ISOLATION OF THE WILD MEASLES VIRUS AND THE IMMUNOLOGICAL COMPARISON WITH SCHWARZ LABORATORY STRAIN

BY

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CERTIFICATION.

This dissertation titled

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With my sincere hope and optimism for the future.
I feel sure that I have learned to swim.
Strong; fearless and full of cheerful enthusiasm.
All of which would help me to keep afloat.
I might need to adapt my strokes, control my
breathing or alter course in the face of currents
but I'm not afraid.

Forth in thy name, O'Lord I go;
My daily labour to pursue;
Thee, only thee, resolved to know;
In all, I think or speak or do.

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ABSTRACT.

The continued outbreaks of measles in vaccinated individuals in Nigeria has prompted this investigation. The study was designed to compare a widely used vaccine strain of measles (Schwarz) with a local isolate (Wild) to determine if there are differences in antigenic and physico-chemical characteristics which could account for the apparent inability of the vaccine antigen to protect against infection with a locally active virus.

The Schwarz vaccine strain of measles was compared with a Wild strain using the growth characteristics in cell culture and thermostability properties. Antibodies raised in monkeys Erythocebus patas to both viruses were used in agar-gel immunodiffusion (AGID) tests, haemagglutination inhibition (HAI) tests and cross neutralization studies.

Both viruses produced identical cytopathic effects (CPE) in the cell cultures used, primarily syncytium, giant and spindle - cell formation. The Wild strain, however, produced 100% CPE in 3 - 5 days whereas the Schwarz strain took 4 - 7 days to reach 100% CPE. The viral yield was lower for Schwarz strain compared with Wild strain in cell cultures used. The Wild strain was characterized by rough, large plaques (>1mm diameter) while the Schwarz strain developed smooth, small plaques (< 1mm diameter). In the thermostability studies,

there was no significant difference in the survival rates of either virus when grown at temperatures from 10°C to 50°C for one hour. There were slight differences in the cross neutralization and the ACID tests, but no differences detectable by the HAI test.

Overall, the differences observed between the two viruses do not appear to provide sufficient explanation for the epidemiological behaviour (vaccination failure) of the virus in Nigeria. The thermostability study is particularly instructive since it has been hypothesized that the inability of the vaccine virus to survive high temperatures may be a major factor in the vaccination failures. It is possible however, that subtle differences detectable by more sophisticated techniques may explain the nature of the causes of vaccination failures.

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CHAPTER 1

INTRODUCTION.

1.1. GENERAL.

Measles is an outstanding, visible and consistently severe disease (Guillbzet, 1979). Wallgren (1965) indicated that it has a worldwide distribution with general susceptibility, marked infectivity, high pathogenicity and life-long immunity, although the issue of life-long immunity is now open for re-investigation. It accounts for a great deal of economic loss due to absenteeism of working mothers having to take care of their ill wards (Krugman, 1971). Miller (1980) have recently reported severe outbreaks especially in developing countries.

Measles infection is known to occur in recipients and non - recipients of a plethora of measles vaccines available (Meyer et al., 1978; Miller, 1980). This has raised some questions on the immunogenicity of the various vaccines, and efforts are being made towards identifying reasons for the apparent failure of measles vaccines. Among the various reasons advanced are: (i) Failure of the vaccine to immunize which could be due to dead vaccines (Eghafona et al., 1979). Vaccines are known to be usually inactivated due to faulty lypholization, improper handling and storage (King, 1978). Heymann et al. (1982) suggested that the availability of a more thermostable live measles vaccine will improve these situation. (ii) Vaccine -

induced disease (vaccine outbreak) as a result of either improper attenuation or reversion to virulence of the strain used (Meyer et al., 1978). In addition, Shahbracli et al. (1980) suggested the existence of strain differences resulting in immunity induced by given vaccine not being protective against all strains. If such strain differences do occur and certain strains are confined to specific geographical locations, distorted results might be obtained if one type of virus is used in vaccine preparation for persons living in an area with an antigenically different wild-type virus (Frank et al., 1981).

One major problem regarding vaccine production or virus propagation concerns the substrate to be used for such a purpose. For measles virus specifically, primary monkey kidney cells represent a satisfactory source of cells (MacArthur et al., 1979), but monkeys have become increasingly more difficult to obtain. Certain countries, for instance the Netherlands, have established local breeding colonies of monkeys providing material sufficient for the national production of vaccines, but the cost is high (Hilleman, 1982). Furthermore, monkeys are sometimes chronically infected with adenoviruses, simian herpes virus (B virus), SV40, foamy virus etc. without showing signs of the disease (Blaskovic and Styk, 1967). The persistent infection of tissues

with monkey viruses significantly lowers the value of monkeys for isolation experiments. The same applies for the use of monkey cells for tissue cultures to be used in isolation or vaccine production. Attempts have been made to substitute the primary monkey kidney cells with other cell-types (Mekino, 1977). Norrby (1983) found that the yield of virus in these systems was relatively poor and therefore the vaccine are expensive. The World Health Organization now accepts the use of non-tumorigenic cells lines for vaccine production with certain provisions (Hilleman, 1982). The cells used e.g. Vero cells, have to be proven not to be tumorigenic in nude mice, they should not show any chromosomal aberrations and the final vaccine product should be purified.

1.2 OBJECTIVE OF STUDY

To explain the reason(s) for the continued outbreaks of measles in recipients of the vaccine, the hypothesis of possible differences in the physico - chemical and antigenic properties between the vaccine (Schwarz) strain and Wild strain was adopted. The present study was designed to:

(1) Isolate the Wild measles strain involved in local epidemics, adapt and propagate in readily available cell cultures (notably Vero and Bovine Kidney cells).

(2) Compare the growth characteristics (cytopathology, plaque morphology, viral yield) of the strains under study.

(3) Compare serologically the Schwarz and Wild strains by haemagglutination inhibition, cross plaque neutralization and immunodiffusion tests.

(4) Study the thermostability property of Schwarz and Wild strains at various temperatures.

CHAPTER 2

LITERATURE REVIEW

2.1. MEASLES VIRUS

(a) Classification:

Measles virus belongs to the family Paramyxoviridiae (Fenner, 1976). It is specifically in the Mobillivirus group which include rinderpest, canine distemper and PPR virus (Yamanouchi, 1980 and Orvell, and Norrby, 1980). Measles is the only member of the mobilliviridae that exhibits hemagglutination activity but lacks neuraminidase (Howe and Schluederberg, 1970).

(b) The Virion:

The measles virion is an enveloped, helical particle containing a single - stranded RNA genome of molecular weight of 6.2×10^6 daltons (Norrby et al., 1964). The virion RNA appears to be similar in size to the 50s RNA isolated from other paramyxoviruses (Choppin and Compans, 1975), migrating a similar distance with other paramyxoviruses in polyacrylamide gels (Hammarkjold and Norrby, 1974), and co-sedimenting with the 50s RNA of Newcastle Disease Virus (NDV) in sucrose gradients (Winston et al., 1973).

Hall and Martins (1973) demonstrated that the virion contains six polypeptides, whose molecular weights range from 38,000 - 79,000 daltons. However, information on electrophoretic gel pattern by Granes et al. (1978) suggests that measles virus

contain seven major structural polypeptides viz: the large (L), hemagglutinin (HA), polymerase (P), nucleocapsid (NP), action fusion (F) and membrane (M) polypeptides. The F-component includes two polypeptide chains, F_1 and F_2 which are linked by disulphide bridges, and only the F_2 polypeptide chain is glycosylated. In this respect measles virus differs from other members of the paramyxovirus family, in which both F_1 and F_2 are glycosylated (Scheid and Choppin, 1977).

Norrby (1964) separated particles that contain primarily hemagglutinating (HA) activity in Cesium chloride from infectious virions that contain both HA and hemolysin (HL) activities. 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., 1975). Ehrnst (1975) found the hemagglutinin and hemolysin to be antigenically distinct.

Different strain of measles virus have been shown to be remarkably similar in molecular weight of their polypeptides. The differences reported concerned small variations in the size of nucleocapsid, polymerase and membrane polypeptides (Hall et al., 1980; Mountcastle and Choppin, 1977).

(c) Stability, Inactivation and Storage:

Krugman (1977) indicated that the measles virus is relatively thermolabile. The half life of the infectivity at 37°C is about 2 hours at pH 7.5 and there is no detectable infectivity after 30 minutes at 56°C (Musser and Underwood, 1960). According to Waterson (1965) storage of measles virus in the cold is greatly improved in the presence of serum of other protein materials and in their absence, the infectivity titer falls, even at - 70°C.

Three complimentation groups of temperature - sensitive mutants have been isolated (Haspel et al., 1975a). Most of the mutants isolated by these workers are classified as RNA - negative because they do not synthesize virion RNA or produce detectable virus antigens but produced syncytia at a temperature of 39°C. 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.

(d) Interferon Induction:

Measles virus has been found to induce the synthesis of interferon during in vitro and in vivo replications (DeMaeyer and Enders, 1965). Mirchamsy and Rapp (1969) demonstrated the inhibition of measles

virus replication by interferon, when it was found that addition of low concentration of Actinomycin D to infected cells inhibited interferon synthesis and enhanced both virus yields and plaque size. The ability of measles virus to induce interferon is under the genetic control of the virion, since plaque-purified clones of measles virus that induce interferon generally retain this characteristic upon subsequent passage (Haspel et al., 1975b).

(e) Isolation of measles virus.

Enders and Peebles (1954) demonstrated that the virus could be isolated from clinical materials in primary cultures of human and monkey kidneys. Some isolations in other tissues notably human amnion, human chorion, dog, sheep and rabbit kidney had been reported (Enders-Ruckle, 1965, Kempe and Fulginiti, 1965 Makino et al., 1970) but monkey kidney remains generally the tissue of choice since the disease is reproducible in this animal (Norrby, 1983).

Measles strains isolated in monkey kidney cells have been adapted to grow in several established human cell lines (notably HeLa, HEP-2, FL and KB) and in chick embryo tissue culture (Waterson, 1965, Okuno et al., 1960.

Enders et al. (1957) indicated that in the continuous human cell lines, growth is generally more rapid and the cytopathic effect occurs earlier.

(f) In Vitro Effects.

I. Cytopathic Effects (CPE).

Enders and Peebles (1954) described a very distinctive cytopathic effect produced by measles virus replication in established cell lines notably Vero. The CPE is characterized by the formation of multinucleated giant cells (Syncytia). In addition, chromosomal aberrations and breakages have been observed in measles virus infected cells (Csonk et al., 1975).

Strand formation is another type of CPE produced by measles virus infection (Oddo et al., 1961). This is characterized by the appearance of spindle shape or stellate cells that have long and irregular cytoplasmic processes, which Parfanovich et al. (1971) associated with a high concentration of virus.

II. Interactions with Erythrocytes:

Measles virus is known to interact with erythrocytes in several ways (Norrby, 1962a,b,c. and Rosen, 1961) resulting in the development of haemagglutination (HA), and haemolysis (HL) assays. These assays have greatly enhanced the ease with which virus replication could be monitored and quantified. Although HA and HL activities can be differentiated, Norrby and Collmar (1975) found that the presence of HA is a necessary pre-requisite for HL activity.

2.2. MEASLES EPIDEMIOLOGY.

(a) Age Distribution:

Measles is a disease of young children especially in pre-school age group (Imam et al., 1965). Hendrickse et al. (1964) showed that 30% of measles cases occurred in children under 1 year of age, 65% in children under 2 years and over 80% in children under 3 years. The occurence of measles in children under 6 months of age was reported by Sinha (1980).

(b) Seasonal Incidence:

Morley (1962) observed the seasonal incidence of measles in both temperate and tropical countries. The author indicated that the percentage of cases and deaths show a rise towards the end of the winter season reaching a peak in the spring and early summer in the temperate regions of the world, while in the tropics, the diesease is noticeable in epidemic proportions during the later part of the dry season when the people come together for festivities, and declines with the onset of rains when the people return to their farms. The high incidence during these periods indicate that person - to - person contact plays a vital role in the transmission of the disease. About 20% of susceptible individuals

in a population is known to maintain the virus at endemic incidence while 25% can initiate a seasonal epidemic (Guillozet, 1979).

(c) Sex Incidence:

The incidence of measles was reported to be more in females than males (Hendrickse et al., 1964). The exact reason for this variation is unknown.

(d) Variation in Child's Environment:

The nutritional state of the child before and during the attack of measles is likely to be an important environmental factor (Whittle et al., 1980), since nutrition was found to be unsatisfactory in those regions where measles is severe particularly amongst children of lower socio-economic background (Ifekwunigwe et al., 1980).

(e) Reservoir Hosts and Carriers:

The incidence of measles in a community is governed by the immune status of the population (Krugman, 1977). There appears to be no natural reservoir of infection other than man (Fulginiti and Kempe, 1963); although MacArthur et al. (1979) reported the incidence of measles in monkeys. The

incidence of clinical disease 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 (Kaplan et al., 1980).

2.3. PATHOGENESIS AND COMPLICATIONS.

(a) Transmission:

Transmission occurs from person - to - person and the carrier state is important (Kaplan et al., 1980). Kempe and Fulginiti (1965) postulated that entry into a new host occurs as a result of virus deposition in the upper respiratory epithelium which seems the most likely site of multiplication of the virus.

(b) Incubation

The first observed event following the introduction of measles virus into the host is its appearance in the blood on the 5th - 7th day (Enders et al., 1959). During this interval, it is presumed that intense viral multiplication occurs in reticulo - endothelial tissue probably following an early viraemia (1st - 2nd Day) which transfers virus from regional respiratory lymphnodes to the entire reticulo - endothelial system and viscera.

Within 3 days following incubation in monkeys, some degree of generalized lymphoid hyperplasia and giant-cell formation was noted by Sergiev et al. (1960). These changes are progressive until the onset of prodromal symptoms.

(c) Symptoms:

The clinical manifestation of measles infection is detailed in several reports, notably Kempe and Fulginiti (1965) and Mordhorst et al. (1968). Generally, the symptoms include severe respiratory symptoms, toxaemia, high fever sometimes associated with febrile convulsions, gastro-intestinal upsets (vomiting and diarrhoea) which might give rise to severe fluid and electrolyte disturbances, sore mouth with mucous membranes presenting a velvety appearance. Numerous Koplik spots and rash are noticed (Hendrickse and Sherman, 1965).

(d) Mortality.

High mortality rate ranging from 5.7% to 30% is noted in Africa compared with lower values recorded for other parts of the world (Morley et al., 1963), with the peak age between 6 - 18 months. Morley and MacWilliam (1961) associated the high mortality with lack of food, principally with protein starvation.

(e) Relationship of Measles to Other Diseases.

The relationship of measles to other diseases is reviewed in various literature. Bech (1965) reported on the exacerbation of pulmonary tuberculosis in natural measles infection. Poliomyelitis has been found on many occasions to follow epidemics of severe

measles infection (Bloch and Massry, 1963). Artenstein and Weinstein (1963) observed a simultaneous infection with the viruses of chickenpox and measles.

(f) Persistent Infections:

Measles like other paramyxovirus infections usually results in widespread cellular destruction in cell cultures, but when surviving cells are selected and subcultured *in vitro*, they are often found to be persistently infected (Choppin and Compans, 1975, Rapp *et al.*, 1974). Barry *et al.* (1976) identified various types of persistently infected cultures due to measles infection viz: (i) the "carrier culture" persistent infection. In this case, Gibson and Bell (1972) demonstrated low levels of infectious virus in the medium. Gould and Linton (1975) showed that such a culture could be maintained for a long period. (ii) The second type of persistent infection reported is the "non-yielder" persistent infection. These non-yielder cultures were found by Knight *et al.* (1972) to be unable to produce infectious virus and the cultures also contain virus-specific intracellular antigens.

