIONS ASSOCIATED WITH MEASLES INFECTIONS

Measles virus is ubiquitous and "classic" measles infection is a common disease of childhood. The virus is highly infectious and spreads by droplet infection. The measles virus initiate infection in the upper respiratory tract of susceptible individuals.

Clinically it has been noticed that symptoms that indicate neurological involvement develop in many patients during measles virus infections. However, whether the virus invades the central nervous system during the secondary viremia is not yet known. Furthermore, during the peak of clinical illness, at least 50% of measles patients have transient electroencephalograhpic changes (Gibbs et al. 1959; Katz, 1972). Pampiglione et al (1971) has also documented aberrant electroencephalographic patterns after vaccination. About 10% of measles patients were found to demonstrate CSF pleocytosis, (that is, increase in number of cerebrospinal fluid lympocytes) (Katz, 1972; Ojala 1947). In actual fact, apart from neurological involvement, many workers have documented a wide variety of complications that can arise during measles infections.

Central Nervous System Disorders

Encephalomyelitis, the most common central nervous system complication may occur after the acute phase of measles infection has begun to subside (Miller et al. 1956). A number of neurological

disorders such as toxic encephalopathy, myelitis, thrombophlebitis of cerebral veins, hemiplegia, optic neuritis, polyradiculoneuritis (Miller et al. 1956; Tyler, 1957) and Bell's Palsy (Pollock et al. 1975) have also been reported.

The onset of encephalomyelitis in measles patients, usually, is marked by seizures, altered states of consciousness, and coma, and the appearance of CSF pleocytosis, an increase in CSF protein levels and electroencephalographic abnormalities (Ehrengut, 1965; Katz, 1972; and LaBoccetta et al. 1964). Between 10% and 30% of all cases are fatal and 20% to 50% of the survivors do develop significant motor, intellectual, sensory or emotional sequelae (Adams et al. 1966; Aicardi et al. 1977; Katz, 1972; and LaBoccetta et al. 1964).

Giant Cell Pneumonia

Giant cell pneumonia (Hech's giant cell pneumonia) is a rare complication of measles infection. It is a fatal interstitial pneumonitis that is characterized by the presence of multinucleated giant cells in the respiratory tract (Enders et al. 1959; and Katz, 1972).

Although most cases occur in children with malignancies or other severe disorders, cases of giant cell pneumonia has also been found in patients who had no symptoms of measles (Enders et al. 1959;

and Mitus et al. 1965). However in others, it developed after the subsidence of a normal measles virus infection (Breitfeld et al. 1973; Mitus et al. 1959). Measles virus has been found to persist for a long period of time in the few survivors, and their antibody response was impaired; no antibody formation was detected in fatal cases (Mitus et al. 1959; 1965).

Measles virus was also isolated from lungs autopsy from patients with leukemia, measles and giant cell pneumonia (Mitus et al. 1959). Before measles was identified as the agent of giant-cell pneumonia, it had been suggested that the disease was caused by canine distemper virus on the bases of the serologic resemblance (Adams et al. 1956; Pinkerton et al. 1945). Canine distemper and measles virus are antigenically related.

Disruption of the Immune System.

Both humoral and cellular responses could be affected in a variety of ways due to measles virus infection. In general, measles virus infections and vaccination with life virus result in antibody formation and life long immunity (Katz, 1972; ter Meulen et al. 1972). Cell - mediated immunity is important, because children with agamma-globulinemia respond normally to measles infections and are immune to reinfection in the absence of detectable antibody formation (Burnet, 1968; Good et al. 1956).

In several fatal cases of measles, immunological complication such as aggregations of thymocytes, formation of large syncytia in the thymus and destruction of the thymic cortex were reported (White et al. 1973).

Cell-mediated immunity may be temporarily impaired by measles virus infections. Von Pirquet (1908) noticed the loss of tuberculin response in children with measles. Cases of tuberculosis were found by Rech (1965) to be reactivated or exacerbated during measles infections. Brody et al (1964); Fireman et al. (1969); Hellman et al (1963); Zweiman et al (1971) have documented a transient loss of delayed cutaneous hypersensitivity to tuberculin and other antigens in measles patients and individuals vaccinated with live measles virus.

The unreactivity of lymphocytes infected with measles virus, or obtained from measles patients, as determined by their inability to mount a proliferative response to stimulating antigens, such as phyto-hemagglutinin (PHA) may be one of the attributed factors to the temporary immunological impairement (Finkel et al. 1973; Sullivan et al. 1975; and Zweiman et al. 1971). Also Overland (1971) found that function helper T-cell from measles virus-infected mice appeared to be suppressed.

Investigators, have found that lymphocytes respond normally to phytohemagglutinins (Graziano et al. 1975; Osunkoya et al 1974; Ruckdeschel et al. 1975), but the response depend on type of antigen used (Fireman et al. 1969) or on the individual lymphocytes donor (Dunmire et al. 1975).

Even though measles virus can infect lymphocytes, both lytic and persistent infections would be obtained as long as the temperature is favourable (Barry et al. 1976) Joseph et al. 1975; Minagawa et al. 1976; and Sullivan et al 1975). Measles virus infected cells can be lysed in the presence of specific antibody and complement (Hicks et al. 1976; Joseph et al. 1975; and Minagawa et al. 1971) but under conditions that strip virus antigens from cells surface (antigen modulation), infected cells become resistant to immune cytolysis (Joseph et al. 1974; 1975).

Hela cells persistently infected with measles virus has been found by radio-immunoassays to bind two to three times less antibody than do acutely infected cells, and these cells were also found to be more resistant to antibody-mediated lysis. The result of Osunkoya et al. (1974) was supported by the work of Joseph et al (1976), who observed that when lymphocytes from measles patients were stimulated with phytohemagglutinin (PHA), measles antigens could be detected by immunofluorescence.

However, the survival of infected lymphocytes and other cells in the presence of specific antibody could have implications for certain chronic disorders such as subacute sclerosing panencephalitis that has been attributed to measles.

Neurological Diseases Linked To Measles Infection

Subacute Sclerosing Panencephalitis:

Subacute Sclerosing panencephalitis - (SSPE) is a rare, inflammatory, slowly degenerative disease of central nervous system which occurs in children and young adults (Johannes et al. 1975; Payne et al. 1971; and terMeulen et al. 1972). The disease begins with a decrease in intellectual skills and terminates in an almost complete loss of cortical function and death (Jabbour et al. 1969; Johannes et al. 1975; Zeman et al. 1968). SSPE - Subacute Sclerosing Panencephalitis produces a diffuse encephalitis of white and grey matter (i.e. panencephalitis) characterized by demyelination, large increases in fibrous astrocytes, infiltration by lymphocytes and plasma cells, perivascular cuffing, and pronounced neuronal loss in the cerebral cortex (Johannes et al. 1975; Johnson, 1970; Paker et al. 1970; terMeulen et al. 1972; and Zeman et al. 1968).

Dawson who in 1933 proposed that SSPE might be of virus origin first noticed eosinophilic Cowdry type A intranuclear inclusion bodies in nerve cells and especially in oligodendroglial cells.

Many other investigators later made the same observation, and found that these inclusions consist of structures resembling paramyxovirus nucleocapsids (Dayan et al. 1967; Freeman et al. 1967; Herndon et al. 1968; Katz et al. 1969; Perier et al. 1968; Shaw et al. 1967; and Tellez et al. 1966).

It has been suggested that SSPE may be a more systemic disease than is clinically apparent, since measles antigens have been detected in lung, kidney and spleen biopsies, and measles virus has been recovered from lymph nodes of some SSPE patients (Horta-Barbosa et al. 1971; Jenis et al. 1973; Johannes et al. 1975). Jabbour et al (1972) and Sever et al (1974), in their epidemiological data, showed that more than 50% of SSPE patients had clinical measles infections before the age of 2 years and that the clinical onset begins at the mean age of 10 years, resulting in an incubation period averaging 6 years. But they also suggested that SSPE might be due to a latent measles infection.

Multiple Sclerosis - (MS)

Multiple Sclerosis is a sub-acute or chronic disease of young adults that usually follows a course of relapses and remissions over a period of years. The severity and variety of neurological symptons produced depend on individuals (McAlpine, 1972; Patersons, 1971).

Paterson (1971) suggested that the demyelination in Multiple Sclerosis might be due to an autoimmune response, but in the epidemiological data based on age distribution curve and geographical distribution, it was thought that MS might be caused by an infectious agent (Acheson, 1972; Alter et al. 1976; Brody, 1972; Eastman et al. 1975; and Poser, 1972).

However, Adams and Imagawa (1962) and several investigators have found high titer of measles antibody in the sera and CSF of MS patients (Brody et al. 1972; 1971; Henson et al. 1970; Norrby et al. 1974; Panelius et al. 1970; and Salmi et al. 1972).

As in SSPE, patients with multiple sclerosis have low serum-to-CSF-antibody ratios, suggesting that the antibody in the CSF must have been produced within the central nervous system (Salmi et al. 1972).

Bacteria Infection

Many workers have reported the occurrence of different type of supperative bacterial infection which complicate measles infection. More common are laryngotracheitis, bronchiolitis, otitis media, mastoiditis, bronchopneumonia and combinations there of (Morley et al. 1963).

The bacterial infection is enhanced by injury to the respiratory mucosa and by the diminution of normal ciliary propulsion of the mucous blanket. Morley et al (1963) reported bronchopneumonia to be responsible for mortality rate as high as 32% in children of about 2 years of age, while laryngitis claimed 8 lives out of 32 cases reported, this gives about 25% mortality rate.

In the tropics such illnesses like diarrhoea, canrum oris and kwashiorkor, often in combinations, complicate measles illness (Morley et al. 1963). Morley (1961) observed that diarrhoea ranked second to bronchopneumonia as the cause of death following measles complication. Catarrhal symptoms and loose stools were amongst the illness that were present in a great frequency in Nigerian children and were absent in children in the United States (Morley et al. 1961). The diarrhoea is more common after the rash has appeared and it is usually followed by kwashiorkor; the sore mouth of measles experienced by some of the cases usually lead to marasmus due to the patient's inability to eat.

IMMUNITY

Immunity to measles is partly dependent on the level of circulating antibodies to measles virus in each individual. Usually newborn infants are protected by maternal antibody for 6-9 months after birth. Albrecht et al (1977) observed in their study that at 12 months, some children still have measurable maternal antibodies to measles virus.

Measles antibodies may first be detected from the first to the twenty-fifth day or earlier after the onset of illness (Anders-Ruckle, 1965). After a maximum titer had been attained, there is a slight drop in titer during the next eight to eighteen months (Norrby et al, 1966), thereafter the antibodies persist for life with a slow decline for more than thirty years after the illness (Ruckle and Rogers, 1957; Black, 1959; and Dekking, 1965). Krugman (1977) reported the persistence of HI antibody for period of 8-10 years. Serological data (Bass et al. 1976; and Yeager, 1967) also supported the existence of waning immunity to measles 8-10 years following vaccination. During this period of waning immunity, if the subjects are exposed to measles, for example during epidemics, the exposure usually results in boostering of antibody level (anamnestic response). Krugman, (1971) observed that children who were not exposed had their titer dropped from 1:512 - 1:16 whereas those exposed had their titers dropped from 1:128 - 1:64 and later raised up to 1:128.

Due to this declining titer with time, one may like to ask of how low the level of antibody may fall before an individual could become susceptible. It was observed by Ender-Ruckle (1965) that clinical immunity was maintained with very low or even non-measurable concentrations of antibodies, such as exist in children with declining maternal antibody at the age of 5-8 months or after injection of preventive doses of gamma globulin. On the contrary, susceptibility to measles seems to exist in those who have neutralizing antibody titers of less than 1:8 (Stokes et al. 1961; Karelitz and Markhan, 1962). It was also observed in the serological data obtained by Weiner et al (1977) that subjects who have HI antibody level of 1:32 were also susceptible to attack during an outbreak of measles amongst the adolescents.

Since any detectable level of HI antibody is indicative of immunity to measles (Krugman, 1977), testing for low antibody levels would help to measure persistence immunity following natural infection or immunization. When the cut-off of HI titer was 1:8, he found that antibody was not detectable 12 years after natural measles infection in 9% of 46 children and 4% of 23 children who had received live attenuated Edmonston B vaccine plus ISG (Immune Serum Globulin) and in 36% of 78 children who received the further attenuated vaccine. Also in the same study, at a cut-off point of

titer 1:2, 12 years later, antibody was measurable in all the children, who had natural infection and who received live attenuated vaccine whereas 9% of those who received further attenuated vaccine had no detectable antibody. In this respect the work of Krugman (1977) was not in full agreement with the works of Ruckle and Rogers (1957), Black, (1959), and Decking, (1965). It is also obvious that it is possible for antibody level to drop to undetectable value 12-14 years after successful immunization. This was observed by Krugman (1957) who found no detectable antibodies in 7 children 12-14 years after immunization but they later gave typical booster response when re-vaccinated.

Secondary measles infections are unusual but they may occur in some instances when probably gamma glubulin is given as prophylactic measure. Such subjects would normally contract measles infection if exposed (Schluenderbug 1965). There is no doubt also that repeated reinfection does occur amongst vaccinated subjects especially during epidemics (McCormick et al. 1977; Shopp et al. 1976; and Wyll and White 1971). This, nevertheless, leads to boostering of the antibody levels. Reinfection of measles virus may also be attributed to the following:

(a) Immunizing children at tender age i.e. before 1 year of age (Albrecht. 1977); and Yeager et al. 1977); which usually results in lack of antibody production due to the fact that existing

maternal antibody circulating in the child prevent the virus from initiating immune response.

(b) Use of killed virus vaccine which had been proved not to give adequate long-term protection in a significant proportion of adolescent (Weiner et al. 1977).

(c) Improper storage of the vaccines which could lead to loss of its antigenicity, thus resulting in its inability to produce immune response if used (Eghafona, 1978).

The degree of antibody response to measles is also influenced by age, nutrition and pre-existing disease of the patient. Children suffering from malnutrition sometimes exhibit a delayed and reduced immune reaction. In this type of people, antibodies, after the usual decline, may be present in minimal concentration (Enders-Ruckle, 1964).

The mechanisms which are responsible for the persistence of humoral immunity have not been fully identified. The factors to be considered (Enders-Ruckle, 1964) are booster effect where there could be possibility of reinfection with circulating measles virus; intracellular persistence of infectious virus or subunits could also be a possibility.

Permanent resistance to further attack, of measles after active immunization or after infection, has been studied by many investigators using the presence and level of circulating measles antibodies in the body fluid as a measure of the state of immunity to measles infection, probably because precise laboratory method for the assessment of acquired cellular immunity to measles is yet to be developed.

The Hemagglutinin. This term has been applied to various forms and components of measles virus and all of them exhibit hemagglutinating activity. It was found by Norrby and co-workers (1964), that the viral envelope bear structures, probably proteins, and possibly involving a carbohydrate, which adhere specifically to erythrocytes of various primate species. It was also found that in the presence of enough viruses the erythrocytes were visibly agglutinated. This phenomenon was also observed on the surface of cells infected with measles virus. Borron, Milgron, Karzon and Whitebsky, (1963) also demonstrated by mixed agglutination the presence of specific viral material on the surface of infected cells.

Measles virion does not have to be infective nor intact to be capable of exhibiting hemagglutination. This point has been supported by the work of Pierie's and Channy (1962), who found that after

sedimentation of all detectable infectivity from the virus suspensions, some hemagglutinating activity remained. This form of particle was referred to as the small hemagglutinin. It lacked nucleic acid and infectivity. It has only small degree of hemolysing and complement-fixing activity. The other form of hemagglutinin is referred to as large hemagglutinin. This was shown to contain nucleic acid (Norrby et al. 1964). It also contains most of the hemolytic and complement-fixing activities. The hemagglutinating activity can be enhanced without denaturing the protein coat by treating the virus with Tween-ether (Norrby, 1962; Waterson, Rott and Ruckle-Enders 1963).

Some basic characteristics of measles virus that differentiate it from most myxoviruses are that when measles virus agglutinates erythrocytes there is no elution of the virus from the erythrocytes. All attempt to remove the virus from the erythrocyte by neuraminidase or proteolytic enzymes has failed (Waterson, 1965).

The highest hemagglutination is obtained at 37°C. The reaction can take place over a wide range of P^H values (P^H6.8 - P^H10.1) and the hemagglutinating activity of the virus is stable after pre-treatment over the same P^H range. Hemagglutination is a property the virus possess which is fairly heat stable relative to

its infectivity. It had also been found that hemagglutinating activity of the particles formed after ether and Tween 80 treatment is more thermostable than that of the intact virion (Waterson, 1965).

Measles virus also exhibit hemolytic activity. Hemolysis by measles virus was observed by Perie's and Channy (1960) and has also been studied extensively by Norrby and Falksveden (1961). Unlike hemagglutinin, the hemolytic activity of the virus is lost on treatment with ether. It is also more sensitive to thermal inactivation.

Antigens: No particular antigens have been isolated in a pure state from measles virus (Waterson, 1965), but it will be important to note that the activities of measles virus against which antibodies are demonstrable are hemagglutination, hemolysis, infectivity and complement-fixation. Thus it simply means that individuals or experimental animals possess in their sera antibodies which will inhibit hemagglutination and hemolysis, which will neutralize infectivity and which will fix complement with the virus or its components.

Waterson (1965) also reported that there is a type of distribution of activities and antigenicities in the virus. Waterson (1965) found that the envelope of the virion was responsible for stimulating the production of hemagglutination-inhibiting

and neutralizing-antibodies and presumably hemolysis inhibiting activity. The envelope is also capable of fixing complement, though the nucleocapsid is responsible for part of the complement-fixing activity (Waterson, 1965).

Preparation of Hemagglutinating Antigen (HA)

Any non-attenuated isolate adapted to tissue culture is suited for the preparation of HA antigen, as long as the virus strain or line produces detectable amounts of hemagglutinin in the chosen cell system (Enders-Ruckle, 1964).

This type of antigen can be obtained if cell cultures are inoculated with high multiplicities of infectious virus which, in turn causes formation of small giant cells along with spindle cell transformation rather than the syncytia type of cytopathic effect. This change is more frequently induced in virus material containing a high proportion of non-infectious spontaneously disintegrated particles (Enders, 1962; Rapp, 1959; Oddo et al. 1961). In the production of HA antigen the successive passages at high multiplicity of the infected cells enhance the spindle cell degeneration and the final titers of the various biologic activities in the product. The preparation is usually incubated at 36°C for some days depending on the source of cell line used for the culture, and after the cytopathic change might have involved about 75-100% of the monolayer, the supernatant and the intra-cellular virus is

harvested. Enders-Ruckle (1964) used Tween-ether-split method to produce the rosette-like Hemagglutinating substructure (diameter 40-60 mm) from the harvested virus culture. This method gives a higher degree of HA activity than even many-folds of concentrated infectious virus preparations.

Indicator: The indicator used was the erythrocytes of monkey. Many workers have found that red blood cells of certain primates excluding man are agglutinable by measles virus. Species from other readily available mammalian families have been tested and their erythrocytes found insusceptible to agglutination by measles virus (Pierie's and Channy, 1960; Der Heio and Gower, 1961; Rosanoff, 1961; Kohn, 1962).

Although there were different opinion about the sensitivity of erythrocytes from different species of monkey, red blood cells from any species of monkey is suitable (Perie's and Channy, 1962). Rosanoff (1961), found that red blood cells from rhesus, cynomogous, patas, green monkey and baboons were equally sensitive in hemagglutination test even when Perie and Channy (1960, 1962) recommended red cells from baboons (cynocephalus) and patas (erythrocebus). It is very important to note here that even when the red blood cells of the same species of monkey are used there could

exist a large range of hemagglutination titers. This point was found by Fundoshi and Kitawaki (1963), when they used red blood cells from several individual cynomolgous monkey and the same stock of virus, but obtained titers ranging from 1:64 - 1:1024. However, Enders-Ruckle (1965) found that, out of the four species tested, (cercopithecus, patas, cynomolgous and rhesus), cercopithecus red blood cells were most sensitive.

Due to this peculiar properties of hemagglutination inhibition, Hemagglutination and Hemagglutination Inhibition (HI) tests have been the most useful serological techniques used all over the world. The Hemagglutination Inhibition test is very single and easy to perform. It also has a very high correlation compared with other tests like neutralization and complement-fixation.

MATERIAL AND METHODS

Collection of Sera Samples

Blood samples were collected from children in selected primary schools from nine Local Government Areas visited. In six of the nine Local Government Areas two schools were selected for specimen collection. In Zaria, Kaduna and Jema'a Federation Local Government Areas where there are many primary schools seven, four and five primary schools were visited respectively for the purpose of specimen collection. Thus 28 schools were visited in all.

Blood samples were collected from approximately sixty pupils in classes one to six, in each school, and the ages of the pupils ranged from six to fifteen (except in schools where there were older children). The total number of sera specimen collected and assayed was 1,607.

Collection of Blood

Venous puncture method was used for the collection of the blood samples and the method is described below.

A rubber tourniquet was tied around the arm above the elbow. This helps make the veins vissible, and also obstruct venous return from the limb, so as to facilitate the withdrawal of blood from vein. Then the selected area where the blood is to be collected

is thoroughly wiped clean with alcohol pad, and a sterile disposable 5 ml syringe with 21 guage needle is used to collect the blood from the chosen vein. The blood is immediately transferred to a sterile 7 ml bottle, kept in a slanting position at room temperature and the blood is allowed to clot.

Before the needle is removed from the vein, the rubber tourniquet is first released, a piece of sterile cotton wool is held over the punctured site, and each child is told to fold over the arm. This prevented excessive loss of blood. Then the blood is clotted, the serum is decanted into a sterile centrifuge tube and centrifuged for 10 minutes at about 1,000 rpm to pack any red blood cells present. Then the serum is aseptically separated into a serum container. The above described procedure was used for the collection of blood for this investigation. This process was performed for each of the over one thousand blood samples collected. The sera were then stored at -20°C.

Collection of Monkey red blood cells for HAI Test

The red blood cells of patas monkey Erythrocebus patas (Schieber) was collected by cardiac puncture method in four times its own volume of alsever solution. The collection was done throughout by a veterinary surgeon weach time it was needed. The monkey was put to sleep by intrasmuscular administration of a

sedative (Ketamine hydrochloride injection - Vetalar), manufactured by Park, Davis and Co., Detroit, Michigan 49232, U.S.A. (121,86900 Vet. SG). This was administered on a volume per weight basis.

The monkey erythrocytes were washed in physiological saline (0.85% sodium chloride in distil water) for, at least, three times and the buffy coat was removed. The packed erythrocytes were then suspended in Alsever's solution to make up 50 per cent. The mixture was then stored at 4°C for not more than one week. A further one percent (1.0%) suspension was made from the stock whenever needed for Hemagglutination Inhibition test.

ANTIBODY TITRATION

Material

The hemagglutination inhibition test was performed in Linbro/ Titertek microtitration multi-well plates (No.76-221-05) with conical bottom wells (1.0 x 0.7 approximately) which have well capacity of 0.25 ml approximately. The plates were obtained from Linbro Scientific, Inc. subsidiary of Flow Laboratories Inc. Hamdem Conn. 06517, U.S.A.

Reagents

The reagents used for the hemagglutination assay are as follows:-

(a) Measles HA antigen (VH03) prepared by Tween-ether treatment of a strain of measles virus grown in monkey kidney cells.

(b) Measles HA negative control antigen (VE03) prepared in a similar way from uninfected control cells.

(c) Measles HAI positive control serum (VG03) prepared from a pool of measles immune sera (this positive control serum inhibits hemagglutinating activity of measles HA antigen).

(d) Measles HAI negative control serum (VJ03) which does not inhibit hemagglutinating activity of measles HA antigen, and was prepared from a pool of sera measles-susceptible children. All of these were obtained from Burroughs Wellcome Reagent Ltd,

Decknham, Kent BR 3BS, England.

The Kaolin used to absorb all the non-specific antibodies that might be present in the sera was obtained from the Pharmacy Department of Ahmadu Bello University Teaching Hospital (ABUTH) Zaria, Nigeria.

Treatment of Sera to remove non-specific Antibodies.

The method used was recommended by Burroughs Wellcome except for a slight modification which will be mentioned below. Twenty-five per cent Kaolin was prepared in physiological saline and this was used in the absorption process.

Serum in 0.1 ml volume was added to each 0.3 ml of Kaolin. The mixture was allowed to stand at room temperature for about 20 mins. (Viral Research Laboratory, Ibadan, Nigeria). After 20 mins incubation, the mixture was centrifuged at 100 rpm to pack the Kaolin. Then, into each of the treated serum, a drop of 5% monkey erythrocytes was added, and this mixture was left at 4^{o}C overnight.

The following day, the mixture was centrifuged at about 1000 rpm for 10 min. to pack the erythrocytes. The supernatant (which now represented 1:4 serum dilution) was removed with a sterile pasteur pipette into a sterile test tube.

