

A Study of Enteroviruses in Families in Lagos, Nigeria

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"Key to control or even eradication of many diseases may be found in a clear understanding of their ecology"

(Gear, 1952b)

"Doctors have tended to underestimate, too, the importance of the studies of human ethology and ecology ..... So they find themselves now intervening busily in the attempt to control individual disease processes before their observational studies have been adequately completed."

(Hubble, 1968)

Summary

Summary-

A study of enteroviruses was carried out in 400 persons, making up 95 families in 15 residential areas of Lagos, Nigeria. The study period was November, 1962 to September, 1963.

1476 faecal specimens from the above persons were passed in primary cultures of monkey kidney, (*Cercopithecus aethiops tantalus*). 238 viruses were isolated, of which 70 were typed as enteroviruses. 37 belonged to the polio group, 15 to the Coxsackie B group, and 18 were Echo viruses. A possible identity was found for some of the non-typable isolates by their association with typable isolates in families, residential areas and the community in general. The remainder were untyped by the methods used.

The isolates were discussed in relation to their patterns in seasons, residential areas, and families, and their possible association with tribes, occupations and sexes.

Their possible relationship to clinical disease in the community was evaluated, and the advisability of vaccination discussed. Alternative plans were described.

Introduction and purpose of the study-

## Introduction and purpose of the study

### Introduction

The epidemiological picture of enteroviruses in tropical areas is one of endemicity rather than epidemicity, (Gear, 1958a). Improved social conditions may change this situation and poliomyelitis epidemics result, with a shift upwards in age-incidence, (Gear, 1958c; Sabin, 1963; Fox, 1964).

A change in epidemiology was seen in some tropical and developing areas. In Jamaica, prior to 1946, poliomyelitis occurred in the 0-4 year-olds but thereafter in this age-group and in those over 15 year, and was associated with a fall in the Infant mortality rate to a level of around 75/1000 live births, (Grant and Peat, 1957a,b). Polio epidemics have occurred since at 3-year intervals, (Charles and Grant, 1962).

In East Africa, especially in Kenya - and in Southern African regions - changes were seen since the second World War, (Fendall, 1960). Epidemics occurred in 1954, 1957 and 1960, (Walker, 1956; Fendall and Lake, 1958; Fendall, 1960, 1962), and oral vaccine was used, in 1962-63, apparently preventing the expected 1963 epidemic, (Fendall and Grounds, 1965b).

The clinical impressions of Collis, (Collis et al, 1961; Huckstep, 1965), suggested that poliomyelitis infection in Nigeria was considerable and on the increase; and, by 1961, the infant mortality rate in Lagos had fallen to around 80/1000 live births, (C.M. Norman-Williams at the W.A.C.M.R. Conference, 1961).

Poliomyelitis oral-vaccine trials were carried out in tropical,

developing countries, e.g. the Belgian Congo, (Lebrun et al, 1960) and subtropical countries with dense populations, e.g. Mexico, (Ramos-Alvarez et al, 1959).

A poliomyelitis vaccination trial was carried out in Nigeria, in young children in Ibadan, (Montefiore et al, 1963). A high percentage of children under one year of age was excreting an enterovirus at the time of the trial, giving rise to viral interference with the vaccine, (Sabin, 1959a; Benyish-Melnick et al, 1959; Ramos-Alvarez et al, 1959; Sabin et al, 1960), and the successful immunisation rate was low.

Vaccine trials had not been carried out in the Lagos area, but it was believed that the picture was similar to that in Ibadan. With the possibility of vaccination in Lagos in the future, it was considered of interest to study the epidemiological picture of enteroviruses in the area and to extend the field to all age-groups; in fact, to a cross-section of the population.



Purpose of the study

(1) (a) To study the overall intestinal viral flora in the population and its distribution, with reference to age-groups, seasons, residential areas, families, etc. before vaccine-virus was introduced into the community - to form a pre-vaccination base for further studies.

(b) To study the distribution of polio and non-polio viruses in the population with reference to age-groups, seasons, residential areas, families, etc., and to determine the pattern of excretion - whether endemic or epidemic.

(2) (a) Other viruses of the enterovirus group (Coxsackies and Echos) can cause illness, in some cases clinically similar to paralytic poliomyelitis, (e.g. Hosier and Newton, 1958; Magoffin et al, 1961; Butler et al, 1962; Bell et al, 1963; Mendez-Cashion et al, 1963; Berkovish and Kibrick, 1964; Hinuma et al, 1964; Newman and Smith, 1965);

and (b), following vaccination with oral polio vaccine, the ecology of the enteroviruses may change, (Dalldorf, 1960; Horstmann, 1963-64); with the removal of polioviruses from the community, viruses, otherwise kept in the background, may come to the fore and give rise to illness, hitherto unseen, (WHO Exp. Comm. Polio., 1957a,b). This could necessitate vaccination against Coxsackie and even Echo viruses, (Gear, 1961-62).

This is a situation to keep in mind, particularly considering the large variety of enteroviruses and the economic factors involved in vaccination programmes.

It was felt necessary to find what these viruses were and whether they occurred in a large variety or in small isolated groups.

(c) New viruses were isolated frequently in different parts of the world; up to 1960, 24 Echo viruses were known, and, by 1965, 33 were listed, (Comm. on the Ent., 1957; Comm. on Ent., 1962; Panel for Picornaviruses, 1963; Melnick et al, 1964; Melnick, 1965). It was possible that new serotypes might be found in Nigeria - or local variants of known types - which would be of importance in the planning of future vaccination programmes, the choice of vaccines and the strains to be included.

(d) Should vaccination be carried out, a study of the seasonal distribution of viruses would be important in choosing the optimum time for vaccination, (Sabin et al, 1960), in the absence of wild viruses, especially those of the Coxsackie B group, (Dalldorf, 1960).

(e) Although the epidemiological pattern is believed to be endemic, and apparently overt clinical outbreaks do not occur, it may be that mild illnesses in the community - particularly those falling into the group of 'pyrexias of unknown origin' - are due to some of the enteroviruses. It was thought to be of interest to attempt to correlate virus isolated with any noted illness in the population under study.

Background to the study

## Background to the Study

### 1. The Enteroviruses

#### History

Clinical poliomyelitis was recognised from early historical times, perhaps as early as 3700 BC - the date of a deformed skeleton found near Cairo by Flinders Petrie - and in Biblical times, (Hutchin, 1932). Hippocrates referred to a possible winter epidemic of paralysis in Thasos, Greece, (Hippocrates, Adams, 1849); but little was documented and records of clinical evidence, showing that the disease was widespread, did not occur in the literature until late in the 18th and early in the 19th centuries.

It has been suggested (Aycock, 1929; League of Nat. Rep., 1930), that the disease occurred in northern countries at remote periods, giving immunisation for several generations, during which time the disease was not heard of, followed by a loss of immunity resulting in the appearance of new cases, particularly towards the end of the 19th century.

Two early recorded outbreaks were in 1795, in England and in 1813, in Italy (Paul, 1951); and in 1823, (Shaw, 1823; Ager, 1918), reference was made to a European child catching infantile paralysis in India where the disease was well-known. Frequent slight paralytic illnesses were seen in English children in India (Goodeve, 1879; Birch, 1879) suggesting, even at that time, the increased susceptibility of the "expatriate"; and it was postulated, (Ager, 1918), that the disease was of Asiatic origin.

Bell, (Bell, 1836) wrote of an epidemic in St. Helena in 1836.

An early account in British literature, with clinical details, (Badham, 1835), described four cases of infantile paralysis affecting the limbs, in an outbreak in Worksop, England. Epidemiologically, this was important and led, in 1840, to the first monograph on poliomyelitis (Heine, 1840): studies by Jacob Heine of Cannstadt, of cases in children aged 6 months to 3 years of age, in good health before infection, showing malaise, pain and resultant paralysis of the extremities. In 1841, Colmer (Colmer, 1843), in Louisiana, U.S.A., described cases of paralysis in "teething" children under 2 years of age.

Study of the disease in Scandinavia, showed that the incidence between around 1800 and 1875 was sporadic and infantile (Bertenius, 1947). In 1877, Medin reported 44 cases in Stockholm, where cases had been infrequent and sporadic, (Medin, 1890); and by the 1890's, particularly after 1895, the epidemic potential of poliomyelitis was recognised, (Paul, 1954). Medin (Medin, 1890) saw it as an "acute infectious disease-an entity", and recognised its epidemic and neurological characteristics; and, by 1896, it came to be known (originally by Wickman) as "Heine-Medin disease".

In 1868, Bull (Leegard, 1890; Leegard, 1914-15) described an outbreak in Norway with non-paralytic cases in family contacts; and in 1885, Cordier in France, noted the infectious nature of the disease, (Cordier, 1888).

Few large epidemics were recorded prior to 1880-except for the isolated one in St. Helena-and Römer (Römer, 1911), in his epidemiological writings, remarked that it was an old disease but that a new epidemiology was emerging.

From the last quarter of the 19th to the early 20th century small epidemics became commoner in Europe and North America, (Hutchin, 1932; Paul, 1954) and were followed by a larger and increasing infection of adults and older children; this was particularly noticeable in Scandinavia, (Bertenius, 1947). In comparative and retrospective studies of cases in the same year in Sweden and the U.S.A., Frost (Frost, 1913) noted that there were more cases under 3 years of age in outbreaks in the U.S.A. than in Sweden in 1905 and that epidemics had occurred earlier in Sweden.

Different areas had either sporadic cases with no epidemics or sporadic cases with epidemics, showing a shift from an endemic to an epidemic pattern, (Paul, 1954). This increase in incidence was not only apparent, but real, (Wells, 1932), beginning in Scandinavia, followed by the northern parts of Europe and North America, and gradually spreading throughout the world in the subsequent 50 to 60 years.

In 1894, Caverly (Caverly, 1894) described an epidemic in north-eastern U.S.A. -in Vermont- in which both paralytic and mild non-paralytic disease occurred together; at the time both were considered to be poliomyelitis, caused by the same agent.

The incidence in England and Wales showed a movement from sporadic to epidemic in the last quarter of the 19th century, (Poliomyelitis, 1937). From around 1890 until 1910, poliomyelitis was seen in hospitals in cities in England and Wales (Gale, 1955) and also in rural districts with several village outbreaks, for example in Upminster, in 1908. By 1910 and 1911 it increased to epidemic level and was widespread in Britain, (Gale, 1955); a steady increase followed



to 1916, when another outbreak occurred (Chalmers, 1925).

The disease was not confined to temperate areas. In 1899, an epidemic occurred in the island of Guam, in the Pacific, (Grunwell, 1900). The disease, previously unknown there, was brought in by a Spanish ship and, meeting "virgin soil", spread rapidly through the island, affecting a large number of young adults who had little or no immunity. Epidemics occurred in Cuba, in 1909, (Lebrede and Recio, 1910), where there was an outbreak of 140 cases; in Nauru Island in the Pacific, in 1910, where an epidemic affected a large number of the population under 40 years of age, (Müller, 1910) and showed an apparent difference in "race" incidence, those of Chinese racial background being spared.

Wickman studied the Swedish outbreak of 1905 (Wickman, 1907; 1913) making important observations. He "challenged the emphasis on paralysis" and discussed the infectious nature of the disease and its spread by humans, the possibility of the existence of carriers, and the possibility that paralysis might be a "complication", that non-paralytic poliomyelitis might be the "typical" disease and that milder illnesses could occur associated with paralytic disease. He noted especially the importance of mild non-paralytic cases in the spread of infection. All this was prior to the discovery of the virus (Landsteiner and Popper, 1908).

The clinical aspects of the Coxsackie viruses-epidemic myalgia, pleurodynia-were described in the 18th century in the report of an epidemic in Schleswig-Holstein, (Hannaeus, 1735; Sylvest, 1933, 1934; Windorfer, 1963b). Epidemics occurred from 1856, in northern European countries, (de Rudder, 1937) with similarities to poliomyelitis

in the seasonal rise in temperate climates; in 1856 and 1863 in Iceland, (Finsen, 1874; Windorfer, 1963a) and in Norway, (Daae, 1872; Homann, 1872).

Cases were seen in the U.S.A. from 1883, first noted in Zealand, Denmark, in 1897 and in Bornholm, Denmark, in 1903, continuing throughout Denmark in 1904. In 1917 the illness returned to Bornholm and in 1927, to Zealand. It may have occurred in England in 1924. Another epidemic started in Bornholm in 1930-31 and spread through Denmark; and there is mention of the disease in Germany and Finland, in 1930 and in Sweden, in 1931. Distribution in male and female, rural and urban, appeared equal and incidence in the under-15 year-olds was twice that in the older groups; incidence rose in summer and fell in winter, (Sylvest, 1933). An outbreak in England, with multiple family cases, was described, (Pickles, 1933).

Outbreaks occurred in the U.S.A. in the early 1930's, (Richter and Levine, 1934; Massell and Solomon, 1935); in Gottland, Sweden, in 1934, in children, (Gard, 1936), followed a few months later by a polio outbreak with a large number of non-paralytic cases. In 1935, there was a large epidemic in children, in the U.S.A., (MacDonald et al, 1937); in 1936, an Australian epidemic spread from town to country and children to young adults, (Doig, 1937); in 1937, there was a small autumn outbreak in Norway, (Torgersen, 1938). Pre-1939 outbreaks in Iceland, Norway, Denmark, Finland, Sweden, Germany, France, Portugal, Great Britain, Australia, the U.S.A. and Argentina, have been reviewed, (Alonso Mujica and Pozzo, 1939).

Possible outbreaks occurred in England in 1941-42, (Beeson and Scott, 1942) and in 1947, (Turnbull, 1947; Bland and Newton, 1947);



and in Belgium, in 1944, (Ronse, 1949). An association between aseptic meningitis and epidemic pleurodynia was noted, (Gsell, 1949).

## Aetiology

### Early studies

From the late 18th century there was controversy and discussion about the clinical aspects and aetiology of poliomyelitis, (Underwood, 1784, 1789). During the period 1836-1907 (Hutchin, 1932) pathological investigations were carried out and the microscopical examinations of Cornil, (Cornil, 1863), were continued by many workers including Charcot (Charcot and Joffroy, 1870). By the end of the 19th century, following the observation of pathological changes in the intestines, it was concluded that the causative agent was transmitted via the intestinal tract, (Rissler, 1888; Bülow-Hansen and Harbitz, 1898, 1899). Wickman, working on data of the 1905 Swedish epidemic, (Wickman, 1907, 1913), established the infective nature of the illness, its human spread and that the pathogenesis was related to the alimentary canal, from which infection spread to the nervous and lymphatic systems.

### Isolation of the poliomyelitis virus

In 1908, the poliomyelitis virus was isolated (Landsteiner and Popper, 1908, 1909) and infection experimentally transmitted to laboratory primates, thereby opening up studies into many aspects of the disease. Similar work was carried out by Flexner and Lewis, (Flexner and Lewis, 1909a), with the serial transmission of infection in monkeys; work which was confirmed by others, (Leiner and von Wiesner, 1909; Römer, 1909; Landsteiner and Prasek, 1909-10).

The "filterability and eo ipso virological nature of human poliomyelitis virus" was recognised, (Landsteiner and Levaditi, 1909) and confirmed, (Flexner and Lewis, 1909c), although work on "Globoid

Bodies" as the aetiological agent went on for years, (Flexner and Noguchi, 1913a,b); a bacteriological cause was considered and many studies carried out with negative results, (Harrington, 1932).

#### Neutralisation

Persons recovering from poliomyelitis developed substances in the blood (antibodies) which neutralised the virus, (Levaditi and Netter, 1910), and these substances were widespread in healthy persons (Kling and Levaditi, 1913; Müller, 1922; Levaditi, 1930; Aycock and Kramer, 1930b). In 1910 a neutralisation test against poliomyelitis virus was carried out on human "convalescent" sera, (Netter and Levaditi, 1910). Neutralisation occurred, using sera from four persons with histories of poliomyelitis attacks from 6 weeks to 3 years previously; serum from an individual who had suffered an abortive attack was also studied.

#### Early attempts at immunisation

Studies on the experimental aspects of poliomyelitis (Landsteiner and Levaditi, 1910; Levaditi and Landsteiner, 1910a), brought up the possibility of vaccination and attempts at immunisation were carried out by many workers. Flexner and Lewis (Flexner and Lewis, 1910a) used virus heated to 55°C, but this was ineffective. Other experiments using virus attenuated by heat had some success (Römer and Joseph, 1910a; Shaughnessy et al, 1930) but attenuation by formaldehyde (Römer, 1911) was initially unsuccessful.

Experiments using the gastro-intestinal route were carried out, (Leake, 1918). Dried and heated poliomyelitis vaccine was used experimentally in monkeys, (Levaditi and Landsteiner, 1910b) with variable results. Live virus was inoculated subcutaneously into a monkey, (Flexner and Lewis, 1910d, 1910e), resulting in some neutral-

isation of virus by convalescent monkey serum; and other studies were made, (Flexner and Lewis, 1909b; 1910a,b,c) on the routes of entry, elimination and transmission to the brain, which was thought to be via the nasopharynx.

In 1925, in Sweden, (Davide, 1928), attempts were made to administer serum prophylaxis for poliomyelitis to humans by administration intramuscularly of serum from cases of the same year. Years later work was carried out showing a parallel between immunity and antibody in serum in animals, (Morgan, 1949b), including chimpanzees, (Melnick and Horstmann, 1947; Howe et al, 1950), and that the inoculation of gamma-globulin produced protection in animals, (Bodian, 1951).

#### Transmission of infection from faeces

The disease was transmitted to monkeys, (Kling et al, 1911-12; 1912; 1912-13), by administration of filtrates of faeces from acute human cases, though experiments on this aspect may have been made earlier, or, at least, considered, (Levaditi and Landsteiner, 1910c).

The 20 to 30 years following 1910 was a period of much work but varying conclusions. Work continued on various aspects of the disease, hampered to some extent by the lack of a stable experimental basis; the use of monkeys, in inadequate numbers, gave variable results, (Salk, 1952). Much of the early confusion in the immunology of poliomyelitis arose from the absence of a quantitative basis for the neutralisation test and the fact that the 3 types of virus were not recognised, (Howe, 1952).

The work of Reed and Muench (Reed and Muench, 1938), on the method of calculating 50% endpoints, led to the standardisation and better interpretation of results. Studies were carried out on physical

characteristics and on the titration of the virus in terms of lethal dosage, (Brodie, 1932). On virulence criteria, differences in strains were recognised at an early stage, (Flexner and Clark, 1911; Flexner, 1912); and when neutralisation tests were used, a definite strain difference was seen.

Work on the distribution of the virus was carried out by many from Landsteiner and Popper onwards, (Landsteiner and Popper, 1908). The existence of passive human "carriers", was noted, with virus being excreted from the naso-pharynx of healthy persons in contact with the disease, (Flexner et al, 1913; Kling and Pettersson, 1914). Further transmission experiments in monkeys, using different routes, were carried out, (Kling et al, 1929), poliovirus being fed to cynomolgus monkeys, resulting in paralytic infection.

#### Portals of entry

The respiratory and alimentary theories of spread became subjects of controversy, (Flexner, 1936). Experiments, (Kling et al, 1929; Levaditi et al, 1931), following on earlier ones, showed that the alimentary tract was the portal of entry and the possibility of infection via the upper respiratory tract had incomplete evidence. Sabin and Olitsky (Sabin and Olitsky, 1937) studied the olfactory route as a means of infection by the poliovirus, infecting monkeys by this route. Later studies established the fact that washed tissues of the alimentary tract were the only sites where virus was regularly found outside the central nervous system, (Sabin and Ward, 1941a; Sabin, 1944), and the olfactory theory was discarded, (Bodian and Horstmann, 1965).

Whether infection occurs by "pharynx to pharynx" spread or by

"faeces to oral" spread is not fully established, although the disease appears to be more infectious in the early stages during which the virus is in the throat. There is much support for the view that it is an enteric infection. Conditions do not parallel respiratory infection spread and the infectious period parallels the period of maximum faecal excretion of the virus. Also, studies following oral vaccination show that spread occurs from vaccinees to contacts without virus in the throat, (Koprowski et al, 1956; Fox et al, 1961). It is possible that both methods of spread operate.

Chimpanzees, (Howe and Bodian, 1940, 1942), and cynomolgus monkeys, (Bodian, 1952b), were susceptible to oral infection with poliovirus. Virus was present in the human oropharynx in the first week of the disease, (Howe et al, 1944); and virus in the nasopharyngeal secretions was mostly on the walls of the pharynx, (Sabin, 1949a). Virus was found in the blood of a patient with abortive poliomyelitis, within less than 6 hours of onset of the illness, (Ward et al, 1946) and was isolated from faeces 2 days later.

Successful oral infection of chimpanzees with human faeces was carried out, (Howe and Bodian, 1941); and chimpanzees could excrete virus in the faeces for up to 2 months after infection, (Bodian and Howe, 1945). Virus was excreted in the faeces of chimpanzees and monkeys following virus inoculation by different routes, (intracerebrally, subcutaneously and intraperitoneally), (Trask and Paul, 1942), before the onset of paralysis (Melnick, 1946), and in the absence of clinical disease, (Bodian and Howe, 1945).

Study of the immune mechanism in poliomyelitis infection (Morgan, 1948), showed that monkeys inoculated with inactive and active virus



became immune with circulating antibodies; chimpanzees infected orally developed circulating antibodies and, in man, circulating antibodies were associated with immunity and were the result of infection.

Viruses were isolated from throat swabs and faecal specimens from poliomyelitis cases, (Steigman and Sabin, 1949; Hammon and Roberts, 1948) and specific antibody was demonstrated in the patients' sera.

Development of antibodies was found in the absence of clinical illness, (Turner et al, 1950; Melnick and Ledinko, 1953); and virus was isolated from children with no disease, (Gear et al, 1951).

However, there may be a failure to isolate virus from cases, especially in adults, (Lennette et al, 1959). In young children, there are probably about 1000 infections for each paralytic case, and in adults only 75 infections to 1 case, (Melnick and Ledinko, 1951a; Olin and Wesslen, 1957).

Horstmann, (Horstmann, 1957-58), discussing pathogenesis and immunisation in poliomyelitis, noted that virus was in the throat and faeces within 24 hours of ingestion and multiplied in the lymphatic and epithelial cells of the alimentary canal. Antibody-negative people became infected, antibody from gamma-globulin and Salk vaccination failed to prevent alimentary infection, but those with a naturally-acquired immunity did not acquire infection. It was questioned what this difference to protect was; was it a quantitative or qualitative difference between naturally-acquired and artificially acquired-immunities? It was postulated that the difference in antibodies was qualitative or that living virus may produce local tissue immunity in the alimentary canal.

### Recognition of type differences

In the 1930's, it was found that all strains of the poliomyelitis virus were not immunologically the same in neutralisation tests, (Paul and Trask, 1933, 1935). This difference was first noted, and the possibility of the existence of at least 2 distinct immunologically different types recognised, during a study of polioviruses from different sources, (Burnet and Macnamara, 1931). Further studies divided the strains into 3 types, (Paul and Trask, 1933; Trask et al, 1937; Kessel et al, 1946; Paul, 1948; Kessel and Pait, 1949; Bodian, 1949a; Morgan, 1948, 1949a; Bodian et al, 1949; Comm.Typ.Nat.Found. Inf.Par., 1951; Koprowski, 1953), and noted the need of 3 antigens for a vaccine. Polio 1 was responsible for most large epidemics, polio 3 was occasionally associated with large outbreaks and polio 2 was not seen in large epidemics, (Comm.Typ.Nat.Found.Inf.Par., 1951, 1953).

Experiments with pooled gamma-globulin, (Bodian, 1949b) showed that antibodies to polio 1, 2 and 3 were present and approximately equal; serum antibody, even passive antibody, was important in preventing infection, (Bodian, 1952a).

Intra-typic strain differences were revealed, based on the use of the neutralization reaction with strain-specific antisera, (Wenner et al, 1956; McBride, 1959; Wecker, 1960), but were not considered sufficient to interfere with cross-protection, (Wenner et al, 1960).

### Experimental use of mice and tissue-cultures

Two factors helped the study of the disease: the discovery that certain mice could be infected with polio virus, later recognised as polio 2, (Armstrong, 1939a,b); and the work of Enders, Weller and



Robbins (Enders et al, 1949) on the growth of polio viruses in "in vitro" cultures of, initially, human tissues.

#### The Coxsackie viruses

Arising out of the use of suckling mice as experimental animals, the first member of what was eventually known as the Coxsackie group of viruses was isolate from clinical cases in Coxsackie, New York, (Dalldorf and Sickles, 1948; Dalldorf et al, 1949). These viruses were subsequently divided into groups A and B, according to the pathology produced in mice, (Dalldorf, 1950a; Gifford and Dalldorf, 1951), and later, into different serological types within the groups, (Sickles and Dalldorf, 1949; Melnick and Ledinko, 1950a; Kraft and Melnick, 1950; Melnick et al, 1949, 1950a; Dalldorf, 1950a, 1953a; Contreras et al, 1952). The term "coxsackievirus" was adopted in 1962, (Int. Ent.Study Group, 1963; Melnick et al, 1963).

Coxsackie viruses were isolated from faeces, and pharyngeal swabs of patients and healthy people, (Kilbourne, 1950; Melnick et al, 1950b; Shaw et al, 1950), from the cerebro-spinal fluid, (Gard, 1950) and the blood, (Findlay and Howard, 1950; Howitt, 1950b).

In all, 23 antigenic types, excluding A 23, (echovirus 9) (Johnsson, 1957; McLean and Melnick, 1957; Boissard et al, 1957; Comm.on Ent., 1962), have been designated in the Coxsackie A group and 6 in group B, (Comm.on Ent., 1962).

Mixtures of types and "prime" variants exist, (Melnick, 1953; Wigand and Sabin, 1962b); and cross-relations have been found in the Coxsackie A group, (Comm. on Ent., 1962).

Some tissue-culture Coxsackie B strains did not cross with the prototype strains, (Wigand and Sabin, 1962; Melnick, 1958), broke

through in tube cultures after a few days and might require plaque techniques for typing, (Davis and Melnick, 1958); some human sera showed heterotypic reactivity in the complement-fixation test, (Kraft and Melnick, 1952; Beeman and Huebner, 1952; Johnsson et al, 1958), as seen in experimentally infected chimpanzees, (Kraft and Melnick, 1953).

#### The Echo viruses

As a result of the use of tissue-culture in experimental work, a further group of enteroviruses was established, the entero cytopathogenic human orphan (ECHO) viruses, (Comm. on the Echo viruses, Nat. Found. Inf. Par., 1955). These were first isolated in the 1950's from healthy children, (Melnick and Ågren, 1952; Ramos-Alvarez and Sabin, 1954; Honig et al, 1956; Hammon et al, 1957a) and from patients with aseptic meningitis, (Robbins et al, 1951; Melnick, 1954).