In vivo, antibodies were implicated to play a role in regulating measles virus persistence and consequent infection by modulating viral antigens expressed on cell surface (Oldstone and Tishon, 1978).

(g) Complications due to Measles Infections.

I. Central Nervous System Disorders.

Clinically, symptoms that indicate neurological involvement have been noticed to develop in many patients during measles virus infections (Katz, 1972, Pampiglione et al., 1971 Aicardi et al., 1977). However, whether the virus invades the central nervous system during the primary or secondary viremia is unknown. Neurological diseases associated with measles infection include (a) Subacute sclerosing panencephalitis (SSPE) (Ter Meulen et al., 1972a,b) and (b) Multiple sclerosis (MS) (Paterson, 1971).

II. Disruption of the Immune System:

Both humoral and cellular responses can be affected in a variety of ways by measles virus infection and vaccination (Katz, 1972). White and Boyd (1973) reported immunological complication such as aggregations of thymocytes, formation of large syncytia in the thymus and destruction of the thymic cortex. Firemen et al. (1969) and Zweiman et al. (1971) documented 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 phytohemagglucinin (PHA) may be responsible for the temporary immunological impairement (Sullivan et al., 1975a,b; Osunkoya et al., 1974a,b).

III. Bacterial Infections Complicating Measles:

Many workers have reported the occurrence of different types of suppurative bacterial infections complicating measles infection. Most common are otitis media, mastoiditis, bronchopneumonia and combinations thereof (Wood and Bernstein, 1978). These authors reported bronchopneumonia to be responsible for mortality rate as high as 32% in children of about 2 years of age, while laryngitis accounts for about 25% mortality rate. In the tropics such illnesses like diarrhoea and kwashiorkor, often in combinations, complicate measles illness (Morley et al., 1963).

2.4. PROPHYLAXIS (IMMUNIZATION).

(a) Active Prophylaxis:

Two different vaccines namely live and inactivated measles vaccines are available commercially all over the world; and both vaccines are derived from the original Edmonston strain (Feldman, 1964).

I. Live measles vaccine:

Two live measles virus vaccines have been studied and both are available as licensed products (live attenuated and live further attenuated) (Krugman, 1971). The live measles vaccines licensed for general use in 1963 were found to be highly effective in producing antibodies and giving protection against the disease (Feldman, 1964). Krugman (1965) reported on the high incidence of febrile reaction and rash following the administration of live measles Edmonston B vaccine. The mild systemic reaction could however, be reduced by either co-administration of a precise amount of gamma globulin with live vaccine or further attenuation of the virus through additional passages in tissue culture.

The advantages and disadvantages of live measles vaccine were documented by Krugman (1965 & 1977). The advantages include (a) high seroconversion (b) stimulation of all arm of immunity. While the

disadvantages are (a) risk of reversion or change of virulence (b) development of atypical measles (c) ineffectiveness in the presence of antibodies (d) difficulty in ensuring adequate attenuation (e) risk of contamination.

II. Inactivated measles vaccine.

The application of measles inactivated vaccines in large scale field trials was reported by Gard et al. (1965). The advantage associated with the use of inactivated measles vaccine include safety, application in individuals with cellular deficiency and effectiveness in presence of antibodies (Feldman, 1964). And the contra-indications associated with the administration of inactivated measles virus vaccine were summed up by Gard et al. (1965) and Kempe and Fulginiti (1965) and include rapid antibody decay rate (b) requirement of adjuvants (c) stimulation of mainly humoral immunity (d) high cost of production due to large amount of antigenic mass (e) induction of immunologic hyper-reactivity such that when recipients were challenged by the virus as it occurs in nature (f) low levels of seroconversion.

Norrby et al. (1975) found that the killed vaccine hitherto employed lacked hemolysin, one of the major viral envelope components. Thus, it appears that new types of killed vaccines containing all essential

envelope antigens could possibly provide more durable protection against measles than the present forms of inactivated vaccines.

III. Combined vaccines.

The combination of inactivated and live measles virus vaccines has proved to be a safe and effective immunizing regimen for protection against the disease (Rod et al., 1970).

Ueda et al. (1966) showed that vaccination with the killed - live measles virus vaccines (KL) has the advantage over the attenuated lives measles virus vaccine used alone in that the development of febrile reaction is reduced without affecting the antibody response to live measles virus vaccine.

It was reported by Brody et al. (1966) that antibody did not persist after the killed - live (KL) method of vaccination, but Ueda et al. (1970) showed that neutralizing antibody arising there - from remained stable for at least 5 years. Sangkawibha et al. (1971a,b) indicated that in the absence of natural measles virus, antibodies titers after the KL vaccination decreased rapidly during the first year following successful vaccination to a level which remained unchanged for at least one more year. However, Chatterji and Mankal (1977) reported the case of a child in whom atypical measles developed despite

the fact that she had received live measles virus vaccine several years after receiving killed vaccine. One reason adduced for the occurrence of the atypical measles by these investigators was the possible destruction of the live attenuated measles virus by the already present antibodies of a prior immunization with killed vaccine virus.

The trivalent combination of live measles, smallpox and yellow fever vaccines which showed identical seroconversion rate with any of the vaccines used monovalently was reported by Meyer (1965). Arakawa et al. (1965) found encouraging results in infants responding with sufficient antibodies to Diphtheria - Pertussis - Tetanus - Measles vaccine (DPTM) and proved that the potency of measles vaccine in DPTM combination is above or the same level of that in single live measles virus vaccine preparation. The remarkable levels of antibodies following the administration of measles - mumps - rubella vaccines was indicated by Weibel et al. (1978) and was found to be similar to that following natural measles infection. Warren et al. (1963) also reported on the possibility of combining measles virus vaccine with polio.

(b) Passive Prophylaxis:

Various preparations notably convalescent serum, placenta extracts globulins, fractionated blood globulin, gamma globulin extracted from plasma have been widely used for prophylaxis against measles infection (Perkins, 1965). There are disadvantages associated with the various preparations. These include principally the occurrence of serum diseases like serum hepatitis, difficulty in obtaining adequate supplies of these materials, low antibody concentration; local inflammation (hypersensitivity), headache and low grade fever following passive prophylaxis. Despite the obvious disadvantages and problems, Perkin (1965) indicated several situations in which it is desirable to prevent all symptoms of measles infection by passive prophylaxis. The situations include children under 3 years of age in whom the mortality rate of measles infection is highest, as well as sick or debilitated children; children who have never been in contact with measles and must travel; children entering hospital who will be exposed to other sick children and non-immune pregnant women.

(c) Present Status of Prophylaxis:

The results so far obtained using the plethora of vaccines (both live and inactivated) coupled with the various programmes of administration (intramuscularly, subcutaneously, intranasally) show no general agreement

about what kind of vaccine should be regarded as the vaccine of choice. According to Wallgren (1965), the ideal vaccine should not necessitate three injections or the use of gamma globulin. It should give sufficiently high and persistent immunity, and be safe and free from side effects. Inactivated measles virus vaccines used alone have lower immunological response and the duration of immunity much shorter than with the attenuated live vaccines (Krugman, 1971).

Inspite of the impressive decrease in the number of reported cases of measles during the last decade which Landrigan and Conrad (1971) and Krugman (1977) attributed to widespread availability of attenuated measles virus vaccines, outbreaks among recipients of the vaccines have continued to occur. (Rod et al., 1970; Wyll and Witte, 1971). In cases where there were outbreak of measles infection in recipients of the vaccines, detailed information was sought to find out the possible factors responsible. The adduced factors were: (a) The possible administration of inactive vaccine with the resultant failure in initial sero-conversion (King, 1978; Eghafona et al., 1979). (b) The lack of herd immunity (Wyll and Witte, 1971). (c) The route of administration was implicated by Wood et al. (1980); which indicated that the intranasal route of vaccine administration was better than the widely used subcutaneous or intramuscular

administration. (d) The inability to produce non-specific inhibitory substances and antibodies against measles by children with chronic disorders of the reticulo-endothelial system such as leukemia or by those with pre-existing disease like sickle-cell, primary tuberculosis and finally by those receiving steroid therapy (Aukrust et al., 1980). (e) The age of vaccination is an important factor affecting measles vaccine efficacy (Shelton et al., 1978, Marks et al., 1978, Brunell, 1978).

The committee on Infectious Diseases of the American Academy of Pediatrics in 1976 reviewed and condensed all pertinent investigation on measles immunization; and recommended that the optimum age for measles immunization be 15 months rather than 12 months. The recommendation was also given, that measles vaccine should be given anytime after 6 months of age during the course of an outbreak, but under these conditions a second vaccination should be repeated after 15 months of age. Vaccination at an early age may be unsuccessful due to the interfering effect of transplacentally acquired antibody (Krugman et al., 1967).

Norrby (1976) indicated that, vaccination should not be carried out too early before the age of one. If done before this age, it may result in restricted viral replication, owing to the presence

of maternal antibodies, and the immunity produced may be of relatively short duration. (h) The possibility that gamma globulin masking the stimulation of active immunity by the vaccine especially in excessive dose was indicated by Herman and Gold (1971). Arbeter et al. (1972) suggested that children who are immunized with live measles vaccine in combination with gamma globulin in the first year of life, should be recalled for revaccination.

The possibility of zooprophylaxis against measles with canine distemper virus was suggested by Wallgren (1965).

2.5. IMMUNITY TO MEASLES.

Black and Rosen (1962) showed that a single attack of measles confers life - long resistance, but Miller (1960) and Cherry (1980) documented second attacks in persons who previously had measles infections. Brody et al. (1966) reported that two years after vaccination inspite of the booster effect of natural measles, the antibody titers among those who received inactivated vaccine plus live vaccine declined to low or undetectable levels in more than half of the vaccinees.

Measles virus is known to stimulate infected tissue culture (De-Maeyer and Sniers, 1965) or human white blood cells (Cresser, 1961) to produce a polypeptide (interferon). This appears capable of inducing as yet unknown change(s) at the subcellular levels which prevent viral replication (Isaacs and Lindermann, 1957). Wilson (1961) described measles immunity as an infection immunity, but noted that the measles virus has not been found to persist in cells of patients as with other infection immunities like tuberculosis, syphilis, histoplasmosis, helminthic infections (e.g. Trichinella) in which constant presence of antigen provided constant antigenic stimulus for antibody production which confers immunity throughout life. However, Osunkoya et al., (1974a, 1974b) not only detected measles virus antigen in

human leukocytes by immuno-fleuresecence, but also observed that measles virus would not only infect human lymphoid cells, but also proceed to actively replicate in such cells.

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 3 - 9 months after birth, but Eghafona et al. (1985) observed that with increase in maternal parity measles HAI antibody titers in children declined. It is known that protective antibodies of such mothers would have been metabolized to such levels, that there is little or no protection for children thus born (Sinha, 1980). Albrecht et al. (1977) found that at 12 months, some children still have measurable maternal antibodies to measles virus. Krugman (1977) studied the persistence of HAI antibody for a period of 8 - 10 years, with dropping titers in children who were not exposed to naturally circulating measles virus.

This decline in antibody titer with time, raises concern about how low the level of antibody can fall before an individual again become susceptible. Odama et al. (1985) studied the neutralization of measles virus and found that HAI titers of 1:16 and above inhibited viral replication for 3 days. It was similarly observed by Weiner et al. (1977) that subjects

who had HAI antibody levels of 1 32 were susceptible to measles infection. In addition, Stokes et al. (1961) noticed reinfection to measles in those who have neutralizing antibody titers of less than 1 8.

There is no doubt that reinfection occurs amongst vaccinated subjects especially during epidemics (McCormick et al., 1977 and Wyll and Witte, 1971) leading to a booster effect on the antibody levels. Antibody functions as an effective barrier to measles virus dissemination while cellular immune mechanisms may prevent or lessen viral growth in the respiratory mucosa (Chiba et al., 1974). The degree of immunological response to measles is influenced by age, nutrition and pre-existing disease of the patient (Weiner et al., 1977).

2.6. METHODS OF CHARACTERIZATION AND DIFFERENTIATION OF VIRUS STRAINS.

A comparison of various properties (physical, chemical, biological, genetical and immunological) is essential in establishing relationships between viruses (McCormick et al., 1983). Generally, many of these properties are used in combination or singly in the characterization of a virus.

Adenovirus strain FL was differentiated from strain K87 on the basis of pathogenicity and tissue tropism (Sugiyama et al., 1967 and Van der Veen and Mes, 1973). Malkova (1974) used significant differences in plaque size, virulence and thermostability to distinguish between wild 181/3 and laboratory - maintained strains of Tahyna viruses. Johnson et al. (1982) compared four strains of subacute sclerosing panencephalitis (SSPE) virus with the wild type measles virus (Edmonston) by considering the titers of extra-cellular virus, morphology of cytopathic effect and occurrence of different measles virus antigens within infected cells as determined by immunoflourescence. Variants of influenza A virus with different pathogenicity for mice were found by Ivanova et al. (1982) using biophysical characteristics namely reproductive activity at optimal temperature, plaque - producing activity in chick fibroblast

and polypeptide composition. Plaque morphology was the sole characteristic used by Mann et al. (1980) in distinguishing between virulent and attenuated measles virus strain.

Two antigenically indistinguishable Kilham rat virus (KRV) strains 171 and 308 were found to have different host ranges and the capsids exhibited different iso-electric points (Mitra et al., 1982). The growth curves have also been used in revealing differences between viruses and strains (Hendricks et al., 1983 and McPhee et al., 1983). Other workers have employed inactivation rates or temperature sensitivity as the parameter for characterizing viruses (Gaunt et al., 1983; Toranzo and Hetrick, 1982). Bedson (1982) used thymidine kinase activity to differentiate between some isolates of Orthopox virus.

Genetically, a myriad of techniques have been employed for studying viruses. For example, the physical map of the DNA of two cytomegalovirus isolates AD-169 and SG were compared by Cross-blot hybridization (Weststrate et al., 1983). Weiss and Leibowitz (1983) studied the characteristics of murine corona virus RNA by hybridization with virus-specific complementary DNA probes. The DNA of various viruses have been easily distinguished from each other by their restriction endonuclease patterns (McIntosh and Ignoffo, 1983

Rohrmann et al., 1982). The genetic and antigenic heterogeneity amongst a collection of 17 poliovirus type 3 strains were detected by Minor et al. (1982) using monoclonal antibodies and T1 oligonucleotide mapping.

Monoclonal antibody technique was employed in distinguishing herpes simplex type 2 glycoproteins (Balachandran et al., 1982, Kumaraswamy and Blough, 1983). Lefrancois and Lyles (1983) selected antigenic variants of vesicular stomatitis virus (VSV), serotypes New Jersey and Indiana (VSV-NJ, VSV-Ind) by using a panel of monoclonal antibodies (MAb) specific for the major surface glycoproteins. Measles polypeptides have been characterized by such panels of monoclonal antibodies secreted from human or mouse hybridomas (Croce et al., 1980; Togashi et al., 1981; McFarlin et al., 1980 and Birrer et al., 1981). Gould et al. (1981) examined the immunological relationship between measles virus and canine distemper virus using monospecific measles antisera. Antigenically distinct strains of other viruses have been detected by monoclonal antibody techniques (Greenberg et al., 1983).