The addition of Kaolin was a minor modification of the method used by Burroughs Wellcome and it also ensured complete removal of non-specific hemagglutinins and other heterophile antibodies (Kano and Millgrom, 1977). When only 50% monkey erythrocyte was used for the absorption process, as recommended by Burroughs Wellcome, it was found that the sera were not properly absorbed. Both the positive and negative control sera were also absorbed in the same way as the test sera.

Titration of Antigen.

The lyophilized HA antigen (VH03) from Burroughs Wellcome was reconstituted with 1 ml sterile deionized water as recommended by Burroughs Wellcome.

The 1:4 dilution of the antigen was made in a test tube by the addition of 0.1 ml of the reconstituted antigen to 0.3 ml of sterile saline which was the diluent in the test.

Saline solution in a 0.025 ml volume was added to well 2-9 of a conical shaped microtitration plate, and into wells 10-12 (0.05 ml) of saline as added. Into the first well 0.05 ml of the diluted antigen was added and with microtitration diluter, 0.025 ml of the diluted antigen was transferred from the first well into the second well, mixed thoroughly by rotating the diluter and 0.025 ml of further diluted antigen was transferred into the third well. This doubling dilution process was continued up to well number nine and the last transfer was discarded.

Then 0.025 ml of sterile saline was added to well numbers 1 to 9, after this exercise, 0.025 ml of one percent (1.0%) monkey erythrocyte made from the 50% stock, was added into all the wells. Thus wells 10-12 contained just the saline and the erythrocytes thereby serving as the cell control wells. This was to guide against any non-specific hemagglutinating reactions that might come from the monkey erythrocytes. The test was run in triplicates. The negative control antigen was treated the same way, and test was incubated at 37°C for one hour.

After one hour incubation period, the antigen titer was read by pattern method. The highest titer of the antigen is taken as one Hemagglutination unit (1HU unit). When this 1HU unit is multiplied by 4, a 4HU unit is obtained which is the dilution of antigen to be used in the test proper.

Conduction of the HAI Test.

Hemagglutination Inhibition Test, the test of choice in this investigation was said to be the most sensitive and accurate method in measuring the humoral antibody to measles virus (Enders-Ruckle, 1965).

In conducting the HAI test, 11 wells were used for each test serum and the 12th well was used for serum control. Thus each test serum was diluted from 1:4 to 1:4096.

With a micro-dropper pipette (Titertek), 0.025 ml of sterile saline was dropped into each well (starting from well number 2) of the conical microtitration multi-well plates. Then 0.05 ml of the treated serum (absorbed serum of 1:4 dilution) was dropped in the first well, and 0.025 ml of the same serum was also dropped into the last (12th) well which already contained 0.025 ml of saline.

Using a microdiluter (Titer Teck) 0.025 ml of the 1:4 treated serum was transferred from the first well into the second well, mixed thoroughly, and transfer and mixing continued in the doubling fashion until the last transfer from the 11th well was discarded. This process was carried out for each of the test sera including both the positive and negative control sera.

The Lab. antigen in 0.025 ml volume was added to all the wells except the last well which is the serum control well, and the mixtures were incubated for 1 hr at room temperature. This allowed the antigen and the antibody enough time to react. During this period of antigen-antibody reaction, one per cent (1%) monkey erythrocytes was made from the 50% stock kept in the refrigerator. After the one hour period, 0.025 ml of the prepared 1% monkey erythrocytes was added to each of the wells including well number 12 which served as the serum control.

Thus all the wells except the last, contained serum-antigen-cell mixture while the last well contained only serum-cell mixture.

The mixtures were then incubated at 37°C for one hour after which the titers were read using an illuminating mirror.

The highest dilution of a serum which gave well-settled red blood cells (i.e. inhibition of hemagglutination) was taken as the titer of the serum. Any small degree of hemagglution was taken as lack of inhibition. Both positive and negative control sera were run each time H.I test was performed. Antigen control also was always run so as to check and make sure that 4HA units was being used for the test. Cell control was also carried out, to check whether the cells were free of nonspecific hemagglutinin. The serum control was also included to make sure that it was properly absorbed and all the nonspecific hemagglutinins were removed.

RESULTS

Figure I is the legend showing the areas covered by the survey. The dotted points (.) are the locations of the primary schools where specimen were collected.

MAP OF KADUNA STATE SHOWING AREAS COVERED BY THE SURVEY



Figure 1

Tables I and II were obtained from the Epidemiological section of Kaduna State Ministry of Health and they show the areas covered by the routine vaccination campaign carried out in Kaduna state. Table II reveals the years such campaigns were carried out, and the number of children vaccinated by either the mobile teams or the static teams. The mobile teams are those who move from one place to the other while the static teams are those who are mainly in the Hospitals and Health Centres and carry on vaccination every year.

The areas covered by this survey which are listed in Table I are Zaria, Kafura and Kaduna.

Table I further shows the Local Government areas and the towns and villages covered by this survey, as well as the months between February, 1976 to November, 1977 when mass vaccination was carried out and the number of people vaccinated.

Table I

DISTRICT BY DISTRICT VACCINATION PROGRAMME IN KADUNA STATE: FROM 1976 - 1977a

Name of District	Month of Vaccination	Total Number of children vaccinated
Kaduna	February 1976	27,658
Mani District - Katsina	May 1976	17,760
Chawai District - Zaria	March 1976	7,535
Bindawa " - Katsina	May 1976	9,468
Ikara District - Zaria	August 1976	8,125
Mashi District - Katsina	September 1976	9,930
Ikara District - Zaria	November 1976	3,708
Makarfi " - Zaria	February 1977	1,333
Kaduna V/Sarkin Pawa	March 1977	95
Kubau Dist. - Zaria	April 1977	2,105
Karvu Dist. - Zaria	March 1977	10,715
Jibia Dist. - Katsina	April 1977	10,602
Rimi District- Katsina	August 1977	17,933
Tsagero Dist. Katsina	November 1977	4,162
Batagarawa Dist. Katsina	November 1977	5,857

The above programme was carried out by the mobile vaccination teams of this unit as maintenance phase Vaccination Campaign in Smallpox eradication and Measles Control.

2/2/78

a - Target Group; 5 months - 5 years.

Table II

YEARLY IMMUNIZATION RETURNS ON MEASLES VACCINATION
1970 - 1977

Years	Number of children vaccinated by the Mobile Teams	Number of children vaccinated by the Static Teams	REMARKS
1970	119,592	-	
1971	319,720	10,956	
1972	63,119	7,762	
1973	86,390	63,119	
1974	133 ,020	7,044	
1975	22,460	15,324	
1976	78,154	7,422	
1977	-	10,945*	* Reports compiled on 9 months only i.e. January-July, October & November of the same year.
Sub-Total	852,455	122,572	
Grand Total	975,027		

From the statistical data (Tables I and II), obtained from the Epidemiological Unit of the Ministry of Health, Kaduna State, it was possible to group the areas covered by this survey into two.

Group 1 - The local government areas where immunization campaigns were carried out.

Group 2 - The local government areas where there was no record of immunization campaign.

Therefore one could assume that the greater percentage of the population in the areas where the immunization campaign had been carried out would have been vaccinated against measles infection, thus, should have high antibody levels.

On the other hand the level of antibodies to measles should be low or non-existence in those areas where there had been no immunization campaigns. Although in these areas high level of antibodies could be recorded which could be as a result of sub-clinical infection. Therefore, the areas under group one are referred to as the "Vaccinated Areas", while the areas under group two are referred to as the "unvaccinated areas" in this paper.

Determination of the differences in the level of homagglutination inhibition antibody titres between the children from the vaccinated areas and those from the unvaccinated areas

Let (H_o) represents our Null hypothesis that there is no significant difference in the level of antibody between the vaccinated and unvaccinated pupils

and

(H_i) represent our alternative hypothesis that there is a significant difference, that is, the pupils in vaccinated areas have higher antibody level than the pupils in unvaccinated areas.

The Tests

Mann-Whitney and T-tests were used for the statistical analysis of the data (Conover W.J. 1971). Mann-Whitney (M) test is a non-parametric test that does not require the data to conform to any particular type of distribution. The T-test is used to test for the difference between two means. It should also be noted here that t-distribution tends to normality when the sample size is large.

Therefore, these two tests have thus taken care of the inequality in the sample sizes seen in the data (Table III).

Table III

Average Titers of Hemagglutination Inhibition antibodies In Each Age Group.

Age Yrs	Vaccinated Area: Sample sizes (No. of Pupils)	Vaccinated Area: Av. Titer X_{i1}	Unvaccinated Area: Sample sizes (No. of Pupils)	Unvaccinated Area: Av. Titer X_{i2}
6	92	112.6	128	52.2
7	110	88.1	111	46.3
8	80	114.2	89	43.9
9	60	126.5	83	43.3
10	81	83.3	68	68.9
11	58	166.8	51	47.0
12	93	164.4	97	51.4
13	50	149.7	86	60.4
14	33	89.1	68	34.7
15	13	138.0	17	36.9
16-21	3	42.7	22	31.1
	Gross Total of Sample size = 669		Gross Total of sample size = 820	
	Average titer = 120.8		Average Titer = 49.3	

S_1 - variance of mean of titer in vaccinated area = 38.23

S_2 - variance of mean of titer in unvaccinated area = 11.11

The test statistics is:

$$U = n_1 n_2 + \frac{n_1(n_1 + 1)}{2} - R_1$$

Where n_1 and n_2 are the sizes of the two samples for (vaccinated and unvaccinated areas) respectively; and R_1 is the sum of the ranks assigned to the values of the first sample (any of the samples could be the first).

To calculate for R_1

The data (average titers in both areas) in table III were ranked jointly as if they were one sample in increasing order of magnitude thus:

Ranking.

31.1	34.7	36.9	42.7	43.3	43.9	46.3	
u	u	u	v	u	u	u	
1	2	3	4	5	6	7	
47.0	51.4	52.2	60.4	68.9	83.3	88.1	89.1
u	u	u	u	u	v	v	v
8	9	10	11	12	13	14	15
112.6	114.2		126.5	138.0	149.7	164.4	166.8
v	v		v	v	v	v	v
16	17		18	19	20	21	22

Σ of ranks for u = 74

Σ of ranks for v = 179

u = Unvaccinated area

v = Vaccinated area

1 - 22 are the ranks.

$R_1 = 74$

$R_2 = 179$

$$M = n_1 n_2 + \frac{n_1(n_1 + 1)}{2} - R_1$$

$$= 11 \times 11 + \frac{11(12)}{2} - 74$$

$$= 121 + \frac{11 \times 6}{2} - 74$$

$$= 121 + 66 - 74$$

$$= 187 - 74$$

$$M = 113$$

$$\sigma_u^2 = \frac{n_1 n_2(n_1 + n_2 + 1)}{12}$$

$$= \frac{11 \times 11\ (23)}{12}$$

$$= 231.92$$

$$\sqrt{\sigma_u} = 15.23$$

$$u_u = \frac{11 \times 11}{2}$$

$$= \frac{121}{2}$$

$$= 60.5$$

$$\therefore Z = \frac{\bar{n} - \mu_n}{\sigma_n}$$

$$= \frac{113 - 60.5}{15.23}$$

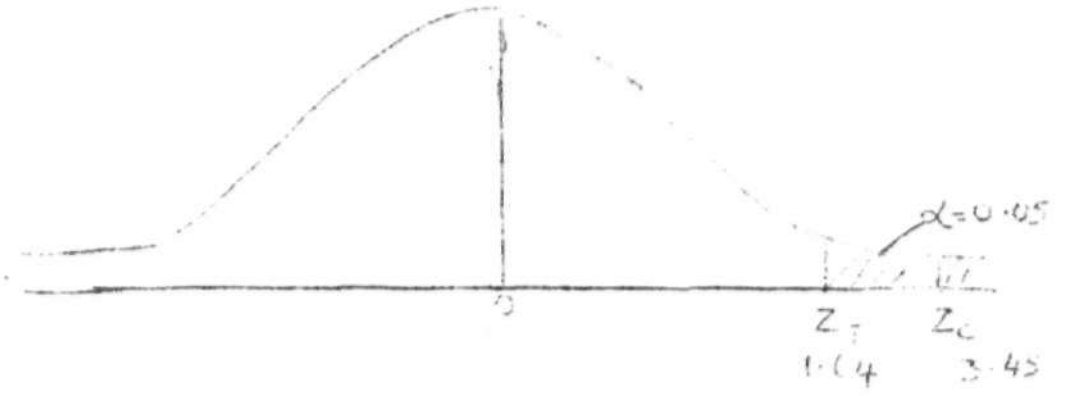
$$Z = 3.45$$

σ_n = variance of n

μ_n = mean of n

Z = standardized normal distribution

Results



Normal Distribution Curve

The abbove curve represents the normal distribution curve. At level of significance $\alpha = 0.05$

Z_T = Z tabulated = 1·64

Z_C = Z calculated from the data (Table III) = 3.45

The shaded area is area of rejection that is, if any value falls within this area, it should be rejected.

Therefore,

since the Z-calculated, the value of which is 3.45 falls within the rejection area, the null hypothesis (H_o) in this case is rejected and (H_1) the alternative hypothesis is accepted.

This means that there is a great significance between the vaccinated and unvaccinated area in terms of the level of hemagglutination inhibition antibodies.

Problem (2) - To test if there is any significant difference in each age group between the vaccinated and the unvaccinated areas.

The test statistics is:-

t - test

$\sigma^2_{\bar{x}_1 - \bar{x}_2} = \sigma^2 = \frac{N_1 S_1^2 + N_2 S_2^2}{N_1 + N_2}$ = variance of difference between x_{i1} and x_{i2} (for large sample sizes)

where N_1 = sample size for vaccinated area

N_2 = sample size for unvaccinated area

S_1^2 = variance of mean of titer in vaccinated area =

$\frac{1}{N_1} \Sigma(x_{i1} - \bar{x}_1)^2 = \frac{1}{N_1} \Sigma(112.6-120.8)^2 + (88.1-120.8)^2 + \ldots\ldots$

S_2^2 = variance of mean of titer in unvaccinated area =

$\frac{1}{N_2} \Sigma(x_{i2} - \bar{x}_2)^2$

where N_1, N_2 = no of age groups = 11

Using this formular, let's calculate t for age 6 year

where

$S_1 = 38.23$

$S_2 = 11.11$

$\sigma^2 = \frac{92(38.23)^2 + 128(11.11)^2}{92 + 128}$

$= 683.0$

$\sigma = 26.13$

Table IV

Statistical analysis using t-test showing the significant difference in the age groups between the vaccinated and the unvaccinated areas.

at α = 0.05 level of significance

Age groups	Calculated (t_c)	Tabulated t (t_n)
6	2.31	1.645
7	1.49	1.645
8	2.56	1.645
9	3.18	1.645
10	0.49	1.645
11	4.14	1.645
12	4.05	1.645
13	3.60	1.645
14	2.30	1.645
15	3.81	1.701
16-21	0.69	1.714

On the other hand

at $\alpha = 0.05$

if $t_c > t_T$

the alternative hypothesis (H_1) is accepted and the null hypothesis (H_o) is rejected. This is to say that in the age groups the pupils in vaccinated areas have higher antibody level than those in the unvaccinated area.

Thus, examining table IV except for ages 7, 10 and (16–21), there is a great significant difference in all the age groups between vaccinated and unvaccinated areas.

The Hemagglutination Inhibition test also revealed in figures II, III and IV the significant difference between the vaccinated and the unvaccinated areas. It could be noticed in figures II and III that even some areas which are grouped under the unvaccinated area seem to record high levels of antibody, this could be due to some reasons which will be discussed later in the paper.

Legend to Figure II.

Figure II represents the percentage of pupils in each age group in the different local government areas with Hemagglutination Inhibition antibody titers of 1:16 and below. In this figure, it will be observed that the low level of antibody titers obtained in different age groups of children vary from community to community. The best picture of possible high level of circulating Hemagglutination Inhibition antibody was obtained in Zaria local government area in almost all the age groups. This is followed by Kaduna. In the remaining local government areas it will be observed that higher percentage of pupils show low antibody levels. Amongst the age group of 6 years 91.7%, 81.8% and 80.0% of pupils with antibody levels of 1:16 and below are recorded for Ikara, Saminaka and Jemaa Federation respectively.

FIGURE II

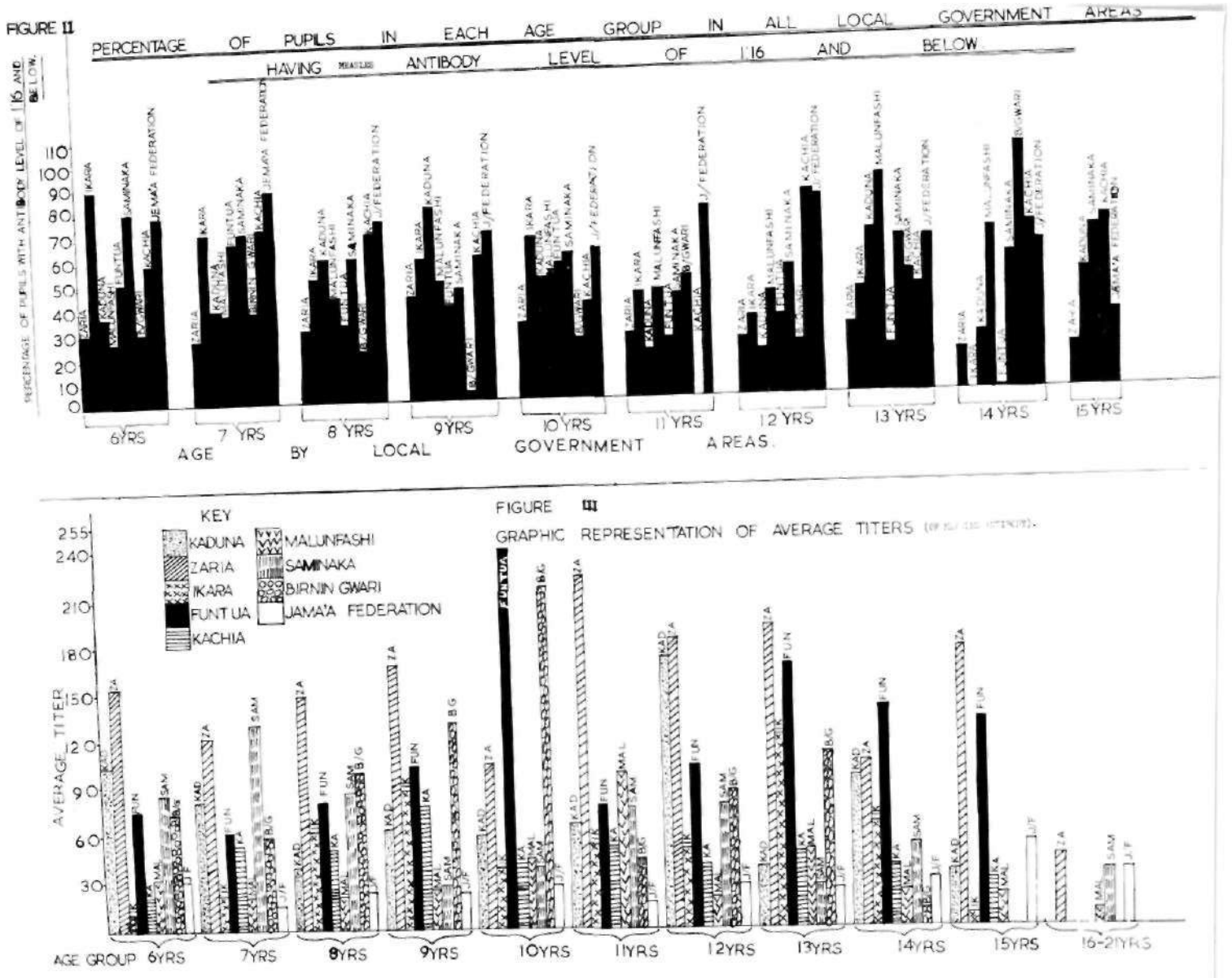

PERCENTAGE OF PUPILS IN EACH AGE GROUP IN ALL LOCAL GOVERNMENT AREAS
HAVING MEASLES ANTIBODY LEVEL OF 1:16 AND BELOW
PERCENTAGE OF PUPILS WITH ANTIBODY LEVEL OF 1:16 AND BELOW
110
100
90
80
70
60
50
40
30
20
10
0
6YRS
7 YRS
8 YRS
9YRS
10YRS
11 YRS
12 YRS
13 YRS
14 YRS
15YRS
AGE BY LOCAL GOVERNMENT AREAS.
KEY
KADUNA
ZARIA
IKARA
FUNTUA
KACHIA
MALUNFASHI
SAMINAKA
BIRNIN GWARI
JAMA'A FEDERATION
FIGURE III
GRAPHIC REPRESENTATION OF AVERAGE TITERS
AVERAGE TITER
255
240
210
180
150
120
90
60
30
AGE GROUP 6YRS
7YRS
8YRS
9YRS
10YRS
11YRS
12YRS
13YRS
14YRS
15YRS
16-21YRS

Table V

Average Antibody Titers In Each Age Group In The Local Government Areas Investigated

	Kaduna	Zaria	Ikara	Funtua	Soninaka
6	102.3	155.7	9.4	74.1	18.9
7	80.5	121.2	18.2	58.7	51.7
8	52.4	148.0	60.0	77.6	48.0
9	59.2	164.2	35.3	100.8	75.8
10	56.0	102.6	35.7	239.2	37.3
11	63.2	220.4	49.8	76.4	49.7
12	166.9	179.4	46.2	101.3	36.3
13	34.2	187.0	117.1	162.7	43.5
14	62.0	102.4	64.0	137.6	37.3
15	32.0	172.6	4.0	128.0	26.7
16-21	-	42.7	-	-	-

Table V Cont'd

	Kachia	Kalumfashi	B/Gwari	J/Modernation
6	30.4	84.9	72.0	28.2
7	17.3	128.8	57.2	12.2
8	18.4	84.0	97.8	20.0
9	21.2	24.0	128.0	15.4
10	26.4	36.8	213.3	23.7
11	96.0	83.3	41.0	15.3
12	14.7	75.7	84.8	27.9
13	46.3	23.6	108.7	21.3
14	19.2	49.3	8.0	26.3
15	16.6	-	-	50.7
16-21	8.0	32.0	-	32.2

Legend to Figure III
(See page 81)

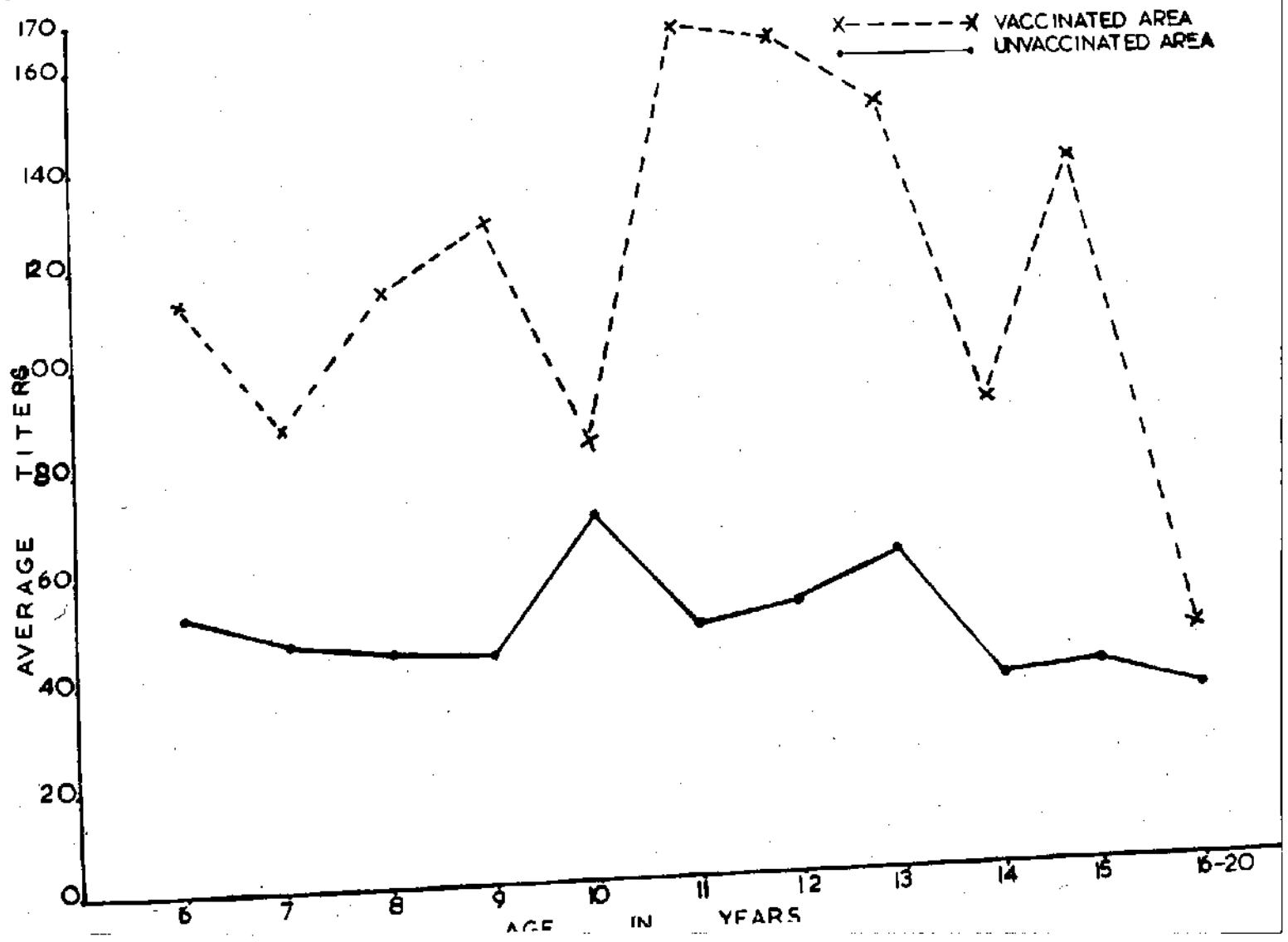
Figure III is a graphic comparison of measles Hemagglutination Inhibition antibody levels in all the nine local government areas per age group. It will be observed that the distribution of low antibody level vary from one locality to the other. In some areas low level of antibodies are more pronounced in the lower age groups that is ages 6 and 7, therefore it appears as if low level of antibodies is also observed in Ikara for age groups 6, 7, 9 and 10 whereas in the higher age groups the antibody levels are high. The story is quite different for Malumfashi, in this locality age groups 6 to 12 appear to have reasonably high level of antibodies but age groups 13 and 14 show marked decline in antibody level giving about 89% and 64% respectively of pupils with antibody level of 1:16 and below. However in Kachia and particularly in Jemaa Federation almost all the age groups had low levels of Hemagglutination Inhibition antibodies.