They occur in the human intestinal tract and, generally, do not produce disease in laboratory animals, though some have been seen to have effects on intracerebral inoculation in monkeys, (Melnick, 1965). Multiple types exist, (Melnick, 1954; Ramos-Alvarez and Sabin, 1954) and by 1965, these numbered 33, (Comm. on the Ent., 1957; Melnick, 1965; Comm. on Ent., 1962; Panel for Picornaviruses, 1963; Melnick et al, 1964). Types 1 and 8 have been placed together as echovirus type 1, (Comm. on Ent., 1962).

Within these types, prime strains exist, e.g. for Echo 1,3,4,5, 6 and 9, (Melnick, 1957c); and variation in one type strain can occur during an epidemic, e.g. Echo 6, (Karzon et al, 1959). Neutralisation difficulties have occurred, e.g. in the case of the prototype strain of Echo 4, (Barron and Karzon, 1961; Yohn and Hammon, 1960); and anamnestic responses can occur among echoviruses, with other entero-

viruses, (von Zeipel and Svedmyr, 1957; Hammon et al, 1958; Neva and Malone, 1959; Halonen et al, 1959; Schmidt et al, 1962; Bell et al, 1964); and in neutralisation in prime strains, (Melnick, 1965).

Multiplication of the Echo viruses occurred mostly in the alimentary tract and all were found in the faeces and many in the oropharynx, (Melnick, 1965); some, e.g. Echo 4,6,9,11,16 and 18, were isolated from the blood, (Neva, 1956; Medearis and Kramer, 1959; Francis and Ceballos, 1959; Lepow et al, 1960; Klein et al, 1960; Yoshioka and Horstmann, 1960; Wigand and Sabin, 1962a; Hsiung et al, 1963); and some, e.g. Echo 2,4,5,6,9,11,14,15,16,18 and 19, from the C.S.F., (Melnick, 1957c; Barron et al, 1958; Henley et al, 1958; McAllister et al, 1959; Eckert et al, 1960; von Zeipel et al, 1960; Howarth et al, 1961), as also Echo 7, (Kleinman et al, 1962b), Echo 23, (Jhala et al, 1961, and Echo 31, (Wenner, 1962).

Some variants of Coxsackie A7 and A14 and some echo viruses produced neuronal lesions in monkeys, whereas variants of polioviruses have lacked this effect. Many properties, such as similar size, cell affinities, multiplication in the human intestinal tract and epidemiological pattern justified the polio, the Coxsackie and Echo viruses being put in the same family, (Comm. on Ent., 1957; 1962), and a common evolution for these viruses has been postulated, (Melnick, 1957c).

## Spread

Many theories were put forward regarding the spread of poliomyelitis infection. It was not until 1884, that a definite hypothesis was put forward by Strümpell, (referred to in Harrington, 1932), in which he considered that infectious diseases in children, including poliomyelitis, formed an aetiological entity. The discovery (Landsteiner and Popper, 1908; Kling et al, 1911-12; 1912; 1912-13) of the causative viral agent and the subsequent advances in laboratory techniques and animal studies, led to the designation of the picorna viruses (Rep. of Int. Study Group, 1963), to the manufacture of vaccines to combat poliomyelitis, and facilitated the study of the disease per se, its epidemiology and ecology.

### Early epidemiological studies

During a poliomyelitis outbreak in 1916-17 in West Virginia, U.S.A., (Leake and Smith, 1917), epidemiological studies concluded that spread other than human was improbable; that contact gave rise to 25% of cases, including non-paralytic, and that there were many unrecognised cases in an epidemic, with symptoms related to the digestive tract; and that other family members, besides the patient, were equally dangerous, especially children, though at this time, it was thought that adults might be more important than children as passive carriers.

Studies in the U.S.A. (Aycock, 1926, 1928a) noted an increase in prevalence since 1907, that transmission was through contact, "carriers" and abortive cases, that distribution of the virus was widespread and that infection took place in concentrated populations with subsequent acquisition of immunity. This immunity was acquired relatively early in life, especially in dense populations, as, at this time in the U.S.A.

poliomyelitis was still a disease of early childhood, with approximately 80% of incidence in the under-10-year-olds. It was recognised, (Aycock, 1928b), that the age-distribution of polio, which was showing a shift to an older age-group in some parts of the world, was a measure of the immunity in the population, as a result of previous exposure to the virus; that recurrences were due to an accumulation of non-immune persons rather than to an increase in the virus virulence; and that virus was always present in the community, bringing about immunity in proportion to the concentration of the population and that only a small proportion exposed was actually susceptible.

Aycock further noted, (Aycock, 1931) the possibility that healthy non-contact "carriers" might exist; and poliovirus was isolated from the tonsils and adenoids of a healthy 2-year-old child who had no contact with a case of polio, (Kramer, 1935).

#### Contact and incubation period

Early work, (Wickman, 1907, 1913; Frost, 1913), recognised that abortive poliomyelitis played a large part in the dissemination of infection and that such cases were numerous. Later epidemiological studies, confirmed that transmission of poliomyelitis was by patient to patient contact, (Casey et al, 1945). Cases of subclinical infection were noted, (Casey et al, 1948) and the possibility that this form was commonest would facilitate spread and resultant immunity. Further studies, (Casey et al, 1950), suggested that the main reservoir of disease in an urban area was the infected ambulatory pre-school child group.

The incubation period was put at 10 to 18 days, (Aycock and Eaton, 1927); between 6 to 15 days, (Casey et al, 1945); and poliovirus was



found in the faeces, (Brown et al, 1945) 19 days before subsequent onset of paralytic disease.

Studies (Casey, 1945) of a 1941 epidemic in a rural area of Alabama, U.S.A., showed that the incubation period was around 12 days, the contact place being 12 days prior to the prodromal stage. The same study found that a contact of several hours, during the infectious period, seemed to be necessary for transmission, and that infection could be traced to one focus directly or indirectly, although spread was scattered. The spread seemed orderly and radial, approximately 1 mile each 10 to 11 days, the contacts for spread being almost entirely under 15 years of age and especially under 4 years of age.

Later studies found the incubation period of the Coxsackie viruses to be about 2 to 5 days in laboratory infections, (Curnen, 1950); less than 48 hours, (Findlay and Howard, 1950); and 3 to 5 days, (Huebner et al, 1950). In general, it was fixed at 1 to 14 days with a mean of 3 to 5 days, (Curnen, 1950; Findlay and Howard, 1950; Huebner et al, 1951; Warin et al, 1953).

Virus excretion in the faeces could last several months (Horstmann, 1955a); in the case of Echo 7 it was on average 24-25 days and could be up to  $3\frac{1}{2}$  months, (Henigst et al, 1961).

Studies in an outbreak in Akron, Ohio, (Francis et al, 1942) recognised that a closer association than ordinary contact was necessary for the spread of infection and observed cases following tonsillectomy. Later (Francis, 1952b) it was concluded that virus-spread during epidemics had a limited, selected, rather than an indiscriminate, distribution and the importance of the family and other intimately-associated groups was realised.

Virus in the faeces

Early work, (Kling et al, 1929; Levaditi et al, 1931), showed that the alimentary tract was the main route of infection and many studies followed confirming this. Experimental infection of cynomolgus monkeys, with poliovirus, (Burnet and Jackson, 1940), suggested that infection of man and monkey was oral, thence to the intestinal wall, abdominal lymph nodes, autonomic nerves and central nervous system. Although virus was found postmortem in the walls of pharynx and intestines, (Sabin and Ward, 1941a) it was not found in nasal mucosa or salivary glands. Droplet transmission was uncommon and alimentary acquisition by faeces, sewage, flies, etc., was more likely, (Bedson, 1943).

Virus was isolated from intestinal contents from a mild poliomyelitis case in a child, (Kling et al, 1939b); from faeces and nasopharyngeal washings during an epidemic of polio, (Trask et al, 1938; Trask and Paul, 1941a) persisting in the faeces for months, though it was in the nasopharynx in the initial 48 hours only; from child contacts with abortive illness, (Lepine et al, 1939) when excretion was claimed for up to 41 to 123 days after infection. Virus was found in the faeces in a case of mild non-paralytic illness, (Kling et al, 1939a); in faeces from healthy contacts in an outbreak, up to 19 days after the outbreak, (Kramer et al, 1939); and in the faeces of contacts of cases up to 1 month after infection, (Gear et al, 1945).

Excretion of virus was noted in healthy individuals, (Trask et al, 1940; McClure, 1941); in faeces from "carriers", (Paul and Trask, 1941; Conybeare, 1946); in typical and atypical cases and in urban sewage during an epidemic, (Paul and Trask, 1941; McClure, 1941), and

during a rural outbreak, (McClure and Langmuir, 1942) virus was found in cases and contacts but not in previous poliomyelitis cases and not in families with no contacts.

Comparison of viral content of pharyngeal swabs, oropharyngeal washings and faeces from patients showed virus was isolated more frequently and for a longer time in the case of faeces, (Horstmann et al, 1946a); virus was excreted for up to 12 weeks and there was no difference between paralytic and non-paralytic excretion; and, inspite of prolonged faecal excretion of the virus in some cases, (Horstmann et al, 1944), no true chronic carriers were found, (Horstmann et al, 1946b). Virus could be isolated from faeces in subclinical cases during the incubation period, (Schabel et al, 1948); and virus excretion from children with only minor symptoms and no paralysis was similar to that from paralytic patients, (Schabel et al, 1950).

A similar picture exists for the other enteroviruses.

The Coxsackie viruses have been found in the faeces and pharyngeal swabs of patients and healthy people, (Kilbourne, 1950; Melnick et al, 1950b; Shaw et al, 1950). Sometimes they were excreted in the faeces with polio virus, (Melnick and Kaplan, 1950). Their excretion pattern was similar to that of polio, (Measroch et al, 1951), with isolation and persistence for at least 1 month.

The Echo viruses, fed to chimpanzees, produced inapparent infection, a transient viraemia and excretion of virus in throat and faeces, persisting in the throat for up to 35 days, (Itoh and Melnick, 1957).

In humans, multiplication of the echoviruses was mostly in the alimentary tract and all were found in the faeces and many in the oropharynx, (Melnick, 1965). They were found mostly in children



under 15 years of age, in summer and autumn in temperate climates and in lower socio-economic groups, (Ramos-Alvarez and Sabin, 1954, 1956; Sabin, 1957a; Melnick, 1957c; Gelfand et al, 1957a, 1963; Reinhard, 1963).

Faecal contamination, in the case of the echoviruses was the most important method of spread; faeces were most infectious early after infection and before onset of illness; for example, the level of Echo 9 in a patient's faeces dropped after the end of the 2nd week, and only traces of virus remained beyond the 5th and 7th week after onset, (Wigand and Sabin, 1962).

Poliovirus in extra-human sources, such as milk, food and particularly drinking-water, was studied, (Kling, 1928, 1939; Gard, 1938; Paul and Trask, 1941; Casey, 1945; Francis et al, 1948); but it was concluded that, although infection could contaminate water supplies, this was not a main method of transmission of infection. Poliomyelitis could be associated with pollution but it was not necessarily a water-borne disease, (Paul, 1941).

#### Virus in Sewage

The knowledge of the faecal excretion of the poliomyelitis virus led to interest in the part played in transmission by sewage, (Kling, 1940; Paul and Trask, 1941). During an epidemic, virus was isolated from sewage samples, (Paul et al, 1939); virus was positive in urban sewage, especially close to an isolation hospital, during an epidemic and negative afterwards, (Paul et al, 1940). But, sewage contamination was not considered a method of spread, (Paul et al, 1940), nor was it likely that viral multiplication took place in sewage, (Kling et al, 1942; Paul, 1941). Polio virus from an infected faecal

specimen could survive in water up to 188 days, (Rhodes et al, 1950a); and polio virus, found in creek water, (Toomey et al, 1945), was transmitted to the cotton-rat. Poliovirus in sewage purification works was studied, (Mundel et al, 1946; Gear and Measroch, 1949).

A study, in Louisiana, U.S.A., from 1928 to 1938, tried to find a link between water-supply, sewage-disposal and poliomyelitis infection rates, (Casey and Aymond, 1940), and concluded that, (a) no water-supply and no sewage-disposal system resulted in a poliomyelitis rate of 40/100,000; (b) a water-supply and a sewage-disposal system gave a rate of 27/100,000; and (c) a water-supply and no sewage-disposal system gave a rate of 84/100,000.

Methods of sewage and refuse-disposal employed in the British Colonies were discussed, (Blacklock, 1944); in this, Poore (in 1893) was in favour of the shallow burial of human excreta and not water-borne sewage; in fact, the bucket system, with no added antiseptic, was used for agricultural purposes. He felt that the risk of epidemics due to disposal by sewage systems was greater than by using superior earth burial.

Studies in tropical areas, (Aycock, 1948), recognised that spread there was also enteric. As virus was widespread in warm climates and conditions better for multiplication and propagation, a relatively high resultant immunity kept rates low. Improving sanitation in some areas was connected with less infection in infancy and an increase in cases and raising of the age-group incidence, (Sabin, 1963).

Studies of epidemic and non-epidemic sewage, (Melnick, 1947), found poliovirus during an epidemic, in Chicago, 1943, and in a period, in New York in 1940-45, when there were only a few sporadic cases,

suggesting a high "carrier" rate. In a Toronto study, (Rhodes et al, 1950c), poliovirus was found in the sewage plant of an area where an outbreak had occurred, mainly at the height of the epidemic. In another plant virus was present before and during an outbreak, suggesting a wide virus dissemination in the community before frank cases appeared.

A continued study in Toronto, (Clark et al, 1951), showed no polio virus but Coxsackie A virus was isolated during June and September. Viruses were found in sewage without overt disease and might be a possible reservoir of infection in the population, (Kelly, 1962; Clark et al, 1951).

Other studies isolated Coxsackie viruses from sewage, (Rhodes et al, 1952; Melnick et al, 1949, 1954a,b; Kelly et al, 1955; Kelly, 1957; Pattyn et al, 1957; Ozere et al, 1961), and confirmed that Coxsackie virus was in the community during the summer and autumn, but was not widely disseminated in the cold months and only rare in the winter, (Huebner et al, 1950, 1951; Cole et al, 1951; Mack et al, 1958). The seasonal distribution in temperate zones was unexplained, (Kelly, 1953; Melnick et al, 1954a,b; Bloom et al, 1959).

Extended studies, (Melnick et al, 1954a), over a 4-year period, on the distribution of Coxsackie viruses in urban sewage and flies, in different parts of the U.S.A., showed that, in urban areas, the incidence varied in amount and type from year to year, in type within a year, sometimes being multiple. Poliovirus type excretion could vary from year to year with no cases, (Fox et al, 1956). Geographical variations occurred as to seasonal excretion, (Honig et al, 1956; Melnick, 1957b) and excretion was greater in the lower socio-economic groups.

In summer and autumn, 1956, sewage samples contained polio, Coxsackie and Echo viruses, (Kelly, 1957), and unidentified agents, showing a change in intestinal viral flora since 1954, when no Echos were isolated. Similar isolations were made from sewage samples, (Bloom et al, 1959), enteroviruses being isolated from all stages of a sewage-treatment plant including the final effluent; and it was considered that sewage samples could form a source from which the viral flora of a population could be determined and monitored; but the small number of viruses in sewage made it an unlikely vector, (Kelly and Sanderson, 1960).

A reciprocity between Coxsackie B and polio viruses was recognised clinically, (Dalldorf and Albrecht, 1955) and later virologically, but, apart from this, an interrelationship was not evident between different types of enteroviruses. It was thought that isolations made in late spring might foretell the dominant strains, and late ones might reflect those which persist, (Dalldorf and Melnick, 1965).

Treatment of sewage might not destroy viruses, (Kelly, 1953; Gilcreas and Kelly, 1954), laboratory studies showing that viruses were still present, (Kelly et al, 1961; Clarke et al, 1961). Virus was found in plant effluents, (Melnick et al, 1954a,b; Kelly et al, 1955, 1961; Mack et al, 1958; Kelly and Sanderson, 1960), and in discharge streams, (Kelly, 1957; Kelly et al, 1957).

The Echo viruses were also recovered from sewage, (Kelly et al, 1957; Gelfand, 1961; Lapinleimu and Penttinen, 1963).

Privies, (Melnick et al, 1954a,b; Horstmann et al, 1959), and septic tanks, (Paffenbarger et al, 1959; O'Connor and Morris, 1955) yielded Coxsackie viruses, which might contaminate nearby water supplies.

Flies as a source of infection

Flies were a possible source of infection and harboured poliovirus in a rural epidemic, (Paul et al, 1941) and in urban epidemics, (Sabin and Ward, 1941c); flies on raw sewage yielded polio virus, (Toomey et al, 1941). Studies in a Texas epidemic showed no connection between flies and the epidemic, although flies were many in association with privies, (Paffenbarger and Watt, 1953; Francis et al, 1953). Excretion of virus in flies was less than in sewage, (Melnick et al, 1954a).

Polio viruses, (Trask and Paul, 1943), Coxsackie viruses, (Melnick et al, 1949, 1954a,b; Melnick and Penner, 1952) and Echo viruses (Riordan et al, 1961) were associated with flies; and Coxsackie viruses were associated, experimentally, with cockroaches, (Fischer and Syverton, 1951, 1957).

Flies carried polio viruses for a period of several months following the peak of an epidemic, in April-May, followed by Coxsackie viruses during the summer and autumn, (Melnick and Dow, 1953); and polio and Coxsackie viruses, (Francis et al, 1953) in flies were proportional to viruses in the privies, the viruses surviving the conditions of heat and exposure to air.

Family and children in the spread of infection

The importance of the family in spread was recognised early, (Flexner et al, 1913; Kling and Petterson, 1914; Taylor and Amoss, 1917), and family members, especially children, were considered as dangerous for spread as the case itself, (Leake and Smith, 1917). Later, the importance of studies in families and small groups was recognised, (Paul, 1941; Francis, 1948).

Some, (Aycock and Eaton, 1927), considered that multiple family cases had a common origin, only a few true secondary cases occurring



in one family, others being abortive. Francis, (Francis, 1948), thought that one person showed the infection, e.g. a child, and that others were subclinically affected, and wondered if virus was in individuals before the disease onset and whether a common source occurred rather than serial infection.

Others thought that infection was not necessarily from a common source, and that inapparent "carrier" cases played a large part in transmission, (Wenner and Tanner, 1948), though subclinical cases were not passive carriers but true cases, (Pearson et al, 1945; Brown et al, 1948).

Burnet, (Burnet, 1945) discussed the possibility that the polio virus was "parasite" in the pharynx and intestine of small children and passed from child to child in non-epidemic times with no symptoms, the paralytic attack being rare. The reason for paralysis was unknown but might be due to balance between virus virulence and individual immunity.

Polio virus in the epidemic community, showed a limited selective, rather than an indiscriminate, distribution, (Francis, 1952b) stressing the importance of the family and intimately-associated groups; infection was from person to person in households, (Bodian and Paffenbarger, 1954), most members becoming faecal carriers.

In multiple cases in families, (Casey et al, 1945), most infection was in the youngest children. Intrafamilial spread was rapid, faecal carriers occurring shortly after the index case, (Zintek, 1947; Siegel and Greenberg, 1953), with 7 to 14 days between each case, (Schabel et al, 1948; Pearson et al, 1945); the interval between onset of multiple polio cases in families was discussed, (Littell and Smith, 1955). The frequency of multiple clinical infections was

related to the number in the household and annual polio attack rate, (Siegel and Greenberg, 1953).

A nursery-school study, (McCarroll et al, 1955), during a polio epidemic, showed a higher rate of infection in families than in schools, and the importance of the family as a unit of spread of infection was again confirmed, (Jordan et al, 1956). Children in the household were a factor in the incidence of paralytic polio in adults, (Siegel et al, 1957). In young children, there were probably about 1000 infections for each paralytic case, and in adults only 75 to 1, (Melnick and Ledinko, 1951a; Olin and Wesslen, 1957); children under 2 years of age being the most successful disseminators of virus, (Gard, 1960).

During the studies of poliovirus excretion by preschool children, (Ravenholt et al, 1962), a high prevalence of non-polio viruses was noted in healthy children in late summer; and early in an epidemic, pre-school children excreted polioviruses, suggesting that such children constituted a great epidemiogenic potential; although earlier, (Langmuir et al, 1952), the 5 to 9 year old child was considered a possible important source of family infection.

The relationship of tonsillectomy to polio was seen in a family outbreak, (Francis et al, 1942) and passage of infection traced in the family, their friends and friends' children, i.e. those in closest contact.

The Coxsackie viruses were isolated from both sexes and more from children than from adults, but insufficient information existed to give the incidence according to sex, race or age, (Dalldorf and Melnick, 1965). No significant difference was found in Coxsackie excretion



between the sexes in the 0 to 5 year-olds, though excretion in the males was greater than in the females, (Gamble, 1962; Spicer, 1961).

Coxsackie infections occurred in families, (Huebner et al, 1950; Armstrong et al, 1950a; Rhodes et al, 1950b; Silverthorne et al, 1951b), with horizontal spread in children followed by vertical spread among older members, (Gelfand, 1961).

Multiple cases were seen in families, (Johnsson, 1954) in both Coxsackie A and B groups, with or without symptoms. About 1 week after the first case, infection was passed on. It was brought in by children and passed to others with an average of one case in 5 days.

Coxsackie A virus infections were found in neighbouring houses, (Huebner et al, 1950), household contacts and children, (Turner et al, 1950; Cole et al, 1951; Melnick and Ledinko, 1953; Fox et al, 1957; Melnick et al, 1957; Gelfand et al, 1957c).

Family infection in Bornholm disease was seen in an Aden outbreak, (Jamieson and Prinsley, 1947). Coxsackie B5 was isolated in a family outbreak, (Chin et al, 1958b); Coxsackie B3 in children, (Kendall et al, 1960), and in an outbreak of subclinical infection in 11 households in which intra-familial spread was greater than inter-familial, (Clemmer et al, 1966).

Polio and Coxsackie viruses occurred and possibly disseminated together, in family groups, (Sabin and Steigman, 1949a; Melnick and Ledinko, 1950b; Melnick et al, 1950b; 1951).

Coxsackie B viruses were seen in the interepidemic faeces of healthy children or those with other illnesses, (Dalldorf and Melnick, 1965).

The Echo viruses were first isolated in the 1950's from healthy

children, (Melnick and Ågren, 1952; Steigman et al, 1953b; Melnick, 1954; Ramos-Alvarez and Sabin, 1954, 1956; Honig et al, 1956; Hammon et al, 1957a). They were seen in family outbreaks, (Faulkner et al, 1957) with inapparent infection in family associates, and most infections in children under 15 years of age.

In epidemics, all age-groups could be attacked in the case of some Echo viruses, (Wenner, 1962), but in the case of others, some age-selectivity seemed to exist, (Davis and Melnick, 1958; Kleinman et al, 1962a). More cases of aseptic meningitis - about 2.5:1 in an Echo 6 outbreak - were seen in males, (Karzon, 1958).

Multiple infection occurred in families, especially in those with young children, (Wenner, 1962); and family infection, e.g. with Echo 4, (Chin et al, 1957), Echo 9, (Nihoul et al, 1957) and Echo 7 (Kleinman et al, 1962a) was seen.

The Southern Louisiana studies on the enteric viral flora in normal children, (Gelfand et al, 1957a) elucidated the viral pattern in a community. Carried out in 1954-55, a variety of viruses was isolated, including polio, Coxsackie, Echo, adeno, Herpes simplex, mumps, influenza and non-typable viruses. There was a seasonal association, with a peak in summer and autumn. The highest rate was in the Negro population, followed by the white lower and then the white higher socio-economic groups. There was no distinct association of excretion with family size, (cf. Siegel and Greenberg, 1953).

Echoviruses were isolated mostly in summer and autumn in temperate climates, and epidemics occurred at this time, though an Echo 9 outbreak was seen in February/March, (Faulkner et al, 1957). The outbreaks varied in size, (Lehan et al, 1957; Sabin et al, 1958) and

some were silent, (Henigst et al, 1961). The incidence was higher in the warmer south of the U.S.A., (Gelfand et al, 1963) although infection was found in the cold climate of the western American Arctic, (Reinhard, 1963).

The Community in the spread of infection

Studies of community distribution were carried out, in the case of the polio viruses, (Francis, 1952a), Coxsackie viruses, (Dalldorf, 1950a; Curnen et al, 1949; Huebner et al, 1950; Weller et al, 1950) and Echo viruses, (Ramos-Alvarez and Sabin, 1954, 1956; Sabin, 1957a; Melnick, 1957c).

In a rural area, polio virus had a limited spread in close relation to sporadic cases, (Pearson and Rendtorff, 1945a); in a small town, polio virus was isolated from families of patients and from children in other families with personal association, (Pearson and Rendtorff, 1945b); and in a large urban area, polio virus was most often recovered from the faeces of close contacts of cases and these were the most persistent "carriers" of the disease, but virus was isolated from those with no known contact, (Pearson, et al, 1945).

Paralytic case passed to paralytic case and non-paralytic to non-paralytic; the chance of paralytic infection being transmitted in household contacts was about 70 times that of its being transmitted in the general public, and that of non-paralytic infection was 100 times, (Siegel et al, 1955); 1 in 850 infections were paralytic, (Fox et al, 1957). Family associates excreted more poliovirus than non-family associates and both more than non-contacts, (Brown et al, 1954).

The majority (61-75%) of susceptible members of an affected family became infected, (Bhatt et al, 1955; Horstmann et al, 1955; Isacson

et al, 1957). Viruses passed from cases to subclinical infection in families, (Brown and Ainslie, 1951; Brown, 1955), establishing less easily where antibody was present and excretion possibly ending with the development of homotypic antibody. Virus type varied from year to year and multiple-type excretion could occur, e.g. polio 1, clinical, and polio 3, subclinical.

The newborn could be infected, (Fox et al, 1955), and the younger members of a family were infected first, though there was a possibility that older non-immune children could bring in virus and pass infection to younger children. Infection occurred in children with maternal antibody, resulting in silent infections passing to older children: so infection could pass in both directions. Infection was more likely if there were other children in the family, less likely if antibody was positive, (Fox et al, 1956), and could occur inspite of Salk inoculation, (Selwyn and Howitt, 1962).

During an outbreak, polio virus spread was greater in households than in daily contacts and less in those over 14 years of age, (Horstmann et al, 1955); all household members under 14 years and 90% of daily contacts excreting virus or developing neutralising antibodies to the virus of the outbreak. The ratio of inapparent to apparent infections was 3:1 and the virus isolation rate was as high in inapparent as in apparent infections. In general, 1 to 2% of polio infections were cases, (Horstmann, 1955a); 4 to 8%, abortive, with recognised mild illnesses; and 90 to 95%, inapparent infections.

In the Louisiana studies, (Gelfand et al, 1957c), in families, 73% of non-immune adults, 96% older and 84% younger non-immune siblings became infected; and 12% immune adults and 37% immune siblings became infected; whereas, transmission of virus from household contacts was

less efficient - 50% in non-immune and 7% in immune.

The poliovirus excretion rate in children was almost as great as in clinical cases, (Isacson et al, 1957). Cycles of activity occurred with different viruses, (Fox et al, 1957), possibly depending on the immune status of young children.