Comparative serological (hemagglutination inhibition and neutralization) characterization and biochemical (isoelectric focussing) were done by Kinney et al. (1983) to distinguish between viruses in the Venezuelan equine encephalitis complex. Trushinskaya et al. (1982)

stulied the hemagglutinin antigenic determinants of Influenza A virus by solid-phase radio-immunoassay. The antigenic relationship of viruses have been determined by neutralization test (CIE inhibition) and enzyme - linked immunosorbent assay (ELISA) (Wigand et al., 1982; Thouless et al., 1982). Nath et al. (1975) observed antigenic variation between SW/73 and SW/31 Influenza viruses using serological test. The variation was detected only when SW/73 antiserum was used in radio-immunoassay technique with purified ^{125}I-labelled viral antigens and it was indicated that it is possible that SW/31 virus, underwent a point mutation, whereby a weak or non antigenic site was replaced by a strong antigenic site in SW/73. Hence antibodies specific to the new antigenic determinant in SW/73 may be unreactive against SW/31. In addition, the degree of displacement of ^{125}I-SW/73 antigen from the SW/73 antiserum in the radio-immunoassay by increasing concentrations of unlabelled antigen was greater with SW/73 than SW/31 antigen, thereby indicating that SW/31 antigen lacked new antigenic determinants needed to compete for the antibodies.

Other serological techniques have been used in the comparison of viruses (Karcher et al., 1981; Goswami and Russel, 1982; Shiraki et al., 1982). The great value of antigen - antibody reactions is their specificity: an antibody will combine only with an

antigen which contains groupings of amino acid sequences similar to that causing its formation in an animal. The production of antiviral serum has been known for a long time, but until recently, most serological tests have been done by end-point titration in liquid medium (Thouless et al., 1982). The Hashimoto and Kawase (1983) agar diffusion techniques has greatly facilitated the testing proceedure, and has made possible an exact method for detecting the quantity, the number and the kinds of antigenic component within and antigen - antibody system.

The choice of an immunological testing proceedure depends upon the skill and interest of the investigator, since each technique contributes to a better understanding of the virus. In this work, the techniques used to characterize and compare the Wild measles virus with Schwarz strain were (a) Cytopathic patterns in Vero - and BK - cells (b) Viral yield, (c) Plague size, (d) Serological tests (HAI, NT, Immunodiffusion) and (e) Thermostability.

CHAPTER 3

ISOLATION AND PROPAGATION.

3.1. INTRODUCTION.

Various clinical specimens viz: serum or plasma, whole blood, lymphocytes, throat and nasal swabs, urine etc. have been used for the isolation of measles virus from infected persons (Enders and Peebles, 1954). The use of human blood for measles virus isolation almost certainly owes its success as a source to the presence of measles in leukocytes (Osunkoya et al., 1974a,b).

Enders (1962) noted that primary cell cultures make more sensitive subtrates than continuous cell lines. Matumoto et al. (1961) indicated that human embryo kidney and monkey kidney were equally sensitive for isolation of measles virus. Once this was accomplished, conversion to growth in continuous cell lines seems to be reasonably easy. This section reports on the isolation of Wild measles strains using Vero; and the propagation of both Wild and Schwarz strains in Vero and Bovine kidney (BK) cells.

3.2. MATERIALS AND METHODS.

(a) Wild measles Virus (MV-W).

Collection and Storage of specimens:

About 5ml of blood was collected from measles patients by the clinicians using venous puncture method. The blood was immediately dispensed into sterile vacuum tubes containing 15 units/ml of heparin. The blood was processed within 12 hours of collection as detailed below.

Lymphocyte seperation:

The heparinized blood was transferred into test tubes, and diluted 1:1 with normal saline. The lymphocytes were isolated by layering the diluted blood into an equal volume of Ficoll - Hypaque gradient (Pharmacia, Uppsala, Sweden) as described by Boyum (1968), and centrifuging at 1,000 x G at room temperature for 30 min. The lymphocyte band was collected into centrifuge tubes, washed three times in 10mls. phosphate buffer saline (PBS) (pH. 7.4).

The washed lymphocytes were subjected to three cycles of freezing and thawing. The homogenates were clarified at 10,000r.p.m. for 10 minutes at 4°C, then the supernate was pelleted at 24,000r.p.m.

for 3 hours using SW41 - rotor (Serial No. E 2193) in Beckman Ultra centrifuge Model L5-50. The final pellet was stored at - 20°C as virus isolate.

(b) Schwarz measles virus (MV-S):

The Schwarz strain measles virus (Dow Lot SA_5 940x) passaged once from the seed virus in chick embryo fibroblast, was obtained from Dr. F. E. Andre's Laboratory (Smith Kline - RIT Biological Division, Rixensart, Belgium) as a lyophilized preparation. The seed virus is used in the preparation of measles vaccine Rixmevax SK and F, the common vaccine used in Nigeria. The virus was re-isolated in our laboratory using Vero cells.

Growth medium.

A single strength Eagle's medium M.E.M. T.C. 46 (Wellcome Reagents Ltd. Beckenham, England) was prepared by dissolving 10gm of dried medium in 950mls. of de-ionized water. This was supplemented with 10% fetal calf serum, 10% tryptose phosphate broth (TPB) (Flow Laboratory, U.K.), antibiotics (Penicillin G 200,000 units/liter and streptomycin sulphate 100,000gm/liter) all supplied by Serva Feinbiochemical Heidelberg and 1ml of Fungizone 250mg/ml. (Flow

Laboratory, U.S.A.).

Maintainance medium:

Cell cultures were maintained in a similar medium but supplemented with 2% fetal calf serum and 2% tryptose phosphate broth.

Tissue culture:

Virus isolation was carried out in Vero cells (P.131) obtained from Nigerian Veterinary Research Institute (NVRI), Vom, Nigeria. Freeze dried Schwarz virus was reconstituted with 1ml PBS while the frozen Wild measles virus was thawed. These were seeded on confluent monolayers of Vero cells which were earlier grown for 48 hours at 37°C in 32oz plastic cell culture bottles using Eagles MEM growth medium. The growth medium was decanted, the cells were washed with PBS, infected with 0.5ml of virus, then incubated at 37°C for 1 hour, and 5ml. maintainance medium was added. The infected monolayer were incubated at 37°C, and observed daily for cytopathic effect (CPE). The cells were ruptured by three cycles of freeze-thawing, and cell debris removed by centrifugation at low speed (10,000 r.p.m.) for 10 min. in Beckman Ultra-centrifuge. The supernate was carefully decanted and the virus was

pelleted by further centrifugation at 30,000 r.p.m. for 2 hours. The pellet thus obtained was resuspended in 1ml. of PBS as stock culture, and stored at - 20°c.

Propagation of measles virus in BKC:

Virus suspensions in 1ml. maintainance medium were seeded into culture flask of confluent BKC (P.5) obtained from NVRI, Vom, Nigeria. The virus was allowed to adsorb for 1 hour at 37°C, then 5 ml. of maintainance was medium added.

Similarly, BKC from confluent monolayer were dispersed with 0.025% trypsin and inoculated with 1 ml. of viral suspension; then 5ml. of maintainance medium added. Cultures were examined daily for CPE for a period of 7 days for the development of CPE.

Purification:

Sucrose (Analar BDH Lab. Chem. Div. Poole, England) was dissolved in distilled water in various concentration (10%, 20%, 30% and 40%). These various concentrations were carefully layered out into Beckman's Polyallomer tube in 1.6, 2.8, 2.8, 2.8ml. respectively; and left overnight in cold to have linear or smooth gradient.

Sucrose gradient columns was run with 1ml virus sample centrifugation in a Beckman Ultra - centrifuge at 35,000 r.p.m. for 2 hours at 4°C as described by Schluederberg (1962). Virus zones were readily located by light scattering UV photometric scanning in CE 202 ultra - violet spectrophotometer. The virus sample was removed from the density - gradient column by inserting a hypodermic needle down through the top of the column to the desired depth, and then slowly removing the sample. The band was washed with PBS under centrifugation thrice and resuspended in 5mls. of PBS.

Spectrophotometry:

For measuring absorbance, cuvettes filled with the virus solution and the PBS (pH 7.4) without the virus (blank) were inserted into the spectrophotometer (Model UNICAM PGE SP8-100). The wavelength dial was set to the required wavelength in the range 320-220mm. The blank was first positioned in the light beam and the scale set to read zero absorbance (= 100% transmittance). After this, the absorbance was read off the cuvette with virus solution. Absorbance patterns were noted at 10mm interval of the whole range of wavelengths.

Determination of Virus titer.

Viral titration was done by inoculating 0.2ml of ten fold virus dilutions into each 60mm petri dishes containing confluent Vero cells. Four cultures per dilution were used. The cultures were incubated at 37°C and observed daily for CPE. Then $TCID_{50}$ was estimated according to the method of Reed and Muench (Basic Virological Methods: Crist et al., 1979).

The infectivity titer of $TCID_{50}$ was further quantified by the plaque counting method of DeMaeyer (1960) with modifications as described below.

Plaque Assay:

Growth and Maintainance medium. As already described.

Overlay medium:- The overlay medium consisted of equal volumes of double (2x) strength Eagles M.E.M. containing 5% tryptose phosphate broth, 2% Ion agar (Oxoid, England) and 0.01% sodium azide. This was supplemented with 0.01% DEAE - Dextran (Pharmacia Fine Chemicals, Sweden) and 0.01% Magnesium chloride (BDH Lab. Chem. Div. Poole, England) to aid plaque development. The overlay was maintained at 45°C in water bath until used.

In details, monolayers of Vero cells were prepared by seeding 60mm plastic petri dishes with 5mls of 3.1×10^6 Vero cells/ml. After overnight incubation at 37°C in a humidified atmosphere of 5% Co_2 in air (standard conditions) the

growth medium was removed and each culture inoculated with 0.2ml. of suitably diluted virus. Inocula were allowed to adsorb for 1 hour at 37°C and overlay medium (5.0mls) added to each dish. After 7 days incubation under standard conditions, 5.0ml of a second overlay medium into which 0.01% neutral red (volume/volume) had been incorporated was poured on dish. Plaques were counted with the aid of a magnifying glass after 24 hr incubation.

3.3. RESULTS.

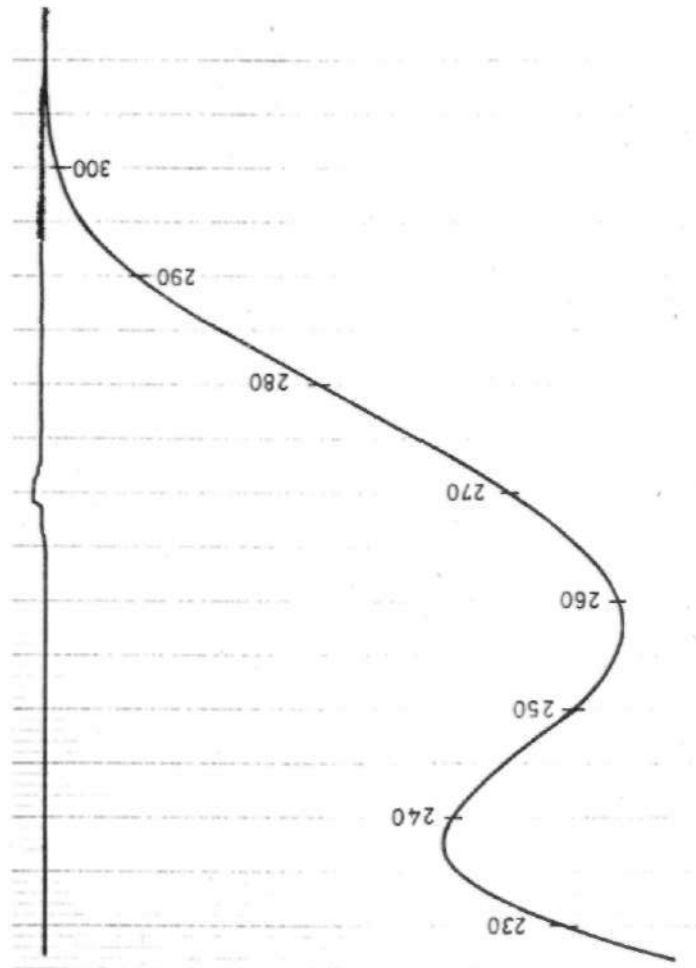
Out of 115 measles patients screened, 14 (12.17%) yielded virus which showed cytopathology in cell cultures (Table 1). Only sample ABUTH No. 539482 was used for further investigation. The development of cytopathology was noted in Vero and BK cells.

The purified virus samples when scanned in a spectrophotomer gave a typical virus spectrum with maximum absorbance at about 260nm and a minimum at about 240nm (Fig. 1) with no correction made for light scattering. The titers for both Schwarz (MV-S) and Wild (MV-W) measles strain were estimated at approximately 1000 pfu/ml.

Table 1: Profiles of patients from whom measles virus was isolated.

Patient No.	Age (months)	Sex	Vaccination	Clinical pictures
545642	18	M	No	Severe
539220	10	F	No	Severe
539491	19	F	Yes	Mild
539060	24	M	No	Mild
389274	18	M	No	Mild
539862	24	F	No	Severe
471976	30	M	No	Mild
548919	7	M	No	Severe
539402*	9	M	No	Severe
545271	18	M	No	Mild
551153	10	M	No	Mild
551280	18	M	No	Mild
551156	12	F	No	Severe
545816	13	F	No	Mild

*Isolate for further study - chosen because of the severe nature of clinical measles.
F - Female
M - Male.


300
290
280
270
260
250
240
230

3.4. DISCUSSION.

Cytopathic effects (CPE) were noted in both Vero and BK cells. The trypsinization of cells seems only to have increased their susceptibility or sensitivity to measles virus, since CPE was noted in both confluent and trypsin-treated cultures at different times. The ability of the virus to form CPE faster in trypsin-dispersed cell was expected. Trypsin has been shown to have an enhancing effect on the plaquing efficency of vaccinia virus (Gifford and Klapper, 1969). Although the mechanism of enhancement is unknown, it was suggested that the trypsin "conditioned" the cells to permit virus entry probably by exposing receptor sites.

It is usual to use the characteristic spectrophotometric spectrum of viruses to ascertain presumptively if a preparation is a virus or not (Noordam, 1978). Most of our preparations absorbed maximally at 260nm and minimally at 240nm which is representative of the absorbance pattern known for viruses. Spectrophotometric data may be indicative of the presence of proteins or nucleic acids but the data do not prove that the absorption is caused by a virus. Isolation of other materials may show absorbance suggestive of viruses. Therefore, a correlation with, for example, infectivity and serological end-point is needed as further indication that the absorbance is caused by a virus.

CHAPTER 4

GROWTH CHARACTERISTICS OF SCHWARZ AND WILD MEASLES STRAINS.

4.1. INTRODUCTION.

The attenuated mutants of several viruses are characterized by well defined in vitro differences when compared to their more virulent counterparts (Schwartz and Roizman, 1969; Figueroa and Rawls, 1969). These differences are genetically stable and can be used as markers for comparing viral strains. For example Gould et al. (1976) identified two strains of SSPE - associated viruses based on the type of CPE formed (syncytial or non-syncytial) and its rate of onset.

Measles virus can be propagated in primary cultures of human or simian origin, and a variety of continuous human cell lines originating from both normal and malignant cells. In the continuous systems, multiplication generally occurs more rapidly with earlier appearance of cytopathic changes consisting of syncytia and spindle-cell structures (Makino et al., 1970).

The different cytopathic effects have proved reliable criteria of monitoring viral multiplication and have also provided the basis for convenient and fairly rapid proceedures for distinguishing a virus, assay its infectivity and in the determination of

neutralizing antibodies (Enlers, 1962). Grist et al. (1979) correlated the development of the cytopathology with the development of virus antigen and formation of infection.