Table VI

Average Hemagglutination Inhibition Antibody Titers In The Age Groups.

	Vaccinated Area	Unvaccinated Area
6	112.6	52.2
7	88.1	46.3
8	114.2	43.9
9	126.5	43.3
10	83.3	68.9
11	166.8	47.0
12	164.4	51.4
13	149.7	60.4
14	89.1	34.7
15	138.0	36.9
16-20	42.7	31.1

THE AVERAGE TITERS IN THE AGE GROUP IN BOTH VACCINATED AND UNVACCINATED AREAS FIGURE 1V



Legend to figure IV.

Figure IV is a graphic representation of the average measles Hemagglutination Inhibition antibody titers of the pupils in the vaccinated and unvaccinated areas within the age groups. It will be observed in this figure that there are significant differences in the levels of measles antibodies between the vaccinated and unvaccinated areas, except for age groups 7, 10 and 16 to 20 years.

CYTOPATHIC EFFECT (CPE) ASSAY

Micro-tissue culture equipment

Sterile plastic, disposable multi-well plates with covers and 96 flat bottom wells of Linbro IS-FB-96-TC-76-003-05 (Linbro Scientific Inc. subsidiary of Flow Laboratories, Inc. Hamden, Conn. 06517, U.S.A.) were used for the micro-neutralization tests.

Sterile micro-pipette droppers (Microtiter, Cooke Laboratory Products, Alexandera, Virginia U.S.A.) calibrated to deliver 0.025 ml were used to dispense diluent, culture medium, cells and sera into wells.

Diluter calibrated to adsorb and deliver 0.025 ml (Microtek diluter 76-742-02 of Flow Laboratories Inc. Hamden Conn. USA) were used for dilution of sera. They were made of stainless steel, they can be flamed to sterilize.

Cells.

Vero cells used for the neutralization tests were obtained from Department of Microbiology, University of Readings, Britain at passage level 150 and it was used as such.

Virus

The measles virus strain in a lyophilized form was obtained from the Immunology Unit, Department of Medicine, A.B.U, Hospital Zaria.

Tissue Culture Medium

The growth medium was Eagle's minimum essential medium containing 10% heat inactivated fetal calf serum, 1.0g/liter sodium bicarbonate streptomycin 100g/ml and neomycin 100g/ml.

Test Medium

Micro-test medium consisting of Eagle's minimum essential medium with 0.1% sodium bicarbonate, 2% fetal calf serum, 0.5% lactalbunin hydrolysate and neomycin was used as diluent for the virus and the sera.

Treatment of Sera.

The sera were inactivated by heating at 56°C for 30 minutes.

A. The Neutralization Test (Micro-method). Some sera with hemagglutination Inhibition titers of 1:4, 1:16 and zero were selected, inactivated and used neat for the neutralization test.

The neat serum was added in to each of 3 - well in a 0.025 ml volume, and 0.025 ml of 10^{-9} tissue culture infective dose (TCID) of the challenge virus was added. The micro-plate containing the virus serum mixture was sealed and incubated at 36°C for 1hr. Then 0.05 ml of 20,000 vero cells diluted in the growth medium was added and then sealed (Kalus et al. 1975). The test was incubated at 36°C for 7 days and reading was taken every day.

B. Two-fold serial dilution of 0.025 ml of serum known to have 1: 1024 titer in the haemagglutination inhibition test was made by diluter transfer in wells containing 0.025 ml of the diluent. The challenge virus suspended in the test medium in a concentration to give 10^{-9} TCID was added to each well in 0.025 ml volume. The test was then incubated at 36°C for 1hr, and 0.05 ml of 20,000 vero cells were added. The micro-plate was sealed and incubated at 36°C for a period of 7 days during which period daily readings were taken. Cells and virus controls were also carried along with the tests and all observations were recorded.

RESULTS OF THE

NEUTRALIZATION TEST

Legend to figures V to VII.

Figures V to VII show the neutralization effect which various level of antibodies have on the measles virus. In the figures pictures a,b,c and d were taken after 18hrs, 42hrs, 72hrs, and 96hrs respectively after virus innoculation. Although the tests were read for seven days, the important thing to note in each figure is the time it took for CPE to develop despite the presence of the antibodies.

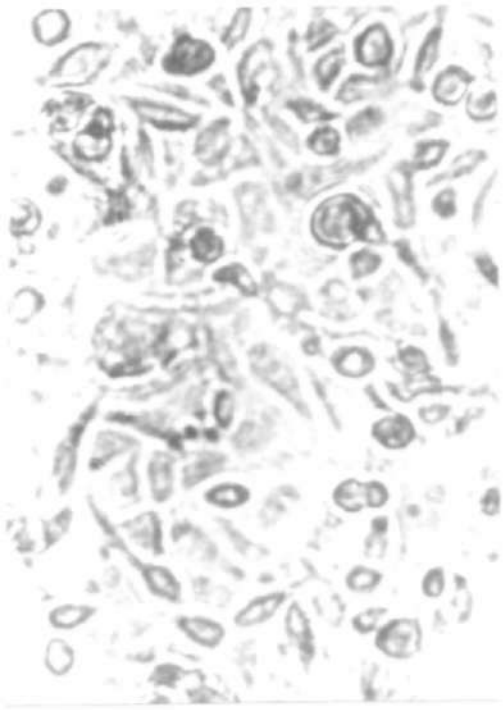
In the presence of hemagglutination inhibition antibody of 1:4 (figure V) slight CPE was evident after about 42hrs and became more prominent as the period of incubation was prolonged. In the presence of HI antibody of 1:16 (figure VI), the virus was kept in check for a period of 72hrs after which cytopathic effect became evident as seen in figure VI (d). However a very pronounced CPE was shown at about 42hrs in the presence of serum that had no measurable hemagglutination inhibition antibody (figure VII). If the pictures seen in figure VII is compared with the virus control (figure VIII) it will be seen that there is no difference between the two, since there was no antibody in the serum which could neutralize the virus.

In summary the pictures revealed that 1:4 HI antibody level would prevent the viral replication for about 42hrs while 1.16 level would prevent replication for at least 72hrs, in an in vitro situation.

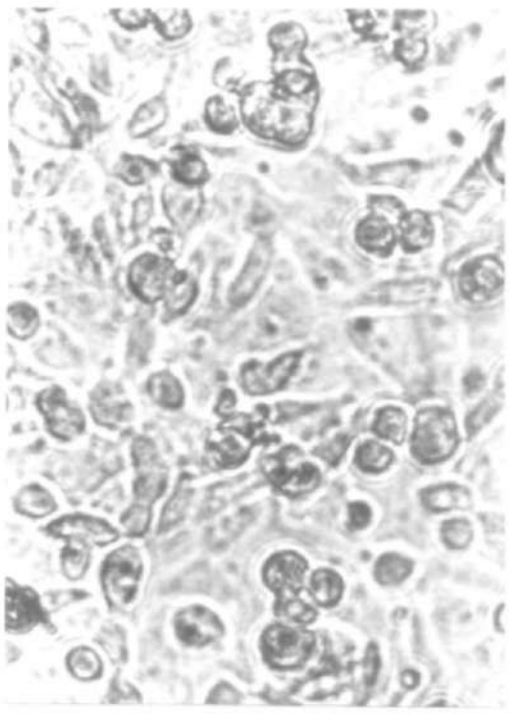
Figure V



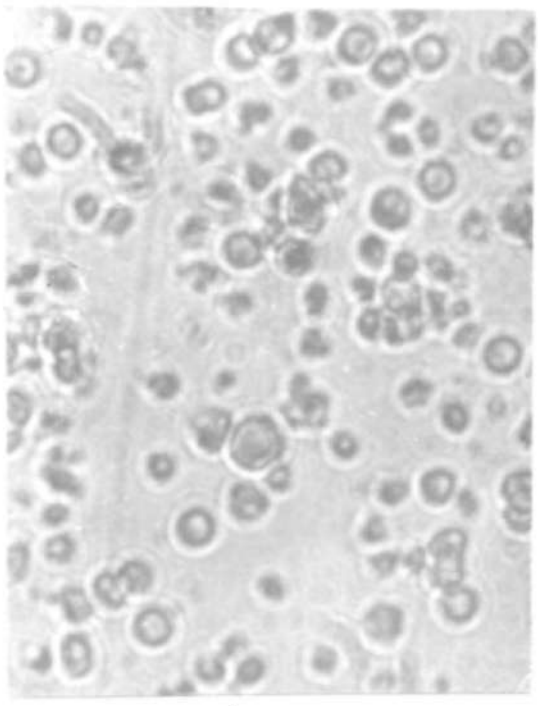
18 hours



42 hours



72 hours



96 hours

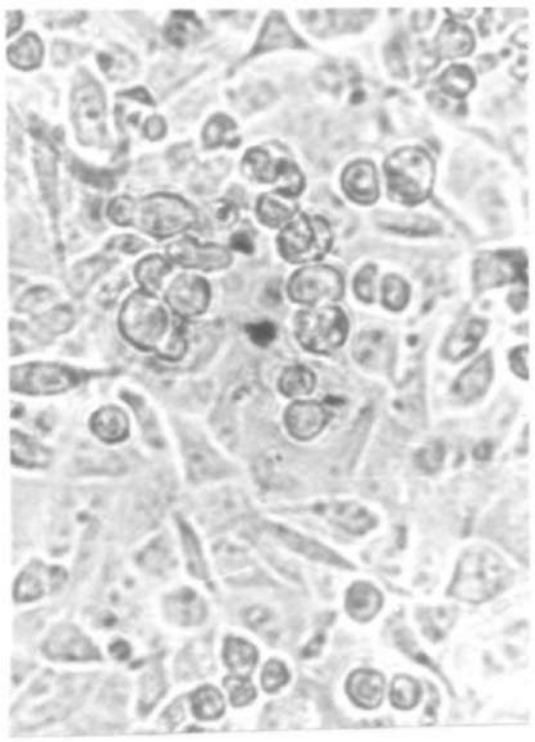
Neutralization effect of Measles virus in the presence of serum with HI titer of 1:4.

Legend to Figure V

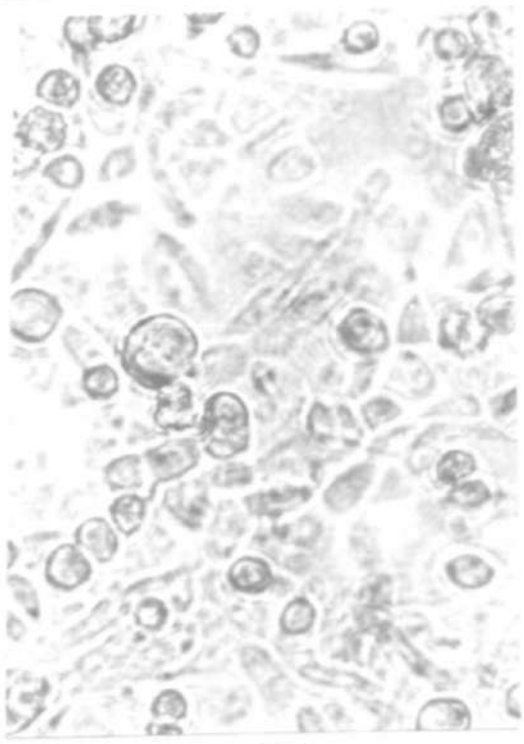
Figure V shows neutralization reaction when neat serum with measles Hemagglutination inhibition antibody titer of 1:4 is used. It will be observed that figure Vb which represents the reaction at about 42hrs after inoculation of the vero cells with live viruses shows a slight CPE. After 72hrs about 75% of the cells were involved in CPE formation (figure Vc) while there was complete CPE at about 96hrs after the inoculation of the live measles virus (figure Vd).

Therefore it can be postulated that if this type of process which occurs in vitro repeats itself in vivo, antibody level of 1.4 would not be enough to neutralize the virus, completely, it could probably only prevent virus replication for some hrs, after which it may be possible for the measles virus to infect the appropriate cells and multiply.

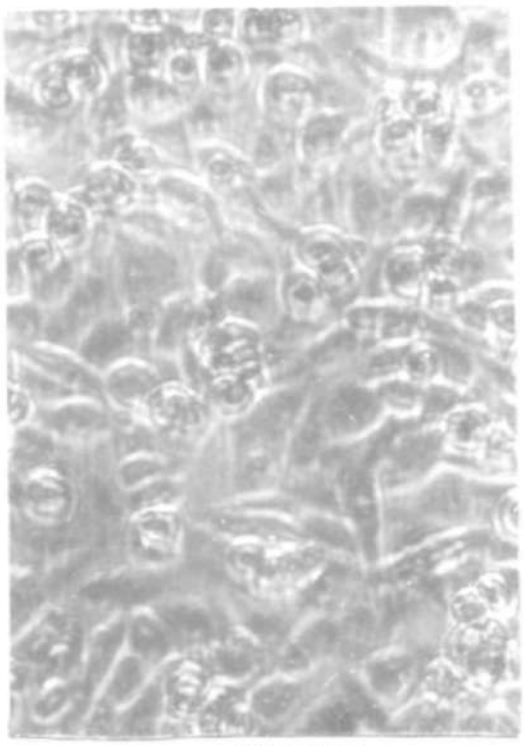
Figure VI



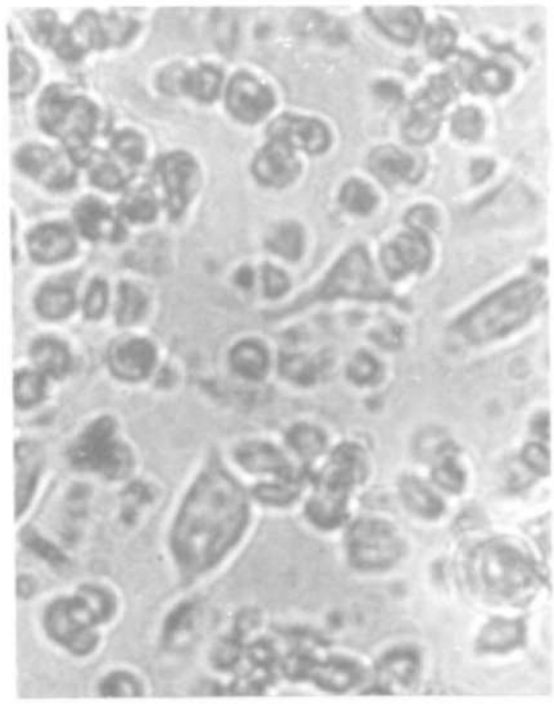
18 hours



42 hours



72 hours



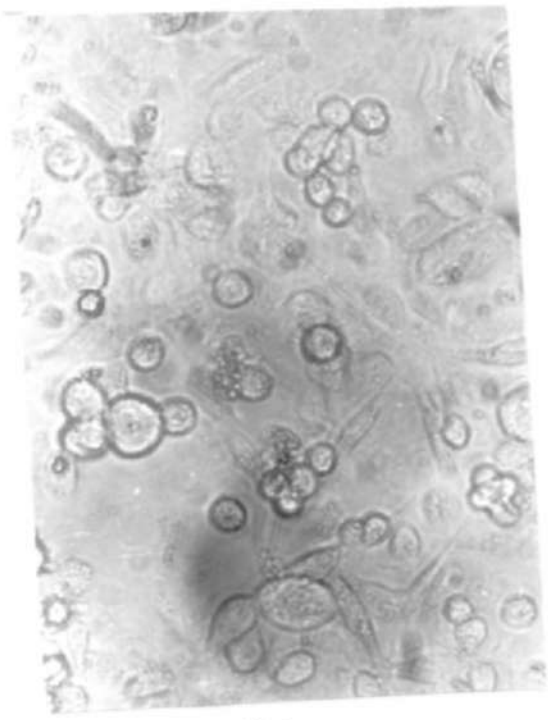
96 hours

Neutralization effect of Measles virus in the presence of serum with HI titer of 1:4.

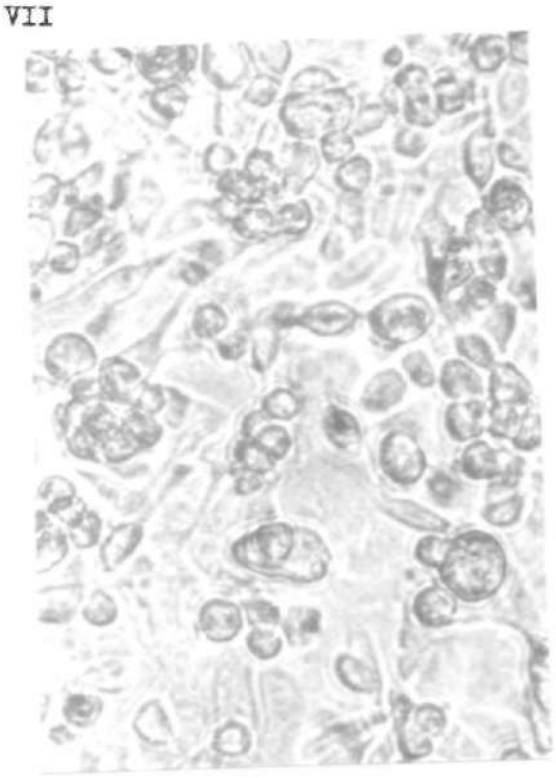
Legend to Figure VI

In figure VI CPE did not start until about 96 hours after inoculation of the vero cells with live measles virus (figure VI d). This figure revealed that Hemagglutination Inhibition antibody titer of 1:16 could prevent viral replication for about 3 days.

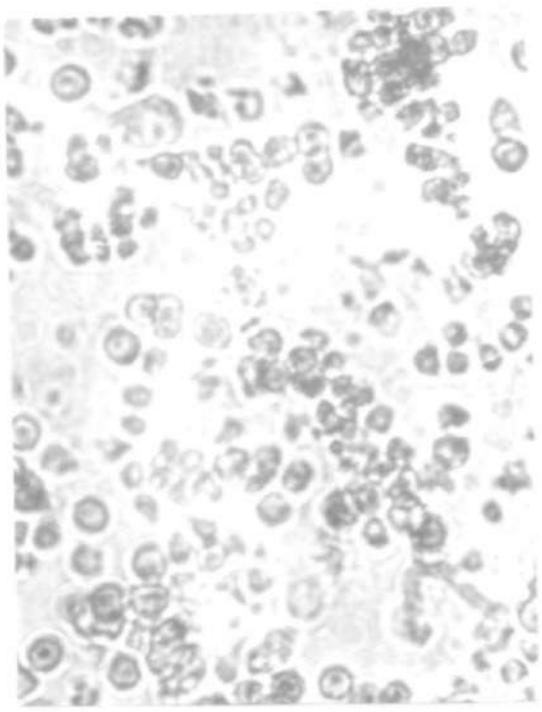
Figure VII



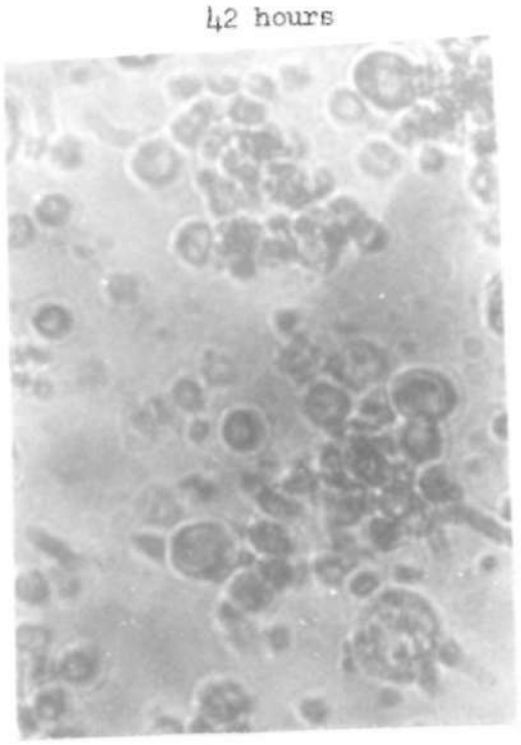
18 hours



42 hours



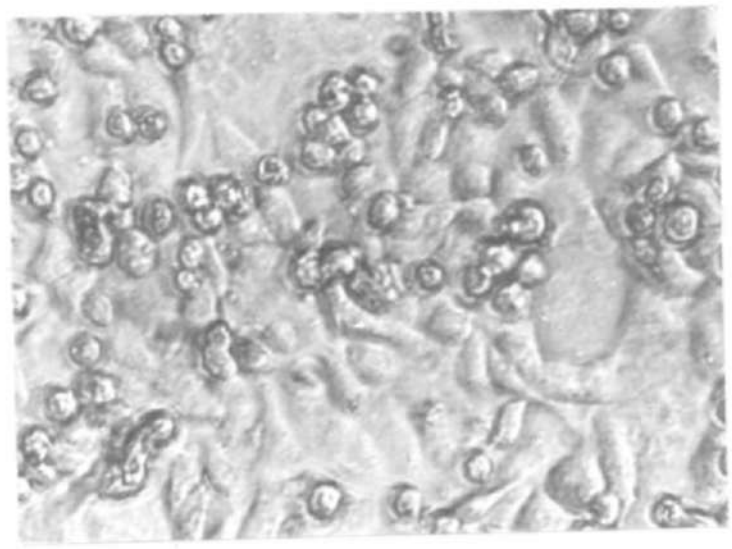
72 hours



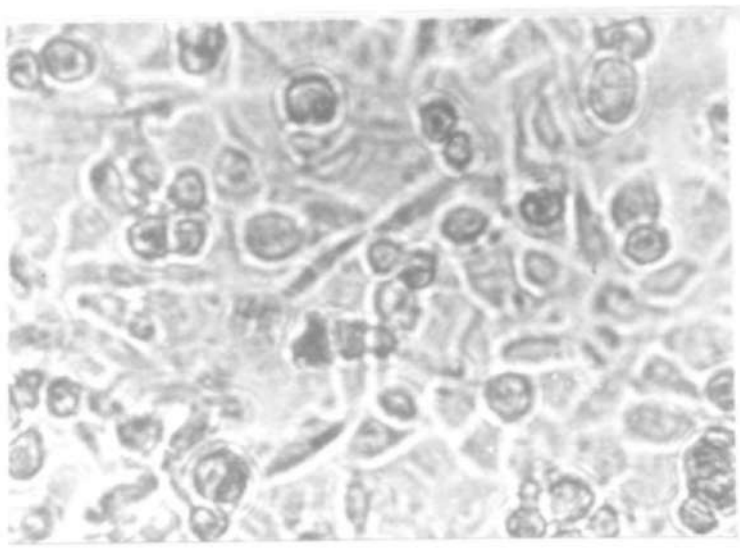
96 hours

Neutralization effect of measles virus on serum with no detectable Hemagglutination Inhibition (HI) antibodies.