Polio virus spread in families was possibly greater from mothers than from fathers, (Kalwij et al, 1959). Studies of faecal and respiratory viruses in families, (Fox et al, 1966), noted illnesses occurred with increasing frequency from fathers, mothers, children over 5 years of age to children under 5 years of age.

Enterovirus studies, (Gelfand et al, 1963), stressed the importance of studying the ecology of enteroviruses and undertaking long-range studies to find quantitative and qualitative variations in healthy children; to study annual, seasonal and geographic differences, and possible variations due to age, sex, and race; to compare enteroviruses in the healthy and the sick, and the prevalence and other factors of polioviruses, before, during and after vaccination with attenuated oral polio vaccine, (Gelfand et al, 1963).

A longitudinal study of virus excretion in Trinidad was compared with a similar one in England, (Sutton, 1965). Minor illness in urban Port of Spain was commoner than in rural Sangre Grande, with a seasonal peak in June - a direct relationship to the rains - opposite in time to England. It was suggested that it was essential to study the distribution of viruses, with pathogenic potentials, in the tropics.

New York family studies, (Spigland et al, 1966), showed excretion of adenos, Coxsackies, rhinos, Echos, parainfluenza, and others. Enteroviruses occurred mainly from August to December; the rhinoviruses

from March to May; parainfluenza and rhinoviruses had a greater isolation rate in faecal than in respiratory specimens. Faeces gave 52 to 68% and respiratory, 20 to 26% isolation rates.



## Clinical

### Association of enteroviruses with disease

#### General

The existence of clinical poliomyelitis in non-paralytic form was noted early, (Caverly, 1894), and, by the 20th century, it was recognised as a proteiform disease. New laboratory techniques evolved, viruses were isolated and linked with disease. The clinical forms of poliomyelitis varied from the frank "major illness" (paralytic or non-paralytic), through the abortive illness, or "minor illness", to the completely inapparent infection, (Paul, 1955). The term "minor illness" was adopted by Paul and other workers, (Paul et al, 1932); it occurred a few days after exposure to infection, and coincided with the period of viraemia and appearance of virus in the throat and faeces; the "major illness" was the second part of the biphasic illness. Horstmann considered the disease in different age-groups, and discussed "childhood" and "adult" types, (Horstmann, 1955b).

Polio virus was isolated from cases of "abortive" illness, (Paul and Trask, 1932), and a general increase in non-paralytic forms was seen, e.g. in America and Denmark, varying in amount in different epidemics, (Aguirre, 1938). Non-paralytic infection was seen in the experimental animal, a chimpanzee excreting polio virus in the faeces with no clinical disease, (Bodian and Howe, 1945).

During a poliomyelitis epidemic, poliovirus was excreted in cases of mild illness, (Brown et al, 1949). Studies in an epidemic of mild polio-like illness showed an increase in non-paralytic forms, (Silverthorne et al, 1949), and a multiple aetiology was considered. Further studies, (Rhodes et al, 1949a), found that 3 out of 5 paralytic cases



and 4 out of 20 non-paralytic cases were due to polioviruses, confirming the possibility of another aetiology.

Non-polio, mouse-pathogenic viruses, similar to that of Dalldorf and Sickles, (Dalldorf and Sickles, 1948), were isolated from non-paralytic poliomyelitis cases, (Melnick et al, 1949), different areas of the U.S.A. rendering different strains; and stored material from an outbreak of epidemic pleurodynia gave an agent, pathogenic for suckling mice, possibly a Coxsackie virus, (Curnen et al, 1949; Weller et al, 1950). Coxsackie viruses were isolated from paralytic and non-paralytic cases, (Melnick and Kaplan, 1950; Melnick, 1951); and from disease similar to non-paralytic poliomyelitis, (von Magnus, 1949; Gard, 1950).

Other studies, (Silverthorne et al, 1951a), showed clinical paralytic poliomyelitis cases excreted both polio and Coxsackie viruses, whereas clinical non-paralytic poliomyelitis cases excreted only Coxsackie viruses; mild illness, during a poliomyelitis outbreak, yielded a non-polio virus, (Steigman et al, 1953b); polio virus was isolated in a low proportion of aseptic meningitis cases, (Tyrrell et al, 1957), Coxsackie viruses being found in both aseptic meningitis and in "polio" cases; and an untypable cytopathogenic agent caused a family outbreak of aseptic meningitis, clinically indistinguishable from polio or Coxsackie infection, (Johnsson, 1955b).

In Boston, U.S.A., in 1951, an outbreak of mild illness with rash - "Boston exanthem"- occurred, mainly in children, from which agents were isolated and later designated Echovirus 16, (Neva and Enders, 1954a,b; Neva et al, 1954). Cases again occurred in Pennsylvania, in 1954, with the same aetiology, (Neva, 1956; Neva and Malone, 1959; Neva and Zuffante, 1957).

During the 1952 poliomyelitis season in the U.S.A. and Canada, (Shelokov et al, 1955), variation in geographic distribution was found by polio and non-polio viruses; all paralytic cases were related to polio 1, 2 or 3, but most non-paralytic cases were related to non-polio viruses.

Association of specific enteroviruses with illness

Studies became more specific in relating enteroviruses to illness, and the importance of controls in the assignment of a virus to a disease was noted, (Huebner, 1957). The World Health Organisation, (W.H.O. Exp.Comm. Polio, 1957a,b), considered that Coxsackie and Echo viruses could cause epidemics resembling non-paralytic poliomyelitis, and that additional research should be carried out, but no specific control measures taken against them, as yet.

Coxsackie viruses, A and B, and Echo viruses were associated with mild paralysis and with aseptic meningitis, (Hammon et al, 1958). Viral agents, mostly polio 1 and Echo 6, were isolated from aseptic meningitis cases, (Davis and Melnick, 1958); and studies in children, (Drouhet, 1960), showed meningitis, paralysis, ataxia and encephalitis associated with Coxsackie B and Echo viruses.

A 4-year study of enteroviruses and the aseptic meningitis syndrome, in Buffalo, New York, (Karzon, 1959), showed type-variation from year to year: 1955, Echo 6; 1956, Echo 4; 1957, Echo 9 and Coxsackie B4; 1958, Coxsackie B5, were the main types with small numbers of others - polio 1, 3, Coxsackie A9, B2, B3, Echo 2 and 14.

The large 1958 Detroit, Michigan, poliomyelitis epidemic of paralytic and non-paralytic cases, was due to polio viruses, but Echo and Coxsackie viruses were commoner in the non-paralytic cases, (Brown

et al, 1960), although Echo 9 and Coxsackie B5 were seen in paralytic cases. Polio incidence was highest in the 0 to 4 year-old group; non-polio viruses were highest in the 0 to 14 year-old group. In comparison, Californian studies, in 1958, on viral central nervous system disease, (Lennette et al, 1962a), found aseptic meningitis was mostly associated with Coxsackie B5, and paralytic polio with polioviruses, but also with non-polio viruses. Poliovirus was mostly in the 1 to 4 year-olds; Coxsackie B viruses mostly in the 4 to 5 year-olds.

Further studies indicated a more specific virus aetiology. Coxsackie viruses were isolated from pleurodynia cases, (Walker et al, 1959; McLean et al, 1958); Coxsackie and Echo viruses, from meningitis, (McLean et al, 1960); and multiple viral types with paralytic "polio" in the tropics, (Paul et al, 1952b; Akers et al, 1960).

Virus types isolated during polio seasons, mostly between June and November, and from aseptic meningitis, were multiple and varied from year to year, (Saslaw et al, 1960; Saslaw and Anderson, 1961; Cooper et al, 1961). Clinical syndromes associated with enterovirus and reovirus infections have been reviewed, (Scott, 1961; Ashkenasi and Melnick, 1962).

A multiple aetiology was seen in aseptic meningitis and encephalitis, (Habel et al, 1957; Lennette et al, 1961; McLean et al, 1962); in children with gastro-enteritis, (Cramblett and Sievers, 1965), most pathogenic organisms coming from infants under 6 months of age; in C.N.S. infections in children, in India, (Jhala, 1962) and in Germany, (Postal et al, 1960); in aseptic meningitis, in Poland,

(Taytsch, 1962); in meningo-encephalitis, in Australia, varying from year to year, (Forbes, 1963); in Russia, (Nikilayev, 1963); possibly in infant diarrhoea in Guatemala, (Dammin, 1964); in serous meningitis, in Scandinavia, (Nielsen, 1964); and in Canada, (McLean et al, 1965).

Interrelationships of polio and non-polio viruses

Dalldorf, (Dalldorf, 1960), debated whether paralysis was always due to polio viruses 1, 2 and 3, and whether the ecology of enteroviruses might not be upset as a result of oral vaccination. Coxsackie A viruses were found associated with polioviruses and he questioned which was the cause of the paralysis. In a poliomyelitis outbreak with non-paralytic disease - was it due to polio virus? Steigman (referred to in Dalldorf, 1960) found Coxsackie B viruses caused paralysis in monkeys in the absence of poliovirus.

Interference was found among the polioviruses; Coxsackie B viruses interfered experimentally with the polioviruses, (Melnick, 1950; Dalldorf, 1951, 1960; Stanley, 1952; Sulkin et al, 1953; Le Bouvier, 1954; de Lustig and Brioux, 1957; Dömök, 1957;), though Coxsackie A viruses did not, (Ledinko and Melnick, 1954).

Frequently Coxsackie B infection and virus isolation appeared inversely proportional to paralytic polio, which was infrequent in pleurodynia epidemics, (Curnen et al, 1949; Dalldorf, 1955; Dalldorf and Albrecht, 1955). Epidemics of Coxsackie B and polio occurred close together but not simultaneously or in the same place; no dual infections were seen, or only very uncommonly, (Dalldorf, 1960).

Experimentally, Coxsackie A viruses did not interfere with polio viruses; in fact, there seemed a possibility of enhancement or summation in paralytic cases, perhaps due to an increase in the number of

affected neurones. In man, one would expect a high paralytic rate and increased severity if a mixed infection existed. The Coxsackie A viruses did not have the inverse relation of Coxsackie B with the incidence of paralytic disease; the original Coxsackie virus was isolated from two patients, both of whom were paralysed, (Dalldorf, 1952a). In an epidemic, 80% of the paralysed excreted polio viruses; 75% excreted Coxsackie A1 and 60% excreted both. Combined excretion was relatively less frequent in non-paralytic cases. It was possible that the Coxsackie viruses could change a non-paralytic to a paralytic infection, (Dalldorf, 1960).

The inter-relationships of the enteroviruses are complex, (Gear, 1961/62; Weigand, 1959) and the above competitive roles affect the ecology and are important.

#### Coxsackie A viruses

The first Coxsackie A virus was isolated in mice, in a poliomyelitis outbreak, from the faeces of children, (Dalldorf and Sickles, 1948); and they have been found in association with polio virus, (Armstrong et al, 1950a; Clark et al, 1950). General clinical features have been reviewed, (Sabin, 1960; Kibrick, 1964).

Herpangina was described clinically, (Zahorsky, 1920, 1924; Shaw et al, 1950; Huebner et al, 1951), and reviewed, (Parrott, 1957). It was associated with Coxsackie A 2,4,5,6,8,10 and 22, (Huebner et al, 1951; Cole et al, 1951; Dempster and Mitchell, 1954; Parrott et al, 1955; Derrick et al, 1956; Hodes, 1960; Cherry and Jahn, 1965).

"Hand, foot and mouth" disease was described in Canada, (Robinson et al, 1958), in England, (Alsop et al, 1960) and in California, Colorado and Hawaii, (Woodward et al, 1960), due to Coxsackie A16;



an epidemic of vesicular exanthem and stomatitis was due to A16, (Robinson and Rhodes, 1961). The "hand, foot and mouth" syndrome was seen in New Zealand, (Seddon, 1961) and in England, where A5 was isolated, (Flewett et al, 1963). A later outbreak was due to A16, (Meadow, 1965).

Central nervous system involvement occurred with Coxsackie A viruses; A 2,4,7,9, and 10 with aseptic meningitis, and A7 and 9 with cases with paralysis. A2 and 4, in Germany, were associated with serous meningitis, non-paralytic poliomyelitis and meningo-encephalitis, (Keller and Vivell, 1952); A4, in Australia, (Stanley and Dorman, 1953; Duxbury et al, 1961), and A7, in Scotland, (Grist, 1962; Grist and Roberts, 1962) in cases resembling clinical poliomyelitis. A7 (polio 4) was isolated from aseptic meningitis, (Chumakov et al, 1956; Johnsson et al, 1957; Habel et al, 1957; Grist, 1962); A9 was seen with aseptic meningitis, (Habel et al, 1957; Melnick, 1957b; Parrott, 1957; Hodes, 1960); unspecified Coxsackie A types were studied in South Africa, (Gear et al, 1956) and surveyed, (Magoffin et al, 1961).

Respiratory disease of a mild variety was associated with Coxsackie A 21, (Eoe virus), (Lennette et al, 1958; Schmidt et al, 1961a). A10 was isolated in acute lymphonodular pharyngitis, (Steigman et al, 1962); A5 and A6 in acute febrile lymphadenitis, (Hodes, 1960; Gear, 1961/62); and a possible relationship was seen between Coxsackie A viruses and an eruptive fever and bullous aphthous stomatitis, in Dakar, Senegal, (Armengaud and Baylet, 1961).

A1, 4 and 10 were isolated from North Alaskan eskimo children, with "minor illness", during a polio-like epidemic, (Banker and Melnick, 1951). Coxsackie A infections in newborn babies, from mothers

with no antibodies, may prove fatal, (Archetti and Bartolozzi, 1954); a case of acute benign pericarditis was due to A1, (Gillett, 1959). They occurred with the following conditions: exanthem, A4,9 and 16; the common cold, A21 and 24; hepatitis, A4 and 9; pneumonitis of infants, A9 and 16; and undifferentiated febrile illness, A2,4,5,6,7,8,9,10, 16, 21 and 24, (Dalldorf and Melnick, 1965).

Experimental inoculation with Coxsackie A7, in cynomolgus monkeys, resulted in paralysis and myocarditis, (Wenner et al, 1961).

### Coxsackie B viruses

The Coxsackie B viruses were linked with "Bornholm disease", epidemic myalgia or epidemic pleurodynia, but came to be associated with other syndromes. The group and its clinical aspects were summarised, (Kilbourne, 1952).

They were associated with various clinical illnesses, (Curnen et al, 1949; Kilbourne, 1950; Weller et al, 1950; Dalldorf and Gifford, 1951), including laboratory infections resulting in pleurodynia, (Findlay and Howard, 1950; Hopkins, 1950;), and abdominal and thoracic pains, (Shaw et al, 1950).

Experimental inoculation in cynomolgus monkeys with Coxsackie B1 to 6 resulted in paralysis; with B2 and 4, in myocarditis; with B1, in pericarditis and with B6, in pancreatitis, (Wenner et al, 1961; Lou et al, 1961).

### Bornholm disease, epidemic myalgia, epidemic pleurodynia

This syndrome was linked with specific Coxsackie B viruses: B1 - in illnesses resembling pleurodynia, in the U.S.A., (Curnen et al, 1949; Finn et al, 1949; Curnen, 1950; Weller et al, 1950; Kilbourne, 1950; Shaw et al, 1950); in Japan, (Yokata, 1955) and in South Africa, after an apparent absence until 1960, (Gear, 1961/62).



B2 - in the U.S.A., (Curnen, 1950; Shaw et al, 1950); in Italy, (Ginevri and Felici, 1959; Felici and Gregorig, 1959) and in Southern Rhodesia, (Wilkins et al, 1955).

B3 - occurred world-wide in 1951, (Dalldorf, 1955); in pleurodynia epidemics, in Sweden, (Gabinus et al, 1952; Johnsson, 1952), showing maximum excretion in 1952 and absence in 1953-54, (Johnsson, 1955c); in England, (Warin, 1953), in children, (Disney et al, 1953); in Scotland, (Swain and Mitchell, 1953); in Canada, (Tempster and Mitchell, 1954); and in the U.S.A., (Huebner et al, 1953; Bell and Meis, 1959; Glick and Stroud, 1962), in a low-polio season, (Lazarus et al, 1952); in Australia, (Derrick et al, 1956); and in Poland, (Gibinski et al, 1960).

B4 - was isolated in an outbreak in the Transvaal, (Patz et al, 1953); in Italy, especially in the under-10 year-olds, (Ginevri and Felici, 1959; Felici and Gregorig, 1959); in Southern Rhodesia, (Wilkins et al, 1955) and the U.S.A. (Swann, 1961).

B5 - was isolated in the U.S.A., (Plager et al, 1962); in children's infections in Canada, following an apparent absence of 8 years, (Walker et al, 1959); and in New Zealand, (Lang et al, 1963).

B6 - was isolated from a pleurodynia case, aged 13 years, in Canada, (Pavilanis et al, 1965).

Orchitis could appear as a complication of pleurodynia, (Sylvest, 1933; Jamieson and Prinsley, 1947; Utz and Shelokov, 1958; Craighead et al, 1962). A rash was noted with B5 infection in infants under 2 years of age, (Cherry et al, 1963a).

Cardiac involvement was seen with Coxsackie B infection. In Denmark, Bornholm disease was associated with pericarditis, (Bing,



1933); in Germany, cases of acute myocarditis in children occurred, between 1937 and 1944 and, in 1952, possibly due to Coxsackie infection, (Stoeber, 1952). Coxsackie B cardiac infections in infants were discussed, (Gear, 1957a, 1958b; Gear and Measroch, 1958), myocarditis, summarised, (van Creveld, 1960), and the literature reviewed, (Lippi et al, 1962a). General studies of Coxsackie B1 to 5 in cardiac infections were carried out, (Lerner, 1965).

B1 was found in neonatal myocarditis, (Gear, 1961/62) and in adult myocarditis, (Glajschen, 1961) in Southern Africa.

B2 was found in adult pericarditis, in Italy, (Lippi et al, 1962a) and in myocarditis of the newborn, in Australia, (Jack and Townley, 1961).

B3 was isolated from acute myocarditis in South Africa, (Javett, et al, 1956); in the U.S.A., from newborn infant with diffuse myocarditis, possibly infected in utero, (Kibrick and Benirschke, 1956); associated with pericarditis, in the U.S.A., (Bell and Meis, 1959); in a newborn child, in Czechoslovakia, (Vanek et al, 1959); with encephalomyocarditis in the newborn, (Hodes, 1960); and with myocarditis and hepatitis, in the U.S.A., (Sun and Smith, 1966).

B4 was isolated from myocarditis in the newborn, (van Creveld and de Jager, 1956; Verlinde et al, 1956), in Southern Rhodesia, (Montgomery et al, 1955); in Australia, during an adult epidemic of Bornholm disease, possibly resulting in "in utero" infection, (Jack and Townley, 1961); from encephalohepatomyocarditis, (Kibrick and Benirschke, 1958); from myocarditis, (Swann, 1961); from fatal encephalomyocarditis in the newborn, in the U.S.A., (Fechner et al, 1963); and from fatal myocarditis in an adult, in Australia, (Cossart et al, 1965).

B5 was isolated in the U.S.A., from myocarditis in an 8-day baby whose mother had the illness, (Delaney and Fukunaga, 1958), from an adult epidemic of pericarditis and myocarditis, (Null and Castle, 1959), from pericarditis, (Gillett, 1959; Lewes and Lane, 1961; Hedlund et al, 1962; Plager et al, 1962); and from pericarditis in New Zealand, (Lang et al, 1963), and in children in Canada, (Walker et al, 1959).

Visceral involvement has been seen. Cocksackie B3 and 5 were associated with abdominal pain, (Walker et al, 1959); hepatomegaly and splenomegaly in German cases possibly were due to Cocksackie infection, (Stoeber, 1952); and B3 was associated with hepatitis, (Sun and Smith, 1966).

"Minor illness" and pyrexias of unknown origin

Cocksackie B2 was isolated in the U.S.A., from "summer grippe" or "sore throat" during an epidemic, (Sabin and Steigman, 1949a), from an outbreak of summer febrile disease, (Heggie et al, 1960), and from an epidemic of febrile illness, (Ager et al, 1964).

B3 was isolated from "minor illness" in Sweden, (Johnsson, 1952); and B4 from a febrile-illness outbreak in America, (Paffenbarger, et al, 1959).

Epidemic respiratory disease, diarrhoea, herpangina

Cocksackie B1 and B4 were associated with herpangina cases: possibly this clinical-pathological response was due to an enterovirus infection rather than to a Cocksackie A virus specifically, (Cherry and Jahn, 1965).

B3 was isolated in epidemic respiratory disease in English children, (Kendall et al, 1960); associated with febrile respiratory illness

and rhinorrhoea, in the U.S.A., (Roberts et al, 1965); possibly associated with epidemic diarrhoea in Italy, (Felici et al, 1960); and isolated in a case of herpangina and pleurodynia with no sign of Coxsackie A virus infection, (Glick and Stroud, 1962).

B5 has been associated with pneumonia, (Dalldorf and Melnick, 1965); and isolated in cases of vesicular pharyngitis, (St.Geme and Prince, 1961).

#### Aseptic meningitis and other Central nervous system involvement

The isolation of a non-polio virus from children with symptoms similar to poliomyelitis, (Dalldorf et al, 1949), suggested the possibility of similar nervous illnesses due to a variety of viruses.

Association was noted between aseptic meningitis and epidemic pleurodynia, (Gsell, 1949); and aseptic meningitis was linked to non-polio viruses similar to those of Dalldorf and Sickles, (Melnick et al, 1949). The aetiology of cases of aseptic meningitis was studied, (Gabinus et al, 1952), Coxsackie viruses in non-paralytic polio, were discussed, (Rhodes et al, 1953), and the evidence of the aetiology of Coxsackie B in aseptic meningitis noted, (Rhodes and Beale, 1956/57; McLeod et al, 1956).

B2, 3, 4 and 5 were found in illnesses resembling mild paralytic poliomyelitis, (Magoffin et al, 1961), and B1 to 5, in meningo-encephalitis, (Dalldorf and Melnick, 1965).

B1 was associated with aseptic meningitis, in South Africa, (Gear, 1961/62), and isolated from the C.S.F., (Girardi et al, 1957).

B2 was associated with aseptic meningitis in the U.S.A., (Kirby and Evans, 1955) and in Canada, (Marchessault et al, 1961), and isolated from the C.S.F., (Melnick, 1957b; Hummeler et al, 1954; Rhodes

and Beale, 1956/57); and in meningo-encephalitis cases, in Southern Rhodesia, (Wilkins et al, 1955).

B3 was isolated from aseptic meningitis, in Sweden, (Johnsson, 1952, 1954, 1955a,c); in meningoencephalitis in a newborn child, in the U.S.A. (Kibrick and Benirschke, 1956); and isolated from the C.S.F., (Gabinus et al, 1952; Gard, 1954; Girardi et al, 1957; Hummeler, 1957).

B4 was associated with aseptic meningitis, (Kirby and Evans, 1955) and with meningo-encephalitis, (Wilkins et al, 1955), and isolated from the C.S.F., (Gard, 1954; Hummeler, 1957; Rhodes and Beale, 1956/57).

B5 was associated with aseptic meningitis in the U.S.A., (Syverton et al, 1957; Rubin et al, 1958; Chin et al, 1958a,b); it caused encephalitis in an adult, (Jarcho et al, 1963) and in a child, (Walker and Togo, 1963); and was isolated from meningo-encephalitis in a New Zealand epidemic, (Lang et al, 1963), in a meningitis outbreak, in Europe, (Schmidt et al, 1965b), in aseptic meningitis, (Furcolow, 1957) and from the C.S.F., (Curnen, 1957).

#### Echo viruses

The clinical spectrum of the Echo viruses was discussed, (Sanford and Sulkin, 1959; McAllister, 1960; Kibrick, 1964; Sabin, 1960; Wenner, 1962; Melnick, 1965). 24 viruses were known up to 1960 and 33 up to 1966; they could be isolated in monkey kidney tissue culture and Echo 9 in suckling mice as well. They did not produce disease in laboratory animals, although some strains of Echo 9 were pathogenic for new-born mice, (Johnsson, 1957; McLean and Melnick, 1957; Boissard et al, 1957) and were sometimes referred to as Coxsackie A 23, (Comm. on Ent., 1962; Lafleur and Martineau, 1962; Archetti et al, 1959).



The viruses of the group were ubiquitous in children and young adults, giving rise to biphasic illnesses. Sometimes they were associated with rashes, lymphadenopathy and splenomegaly, and C.N.S., myocardial, renal and hepatic involvement. Infant diarrhoea has been seen with Echo 18 excretion. The relationship of diarrhoeas in adults to viruses was not proven, (Gordon and Whitney, 1956); diarrhoea of the newborn and older infants, (Hodes, 1956) was possibly due to a number of organisms including viruses; and children's diarrhoea studies, (Ramos-Alvarez, 1957) did not show sufficient evidence to link viruses and disease, but showed some possible connection with the later-designated Echo 18.

They produced transitory infections of the alimentary tract and were found in faeces of healthy humans not in contact with febrile illness, varying with age, season and socio-economic status. Studies showed a high "carrier" rate in children. Some Echo viruses gave more inapparent infections than others and Echo 4, 6, 9, 16 and 18 were rarely found in healthy children, (Sabin, 1960).

Polio-like illness, clinical "poliomyelitis"

Paralytic disease was associated with Echo viruses, (Wenner, 1962; Kibrick, 1964), viruses being isolated from faeces or parenterally from patients.

Echo 1 was isolated from paralytic disease, (Meyer et al, 1960).

Echo 2 was isolated from the spinal cord of a child with fatal "poliomyelitis". Cellular involvement was similar to that in paralytic poliomyelitis, and no polio virus was found, (Steigman et al, 1953a; Steigman, 1958).

Echo 4, (Karzon et al, 1961), Echo 6, (Davis and Melnick, 1956;

Kibrick, 1959; Francis and Ceballos, 1959; Karzon et al, 1962) and Echo 9, (Sabin et al, 1958; Foley et al, 1959) have been isolated. The possibility was discussed of a synergistic action on the C.N.S. of Echo 9 and polio 2 viruses, (Pette et al, 1961). A mixture of polio 2 and Echo 9 was found in a fatal C.N.S. infection in an infant, (Verlinde et al, 1961) and Echo 9 was isolated from paralytic "polio-myelitis" in a child, (Wilterdink and van der Maesen de Sombreff, 1965).

Others isolated were: Echo 7, (Kleinman et al, 1962a); Echo 11, (McAllister, 1960; Brown et al, 1960), from a fatal bulbospinal "polio-myelitis" case, (Steigman and Lipton, 1960); Echo 16, (Hammon et al, 1958); Echo 18, (Grist, 1961); Echo 30, (Duncan, 1961; Cooney et al, 1962).

Paralysis was transient or severe and permanent, (Kibrick, 1959; Steigman, 1958; McAllister, 1960; Magoffin et al, 1961; Lepow et al, 1962b).