Mann et al. (1980) indicated that virulent Edmonston strain can be differentiated from the attenuated strain on the basis of plaque morphology and size with the virulent strain larger in size and rougher in morphology. Various strains of pseudorabies virus have been classified into three categories namely medium size plaques, large plaques and well formed small plaques by Golais et al. (1982).

The growth characteristics of Schwarz and Wild measles strains were examined in this section, by cytopathology, plaque size. Comparative viral yield in Vero and Pk-cells were also studied.

4.2. MATERIALS AND METHODS.

Tissue culture:

Bovine Kidney (BK) (P.5-8) and Vero (P.136-140) cells were used in this investigation. Both were supplied by Nigerian Veterinary Research Institute (NVRI), Vom, Nigeria.

Growth and Maintainance medium:

Same as in chapter 3 pages 37 and 38 (Materials and Methods).

Overlay medium:

Same as in Chapter 3 page 41 (Materials and Methods).

Both confluent and dispersed cultures of cells in 25cm^2 tissue culture flasks, were inoculated with approximately 200pfu/0.2ml. of Schwarz and Wild samples. The cultures were allowed to adsorb for 1 hour at 37°C. Then 5ml. of maintainance medium was added and observed for evidence of CPE daily. At regular intervals of 12, 24, 36, 48, 60 and 72 hours, 0.2ml aliquots of the culture medium were taken from triplicate cultures after rocking of culture flasks. The fluids, thus derived

from cultures infected with the same strain were titrated and the amount of infectious particles present was determined by plaque assay methods already described. All experiments were done in triplicate. The morphology of plaques thus developed in various cell cultures were also noted.

4.3. RESULTS.

Cytopathology:

The Vero cells showed syncytial and giant cell formation, while the BKC mainly spindle cells when inoculated with Schwarz and Wild measles strains (Plate 1AB). These early changes were often accompanied by the following phenomena cell rounding, pyknosis and disirtegration.

The cytopathology of Schwarz strain become evident in 7 days and Wild strain in 5 days in confluent Vero cells, while the dispersed Vero cells showed CPE at 5 and 4 days for Schwarz and Wild strains. The Bovine kidney cells were more sensitive to the virus than Vero, showing cytopathology in 6 and 4 days confluent cultures for Schwarz and Wild strains, and 4 and 3 days in dispersed cultures for Schwarz and Wild strains (See Table 2.1).

Viral Yield:

The viral yield for Schwarz and Wild strain are presented in Table 2.2. Viral yield was lower for Schwarz strain compared with Wild in all

cells by analysis of variance (F * 9£743). Both Vero and BK cells showed no significant differenc- in viral yield (F • 1.169),

£}• a< 3 ua. ^10 r P h0 ^° V *"

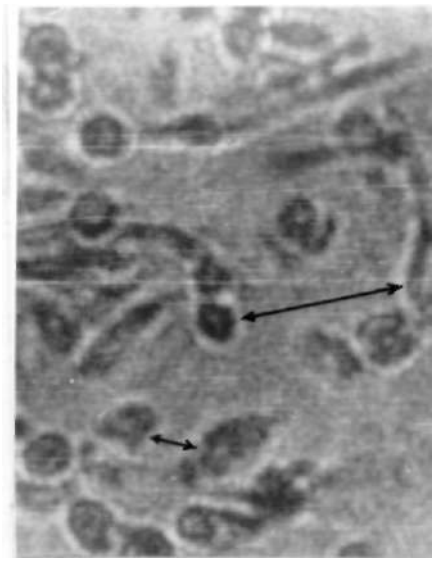
Schwarz strain developed jsnooth plaquos of less than 1mm in size, while the Wild strain showed rough plaques greater than IKUII in size.

Table 2.1: Time (days) for 100% CPE Development in various cell cultures by Schwarz (MV-S) and Wild (MV-W) Measles strains.

Strain	Confluent		Dispersed	
	Vero	BKC	Vero	BKC
Schwarz (MV-S)	7	6	5	4
Wild (MV-W)	5	4	4	3

Plate 1A: Cytopathic effect of Schwarz (MV-S) and Wild (MV-W) measles strains on Vero cells. Arrows show syncytial and giant cell formation. Magnification x 100.

c:S



m

'4

'i

1

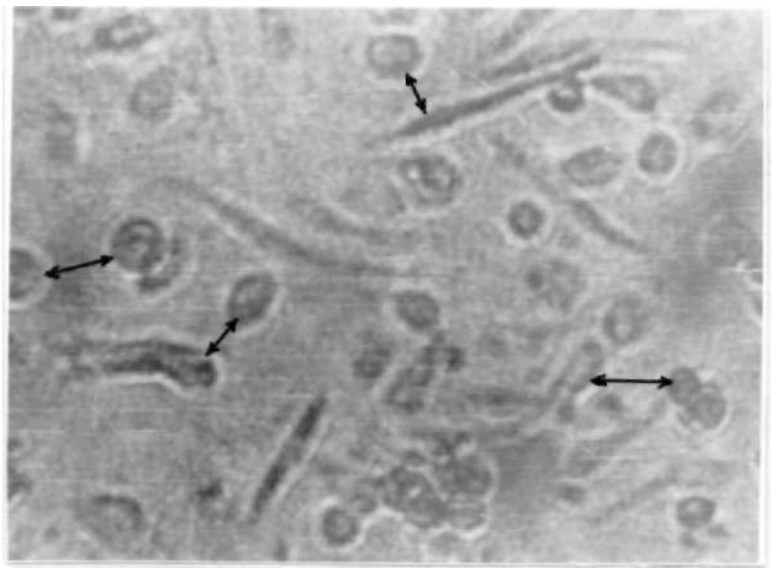


Plate IE? Cytopathic effect of Schwarz (MV-S) **and Wild** (WV-W) **measles strains on** fjovine **K id ne y Ce1Is** (**BKC**). **Arrows s how spi ndle cell formation. Magnification x 100.**

Table 2.2: Viral Yield (pfu/0.2ml) of Schwarz (MV-S) and Wild (MV-W) measles strains in Vero and Bovine kidney cells.

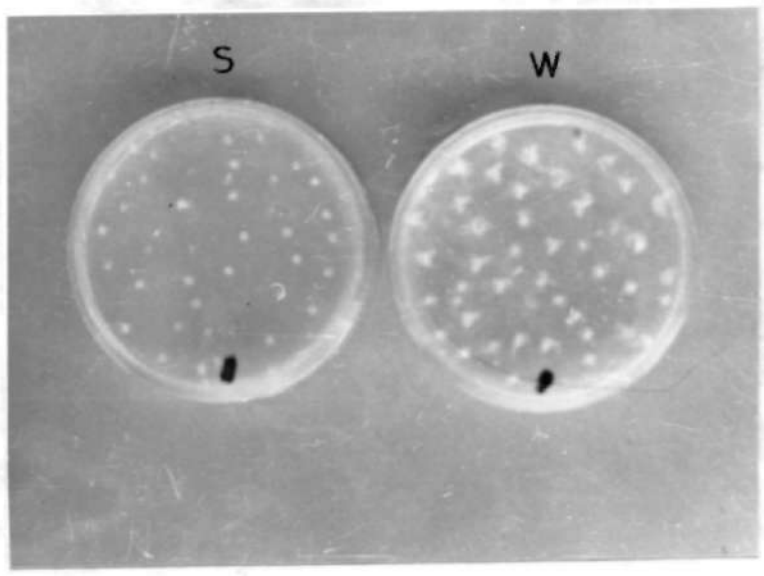
Incubation Time (Hours)	Schwarz (MV-S)		Wild (MV-W)	
	Vero	BKC	Vero	BKC
12	2,7,8: 6.00[a] (0.78)	3,0,5: 3.00[a] (0.48[a])	8,9,11: 9.00[a] (0.95)	11,9,15: 12.00[a] (1.08)
24	14,11,10: 12.00 (1.08)	8,9,11: 9.00 (0.95)	19,18,24: 20.00 (1.30)	24,16,18: 19.00 (1.32)
36	13,19,24: 19.00 (1.28)	12,15,20: 16.00 (1.20)	28,23,31: 27.00 (1.43)	24,38,30: 31.00 (1.58)
48	22,29,31: 27.00 (1.43)	16,23,28: 22.00 (1.34)	43,37,52: 44.00 (1.64)	51,40,36: 42.00 (1.62)
60	36,30,40: 35.00 (1.54)	31,35,30: 32.00 (1.51)	63,74,58: 65.00 (1.81)	59,48,38: 48.00 (1.68)
72	57,61,49: 56.00 (1.75)	47,38,44: 43.00 (1.63)	69,77,73: 73.00 (1.86)	60,66,53: 60.00 (1.78)

[a] Mean of triplicate counts

( ) Mean expressed in $\log_{10}$

F virus = 9.743

F cell = 1.169.



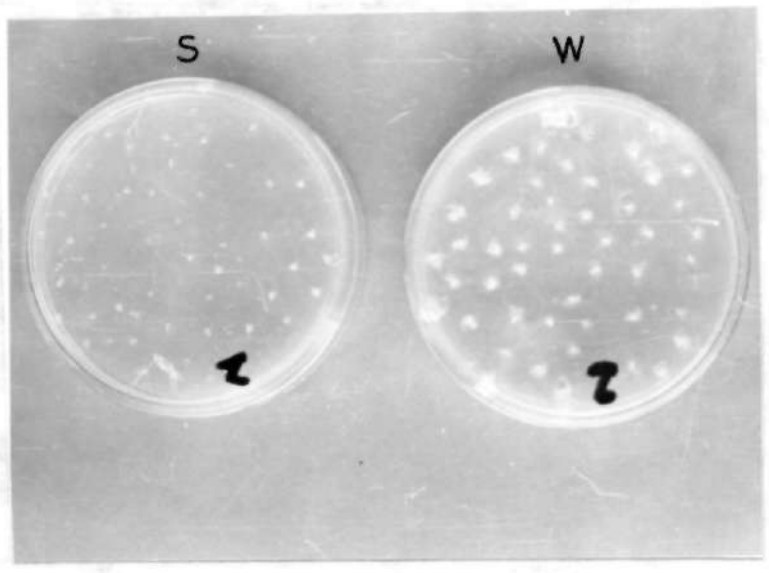


Plate II: Plaques of Schwarz (MV-S) and Wild (MV-W) measles strains on (A) Vero cells and (B) Bovine kidney cells.

4.4. DISCUSSION.

Schwarz and Wild measles strain produced typical cytopathologic lesions in Vero and BK cells indicating their ability to replicate and produce CPE in these cultures. However, the growth of the Schwarz strain was slower in all cells compared with Wild strain. In measles virus, two variants of the Edmonston strain have been established based on time and type of CPE development (Chourini and Norrby, 1970). A similar study carried out by Matumoto et al. (1961) distinguished the Japanese strains of measles virus from the Edmonston strain on the basis of time of CPE development. These Japanese strain required 3 days for CPE development, as against 5 - 6 days for Edmonston in renal cell cultures.

The growth of the measles virus on BKC provides a ready substrate for the propagation of the virus in our laboratory, especially when the medium is changed at 3 days intervals and 0.02% trypsin is added to culture. This observation supports that by Makino (1977) which indicated that sheep kidney cells were found to be equally susceptible to measles virus as human or monkey kidney cells. The viral yield in both cell types were comparable, with no significant difference by the statistical analysis done.

The overall picture then, emerging from these comparative growth yield is a follows: for unknown reasons the Schwarz strain grows less well in all cells used. Various conditions are known to increase the yield of measles virus in cell culture systems namely cell type, temperature of incubation and the use of low passaged virus (Scott and Choppin, 1982). There are evidences that all laboratory isolates of measles are not identical in plaque formation. Rapp (1964) showed that large plaques (>1mm) were formed with virulent Edmonston strain and small plaques (<1mm) with the attenuated Edmonston strain. Schumacher et al. (1972) recorded that plaque size in Vero cells was a significant factor in distinguishing Wild measles strain from vaccine strains. It is known that some stable plaque variants of measles virus exist (Frazer and Martin, 1978).

Ruckle - Enders (1962) indicated that the different growth pattern in cell cultures represented variant forms of measles.

CHAPTER 5

SEROLOGICAL COMPARISON OF SCHWARZ AND WILD MEASLES STRAINS.

5.1. INTRODUCTION.

Mammalian viruses have been classified into serotypes by a variety of serological tests, notably immunodiffusion, passive hemagglutination, hemagglutination inhibition, neutralization and indirect immunoflourescent techniques (Geder and Skinner, 1971; Schneweis and Nahmias, 1971). Serological variants of measles virus are not numerous, probably because only a limited number of isolates have been studied. However, serological analysis of the measles virus isolates can lead to an understanding of the reason of vaccine failures especially in circumstances where an antigenically different strain is used for immunization.

This section describes a serological comparison os Schwarz and Wild strains by haemagglutination inhibition, plaque neutralization and immunodiffusion assay.

5.2. MATERIAL AND METHODS.

Animal Model:

Young patas monkeys Erythrocebus patas (Schreber) obtained locally from Thomy Brothers Enterprises (Zoological Gardens) Kano, Nigeria were used for this study. For experimental inoculations, the monkeys were divided into 2 groups of 5 each and kept in strict quarantine for a period of 4 weeks. Quarantine and maintainance of the monkeys were conducted in accordance with the proceedures desrribed by Takasaka et al. (1964), supplemented with the advice given by the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

Inoculation:

All the monkeys were marked and numbered. The animals were sedated with Ketamine hydrochloride (Vetalar, Park-Davis and Co. Detroit Michigan 49232, U.S.A.), A 500pfu/0.5ml. of measles strain was mixed with equal volume of Freund's incomplete adjuvant (Behringwerke AC, Marburg, Lahn West Germany) and inoculated intra-muscularly into each animal. The animals were re-inoculated thrice at 2 weeks interval with appropriate antigen.

Sera Preparation:

Five milliliters of blood obtained in the cardiac bleeding was transferred to a sterile MacCartney bottle and allowed to clot overright at 4°C. After clotting, serum was separated by centrifugation at 1,000 x G for 30 minutes. Each serum sample was decanted into a sterile bijoux bottle and 2 drops of 0.1% sodium azide added as a bacteriostatic preservative. The samples were labelled and stored at - 20°C until titrated.

(a) Haemagglutination Inhibition (HAI) Test:

The Haemagglutination Inhibition test was performed as described by Rosen (1961) and recommended by Wellcome Research Laboratories, Beckenham, Kent, England. This method was, however, modified by using 1% monkey erythrocytes instead of the recommended 0.5% to enhance sensitivity of HAI pattern. The test was performed in Linbro/Titertek micro-titration 96 well V-bottom plates (Linbro Scientific, Inc., Hamden Conn., U.S.A.).

Diluent:

Normal physiological sterile saline solution was used.

Monkey Erythrocytes:

The erythrocytes used as test indicator were collected by cardiac puncture. Monkey erythocytes (patas) were collected in at least four times its own volume of Alservers solution (1:4) as anticoagulant and used within one week of collection. Before use, the erythrocytes were washed at least three times in normal saline.

The buffy coat (white blood cells) was carefully removed after each washing. The packed cell volume (PCV) was noted and 50% of the erythrocytes prepared by adding an equivalent volume of saline. One percent (1.0%) of this suspension was further prepared in sufficient amount and used for HAI test.

Reagents:

The other reagents were (a) measles HA antigen (VH03) prepared by Tween - ether treatment of a strain of measles virus grown in monkey kidney cells (b) Measles HA positive control serum (VE03) prepared from a pool of measles immune sera (this reagent inhibits the haemagglutinating activity of measles HA antigen) (c) measles HAI negative control serum (VJ03) prepared from a pool of sera from measles

negative persons. These reagents were purchased from Burroughs Wellcome Reagents Limited, Beckenham, England. Kaolin used to adsorb all the sera was obtained from the Pharmacy Department of Ahmadu Bello University Teaching Hospital (ABUTH) Zaria, Nigeria. Twenty-five percent (25%) (w/v) was made up in sterile saline solution and used for adsorption.