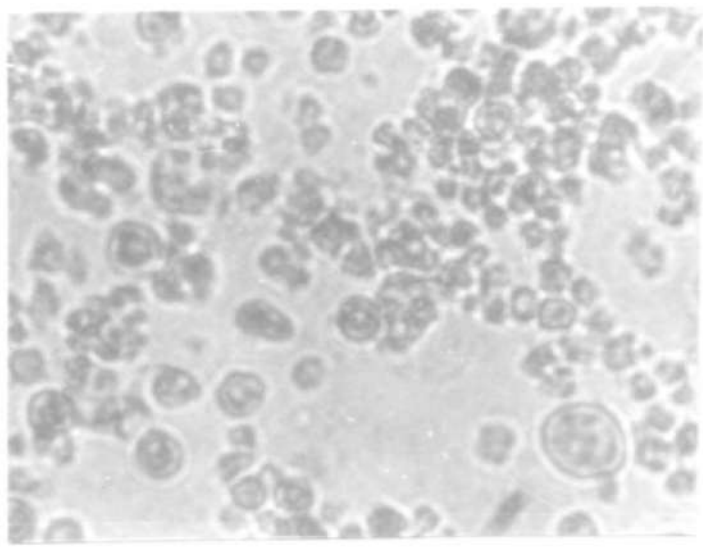
Figure VIII



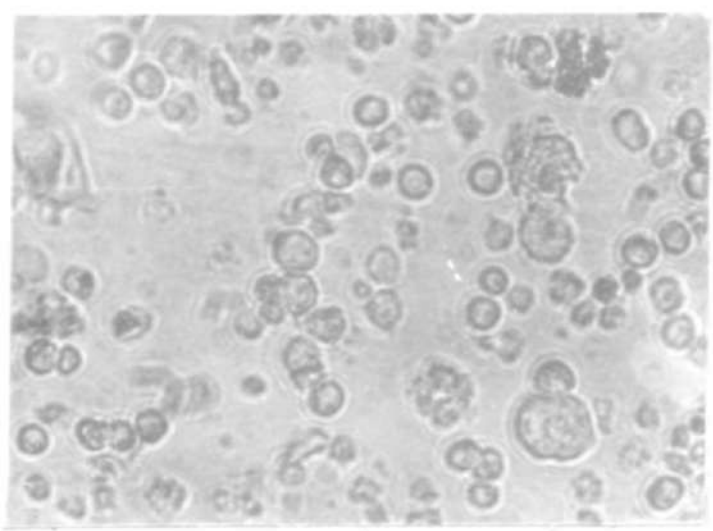
18 hours



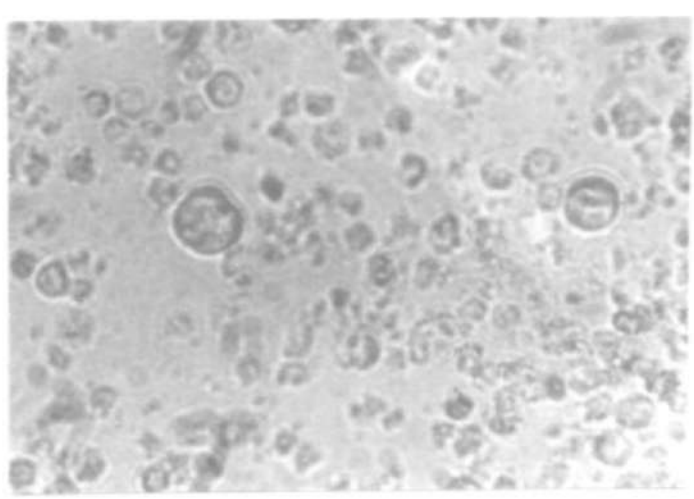
42 hours



72 hours



96 hours



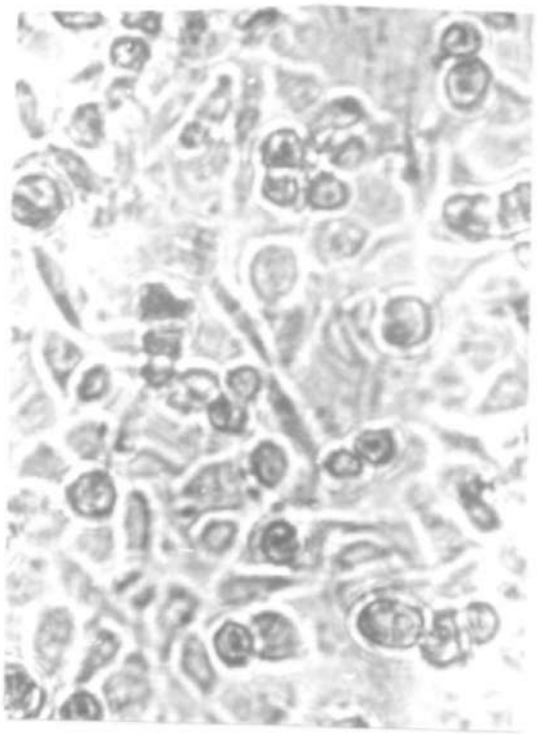
144 hours

The virus control at various stages.

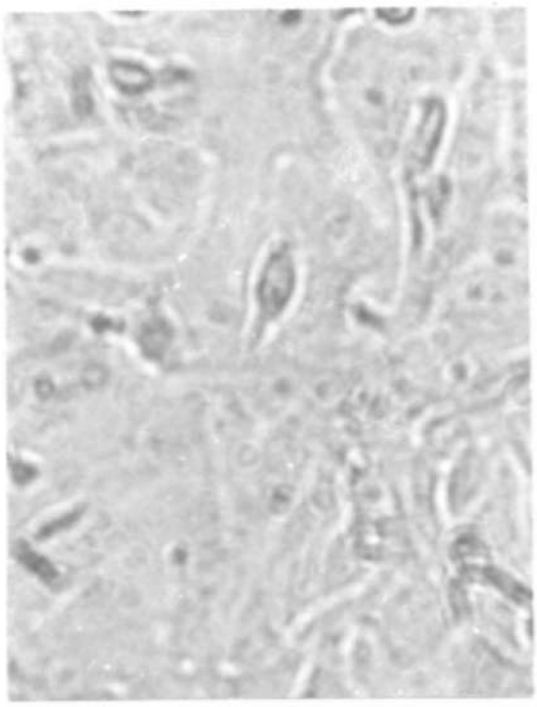
Legend to figure IX.

Figure IX is the cell control. The cell control wells were also observed throughout the period of the experiment. Figures IX a, b, c and d represent 18 hours, 48 hours 96 hours and 144 hours respectively. It will be observed that apart from the cell degeneration in the later picture (d) there is no sign of cytopathic effect.

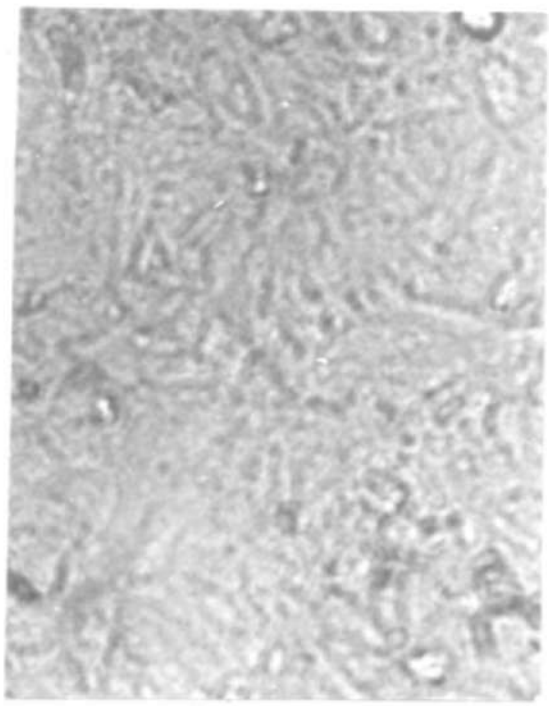
Figure IX



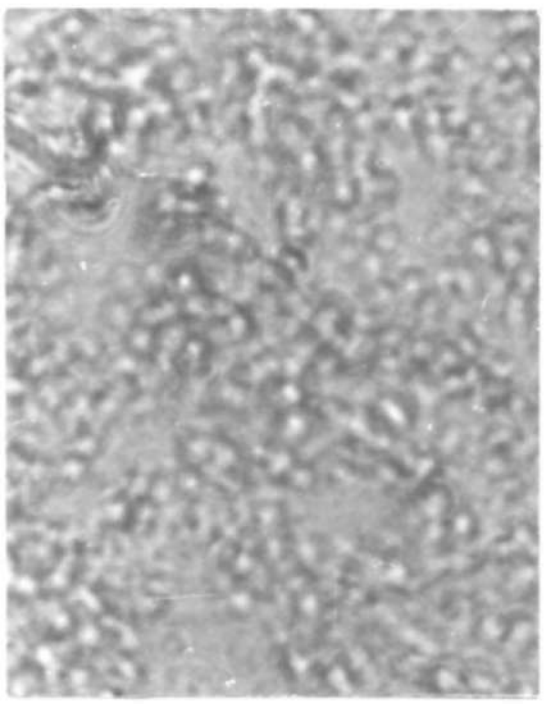
18 hours



48 hours



96 hours



144 hours

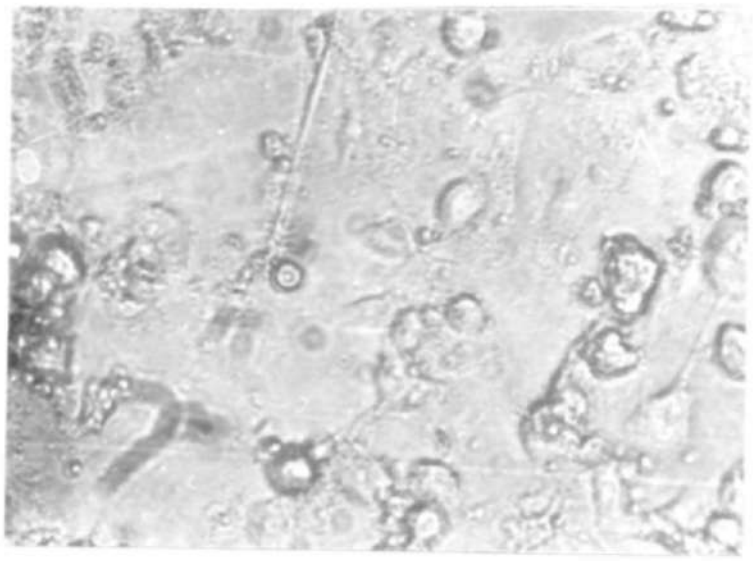
The Cell Control at various stages.

Legend to figures X-XVIII

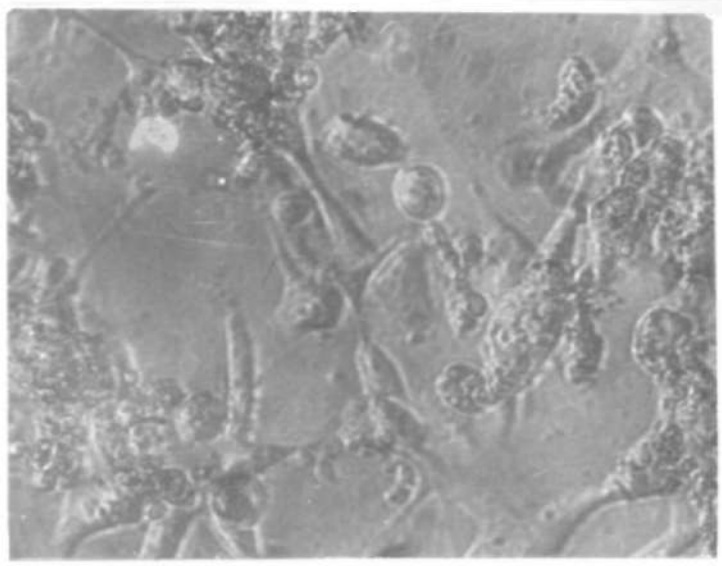
Figures X to XVIII show the complete neutralization test on a selected serum which has measles Hemagglutination Inhibition antibody titer of 1:1024. These figures also revealed the fact that the Hemagglutination Inhibition and Virus Neutralization tests correlate very well.

In the virus neutralization test there was complete neutralization of the virus from well number 1 to 6. CPE is observed after 120 hours in well number 7 which contained antibody dilution of 1:256 and after 96 hours in well number 8 containing antibodies dilution of 1:512 while the last dilution of 1:1024 showed CPE at 48 hours after innoculation with the virus.

Figure X



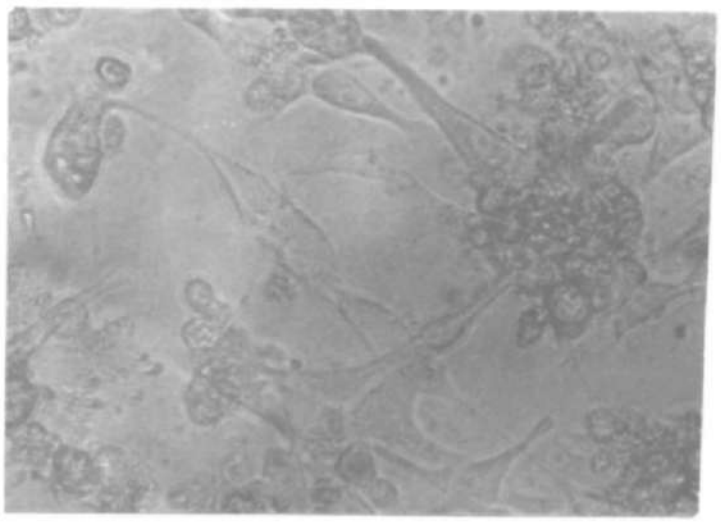
24 hours



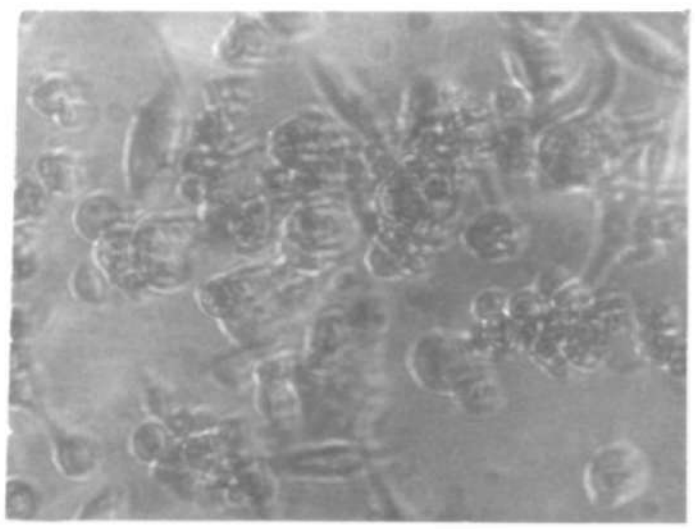
96 hours



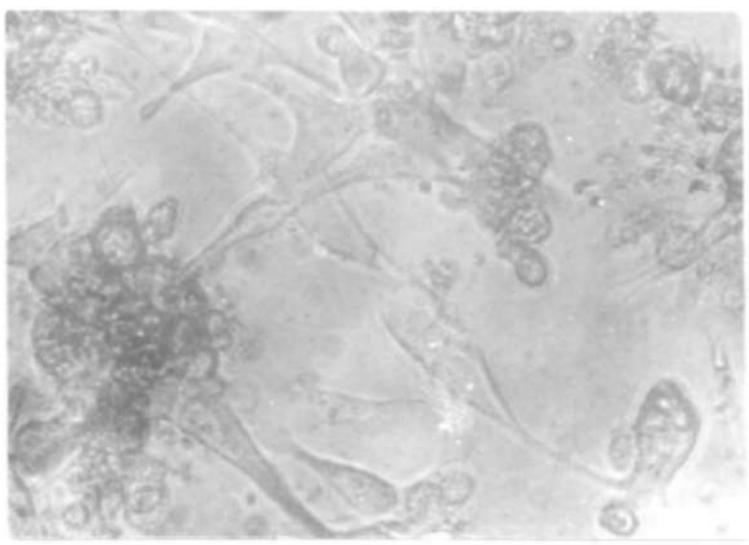
48 hours



120 hours



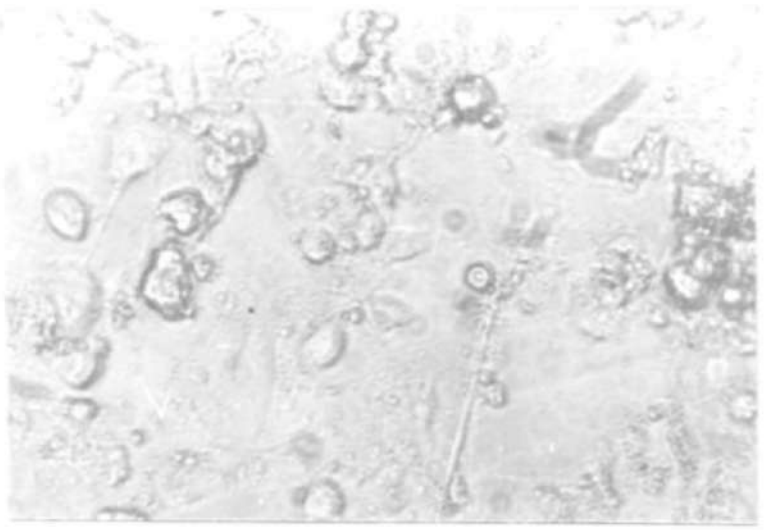
72 hours



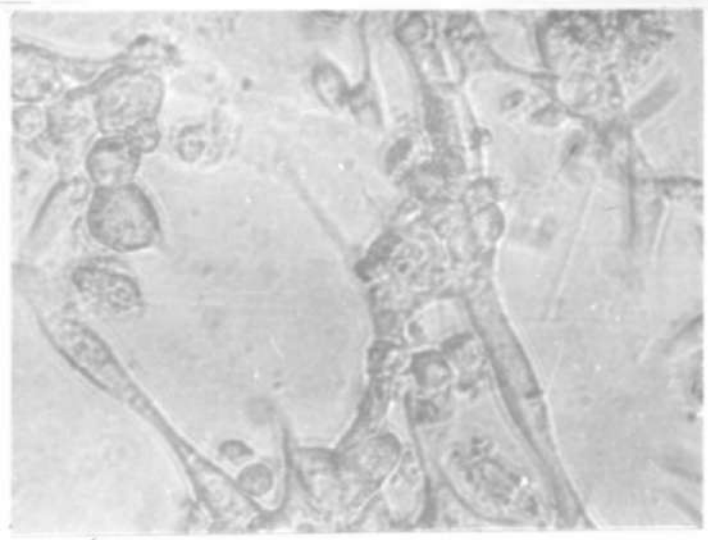
144 hours

Well number 1 containing antibodies diluted out to 1:4

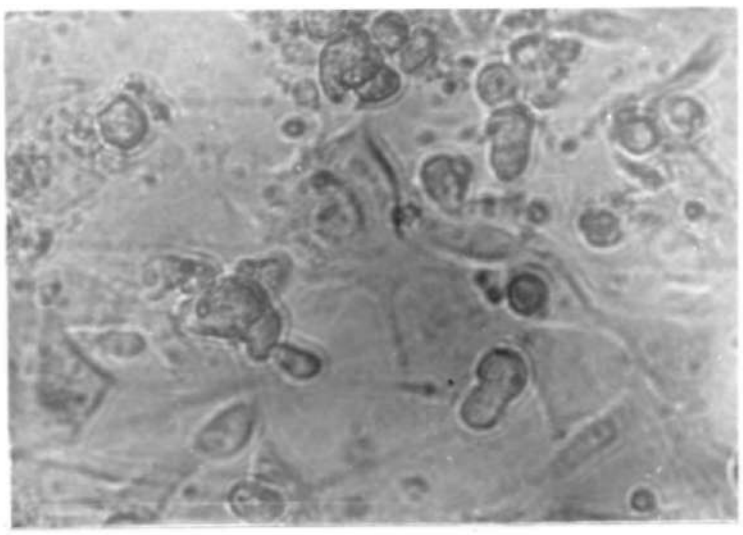
Figure XI



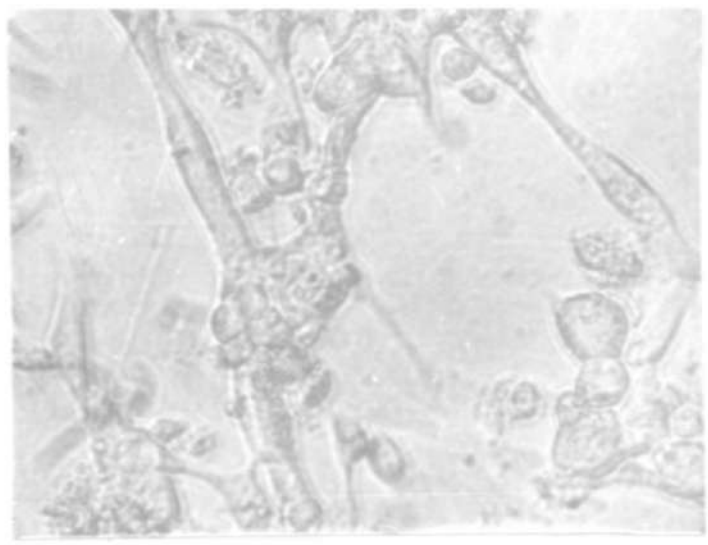
24 hours



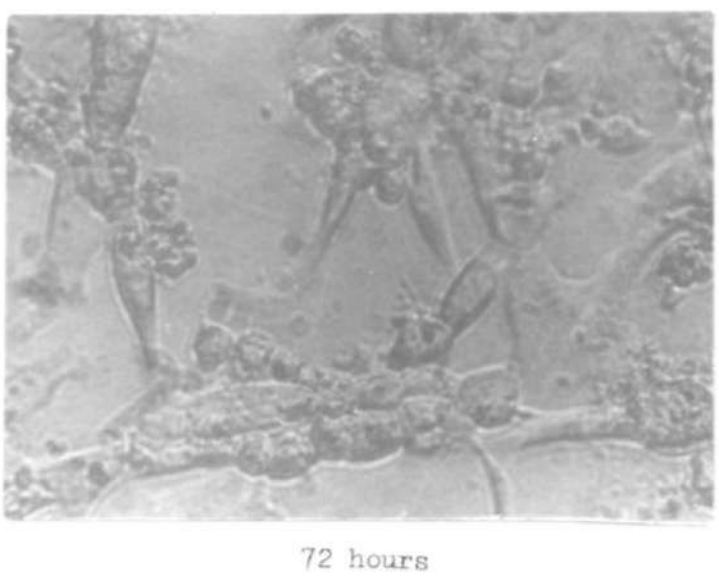
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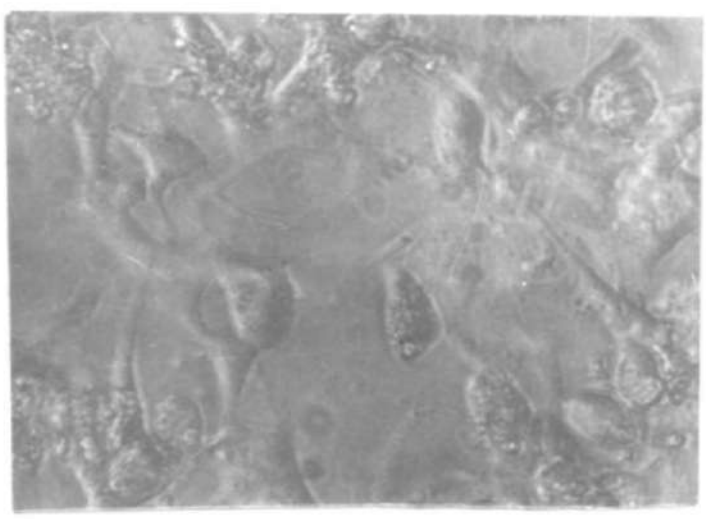
48 hours



120 hours



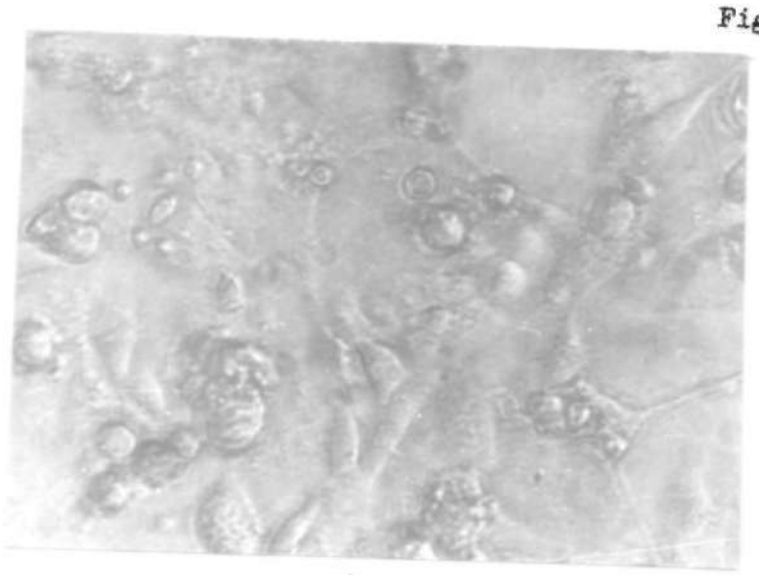
72 hours



144 hours

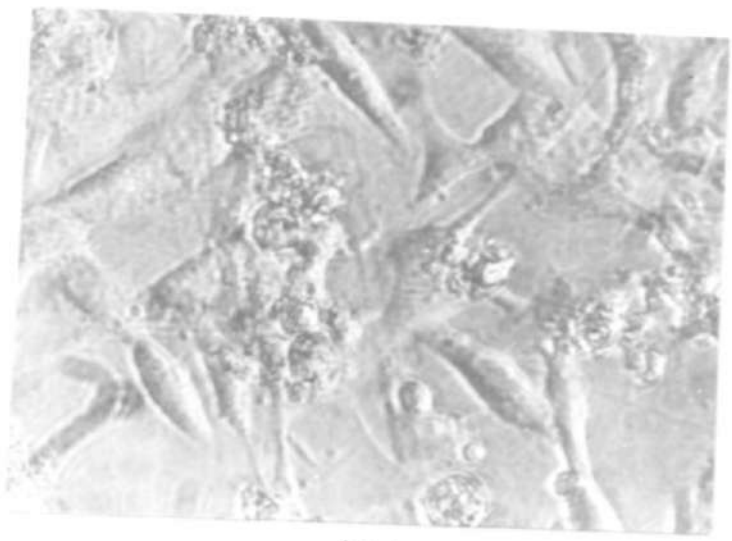
Well number 2 containing antibodies diluted out to 1:8.