#### Aseptic meningitis

The Echo viruses were a common cause of aseptic meningitis, with or without a rash, (Robbins et al, 1951; Melnick, 1954; Comm.on Echo viruses, 1955). This was the commonest syndrome, 24 of the first 30 echo viruses having been found associated with it, (Melnick, 1965). Up to 1965, all except Echo 24, 26, 27 and 29 were isolated from aseptic meningitis, (Melnick, 1965); Echo 2, 4, 5, 6, 7, 9, 11, 14, 15, 16, 18, 19, 23 and 31 were isolated from the cerebro-spinal fluid, (Sabin, 1960; Melnick, 1965); and these and Echo 17, 20 and 30 from other parenteral sources, (Melnick, 1965).

#### Isolations from the C.S.F.

Echo 2, (Barron et al, 1958); Echo 4, (Johnsson et al, 1958;



Karzon et al, 1961; Kopel et al, 1965); Echo 5, (Melnick, 1958); Echo 6, (Karzon and Barron, 1962); Echo 7, (Kleinman et al, 1962b); Echo 9, (Sabin et al, 1958; Solomon et al, 1959); Echo 11, (von Zeipel et al, 1960); Echo 14, (Melnick, 1958); Echo 15, (Johnson et al, 1960a); Echo 16, (Saslaw and Anderson, 1961); Echo 18, (Eckert et al, 1960); Echo 19, (Faulkner and Ozere, 1960); Echo 23, (Jhala et al, 1961); and Echo 31, (Wenner, 1962).

Echo 4 has been seen in outbreaks, in the U.S.A., (Chin et al, 1957; Karzon et al, 1961; Lehan et al, 1957; Barron and Karzon, 1961), South Africa, (Malherbe et al, 1957), Europe, (Johnsson et al, 1958), Holland, (Strik, 1966), and in Japan, (Hinuma et al, 1966).

Echo 6 was associated with epidemics, (Habel et al, 1957; Kibrick et al, 1956/57; von Zeipel and Svedmyr, 1957), in the U.S.A., (Davis and Melnick, 1956; Karzon et al, 1956; Karzon, 1957; Winkelstein et al, 1957; Karzon and Barron, 1962); in an epidemic, with other viruses, (Johnsson et al, 1957; Lahelle, 1957b; Karzon et al, 1962; King and Karzon, 1962) in the U.S.A., Sweden and Norway.

Echo 6 and 16, experimentally inoculated (intrathecally and intraspinally) into rhesus and cynomolgus monkeys produced mild muscular weakness, (Arnold and Enders, 1959), raising the possibility of paralysis or muscular weakness in man.

Echo 9, occurred in outbreaks, in the U.S.A., (Sabin et al, 1958; Kibrick and Enders, 1958; Prince et al, 1958; Solomon et al, 1959; Oren et al, 1961), Canada, (Sultanian and Rhodes, 1958), Britain, (Garnett et al, 1957; Boissard et al, 1957; Rotem, 1957), Switzerland, (Baumann et al, 1957), Belgium, (Nihoul et al, 1957), Denmark, (Godfredsen and von Magnus, 1957), Italy, (Felici and Archetti, 1958),

England, (McLean and Cameron, 1957) and Germany, (Hennessen, 1957).

Other Echo viruses were associated with aseptic meningitis in sporadic cases or outbreaks; Echo 2, in the U.S.A., (Barron et al, 1958); Echo 7, in Scotland, (Bell et al, 1963); Echo 11, in the U.S.A., (Elvin-Lewis and Melnick, 1959) and in Sweden, (von Zeipel et al, 1960); Echo 16, (Kibrick et al, 1956/57), Echo 18, (Eckert et al, 1960) and Echo 19, (Faulkner and Ozere, 1960), in the U.S.A.; Echo 23, in the U.S.A., (Jhala et al, 1961); Echo 25, in Scotland, (Bell et al, 1965), Germany, (Rosen et al, 1964a; Henigst, 1963), and Russia, (Podoplekin and Idina, 1963); Echo 27 and Echo 31, in Canada, (Kelen et al, 1964) and the U.S.A. (Lennette et al, 1962b; Wenner et al, 1963); Echo 29, in the U.S.A. (Rosen et al, 1964b); and Echo 30, in Scotland, (Duncan, 1961) and the U.S.A. (Cooney et al, 1962).

Echo 16, 18, 19, 23 and 25 have been seen in sporadic cases with other sero-types, (Melnick, 1958; Hammon et al, 1958; Wigand and Sabin, 1961; Wenner, 1962; Lennette et al, 1962a,b; Lepow et al, 1962a; Kibrick, 1964).

Encephalitis, ataxias and other clinical C.N.S. disturbances have occurred in outbreaks or sporadic cases and been associated with the following Echo viruses, in the U.S.A.; Echo 3, 4, 6 and 7, (Lepow et al, 1962a); Echo 9, (Sabin, 1960); Echo 11, (McAllister, 1960); Echo 14, (Lennette et al, 1962a); Echo 18, (Sabin, 1960); and Echo 19, (Meyer et al, 1960).

Cerebellar involvement has been associated with Echo 9, (Sabin et al, 1958; McAllister et al, 1959).

Cardiac involvement

Pericarditis occurred in sporadic cases with Echo 1, 9 and 19,

(Johnson et al, 1961; Kavelman et al, 1961).

Echo 3 was associated with myocarditis and hepatitis, in Scotland, (Selwyn et al, 1963); Echo 6 and 9 was seen with myocardial changes in E.C.G's, (Kibrick, 1964); Echo 25 infection was associated with myocarditis in Scotland, (Bell et al, 1965).

In cynomolgus monkeys, experimental myocarditis was seen with Echo 2 and 14, and pericarditis with Echo 9 and 16, (Lou and Wenner, 1962).

#### Foetal abnormalities

It was thought that Echo 9 might be associated with foetal abnormalities and abortions, (Sabin et al, 1958), but later studies found no association with abortions, stillbirths and congenital abnormalities, (Kleinman et al, 1962b).

Echo 19 was isolated from cystic emphysema in a premature infant, during an outbreak, (Butterfield et al, 1963) and Echo 25 was found associated with hydrocephalus, (Bell et al, 1965).

#### Diarrhoea and gastro-enteritis

Enteroviruses were found on many occasions in healthy subjects, in the U.S.A. and Mexico, (Ramos-Alvarez and Sabin, 1954, 1956; Honig et al, 1956; Gelfand et al, 1957a); Echo 8 was found in Germany, in asymptomatic infection in children, (Henigst, 1962); Echo 13, in healthy children in the Philippines, (Hammon et al, 1959, 1961); and Echo 14, in Japan, in a symptomless outbreak in babies, (Hinuma et al, 1965).

Diarrhoea was noted during Echovirus outbreaks, (Kibrick, 1964) and Echoviruses isolated in large numbers of sporadic diarrhoea cases, (Ramos-Alvarez and Sabin, 1958; Sommerville, 1958), and outbreaks of

gastro-enteritis, due to Echo 11, in children in Italy, (Bergamini and Bonetti, 1960) and laboratory personnel, (Klein et al, 1960); Echo 13, in an outbreak in which diarrhoea was a symptom, (Lambert et al, 1963); Echo 14 in children, (Lepine et al, 1960); Echo 18, (Eichenwald et al, 1958); Echo 19, in diarrhoeal disease of infancy and early childhood, (Ramos-Alvarez and Sabin, 1958) and possibly in children with diarrhoea, (Cramblett et al, 1962); and Echo 22, in infant enteritis, (Bauer et al, 1963).

It was difficult to determine a definite association. Enteroviruses were present, (Ramos-Alvarez and Sabin, 1958), in almost 50% of the diarrhoea cases, the incidence being the same for Coxsackie and polio viruses in cases and controls, but 6 times as many with echoviruses, (Echo 1, 2, 6, 7, 11, 12, 14, 18, 19, 22, 23 and 24) in the diarrhoeas as in the controls.

Echo 6, 7, 9, 11 and 13 were found in 8.5% of cases with diarrhoea compared with 2.5% with respiratory infections, (Sommerville, 1958).

Echo 1, 5, 6, 7, 12, 14, 15 and 21 were excreted in cases of diarrhoea, (Yow et al, 1963) but there were no significant differences in the controls.

Children with diarrhoea showed a higher isolation-rate of adenoviruses (8.5%) and echoviruses (8.5%) than did controls in a Mexico City study, (Ramos-Alvarez and Olarte, 1964).

#### Respiratory-enteric illness

Echo viruses were involved in illness with respiratory and enteric symptoms, (Sabin, 1960; Wenner, 1962).

Echo 11 infection resulted in respiratory symptoms in some,

(Philipson and Wesslen, 1958; Philipson, 1958a,b,c) and gastroenteritis in others, (Buckland et al, 1959; Klein et al, 1960).

Echo 19 and 20 were seen in simultaneous respiratory and enteric infections, (Cramblett et al, 1962; Rosen et al, 1958).

#### Respiratory illness

Respiratory illness was associated with Echo viruses, (Kibrick, 1959; Bell et al, 1961; Cramblett et al, 1962).

Echo 1 and 6 were seen in children and adults in Japan, (Matumoto, 1963); Echo 3 and 20, in children, (Cramblett et al, 1958; Rosen et al, 1958, 1964c); Echo 11, in children and adults, (Philipson, 1958a,c; Philipson and Wesslen, 1958) and experimentally in adults, (Lippi et al, 1962b; Philipson, 1958b); Echo 13, in children, (Lambert et al, 1963); Echo 19, in a possible association, (Cramblett et al, 1962); and Echo 25, (Reilly et al, 1963; Bell et al, 1965).

Eye infections occurred, with photophobia in some infections, with Echo 4, (Karzon et al, 1961), Echo 6, (Karzon et al, 1962) and Echo 9, (Lerner et al, 1963).

Lymphadenopathy occurred occasionally, (Lyle, 1956; Sabin, 1960); and sporadic hepatic involvement was seen in outbreaks of Echo 4, (Malherbe et al, 1957; Karzon et al, 1961) and Echo 9, (Sabin et al, 1958; Solomon et al, 1959).

Epidemic myalgia occurred, mainly in the extremities, (Kibrick, 1959); and pleurodynia, in infections with Echo 1, (Kantor and Hsiung, 1962; Karzon et al, 1962) and Echo 9, (Solomon et al, 1959).

Echo 9 was isolated in cases of "minor illness", (Petersen, 1957).

Rashes of various types, (urticarial, rubelliform, morbilliform, maculo-papular, vesicular, petechial or mixed), occurred in outbreaks



and sporadic cases, with or without aseptic meningitis, and were reviewed in detail, (Lerner et al, 1963; Wenner and Lou, 1963). They occurred at various times in the illness, (Melnick, 1965), and were associated with adenopathy, in the case of Echo 2, 4, 9 and 16, (Rendtorff et al, 1964; Lerner et al, 1963).

Epidemics with rashes were associated with Echo 4, 9 and 16; and sporadic cases with Echo 2, 6, 11, 14 and 18, (Kibrick, 1964). Echo 2 was seen in children, (Rendtorff et al, 1964); Echo 4, (Karzon et al, 1961; Johnsson et al, 1958); and Echo 6, in an outbreak with varied symptoms including rash, in Biak and Netherlands New Guinea, (Neeb et al, 1959).

Echo 9 was isolated from aseptic meningitis with rash, in Belgium, (Nihoul et al, 1957); from aseptic meningitis, with rubelliform rash, in Canada, (Sultanian and Rhodes, 1958); in the U.S.A., in a widespread epidemic of morbilliform rash, with or without aseptic meningitis, (Prince et al, 1958), clinically similar to epidemics in Belgium, in England, in 1955-56, (McLean and Melnick, 1957), and in Canada, in 1956, (Laforest et al, 1957). It was isolated, along with Echo 2, 14 and 16, in an epidemic of aseptic meningitis with maculopapular rash, in the U.S.A.

Other outbreaks with rashes were associated with Echo 9, (Tyrrell et al, 1960), in England, (Boissard et al, 1957; Garnett et al, 1957; Lyle, 1959); and in the U.S.A., (Solomon et al, 1959; Takos et al, 1960; Lepow et al, 1960).

Echo 11 was associated with rashes, (Cherry et al, 1963c; Kibrick, 1964). Echo 16 was isolated in "Boston exanthem" cases, (Neva and Enders, 1954a,b; Neva et al, 1954; Neva, 1956; Neva and Zuffante, 1957;



Neva and Malone, 1959) affecting adults and children. Echo 18 was found in children with rashes, (Medearis and Kramer, 1959); Echo 25 in cases with macular rashes, (Bell et al, 1965); and, in Dakar, Senegal, studies carried out on cases with rashes, not due to measles, (Armengaud and Baylet, 1961; Armengaud et al, 1961b) suggested an association with Echo, or Coxsackie A, viruses.

Enantheims, of papules, vesicles and ulcers, occurred in infections of Echo 6, (Sabin, 1960), Echo 9, (Lerner et al, 1963) and Echo 16, (Neva, 1956).

## Vaccination

Following the early experiments on vaccination in poliomyelitis, (Flexner and Lewis, 1910d,e; Levaditi and Landsteiner, 1910b), later attempts at vaccination, (Kolmer et al, 1935; Brodie and Park, 1936), were carried out. Studies on influenza vaccines led to work on polio vaccine, (Salk, 1953a). Work on killed-virus vaccine began with animal experiments, (Boyd, 1953; Horstmann, 1953; Hammon, 1953; Cox, 1953), in pre-tissue-culture days, and inactivated virus was used in experiments on monkeys, mice and cotton rats, producing some immunity.

Inactivation was carried out by ultra-violet light and formalin, (Poliomyelitis, 1955, 1961). Tests on humans were carried out, (Salk, 1953b,c), using a formalinised vaccine, antibody being produced, and using a U/V-light-inactivated vaccine, (Milzer et al, 1954). Both were triple-polio vaccines, cultivated on monkey kidney tissue culture and led to further studies which produced the inactivated "Salk" poliomyelitis vaccine.

Following "Salk"-type vaccination, a change in the epidemiology of poliomyelitis was seen; there was a reduction in epidemics and incidence, and the disease occurred in lower age-groups and lower socio-economic groups, (Chin and Marine, 1961; Poliomyelitis, 1962). Immunisation with inactivated vaccine did not prevent infection with polioviruses, but there was evidence that some suppression of wild-virus circulation occurred in the vaccinated population, (Gard, 1961-62; Poliomyelitis, 1962), bringing about a decline in cases in non-vaccinated and in vaccinated individuals. But antibodies from inactivated vaccines did not hinder infection and spread of polioviruses, from naturally occurring or live-virus vaccine strains, (Sabin, 1959a; Paul et al, 1957; Gelfand et al, 1959; Lepow et al, 1960).

Further work brought out the existence of attenuated virus strains. An early virus, experimentally modified for virulence, was polio 2 in mice, (Theiler, 1941; Gear, 1952a).

Studies continued on the attenuation of strains for use in vaccines. Experiments were carried out, administering type 2TN rodent-adapted poliovirus orally to humans, (Koprowski et al, 1952), resulting in infection, those fed the vaccine becoming virus-carriers and developing antibody but no disease. Other animal-adapted strains included a mouse-pathogenic type 1 Mahoney mutant, (Li and Shaeffer, 1953) and a type 2 MEF1 chicken-embryo-adapted virus, (Cabasso et al, 1952). Work was carried out on non-neural tissue-culture-adapted Brunhilde polio 1 virus, (Enders et al, 1949, 1952; Enders, 1952; Sabin, 1953), with varying results in subsequent animal experiments.

Other studies, (Koprowski et al, 1956) used virus strains attenuated by passage in non-primate laboratory animals, and in cynomolgus monkey kidney and other tissue cultures, (Sabin, 1953, 1955a, 1957; Sabin et al, 1953, 1954; Cox et al, 1959; Koprowski et al, 1952, 1956), searching for suitable oral-vaccine strains. Tissue-culture work accelerated the development of live attenuated vaccines; the development of oral vaccines was reviewed, (Sabin, 1959a; Koprowski, 1961; Paul, 1961) and discussed at conferences, (Live Polio.Vacc., 1959, 1960).

Variation in neurovirulence of naturally-occurring strains, as measured in the nervous systems of monkeys, was noted, (Sabin, 1957b) leading to their use in vaccines. The plaque technique was used for the selection of attenuated clones and led to the use of viruses with a reduced neurovirulence, (Sabin, 1957c). Marker characteristics

for the classification of virus strains were reviewed, (Plotkin et al, 1962), and studied, (Dubes and Wenner, 1957; Lwoff and Lwoff, 1960; Vogt et al, 1957).

During oral-vaccine studies, (Horstmann et al, 1957), using an attenuated tissue-culture polio 3 Sabin strain, it was found that people with homotypic antibody could still be infected. After vaccination, individuals excreted viruses which had increased slightly in their neurotropic characters, and one of which was equivalent to a virulent strain. This raised the question of future complications.

It was particularly important to know the outcome of ingested viruses, (Koprowski et al, 1960; Melnick et al, 1959); whether disease was caused or whether there was a change in characteristics of the excreted viruses, with a return to virulence after passage in humans. Much work on these aspects was carried out over the years, (Koprowski, 1960, 1961; Melnick et al, 1959; Paul, 1960, 1961, 1963; Sabin, 1959c; Chumakov, 1961; Horstmann et al, 1961; Goffe, 1962; Dame, 1963).

Attenuated virus fed to humans resulted in an increase in virus in the throat secretions and in the faeces in 1 to 3 days, (Sabin, 1955a). Inhibition of virus in the throat, (Wehrle et al, 1961; Howe, 1962), required less antibody than inhibition in the intestine, both in man and in the chimpanzee, (Bodian and Nathanson, 1960; Marine et al, 1962). Possibly, in areas of high socio-economic conditions and good hygiene, transmission of virus was mainly by the pharyngeal-  
oropharyngeal route. Even a low level of antibody would reduce circulating virus, and protection was present unless highly virulent virus was brought in. Disease and infection, in this case, were mainly in the unvaccinated, but virus circulated in the vaccinated as well, (Marine et al, 1962).

In low-sanitation areas, infection was mainly faecal-oro-pharyngeal and viruses continued to circulate and infect, with some cases of paralysis. Therefore it was considered that there were still drawbacks in killed-virus vaccines.

Response to oral vaccine was similar to infection with natural virus. Age affected the reaction to the vaccine, conversion being less in adults, in whom intestinal resistance and immunity might occur, even with low circulating-antibody. Protection was serologic, with local resistance in the intestines; intestinal resistance prevented re-infection, (Koprowski et al, 1956; Fox et al, 1958; Paul et al, 1959), but there was no definite data to establish whether intestinal resistance was due to antibody or to cellular factors.

Spread of viruses after vaccination was the same as in the case of wild viruses, (Smorodintsev et al, 1959; Horstmann et al, 1959; Plotkin et al, 1960c). Oral vaccine showed a spread from vaccinees to contacts without virus being in the throat, (Koprowski et al, 1956; Fox et al, 1961). Family contact was common; extra-familial much less so, community spread ceasing before the supply of susceptibles was exhausted, (Paul, 1961; Fox et al, 1961). The vaccine-virus circulated for several months and then disappeared, (Sabin, et al, 1960).

#### Interference

The phenomenon of interference was studied in the laboratory, (Lycke, 1958; Kono et al, 1963). Wild enteroviruses, (Sabin, 1959a; Benyesh-Melnick et al, 1959; Sabin et al, 1960), could interfere with vaccine viruses, (Ramos-Alvarez et al, 1959), and conversely, in the case of the polio viruses, (Hale et al, 1959, 1961; Sabin, 1962) when polio vaccine was used to control epidemics; or the Coxsackie and Echo viruses, (Fox et al, 1961).

Interference occurred between vaccine strains, type 2 being dominant in the Sabin and type 3 in the Lederle-Cox vaccine. Large-scale vaccination was advisable, covering as much of the child - especially the pre-school child - and young adult population as possible, as quickly as possible. At least 80 to 85% of the 0 to 4 year-old group should be vaccinated, with others in the population, according to the ages of susceptibles in the particular area. Continuation and follow-up programmes were essential; especially for children born into the community, otherwise a dangerous situation would develop, building-up a susceptible young population. They would not get their natural immunity in the community as before, and be very vulnerable to an introduced virus.

It was seen in studies of epidemiology and ecology that, with the possible relative departure of polio viruses as a result of vaccination, the Cocksackie and Echo viruses could come to play a more important part in disease-production and possibly preparation should be made for vaccination with viruses of these groups, (Gear, 1961-62).

Experiments in rhesus monkeys, orally infected with Cocksackie B1 to 6 and A9, found that all but B2 gave infection, (Schmidt et al, 1965c); antibody pattern in man being similar to that in monkeys and no clinical or pathological effects resulting from "infection".



## Geographical

### General

Early cases of poliomyelitis were sporadic, with or without small outbreaks, initially "infantile" and "paralytic" in character, later affecting those in older age-groups. Low hygienic standards in a large area of the world, up to the end of the 19th century, gave rise to an inbuilt immunity. A gradual improvement of conditions over the years led to a loss of this immunity and the occurrence of outbreaks and epidemics in different areas. Prior to this, mainly newly-arrived individuals, from lower-immunity areas, succumbed to infection, while the infection was circulating in the indigenous population.

There was a general increase in most regions of the world, starting at different times, with outbreaks of varying severity, length and time intervals. In temperate countries outbreaks usually occurred between August and October; in tropical areas they occurred at any time of the year, although a relationship was noted with the rainy seasons.

Coxsackie viruses were found in temperate areas, mostly in summer and early autumn, (Huebner et al, 1950, 1951; Clark et al, 1951; Cole et al, 1951) with less dissemination of the virus in the cold months and rarely in winter. They were seen occasionally in winter months, but this seasonal distribution in temperate zones was unexplained, (Kelly, 1953; Melnick et al, 1954a,b; Kelly et al, 1955; Mack et al, 1958; Bloom et al, 1959).

The study of enteroviruses in different countries varied according to geographical position and the state of social development. The

reliability of medical records varied from place to place, especially in countries where facilities were not fully developed. It was only in relatively recent years that poliomyelitis was noted with any degree of accuracy and many areas exist where this degree of efficiency has not yet been reached. Laws governing compulsory notification of poliomyelitis in various countries were laid down, (League of Nat.Mon.Epid.Rep., 1930) and reporting continued in the W.H.O. Epidemiological and Vital Statistics Reports.

General geographical studies were carried out, in some cases by antibody studies in the population, (Gear, 1948b,c, 1954; Sabin, 1949b; Hammon et al, 1950; Paul and Riordan, 1950; Hammon and Sather, 1953; Melnick and Ledinko, 1953) including the tropics, (Rhodes, 1948). The poliomyelitis status in different countries was noted in general works (Simmons et al, 1944, 1951) and considered as a world problem, (Payne, 1954). The world-wide distribution of antibodies to the Coxsackie viruses and their presence in gamma-globulin were studied, (Banker and Melnick, 1951).

Other world-wide summaries of the state of poliomyelitis were undertaken, (Freyche and Nielsen, 1955); world Conferences on poliomyelitis and the other enteroviruses were held and the reports of attending delegates summarised the state of the disease at those times, (Poliomyelitis, 1948; 1951; 1954; 1957).

During world studies of poliomyelitis, the relationship of its incidence to the infant mortality rate (I.M.R.) was noted; a low I.M.R. was seen with an increase in poliomyelitis, (Payne, 1954). However, areas existed where both remained high; for example, the Belgian Congo and Costa Rica, which had high poliomyelitis rates with

an I.M.R. of over 110/1000 live births, (Paul, 1958). It was still an infantile disease in those areas where poliomyelitis increased inspite of continued poor hygienic conditions, although this situation seemed to have had an immunising effect in temperate zones.

Geographically-isolated island communities were exceptions to the rule that an I.M.R. below 80/1000 live births was followed by an increase in paralytic poliomyelitis, (Melnick, 1962).

Studies of the state of infection with polioviruses prevalent in the first few months of life, (Sabin et al, 1960), showed that the increase in the endemic rate, in some areas, was not due to a diminished exposure early in life. There was a high infection-rate in mothers also - 5% for polio viruses and 16% for other enteroviruses. Therefore, perhaps hygiene was not so important in the emergence of the disease, although contributory to a higher age-incidence in more advanced countries. It was possibly due to population shifts and the introduction of different strains, (Gear, 1948b): the existence of territories "virgin" for viral strains rather than for the disease itself.

Enteroviruses were found in young children in tropical areas, (Sabin et al, 1960), but not in older persons in countries with better sanitation and hygiene, (Sabin, 1956). There was more interference to incoming strains in the case of the children, (Payne, 1954, 1957; Paul, 1958).

Some detail will be given of areas where more extensive studies were, and continue to be, carried out, areas which are of epidemiological importance, and those areas of interest in the present study, and which may be compared and contrasted with it.

## Europe

### Northern Europe

It was in the Scandinavian countries, particularly Sweden and Norway, and in North America, that poliomyelitis and the other enteroviruses made an early appearance in epidemic form, (Nissen, 1935; Horstmann, 1946; Bertenius, 1947; Sigurjonsson, 1950; Oker-Blom and Pohjanpelto, 1953).

Poliomyelitis increased in the early 20th century, (Sabin, 1963) and outbreaks were noted in Norway and Sweden, in 1905; in Denmark in 1911, with a later increase by 1931; in Iceland in 1904, 1914-15 and 1924. Finland had a lower incidence than other north European countries, (Oker-Blom and Pohjanpelto, 1953) but showed an increase in 1931. These outbreaks were followed by others at varying intervals.

From the 1930's onwards, a noticeable increase in age-group-incidence in paralytic disease was seen in these areas, although, initially, not in aparalytic cases, (Horstmann, 1946).

### Sweden

The poliomyelitis epidemic pattern in Sweden between 1905 and 1950 was studied, (Olin, 1952). Although the age-incidence was stable from 1905 to around 1911-13, there was an increase in this thereafter to 1944, (Wickman, 1907; Wernstedt, 1919, 1949; Olin and Heinertz, 1943). Older age-groups were affected in rural areas, whereas infection was earlier in towns.

In 1936, a polio epidemic occurred with multiple family cases, showing paralytic and non-paralytic peaks. The highest incidence in rural areas was in the 15 to 30 year age-group; in the towns it was under 5 years of age, (Gard, 1938).

Swedish studies of poliomyelitis between 1930 and 1939 showed an increase in cases from 1936, (Olin and Heinertz, 1943), with the possibility of changes in epidemiology in some areas. Rural incidence was greater than urban and the greatest attack-rates in Sweden were in the 0 to 4 year-old and 5 to 9 year-old groups.

In 1931 a massive outbreak of epidemic myalgia occurred in coastal Sweden, affecting the 15 to 30 year age-group, (Huss, 1933); this was preceded by sporadic cases in 1927 and 1929 and followed by sporadic cases in 1932-33, (Huss, 1934), the highest incidence being again in the 15 to 30 year age-group. In 1934, a further epidemic of myalgia occurred in Gottland followed later by poliomyelitis cases, (Gard, 1936). A 5-year study of aseptic meningitis and pleurodynia in Sweden (Johnsson, 1955c) showed a variation of viruses from year to year.