Treatment of Sera for Removal of Heterophile Antibodies:

The sera collected were adsorbed with 25% kaolin, then with 50% (w/v) of patas monkey erythrocytes at 4°C overnight. Kaolin (0.3ml) was mixed with 0.1ml of serum, left at 4°C for 20 mins., the mixture was then centrifuged at 1000 x G for 10 minutes to pack the kaolin. A drop of the 50% preparation of monkey erythrocytes was added to the resulting supernatant serum and left at 4°C overnight. The serum was, subsequently centrifuged at 1000 x G for 10 mins. and decanted into a clean test tube. This treatment of mixing 0.3ml of kaolin and 0.1ml of serum, left the serum at 1:4 dilution. The addition of kaolin was a minor modification of the method used by Burroughs Wellcome, and this modification ensured removal of non-specific haemagglutinins and other heterophile antibodies (Kano and Millgrom, 1977).

This adsorption process also applied to the HAI positive and negative control sera after each had been reconstituted with 1.0 ml. of de-ionized water.

Antigen Titration:

The lyophilized HA antigen was reconstituted with 1.0 ml of de-ionized water and titrated as detailed below. A preliminary 1:4 dilution of the antigen was prepared by adding 0.1 ml. to 0.3 ml. of sterile saline. Saline solution (0.025ml.) was added to wells 2 - 9 in each of three rows of wells in a V-type microtitration plate and 0.05 ml. was added to three additional wells for cell control. Antigen (0.05ml. of 1:4 dilution) was added to the first wells of the three rows. With the aid of microdiluter 0.025ml was transferred from the first well to the second well in each row and the series was continued till the end of each row. The antigen dilution now ranged from 1:4 in well 1 to 1:1024 in well 9 with a two fold difference between wells. Saline solution (0.025ml.) was added to each well of the diluted antigen and 0.025ml. of a 1% suspension of washed monkey erythrocytes added to all wells including cell controls. The plates were gently tapped to ensure even distribution of erythrocytes, then the

plates were sealed to prevent evaporation and were then incubated for 1 hour at 37°C. The HA pattern was found and the titer recorded. The 4HA unit was calculated from the highest titer by dividing this titer by 4 to obtain the dilution for use in the HAI test. The antigen titration was carried out regularly to ensure that 4HA unit was used in each test.

A row of 10 wells in a microtitration plate was used to dilute out the sera obtained from the children. The first 9 wells were for the serum dilution 1:4 to 1:1024. The 10th and 11th wells served as the positive and negative control wells. First, 0.025ml of saline solution was placed in each well except the first. Diluted serum (0.05ml. of 1:4 dilution) was placed in the first well while 0.025ml. was placed in the serum control well.

With the aid of a microdiluter, 0.025ml. of 1:4 diluted serum was transferred from the first well to the second and after mixing 0.025ml. of 1:8 diluted serum transferred to the third well. This process was continued in this manner to the 9th well (serial doubling dilution) and 0.025ml. of the 1:1024 diluted serum was discarded from the 9th well to ensure equal volumes being dispensed in all the wells. Antigen dilution (0.025ml.) containing 4HA unit was added to all the wells except the 10th and 11th, then covered

and incubated at room temperature for 1 hour, to give enough time for the antigen - antibody to react.

Thereafter, 0.025ml. of 1% washed monkey erythrocytes was added to each well and incubated at 37°C for another one hour and the resulting titer read with the aid of a magnifying glass. All the controls earlier on mentioned together with the cell and test serum controls were run along with the test. A reciprocal of the serum dilution which showed a complete inhibition of the haemagglutination was expressed as HAI antibody titer.

(b) Plaque Reduction Neutralization test:

Antibodies were assayed by reacting a constant amount of virus (approximately 50 or 100pfu/0.2ml) with varying serum dilutions, in standard plaque assay technique as described by Rawls et al. (1970). The antisera used were obtained 4 weeks post - inoculation with measles strains.

Tissue culture:

Vero cells (NVRI, Vom, Nigeria) were used for plaque neutralization assay.

Growth/Maintainance/Overlay media

As already described (Chapter 3 - Materials and Methods).

Serial doubling dilutions of immune measles serum was made with maintainance medium starting from 1:4. Schwarz (MV-S) and Wild (MV-W) measles virus stock were mixed with equal volume (0.5ml each) of each serum dilution, and incubated at 37°C for 1 hour. 0.2ml. of each mixture were then inoculated into monolayers of the Vero cells grown in 60mm petri dishes. Triplicate cultures were inoculated for each mixture. Plaque assay was done on inoculated cultures as already described, and 50% plaque reduction was the neutralization titer.

The plaque neutralization test was repeated using 6 patas monkeys divided into 3 groups of 2 monkeys each. Two groups were inoculated with 0.5ml of 1000 pfu/ml of Schwarz and Wild measles strains mixed with 0.5ml of Freund's incomplete adjuvant. The third group which received 1ml of saline served as control. Sera collected 3 weeks post-inoculation were titrated by HAI test. These were further titrated for neutralizing antibodies using the plaque neutralization technique already described. Serial doubling dilutions of sera starting from 1.10 were used. Experiments were done in duplicates.

Gel Plates:

Ionagar No.2 (Oxoid Ltd., London) was prepared 1gm per 100mls. of phosphate buffer saline (pH 7.2) to give 1% (w/v) of agar. Sodium azide (0.1g) was added, and sterilized by autoclaving at 15lb sq in for 15 mins. Five (5) mls. of this preparation was poured into the bottom of 50mm plastic petri dish. The agar was allowed to solidify and a series of wells made using standard gel puncher of diameter 5.0mm. A circular pattern was made with a central well and six surrounding wells 4.5mm equidistant from one another. The plates were appropriately labelled at the bottom.

Measles Sera:

Measles positive control serum (HAI titer 1:512) (Behring Institute, Marburg - Lahn, West Germany) was used against Schwarz (MV-S) and Wild (MV-W) strains.

Measles Antigen:

Measles antigen (HA titer 1:128) (Berhring Institute, Marburg - Lahn, West Germany) was used against anti-Schwarz and anti-Wild sera.

The center well was filled to the brim without allowing spillage with measles positive sera and the peripheral wells charged with the purified virus strains (measles antigen). The plates were placed in a moist chamber and incubated at room temperature for 3 days. The gel was washed in two changes of 0.85% Sodium chloride for 6 hours; followed by distilled water overnight; and dried before staining in 0.01% Coomassie blue stain and finally dried again then viewed with magnifying glass.

The entire proceedure was repeated using measles antigen in the central well and the peripheral wells filled with immune sera.

5.3. RESULTS.

Hemagglutination Inhibition:

The HAI titers of monkeys sera inoculated with Schwarz and Wild measles strain are shown in Table 3.1. The HAI titer ranged from 128 - 512 and from 16 -1024 for Schwarz and Wild strains respectively. The geometric mean titer (GMT) analyzed by the Mann-Whitney test at $\alpha = 0.05$ showed a no significant difference in the titers for HAI antibodies stimulated by Schwarz and Wild strains.

Plaque reduction neutralization test:

The neutralization titers of the antisera measured by 50% plaque reduction as compared with control sera are given in Tables 3.2 - 3.7. Antisera to both strains exhibited cross neutralizing activity but the dilution of serum which reduced the pfu by 50% were different for the two strains by the Mann - Whitney statistical analysis at $\alpha = 0.05$.

Immunodiffusion.

The Schwarz and Wild antigens developed 2 sharp lines in addition to a spur indicative of partial relatedness against a commercial antisera (Plate IIIA). With antisera collected from monkeys inoculated with

Schwarz and Wild strain against homologous commercial measles antigen, only one solid precipitin line appeared to be common (Plate IIIB) but with a spur.

Table 3.1: Hemagglutination inhibition titers of monkey sera (Pre - and Post - inoculation) Schwarz (MV-S) and Wild (MV-W) measles strains

Sera	Mean hemagglutination inhibition end-point							
	Schwarz (MV-S)				Wild (MV-W)			
		Post				Post		
	PRE	2WK	4WK	6WK	PRE	2WK	4WK	6WK
1.	0[a]	8[a]	32[a]	128[a]	0[a]	16[a]	256[a]	512[a]
2.	0	16	64	512	0	64	256	1024
3.	8	32	128	256	0	32	128	256
4.	0	16	128	512	0	32	256	1024
5.	0	16	256	512	0	16	128	512
	1.51	15.85	97.79	508.84	0	28.18	194.99	588.84[b]

[a]HAI titer of triplicate run of sera collected: Highest dilution showing hemagglutination inhibition activity to a fixed amount of virus antigen.

[b]Geometric mean titer.

Table 3.2: Plaque neutralization of Schwarz (MV-S) and Wild (MV-W) measles strains (100 pfu/0.2ml) by anti-Schwarz serum (FAI 1/256).

Anti-body Dilution	Schwarz (MV-S)		Wild (MV-W)	
1:4	11,14,10:12.00[a]	82.33[b]	13,11,15:13.00	87.00[b]
1:8	17,14,20:17.00	83.00	18,21,14:18.00	82.33
1:16	22,15,24:20.00	79.67	16,24,22:21.00	79.33
1:32	28,18,26:24.00	76.00	21,23,28:24.00	76.00
1:64	29,23,36:29.00	70.67	49,56,47:51.00	49.33
1:128	43,40,35:39.00	60.67	64,58,60:62.00	36.67
1:256	65,53,59:59.00	41.00	74,69,77:73.00	26.67
1:512	78,81,75:78.00	22.00	87,79,73:80.00	19.67
1:1024	93,85,87:88.00	11.67	91,94,89:91.00	8.67
	192.00*		64.00*	

[a] Mean of triplicate counts.

[b] Percentage plaque reduction $\left(\frac{\text{Control} - \text{Test}}{\text{Control}} \times 100\right)$

* 50% Neutralization titer.

Table 3.3: Plaque neutralization of Schwarz (MV-S) and Wild (MV-W) measles strains (50pfu/0.2ml) by anti-wild serum (PAI 1/128).

Antibody dilution	Schwarz (MV-S)			Wild (MV-W)		
1:4	4,3,3	3.00[a]	93.34[b]	3,3,0	2.00[a]	96.00[b]
1:8	4,6,3	4.00	91.34	7,4,8	6.00	88.66
1:16	8,13,6	9.00	82.00	7,5,9	7.00	86.00
1:32	15,11,17	14.00	71.34	9,11,13	11.00	78.66
1:64	19,24,23	22.00	56.66	15,11,19	15.00	70.00
1:128	30,23,25	26.00	48.00	24,18,21	21.00	58.00
1:256	35,28,28	30.00	39.34	30,23,28	27.00	46.00
1:512	37,42,31	37.00	26.66	40,39,34	38.00	25.26
1:1024	49,48,44	47.00	6.00	45,42,44	44.00	12.66
	128.00*			256.00*		

[a] Mean of triplicate counts.

[b] Percentage plaque reduction. $\left(\frac{\text{Control} - \text{Test}}{\text{Control}} \times 100\right)$

* 50% Neutralization titer.

Table 3.4: Plaque neutralization of Schwarz (MV-S) and Wild (MV-W) measles strains by anti-wild serum (HAI 1/256).

Antibody dilution	Schwarz (MV-S)		Wild (MV-W)	
CONTROL				
(1:10)	54,37 46.00[a]		61,52:54.00[a]	
1:10	0,0 0.00[a]	100.00[b]	0,0:0.00[a]	100.00[b]
1:20	11,8:10.00	78.30	3,4:4.00	93.80
1:40	17,22.20.00	56.50	9,5:7.00	87.60
1:80	28,24:26.00	43.50	18,13:16.00	72.60
1:160	39,43:41.00	11.80	26,22:24.00	57.50
1:320	44,46:45.00	1.30	37,41:39.00	30.70
	80.00*		208.00*	

[a]Mean of duplicate counts.

[b]Percentage plaque reduction $\left(\frac{\text{Control} - \text{Test}}{\text{Control}} \times 100\right)$

* 50% Neutralization titer.

Table 3.5: Plaque neutralization of Schwarz (MV-S) and Wild (MV-W) measles strains by anti wild serum (HAI 1/512).

Anti-body Dilution	Schwarz (MV-S)		Wild (MV-W)	
CONTROL				
(1:10)	54,47:50.50		61,52:56.50	
1:10	0,0:0.00[a]	100.00[b]	0,0:0.00[a]	100.00[b]
1:20	3,0:2.00	97.00	0,0:0.00	100.00
1:40	7,11:9.00	82.20	5,3:4.00	92.90
1:80	19,23:21.00	59.40	12,9:11.00	89.00
1:160	27,31:29.00	42.60	22,26:24.00	57.50
1:320	42,46:44.00	12.90	28,33:31.00	46.00
	132.00*		264.00*	

[a] Mean of duplicate counts.

[b] Percentage plaque reduction $\left(\frac{\text{Control} - \text{Test}}{\text{Control}} \times 100\right)$

* 50% Neutralization titer.

Table 3.6: Plaque neutralization of Schwarz (MV-S) and Wild (MV-W) measles strains by anti-Schwarz serum (1:128).

Anti-body Dilution	Schwarz (MV-S)		Wild (MV-W)	
CONTROL				
(1:10)	51,55:53.00[a]		48,52:50.00[a]	
1:10	0,0:0.00	100.00[b]	3,2:3.00	95.00[b]
1:20	0,0:0.00	100.00	13,9:11.00	78.00
1:40	5,8:7.00	87.70	21,26:24.00	53.00
1:80	14,19:17.00	68.90	30,24:27.00	46.00
1:160	26,22:24.00	54.70	29,35:32.00	36.00
1:320	38,41:40.00	25.50	46,49:48.00	5.00
	184.00*		56.00*	

[a]Mean of duplicate counts.

[b]Percentage plaque reduction $\left(\frac{\text{Control - Test}}{\text{Control}} \times 100\right)$

* 50% Neutralization titer.

Table 3.7: Plaque neutralization of Schwarz (MV-S) and Wild (MV-W) measles strains by anti-Schwarz serum (HAI 1/64).

Antibody Dilution	Schwarz (MV-S)		Wild (MV-W)	
CONTROL				
(1:10)	51,55:53.00[a]		48,52:50.00[a]	
1:10	0,0:0.00[a]	100.00[b]	0,0:0.00[a]	100.00[b]
1:20	3,4:4.00	93.40	8,6:7.00	86.00
1:40	10,8:9.00	83.10	13,18:16.00	69.00
1:80	18,15:17.00	68.90	26,29:28.00	45.00
1:160	29,34:32.00	40.60	41,36:39.00	23.00
1:320	38,42:40.00	24.50	43,47:45.00	10.00
	128.00*		70.00*	

[a]Mean of duplicate counts.

[b]Percentage plaque reduction $\left(\frac{\text{Control} - \text{Test}}{\text{Control}} \times 100\right)$

* 50% Neutralization titer.