Figure XII



24 hours

96 hours



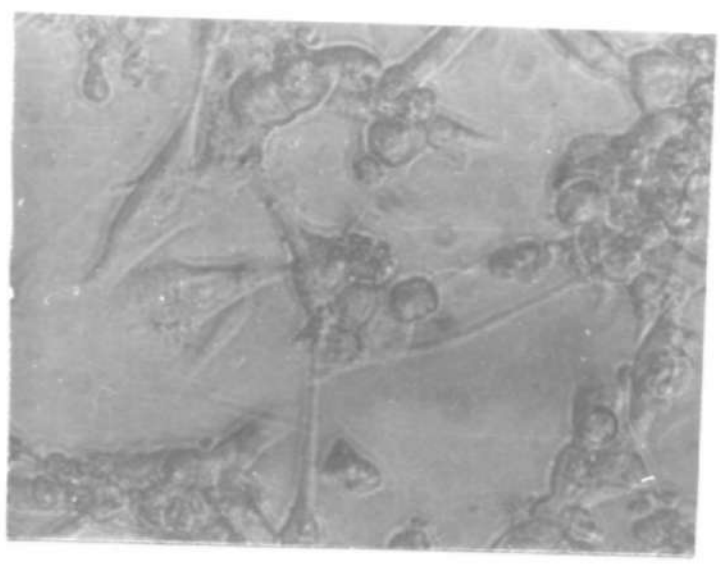
48 hours



120 hours



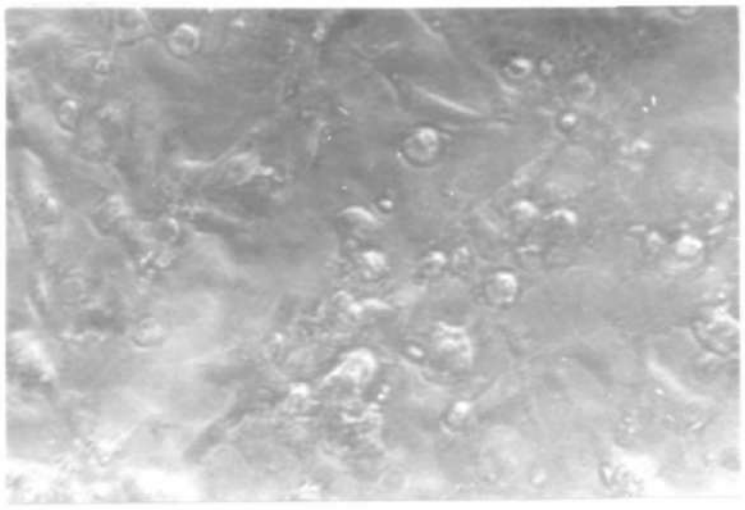
72 hours



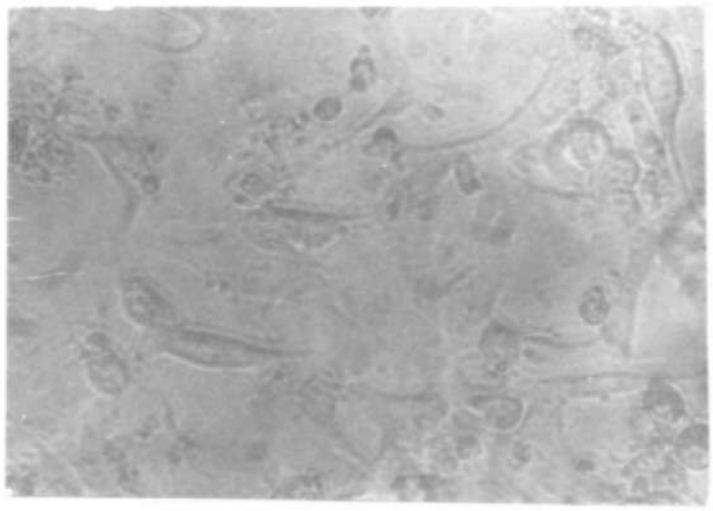
144 hours

Well number 3 containing antibodies diluted out to 1:16.

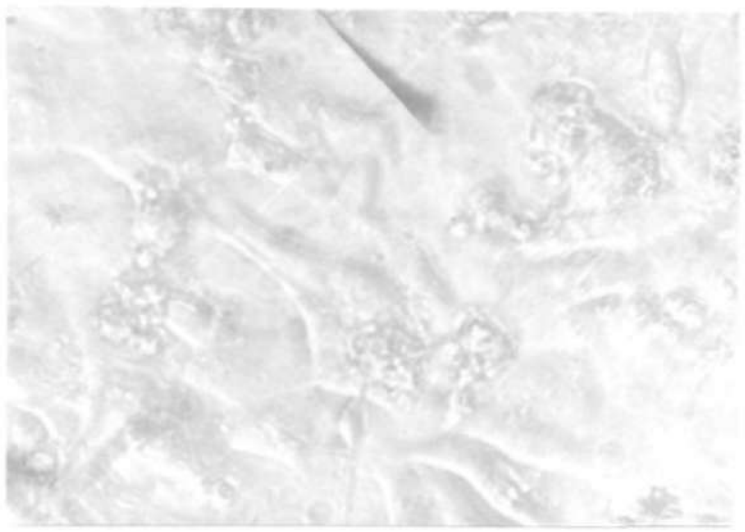
Figure XIII



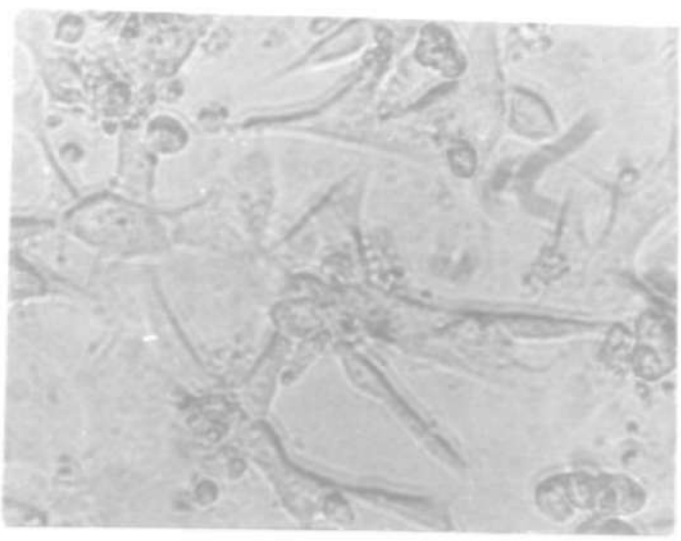
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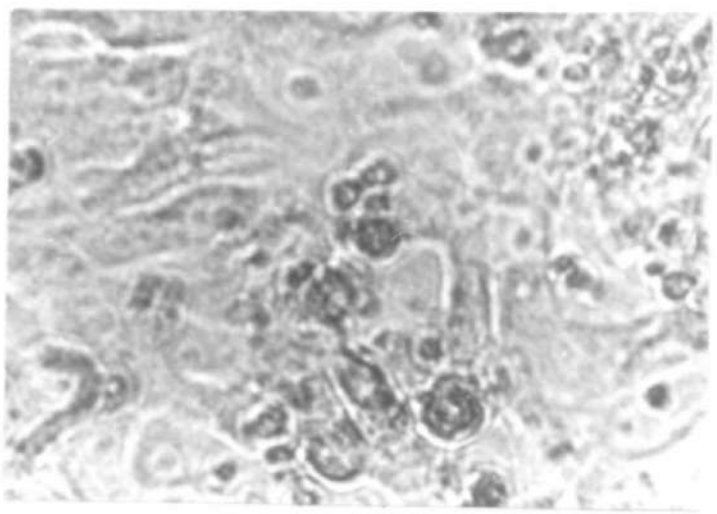
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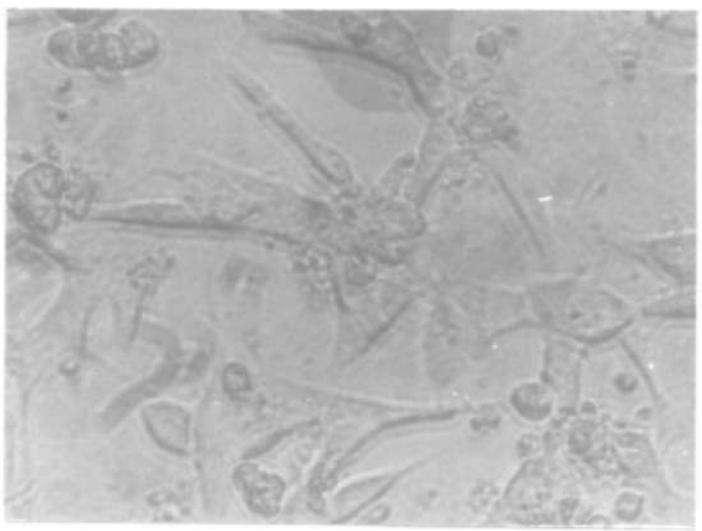
48 hours



120 hours



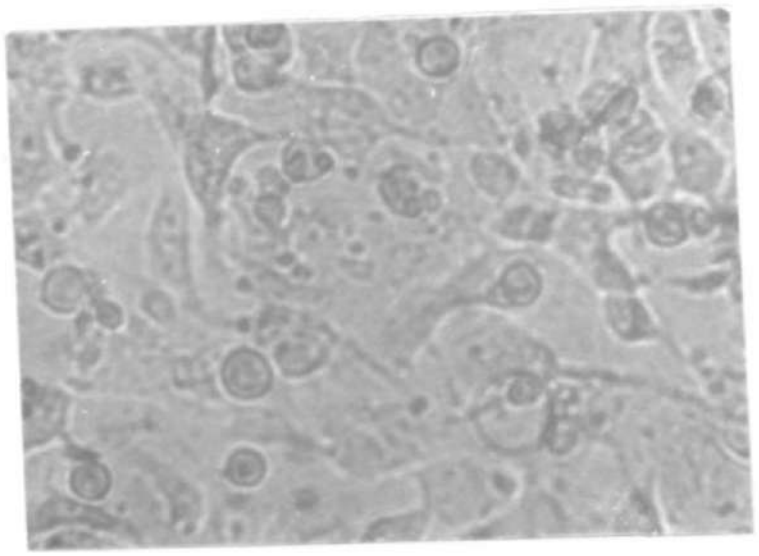
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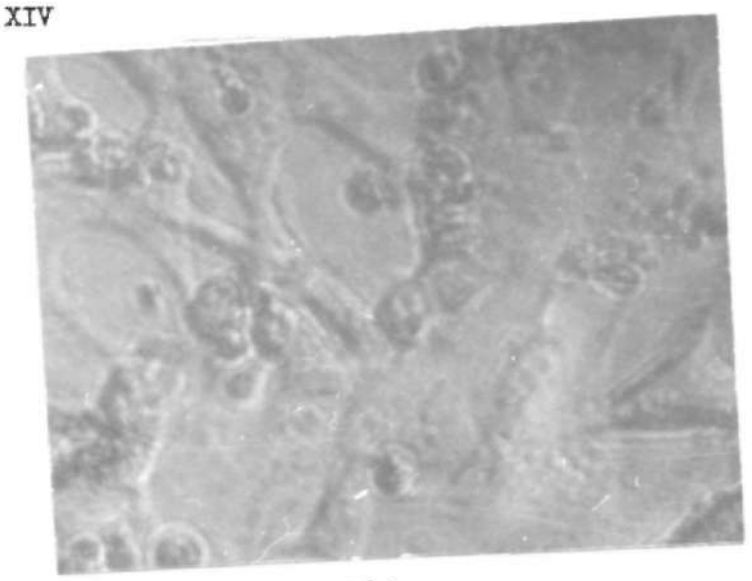
144 hours

Well number 4 containing antibodies diluted out to 1:32.

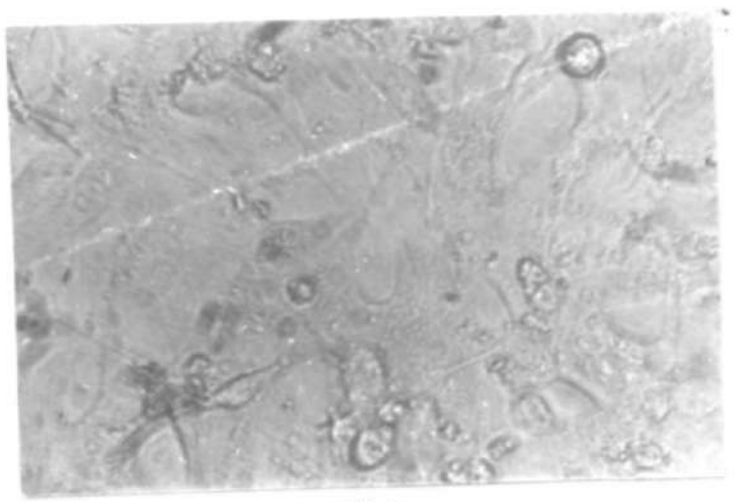
Figure XIV



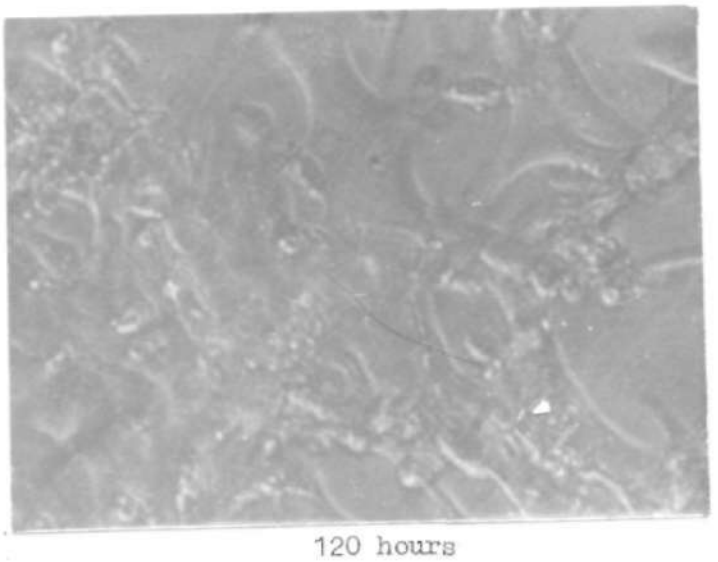
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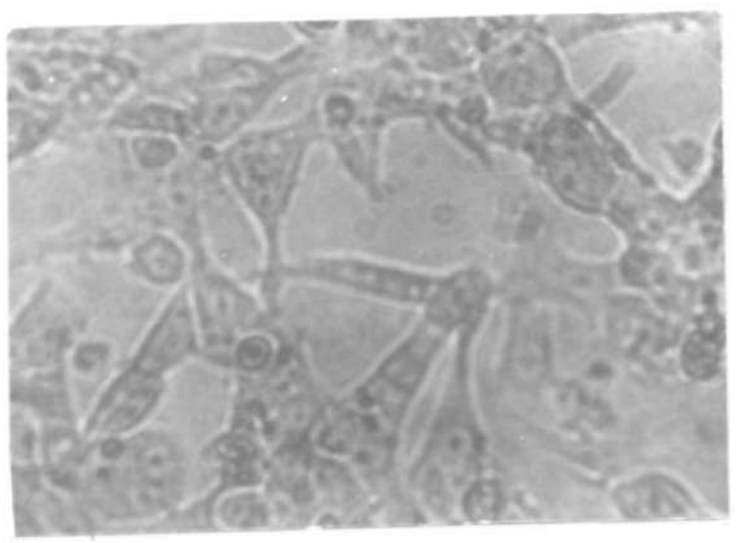
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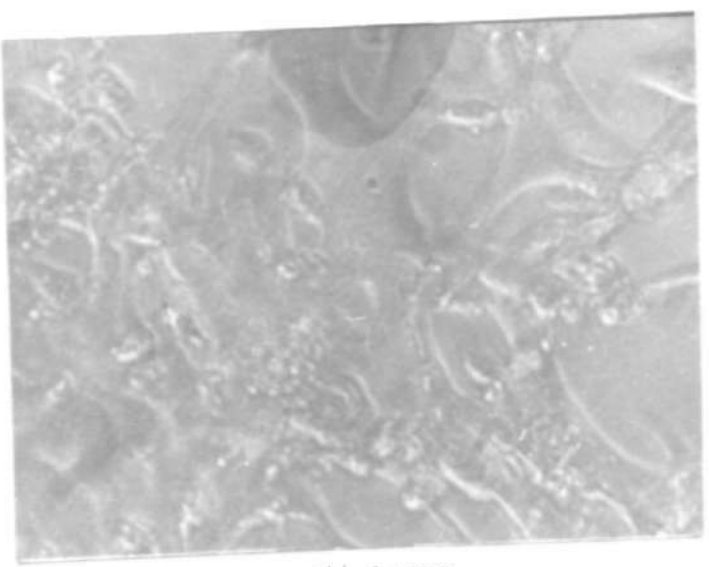
48 hours



120 hours



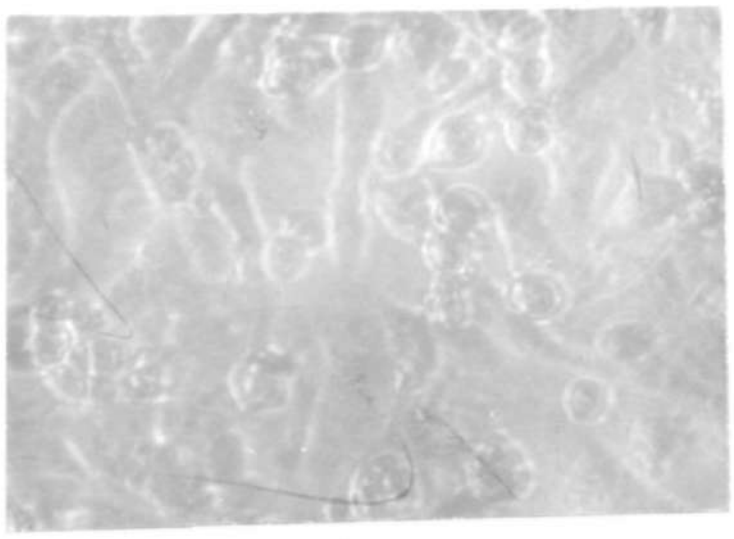
72 hours



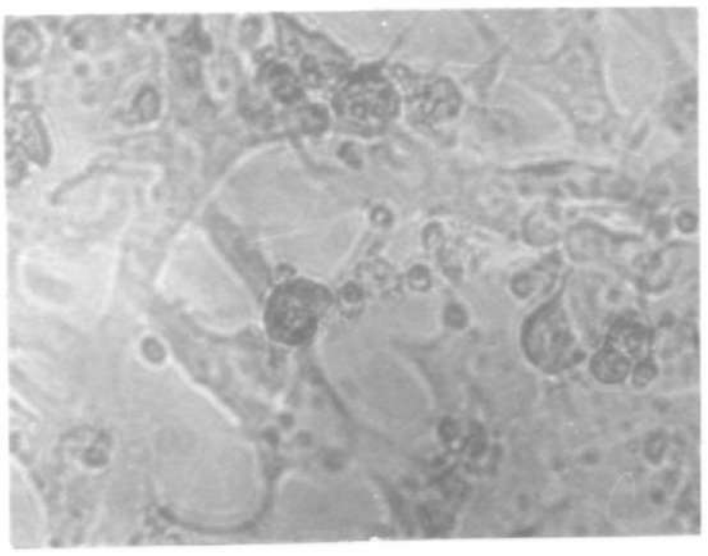
144 hours

Well number 5 containing antibodies diluted out to 1:64.

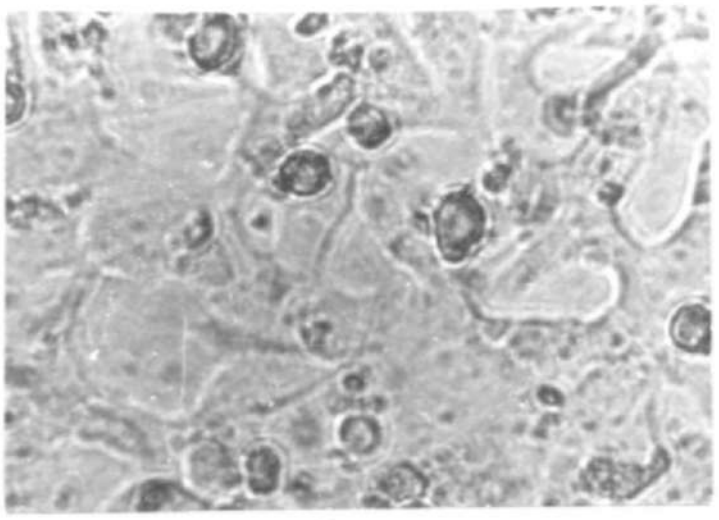
Figure XV



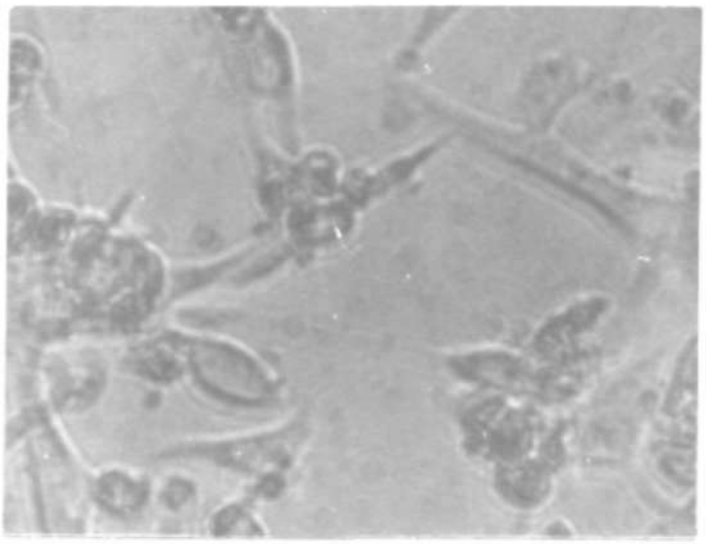
24 hours



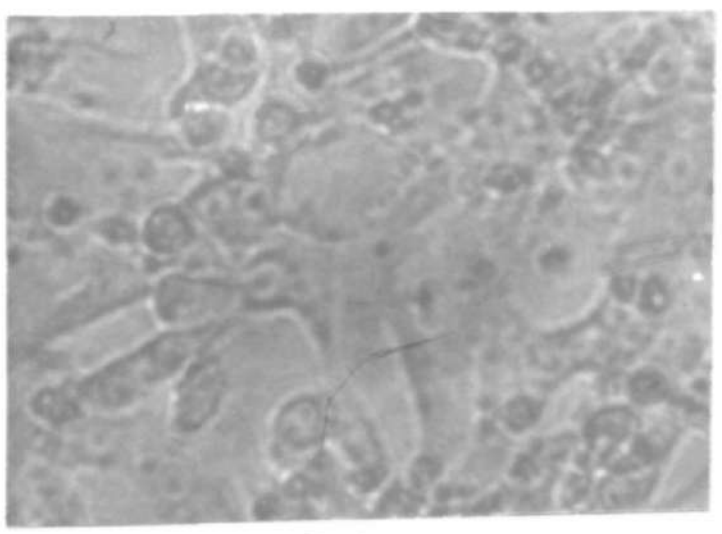
96 hours



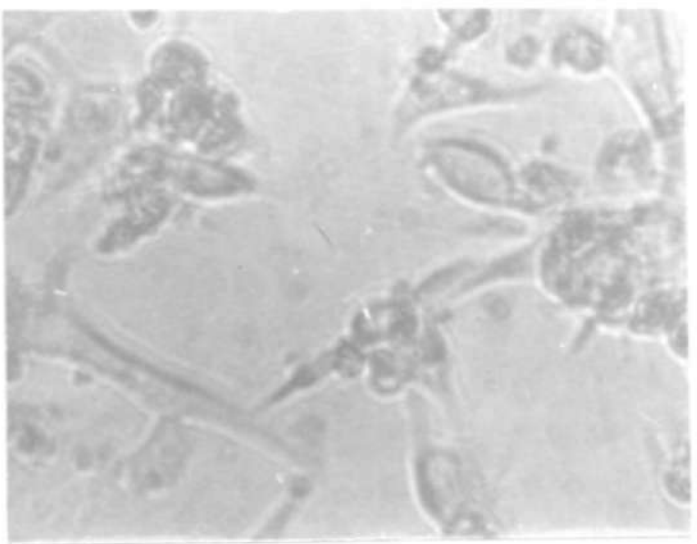
48 hours



120 hours



72 hours



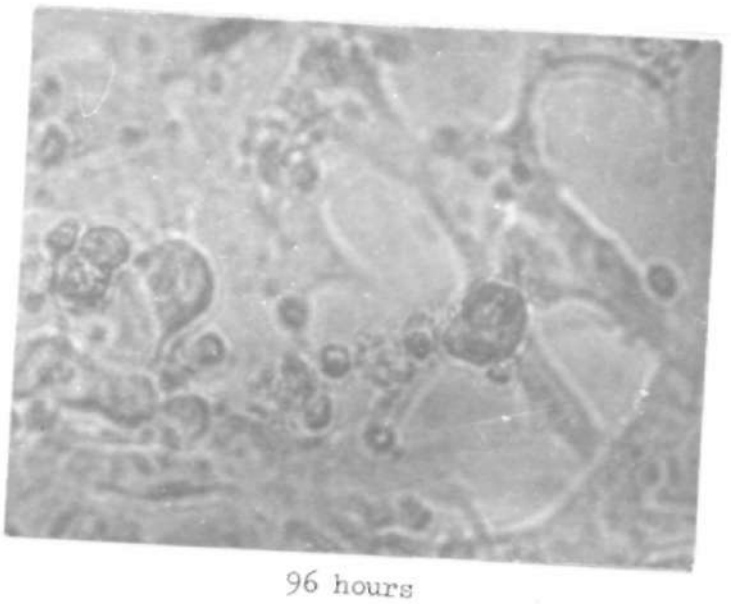
144 hours

Well number 6 containing antibodies diluted out to 1:128.