An increase in aparalytic cases occurred in Denmark from around 1933, (Nissen, 1935); a marked shift being noted from 1919 when no aparalytic cases were seen, to 1934, when aparalytic cases outnumbered the paralytic.

#### Great Britain and Northern Ireland

In England and Wales, a move from sporadic to epidemic poliomyelitis was noted in the last quarter of the 19th century; an epidemic occurred around 1910, later than in the U.S.A. and Scandinavia, (Chalmers, 1925). A gradual age-shift to include older people was noted over the years from 1912-19 to 1944-48, (Benjamin and Gale, 1949), as in Sweden; particularly from the late 1930's, (Hill, 1954) with outbreaks in the 1920's and 1930's, (Hamer, 1924; Gale, 1949; Poliomyelitis, 1937). By the 1947 epidemic there was a notable age-incidence increase, (Daley and Benjamin, 1948).



Prior to 1946, one strain of poliomyelitis virus was isolated in Britain, (MacCallum, 1950). Between 1947 and 1953, there were several epidemics, (Benjamin and Logan, 1953). 1951 saw an epidemic in Liverpool, following annual sporadic cases. Serological studies there and in rural Wales, (Fallon, 1956), comparing antibody levels with those found in studies in the U.S.A., (Melnick and Ledinko, 1953; Paul et al, 1952b), found that there was more "infection" and therefore possibly more immunity in these areas of England and Wales than in the U.S.A.

In 1960, in England, (Hobson et al, 1962) and Wales, (Guy, 1961), there was an increase in non-poliomyelitis virus infection, possibly showing a shift in virus type.

In Scotland, an early major epidemic was seen in Glasgow in 1928 and was the most important in Britain for some time, (Halliday, 1929). Most cases were under 5 years of age and apparently poliomyelitis was becoming commoner: "the more constant and extensive the reservoir, the younger is the predominating age-group affected", (Halliday, 1931). Outbreaks continued, the largest being in 1947 and 1950, and an increase in non-paralytic cases was noted, (Munro, 1950; Dewar, 1960).

Laboratory studies, (Grist et al, 1958; 1960) showed polio 1, 2 and 3 and other viruses occurred in different proportions from year to year and that paralytic poliomyelitis was greatest in the under-5's and aseptic meningitis in the over-5's.

In Northern Ireland the incidence was always lower than in England, Wales and Scotland; there was an increase from 1947 onwards. Antibodies were acquired early, (Dane et al, 1956), more rapidly in towns than in rural areas and a 1957 epidemic occurred in young children, (Donaldson, et al, 1960).



Eire

Eire appeared to have fewer outbreaks; perhaps geographical borders originally tended to limit spread, (Freysche and Nielsen, 1955).

Central Europe

In Germany, there was an increase in incidence from 1909, when notification became compulsory, epidemics occurring every 3 to 5 years with increasing severity in the 1920's and 1930's. There was a marked rise in 1947, with epidemics that year and in 1952, and non-polio viruses were also noted, (Windorfer, 1953; Petzelt and Schneeweis, 1954; Gibbels and Scheid, 1963; Luthardt et al, 1965).

Switzerland showed an increase in poliomyelitis by 1931; Austria and Hungary by 1936, with minor outbreaks in the 1930's, increasing in the 1940's. In Hungary, before World War II, there were epidemics of poliomyelitis at intervals of 5 to 6 years. Between 1954-59 there were four outbreaks, with polio viruses 1, 2 and 3 in different years, (Rudnai, 1960); and in 1958, there was a Coxsackie B epidemic with low poliovirus incidence, followed by a severe polio epidemic in 1959, (Dömök et al, 1960).

An increased incidence of poliomyelitis was evident in Czechoslovakia by 1941 and a large outbreak occurred in 1948, (Freyche and Nielsen, 1955).

In 1927, in Rumania, there was a large poliomyelitis epidemic, mainly infantile, (Jonesco-Mihaesti, 1928; Marinesco, 1928). Epidemiological studies in 1962-63 on young children in Rumania showed a decline in virus excretion, possibly due to oral vaccination, (Predescu et al, 1964).

There was more recorded poliomyelitis in Bulgaria than in Poland up to the 1950's, (Freyche and Nielsen, 1955). In Silesia, Bornholm disease was connected with Coxsackie virus isolations between 1954 and 1957, (Gibinski et al, 1960).

There was little study of the epidemiology of poliomyelitis and related diseases in Yugoslavia. Between 1952 and 1956 there was an increase in non-polio viruses isolated. An epidemic in Slovenia in 1956 was due to Echo 9, (Todorovich, 1959).

Poliomyelitis became notifiable in the Soviet Union in 1924, but little was known of its history there. It occurred in 1905 in St.Petersburg and spread via European Russia to the Urals in the 1930's. There were 3 waves: from Scandinavia via the Baltic States; from Berlin to Warsaw to White Russia and Moscow and from the Balkans to the Black Sea, Ukraine and Crimea. It increased by 1950 and after, and became endemic with sporadic outbreaks. Little was known of the incidence in the civil population and it was possible that the epidemiology there was 20 years behind that of Central Europe, (Anders, 1955; Baroyan and Gailonskaya, 1960).

#### Western Europe

Holland and Belgium had an increased incidence from the 1930's, with epidemics in the 1940's and 1950's. Recorded incidence was less marked in France, with a rise since 1943, but no epidemics until 1959-60, when children under 5 years of age were mostly affected, (Chassagne and Gaignoux, 1961); a variation was seen in poliovirus types from year to year, (Maurin et al, 1960; Lepine et al, 1956).

#### Southern Europe

Poliomyelitis was seen frequently in Italy, with an increased

incidence from 1936 onwards; the country taking an intermediate place in the European incidence-level, with the United Kingdom and the Netherlands. Outbreaks occurred in low-density populations but were rarer in high-density areas, (Garaci and Pietrogrande, 1949).

Local outbreaks occurred from 1956, infection being endemic on the islands and Sicily, occurring mostly in summer and with infantile distribution, especially in rural areas, (Siggia and Pinto, 1959). Poliovirus antibodies were acquired early in Palermo children, (Oddo and Grazia, 1956); and viruses, especially Echoviruses, were endemic in infants, (Dardanoni et al, 1961), incidence being greater in the lower classes, (Brancato et al, 1962).

Enteroviruses were isolated from infantile diarrhoeal cases in Sicily, (Reitano and Dardanoni, 1961).

Between 1940 and 1959, in various regions of the mainland, there was an increased incidence, with small epidemics, infantile in distribution, in Ancona, Mantua and Naples, (Balice, 1961; Buffa, 1961; Tecce and Pane, 1957; Chignoli and Fraenza, 1959). Non-polio virus outbreaks were documented; in Ancona in 1955, (Balice, 1957); in Teramo province in 1957, (Ginevri and Felici, 1959); in Gorizia, 1958, (Felici and Gregorig, 1959).

Healthy children in Rome showed a high enterovirus excretion rate, (Moscovici et al, 1957; Ginevri et al, 1957) and outbreaks had an infantile distribution, (Provvidenza et al, 1959; Cerruti, 1961).

Incidence in Portugal and Spain was low and infantile in age-distribution, as in Greece until the late 1940's and 1950's, when small outbreaks were noted and studies showed infection was endemic and infantile, (Pavlatou et al, 1965).

### Israel

An unusual epidemiological state arose in Israel as a result of population movements after the 2nd World War, when Jewish people arrived from different parts of the world in 1948-49. Until then cases were sporadic, but thereafter epidemics occurred, possibly due to the incoming population being non-immune to the local viruses. The age-incidence in epidemics was mainly infantile. The need for vaccination was imperative and Salk vaccine was used in 1957 and oral vaccine in 1961, (Yekutiel et al, 1955; Levy and Falk, 1960; Goldblum et al, 1965).

### North America

An early epidemic in the U.S.A. occurred in Vermont in 1894, followed by an increased incidence in New York and Massachusetts in 1907; in Wisconsin in 1908; Nebraska in 1909 and Massachusetts, Pennsylvania, Minnesota and Iowa in 1910, with a general major epidemic in 1916, (Serfling and Sherman, 1953).

During the increase to epidemic level, around 1910-12, the age-incidence was infantile, most cases in cities being under 3 years of age; cases in rural areas were fewer, possibly due to less contact with a resultant lower immunity until later in life, (Frost, 1913).

In the 1916-17 outbreaks, in the northern U.S.A., the epidemiological importance of contact and families, especially children, in the spread of infection, was noted along with the existence of many unrecognised cases with symptoms referred to the digestive tract, (Leake and Smith, 1917; Lavinder et al, 1918); in a 1923 Kansas epidemic, the possibility of "carriers" and mild forms of poliomyelitis, was evident, (Diveley, 1925).

During the late 1920's and the 1930's outbreaks occurred at closer intervals, affecting different areas and extents of the U.S.A. at different times, (Geiger and Gray, 1932; Fischer and Stillerman, 1937; Hampton, 1938; Casey and Aymond, 1940), with attacks in older persons becoming commoner, (Geiger and Gray, 1932; Fischer and Stillerman, 1937).

This was followed by epidemics in 1931 and 1944 with the increased incidence continuing to a further major epidemic in 1952. Throughout these outbreaks there was an apparent change in geographical distribution, the incidence moving westwards, (Serfling and Sherman, 1953).

In the 1940's a definite increase occurred with a rise of 40% in 1940 over 1939, (Dauer, 1941), epidemics occurring in areas in 1940 where mild outbreaks had taken place the previous year; a small increase in autumn and winter seeming to lead to an increase the following summer, (Dauer, 1941). 1943 saw outbreaks on the Pacific Coast and the Northwest; 1944, in the Northeast and New York State; 1945, small epidemics all over. This was followed in 1946 by a major countrywide epidemic which was the largest since 1916, (Dauer, 1947).

At the same time an increase to epidemic proportions was noted in Canada, the West Indies and Central and South America, followed, in 1948, by scattered outbreaks in different areas of the U.S.A., (Dauer, 1949).

During the 30 years prior to 1948 there was an increase in age-incidence, (Dauer, 1948b), similar to that seen in Scandinavia, England, and Australasia. A 4-decade age-distribution study, (Dauer, 1955), showed a decrease in the under-5 year-old group between 1920 and 1945, and an increase in the 5 to 9 year-old and 10 to 19 year-



old groups until 1935, when these became fairly constant. The over-20 year-old group was constant until 1940, when there was a marked increase, especially between 1948 and 1952. Paralytic cases were greater than non-paralytic in the 0-4 year-old and over-20 year-old groups since 1935, but the non-paralytic were greater than the paralytic in the 5 to 9 and 10 to 19 year-old groups: perhaps a multiple aetiology was at work.

In the 1916 epidemic, cases amongst 2-year-olds were highest with a sharp fall to the 9 year-old level. Distribution appeared to be different in spinal and bulbar cases, with a parallel between non-paralytic and bulbar cases: again possibly due to a different aetiology. Distribution was more infantile in the Southern states with a greater concentration in the under-5 year-olds, compared with the northern part of the country, (Dauer, 1955).

Much work was carried out in the U.S.A. into many aspects of the enteroviruses, details of which are noted in other sections. As a result of this much general epidemiological and ecological information came to light and was documented.

By 1953, Ohio studies showed many enteroviruses were excreted, especially by younger children, (Ramos-Alvarez and Sabin, 1954); later (Ramos-Alvarez and Sabin, 1956) it was recognised that enterovirus infection in this part of the U.S.A. was less than in less-developed areas, such as Mexico. The disease incidence between 1945 and 1954, (Puffer, 1955), showed that in the U.S.A. and Canada it was greater than in Central and South America, the U.S.A. having an epidemic in 1952 and Canada in 1953, although outbreaks did occur in 1954 in Costa Rica, after years of low incidence and in Jamaica and Trinidad.



Following their virological recognition, the Coxsackie viruses were found and studied in different regions of the U.S.A. and Canada, (Clark et al, 1950, 1951; Armstrong et al, 1950a,b; Silverthorne et al, 1951b; Rhodes et al, 1950b;) and in Alaska, (Banker and Melnick, 1951).

The Louisiana studies in healthy children in 1954-55 showed considerable excretion of polio and non-polio viruses, (Gelfand et al, 1957a). In a 1957 Washington, D.C. epidemic, cases of paralytic polio, associated with polio viruses, mainly polio 3, and aseptic meningitis, associated with Coxsackie and Echo viruses, were seen simultaneously, (Siedler et al, 1960).

The 1958 Detroit, Michigan, epidemic was due to polio 1 and 3 viruses, with Echo and Coxsackie viruses more common in the non-paralytic cases; and polio viruses mainly affecting the 0 to 14 year-old group, (Brown et al, 1960).

In the 1959 Iowa epidemic, polio 1 was seen in paralytic cases, but Echo, Coxsackie and polio 3 in non-paralytic cases as well. The highest incidence in this epidemic was in the 0 to 4 year-old group; in 1952 and 1954, it had been in the 5 to 9 year-old group; and an increase was also seen in the number of cases in Negroes. This had not been evident prior to 1956, (Speers et al, 1960). All this was evidence of a change in epidemiological pattern to older age-groups and an increase in non-paralytic illness, with isolation of other aetiological agents besides the polioviruses.

Studies, in 1960-61, on healthy children in 6 cities of the U.S.A.: Atlanta, Miami, Minneapolis, Buffalo, Seattle and San Francisco, (Gelfand et al, 1963), showed a geographic variation in the pattern of

virus excretion, associated with age, sex and possibly race. The highest endemicity was found in Atlanta, Georgia and in Miami, Florida, with virus transmission all the year, although with a drop in winter and early spring. In the Northern cities no virus was seen in the winter and, in San Francisco, it was low at this time.

In 1961, an earlier appearance in Atlanta and Miami than in 1960 showed a possible variability and even a possible "cyclic appearance of enteroviruses". The incidence in males was greater than in females and greater in non-whites than in whites, possibly due to social and economic factors.

A variation in type-excretion was seen; for example, Coxsackie B4 was plentiful in 1960 but rare in 1962, possibly due to loss of susceptibles. An absence of Coxsackie B1 was noted; comparable to the findings of Gear (Gear, 1961-62) in South Africa. In the Louisiana surveys, an absence of Coxsackie B1 was noted prior to 1959, leading to a possible lack of immunity and the potential threat of an epidemic outbreak.

In 1962, there was an increase in certain enteroviruses, for example, Coxsackie A9, B3, Echo 9, 11 and 14, previously absent, (Gelfand et al, 1963). The coincidence of a susceptible population and the introduction of an agent could give conditions for transmission.

A 1960-63 study, in healthy children (Froeschle et al, 1966) following on the above, showed a seasonal distribution of viruses with frequency in summer and autumn; infection rates were highest in the 1 year-olds, decreasing with increasing age; infection was greater in males than in females; and a large number of enterovirus types were prevalent each year, the most prevalent varying annually and

between different cities. In isolations from C.N.S. disease there was a seasonal distribution of viruses; male infection was greater than female in the under 10's; and if enteroviruses were positive in the under-1 year-olds, there was the greatest risk of C.N.S. disease, and if the incidence was low in the 1 year-olds, there was a later rise of infection. There was a variation in prevalent types, as in the excretion from healthy persons, changing from year to year and in different places; and it seemed possible that polio 1 and 3, Coxsackie A9, B1 to 5, and Echo 4, 9, 11, 14 and 17 were more likely to give rise to C.N.S. disease than polio 2, Echo 1/8, 7, 12, 15, 20 and 27.

#### Arctic regions

Poliomyelitis outbreaks occurred in these seemingly "isolated" regions and were well studied, especially in the 1940's and 1950's, in Alaska, Greenland and the North West Territories (N.W.T.) of Canada, (Epid. and Vit.Stats., 1951, 1953; Freyche, 1952). Coxsackie Viruses were studied in North Alaskan Eskimos, (Banker and Melnick, 1951).

In 1948-49, there was an outbreak in nomadic people of the East Arctic, (N.W.T.), (Adamson et al, 1949; Peart, 1949), due to poliovirus, (Rhodes et al, 1949b) later confirmed as polio 1 (Bodian et al, 1949; Wood et al, 1953). Studies in Lake Harbour, Baffin Land Eskimos, showed antibodies to polio 2 were present in 75% of sera taken in 1950, possibly due to earlier infection in 1942, (Clark and Rhodes, 1952). Similar tests in the Pangnirtung, Baffin Land Eskimos showed positive antibody to polio 2 in 18 year-olds and over, with no history of outbreaks. Further studies for polio 1 and 3 antibodies (Wood et al, 1954) in Baffin Land Eskimos, showed no evidence of clinical polio in this

area and there was no significant number of positive sera in those under 45 years of age. Possibly there had been polio infection in Pangnirtung Eskimos many years before.

In 1953, there was an outbreak at Maguse River, N.W.T., (Johnsen and Wood, 1954), showing some possible previous immunity in the indigenous population.

A polio 1 epidemic occurred in 1953 in the Yukon where there were previous outbreaks in 1940, 1945 and 1952. All age-groups were attacked, 50% of cases being over 20 years of age, (Adamson et al, 1954).

Evidence of past infection was seen in antibody surveys in North Alaska, (Paul et al, 1951a); polio 2 and 3 antibodies were found only in those over 30 years of age and polio 1 only in the over-20's, (Paul et al, 1951b).

During the winter of 1953-54, St. Paul Island, Alaska, had a polio 3 epidemic. Polio 1 antibody was absent under 33 years of age and polio 2 in those under 20 years of age. Most cases were in the 10-14 year-old group, (Eklund and Larson, 1956). Later studies were made into epidemiology and ecology in Alaskan Eskimos, (Reinhard and Gibson, 1960; Reinhard and Gerloff, 1960; Reinhard et al, 1960).

There may have been an epidemic in Greenland as early as 1858, followed by other outbreaks in 1913-14, 1920, 1925 and 1932-33, affecting young people born in the district, (Eskesen and Glahn, 1955). An epidemic occurred in June, 1932, in Sukkertoppen. Prior to this there was an outbreak in 1914, perhaps due to the same virus, as the oldest patient in 1932 was 17, (Hrolv, 1934). It was followed in January, 1933 by an epidemic in Godthaab, which may have come from

Sukkertoppen by healthy carrier, the last contact being late in November, (Christensen, 1934).

In 1953, a polio 1 epidemic spread to Greenland from the 1952-53 epidemic in Denmark, (Eskesen and Glahn, 1955) and lasted a year.

In the view of Fog-Poulsen, (Fog-Poulsen, 1955) infection in Greenland was never endemic, but was introduced at long intervals and gave universal immunisation at the time, which sometimes lasted for years: a situation to be contrasted with that of the Canadian Eskimos, where sporadic cases occurred, (Eskesen and Glahn, 1955), and compared with that in the North Alaska regions, where a situation similar to that in Greenland existed, (Fog-Poulsen, 1955). Incidence in the 1952-53 epidemic showed 9% under 5 years of age, 60% between 5 and 14 years and 31% over 15 years of age, (Fog-Poulsen, 1955).

#### Central America

The first epidemic in the "Deep South" of the U.S.A., occurred in the 1930's and in Florida in 1946. In Florida most cases were in the 5 to 14 year-old group: a possible age-shift upwards, (Paul et al, 1949); that is, not a "tropical distribution".

Polio was sporadic in Mexico from the early 1920's, (Ortiz Mariotte et al, 1953). There was an increase in 1944 with an epidemic in 1946 and outbreaks in 1950, 1951 and 1952, showing a trend to epidemic incidence. Later studies, (Campillo et al, 1961; Sabin et al, 1960) showed enterovirus excretion was high, in the general population.

There was an outbreak of polio in Guatemala in 1949, (Poliomyelitis, 1955); an immunity and enterovirus survey (Horstmann et al, 1960) showed most cases were under 4 years of age and many enteroviruses were excreted by children, 10% of whom were excreting polioviruses.



An infantile pattern was noted in later studies of acute diarrhoeal disease, (Gordon et al, 1964a,b). During studies into causes of death, viruses were isolated from cases of acute diarrhoea, (Dammin, 1964.

In 1943, there was a polio epidemic in El Salvador, with 64 paralytic cases, (Allwood-Paredes, 1944); previously, it was considered a rare disease in this country which had 90% immunity under 5 years of age. This was followed by an outbreak of 78 cases in 1953, (Utz, 1955); the disease was mild, 85% being under 3 years of age; comparable with "infantile paralysis" in pre-1880 Europe.

In Nicaragua, a polio 2 epidemic occurred in 1958, followed by one due to polio 1 in 1960, (Alcocer et al, 1960).

There were epidemics in Costa Rica in 1944, and in 1954, after years of low incidence, followed by a higher endemic rate, (Nunez et al, 1960).

In 1950-51, there was a polio epidemic in Panama with a high incidence in the very young, 81% being under 5 years of age, (Rodaniche and de Rodaniche, 1952). This followed an increased trend since 1947.

#### South America

Antibody surveys in Peru and Colombia in 1955-56 showed a possible increase in age-incidence, (Gelfand et al, 1957b) which might necessitate immunisation, although later serological surveys, (Kalter, 1962) in Colombia and different areas of Peru showed early antibody-acquisition.

Ecuador had a low incidence, but the I.M.R. fell between 1946 and 1957 from 148 to 108/1000 live births; polio antibodies were positive by 4 years of age and rose to 100% by 10 to 14 years of age, (Melnick, 1959).



In Chile, the disease was unknown until 1912; there was an outbreak in 1921-22 and an increase after 1935. The incidence was infantile, (Romero, 1948).

In Argentina outbreaks were noted in the 1940's, followed by an epidemic in 1956 and a movement of incidence, from the coast in 1942 to inland areas by 1953. Epidemics occurred more frequently and the age-incidence increased, with a shift from infantile distribution and an apparent move from endemic to epidemic, (Bottinelli, 1956).

Incidence in Uruguay was endemic in rural and urban areas, and infantile in age-distribution, (Lepine et al, 1955). There were outbreaks in 1935-37, and between 1941 and 1947, (Poliomyelitis, 1955).

Polio was sporadic in Brazil until 1953, when an epidemic occurred, followed by another in 1954, incidence being infantile, (da Silva and Syverton, 1956). Later studies showed Echo viruses were active in the Rio de Janeiro population, (Lacorte et al, 1965).

Sabin (Sabin, 1961/62) studied polio in certain South American areas, and noticed the increasing frequency of epidemics; the endemic incidence in Brazil was infantile in age-distribution, and occurred throughout the year. Uruguay, Chile and Argentina had cases in the hot season and epidemics with varying periodicity: in

Uruguay	90%	cases	were	under	20-30	years	of	age;	in
Argentina	90%	"	"	"	10-13	"	"	":	in
Chile	90%	"	"	"	7- 9	"	"	":	and this

must be taken into account when planning immunisation.

The situation in Surinam was of interest in that the I.M.R. was 75/1000 live births from 1935 and 43/1000 live births from 1950 with no reported polio, (Melnick et al, 1962). It had a similar pattern

to areas of the Caribbean and depressed areas of the U.S.A. before vaccination.

In British Guiana (Guyana) the first epidemic occurred in 1957, although isolated cases occurred in previous years, (Melnick, 1959). The I.M.R. was constant at about 200/1000 live births from 1870 to 1920. Then it fell to 101 by 1945, with a further decline to 70 in 1957, with a polio 1 outbreak of 87 cases around Georgetown, mostly under 5 years of age. Polio infection was positive and widespread in indigenous Rupununi Savannah Indians, who lived in a remote area and had few outside contacts and the infection occurred as early in life as in urban tropical regions, (Melnick, 1959). A further polio 1 epidemic, occurred in 1962-63; infantile in age-distribution, especially in 1 year-olds; vaccine was used to combat it, (Witte et al, 1965; Feldman et al, 1965).

Epidemics occurred in Venezuela in 1941, 1953 and 1955 in the large cities, (Osuna and Guzman, 1957).

#### The Caribbean

Cuba had epidemics as early as 1903, 1906 and 1909, (Lebrede and Recio, 1910; Ramirez-Corria, 1948), followed by outbreaks in 1934, 1942, 1946 and 1952, (Ramirez-Corria, 1948; Paul et al, 1949; Poliomyelitis, 1955).

In Jamaica, prior to 1946, most cases were in the 0 to 4 year-old group; during the period 1947 to 1953, cases in the 0 to 4 year-old and under 15 year-old groups were equal, showing an upwards shift in age-incidence. After 1928 the I.M.R. fell and in 1953 reached a danger-level of 75/1000 live births, (Grant and Peat, 1957b): polio epidemics possibly occurring when the I.M.R. reached 80/1000 live

births, (Charles and Grant, 1962). Cases were sporadic up to 1954, when the first epidemic due to polio 1, occurred, affecting the 0 to 5 year-olds and the 20 to 30 year-olds, (Charles and Grant, 1962): the I.M.R. was 64/1000 live births. The incidence in the 20 to 30 year-old group was different to that in Costa Rica and Trinidad in the same year. There were many cases and several deaths in the 0 to 6 month-old group, showing a lack of immunity in the mothers, (Grant and Peat, 1957a). The epidemic, mostly urban, spread from Kingston to rural areas; the incidence was highest in the warmer months and fell in the cooler. After 1954, epidemics occurred at 3-year intervals, in 1957 and 1960, (Charles and Grant, 1962).

A small outbreak occurred at Cap Haitien, in Haiti, in late 1956-57, mostly in those under 5 years of age; and 99% of the 1 to 4 year-old group had antibodies to at least one type of poliovirus, (Melnick, 1959).

Puerto Rico had a small outbreak in 1928, (Morales, 1930). Sporadic cases followed, with minor epidemics in 1942, 1946 and 1951. In 1954 polio 1 increased in a rural area, with infantile tropical endemic distribution, (Pons, 1956; Paul et al, 1949; Busquets, 1947).

Antigua had no record of an epidemic; 1 case in a child occurred in 1950; the I.M.R. had fallen since 1936, from 132/1000 to 82/1000 live births in 1958. Infection with polio 1 occurred early in the population; with polio 2 mostly in the 4 year-old group; and polio 3 delayed to the 5 year olds and over, (Melnick, 1959). Payne (Payne, 1954) suggested that the non-occurrence of epidemics was due to the small population involved.

In Barbados, the I.M.R. had fallen since 1919, to 174/1000 in

1948 and to 82/1000 in 1958, with an epidemic of 61 cases, mostly under 5 years of age, in 1933, when the I.M.R. was as high as 233/1000 live births, (Payne, 1954). Polio 1, 2 and 3 distribution was similar to that in Antigua with a delay in polio 2 and 3 infection, although antibody levels were higher in Barbados, (Melnick, 1959). In 1963, a polio 1 epidemic occurred, of an infantile distribution, (Leedom et al, 1963).

An antibody survey on St.Vincent showed polio infection was endemic and infantile in the under-5 year-olds, without outbreaks. It was part of "Caribbean zone of endemicity", but as outbreaks occurred elsewhere, vaccination of children might be necessary to anticipate epidemics, (Proctor et al, 1961).