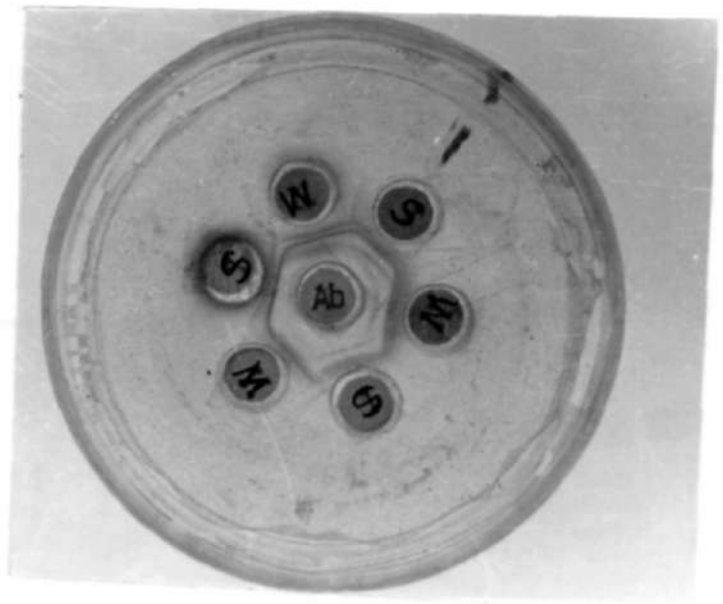


Plate IIIA: Immunodiffusion reactions of Schwarz (MV-S) and Wild (MV-W) measles virus against commercial measles antisera (HAI titer 512). Central well-measles antisera; Outer wells - measles strain (Schwarz - MV-S, Wild - MV-W).

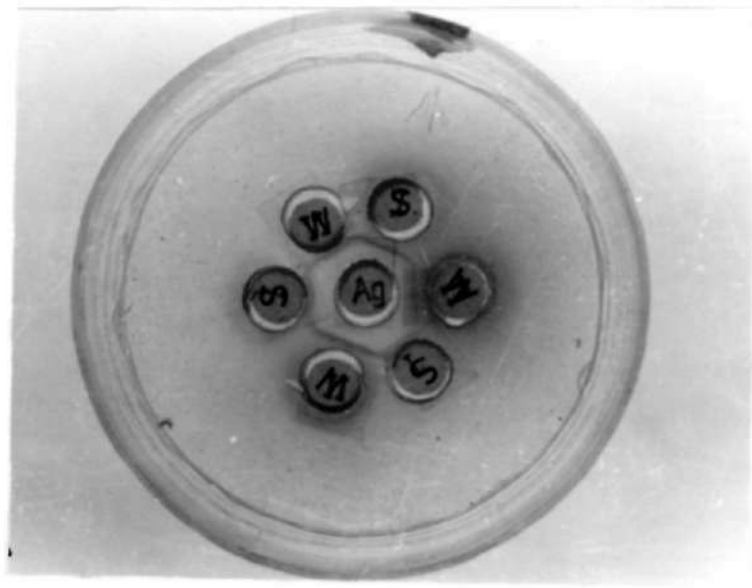


Plate IIIB. Immunodiffusion reactions of Schwarz (MV-S) and Wild (MV-W) measles virus immune sera against commercial soluble measles antigen (EIA titer 128). Central well- measles antigen; Outer well immune sera (sera collected from monkeys 2 weeks post - inoculations with the respective measles strain.

5.4. DISCUSSION.

The various serological techniques - HAI, plaque reduction neutralization measured measles antibodies in the sera to each of the measles strains. There was no difference between the two strains when HAI test was used.

In the plaque reduction neutralization test, the neutralization titers measured by 50% reduction in plaque showed differences between the measles strains. Norrby and Collmar (1975) indicated that the two glycoproteins on the surface of measles virus, responsible for biological activities are the hemagglutinin (HA) and hemolysin (HL). The larger glycoprotein (C) contains the active site for HA, but it is not yet established whether the HL activity resides solely in the smaller glycoprotein (C). In contrast to the HA activity the presence of lipid is important for hemolytic activity i.e. HL activity can only be expressed when the glycoproteins is complexed in a particular manner with virus lipids.

Norrby and Collmar (1972) identified antibody specifically to measles capsid, hemagglutinin and

hemolysin. Virus neutralization is associated with anti-hemolysin (Norrby and Gollmar, 1975). It should be noted that the hemolysin activity is not always proportional to the hemagglutination property of the virus, for example the hemolysin activity could be inactivated at a temperature of 58°C, at which temperature hemagglutinin is not affected (Norrby and Falksveden, 1973). What is unclear is how the effectiveness of neutralization may differ by disproportion between the amount of each kind of antibody present.

The pattern of precipitin lines indicated in Plates IIIAB showed that the two strains being compared, have slightly different antigenic determinants. The internal nucleocapsid antigen(s) reacting with antibody in gel diffusion tests (Norrby and Gollmer, 1972) is known to slightly vary with measles strains.

6.1. INTRODUCTION.

Thermostability studies have been used for characterizing a virus (Smith et al., 1971). Haspel et al. (1975) isolated three complimentation groups of temperature - sensitive measles mutants. Two mutants of measles virus based on the growth or no growth at 39°C were identified by Gould and Linton (1975). The hemolysin (HL) component of measles virion is known to be more sensitive than hemagglutination (HA) component to thermal inactivation (Norrby et al., 1975).

The thermostability property of Schwarz and Wild measles strains was examined by their plaquing capacity after incubation at 0°C, 10°C, 20°C, 30°C, 40°C and 50°C for 1 hour.

6.2. MATERIALS AND METHODS.

Virus:

The viral stock (5×10^{10} pfu/ml) was used.

Thermal Inactivation:

Stock of virus (1ml) was dispensed in a standard Kahn test-tube, and incubated at various temperatures ranging from 10 to 50°C for 1 hour in a water-bath. Controls kept at 0°C for 1 hour were set up alongside tests. All water-baths were covered throughout the experiment to prevent heat loss.

Plaque Assay:

After incubation for 1 hour at the various temperatures, 0.1ml of each virus preparation was inoculated into Vero cell monolayer prepared by seeding 5mls of 2.13×10^5 cells/ml into 60mm plastic petri-dishes. Inocula were allowed to adsorb for 1 hour at 37°C, and plaque assay was performed as already described. The experiment was run in duplicates. The thermostability of virus was estimated by the percentage survival relative to mean count of control culture at 0°C.

6.3. RESULTS

The thermostability of Schwarz and Wild measles strains shown by the percentage survival of the measles strains at different incubation temperatures are presented in Table 4. The results inlicated that the Wild strain did not significantly differ in thermostability property at various temperatures than the Schwarz strain.

Table 4: Thermal stability of Schwarz (MV-S) and Wild (MV-W) measles strains at different incubation temperature (10° - 50°C).

Temperature °C	S c h w a r z (MV-S)		W i l d (MV-W)	
		% Survival $\left(\frac{N}{No} \times 100\right)$		% Survival $\left(\frac{N}{No} \times 100\right)$
Control	57,61:59.00[a]	100.00	66,63:64.50[a]	100.00
10	53,56:54.50	92.37	64,58:61.00	94.60
20	50,47:48.50	82.20	61,54:57.50	89.15
30	48,43:45.50	77.12	47,56:51.50	79.85
40	39,32:35.50	60.12	43,38.40.50	62.79
50	19,14:16.50	27.79	23,28:25.50	39.54

[a]Mean of duplicate counts.
Control (Preparation kept at 0°C).

6.4. DISCUSSION.

The inactivation data showed no difference in the thermal stability between Schwarz and Wild measles strains. The thermosensitivity property of both strain may be reflective of a selection/ adaptive phenomena of the virus. Albrecht and Schumacher (1972) found that hamster - brain - adapted virus is more heat-sensitive than Wild virus.

Strains of measles virus which are temperature-sensitive have been recorded from persistently infected cultures (Haspel et al., 1973). It is not certain that these 'naturally occurring' variants are stable, but induced temperature - sensitive mutants have been prepared (Haspel et al., 1975a). Mutants from same stock of virus as a result of temperature effect are known to show slightly different pathogenic effects on intra-cerebral inoculation into hamster (Haspel et al., 1975b). Cernescu et al. (1973) indicated that cold measles variants leads to loss of pathogenicity and formed smaller plaque on Ceroopithecus R_6-CA cells.

Several live measles vaccines have been found to be relatively heat-labile, resulting in their ready inactivation, when used under primitive field conditions. There is a distinct and steady loss of infectivity even at incubation temperature, once the virus is released from the cell (Norrby et al. (1975). It is well known that measles virus yield is lower at 37°C than respective adapted lower temperature (Makino, 1977).

7. GENERAL DISCUSSION AND CONCLUSION.

Essentially, some differences were observed between the Schwarz and Wild measles strains. The differences may not be great. Dietzschold et al. (1983) demonstrated that a single amino acid substitution of the glycoprotein molecule results in non-pathogenic strain of rabies virus. Evidences for measles virus variants between Wild strains and the prototype laboratory strains have been reported (DeMaeyer and Enders, 1965; Oddo et al., 1961). Makino (1977) indicated that measles virus strains appear to share some common antigen(s) and in addition have one or more type - specific antigen. Whether the differences in the expression of the antigen are qualitative or quantitative or is of sufficient stability to warrant type distinction is a matter of further investigation by more sophisticated techniques such as polyacrylamide gel electrophoresis or electrofocusing or analysis with monoclonal antibodies. Such techniques would amplify the subtle differences being observed by the methods used in the study.

It is important that such a sophisticated typing scheme should not be based on the study of a limited number of strains by a few investigators. It will require a much larger number of strains for several markers that vary independently to determine whether the differences are indicative of types or whether they represent the extremes - a continuum of variation.

Measles vaccination campaign in Nigeria are beset with several problems. The presence of measles variants in Nigeria could be an important factor in vaccination failures. Perhaps, the most critical is the use of impotent vaccines. Immunization with heat-stable vaccine is needed in the tropics, where the effect of severe climatic conditions notably high temperatures on measles vaccines and other biologic preparations is high. The isolation of temperature - resistant strains of measles virus that can be used to develop vaccines able to withstand adverse climatic conditions would require extensive investigation.

6. BIBLIOGRAPHY.

Aicardi, J., Goutieres, F., Aresenionunes, M.L. and Lebon, P. (1977). Acute measles encephalitis in children with immunosuppression. Pediatrics 59, 232-239.

Albrecht, P., Ennis, F.A., Saltzman, E.J. and Krugman, S. (1977). Persistence of maternal antibody in infants beyong 12 months Mechanism of Measles vaccine failure. J. Pediat., 91(5), 715-718.

Albrecht, P. and Schumacher, H.P. (1972). Markers for Measles virus 1. Physical properties. Arch. Ges. Virusforch 36, 23-35.

Arakawa, S., Kaneko, T., Iijima, T., Noro, T., Iwakawa, K., Yoshida, Z., Chang, C.T. and Akama, K. (1965). A study of diphtheria, pertussis, tetanus and measles combined vaccine. Jap. J. Exp. Med. 35(6), 481-489.

Arbeter, A.M., Arthur, J.H., Blakeman, G.J. and Mcintosh, J. (1972). Measles Immunity: Re-immunization of children who previously received live measles vaccine and gamma globulin. J. Pediat. 81(4), 737-741.

Artenstein, M. and Weinstein, L. (1963). Simultaneous infection with the viruses of Chickenpox and Measles. J. Pediat. 62, 156.

Aukrust, L., Almeland, T.L., Refsum, D. and Aas, K. (1980). Severe hypersensitivity or intolerance reactions to measles vaccine in 6 children-Clinical and Immunological studies. Allergy 35(7), 581-587.

Balachandran, N., Harnish, D., Rawls, W.E. and Bacchetti, S. (1982). Glycoproteins of herpes simplex virus type 2 as defined by monoclonal antibodies. J. Virol. 44(1): 344-355.

Barry, B., Sullivan, J.L., Lucas, S.J., Dunlap, R.C. and Albrecht, P. (1976). Acute and chronic infection of human lymphoblastoid cell lines with measles virus. J. Immunol. 116, 89-98.

Bech, V. (1965). The Measles Epidemic in Greenland in 1962. Arch. Gesamte Virusforchung. 16, 53-56.

Bedson, H.S. (1982). Enzyme studies for the characterization of some orthopox virus isolates. Bull. WHO 60(3), 377-380.

Birrer, M.J., Bloom, B.R. and Udem, S. (1981). Characterization of measles polypeptides by monoclonal antibodies. Virol. 108(2), 381-390.

Black, F.L. and Rosen, L. (1962). Pattern of measles antibodies in residents of Tahiti and their stability in the absence of re-exposure. J. Immunol. 88, 725-731.

Blaskovic, D. and Styk, B. (1967). Laboratory methods of virus transmission in multicellular organisms. In Method in Virology (Ed. K. Maramorosch and H. Koprowski). Vol. 1, Ch. 6 pp.163-235. Acad. Press New York, London.

Bloch, A. and Massry, S. (1963). Poliomyelitis following recent measles. Pediatric 31, 929.

Boyum, A. (1968). Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation and of granulocytes by combining centrifugation and sedimentation at 1g. Scand. J. Clin. Lab. Invest. 21 (supp 97) 77.

Brody, J.A., Alexander, E.R. and Hanson, M.L. (1966). Measles vaccine field trials in Alaska III Two year follow-up of inactivated vaccine followed by live, attenuated vaccine and of immune globulin with live, attenuated vaccine. J. Amer. Med. Assoc. 196(9), 757-760.

Brunell, P.A. (1978). Measles Immunization: 12 or 15 months. Pediatric 62(6), 1038-1041.

Buynak, E.B., Peck, H.M., Creamer, A.A., Coldner, H. and Hilleman, M.R. (1962). Differentiation of virulent from avirulent measles strains. Am. J. Dis. Child. 103, 460-473.

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

Cernescu, C., Cajal, N., Cepleanu, M. and Sorodoc, Y. (1973). Approaches to attenuation of ~~an~~ autochthonous measles virus strain. Rev. Roum. Virol. 10, 117-126.

Chatterji, M. and Mankad, V. (1977). Failure of Attenuated Viral Vaccine in Prevention of Atypical Measles. J. Amer. Med. Assoc. 238(24), 2635.

Cherry, J.D. (1980). The new epidemiology of measles and rubella. Hosp. Pract. 15(7), 49-57.

Chiba, S., Yamanaka, T. and Nakao, T. (1974). Detection of cell mediated immunity to measles virus with $^{51}_{Cr}$ release Cytotoxic assay in patients with natural measles. Tohoku J. Exp. Med. 112, 285-288.

Choppin, P.W. and Compans, R.W. (1975). Reproduction of paramyxoviruses, P. 95-178. In Comprehensive Virology (Ed. H. Fraenkel-Conrat and E.R. Wagner). Vol.4, pp.95-178. Plenum Press, New York, London.

Chourini, A. and Norrby, E. (1970). Seperation and characterization of products of two measles virus variants. Arch. Virol. 29, 205-214.

Croce, C.M., Linenbach, A., Hall, W., Steplewski, Z. and Koprowski, H. (1980). Production of human hybridomas secreting antibodies to measles virus. Nature 288(5790), 488-489.

Csonk, E., Ruzickska, P. and Koch, A.S. (1975). Chromosome abberations in cells infected with various strains of measles virus. Acta Microbiol. Acad. Sci. Hung. 22, 41-44.

De-Maeyer, E. (1960). Plaque formation by measles virus. Virology 11, 634-638.

De-Maeyer, E. and Enders, J.F. (1965). Growth characteristics, interferon production and plaque formation with different lines of Edmonston measles virus. Arch. Gesamte. Virusforsch. 16, 151-160.

Dietzschold, B., Wunner, W.H., Wiktor, J.T., Lopes, A.D, Lafon, L., Smith, C.L. and Koprowski, H. (1983). Characterization of antigenic determinant of the glycoprotein that correlates with pathogenicity of rabies virus. Proceedings of the National Academy of Science. U.S.A. 80, 70-74.