Figure XVI



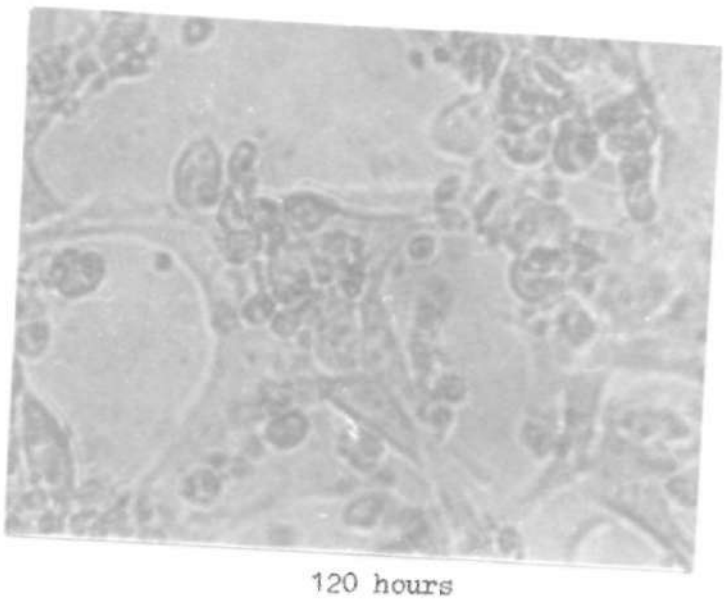
24 hours



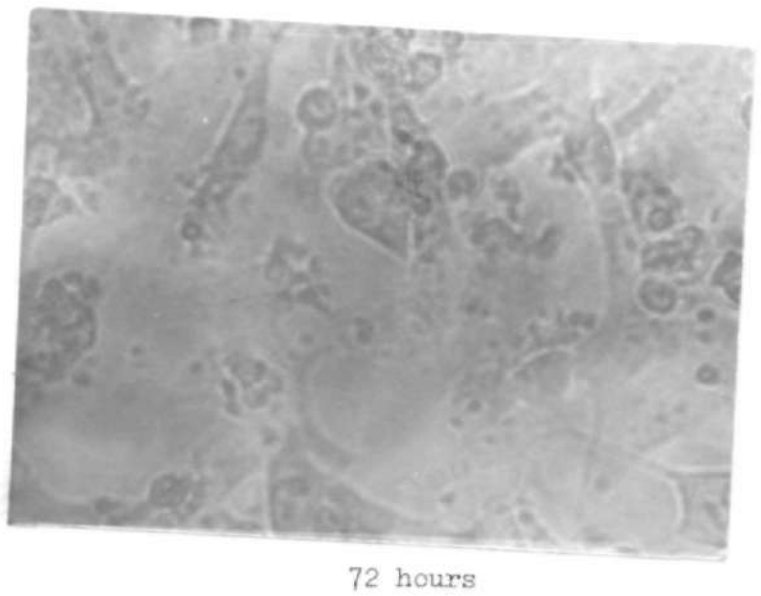
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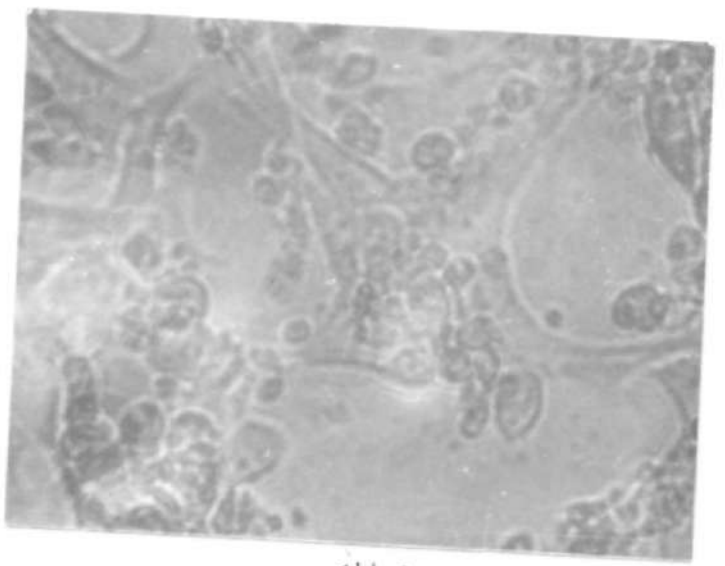
48 hours



120 hours



72 hours



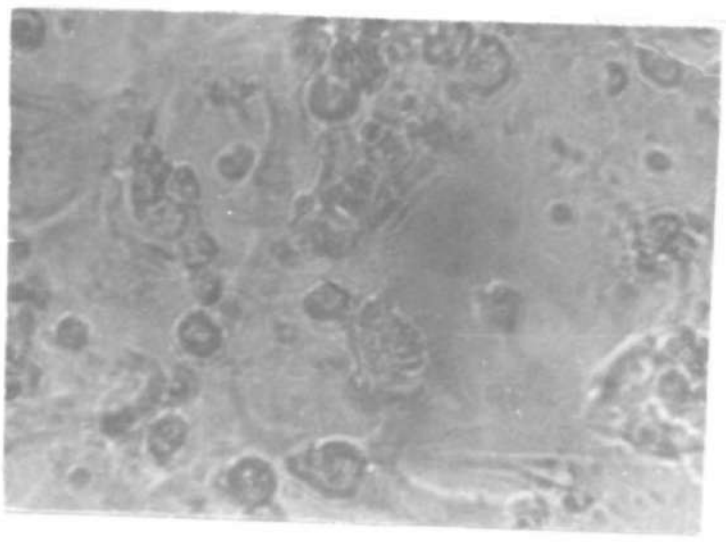
144 hours

Well number 7 containing antibodies diluted out to 1:256.

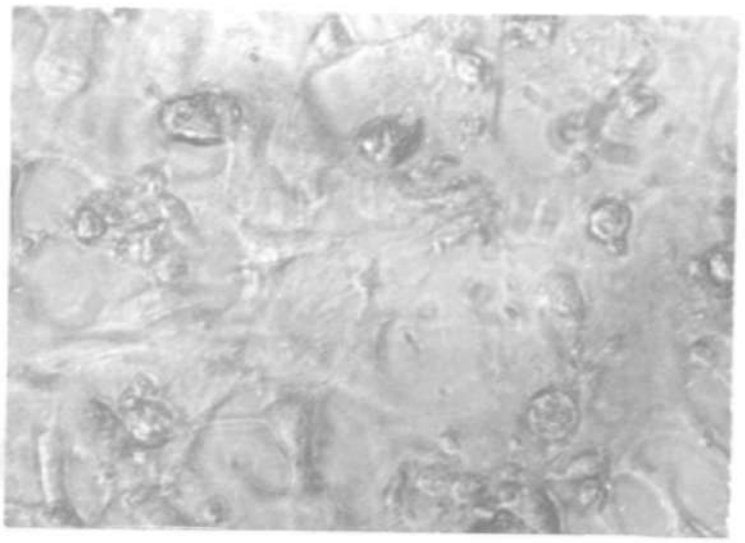
Figure XVII



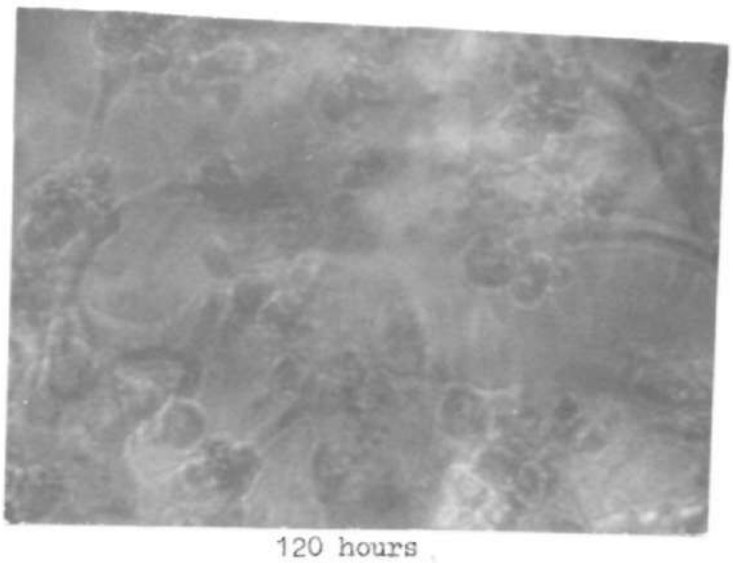
24 hours



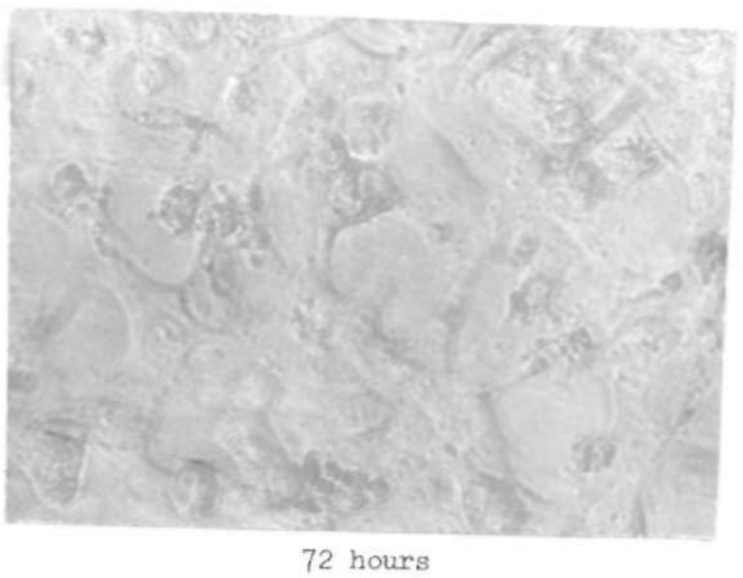
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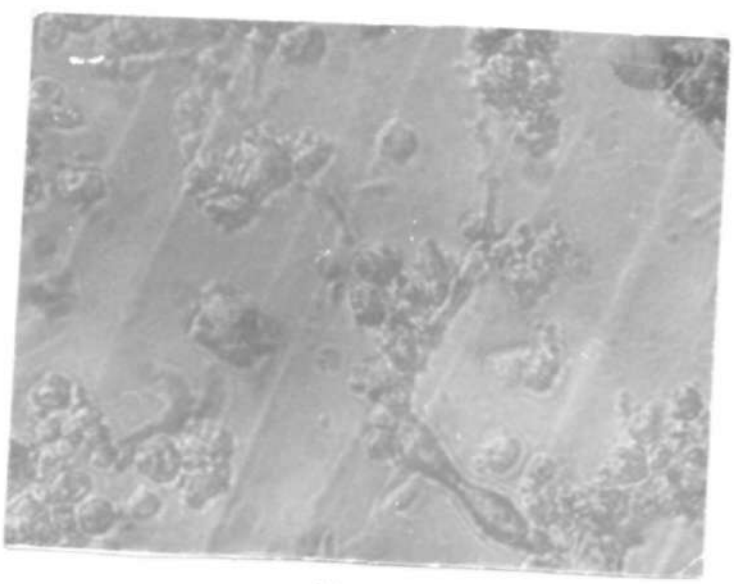
48 hours



120 hours



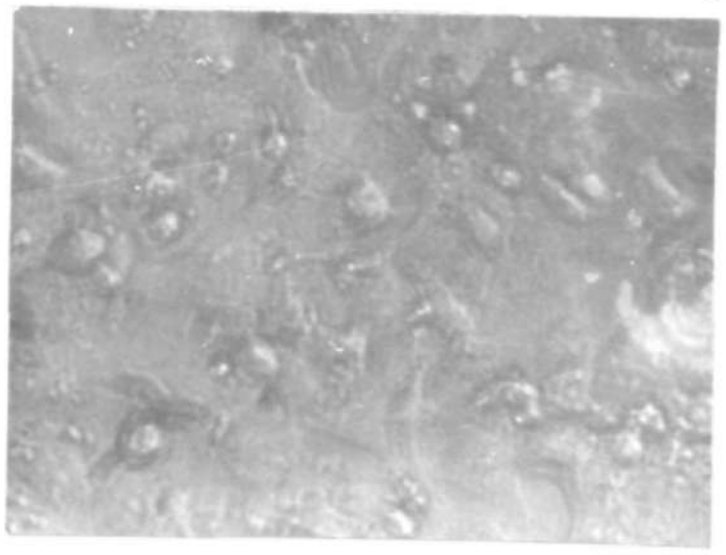
72 hours



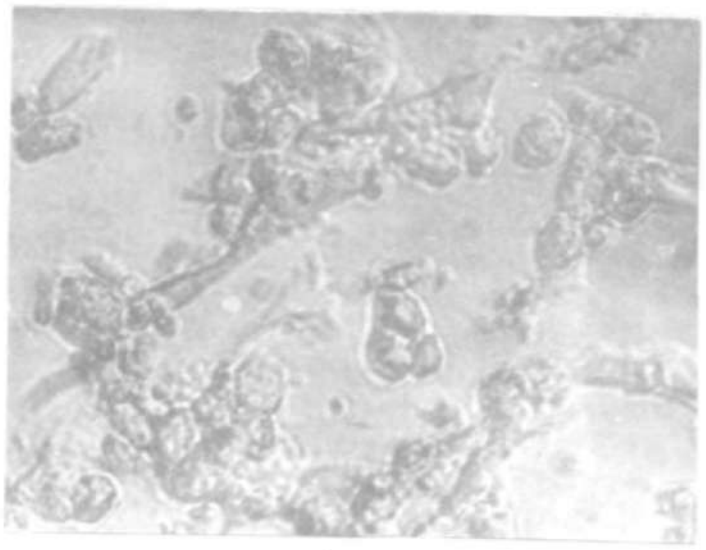
144 hours

Well number 8 containing antibodies diluted out to 1:512.

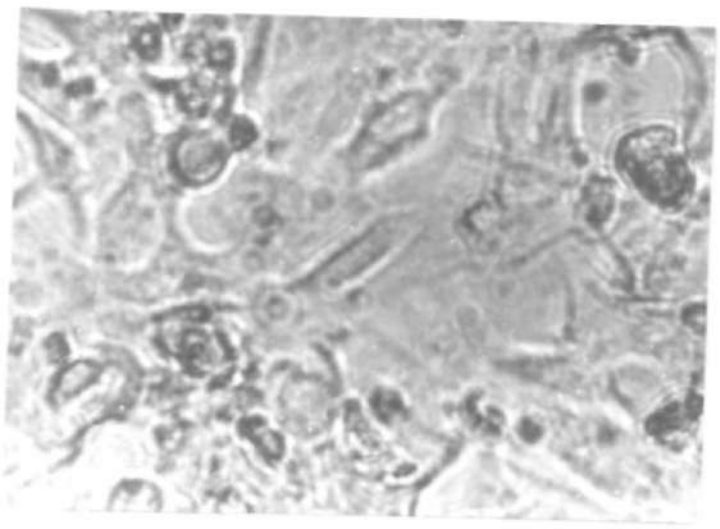
Figure XVIII



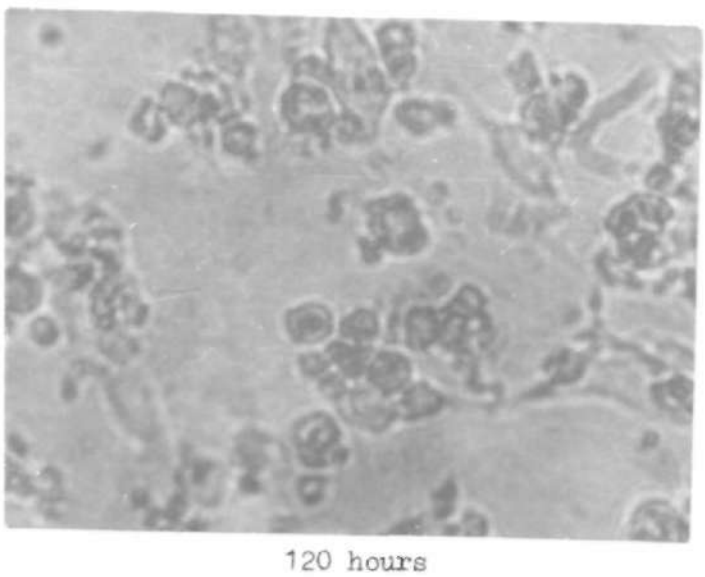
24 hours



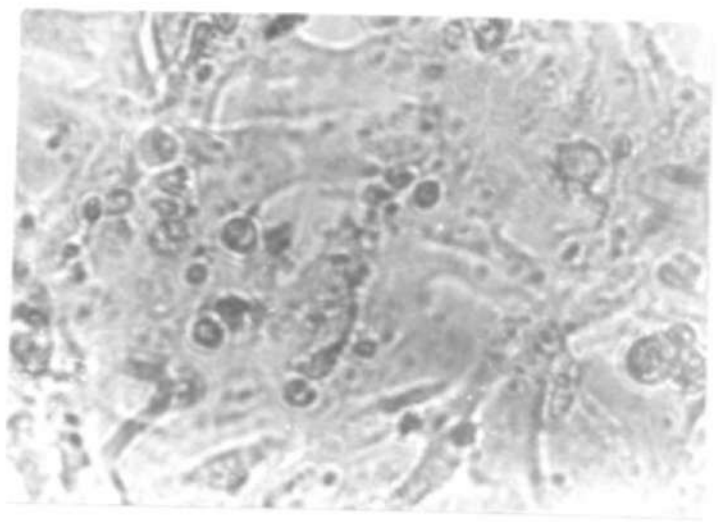
96 hours



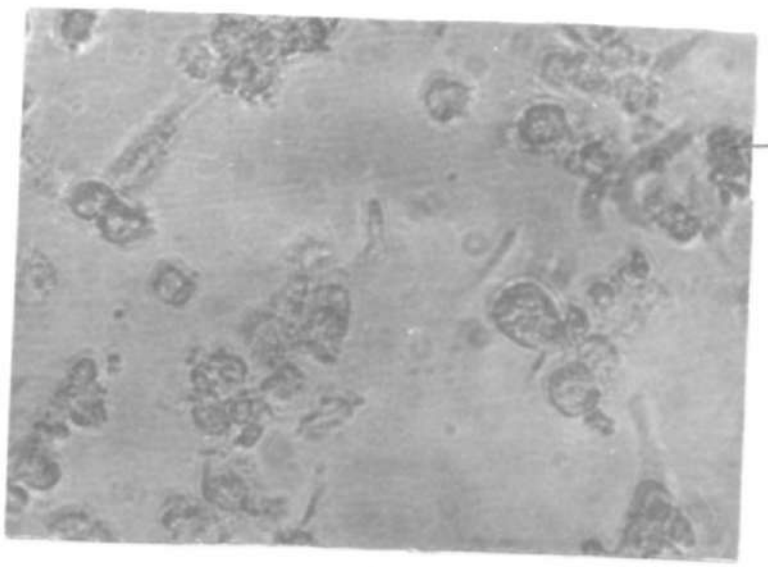
48 hours



120 hours



72 hours



144 hours

Well number 9 containing antibodies diluted out to 1:1024.

DISCUSSION

In measles infection circulating antibodies are detected 10 to 14 days after infection, that is to say when the rash appears or shortly thereafter (Bech, 1959), and the antibody titer reaches maximum by the time the exanthema disappears. Antibody titers (neutralising, complement fixing, and hemagglutination inhibiting) remain high following infection, and it is thought that immunity associated with the presence and level of these antibodies often persists for a life time (Krugman et al. 1965). It is of course to be remembered that measles re-infection in children is a probability in this community, although the proof and the extent of the problem are still to be documented.

In monkeys, re-infection can be produced 3 to 6 months after a primary infection although clinical disease does not appear to ensue.

Morley et al (1963) writings on their experience in Ilesha, stated that clinical and epidemiological characteristics of measles in West Africa today are quite unlike experience of the disease in England. They suggested that the pattern of the disease in West Africa resemble closely measles as seen in England before the 20th century. The information now available in the literature and the disease pattern and intensity highlights the need for concerted research effort on the problem.

Measles antibodies do persist up to eight years after a single immunization (Lepow et al. 1969 and McCrumb et al. 1962). The correlation between vaccine-induced antibodies and protection against clinical disease is well established (Katz et al. 1960; Stokes et al. 1961; McCrumb et al. 1962 and Weibel et al. 1962).

However, reinfection of previously vaccinated children have been reported by many researchers (Lerman et al. 1971; Baratta et al. 1970; Linnemann et al. 1972; 1973; Weiner et al. 1977; and Wyll et al. 1971). Although the reinfection was partly attributed to vaccine failure it could also be due to vaccination of children at early age because maternal antibodies may persist and interfere with immune response to virus vaccine (Lerman et al. 1971 and Reynolds et al. 1972).

It has been shown that some of the vaccines used in this area may not be immunogenic enough to induce antibody formation (Eghafona 1978). However, this study shows that there is a significant difference in hemagglutination inhibition antibody levels between the vaccinated and the unvaccinated areas thus suggesting that some of the vaccines used during mass immunization campaigns might have been used in a potent form thereby gave rise to the high level of hemagglutination inhibition antibodies observed in the vaccinated areas.

The vaccinated areas consist of Zaria and Kaduna, the most thickly populated urban towns in the areas surveyed. The age comparison data (table III) shows that in vaccinated areas antibody level is relatively high in all ages except for ages 7, 10 and 16-20 where there is no significant difference in hemagglutination inhibition antibody level between the two areas.

The possibility of efficient public health services in Kaduna and Zaria in particular is seen in figure II where percentage of pupils with high level of hemagglutination inhibition circulating antibody is much higher in all age groups than the rest of the local government areas. This picture is also true of figure III which shows the average titer in each age group in the different local government areas. In figure II and III it will be observed that the vulnerability to measles infection in the different age group of children vary from one locality to the other, if the level of circulating hemagglutination inhibition antibody is assumed to represent some degree of protection against infection. Furthermore our observation records that in case of a wide spread measles epidemics in the areas studied, more children of 6 years of age are likely to succumb to the infection especially in Ikara, Saminaka Kachia and Jema'a Federation (see figure II), although other age groups may also be affected in an alarming numbers particularly in

Jema'a Federation (see table III and IV). Here again the assumption in this statement is that the level of haemagglutination inhibition and/or *in vitro* neutralization antibodies can be related to protection against infection.

The immunization record (table II) shows that vaccination of children against measles infection in these areas started in 1970, and that the target group (table I) was set as from 5 months to 5 years of age. For the target group, the children who were 12 years at the time when the samples for this work were collected must have benefited from the immunization campaign. Those who were from 13 years upwards at the time of sample collection must have escaped being vaccinated since in 1970 they were above the age limit set.