Surveys carried out in Trinidad showed the importance of further studies of the part played by viruses in the production of illnesses in the Tropics, (Sutton, 1965; Milam and Smillie, 1931). There were periodic major outbreaks of polio preceded by an interval of low incidence, the first epidemic being approximately at the end of the 19th century, when the I.M.R. was 160/1000 live births, (Payne, 1954).

Payne, (Payne, 1954) discussed this high I.M.R. with the polio case-rate pattern in Trinidad and compared it with the Maoris in New Zealand, (Coughey et al, 1958), where epidemics occurred in 1916 and 1925, when the I.M. rates were over 100/1000 live births: possibly due to variations in the population structure; and discussed it with reference to the state in Antigua and Barbados mentioned above. Epidemics followed in 1954 and 1957 with a fall in I.M.R. In 1959, an epidemic of 301 polio 1 cases occurred, under 3 years of age in distribution, (Melnick, 1959).

Between 1937 and 1948, there were a few cases of poliomyelitis in the Dutch West Indies, under 15 years of age; a possible age-shift upwards, (van Creveld, 1948). An antibody study in Curacao showed distribution-levels equivalent to those in Cairo, Egypt, (Hofman and Wilterdink, 1960).

### Oceania

Australia and New Zealand followed a pattern similar to that in North America: endemic, with occasional epidemic and lower inter-epidemic incidence. Both countries had outbreaks in the late 1930's and repeated epidemics of varying size in the 1940's and 1950's.

In Australia, by 1937-38, originally localised outbreaks were moving to epidemics, mostly infantile in distribution, (Bull, 1937-38; Burnet, 1940), but an upwards age-shift took place over the previous 20 years, (Helms, 1941). Serological studies in aborigines in the Northern Territory showed that antibodies to polio 2 were present in all areas in the over-5 year-olds, (Miles, 1953), but that polio 1 and polio 3 antibodies varied in different areas, (Stokes et al, 1955). Coxsackie A and B infections were studied in Australia, (Derrick et al, 1956) and in the period 1963-65 there was less polio, but an increase in Coxsackie and Echo viruses in aseptic meningitis cases, with the possibility of epidemics of these occurring, (Nat.Health and Med.Res. Counc., 1965).

New Zealand had a small epidemic in 1916, followed by a major one in 1924-25, especially in North Island. The age-incidence was mainly in the under-20's, showing a shift upwards in age. Epidemics occurred at about 10-year intervals, (Murphy, 1955) with sporadic cases in between.



Children were immune to polio 1, 2 and 3, with a low immunity in those under 15 years of age, possibly due to the good hygienic conditions existing in the country. Epidemics in 1916, 1925, 1937 and 1948-49 to 1952-53 (Maclean, 1955) showed an upward age-shift; in the 1916 and 1925 epidemics, the majority affected was under 5 years of age; in the 1952-53 outbreak the majority was over 15 years of age.

#### Pacific Islands

There was an epidemic in Guam as early as 1899; previously, poliomyelitis was unknown and the main incidence was between 15 and 50 years of age, (Grunwell, 1900).

In 1929, a polio epidemic occurred in the Solomon Islands, (James, 1938), an area of low natural immunity. In 1946-47, Coxsackie virus infection was described in the Friendly and Cook Islands, (Matheson, 1947). An epidemic of polio occurred in the Gilbert and Ellice Islands in 1952, (Poliomyelitis, 1955).

In Tahiti, few polio cases were noted between 1947 and 1950, but an epidemic occurred in 1951, especially in the 10 to 19 year age-group, the pattern being that of a "virgin soil" territory, (Rosen and Thooris, 1953). A serum-survey in Tahiti and Raiatea after this epidemic, (Horstmann and Kraft, 1955) showed an endemic antibody-pattern to polio 1 and 3 such as is seen in underdeveloped areas. As polio 1 caused the epidemic, it was possible that polio 1 infection had not been known in the area for over 30 years; antibodies to polio 1 were present in the over-30's, who were not infected during the epidemic. Studies in the Society Islands, (Kessel et al, 1956) confirmed this.

In Hawaii, nothing was reported prior to 1922; then sporadic cases occurred annually with increases in 1927, 1931 and 1936. In



1940, an epidemic occurred in which the incidence was lower in those of Filipino, Chinese and Japanese blood than in Caucasians or part-Caucasians and the under-14 year-olds were mostly affected, (Lee, 1941), although cases were scattered over all age-groups.

Between 1938 and 1947, most cases were infantile and "racial" incidence-differences were noted, as above, (Enright, 1948). An epidemic of mixed aetiology - polio, Coxsackie and Echo viruses- occurred in 1958, (Johnson et al, 1960a, 1960b).

### Asia

Poliomyelitis was widespread in Asia.

In Indonesia, cases occurred in the literature showing that, pre-World War 2, the illness was endemic with small outbreaks in cities, especially in children; Chinese, Indonesian and European. From 1947 to 1949, there was an increase in Batavia, especially in older age-groups: possibly the first "epidemic" in the Far East, excluding Australia, (Verhaart, 1949). A 1957-58 survey in Bandung, Java, (Lie-Khing-Ting, 1960), showed polio 1, 2 and 3 antibodies were present in children and adults and clinical polio was rare.

In Singapore, an epidemic of polio occurred in 1958-59, (Hale et al, 1959), and a recurrence in 1960, (Sabin, 1963).

An epidemic occurred in North Vietnam in 1952, with an increase in polio 2 afterwards, (Fam-Ngok-Tkhak, 1961).

Reports from China around 1930 showed that clinical polio was rare in places such as Peking, Tientsin, Shanghai and Seoul and was infantile in distribution when it did occur in the local population, (Zia, 1930). In 1938, it was endemic, rare and infantile in distribution, (Scott, 1938); in 1952, polio 2 was prevalent, epidemics occurring

in the cities in the following years, (Gu, 1960). In 1959-60, aseptic meningitis in Peking, (Ch'iu et al, 1965), had an aetiology of different viruses, including polio, Coxsackie and Echo viruses.

In Taiwan, (Yang et al, 1960) the normal population in the Taipei area showed early infection with enteroviruses; and illness between 1957 and 1959, showed an aetiology of polio, Coxsackie and Echo viruses, (Lee et al, 1961).

In Hong Kong, notifications since 1958 showed paralytic disease in very young children. The faecal-virus "carrier" rate was high under 5 years of age, particularly in the under-2 year-olds, and antibody levels were high, (Chang and Shum, 1962). In the summer of 1960 there was a polio 1 outbreak and later in the year one due to polio 2 infection.

Japan had small epidemics from 1921, with a high endemic rate and increased incidence in urban areas, for example, Kobe-Osaka and Tokyo; age-incidence during 1938-40 was under 5 years of age, (Paul, 1947a). In Tokyo, from 1927 to 1942, polio infection was steady and since 1947 no increase was noted as in Europe and America. 80% was in the under-5 year-olds, as in the West before the age-shift in the late 1930's, (Matsuda et al, 1952).

In general the antibody patterns were as for low socio-economic groups, (Paul et al, 1952a) and paralytic cases were mostly in the 1 to 3 year age-group; the acquisition of polio 1, 2 and 3 immunity was rapid and between 1954-56 there was an increasing isolation of polioviruses, (Kono et al, 1958).

Coxsackie B1 was seen in Japan, associated with pleurodynia, (Yokota, 1955).

In general, the position in China and Japan at the end of the 1940's was equivalent to that in the U.S.A. before 1890 to 1900; possibly small intense outbreaks, rather than widespread epidemics, (Dauer, 1948a) but a transition from endemic to epidemic was occurring, (Paul, 1947a).

Studies in the Philippines, in 1933, (Doull et al, 1935), showed that polio antibodies were present and the clinical disease unknown. In 1952-53, a study in expatriate American families and their local servants, showed that clinical polio occurred frequently in new arrivals with none in the indigenous population, (Hammon et al, 1957a); other enteroviruses circulated in the population, (Hammon et al, 1957a, 1961).

In Ceylon, an increase in poliomyelitis cases was seen after 1948, infantile in distribution and greater in times of high humidity, (De Silva, 1951). In 1962, a polio 1 outbreak occurred all over the island with most cases under 10 years of age, (Arumanayagam and Mendis, 1965).

A polio 1 outbreak occurred in 1957 on the Andaman Islands, of infantile distribution and paralytic; polio was endemic on the islands, (Seal and Gharpure, 1961).

Before 1947, polio was unknown in the Nicobar Islands. Infection, brought from the Andaman Islands affected the islanders in all age-groups: a "virgin soil" outbreak, with massive family infections, (Moses, 1948). Although infection was rife in local Nicobarese and spread to other islands, there was no infection in Indians, (van Loghem, 1949).

Reference has been made to early infections in India, occurring in expatriates, (Shaw, 1823; Ager, 1918; Goodeve, 1879; Birch, 1879).

Between 1913 and 1937, (McAlpine, 1945) no cases were observed in the Army in India, but from 1938 to 1940 cases occurred in British troops. Cases in the indigenous population were mostly in children, rare in adults and seldom epidemic; when outbreaks did occur they were in newcomers, especially officers and mostly from March to October: perhaps relative to the Monsoons.

Outbreaks occurred in Bombay in 1938 and in 1949 and 1952, (Vora, 1949; Bhardwaj and Iyer, 1953; Gharpure, 1954), but cases were generally sporadic. In 1949, in Bombay and other cities there was a wide dissemination of the disease in children under poor sanitary conditions, (Gharpure, 1954), antibodies to polio 1, 2 and 3 being present in those over 9 years of age. The 1949 outbreak was infantile in distribution, (Vora, 1949); the 1952 one was also infantile but, at its peak, showed a distribution in older age-groups, (Bhardwaj and Iyer, 1953).

In healthy people, in Bombay, all enterovirus groups were excreted, mostly in young children and in greatest amount in the monsoon months, (Meherhomji and Gharpure, 1961). C.N.S. infection studies in children under 15 years of age, in Ahmedabad, in 1959-60, linked all types of enteroviruses with different clinical types of disease, (Jhala, 1962); aseptic meningitis, polio, encephalitis, etc. More clinical cases were seen with an older age-incidence: a possible change from endemic to epidemic pattern.

Coxsackie outbreaks occurred in some areas of Asia. In 1946, there was an epidemic of Bornholm disease amongst European Naval ratings in Singapore, (Smith, 1947), followed some weeks later by an outbreak in Aden, mostly affecting Europeans, officers more than ranks with noticeable family infections, (Jamieson and Prinsley, 1947).

In 1947-48, an epidemic of Bornholm disease occurred in South India affecting Europeans and only 2 Indians, (Hamburger and McNeil, 1947). In the early 1950's, a possible outbreak occurred in New Guinea in Europeans, showing four different clinical types, (Gunther, 1952).

### Africa

Africa has shown a changing epidemiological pattern, varying in different regions, according to socio-economic pattern, racial make-up and degree of contact with the outside world; sporadic cases and local outbreaks occurred in most areas, being seen first in parts of tropical Africa around World War 2.

The earliest-noted epidemic related to the African continent, and already referred to, occurred in 1836 in the island of St. Helena, (Bell, 1836), and was followed over a hundred years later, in 1945-46 by a second, (Nissen, 1947). The island had a mixed population of East Indians, West Africans and British, and was overcrowded. The infection of the latter outbreak may have spread from South Africa, as a ship from South Africa, where an epidemic was rampant in 1945, visited the island prior to the onset of infection. The infection affected mostly the 5 to 19 year-olds, with little effect on the under-5's; the higher age-incidence was in keeping with a "virgin soil" outbreak, with an increase of paralysis and mortality in older persons, (Burnet, 1952; Poliomyelitis, 1955).

In Madagascar, there was a steady increase after 1924, (Fontoyant and Raharijaona, 1930), with rare sporadic cases in infants and overt illness showing mainly in the expatriate adult population, (Sureau, 1959). In 1952, an increase in polio was noted, (Epid. and Vit. Stat. 1953) and in the 1960's, polio and Coxsackie viruses gave rise to out-



breaks, (Sureau and Kauffman, 1965), with an increase in epidemics due to Coxsackie A and B viruses. This might be due to a shift in immunity, resulting in a change from polio to Coxsackie infections.

Large epidemics occurred in Mauritius in 1945, 1949 and 1952, (Poliomyelitis, 1955). The 1945 epidemic followed a cyclone, with most of the cases in the under-10 year-olds; the Chinese population showing a larger incidence than the Indian, and families a greater incidence than the general population, (McFarlan, 1946). Distribution was infantile following on basic endemic polio, and the situation was compared with the New York outbreak in 1916 and that in Malta in 1943-43, (McFarlan et al, 1946).

In 1950, the polio incidence was down, in Mauritius, (Epid. and Vit.Stat.Rep., 1951) but rose in 1952, (Epid. and Vit.Stat.Rep., 1953) and in 1959 there was another polio 1 epidemic, still with an infantile distribution, (Teelock, 1960). La Reunion Island had an outbreak in 1949, (Poliomyelitis, 1955).

During World War 2, there was a high morbidity rate in Egypt and the Sudan amongst New Zealand troops, with a high non-paralytic rate, (Caughey and Porteous, 1946). In Cairo in the early 1950's, (Paul et al, 1952b), polio 1, 2 and 3 antibodies were present by the age of 4 years in 70% to 100%; the disease was endemic in Egypt and in the vicinity of Cairo was "paralytic infantile" polio.

In the Cairo vicinity, (Melnick and Agren, 1952), viruses were isolated from normal infants; including polio, Coxsackie viruses and, at that time, unidentified cytopathogenic agents. In Cairo, in 1957-58, (Akers et al, 1960) cases of paralytic polio were mostly due to polio 1, 2 and 3, but other enteroviruses were involved. In 1955, the proportion



of incidence of polio 1, 2 and 3 was different and virus isolations took place all the year. The endemicity was high, 90% of the paralytic cases being in infants and young children and 100% immunity being acquired by 5 years or so, (El-Messih, 1960). Two seasonal peaks occurred, one between April and June and the second in September. A polio antibody-survey in Cairo, (Imam et al, 1962) still showed an "infantile" distribution, but paralytic polio increased in urban regions and spread to older children, suggesting vaccination might be extended to those of 5 years and over. There was a shift in virus types over the years.

Khartoum had an epidemic in 1959, with a peak in August mainly in young children and to a large extent paralytic, (Hassan and Haseeb, 1960). Polio, Coxsackie and unknown enteroviruses were isolated; the disease was endemic, this being the first epidemic in this area: possibly an imminent change from endemic to epidemic pattern.

Infection in Ethiopia was endemic, as shown by serological studies in 1960 in Asmara, (Garcia-Gancedo et al, 1961) and by the absence of clinical cases in the area. The pattern was possibly similar in Somaliland and in the Seychelles Islands; these areas showed an increased incidence in 1952, (Epid. and Vit.Stat.Rep., 1953).

The incidence of polio rose in Kenya, Uganda and other parts of Africa after 1950, (Laufer, 1958). It fell in Tanganyika in 1950, (Epid. and Vit. Stat.Rep., 1951); rose in Uganda in 1951, (Freyche, 1952), and again in Uganda, Tanganyika and Kenya in 1952, (Epid. and Vit.Stat.Rep., 1953).

In Kenya, there was an increase since the early 1940's, (Polio-myelitis, 1955) particularly from 1944, (Fendall, 1960). From late

1953 to 1955, there was a major epidemic of polio 1, with many cases in 1954; it affected European adults, especially expatriate immigrants, and African and Asian children, (Walker, 1956) and the importance of the African servant was noted in the spread of infection.

Epidemics attacking European adults, Asians under 5 years of age and Africans under 3 to 5 years of age, (Fendall and Lake, 1958) showed a potential danger for a European community and the possible advisability of vaccination of European adults and children, African children under 2 to 3 years of age and Asians under 5 years of age. A further epidemic in 1957 stressed this necessity, (Fendall, 1960). This vaccination was undertaken, with killed-virus vaccine, but had defects as it was not effective against non-paralytic infection: possibly as this may have been due to non-polio viruses. And so, in Kenya, polio, apparently originally sporadic, (Fendall, 1962), became epidemic from 1954, occurring in 3-year cycles, because of the accumulation of susceptible infants: cf. in Jamaica, (Charles and Grant, 1962).

Between 1954 and 1960, the incidence in Europeans fell but that in Africans rose and from 1957 to 1960 that in Asians increased also. In 1960 most cases (83%) were in Asians and Africans and infantile in distribution: 93 to 97% being under 5 years of age.

The aggregation of populations in towns led to increasing epidemics there, and spread was from urban to rural areas, (Fendall, 1962). As a result of the shift from endemicity to epidemicity, with 3-yearly outbreaks, Sabin oral vaccine was used in 1962-63 and apparently prevented the expected 1963 epidemic, (Fendall and Grounds, 1965b).

In 1954, an outbreak, "infantile" and "paralytic" in nature, occurred in Tanganyika, in the local population and possibly due to

an incoming strain to which there was little immunity, as there were no improved hygienic conditions, (Kerr and Pease, 1956). A further epidemic of polio 1 followed in 1956 in West Tanganyika, in those under 5 years of age, (Laufer, 1958). This was a marked increase in epidemicity; possibly the "tropical endemicity" of polio was changing and giving way in places to epidemics as in the Western World, 80 to 90 years previously.

In 1940, in the Belgian Congo, an epidemic occurred in Europeans and Africans, (van Hoof, 1940), infection probably coming from the Middle East, as in the case of outbreaks in Northern Rhodesia, (vide infra), (Gear, 1948b). A further increase was noted after 1945, (Poliomyelitis, 1955); between 1935 and 1949, 397 cases, with 29 deaths, occurred in Africans, (Lebrun et al, 1959). The increased incidence was more noticeable in 1950, (Epid. and Vit.Stat.Rep., 1951), and major outbreaks occurred in 1951, (Poliomyelitis, 1955; Freyche, 1952), and in 1952 (Poliomyelitis, 1955).

From 1950 to 1958, 6,233 cases, with 331 deaths, occurred in Africans, (Lebrun et al, 1959). In Leopoldville, most European cases were in adults and the possibility was entertained of inoculating European adults and indigenous children under 2 years of age, (Lebrun, 1956).

Enteroviruses, especially polioviruses, were endemic in Leopoldville children, (Vandeputte, 1960), and increased after 1951, (Lebrun et al, 1960) in Africans under 5 years of age; antibody surveys showed that a large number under 3 years were negative to one or more types, (Lebrun et al, 1960). Therefore, it was decided to protect Africans under 3 years of age with oral vaccine. This was followed 2 months

later by a polio 1 epidemic: low conversion possibly being due to interference from other viruses in the population, (Plotkin et al, 1960a).

In 1954-55, in Elizabethville, during the rainy season, an outbreak of polio 1 with Coxsackie A, occurred; rare in Africans, except under 2 years of age, but affecting Europeans of all ages, (Delville and Pattyn, 1957). Between 1957 and 1959, in Elizabethville, 50% of healthy children excreted viruses, with a high rate at all times of the year, an important fact in oral-vaccine campaigns, (Pattyn et al, 1960).

French Equatorial Africa had an increased incidence in 1951, (Freyche, 1952), infection probably having spread from the Belgian Congo, (Poliomyelitis, 1955). There were previous polio cases in 1943, in Brazzaville, (Poliomyelitis, 1943). Serological studies in the city showed immunity was acquired at an early age: between 5 and 15 years, although no clinical disease was seen in Africans, (Pellisier et al, 1954).

The incidence in Angola increased from the 1940's and an outbreak occurred in 1951, possibly spread from the Belgian Congo, followed by an increased incidence in 1952 and 1953, (Poliomyelitis, 1955; Freyche, 1952).

Clinical polio was rare in Northern Rhodesia, an early outbreak occurring in 1943-44 in Europeans and Africans in Nkana, (Gear and Rodger, 1946), "on the overland route from South Africa to the Middle East theatre of War", (Gear, 1948b). Infection may have entered from the Middle East, but there was little spread, possibly due to the fact that there were no school-children travelling at the time. This was

followed by an outbreak at the Roan Antelope Company at Luanshya in 1946 which spread to Lusaka and Nkana. It was severe and first occurred in houses with no water-borne sewage; spread was related to cases and their families; the part played by indirect contacts was very important, and apparently infection was present in Luanshya before the first case, (Gear and Rodger, 1946).

In Southern Rhodesia there was a high endemicity (Poliomyelitis, 1955) but an increase in cases was noted in 1951, as in other areas, (Freyche, 1952) and in 1954-55 an epidemic of polio 1 occurred in Europeans and Africans. Older age-groups were affected in Europeans than in Africans, with distribution of infection over all ages in Europeans but confined to an infantile distribution in Africans, (Kotze, 1955).

A further outbreak occurred in 1957-58, in which African mortality increased, distribution in Africans moved into a higher age-group and non-paralytic disease was rare in Africans, (Blair, 1959).

Studies in South Africa were more extensive than in other parts of the continent and, with the racially-mixed population, of great epidemiological interest; studies there, as in the U.S.A., have thrown light on many epidemiological and other factors. Epidemics were not known there before the 1st World War, (Gear, 1946); the first outbreak was in 1917-18, with localised outbreaks almost yearly thereafter. The endemic years were followed by epidemics in 1918, 1944-45 and 1948, in which attacks in Europeans were greater than in Africans, (Gear, 1948a).

During the 2nd World War, the disease became more prevalent, (Gear, 1946) and in 1944-45 a polio epidemic occurred affecting mostly Europeans under 10 year of age, (Henson and Port, 1945), with later spread



to Mauritius, Madagascar and St. Helena, (Gear, 1946). The infection may have come from the Middle East, as in the case of the Northern Rhodesian outbreak of 1946, (Gear, 1948b). The 1944 Durban epidemic may have originated in the Middle East or the U.S.A. There were few cases in native territory, European cases numbering ten times the African, being mostly in children of 1 to 4 years of age and in older people.

In 1945, during an outbreak in Johannesburg, (Gear and Mundel, 1946), virus was isolated in the local South African vervet monkey (*Cercopithecus aethiops pygerythrus*), from faeces and sewage; the importance of multiple cases in families and personal contact, and the possibility of a large number of symptomless "carriers" were realised, (Gear, 1946; Gear et al, 1946). Virus was isolated from sewage purification works, (Mundel et al, 1946).

Serological surveys on polio 2 showed that the African adult was highly immune and the European adult immune, but less so. This pertained in all ages, the African being more immune than the European, with more cases in Europeans but more infection in Africans, and silent cases in the latter. Children excreted virus where no cases were noted; in 1946, virus was in the sewage in "summer" but not in "winter", was absent in a non-epidemic year (1946-47), but reappeared during the 1948 epidemic, (Gear, 1948a).

Silent, immunity-giving, infection was seen in Bantus in the 1944, 1945 and 1948 epidemics and by 6 years of age, Bantus had antibodies; Europeans had more cases than Bantus and infection in the 5 to 10 year-old group was equal to that in the 0 to 5 year-old group, in the case of the former, whereas infection in the Bantu 0 to 5 year-old group



was greater, showing the "racial" socio-economic immunity difference and age-shift.

In non-epidemic years, although there were no illnesses, Bantu infants excreted viruses in the faeces, (Gear et al, 1951). Healthy Bantu infants excreted Coxsackie viruses, which persisted at least a month in an apparently symptomless epidemic, (Measroch et al, 1951).

Although Bantus had universal infection over 6 years of age, about one third of Europeans, both in rural and urban areas, were without immunity and with a drop in the I.M.R., the likelihood of epidemics increased, (Poliomyelitis, 1954).

In 1956, (Gear, 1957b), epidemics occurred with an increased incidence in Africans, although antibodies in Africans were still greater than those in Europeans, (Gear, 1958a); possibly this was the beginning of epidemic waves, as in the northern hemisphere, and a change in epidemiology, due to a reduced basic immunity following on improved hygiene, and an increase in recently circulating virulent strains.

Polio 1, 2 and 3 were prevalent in South Africa, different types occurring at different times and places, and in the same place in the same season, succeeding waves of infection being due to each of the 3 types in turn, (Gear, 1957b).

Epidemics occurred after heavy rains and children were reservoirs of infection. Polio 1 caused epidemics since 1956, but polio 2 and 3 were present in smaller outbreaks, (Gear, 1958a).

The Coxsackie viruses were seen in South Africa, (Gear, 1958b). They were isolated from cardiac infections in newborn babies; part of an apparent increase in myocarditis in children since 1941.

In 1960-61, Coxsackie B1, was first isolated in relation to an

increase in epidemic pleurodynia, aseptic meningitis and neonatal myocarditis, suggesting a lack of immunity, and the possible necessity of future vaccination against Coxsackie viruses, (Gear, 1961-62).

#### North Africa

Between 1921 and 1941, there were only 61 cases of polio in Malta, (Seddon et al, 1945). This was followed in 1942 by an epidemic; the first cases were in members of the Armed Services but eventually 82% were in the under 5 year-olds and most cases, paralytic. Many cases occurred in Malta and Gozo, almost all under 5 years of age, (Agius et al, 1945), and no Maltese troops and few Maltese adults were affected, (Bernstein et al, 1945).

Other outbreaks occurred in 1945-46 and 1950-51, (Poliomyelitis, 1955). During the later epidemics there were no local-population cases over 5 years and all adult cases occurred in expatriate Service personnel, (Galea, 1953).

Polio increased in Tripoli, Libya, in 1952, (Epid. and Vit.Stat. Rep., 1953) and in French North Africa in 1950, (Epid. and Vit.Stat. Rep., 1951). It was endemic and infantile in Tunisia, with no epidemic rise in the 10 years to 1956-57, (Didier, 1957). In Algiers, since 1955, the disease was sporadic, but with scattered mild outbreaks, with age-incidence under 5 years of age, (Massonnat et al, 1957). This slight increase in the 2 to 3 years prior to 1957 was a possible movement into a pre-epidemic phase, necessitating vaccination, (Massonnat et al, 1957).

In Morocco, (Paul and Horstmann, 1955), clinical incidence in children was rare; most cases were in Europeans with a higher age-incidence; early and almost universal infection with polio virus occurred after the disappearance of maternal antibodies; and adult infec-

tion and reinfection were rare, (Paul and Horstmann, 1955).

### West Africa

Studies in the Cape Verde Islands, (Portuguese West Africa), showed a high incidence of polio 1, 2 and 3, especially in children, with the possible occurrence of a polio 1 outbreak, (De Sousa, 1960).

In Dakar, (French West Africa, (Senegal)), immunity to polio 1, 2 and 3 was acquired early, typical of the tropical situation, (Said et al, 1959).

An epidemic of considerable size, with 357 notified cases, was reported from the French Cameroons as early as 1936, (Poliomyelitis, 1955). There was no recorded polio epidemic in other parts of West Africa (Findlay et al, 1946), but the disease was known to the indigenous population, clinical evidence was present, and infection was widespread in children and rare in adults, (Turner, 1932).

During World War 2, cases in British troops were more frequent than those in West African troops, more severe, generally paralytic and often fatal; and, as in India, (McAlpine, 1945), officers were more often affected than men.