Eghafona, N.O., Obineche, E.N., Odama, L.E., Mu'azu, S., Gomwalk, N.E. and Emejuaiwe, S.O. (1979). Importance of measles and potency of imported live measles vaccine. New Developments in Vaccines. (Ed. F.G. Andre), 239-257 Smith Kline RIT, Belgium.

Eghafona, N.O., Odama, L.E., Onuora, C., Gosham, L.T., Emejuaiwe, S.O. and Woghiren, E.I. (1985). The levels of measles antibodies in children age 0 – 12 months and its relationship with maternal parity. Accepted in Nigerian Journal of Microbiology Vol. 5(1), 2/6/85.

Ehrnst, A. (1975). Characterization of measles virus – specific cytotoxic antibodies by use of a chronically infected cell line. J. Immunol 114, 1077-1082.

Enders, J.F. (1962). Measles virus: Historical Review, Isolation and Behaviour in Various Systems. Am. J. Dis. Child. 103, 282-287.

Enders, J.F., Katz, S.L. and Medearis, D.N. (Jr. (1959). Recent Advances in Knowledge of the Measles virus. In Perspective in Virology, (Ed. M. Pollard) p.103-116. John Wiley and Sons, Inc. New York.

Enders, J.F. and Peebles, T.C. (1954). Propagation in Tissue cultures of cytopathogenic agents from patients with measles. Proc. Soc. Exp. Biol. Med. 86, 277-288.

Enders, J.F., Peebles, T.C., McCarthy, K., Milovanovic, M., Mitus, A. and Holloway, A. (1957). Measles Virus: A summary of experiments concerned with isolation, properties and behaviour. Amer. J. Pub. Hlth. 47, 275-285.

Enders-Ruckle, G. (1965). Methods of determining immunity, duration and character of immunity resulting from measles. Archiv. F. Viruserschung. Bd XVI, H1-5, 187-205.

Feldman, H.A. (1964). Measles Immunization. Bacteriol. Review 28(4), 440-443.

Fenner, F. (1976). Classification and nomeclature of viruses. Second report of the international committee on taxonomy of viruses. Intervirology 7, 59-60.

Figueroa, M.E. and Rawls, W.E. (1969). Biological markers for differentiation of herpes virus strains of oval and genital origins. J. Gen. Virol. 4, 259-267.

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

Frank, J.A. Jr., Hoffman, R.E., Mann, J.M., Crowe, J.D. and Hinman, A.R. (1981). Imported measles A potential control problem. J. Am. Med. Assoc. 245(3), 264-266.

Fraser, J.B. and Martin, S.J. (1978). Biological characters and Genetic markers of measles virus. In Measles virus and its Biology. pp.25-40. Academic Press. London, New York. San Francisco.

Fulginiti, V.A. and Kempe, C.H. (1963). Measles exposure among vaccine recipients. Response to measles exposure and antibody persistence amongst recipients of measles vaccines. Amer. J. Dis. Child. 106(5), 450-461.

Gard, S., Carlstrom, C., Lagercrantz, R. and Norrby, E. (1965). Immunization with Inactivated measles virus vaccine. Archiv. F. Virusforschung Bd XVI, H1-5, 315-323.

Gauntt, C.J., Trousdale, D.M., Lee, J.C. and Paque, R.E. (1983). Preliminary characterization of coxsackie virus B3 temperature - sensitive mutants. J. Virol. 45(3), 1037-1047.

Geder, L. and Skinner, G.R. (1971). Differentiation between type 1 and type 2 strains of herpes simplex virus by an Indirect immunofluorescent technique. J. Gen. Virol. 12, 179-182.

Gibson, P.E. and Bell, T.M. (1972). Persistent infection of measles virus in mouse brain cell cultures infected in vivo. Arch. Gesamte Virusforsch. 37, 45-53.

Gifford, G.E. and Klapper, D.C. (1969). Effect of proteolytic enzymes on vaccinia virus replication. Arch. ges. Virusforsch. 26, 321-333.

Golais, F., Sabo, A., Weismann, P. and Mucha, V. (1982). Plaque characteristics of virulent and attenuated strains of pseudorabies virus in some cell cultures. Biologia (Bratisl) 37(7), 695-700.

Goswami, K.K.A. and Russel, W.C. (1982). A comparison of paramyxoviruses by immunoprecipitation. J. Gen. Virol. 60(1), 177-184.

Gould, E.A., Armstrong, M., Shirodaria, P.V. and Deornella, D. (1981). Examination of the Immunological relationship between measles virus and canine distemper virus using monospecific measles antisera. J. Med. Virol. 7(1), 51-60.

Could, E.A. and Linton, P.E. (1975). The production of a temperature-sensitive persistent measles virus infection. J. Gen. Virol. 28, 21-28.

Granes, M.C., Silver, S.M. and Choppin, P.W. (1978). Measles virus polypeptide synthesis in infected cells. Virology 86, 254-263.

Greenberg, H., Mcauliffe, V., Valdesuso, J., Wyatt, R., Flores, J., Kauca, A., Yoshino, Y. and Singh, N. (1983). Serological analysis of the subgroup protein rotavirus, using monoclonal antibodies. Infect. Immun. 39(1), 91-99.

Gresser, J. (1961). Production of Interferon by suspensions of human leukocytes. Proc. Soc. Exp. Biol. Med. 108, 799.

Grist, N.R., Bell, E.J., Follett, E.A.C. and Urquhart, G.E.D. (1979). Cell culture. In Diagnostic Methods in Clinical Virology. 3rd Edition pp.60-80. Blackwell Scientific Publications Oxford, London, Edinburgh Melbourne.

Guillozet, N. (1979). Measles in Africa: A deadly disease. Clin. Pediatrics 18(2), 95-100.

Hall, W.W., Lamb, R.A. and Choppin, P.W. (1980). The polypeptides of canine distemper virus: Synthesis in infected cells and relatedness to the polypeptides of other morbilliviruses. Virology 100, 433-449.

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

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

Hashimoto, Y. and Kawase, S. (1983). Characteristics of structural proteins of Infectious Flacherie virus from silk worm. J. Invertebrate Pathol. 41(1), 68-76.

306201

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

Haspel, M.V., Duff, R. and Rapp, F. (1975b). Experimental measles encephalitis: A genetic analysis. Infect. Immun. 12, 785-790.

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

Hendricks, D.A., Hardy, J.L. and William, C.R. (1983). Comparison of biological properties of St. Louis encephalitis and Rio Bravo viruses. Am. J. Trop. Med. Hyg. 32(3), 602-609.

Hendrickse, R.G. and Sherman, P.M. (1965). Morbidity and mortality from measles in children seen at University College Hospital Ibadan. Archiv. f. Virusforschung Bd. XVI, H1-5, 27-34.

Hendrickse, R.G., Montefiore, D., Sherman, P.M. and Van Der Wall, E.M. (1964). Studies on measles vaccination in Nigerian children. Brit. Med. Journ. 5381, 470-474.

Heymann, D.L., Smith, E.L., Nakano, J.H., Jato, J.G., Martin, G.E. and Maben, G.K. (1982). Further field testing of the more heat stable measles vaccines in Cameroun. Brit. Med. J. 285, 531-534.

Hilleman, M.R. (1982). Vaccines for the decade of the 1980s and beyond. S. A. Med. J. 61, 691-698.

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

Ifekwunigwe, A.E., Grassft, N., Glass, R. and Foster, S. (1980). Immune response to measles and smallpox vaccinations in malnourished children. Am. J. Clin. Nutr. 33(3), 621-624.

Imam, Z.E.I., Hosny, A., Alfy, M.E. and El-Rai, F. (1965). Medical importance of measles in U.A.R. (Egypt). Archiv. f. Virusforchung Bd. XVI, H1-5, 49-52.

Isaacs, A. and Lindermann, J. (1957). Viral Interference I. The Interferon II. Some properties of Interferon. Proc. Roy. Soc. London 147, 258-273.

Ivanova, V.T., Zakstel'Skaya, L.Y., Podche-Snyaeva, R.Y., Klinova, V.F., Sokolov, I.P. and Zhbanov, V.M. (1982). Comparative analysis of biophysical characteristics of variants of influenza A viruses with varying pathogenicity for mice. Vopr. Virusol. 0(3), 276-279.

Johnson, K.P., Norrby, E., Swoveland, P. and Carrigan, D.R. (1982). Expression of 5 viral antigens in cells infected with wild - type and subacute sclerosing pan-encephalitis strains of measles virus: Correlation with cytopathic effects and productivity of infections. Arch Virol. 73(3/4), 255-262.

Kano, K. and Millgrom, F. (1977). Heterophile Antigens and Antibodies in Medicine. Current Topics in Microbiol. and Immunol. Springer - Verlag, 43-63.

Kaplan, J.S., Larrick, J.W., Yost, J., Farrel, L., Greenberg, H.B., Herrmann, K.L., Sulzer, A.J., Walls, K.W. and Pederson, L. (1980). Infectious disease patterns in Waorani. An isolated Amerindian population. Am. J. Trop. Med. Hyg. 29(2), 298-312.

Karcher, D., Iowenthal, A., Thomer, H. and Noppe, M. (1981). Serological identification of viral antigens after electrophoretic transfer. J. Immun. Methods. 43(2), 175-179.

Katz, S.L. (1972). Measles. In Pediatrics. (Ed. H. L. Barnet and A.N. Einhorn) 15th Edition PP.729-737. Appleton - Century-Crofts, New York. U.S.A.

Kempe, C.H. and Fulginiti, V.A. (1965). The Pathogenesis of measles virus infection. Archiv. f. Virusforschung. Bd XVI, H1-5, 103-128.

King, B. (1978). Measles vaccination in a rural Tanzanian community. E. Afri. Med. J. 55(8), 252-255.

Kinney, R.M., Trent, D.W. and Monroe, J.K. (1983). Comparative immunological and biochemical analysis of viruses in the Venezuelan equine encephalitis complex. J. Gen. Virol. 64(1), 135-148.

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

Krugman, S. (1965). Measles Immunization: Combined vaccinations. Archiv. f. Virusforschung. Bd XVI, H1-5, 339-342.

Krugman, S. (1971). Present status of Measles and Rubella Immunization in the United States: A Medical Progress Report. J. Pediatrics 78, 1-16.

Krugman, S. (1977). Present Status of Measles and Rubella Immunization in the United States: A Medical Progress Report. J. Pediatrics 90(1) 1-12.

Krugman, S., Giles, J.P., Friedman, H. and Stone, S. (1965). Studies on Immunity to Measles. J. Pediatrics. 66, 471-488.

Kumarasamy, R. and Blough, H.A. (1983). Characterization of oligosaccharides of highly purified glycoprotein gC of herpes simplex virus type 1. Biochem. Biophys. Res. Comm. 109(6), 1108-1115.

Landrigan, P.J. and Conrad, J.L. (1971). Current status of measles in the United States. J. Infect. Dis. 24, 620.

Lefrancois, L. and Lyles, D.S. (1983). Antigenic determinants of vesicular stomatitis virus: Analysis with antigenic variants. J. Immunol. 130(1), 394-396.

Lerman, S.J. and Gold, E. (1971). Measles in children previously vaccinated against measles. J. Amer. Med. Assoc. 217, 1311-1314.

MacCarthur, J.D., Mann, P.C., Oreffo, D. and Scott, G.E.D. (1979). Measles in monkeys. J. Hyg. 83(2), 207-212.

Makino, S. (1977). Measles virus mutants and their implications in Basic and Applied Virology. Ann. Microbiol. (Inst. Pasteur) 128 B, 507-515.

Makino, S., Sasaki, K., Nazari, F., Nakagawa, M., Nakamura, N. and Kasahara, S. (1970). Cultivation of Measles virus in sheep kidney cells. Jap. J. Microbiol. 14, 501-504.

Malkova, D. (1974). Comparative Study of two variants of Tahyna virus. Acta Virologica 18, 407-415.

Mann, G.F., Allison, I.M.C., Copeland, J.A., Agostini, C.E.M. and Zuckerman, A.J. (1980). A simplified plague assay system for measles virus. J. Biol. Stand 8(3), 219-225.

Marks, J.S., Halpin, T.J. and Orenstein, W.A. (1978). Measles vaccine efficacy in children previously vaccinated at 12 months of age. Pediatrics 62(6), 955-960.

Matumoto, M., Mutai, M., Ogiwara, M. and Nakamura, M. (1961). Proliferation du virus rougeoleux en cuture de cellules renales bovine. C. R. Soc. Biol. (Paris) 155, 1192-1195.

McCormick, J.B., Bauer, S.P., Elliott, L.H., Webb, P.A. and Johnson, K.M. (1983). Biological differences between strains of Ebola viruses from Zaire and Sudan. J. Infect. Dis. 147(2), 264-267.

McCormick, J.L., Halsey, N. and Rosenberg, R. (1977). Measles vaccine efficacy determined from secondary attack rates during a severe epidemic. The J. Ped. 91(1), 13-16.

McFarlin. D.E., Bellini, W.J., Cinqioli, E.S., Behar, T.N., Trudgett, A. (1980). Monospecific antibodies to hemagglutinin of measles virus. J. Gen. Virol. 48(2), 425-429.

Mcintosh, A.H. and Ignoffo, C.M. (1983). Restriction endonuclease patterns of baculoviruses isolated from Heliothis spp. J. Invertebr. Pathol. 41(1), 27-32.

Mcphee, D.A., Parsonson, I.M. and Della-Prta, A.J. (1983). Comparative studies on the growth of Australian blue-tongue virus serotypes in continuous cell lines and embryonated chicken eggs. Vet. Microbiol. 7(5), 401-410.

Meyer, H.M. (1965). Field Experience with combined Live Measles, Smallpox and Yellow Fever Vaccine. Archiv. f. Virusforschung Ed XVI, H1-5, 365-366.

Meyer, H.M. Jr., Hopps, H.E., Parkman, P.D. and Ennis, F.A. (1978). Control of measles and rubella through use of attenuated vaccines. Am. J. Clin. Pathol. 70(1) Suppl. 128-135.

Miller, C. (1980). Measles again. Br. Med. J. 280, 6229.

Minor, P.D., Schild, G.C., Ferguson, M., Mackay, A., Magrath, D.I., John, A., Yates, P.J. and Spitz, M. (1982). Genetic and antigenic variation in type 3 poliovirus: Characterization of strains by monoclonal antibodies and T1 Oligonucleotide mapping. J. Gen. Virol. 61(2), 167-176.

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

Mitra, S., Snyder, C.E., Bates, R.C. and Banerjee, P.T. (1982). Comparative physiochemical and biological properties of 2 strains of Kilham rat virus, a non-defective parvovirus. J. Gen. Virol. 61(1), 43-54.

Mordhorst, C.H., Tulinius, S. and Von Macnus, H. (1968). Use of two live measles vaccines in virgin populations in Arctic Greenland. Clinical and serological reaction in children and adults. Dan. Med. Bull. 15(9), 263-270.

Morley, D.C. (1962). Measles in Nigeria. Amer. J. Dis. Child. 103, 230-233.

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.

Morley,D.C. and MacWilliam, K.M. (1961). Measles in Nigerian Community. W. Afri. Med. J. 10, 146-253.

Morley, D.C., Woodland, M. and Martin, W.J. (1963). Measles in Nigerian Children. J. Hyg. Camb. 61, 115.

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

Musser, S.J. and Underwood, G.E. (1960). Studies on measles Virus II. Physical properties and Inactivation studies of measles virus. J. Immunol. 85, 292-297.

Nath, D.M., Rodkey, L.S. and Minocha, H.C. (1975). Antigenic comparison of swine Influenza virus isolates. Archiv. Virol. 48, 245-252.