This suggests that children who were 13 years and above at the time of sample collection were never vaccinated, and the high level of antibodies recorded in these age groups in vaccinated area could be as a result of epidemics which might have occurred much more constantly in these areas , or subclinical infection which might have affected this group. The constancy of epidemics in these thickly populated cities would certainly be more than that in the remaining areas where poeple live in isolated villages, since in the thickly populated areas there are more susceptible individuals present at any given time. Furthermore since these

cities are thickly populated, it can be postulated that more people as many as are susceptible would contact the infection during epidemics.

Thus, since the children in these areas experience constant epidemics than those in isolation many who might have escaped the clinical illness must have had their antibody level increased due to anamnestic response following earlier clinical or subclinical infection.

This then suggests that the high level of antibodies recorded for these areas might not have been as a result of vaccination alone. There is no doubt that villages in the unvaccinated areas do experience epidemics but the constancy and the number of susceptible individuals at a given time may not be as high as that found in the thickly populated areas.

Furthermore, if vaccination campaign against measles started in some parts of Kaduna state in 1970 as Table II indicates, and that as of 1970 at least in some parts of Kaduna state children between 5 months and 5 years were vaccinated, the lack of significant difference in hemagglutination inhibition antibody levels in age groups 7 years, 10 years and 16-21 years between the vaccinated and the unvaccinated areas can be interpreted in several ways. Firstly in 1977 when this survey was carried out it is possible that only a small percentage of the total births in the

vaccinated areas were up to 5 months old at the time of immunization campaign and therefore majority of the children born in 1970 were less than five months old at the time of mass immunization campaign and therefore were not vaccinated.

Secondly it is also possible that the viral vaccines used during the campaign in 1970 had lost their potency, and therefore the levels of hemagglutination inhibition antibody observed in the vaccinated areas during this period represents an identical situation as observed in the unvaccinated areas, hence the hemagglutination inhibition antibody in the two areas were almost identical, or at least no statistical different levels of hemagglutination inhibition measles antibodies were observed. Thirdly since the table under reference did not specify the towns and villages where mass immunization campaigns were carried out in 1970 as is the case in 1976, it is possible that we had infact investigated two identically unimmunized areas and assumed that one area represents vaccinated area, just because the data in 1976 showed that this particular area was immunized whereas the 1970 campaign might not have covered the areas under reference.

Children in the age group of 10 years, must have been about three years old at the time of the first mass immunization campaign in Kaduna state and therefore the lack of differences in the hemagglutination inhibition antibody levels in this group could be due

to either that the areas we have called vaccinated areas were not infact vaccinated in 1970 and in subsequent years that followed, or if the mass campaign in 1970 or in the years that followed did reach these areas the most probable reason for the lack of differences in haemagglutination inhibition antibody levels could be due to vaccine failure or vaccine impotency.

On the other hand the pupils who were between 16 years and 21 years at the time of our investigation, must have been between 9 years and 14 years of age at the time of first immunization campaign in 1970, therefore even if these groups of pupils were resident in the vaccinated areas, they still would have been missed throughout the course of the immunization campaign because they had exceeded the target age range for immunization at these periods. One can then assume that like the same age groups in the unvaccinated areas, the levels of Haemagglutination Inhibition antibody observed in these groups can be as a result of subclinical or earlier measles infection, or infections by some other viral organism(s) showing similar antigenic determinants.

The tissue culture test clearly revealed the length of time certain levels of antibodies can prevent multiplication of the measles virus.

The *in vitro* neutralization test of serum with haemagglutination

inhibition antibody (HI) titer of 1:4 shows cytopathic effect (CPE) after about 36 hours (figure V) while serum with 1.1[illegible] hemagglutination inhibition antibody titer showed CPE the third day after viral innoculation (figure VI). However the serum with no detectable HI antibodies showed CPE barely after 24 hours (fig. VII).

If this in vitro process could be equated to what may happen in vivo, it suggests that during measles infection, any child with antibody levels of 1:4 or 1:16 would be able to keep the virus replication under control for 36 hours or 3 days respectively, after which virus multiplication may start in the absence of complete in vivo neutralization and or secondary immune response.

Although any measurable hemagglutination inhibition antibody level was supposed to be indicative of immunity (Anders-Ruckle 1965) it is not known to us that any work has been done which could presume to establish the period it takes a subject to start antibody production after infection with measles virus. Therefore we could only assume as of now that if the appearance of rash is associated with appearance of antibodies (Bech, 1959), the antibody level of 1:4 and 1:16 may not be enough during an infection to prevent viral multiplication. On the other hand if antibody production in measles infection follows immunological patterns, antibody level of 1:16 could inhibit the viral multiplication

until the initiation of anammestic response which usually leads to the boostering of the antibody level subsequently leading to complete neutralization of the viruses.

However, more work is required in this area so as to establish specifically the period of antibody production in measles infection and shade more light in an *in vivo* situation as to the lowest antibody level that will prevent reinfection in case of measles epidemics, this is particularly very important since there are many reports in the literature pointing to failures of active immunization against measles in many parts of the world and in Nigeria particularly Fabiyi and Tomori in 1973 reported an outbreak of measles disease in vaccinated children.

It has been observed that measles antibody level declines with time (Bass et al. 1976; Yeager, 1976). The picture observed in Malumfashi (Figure III) showed a decline in antibody level with increasing age. Although Malumfashi was grouped under unvaccinated area, there is a possibility that children in this local government area have good health care. In this small town surveyed there is an arm of Ahmadu Bello University Teaching Hospital. Since it is a routine in the Hospital to give appropriate immunization to children who attend their clinics at the appropriate time, greater percentage of the children in this village must have been actively immunized before they reach school age. The same story goes for Birnin Gwari and Funtua; these towns are the only major towns in these local government areas. The rest are small villages some of which could not be reached because the roads leading to them are not motorable. However these towns have hospitals which can cater for the small populations.

In Jema'a Federation and Kachia local government areas where very low level of antibodies were observed in all age groups (figure II and III) the most tempting interpretation could be as a result of either inadequate health care or lack of proper health education.

Figure IV shows that measles vaccine could be reliable as a good preventive measure as has been reported in the developed countries, the periodic vaccine failures notwithstanding. Although there is an ample evidence to show that it may not be unlikely that some of the batches of measles vaccines delivered to various public health units in Nigeria may have lost their potency and therefore their antigenic values may be in doubt, nevertheless there is enough scientific evidence as revealed by this study and other clinical studies reported earlier, the advantages of vaccination campaign against measles in Nigeria outweighs the disadvantages arising mainly from cases of loss of potency (Eghafona 1978) and outbreaks of measles in vaccinated children (Fabiyi and Tomori, 1973). There are no doubts to the fact that with improving physical facilities and good net work of roads as well as the availability of improved measles vaccines that can stand higher temperatures than was the case earlier, the sources of problems leading tc the loss of potency through temperature effects and bad handling will be on the decline with time.

Our results also point to the need for active participation by Nigerian scientists in quality control of biological materials, imported and locally produced, monitoring of the effectiveness of such biological preparations in use as well as surveillance of the patterns of epidemics, in areas where such preparations have been prophylactically used.

REFERENCES

1. Adams, J.M., C. Baird, and L. Filloy, 1966. Inclusion bodies in measles encephalitis. J. Am. Med. Assoc. 195:290-298.

2. Ricardi, J., P. Coutieres, M.L. AresenioNunes, and P. Lebon. 1977. Acute measles encephalitis in children with immunosuppresion. Pediatrics 59:232-239.

3. Anderson, C.D., and J.G. Atherton. 1964. Effect of actinomycin D on measles growth and interferon production. Nature (London) 203:671.

4. Atkins, G.J., and C.L. Lancashire. 1976. The induction of interferon by temperature-sensitive mutants of Sindbis virus: its relationship to double-stranded RNA synthesis and cytopathic effect. J. Gen. Virol. 30:157-165.

5. Babbot, F.L. Jr., and Gordon, J.E. (1954). Modern measles. Amer. J. Med. Sci. 228, 334.

6. Baker, R.F., J. Gordon, and F. Rapp. 1960 Electron dense crystallities in nuclei of human amnion cells infected with measles virus. Nature (London) 185:790-791.

7. Baker, R.F., J. Gordon, and D. Stevenson, 1965. Electron microscope study of hemadsorption in measles virus infection. Virology 27:441-445.

8. Baratta, R.O., Gunter, M.C., Prince, M.L., Walker, J.F., Brunner, M.G., Prather, E.C. and David, J.K. (1979) Measles (Rubeola) in previously immunized children Pediatrics 46(3), 397-402.

9. Barry, D.W., J.L. Sullivan, B.J. Lucas, R.C. Dunlap, and P. Albrecht. 1976. Acute and chronic infection of human lymphoblastoid cell lines with measles virus. J. Immuno. 116:89-98.

10. Bass, J.W., Halstead S.B., Fischer G.W., Podgore, J.K., Pearl, V.R., Schydlower, M., Weibe R.... and Ching, F.H. (1976) Booster vaccination with further live attenuated measles vaccine. J. Amer. Med. Assoc. 235(1) 31-34.

11. Bech, V. (1959) Studies on the Development of complement fixing antibodies in measles patients. Observations during a measles epidemics in Greenland. J. Immunol. 83 267-275.

12. Bech, V. 1965. The measles epidemic in Greenland in 1962. Arch. Gesamte Virusforsch. 16:53-56.

13. Bellanti, J. . 1975. Adverse effects of viral vaccines, p. 327-399. In A.L. Notkins (ed), Viral immunology and immunopathology. Academic Press Inc., New York.

14. Bergholz, C.M., H.P. Kiley, and F.E. Payne. 1975. Isolation and characterization of temperature-sensitive mutants of measles virus. J. Virol. 16:192-202.

15. Breitfeld, V., Y. Hashida, F.B. Sherman, K. Odagiri, and M.J. Yunis. 1973. Fatal measles infection in children with measles. Lab. Invest. 28:279-291.

16. Breschkin, A.M., H.V. Haspel, and F. Rapp. 1976. Neurovirulence and induction of hydrocephalus with parental, mutant, and revertant strains of measles virus. J. Virol. 18:809-811.

17. Breschkin, A.M. H. Walmer, and F. Rapp. 1977. Hemagglutination variant of measles virus. Virology, in press.

18. Brody, J.A. 1972. Epidemiology of multiple sclerosis and a possible virus aetiology. Lancet 2:173-176.

19. Brody, J.A., J.L. Sever, A. Edgar, and J. McNew. 1972. Measles antibody titers of multiple sclerosis patients and their siblings. Neurology 22:492-499.

20. Brown, P., P. Cathala, D.C. Gajdusek, and C.J. Gibbs, Jr. 1971. Measles antibodies in the cerebrospinal fluid of patients with multiple sclerosis. Proc. Soc. Exp. Biol. Med. 127:956-961.

21. Burnet, F.M. 1968. Measles as an index of immunological function. Lancet 2:610-613.

22. Bussell, R.H., D.J. Waters, H.K. Seals, and W.B. Robinson. 1974. Measles, canine distemper and respiratory syncytial virions and nucleocapsids. A comparative study of their structure, polypeptide and nucleic acid composition. Med. Microbiol. Immunol. 160:105-124.

23. Carter, C.F.L. Black, and A. Schluederberg. 1973. Nucleus-associated RNA in measles virus-infected cells. Biochem. Biophys. Res. Commun. 54:411-416.

24. Cascardo, A.R., and D.T. Karzon. 1965. Measles virus giant cell inducing factor (fusion factor) Virology 26:311-325.

25. Choppin, P.W., and R.W. Compans. 1975. Reproduction of paramyxoviruses, p. 95-178. In H. Fraenkel-Conrat and R.R. Wagner (ed.), Comprehensive virology, vol. 4. Plenum Press, New York and London.

26. Compans, R.W., and P.W. Choppin. 1971. The structure and assembly of influenza and parainfluenza viruses, p. 407-432. In K. Maramorosch and P. Kurstak (ed.), Comparative virology. Academic Press Inc., New York.

27. Compans, R.W., H.V. Holmes, S. Dales, and P.W. Choppin. 1966. An electron microscopic study of moderate and virulent virus-cell interactions of the parainfluenza virus SV5. Virology 30:411-426.

28. Conover, W.J. (1971). Practical non-parametric Statistics. John Wolley and Sons Inc. New York.

29. Csonka, E., P. Ruzicksa, and A.S. Koch. 1975. Chromosome abberations in cells infected with various strains of measles virus. Acta Microbiol. Acad. Sci. Hung. 22: 41-44.

30. Dawson, J.R. 1933. Cellular inclusions in cerebral lesions of lethargic encephalitis. Am. J. Pathol. 9:7-16.

31. Dayan, A.D., J.V.T. Gostling, J.L. Greaves, D.M. Stevens, and A.A. Woodhouse. 1967. Evidence of a pseudomyxovirus in the brain in subacute sclerosing leucoencephalitis. Lancet 1:890-981.

43. Ehrnst, ..C. 1975. Characterization of measles virus-specific cytotoxic antibodies by use of a chronically infected cell line. J. Immunol. 114:1077-1082.

44. Ehrnst, A., and K.G. Sundqvist. 1975. Polar appearance and non-ligand induced spreading of measles virus hemagglutinin at the surface of chronically infected cells. Cell 5:351-359.

45. Ehrnst, A., I. Weiner, and E. Norrby. 1974. Fluctuations and distribution of measles virus antigens in chronically infected cells. Nature (London) 248:691-693.

46. Enders-Ruckle, G. (1965). Methods of determining immunity, duration and character of immunity resulting from measles. Arch. ges. virusforsch. 16:182-207.

47. Enders, J.F., and T.C. Peebles. 1954. Propagation in tissue cultures cytopathogenic agents from patients with measles. Proc. Soc. Exp. Biol. Med. 86:277-286.

48. Enders, J.., I. McCarthy, A. Mitus, and W.J. Cheatham. 1959. Isolation of measles virus at autopsy in cases of giant-cell pneumonia without rash. N. Engl. J. Med. 261:875-881.

49. Fabiyi, A. and Tomori, O. 1973. Outbreak of measles Disease in vaccinated children. Paper presented at the East African Medical Research Council, Annual Scientific Conference, Nairobi, Kenya.

50. Fenner, F. 1976. Classification and nomenclature of viruses. Second report of the international committee on taxonomy of viruses. Intervirology 7:59-60.

51. Finkel, ..., and P.B. Dent. 1973. Abnormalities in lymphocyte proliferation in classical and atypical measles infection. Cell. Immunol. 6:41--48.

52. Fireman, P., G. Friday, and J. Kumate. 1969. Effect of measles vaccine on immunologic responsiveness. Pediatrics 43:264-272.

53. Fjelde, A., and O.K. Holtermann. 1962. Chromosome studies in HEp-2 tissue culture cell line during infection with measles virus. Life Sci. 12:683-692.

54. Flanagan, J.B., and J.H. Henna. 1976. Induction of measles virus hemagglutinin in a persistently infected, non-virogenic line of cells (BCH/HV). J. Virol. 17:1052-1055.

55. Follett, E.A.C., C.R. Pringle, T.N. Pennington, and S. Shirodaria. 1976. Events following the infection of enucleate cells with measles virus. J. Gen. Virol. 32:163-175.

56. Frankel J.W., and M.K. West. 1958. Cultivation of measles virus in stable line of human amnion cells. Proc. Soc. Exp. Biol. Med. 97:741-742.

57. Freeman, J.A., R.L. Magoffin, E.H. Lennette, and R.A. Herndon. 1967. Additional evidence of the relation between subacute inclusion body encephalitis and measles virus. Lancet 2:129-131.

58. Gans H., Macnamara F.N., Horley D.C., Thomson C.T. and Watt A. (1961). Some observation on the Epidemiology of measles in West Africa. The West Afr. Med. J. 10, 253-262.

59. Gibbs, F.A., E.L. Gibbs, P.R. Carpenter, and H.T. Spies. 1959. Electro-encephalographic changes in "uncomplicated" childhood disease. J. Am. Med. Assoc. 171:1050-1055.

60. Gibson, J.A., and T.M. Bell. 1972. Persistent infection of measles virus in mouse brain cell cultures infected in vivo. Arch. Gesamte Virusforsch. 37:45-53.

61. Gillespie, J.H., and D.T. Karzon. 1960. A study of the relationship between canine distemper and measles in the dog. Proc. Soc. Exp. Biol. Med. 105:547-551.

62. Good, R.A., and S.J. Zak. 1956. Disturbances in gamma globulin synthesis as "experiments of nature." Pediatric 18:109-149.

63. Gould, E.A., and P.M. Linton. 1975. The production of a temperature-sensitive persistent measles virus infection. J. Gen. Virol. 28:21-28.

64. Gould, E.A., S.L. Cosby, and P.V. Shirodaria. 1976. Salt-dependent hemagglutinating measles virus in S.S.P.E. J. Gen. Virol. 33:139-142.

65. Graziano, K.D., J.C. Rukdeschel, and M.R. Hardiney Jr. 1975. Cell-associated immunity to measles (rubeola). The demonstration of in vitro lymphocyte tritiated thymidine incorporation in response to measles complement fixation antigen. Cell. Immunol. 15:345-359.

66. Hall, W.W., and S.J. Martin. 1973. Purification and characterization of measles virus. J. Gen. Virol. 19:175-188.

67. Hall, W.W., and S.J. Martin. 1974. Structure and function relationships of the envelope of measles virus. Med. Microbiol. Immunol. 160:143-154.

68. Hall, W.W., and S.J. Martin. 1974. The biochemical and biological characteristics of the surface components of measles virus. J. Gen. Virol. 22:363-374.

69. Hall, W.W., and V. terMeulen. 1976. RNA homology between subacute sclerosing panencephalitis and measles viruses. Nature (London) 264:474-477.

70. Hall, W.W., and V. terMeulen. 1977. The effects of actinomycin D on RNA synthesis in measles virus-infected cells. J. Gen. Virol. 34:391-396.

71. Hall, W.W., S.J. Martin, and E. Gould. 1974. Defective interfering particles produced during the replication of measles virus. Med. Microbiol. Immuno. 160:155-164.

72. Hammarskjold, B. and E. Norrby. 1974. A comparison between virion RNA of measles virus and some other paramyxoviruses. Med. Microbiol. Immunol. 160:99-104.

73. Hardwick, J.M., and R.H. Bussell. 1976. Glycoproteins of measles virus under reduced and non-reduced contions, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, S166, p. 232.

74. Hartfield J. and Morley D. (19763). Eficacy of measles vaccine. J. Hyg. 61, 143-147.

75. Haspel, M.V., P.R. Knight, R.G. Duff, and F. Rapp. 1973. Activation of a latent measles virus infection in hamster cells. J. Virol. 12:690-695.

76. Haspel, M.V., R. Duff, and F. Rapp. 1975. Isolation and preliminary characterization of temperature-sensitive mutants of measles virus. J. Virol. 16:1000-1009.

77. Henson, T.E., J.A. Brody, J.L. Sever, M.L. Dyken, and J. Cannon. 1970. Measles antibody titers in multiple sclerosis patients, siblings and controls. J. Am. Med. Assoc. 211:1985-1988.

78. Herndon, R.M., and L.J. Rubinstein. 1968. Light and electron microscopy observations in the development of viral particles in the inclusions of Dawson's encephalitis (subacute sclerosing panencephalitis). Neurology 18:8-20.

79. Hicks, J.T., and P. Albrecht. 1976. Cytolytic, complement-dependent antibodies to measles virus in rhesus monkeys after administration of live or killed virus. J. Infect. Dis. 133:648-654.

80. Hicks, J.T., M.J. Klutch, P. Albrecht, and M.M. Frank. 1976. Analysis of complement-dependent antibody-mediated lysis of target cells acutely infected with measles. J. Immunol. 117:208-215.

81. Ho, M., and J.F. Enders. 1959. Further studies on an inhibition of viral activity appearing in infected cell cultures and its role in chronic viral infections. Virology 1:446-477.

82. Horta-Barbosa, L., R. Hamilton, B. Witting, D. Fuccillo, and J.L. Sever. 1971. Subacute sclerosing panencephalitis: isolation of suppressed measles virus from lymph node biopsies. Science 173:840-841.

83. Howe, C., and A. Schluederberg. 1970. Neuraminidase associated with measles virus. Biochem. Biophys. Res. Commun. 40: 606-607.

84. Huang, A.S. 1973. Defective interfering viruses. Annu. Rev. Microbiol. 27:101-117.

85. Huang, A.S., and D. Baltimore. 1970. Defective viral particles and viral disease processes. Nature (London) 226:325-327.

86. Huang, A.S., D. Baltimore, and M.A. Bratt. 1971. Ribonucleic acid polymerase in virions of Newcastle disease virus: comparison with the vesicular stomatitis virus polymerase. J. Virol. 7:389-394.

87. Imagawa, D.T. 1968. Relationships among measles, canine distemper and rinderpest viruses. Prog. Med. Virol. 10:160-193.

88. Jabbour, J.T., J.H. Garcia, H. Lemmi, J. Ragland, D.A. Duenas, and J.L. Sever. 1969. Subacute sclerosing panencephalitis. A multidisciplinary study of eight cases. J. Am. Med. Assoc. 207:2248-2254.

89. Jabbour, J.T., D.A. Duenas, J.L. Sever, H.M. Krebs, and L. Horta-Barbosa. 1972. Epidemiology of subacute sclerosing panecenphalitis (SSPE): a report of the SSPE registry. J. Am. Med. Assoc. 220:959-962.

90. Jenis, E.H., M.R. Knieser, P.A. Rothouse, G.S. Jensen, and R.H. Scott. 1973. Subacute sclerosing panencephalitis. Immunoultra-structural localization of measles-virus antigen. Arch. Pathol. 95:81-89.

91. Johannes, R.S., and J.L. Sever. 1975. Subacute sclerosing panencephalitis. Annu. Rev. Med. 26:589-601.

92. Johnson, R.T. 1970. Subacute sclerosing panencephalitis. J. Infect. Dis. 121:227-230.

93. Joseph, B.S., and M.B.A. Oldstone. 1974. Antibody-induced redistribution of measles virus antigens on the cell surface J. Immunol. 113:1205-1209.

94. Joseph, B.S., and M.B.A. Oldstone. 1975. Immunologic injury in measles virus infection. II. Suppression of immune injury through antigenic modulation. J. Exp. Med. 142:864-876.

95. Joseph B.S., M.R. Cooper, and M.B.A. Oldstone. 1975. Immunologic injury of cultured cells infected with measles virus. I. Role of IgG antibody and the alternative complement pathway. J. Exp. Med. 141:761-774.

96. Joseph, B.S., P.W. Lampert, and M.B.A. Oldstone. 1975. Replication and persistence of measles virus in defined subpopulations of human leukocytes. J. Virol. 16:1638-1649.

97. Joseph, B.S., L.H. Perrin, and M.B.A. Oldstone. 1976. Measurement of virus antigens on the surfaces of HeLa cells persistently infected with wild type and vaccine strains of measles virus by radioimmune assay. J. Gen. Virol. 30:329-337.

98. Katz, S.L., Kempe. C.H., Black, F.L., Lepow, M.L., Krugman S., Haggerty, R.J. and Enders, J.F. 1960. Studies on an attenuated measles-virus vaccine VIII: General summary and evaluation of the results of vaccinations. New Engl. J. Med. 263, 27-31.

99. Katz, S.L., Morley D.C. and Krugman S (1962). Attenuated measles vaccine in Nigerian children. Amer. J. Dis. Child. 103, 402-405.

100. Katz, M.S. Oyanagi, and H. Koprowski. 1969. Subacute sclerosing panencephalitis: structures resembling myxovirus nucleocapsid in cells cultured from brains. Nature (London) 222:888-890.

101. Katz, S.L. 1972. Measles, p. 729-737. In H.L. Barnet and A.H. Einhorn (ed), Pediatrics, 15th ed. Appleton-Century-Crofts, New York.

102. Kiley, M.P., and F.E. Payne. 1974. Replication of measles virus: continued synthesis of nucleocapsid RNA and increase synthesis of mRNA in the presence of cycloheximide. J. Virol. 14:758-764.

103. Kingsbury, D.W. 1972. Paramyxovirus replication. Curr. Top. Microbiol. Immunol. 59:1-33.

104. Kingsbury, D.W. 1973. Cell-free translation of paramyxovirus RNA. J. Virol. 12:1020-1027.

105. Kingsbury, D.W. 1974. The molecular virology of paramyxoviruses. Med. Microbiol. Immunol. 160:73-83.

106. Kingsbury, D.W., A. Portner, and R.W. Darlington. 1970. Properties of incomplete Sendai virions and subgenomic viral RNAs. Virology 42:857-871.

107. Klaus, S., Kenny, M.T., and Jackson, J.E. 1975. Serological evaluation of measles virus (Schuarz strain), mumps virus (Jeryl Lynn strain) and rubella virus (Cendehill strain) combination vaccines. J. Biolog. standard. 3, 231-239.