The majority of cases occurred during the "rains": i.e. March to October, in keeping with its being considered a faeces-spread disease.

West African monkeys were susceptible to poliovirus infection (Papio papio, cercopithecus aethiops centralis, cercopithecus mona roloway, cercocobus torquatus, erythrocebus patas).

Sera from West Africans neutralised polio 2 virus (Findlay et al, 1946); and serological studies in healthy people of British West African territories, aged 1 to 40 years of age, all showed antibodies to polio 2, (Olitsky and Findlay, 1946), suggesting widespread infection.

In Liberia, in the early 1930's, polio antibody was found in members of scattered inland tribes who had little contact with Europeans or Americans, (Hudson and Lennette, 1933); and mention was made of clinical disease in Nigeria, the Gold Coast, the Belgian Congo and Angola, (League of Nat.Epid.Rep., 1924-33).

Antibody surveys, made more than 20 years later in Liberia, (Gelfand and Miller, 1956; Kalter, 1962), showed 90% had antibodies to polio 1, 2 and 3 by the age of 5 to 6 years of age, that infection was endemic and that vaccination was advisable for expatriates, (Gelfand and Miller, 1956).

### Nigeria

Hudson and Lennette, (Hudson and Lennette, 1933) quoted A.B. Aitken as having treated 2 cases of polio in Europeans in Nigeria, but he made no observation of acute attacks in Nigerians. They quoted W.B. Johnson, (Director of Medical and Sanitary Services of Nigeria) as recording acute polio in Europeans and Africans in Nigeria, as follows:

	1927	1928	1929	1930	1931
European	1	0	1	1	0
African	26	23	34	8	10

and that, at that time, there was an almost complete exclusion of white children from West Africa.

In 1925, the I.M.R. in Lagos, then a town of 109,076 inhabitants, was 238/1000 live births, (Infant Mortality and Infant Welfare and Maternity work in Tropics), deaths in children under 5 years of age accounting for 41% of all mortality.

Sporadic cases occurred. In 1960, there was a case of severe polio in a European in Northern Nigeria, but the disease was almost

unknown in the adult West African, though possibly there was widespread infection in children and advice on immunisation was given to expatriates, (Scarborough, 1960).

By 1961, the I.M.R. in Lagos, then with a population around 600,000, was about 80/1000 live births and peak polio in nearby Ibadan was in the local population of up to 2 years of age, usually in the limbs, and after that, virtually unknown, (Dr.C.M.Norman-Williams, at W.A.C.M.R. Conference, 1961).

In 1961, (Collis et al, 1961) the polio incidence throughout the country for all ages, was estimated at 100,000; in 1965, this was put at nearer 200,000 to 300,000, (Collis, quoted in Huckstep, 1965).

In 1961-62, a trial of polio 1 oral vaccine was carried out in Ibadan, giving a low conversion rate, possibly due to interference from other enteroviruses, (Montefiore et al, 1963).

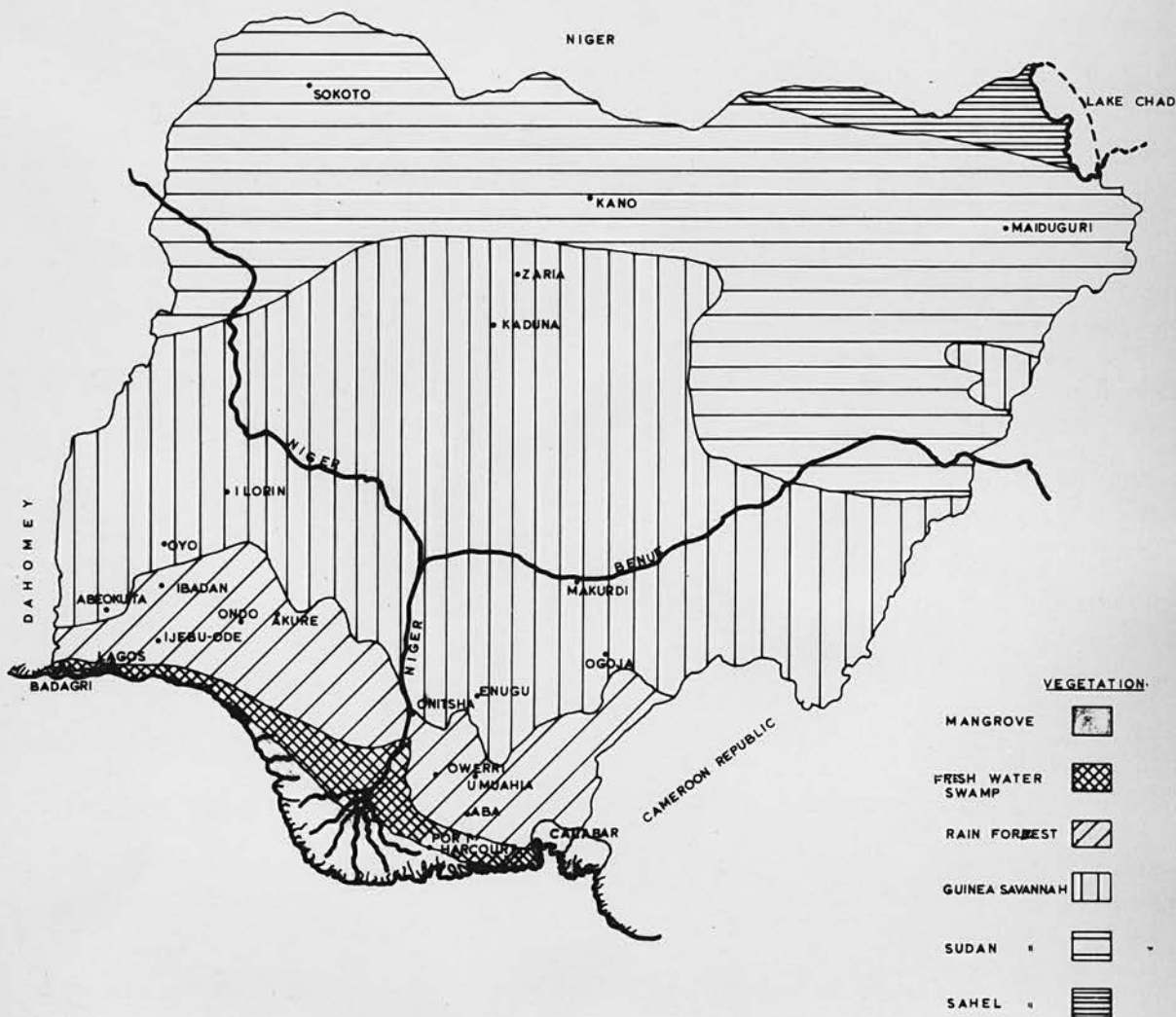
In 1946, an outbreak of epidemic myalgia occurred in West Africa, (Goldstone and LeMarquand, 1946), presenting as "acute abdomen" and possibly due to a Cocksackie infection, (cf.Hamburger and McNeil, 1947).

Map of Nigeria



Map of Nigeria

(Based on maps of Survey Department, Lagos, Nigeria)



## Background to the study

### 2. Nigeria

Nigeria lies between latitude  $4^{\circ}$  and  $14^{\circ}$  North and longitude  $3^{\circ}$  and  $15^{\circ}$  East, covers an area of 356,669 square miles and stretches over 700 miles from east to west and over 600 miles from north to south. It is bound on the south by over 500 miles of Atlantic coastline, on the west by Dahomey, on the north by Niger and on the east by the Cameroon Republic.

#### Vegetation

The coastal strip, from 3 to 60 miles wide, which has no elevation, consists of mangrove swamps, (*Rizophora racemosa*), with sandbanks up to several miles wide, creeks and lagoons, open to navigation but often choked with sudd, (*Pistia stratiotes*).

This strip merges with fresh-water swamps and the tropical rain forest belt, 50 to 100 miles wide, of evergreens and oil palm (*Elaeis guineensis*) and more undulating ground, interlaced with "bush paths" but few roads.

The forests thin out at a line through Abeokuta, Ondo, Onitsha and Afikpo; the countryside becomes park-like with deciduous forest (guinea savannah) and hills north of Ondo. North of this lies an undulating plateau, about 2000 feet above-sea-level, rising to the Bauchi Plateau, which reaches 6000 to 7000 feet.

North of Zaria, the country opens out (Sudan savannah) decreasing in altitude and becoming sandier with the encroaching desert, (Sahel savannah).

The study area in Lagos lies in territory made up of mangrove and fresh-water swamp on the lagoon side, with adjoining rain-forest

on the landward side.

### Geology

Detailed descriptions are given in specialised works, (Buchanan and Pugh, 1955; Maps of Nigerian Survey Department).

In general, the land consists of crystalline rocks, chiefly granites, often overlain by sandstones, which may be the same age as the Congo basin, (Talbot, 1926, who quotes information from former Mineral Survey (Nigeria) and papers by A.E.Kitson and J.Parkinson); mainly Archaean or Palaeozoic and upper Cretaceous sedimentary on the Coast and centrally along the Niger River. The region is possibly a continuation of rocks in Brazil extending to Arabia and South India: the continent of Gondwanaland, (Talbot, 1926).

The Lagos region consists of (a) recent Pleistocene River alluvium and Delta deposits on the coast and (b) Tertiary Eocene rocks on the landward side.

### Climate

The climate of Nigeria is tropical, varying in different areas with the physical features of the country, (Buchanan and Pugh, 1955; Maps of the Nigerian Survey Department.)

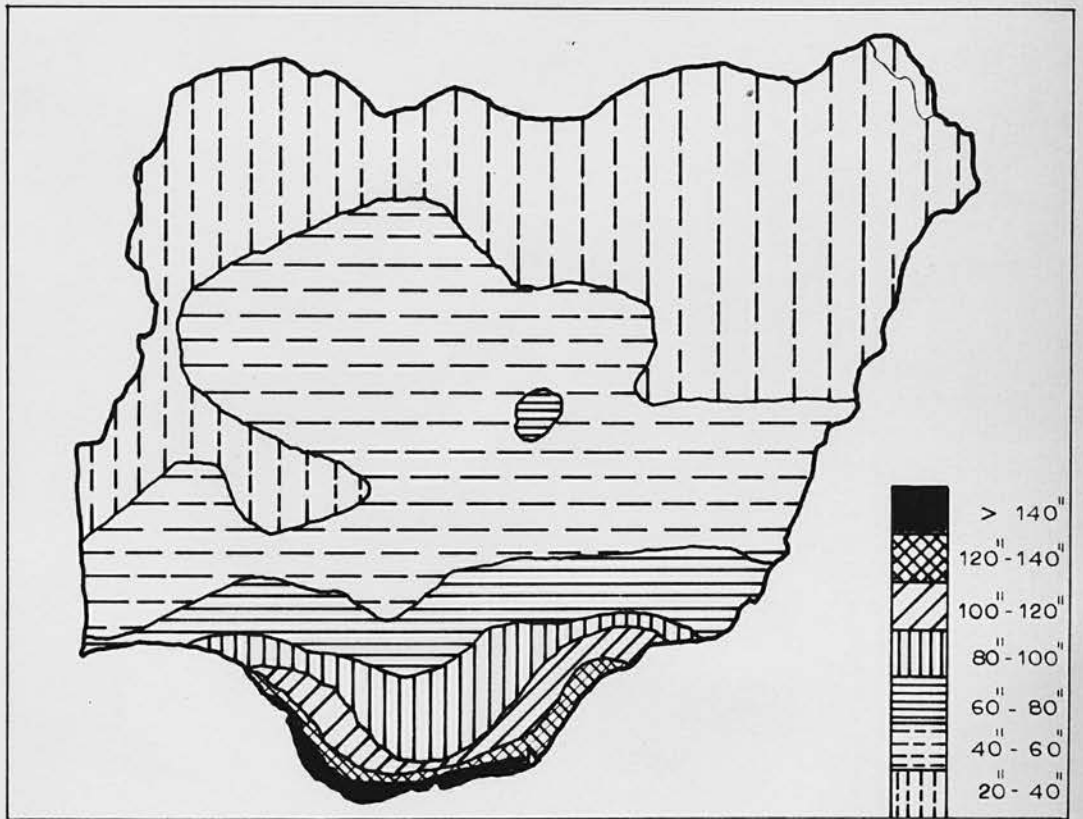
In the Lagos area, the Dry season lasts from November to March, the Harmattan wind blowing from the north-east during the middle of this period. Tornadoes occur before and after the rains, South-west Monsoons, which come between March and October, with a break in August. The mean annual rainfall in Lagos is 70 to 75 inches; elsewhere, it varies from 20 to 30 inches in the north to more than 160 inches in the Delta region and east.

In the south the lowest mean temperature is in July and August;

Map of Nigeria - Rainfall - Annual total

Map of Nigeria - Rainfall - Annual total

(from Buchanan and Pugh, 1955)

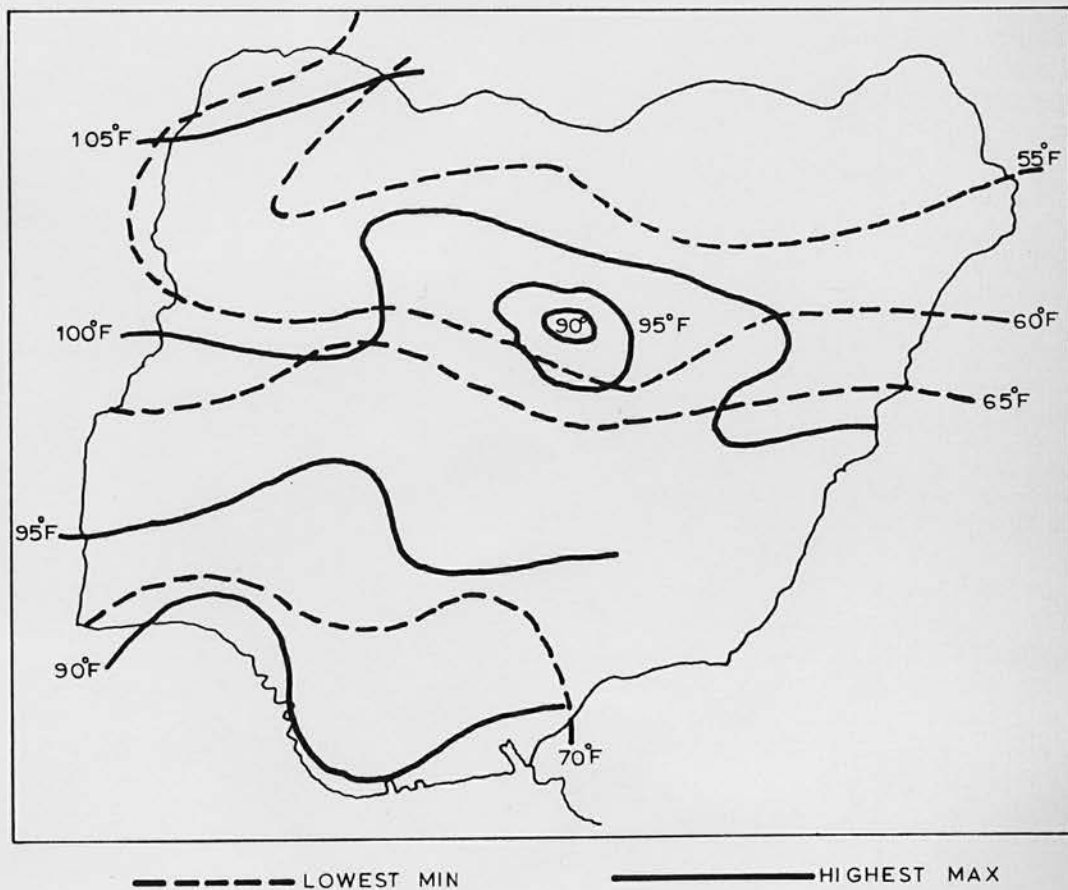


Map of Nigeria - Temperatures - (monthly means)



Map of Nigeria - Temperatures - (monthly means)

(from Buchanan and Pugh, 1955)



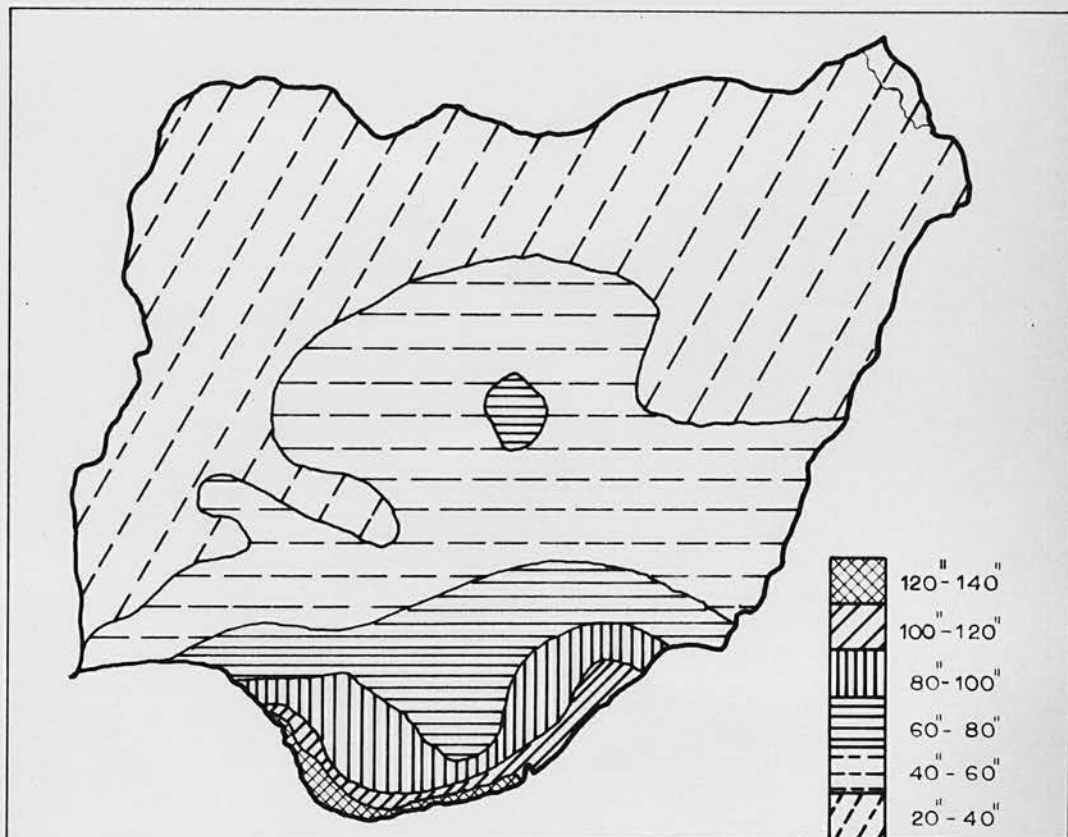
Map of Nigeria - Rainfall

- (a) Wet season - April to October inclusive
  
- (b) Dry season - November to March inclusive

Map of Nigeria - Rainfall

(a) Wet season - April to October inclusive

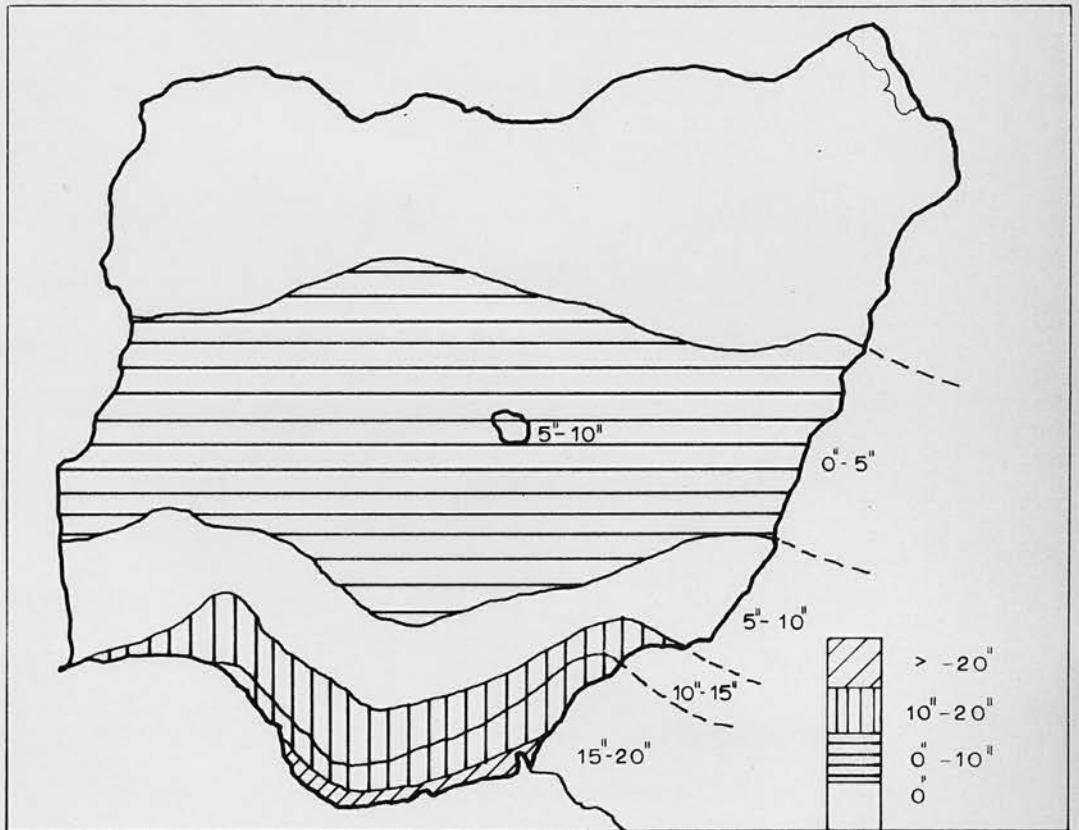
(from Buchanan and Pugh, 1955)



Map of Nigeria - Rainfall

(b) Dry season - November to March inclusive

(from Buchanan and Pugh, 1955)



the lowest minimum in December and January; the highest mean and maximum temperatures in March and April.

The annual mean temperature in Lagos is about 80°F, with an absolute minimum of about 60°F and absolute maximum of about 95°F. The relative humidity is high, reaching 95% in the Lagos area, with an overall average of 80%.

#### History and social background

Darwin believed that Africa was the possible birthplace of mankind. A considerable proportion of southern Nigerians are yellow-skinned and Mongoloid eyes and cheekbones are occasionally seen, possibly due to Cro Magnon influence, (Talbot, 1926).

Nigeria contains a great diversity of tribes, on a linguistic, rather than an anthropological, basis; southern Nigeria being the meeting-place of three principal African race-groups, the Bantu, Semi-Bantu and Sudanese Negroes. The Ijaws, one of the most ancient Sudanese tribes and possibly remnants of the original people of Nigeria, are still seen on the coastal Creeks and Delta regions.

The Semi-Bantu, influenced by contact with the Brown Mediterranean race, consist of the Ibibio, Ekoi, Boki and other tribes in the eastern part of the country; the Ibibio, possibly the most ancient descendants of the Semi-Bantu, inhabit mainly the Cross River area and "pockets" further north.

The Bantu live in the extreme southeast and Cameroon Republic. The Semi-Bantu and Bantu tongues developed in or near Nigeria, and the people were probably driven south by the increasing aridity of the Sahara region. Other Sudanic people came later, around 7000 BC, and the Edo, Ewe, Ibo and Yoruba tribes around 2000 BC, (Talbot, 1926).

Nigeria is an agricultural country, and may have been the centre of the first agriculture in West Africa, but many of the people live in towns, especially in the south-west. The population density varies, being greatest between the Niger and Cross Rivers, the reason for which is unknown.

The north is peopled by over 230 tribes. The most important are the Hausa, a Sudanic people; the pastoral or "cow Fulani" (Borroroje), a Berber-Negroid Hamitic people; the "Town Fulani" (Fulani Gidda), a Hamitic people, mixed with the Negro Hausa; the Kanuri, who inhabit the lands from the Nile Valley to the Chad; the Shuwa Arabs, in the Lake Chad area; a mixture of Berber, Bantu and Nilotic strains; and many other Sudanic and Semi-Bantu tribes, (Meek, 1925).

The north had a separate development from the Coastal areas, with a tribal influx from the northern and north-eastern desert regions. The indigenous primitive peoples were driven to the hill areas, where they can still be found: for example on the Bauchi Plateau. Local dynasties or Emirates grew up. The people were originally pagan, or may have had a form of Christianity, before Mohammedanism reached the Hausa in the 15th century, by way of the Niger Valley.

The west is peopled mainly by the Yoruba, in clans or kingdoms: Oyo, Egba, Ife, Ijebu, Owu, Ijesha, Ekiti, Ondo. The indigenous inhabitants of Lagos are of Yoruba origin with sub-tribes of Eko, Egba, Awori and Ijebu. They may have reached the area 1000 years ago and spread over to Dahomey, where the "Amazons" were Yoruba.

The origin of these Sudanic people is unknown, (Johnson and Johnson, 1921), but they may have come from the north-east, starting the migration of Bantu-speaking people across all Africa. In Nigeria,



the Stone Age and Iron Age appear to exist simultaneously in different areas, (Talbot, 1926). The only evidence of a Bronze Age was seen in connection with the Yoruba in Benin and Ife, although this may have been later Arab and Portuguese influences in these regions.

Further east, the Ibo inhabit the lands east of the Niger River and south of the Benue River and Asaba. They have little tribal cohesion, living in independent villages and speaking different dialects. Also in this area are the Ibibio, Ekoi, Efik, Kwa, Ogoni, Kalabari and many others. North of Calabar are empty uninhabited areas.

Between the Niger and Benue Rivers live the Igara, Okpoto, Tiv and, bordering on the Bauchi Plateau, many pagan tribes, including the Gwari, Nupe and Borgu, (Meek, 1925).

Trading was carried on with North Africa from early times and cultural influences of this area are seen in the Ekoi, Ibo, Ibibio, Yoruba and Bini, (Talbot, 1926). Owing to the tribal movements from the north-east, indirect trade must have occurred with Egypt.

The history of Nigeria was closely connected with trade: in goods and in people, within and outside Africa. The Phoenicians and Carthaginians traded along the Coast, perhaps as early as 600 to 500 BC, (Talbot, 1926). Between 500 and 400 BC the fluvial system from the Senegal River to the Bahr-al-Ghazal, east of Lake Chad, was known to the Greeks and, around 100 AD, to the Romans.

The Fulani moved into the north-west of the country around 320 AD. In the 8th and 9th centuries AD invasions from the north came, via Borgu and Nupe, into the Yoruba country, the invaders later setting up the ruling dynasties. By 1000 AD, Arab conquests in North Africa started tribal movements southwards, resulting in further tribal wars.

All this resulted in great movements of people, not only out of Nigeria, but into the country. As well as the indigenous population, account must be taken of people of African descent returning, in the last century or so, from overseas; for example, Brazilians of Yoruba background; those from Sierra Leone (Creoles); ~~E~~mancipados from Havana, Cuba; and Liberians, particularly in Lagos, (Talbot, 1926; Thorp, 1956). Co-existent with this may have been a movement of disease; the indigenous diseases and unhealthy climate led to the naming of the region: "the White Man's Grave".