Noordam, D. (1978). Spectrophotometry. Identification of plant viruses (Methods and Experiments) pp.88-102. Centre for Agric. Publ. and Documentation, Wageninger, U.S.A.

Norrby, E. (1962a). 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.

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

Norrby, E. (1962c). 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.

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

Norrby, E. (1976). Measles Vaccination. Bull. Pan. Amer. Health Organ. 10(3), 196-197.

Norrby, E. (1983). Viral Vaccines: The use of currently available produces and future developments. Arch. Virol. 76(3), 161-177.

Norrby, E., Enders-Ruckle, G. and Ter-Meulen, V. (1975). Difference in the appearance of antibodies to structural components of measles virus after immunization with inactivated live virus. J. Infect. Dis. 132, 262-269.

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

Norrby, E. and Gollmar, Y. (1973). Appearance and persistence of antibodies against different viral components after regular measles infection. Infect. Immunol. 6, 230-247.

Norrby, E. and Gollmar, Y. (1975). Identification of measles virus-specific hemolysis-inhibiting antibodies seperate from hemmagglutination - inhibiting antibodies. Infect. Immunol. 11, 231-239.

Norrby, E., Magnusson, P., Falkåvedon, L.G. and Cronberg, M. (1964). Separation of measles virus components by equilibrium centrifugation in CsCl gradients. I. Studies on the large and small hemagglutinin. Arch. Gesamte Virusforsch. 14, 462-473.

Odama, L.E., Eghafona, N.O. and Emejuaiwe, S.O. (1985). In Vitro effect of haemagglutination inhibition antibodies on measles virus replication. Microbios 43, 161-163.

Odama, L.E., Obinecho, E.N., Eghafona, N.O. Comwalk, N.E. and Emejuaiwe, S.O. (1980). Sero-epidemiological survey of the level of measles antibody in Primary school children in Kaduna State of Nigeria. In Global Impacts of Applied Microbiology. Sixth International Conference GIAM VI (Edited by S.O. Emejuaiwe et al) pp.315-333. Academic Press. London.

Oddo, F.G., Flaccomio, R. and Sinatra, A. (1961). Giant cell and strand-forming cytopathic effect of measles virus lines conditioned by serial propagation with diluted or concentrated inoculum. Virology 13, 550-552.

Okuno, Y., Takahashi, M. and Toyoshima, K. (1960). Studies on the prophylaxis of measles with attenuated living virus III Inoculation tests in man and monkey with chick embryo passage measles virus. BIKEN J. 3, 115-122.

Oldstone, M.B.A. and Tishon, A. (1978). Immunologic injury in measles virus infection IV. Antigenic modulation and abrogation of lymphocyte lysis of virus infected cells. Clin. Immun. and Immunopathol. 9, 55-62.

Orvell, C. and Norrby, E. (1980). Immunological relationships between homologous structure polypeptides of measles and canine distemper. J. Gen. Virol. 50(2), 231-245.

Osunkoya, B.O., Cooke, A.R., Ayoni, O. and Adejumo, T.A. (1974a). Studies of Leukocyte cultures in measles I. Lymphocyte Transformation and Giant cell Formation in Leukocyte Cultures from Clinical cases of measles. Archiv. f. Virusforschung. 44, 313-322.

Osunkoya, B.O., Adeleye, C.I., Adejumo, T.A. and Salimonu, L.S. (1974b). Studies on Leukocyte cultures in measles II. Detection of measles virus antigen in Human Leukocyte by Immuno-flourescence. Archiv. f. Virusforschung. 44, 323-329.

Pampiglione, G., Griffith, A.P. and Bramwell, E.C. (1971). Transient cerebral changes after vaccination against measles. Lancet 2, 5-8.

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

Paterson, P.Y. (1971). The demyelinating diseases-clinical and experimental correlates. In Immunological Diseases (M. M. Samter). Vol. II 2nd ed. Little Brown and Co., Boston.

Pereira, M.S., Pereira, H.G. and Allison, A.C. (1959). Use of Gel diffusion precipitation test in the diagnosis of adenovirus infections. Lancet 1, 551.

Perkins, F.T. (1965). Passive prophylaxis of measles. Archiv. f. Virusforschung. Bd XVI, H1-5, 210-217.

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

Rapp, F., Gordon, I. and Baker, D.P. (1974). 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.

Rawls, W.E., Iwamoto, K., Adam, E. and Melnick, J.L. (1970). Measurement of antibodies to herpes virus types 1 and 2 in human sera. J. Immunol. 104, 599-606.

Rod, T., Haug, K.W. and Ilistrup, J.C. (1970). Atypical measles after vaccination with killed vaccine. Scand. J. Infect. Dis. 2(3), 161-165.

Rohrmann, G.F., Melgaard, S., Beaudreau, G.S. and Martignoni, M.E. (1982). Characterization of DNA from 3 nuclear polydrosis viruses pathogenic for choristoneura sp. J. Invertebr. Pathol. 40(2), 237-241.

Rosen, L. (1961). Haemagglutination and Haemagglutination - Inhibition with Measles Virus. Virology, 13, 139-141.

Ruckle-Enders, G. (1962). Comparative studies of monkey and human measles virus strains. Am. J. Dis. Child. 103, 297-307.

Sangkawibha, N., Sombodhi, D., Osatanonda, P., Ueda, S. and Okuno, Y. (1971a). Studies on measles in Thailand IV. Persistence of measles antibody in the absence of natural virus two years after vaccination by the 'KL' method. Biken J. 14, 149-154

Sangkawibha, N., Tuchinda, P., Sombodhi, D., Osatanonda, P., Ueda, S. and Okuno, Y. (1971b). Studies on measles in Thailand V. Protection against measles outbreak by previous vaccination in an isolated institution. Biken. J. 14(4), 413-417.

Scheid, A. and Choppin, P.W. (1977). Disulfide linked polypeptide chains constitute the active F-protein of paramyxoviruses. Virology 80, 54-66.

Schluederberg, A.E. (1962). Separation of measles virus particles in density gradients. Amer. J. Dis. Child. 103, 291.

Schneweis, K.E. and Nahmias, A.J. (1971). Antigens of herpes simplex virus types 1 and 2: Immunodiffusion and Inhibition Passive Hemagglutination studies. Z. Immun. Forsch. Exp. Klin. Immunol. 141, 471-487.

Schumacher, H.P., Albrecht, P. and Tauraso, N.M. (1972). Markers for measles virus. Tissue culture properties. Arch. ges. Virusforch. 36, 296-310.

Schwartz, J. and Roizman, B. (1969). Similarities and differences in the development of laboratory strains and freshly isolated strains of herpes simplex virus in HEp-2 cells. Electron Microscopy. J. Virol. 4, 879-889.

Scott, T.V. and Choppin, P.W. (1982). Enhanced yields of measles virus from cultured cells. J. Virol. Methods. 5(3/4), 173-180.

Sergiev, P.G., Ryazantseva, N.E. and Shroit, I.G. (1960). The Dynamics of pathological processes in experimental measles in monkeys. Acta Virol. 4, 265-273.

Shahbrabachi, M.S., Mirchamy, H., Shafyi, A., Bahrami, S. and Kamali, M. (1980). Morphological properties of different measles virus vaccine strains. Microbios. 14(53), 39-46.

Shelton, J.D., Jacobson, J.E., Orenstein, W.A., Schulz, K.F. and Donett, H.D. Jr. (1978). Measles vaccine efficacy: Influence of age of vaccination and duration of time since vaccination. Pediatrics. 62(6), 961-964.

Shiraki, K., Okuno, T., Yamanishi, K. and Takachi, M. (1982). Polypeptides of varicella-zooster virus and immunological relationship of varicella - zooster virus and herpes simplex virus. J. Gen. Virol. 61(2), 255-270.

Sinha, N.P. (1980). Measles in children under 6 months of age: An epidemiological study. J. Trop. Med. Hyg. 83(6), 255-257.

Smith, J.W., Rodriquez, J.E. and Mobee, A.P. (1971). Biological characteristics of cloned populations of herpes simplex virus types 1 and 2. App. Microbiol. 21, 350-351.

Stokes, J. Jr., Hilleman, M.R., Weibel, R.E., Buynak, E.B., Halender, R. and Goldner, H. (1961). Efficacy of live attenuated measles virus vaccine given with human immune globulin: A preliminary report. New Eng. J. Med. 265, 507-512.

Sugiyama, T., Hashimoto, K. and Sasaki, S. (1967). An adenovirus isolated from the feces of mice II Experimental Infection. Jap. J. Microbiol. 11, 33-42.

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

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

Ter-Meulen, V., Katz, M. and Muller, D. (1972a). Subacute sclerosing panencephalitis: A review. Curr. Trop. Microbiol. Immunol. 57, 1-38.

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

Thouless, M.E., Leards, C.M. and Flewett, T.H. (1982). Serotyping and subgrouping of rotavirus strains by the enzyme linked immunosorbent assay test. Arch. Virol. 73(3/4), 219-230.

Togashi, T., Orvell, C., Vartdal, F. and Norrby, E. (1981). Production of antibodies against measles virions by use of the mouse hybridoma technique. Arch. Virology 67, 149-157.

Toranzo, A.E. and Hetrick, F.M. (1982). Comparative stability of 2 salmonid viruses and poliovirus in fresh, estuarine and marine waters. J. Fish. Dis. 5(3), 223-232.

Trushinskaya, G.N., Lfrezina, O.N., Rovnova, Z.I., Grigor'Eva, T.A., Isaeva, E.I., Lelkina, T.S. and Zhdanov, V.M. (1982). Studying the antigenic determinants of haemagglutinin of Influenza A viruses, sub type H1N1 by indirect solid - phase radioimmunoassay - VOPR VIRUSOL 0(3), 280-287.

Ueda, S., Hosai, H., Minekawa, Y. and Okuno, Y. (1966). Studies on the combined use of killed and live measles vaccines III. Conditions for the 'take' of live vaccine. Biken J. 9, 97-101.

Ueda, S., Takahashi, M., Kurimura, T., Ogino, T., Suzuki, N., Yamanishi, K., Papa, K. and Okuno, Y. (1970). Studies on the combined use of killed and live measles vaccines V. Five years follow-up. Biken J. 13(3), 179-183.

Van Der Veen, J. and Mes, A. (1973). Experimental infection with mouse adenovirus in adult mice. Arch. ges. Virusforsch. 42, 235-241.

Wallgren, A. (1965). The future of measles vaccination. Archiv. f. Virusforschung. Bd XVI, H1-5, 367-374.

Warren, J., Feldman, H.A., Lezzoni, D., Fulginiti, V.A., Conway, T. and Callian, M.J. (1963). The response of children to combined measles - poliomyelitis vaccine. J. Amer. Med. Assoc. 186(6), 533-536.

Waterson, A.P. (1965). Measles virus. Archiv. f. Virusforschung. Bd XVI, H1-5, 57-80.

Weibel, R.E., Buynak, E.I., Mclean, A.A. and Hilleman, M.R. (1978). Persistence of antibody after administration of monovalent and combined attenuated measles, mumps and rubella virus vaccines. Pediatric 61, 5-11.

Weiner, L.B., Corwin, R.M., Nieburg, P.I. and Feldman, H.A. (1977). A measles outbreak among adolescents. J. Pediatrics. 90(1), 17-20.

Weiss, S.R. and Leibowitz, J.L. (1983). Characterization of murine coronovirus RNA by hybridization with virus - specific complementary DNA probes. J. Gen. Virol. 64(1), 127-134.

Westrate, M.W., Geelen, J.L.M., Wertheim, P.M.E. and Van Der Noordaa, J. (1983). Comparison of the physical maps of DNA of 2 cytomegalovirus strains. J. Gen. Virol. 64(1), 47-56.

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

Whittle, H.C., Mee, J., Werblinska, J., Yakubu, A., Onuora, C. and Gomwalk, N.E. (1980). Immunity to measles in malnourished children. Clin. Exp. Immunol. 42(1), 144-151.

Wigand, R., Koller, D. and Werling, I. (1982). Immunological relationships among adenoviruses of subgenus D. Arch. Virol. 72(3), 199-210.

Wilson, G.S. (1961). Future of measles vaccines: Scientific problems remaining unanswered (Part I). Amer. J. Dis. Child. 103 (March), 348-350.

Winston, S.H., Rustigian, R. and Bratt, M.A. (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.

Wood, B.P. and Bernstein, R.M. (1978). Pulmonary nodular pneumonia during atypical measles illness. Ann. Radiol. 21(2-3), 193-198.

Wood, P.B., Soheranela, K.S., Bracken, P.N. and Houser, F.E. (1980). Measles vaccination in Zaire - When and How?. Trans. R. Soc. Trop. Med. Hyg. 74(3), 381-382.

Wyll, S.L. and Witte, J.J. (1971). Measles in previously vaccinated children: An Epidemiological study. J. Amer. Assoc. 216(8), 1306-1310.

Yamanouchi, K. (1-80). Comparative aspects of pathogenicity of measles, canine distemper virus and rinderpest viruses. Jap. J. Med. Sci. Biol. 33(2), 41-66.

Zweiman, B., Appagianis, D., Maibach, A. and Hildreth, E.A. (1971). Effects of measles immunization on tuberculin hypersensitivity and *in vitro* lymphocyte reactivity. Int. Arch. Allergy. 40, 834-841.

9. STATISTICAL ANALYSIS.

Mann-Whitney test:

Objective

The results obtained by some methods used in determining relationships between Schwarz and Wild measles virus strains were tested statistically to ascertain any significant difference at 5% level of significance ($\alpha = 0.05$).

Hypothesis

To test the null hypothesis (H_0) that there is no significant difference between the observed values for the two strains versus the alternative (H_A) - that there is a significant difference i.e.

$$H_0 = \bar{X}_1 = \bar{X}_2$$

$$H_A = \bar{X}_1 \neq \bar{X}_2.$$

Mann-Whitney test is a non-parametric test that does not require the data to conform to any particular type of distribution. In this method, all observations in both groups are put into a single array, care being taken to tag the numbers of each group so that they can be distinguished. Ranks are then assigned to the combined array. The test statistic is

$$\alpha = N_1 N_2 + \frac{N_1 (N_1 + 1)}{2} - R$$

where N_1, N_2 are the sizes of the two samples (Schwarz and Wild) respectively, and R is is the sum of the ranks assigned to the values of any of the samples.

Note:

The data were ranked jointly as if they were one sample in increasing order of magnitude.

EK_1 = Ranks for Schwarz.

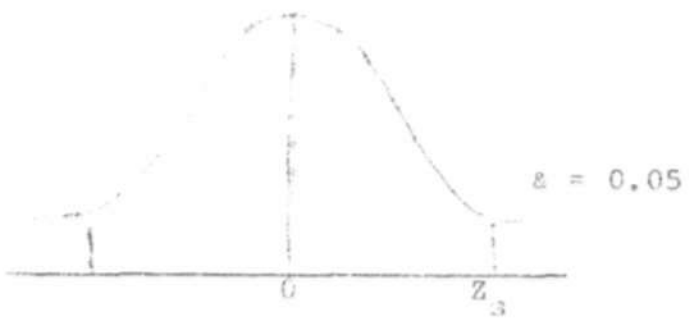
EK_2 = Ranks for Wild

$$\text{Standard Deviation (d)} = \frac{N_1 N_2 (N_1 + N_2 + 1)}{N + 1}$$

$$\text{Standard Error (c)} = \sqrt{d}$$

$$\&_0 = \frac{K_1 + K_2}{2}$$

$$Z_c = \frac{\& - \&_0}{d}$$



Both calculated (Z_c) and standard (Z_s) valu
were compared to accept or reject hypothesis.