108. Knight, P., R. Duff, and F. Rapp. 1972. Latency of human measles virus in hamster cells. J. Virol. 10:995-1001.

109. Kohn, A. 1962. Hemadsorption by measles syncytia. Nature (London) 193:1088-1089.

110. Kolakofsky, D. 1976. Isolation and characterization of Sendai virus DI-RNAs. Cell 8:547-555.

111. Krugman, S. 1977. Present status of measles and rubella immunization in the United States: a medical progress report. J. Pediatr. 90:1-12.

112. Krugman , S., Giles, J.P., Freedman H. and Stone, S. 1965. Studies on immurity to measles. J. Pediatric 66, 471-488.

113. LaBoccetta, A.C., and A.S. Torney. 1964. Measles encephalitis. Report of 61 cases. Am. J. Dis. Child. 107: 247-255.

114. Lai, M.T., and W.K. Joklik. 1973. The induction of interferon by temperature-sensitive mutants of reovirus, UV-irradiated reovirus, and subviral reovirus particles. Virology 51:191-204.

115. Lepow, M.L. and Nankervis, G.A. 1969. Eight year serologic evaluation of Edmonston live measles vaccine. J. Pediatrics 75 (3). 407-411.

116. Lerman S.J. and Gold E. 1971. Measles in Children Previously vaccinated Against measles. JAMA 216, 8. 1311-1314.

117. Levenshtam, M.A., N.S. Radyshich, E.N. Zakharchenko, and T.G. Shroit. 1969. Chromosomal aberrations in human amniotic cells before the completion of a single growth cycle of measles vaccine virus L-16. Acta Virol 13.401-406.

118. Linnemann, C.C., Dine, M.S., Bloom J.E. and Schiff, G. . 1972. Measles antibodies in previously immunized children. The need for revaccination. Amer. J. Dis. Child 124(1) 53-57.

119. Linnemann, C.C., Heggs, M.B., Rotte, T.C., Phair, J.P. and Schiff, G.M. 1973. Measles IgM response during reinfection of previously vaccinated children. J. Pediatrics 82:5, 798-801.

120. Llanes-Rodas, R., and C. Liu. 1965. A study of measles virus infection in tissue culture cells with particular reference to the development of intranuclear inclusion bodies. J. Immunol. 95:840-845.

121. Lomniczi, B., and D.C. Burke. 1970. Interferon production by temperature-sensitive mutants of Semliki Forest virus. J. Gen. Virol. 8:55-68.

122. MacIntyre, E.H., and J.A. Armstrong. 1976. Fine structural changes in human astrocyte carrier lines for measles virus. Nature (London) 263:232-234.

123. McAlpine. D. 1972. Symptoms and signs, p. 132-196. In D. McAlpine C.E. Lumsden, and E.D. Acheson (ed), Multiple sclerosis, a reappraisal, 2nd ed. Churchill Livingstone, Edinburgh and London.

124. McCormick J.B., Halsey N. and Rosenberg R (1977). Measles vaccine efficacy determined from secondary attack rates during a severe epidemic. The J. Ped. 90, 1. 13-16.

125. McCrumb, F.R. Jr; Kress, S and Snyder, M.J. 1962. Quantitative Aspects of Attenuated Measles virus infection. Amer. J. Dis. Child. 103, 443-444.

126. McFarland, H.F. 1974. The effect of measles virus infection on T and B lymphocytes in the mouse. I. Suppression of helper cell activity, J. Immunol. 113:1978-1983.

127. McKinn, J., and F. Rapp. 1977. Variation in ability of measles virus plaque progeny to induce interferon. Proc. Natl. Acad. Sc. U.S.A. 74:3056-3059.

128. Matumoto, M. 1966. Multiplication of measles virus in cell cultures. Bacteriol. Rev. 30:152-176.

129. Mellman, W.J., and R. Wetton. 1963. Depression of the tuberculin reaction by attenuated measles virus vaccine. J. Lab. Clin. Med. 61:453-458.

130. Menna, J.H. A.R. Collins, and T.D. Flanagan. 1975. Characterization of an in vitro persistent-state measles virus infection establishment and virological characterization of the BGH/HV cell line. Infect. Immun. 11:152-158.

131. Miller, H.G., J.B. Stanton, and J.L. Gibbons. 1956. Parainfectious encephalomyelitis and related syndromes. A critical review of the neurological complications of certain specific fevers. Q.J. Med. 25:427-505.

132. Milovanovic, M.V., J.F. Enders, and A. Mitus. 1957. Cultivation of measles virus in human ammnion cells and developing chick embryos. Proc. Soc. Exp. Biol. Med. 95:120-127.

133. Minagawa, T. 1971. Studies on the persistent infections with measles virus in HeLa cells. I. Clonal analysis of cells in carrier cultures. Jpn. J. Microbiol. 15:325-331.

134. Minagawa T., and M. Yamada 1971. Studies on the persistent infection with measles virus in HeLa cells. III. Immunolysis of cells in carrier state by anti-measles sera. Jpn. J. Microbiol. 15:341-350.

135. Minagawa, T., T. Sakuma, S. Kuwajima, T.K. Yamamoto, and N. Iida. 1976. Characterization of measles viruses in establishment of persistent infections in human lymphoid cell line. J. Gen. Virol. 33:361-379.

136. Mirchamsy, H., and F. Rapp. 1969. Role in interferon in replication of virulent and attenuated strains of measles virus. J. Gen. Virol. 4:513-522.

137 Mitus, A., J.F. Enders, J.M. Craig, and A. Holloway. 1959. Persistence of measles virus and depression of antibody formation in patients with giant-cell pneumonia after measles N. Engl. J. Med. 261:882-889.

138. Mitus, A., J.F. Enders, G. Edsall, and A. Holloway. 1965. Measles in children with malignancy: problems and prevention. Arch. Gesamte Virusforsch. 16:331-337.

139. Morgan, C., and C. Howe, 1968. Structure and development of viruses as observed in the electron microscope. IX. Entry of parainfluenza 1 (Sendai) virus. J. Virol. 2:1122-1132.

140. Mountcastle, W.E., and P.W. Choppin. 1977. A comparison of the polypeptides of four measles virus strains. Virology 78:463-474.

141. Morley D.C. 1962. Measles in Nigeria. Amer. J. Dis. Child. 103:230-235.

142. Morley D., Katz S.L. and Krugman S 1963. The clinical reaction of Nigerian children to measles vaccine with and without gamma globulin. J. Hyg. 61, 135-141.

143. Morley D.C. and MacWilliam K.M. 1961. Measles in Nigerian Community. W. Afr. Med. J. 10, 246-253.

144. Morley D., Wooland M. and Martin W.J. 1963. Measles in Nigerian Children. J. Hyg. 61, 115-134.

145. Nagata, I., Y. Kimura, Y. Ito, and T. Tanaka. 1972. Temperature-sensitive phenomenon of viral maturation observed in BHK cell persistently infected with HVJ. Virology 49:453-461.

146. Nakai, M., and D.T. Imagawa. 1969. Electron microscopy of measles virus replication. J. Virol. 3:187-197.

147. Nakai, T., F.E. Shand, and A.F. Howatson. 1969. Development of measles virus in vitro. Virology 38:50-67.

148. Nichols, W.W., and A. Levan. 1965. Measles associated chromosome breakage. Arch. Gesamte Virusforsch. 16:168-174.

149. Norrby, E. 1962. Hemagglutination by measles virus. I. The production of hemagglutinin in tissue culture and the influence of different conditions on the hemagglutinating system. Arch. Gesamte Virusforsch. 12:153-163.

150. Norrby, E. 1962. Hemagglutination by measles virus. II. Properties of the hemagglutinin and of the receptors on the erythrocytes. Arch. Gesamte Virusforsch. 12:164-172.

151. Norrby, E. 1962. Hemagglutination by measles virus. 4. A simple procedure for production of high potency antigen for hemagglutination-inhibition (HI) tests. Proc. Soc. Exp. Biol. Med. 8:814-818.

152. Norrby, E. 1964. Separation of measles virus components by equilibrium centrifugation in CsCi gradients. 1. Crude and Tween and other treated concentrated tissue culture material. Arch. Gesamte Virusforsch. 14:306-318.

153. Norrby, E. 1967. A carrier cell line of measles virus in LU 106 cells. Arch. Gesamte Virusforsch. 20:215-224.

154. Norrby, E. 1972. Intracellular accumulation of measles virus nucleocapsid and envelope antigens. Microbios 5:31-40.

155. Norrby, E. 1975. Occurrence of antibodies against envelope components after immunization with formalin inactivated and live measles vaccine. J. Biol. Stand. 3:375-380.

156. Norrby, E.C.J., and L.G. Falksveden. 1964. Some general properties of the measles virus hemolysin. Arch. Gesamte Virusforsch. 14:474-486.

157. Norrby, E.C.J., and Magnusson. 1965. Some morphological characteristics of the internal component of measles virus. Arch. Gesamte Virusforsch. 17:443-447.

158. Norrby, E., and Y. Gollmar. 1972. Appearance and persistence of antibodies against different virus components after regular measles infections. Infect. Immun. 6:240-247.

159. Norrby, E., and B. Hammarskjold. 1972. Structural components of measles virus. Microbios 5:17-29.

160. Norrby, E., and Y. Gollmar. 1975. Identification of measles virus-specific-hemolysis-inhibiting antibodies separate from hemagglutinating inhibiting antibodies. Infect. Immun. 11:231-239.

161. Norrby, E. C.J., E. Magnusson, L.-G. Falksveden, and L. Gronberg 1964. Separation of measles virus components by equilibrium centrifugation in CsCl gradients. II. Studies on the large and small hemagglutinin. Arch. Gesamte Virusforsch. 14:462-473.

162. Norrby, E., B. Link, and J.-E. Olsson. 1974. Measles virus antibodies in multiple sclerosis. Arch. Neurol. 30: 285-292.

163. Numazaki, Y., and D.T. Karzon. 1966. Density separable fractions during growth of measles virus. J. Immunol. 97:458-469.

164. Oddo, F.G., R. Flaccomio, and A. Sinatra. 1961. "Giant-cell" and "strand-forming" cytopathic effect of measles virus lines conditioned by serial propagation with diluted or concentrated inoculum. Virology 13:550-553.

165. Ojala, A. 1947. On changes in the cerebrospinal fluid during measles. Ann. Med. Fenn. 36:321-331.

166. Orvell, C., and E. Norrby. 1974. Further studies on the immunologic relationships among measles, distemper, and rinderpest virus. J. Immunol. 113. 850- 858.

167. Osunkoya, B.O., A.R. Cooke, O. Ayeni, and T.A. Adejumo. 1974. Studies on lymphocyte cultures in measles. I. Lymphocyte transformation and giant cell formation in leukocyte cultures from clinical cases of measles. Arch. Gesamte Virsusforsch. 44:313-323.

168. Osunkoya, B.O., G.I. Adeleye, T.A. Adejumo and L.S. Salimonu. 1974. Studies on leukocyte cultures in measles II. Detection of measles virus antigen in human leukocytes by immunofluorescence. Arch. Gesamte Virusforsch. 44:323-329.

169. Oyanagi, S., V. terMeulen, D. Müller, H. Katz, and H. Koprowski. 1970. Electron microscopic observations in subacute sclerosing panencephalitis brain cell cultures: their correlation with cytochemical and immunocytological findings. J. Virol. 6:370-379.

170. Oyanagi, S., V. terMeulen, H. Katz, and H. Koprowski. 1971. Comparison of subacute sclerosing panencephalitis and measles virus: an electron microscope study. J. Virol. 7:176-187.

171. Pampiglione, G., A.H. Griffith, and E.C. Bramwell. 1971. Transient cerebral changes after vaccination against measles. Lancet 2:5-8.

172. Panelius, M., A. Salmi, and P. Halonen. 1970. Gel precipitation reactions between measles antigens and sera of patients with multiple sclerosis. Acta Pathol. Microbiol. Scand. 78B:588-592.

173. Parfanovich, M, B. Hammarskjold, and E. Norrby. 1971. Synthesis of virus-specific RNA in cells infected with two different variants of measles virus. Arch. Gesamte Virusforsch. 35:38-44.

174. Parkers, J.C., G.K. Klintworth, D.G. Graham, and J.P. Griffith. 1970. Uncommon morphologic features in subacute sclerosing panencephalitis (SSPE). Am. J. Pathol. 61:275-284.

175. Paterson, P.Y. 1971. The demyelinating diseases: clinical and experimental correlates, p. 1269-1299. In H. Samter (ed.) Immunological diseases, vol. II, 2nd ed. Little Brown and Co., Boston.

176. Payne, F.E., and J.V. Baublis. 1971. Measles virus and subacute sclerosing panencephalitis. Perspect. Virol. 7:179-195.

177. Perier, O., L. Thiry, J.J. Vanderhaeghen, and S. Pelc. 1968. Attempts at experimental transmission and electron microscopic observations in subacute sclerosing panencephalitis. Neurology 18:138-143.

178. Peries, J.R., and C. Chany. 1960. Activite hemagglutinante et hemolytique du virus morbilleux. C.R. Acad. Sci. 251:820-821.

179. Peries, J.R., and C. Chany. 1962. Studies on measles viral hemagglutination. Proc. Soc. Exp. Biol. Med. 110:477-482.

180. Pertralli, J.K., T.C. Merigan, and J.R. Willbur. 1965. Circulating interferon after measles vaccination. N. Engl. J. Med. 273:198-201.

181. Pollack, M.A., C. Crose, and H. Friend. 1975. Measles associated with Bell's palsy. Am. J. Dis. Child. 129:747.

122. Portner, A., and D.W. Kingsbury. 1972. Identification of transcriptive and replicative intermediates in Sendai virus-infected cells. Virology 47:711-725.

183. Portner, A., and R.H. Bussell. 1973. Measles virus ribonucleic acid and protein synthesis: effects of 6-azauridine and cycloheximide on viral replication. J. Virol. 11:46-53.

184. Poser, C.M. 1972. Recent advances in multiple sclerosis. Med. Clin. N. Am. 56:1343-1362.

185. Preble, O.T., and J.S. Youngner. 1972. Temperature-sensitive mutants isolated from L-cells persistently infected with Newcastle disease virus. J. Virol. 9:200-206.

186. Preble, O.T., and J.S. Youngner. 1973. Selection of temperature sensitive mutants during persistent infection: role in maintenance of persistent Newcastle disease virus infections of L cells. J. Virol. 12:481-491.

187. Preble, O.T., and J.S. Youngner. 1975. Temperature-sensitive viruses and the etiology of chronic and inapparent infections. J. Infect. Dis. 131:467-473.

188. Raine, C.S., L.A. Feldman, R.D. Sheppard, and M.B. Bronstein. 1969. Ultrastructure of measles virus in cultures of hamster cerebellum. J. Virol. 4:169-181.

189. Rapp, F. 1964. Plaque differentiation and replication of virulent and attenuated strains of measles virus. J. Bacteriol. 88:1448-1458.

190. Rapp, F., I. Gordon, and R.F. Baker. 1960. Observations of measles virus infection of cultured human cells. I. A study of development and spread of virus antigen by means of immunofluorescence. J. Biophys. Biochem. Cytol. 7:43-48.

191. Rapp, F., L.V. Haspel, R. Duff, and P. Knight 1974. Measles virus latency and neurovirulence, p. 199-212. In W.S. Robinson and C.F. Fox. (ed), Mechanisms of virus disease W.A. Benjamin, Inc., Menlo Park, Calif.

192. Reynolds, D.W. and Start, A. 1972. Immunity to measles in children vaccinated before and after one year of age. Am. J. Dis. Child. 124(6), 848-850.

193. Robinson, W.S. 1971. Ribonucleic acid polymerase activity in Sendai virions and nucleocapsid. J. Virol. 8:81-86.

194. Robinson, W.S. 1971. Sendai virus RNA synthesis and nucleocapsid formation in the presence of cycloheximide. Virology 44:494-502.

195. Roman, J.M., and E.H. Simon. 1976. Defective interfering particles in monolayer-propagated Newcastle disease virus. Virology 69:298-303.

196. Rosen, L. 1961. Hemagglutination and hemagglutination-inhibition with measles virus. Virology 13:139-141.

197. Savage, F.M.A. 1967. A year of measles. Med. J. Zambia. 67-77.

198. Ruckdeschel, J.C., K.D. Graziano, and M.R. Mardiney, Jr. 1975. Additional evidence that the cell-associated immune system is the primary host defence against measles (rubeola). Cell. Immunol. 17:11-18.

199. Rustigian, R. 1962. A carrier state in HeLa cells with measles virus (Edmonston strain) apparently associated with noninfectious virus. Virology 16:101-104.

200. Rustigian, R. 1966. Persistent infection of cells in culture by measles virus. I. Development and characteristics of HeLa sublines persistently infected with complete virus. J. Bacteriol. 92.1792-1804.

201. Rustigian, R. 1966. Persistent infection of cells in culture by measles virus. II. Effect of HeLa sublines and recovery of a HeLa clonal line persistently infected with incomplete virus. J. Bacteriol. 92:1805-1811.

202. St. Geme, J.W., Jr. 1964. Evidence for the nucleic acid composition of measles virus. Pediatrics 33:71-74.

203. Salmi, A.A., E. Norrby, and M. Panelius. 1972. Identification of different measles virus-specific antibodies in the serum and cerebrospinal fluid from patients with subacute sclerosing panencephalitis and multiple sclerosis. Infect. Immun. 6:248-254.

204. Salmi, A.A., M. Panelius, P. Halonen, U.K. Rinne, and K. Penttinen. 1972. Measles virus antibody in cerebrospinal fluid from patients with multiple sclerosis. Br. Med. J. 1:477-479.

205. Schluederberg, A. 1962. Separation of measles virus particles in density gradients. Am. J. Dis. Child. 103:121-126.

206. Schluederberg, A. 1971. Measles virus RNA. Biochem. Biophys. Res. Commun. 42:1012-1015.

207. Schluederberg, A., and M. Nakamura. 1967. A salt dependent hemagglutinating particle from measles-infected cells. Virology 33:297-306.

208. Schluederberg, A., and S. Chavanich. 1974. The role of the nucleus in measles virus replication. Med. Microbiol. Immunol. 160:85-90.

209. Schluederberg, A., C.J. Williams, and F.L. Black. 1972. Inhibition of measles virus replication and RNA synthesis by actinomycin D. Biochem. Biophys. Res. Commun. 48: 657-661.

210. Sever, J.L., J.M. Ellenberg, H.M. Krebs and J.T. Jabbour 1974. Subacute sclerosing panencephalitis and measles vaccine. Pediatr. Res. 8:114

211. Shaw, C.M., G.C. Buchan, and C.B. Carlson. 1967. Myxovirus as a possible etiologic agent in subacute inclusion-body encephalitis. N. Engl. J. Med. 277:511-515.

212. Shirodaria, P.V., B. Dermott, and E.A. Gould. 1976. Some characteristics of salt-dependent hemagglutinating measles viruses. J. Gen. Virol. 33:107-115.

213. Stanwick, T.L., and J.V. Hallum. 1976. Comparison of RNA polymerase associated with Newcastle disease virus and a temperature-sensitive mutant of Newcastle diesease virus isolated from persistently infected L cells. J. Virol. 17:68-73.

214. Stokes J., Reilly, C.M., Buynak. E.B. and Hilleman, M.R. 1961. Immunological studies of measles. Amer. J. Hyg. 74, 293-303.

215. Stone, H.O., A. Portner, and D.W. Kingsbury. 1971. Ribonucleic acid transcriptases in Sendai virions and infected cells. J. Virol. 8:174-180.

216. Sullivan, J.L., D.W. Barry, P. Albrecht, and S.L. Lucas. 1975. Inhibition of lymphocyte stimulation by measles virus. J. Immunol. 114:1458-1461.

217. Sullivan, J.L., D.W. Barry, S.J. Lucas, and P. Albrecht. 1975. Measles infection of human mononuclear cells. I. Acute infection of peripheral blood lymphocytes and monocytes. J. Exp. Med. 142:773-784.

218. Tellez-Nagel, J., D.H. Harter. 1966: Subacute sclerosing leucoencephalitis. I. Clinicopathological electron microscopic and virological observations. J. Neuropathol. Exp. Neurol. 25:560-581.

219. Tellez-Nagel, J., and D.H. Harter. 1966: Subacute sclerosing leucoencephalitis: ultra-structure of intranuclear and intracytoplasmic inclusions. Science 154: 899-901.

220. ter Meulen, V., 1974: Pathogenic aspects of measles virus infections. Med. Microbiol. Immunol. 160: 165-172.

221. ter Meulen, V., and AS.J. Martin. 1976: Genesis and maintenance of a persistent infection by canine distemper virus. J. Gen. Virol. 32: 431-440.

222. ter Meulen, V., M. Katz, and D. Muller. 1972: Subacute sclerosing panencephalitis: a review. Curr. Top. Microbiol. Immunol. 57: 1 - 38.

223. ter Meulen, V., H. Koprowski, Y. Iwasaki, Y.M. Kackell, and D. Muller. 1972: Fusion of cultured multiple sclerosis brain cells with indicator cells: presence of nucleocapsids and virions and isolation of parainfluenza-type virus. Lancet Lancet 2: 1-5.

224. Tyler, H.R. 1957: Neurological complications of rubeola (measles). Medicine 36: 147-167.

225. Underwood, B., and F. Brown. 1974: Physicochemical characterization of rinderpest virus. Med. Microbiol. Immunol. 160: 125-132.

226. Von Pirquet, 1908: Das Verhalten der Kutanen Tuberculinreaktion wahrend der Masern. Deutsch. Med. Wschr. 34: 1297-1300.

227. Waters, D.J., and R.H. Bussell. 1973: Polypeptide composition of measles and canine distemper viruses. Virology 55:554-557.

228. Waters, D.J., and R.H. Bussell. 1974: Isolation and comparative study of the nucleocapsids of measles and canine distemper viruses from infected cells. Virology 61:64-79.

229. Waters, D.J., R.T. Hersh, and R.H. Bussell. 1972: Isolation and characterization of measles nucleocapsid from infected cells. Virology 48: 278-281.

230. Waterson, A. P. 1962: Two kinds of myxovirus. Nature (London) 193: 1163-1164.

231. Waterson, A.P. 1965: Measles virus. Arch. Gesamte Virusforsch. 16: 57-80.

232. Waterson, A.P., J.G. Cruickshank, G.D. Lawrence, and A.D. Kanarek. 1961: The nature of measles virus. Virology 15: 379-382.

233. Waterson, A.P., R. Rott, and G. Ruckle-Enders. 1963: The components of measles virus and their relations to rinderpest and distemper Z. Naturforsch. 18b:377-384.

234. Weibel, R., Halend, R., Stokes, J. Jr., Hilleman, M.R., and Buynak, E.B. 1962: Persistence of measles antibodies after vaccine administration. J. Amer. Med. Assoc. 180: 1086

235. Weiner, L.B., Corwin, R.M., Nieburg, P.I., and Feldman, M.A. 1977: A measles outbreak among adolescents. The J. Ped. 90; 1. 17-20.

236. White, R. G., and J.F. Boyd. 1973: The effect of measles on the thymus and other lymphoid tissues. Clin. Exp. Immunol. 13: 343-357.

237. Winston, S.H., R. Rustigian, and M.A. Bratt. 1973: Persistent infection of cells in culture by measles virus. III. Comparison of virus-specific RNA synthesized in primary and persistent infection in HeLa cells. J. Virol. 11: 926-932.

238. Wyll, S.A., and Witte, J.J. 1971: Measles in Previously Vaccinated children. JAMA, 216; 8 1307-1310.

239. Yamanouchi, K., Kobune, A., Fukuda, M., Hayami, and A. Shishido 1970: Comparative immunofluorescent studies on measles canine distemper and rinderpest viruses. Arch. Gesamte Virusforsch. 29: 90-100.

240. Yamazi, Y., and F.L. Black. 1972: Isolation of temperature-sensitive mutants of measles virus. Med. Biol. (Tokyo) 84:47-51.

241. Yeager, A.S. 1976: Measles Immunity Clin. Res. 24, 186.

242. Yeh, J. 1973: Characterization of virus-specific RNAs from subacute sclerosing panencephalitis virus-infected CV-1 cells. J. Virol. 12: 962-968.

243. Youngner, J.S., and D.O. Quagliama 1975: Temperature-sensitive mutants isolated from hamster and canine cells lines persistently infected with Newcastle disease virus. J. Virol. 16: 1332-1336.

244. Zeman, W., and O. Kolar, 1968: Reflections on the etiology and pathogenesis of subacute sclerosing panencephalitis. Neurology 18: 1- 7.

245. Zweiman, B. 1971: In vitro effects of measles virus on proliferating human lymphocytes. J. Immunol. 106:1154-1158.

246. Zweiman, B., D. Pappagianis, H. Maibach, and E.A. Hildreth, 1971: Effects of measles immunization on tuberculin hypersensitivity and in vitro lymphocyte reactivity. Int. Arch. Allergy 40: 834-841.