The Portuguese reached southern Nigeria in 1472 and visited the city of Benin in 1485. By 1516, the slave trade to America was in full force with an estimated 10,000 to 20,000 slaves a year passing through Lisbon. In 1588, a Portuguese trading station existed at Lagos.

By the 17th century Portugal became more interested in trade in India, and England, France, Spain, Denmark, Holland and others took their place in trade with Nigeria. By 1631, England was involved in the slave trade, which was supplied by the continual tribal wars being carried on in the interior; the notorious "Slave Coast" was Dahomey and Nigeria. There was a calculated possible loss to Nigeria of 20 million people and progeny by the slave trade, (Niven, 1967).

Trading eventually necessitated the appointment of British Consuls to look after interests, backed by the British Naval Squadron in the Bights of Benin and Biafra. One of these Consuls, in 1861, was Richard <sup>B</sup>urton, who served as Consul of the <sup>B</sup>ight of Biafra, (Niven, 1967).

Although slavery was made illegal in Britain in 1807, it flourished on the Coast until at least the middle of the 19th century.

In 1815, Lagos was a large slave-port and there was a slave-market there until 1861.

The southward movement of the Fulani led to the disruption of the Yoruba towns, including Ibadan, and the formation of a refugee settlement, Abeokuta, ("refuge on the rocks"), (Johnson and Johnson, 1921). Local wars continued in Yorubaland, largely due to French influence, and supplied an increase in slave-trading in Ouidah, in the contiguous French territory of Dahomey, (Talbot, 1926). Lagos was captured by the British in 1851; in the period 1858-59, slave-trading increased near Lagos, and nearby Badagri became an important slave-port. In 1861, the port and island of Lagos were handed over to Britain in perpetuity, by Treaty, (Niven, 1967).

The history of the eastern part of the country was bound up with trade on the Niger River, the Delta and surrounding country; largely in palm-oil, and, more recently, in petroleum.

Europeans first settled at the coastal Calabar, Brass, Bonny and Opobo. Missions, of different denominations and nationalities, played an important part in the development of the area; one of the earliest, set up in 1846, was the Church of Scotland Mission under the Rev. Hope Waddell, in Calabar.

1841 saw the opening-up of the Niger regions; and Onitsha, 200 miles from the sea, was a town with over a quarter of a million people, mostly Ibo, (Niven, 1967).

The western part of the country had a less difficult terrain than the east. Missions again played a large part in the opening-up of the hinterland; early ones were set up in 1842 at Abeokuta and in 1850 in Ibadan. But the Yoruba wars of the 19th century resulted in

years of unrest, the full story of which can be found in historical works, (Burns, 1963; Niven, 1967).

In 1965, political differences of opinion led to riots in the northern suburbs of Lagos, in which area most of the population in the present study lived.

The history and development of the northern part of the country was linked with the Army and the Royal Niger Company. The former, the West African Frontier Force, under Lugard, was based at Lokoja, at the junction of the Niger and Benue Rivers, and its Army Medical Corps later formed the basis of the Government Medical Service in Nigeria, (Schram, 1966). Early in the 20th century, the Railways played a large part in the opening-up of all the country.

In general, much of the transport is by foot between farms in the "bush" and the market towns. Most food is locally produced, even for consumption in large towns; the staple crops are guinea corn or millet and ground nuts in the dry north, and the root crops of cassava and yams in the damp south, (Niven, 1967). Standards of food and nutrition are low with semi-starvation or relative starvation in some areas, taking very little to upset the balance, (Simmons et al, 1951).

#### Lagos and environs

Lagos Island lies at the mouth of the Ogun River, and is 5 miles long by 1 mile wide, with the densest population at the western end and the Government "reservation" at the eastern end. The population, of between 600,000 and 700,000 (in 1965) expanded northwards to take in communities on the Mainland, including Ebute Metta and Yaba; and the City environs stretch more than ten miles inland.

Yorubas originally settled at Isheri, on the Ogun River, about

12 miles from Lagos, later moving to Ebute Metta, where they built a town and farmed. They then moved to Iddo Island, which was safer, and then to Lagos Island. The area was sparsely populated, possibly by the Awori sub-tribe of the Yoruba and by the Ijebu. On the coast were the Edo and Popo Ewe, (Talbot, 1926; Niven, 1967).

There was an influx of Bini from Benin, bringing their cultural influence; and, around 1450, the area was under the Benin Army. The Portuguese arrived in 1472 and the first known British contact occurred when James Welsh landed in 1591, (Talbot, 1926; Thorp, 1956).

Originally a swampy island, by about 1550 the bush was cleared and houses built. The town, at first known as Ile Eko, was re-named Lago di Kuramo by the Portuguese, after a small village which still exists to the east of the town. By 1660, gradual colonisation took place in the Lagos area: by Yoruba hunters, Awori fishermen and Ijebu.

In the 1700's, the nearby areas, Ouidah in Dahomey, and Badagri, expanded from the slave-trade, and Badagri was divided into French, Spanish, Portuguese and English quarters. As previously mentioned, local wars were in full swing. By 1838-39, Sierra Leone slaves of Yoruba origin, from Freetown, returned to Badagri and Abeokuta, in the Lagos hinterland.

In 1845, the Rev. Gollmer of the Church Missionary Society arrived at Badagri from Sierra Leone. He became known as "Alapako" ("owner of the boards, or house") because he arrived with two frame-houses in sections: possibly the first pre-fabricated dwellings on the Coast.

Generally, housing was poor. At the British annexation of Lagos in 1851, there was gross over-crowding, lack of hygiene and swamps. Some trees were being planted and plans undertaken for setting-up a



Marina along the Lagoon, (Thorp, 1956).

In 1854, Consul Campbell wrote of Lagos: "The whole town excepting the Church Missionary House,.....is but an assemblage of huts constructed not of clay, but of the mud deposit of the creeks, containing a quantity of decomposed vegetable matter and other unhealthy abominations", (Talbot, 1926).

In 1855, an iron house, was sent out in sections for use of the Lagos Consul, and about 100 European-style houses were built. At this time the population was estimated at 20,000 and included American trading firms. By the following year, buildings improved and trade increased. By 1861, the population was around 30,000 and construction had started on the Marina, along the Lagoon-side.

Burton described the Lagos of 1862, even then showing a cosmopolitan picture: "The thin line of European buildings.....are first the French Comptoir's, prettily surrounded with gardens; then a large pretentious building, white and light yellow, lately raised by M. Carrena, a Scandinavian merchant-it is said to be already decaying; then the Wesleyan Mission House; the Hamburger's factory; the Wesleyan chapel with almost five times its fair amount of ground; the British Consulate, like that at Fernando Po, a corrugated iron coffin or plank-lined morgue, containing a dead Consul once a year; the Church Missionary House, whose over-grown compound caused such petty squabbles in days gone by, and which, between whiles, served as a church; another Sardinian factory; a tall white-washed and slated house, built by Mr. McCoskry; and at the furthest end another establishment of Hamburgers, who at present have more than their share of the local commerce.....The cumbered sands are alive with impurities; the Acting



Governor, however, has wisely determined to have a decent walk," - that is, Mr. McCoskry's Marina, (quoted in Talbot, 1926).

The region between Lagos Lagoon and the sea was thickly scattered with the villages of fishermen and salt collectors. In 1864, Burton described Lagos as "pestilential", 9 of 70 Europeans having died in 13 days.

In spite of improvements and the laying-down of Public Health ordinances in 1876-8, Griffith said, in 1878 (Talbot, 1926) "upon... the Marina...garbage, refuse.....Government land...was a swamp in the wet season". In 1886, the Five Cowrie Creek Bridge was completed and the Marina embankment started.

The recording of Vital Statistics became compulsory in Lagos, in 1863, and the registration of births and deaths since 1867.

By 1898, there was electricity in Lagos streets and shortly afterwards in some houses and offices, (Thorp, 1956). The 2,600-foot-long Carter Bridge, linking Lagos Island to Iddo Island was finished in 1900, as also the 917 feet Denton Bridge (later Causeway) linking Iddo to the Mainland.

The reclaiming of swamps was undertaken and at the beginning of the 20th century, a drainage canal, the MacGregor Canal, was built between the town and Ikoyi. The low-lying swamps resulted in pollution and, in 1897, controversy arose regarding "nightsoil" disposal. This is still an unsolved problem. In the early 1900's, Professor W.J.R. Simpson made recommendation for "slum clearance, sewerage system, plan for surface drainage, refuse destruction, a new Building Act and a piped water supply", (Schram, 1966).

In 1902, a tramline from Iddo to Lagos Island carried night-soil, ending 65 feet from the shore at Wilmot Point, south of Five Cowrie Creek Bridge. This continued until 1933. Nightsoil is still carried in trucks, and methods of sewage-disposal still discussed, (Weir, 1960).

No modern sewage-disposal systems exist in Nigeria. Latrines are used with disposal by burial, compost or dumping in the sea and incineration. Septic tanks are increasingly used in modern living-quarters and newer buildings. The Federal Nigeria-Annual Report of 1957 stated that, "although septic tank installations are becoming increasingly popular with the higher income-groups of town dwellers, the main methods of night-soil disposal continues to be the bucket conservancy system", (Fed.Nigeria-Ann.Rep., 1957).

Throughout the country the water-supply is obtained from streams, rivers and wells. In 1916, the Lagos waterworks were finished. Underground streams feed the reservoir at Iju, 18 miles from Lagos. The water is piped to an open reservoir before distribution to community pumps and houses, (Simmons et al, 1951).

This reservoir produced "pipe-borne water, which is soft, palatable, safe and adequate in quantity. The water, obtained from adjacent streams, is subjected to aeration, coagulation, slow sand-filtration and chlorination. It is then pumped into a clean water-reservoir from which it flows to the town", (Fed. Nigeria-Ann.Rep., 1957).

Many roads are unmade and open drains and overcrowding still exist. The town gradually expanded to other islands, for example Ikoyi, as Lagos Island was considered less healthy, because of malaria and Yellow fever infection in the indigenous population, especially in the children. This was recognised by Lugard in the 1920's, (Lugard, 1923; Schram, 1966).

In 1924, bubonic plague broke out, lasted a year in Lagos and Iddo and increased the necessity for slum clearance. It also spread up-country, where it smouldered for several years.

1929 saw the setting-up of the Lagos Executive Development Board, which laid down plans for reforms, demolished old buildings in the region of the Idunmagho Lagoon and built a Market. In 1930, the Yaba Town Planning scheme was started and completed in 9 years, and the first planned roads in Lagos were built, (Schram, 1966). This was followed by new estates in Surulere, Apapa, Lagos Island Centre, Victoria Island, Itire Road and Ikoyi, (Nigerian Year Book, 1957). But the rehousing from Lagos Island to the Mainland at Surulere, (Marris, 1961), was not as successful as planned and led to further overcrowding.

From a health point of view this part of the Coast was not a good area. Schram quotes Surgeon Comdr. H.B. Padwick, in 1922, as commenting, "this coast is certainly the father and mother of all fevers,.."; and earlier, in 1831, James Boyle, in "A practical medico-historical account of the Western Coast of Africa, together with the causes, symptoms and treatment, of the fevers of Western Africa", regarded the Niger Delta worse than the Gold Coast and the Bight of Benin the worst of all West African Squadron stations, (Schram, 1966).

During World War 2, Nigeria was used as a transit area, particularly for aircraft, to the Middle East, the Sudan and Egypt. The use of Lagos Harbour, first opened to seagoing vessels in 1913, and the setting-up of airports at Apapa and later at Ikeja, with British and Allied Service personnel movements, no doubt increased the possibility of transmission of infections. Expatriate troops showed higher infection rates, for example, to poliomyelitis, than did indigenous West

African troops, (Findlay et al, 1946; Gear, 1948b).

Crippling is commonly seen in Nigeria and may have been due, in part, to poliomyelitis. For all ages, this was estimated at 100,000 cases, (Collis et al, 1961); an estimate revised by 1965 to 200,000 to 300,000 (Collis, in Huckstep, 1965).

Censuses were carried out in Nigeria in 1921, 1932, 1952 and 1962, but Vital Statistics are collected only in Lagos.

The infant mortality rate over a period of 50 years, in Lagos, showed a decrease:

1910	-	324/1000	live	births
1916	-	261/1000	"	"
1932	-	154/1000	"	"
1945	-	128/1000	"	"
1947	-	126/1000	"	"
1957	-	80/1000	"	"
1960	-	62.9/1000	"	"

(Fed.Nigeria-Ann.Rep., 1957; Schram, 1966).

The Province of Katsina, in the north, where records have been well kept, showed an I.M.R. in 1946 of 173/1000 and in 1960 of 80/1000. But much of the country varied between 150-200/1000 live births.

Overall general and medical studies in the country have felt that the future accent should be on preventive medicine, (Ashby, 1964; Bull, 1960).

Miscellaneous viruses

Monkeys

Tissue Culture

3. In a study such as this, other viruses may be isolated, either from the faecal specimens or from the cultures of monkey kidneys, (Gelfand et al, 1957a). The most important of these are noted.

#### Adenoviruses

Adenoviruses have been found in, and possibly multiplied in, the intestinal tract and have been excreted in the faeces. HeLa and KB cells were mostly used for their growth, but they also multiplied in monkey kidney tissue culture, although less well, (Ginsberg and Dingle, 1965). Adaptation to monkey tissue cultures occurred, (Hartley et al, 1956); primary monkey kidney tissue cultures of erythrocebus patas were sensitive to adenoviruses, although some other monkey kidney tissue cultures were not, (Duncan, 1960b).

Types above adeno 9, except 14 and 21, were recovered almost wholly from the intestinal tract. In infections in infants and children, (Vargosko et al, 1965), a large number of different adenoviruses were isolated; 2 to 3 times more were isolated from anal swabs than from throat swabs; but only 1-2% were isolated in monkey kidney tissue cultures.

The group consists of ether-resistant, DNA viruses, first isolated from human adenoid tissue culture, (Rowe et al, 1953; Hilleman and Werner, 1954). 28 to 30 of them are of human origin and at least 17 of animal origin, (Ginsberg and Dingle, 1965).

12 simian types were studied, (Pereira et al, 1963); 11 were isolated from monkey kidney tissue culture or monkey faeces, (Hull et al, 1956, 1958; Hoffert et al, 1958). 1 was found in the faeces of a chimpanzee with mild upper respiratory disease, (Rowe et al, 1956).

Those simian adenoviruses, designated SV, were found in chimpanzees,



rhesus and cynomolgus monkeys, (Hull et al, 1956, 1958; Hull and Minner, 1957; Pereira et al, 1963).

Those designated SA were found in African green monkeys, (Malherbe and Harwin, 1957; Malherbe et al, 1963); in African and Asian monkeys, (Felici et al, 1959; Mancini et al, 1959); and 1 strain in a chimpanzee, (Pereira et al, 1963).

The adenovirus group was studied in different tissue culture systems, (Rowe et al, 1955; Ginsberg et al, 1955; Grayston et al, 1958); and their characteristic effects in susceptible cells noted, (Syverton, 1961). They produced a characteristic cytopathogenic effect in from 2 to 21 days. Multiplication in the nuclei with no changes in the cytoplasm, resulted in an increased acidity of the culture-medium, (Ginsberg and Dingle, 1965).

#### Reoviruses

Echo 10 was considered identical to reovirus 1, (Sabin, 1959d). It was considered originally as an echovirus, (Comm.Echo viruses, 1955), as it was isolated from human faeces, in primate cell cultures. It was non-pathogenic for laboratory animals and not related to any other virus group, (Rosen, 1965b). But it differed in certain respects, including the type of cytopathogenic effect produced, the virus size and the production of cytoplasmic inclusions, (Malherbe and Harwin, 1957; Drouhet, 1958; Shaver et al, 1958). The cytopathogenic effect was different to that of the picornaviruses; the cells became granular and did not slough off the glass readily. They remained attached to the glass by one process and moved in the medium; and the cytopathogenic effect of all 3 types was enhanced by rolling, (Lerner et al, 1962).

Reoviruses 1, 2 and 3 had world-wide distribution and were found in man, and in some animals, although their relation to disease in man and animals was unknown, (Rosen, 1965b). SV12 and SV59, (Hull et al, 1956, 1958), were found to be the same as reoviruses 1 and 2, (Rosen, 1960); and the hepato-encephalitis virus, (Stanley et al, 1953) the same as reovirus 3, (Stanley, 1961a).

The viruses multiplied and produced a cytopathogenic effect in many cell-systems, of animal and primate tissue culture, (Hsiung, 1958; Lenahan and Wenner, 1960). Reovirus 1 could exist in the latent state in monkey kidney cultures, (Hull et al, 1956; Hull and Minner, 1957; Malherbe et al, 1963).

Their hosts covered a wide range, (Rosen, 1965b); including, mice (types 2 and 3), dogs, (type 1), cattle (types 1, 2 and 3), macaca (types 1 and 2), cercopithecus (type 1), chimpanzees (type 2), as well as man.

They were recovered from the faeces of naturally-infected humans, (Stanley et al, 1953; Ramos-Alvarez and Sabin, 1954); and from cercopithecus monkeys, (Malherbe et al, 1963). The finding of reovirus 1 in uninoculated cultures of macaca, (Hull and Minner, 1957) and cercopithecus, (Malherbe et al, 1963), monkey kidney cultures, suggested that the viruses originated in the kidneys and were not introduced from outside sources.

The system of choice for isolation is rhesus kidney tissue culture and it is important to use serum-free maintenance media because of ubiquitous antibodies; "blind" passages are important, (Rosen, 1965b).

Little was known of the epidemiology; human infection with the 3 types was common from the results of sera surveys, (Ramos-Alvarez and Sabin, 1956), and were acquired early in life. Institution

outbreaks were seen in the late summer, autumn and winter, (Rosen et al, 1960b, 1960c); and in a community study, in the winter months of January, February and March, (Gelfand, 1959). Cattle might be an important source, as the viruses were in the faeces for a long time, (Rosen, 1965b).

Measles virus was isolated and gave "syncytial giant cells" in primary monkey kidney cells, (Enders and Peebles, 1954). It could infect Old World monkeys, (Katz and Enders, 1965).

Mumps, herpes simplex and influenza viruses were found and might multiply in the intestinal tract, (Gelfand et al, 1957a); some influenza viruses were grown in primary kidney cells of monkeys, (Mogabgab et al, 1955), with the production of a cytopathogenic effect.

Record of para-influenza viruses, types 1, 2, 3 and 4, in the faeces was not found. Type 2 produced focal syncytial changes in primary monkey tissue cultures; types 1, 3, and 4 produced little or no Cytopathogenic effect, (Chanock and Parrott, 1965).

Echo 28 was classified originally with the echoviruses, because of certain similarities, but later was included with the rhinoviruses, (Price, 1956; Pelon et al, 1957; Jackson et al, 1960; Andrewes and Tyrrell, 1965); it gave an antigenic overlap with the M strain, B632, (Andrewes and Pereira, 1967).

It was isolated from the respiratory tract and produced "colds". It differed from other picorna viruses in that it was acid-labile, (Dimmock and Tyrrell, 1962; Ketler et al, 1962), and was not found in the faeces. It produced a cytopathogenic effect in monkey kidney cultures.

Rhinoviruses were divided into 2 groups, "H" and "M", according to their affinity for human and monkey tissue cultures. 50 types

of "H" and over 7 types of "M", were isolated, (Andrewes and Tyrrell, 1965).

### Simian viruses

Many viruses were isolated from rhesus and cynomolgus monkeys, (Hull et al, 1956, 1958; Hull and Minner, 1957; Cheever, 1957), in tissue culture and from faeces, and designated SV viruses.

Viruses were isolated from South African vervet monkeys (*cerco-pithecus aethiops pygerythrus*), (Malherbe and Harwin, 1957; Malherbe et al, 1963), from uninoculated monkey kidney cultures and from faeces, and were designated SA1 to SA15. SA1 was similar to the monkey kidney viruses of Rustigian and others, (Rustigian et al, 1955); SA3 was considered to be a reovirus. "The use of small tissue batches consisting of kidneys from 1 or 2 monkeys only, has led to marked reduction in the number of isolations of SA3 and SA4. SA1 continues to be encountered and can be misleading", (Malherbe and Harwin, 1957).

Hull divided them into groups I to VIII on a basis of their cytopathogenic effects, (Andrewes and Pereira, 1967).

Rhesus and cynomolgus monkeys in captivity developed diarrhoeal diseases with a high fatality rate. Enteric viruses of simian origin, (enteric cytopathogenic monkey orphan - ECMO), were isolated in tissue culture and mice, (Hoffert et al, 1958), from normal and diarrhoeal monkeys; 90% of specimens gave a cytopathogenic effect (CPE) in monkey kidney cultures. None were related to polio 1, 2, 3 or Echo 1 to 13, but possibly some Coxsackie cross-relationship existed. They were divided into prototypes P1 to P13, some from faeces, some from kidneys and one from kidney and tonsils.

By CPE, they were divided into 2 groups, (a) as for adenoviruses, (Rowe et al, 1955), and (b) as for polio, (Enders, 1954) and Echo, (Melnick, 1954) viruses. Prototypes were recognised and related to viruses already known: the Hull SV group, adenos in monkeys, (Rowe et al, 1958) and other monkey enteroviruses, (Melnick, 1957c).

Enteroviruses in cynomolgus monkeys were studied in Saigon, (Andre and Audebaud, 1959) and divided into 2 groups by CPE, again resembling those of adeno and polio viruses. Similar CPE's were found in studies of viruses in kidneys and faeces of African and Asian monkeys, (Felici et al, 1959; Mancini et al, 1959).

Animal "orphan" enteroviruses were studied, (Kalter, 1960), postulating a possible relationship with each other and with human enteroviruses.

A vacuolating virus was isolated and designated SV40, (Sweet and Hilleman, 1960a,b), causing a CPE in cercopithecus aethiops cultures. A vacuolating agent was found in rhesus and cynomolgus monkey kidney cultures, producing no CPE in those cultures, but producing a CPE in vervet monkey kidney cultures, (Magrath et al, 1961) and possibly existed in vaccines. The presence of extraneous agents was recognised and the control, by quarantining of animals, put forward, (Tobin, 1960). SV40 might infect those handling infected monkeys and monkey kidney cultures, (Horvath, 1965).

Monkey kidney cultures of green monkeys, (a species seen to be infected naturally with SV40 and measles viruses) were experimentally infected with SV40, measles and SV5 viruses, in combinations, and could still be infected with polio virus, (Hsiung et al, 1966). The extraneous agents can be eliminated by formalinisation, (Andrewes and Pereira, 1967).

A "Foamy agent" was isolated from monkey kidney tissue cultures, ((Enders and Peebles, 1954; Rustigian et al, 1955), producing syncytia in cells. It was the commonest contaminant of monkey kidney cultures and was isolated from 40 to 65% of kidney tissue cultures of rhesus, cynomolgus and cercopithecus pygerythrus, (Andrewes and Pereira, 1967).

A longitudinal study of monkey enterovirus excretion, (Heberling and Cheever, 1967) found that rhesus monkeys excreted viruses on arrival in the U.S.A. from India and continued to excrete them for over 11 weeks after arrival. They were associated with intestinal disease, but of low virulence.



### Monkeys

Monkeys played an important part as susceptible animals in the original isolation of the poliomyelitis viruses; the catarrhine, (Old World) monkey appearing more susceptible than the platyrrhine, (New World) monkey, perhaps due to a closer relationship with man, (Poliomyelitis, 1932).

*Macacus cynomolgus* was susceptible to human polio viruses, (Burnet et al, 1939); *cercopithecus aethiops sabaeus* (African green monkey) was as sensitive as *macacus rhesus* to oral administration of polio viruses, (Trask and Paul, 1941b).

*Cercopithecus aethiops pygerythrus* (South African vervet monkey), (Gear et al, 1945; Gear, 1946); *cercopithecus aethiops centralis*, (Kenyan vervet monkey), (Paul et al, 1944; Melnick and Ward, 1945) and *cercopithecus griseoviridis*, *erythrocebus patas*, *papio hamadryas* and *macaca radiata*, (Paul et al, 1944); *cebus capucina* (South American ringtail monkey) and *cercopithecus cephus* (African mustache monkey), (Melnick and Paul, 1943); and the West African *papio papio*, *cercopithecus mona rolway*, *cercopithecus torquatus* were susceptible, (Findlay et al, 1946).

### Tissue culture

Polioviruses were cultivated in vitro in human, (Enders et al, 1949; Smith et al, 1950; Weller et al, 1949) and monkey, (Smith et al, 1951; Syverton et al, 1951) extra-neural tissues, with the production of a cytopathogenic effect (CPE), (Robbins et al, 1950); resulting in rounding, irregularity, granulation and finally fragmentation of cells, (Enders, 1954). Other cytopathogenic effects

produced by viruses fall into 2 categories: inclusion bodies in cytoplasm or nucleus, and giant cells or coalescence of cells to give a syncytial mass.

Basic technical details (Robbins et al, 1952) are still used, although variations have resulted from many studies carried out in relation to particular work or conditions. The present work incorporated information from the following literature.

Primate tissue cultures susceptible to polioviruses, were reviewed, (Plotkin et al, 1962; Luginbuhl and Black, 1960-61; Henderson, 1960-61; Hsiung, 1960-61) and studies on monkey kidney tissue culture sensitivity (Wenner and Miller, 1954), showed that tissue culture was as good, as if not better than, monkey inoculation for poliovirus isolation.

Monkey testis and kidney cultures were used most often, (Ledinko et al, 1951; Salk et al, 1953; Youngner et al, 1952); and testicular tissue from immune monkeys was as susceptible to poliovirus as non-immune monkey tissue, (Ledinko and Melnick, 1952).

Different animals and cultures of human and animal tissues varied in their susceptibility to polioviruses, (Kibrick, 1955). In a comparative study of different cell systems, monkey kidney tissue culture was the most generally sensitive for enterovirus isolation, (Kelly and Sanderson, 1962).

Toxicity from faeces did not interfere with virus isolation, (Stokes and Macrae, 1957).

The growth of Coxsackie viruses in tissue culture was summarised, (Melnick et al, 1964). The proportion of isolation of Coxsackie A's in suckling mice and monkey kidney cultures was about 19 to 1, (Dall-dorf, 1957), varying according to the number of cytopathogenic strains

in circulation; therefore it was important in studying Coxsackie viruses to inoculate mice. Coxsackie A viruses studied in tissue culture, (Sickles et al, 1955), showed that A9 was the only positive one in monkey kidney cultures; but later studies on more recent Coxsackie A strains, gave variable results, (Sickles et al, 1959). Group B viruses were more suitable for isolation in tissue culture.

The Echo viruses generally produced a CPE in tissue culture, (Melnick, 1954, 1955a; Reissig et al, 1956; Barski, 1962; Jamison et al, 1963). They produced the same type of CPE as the polio and Coxsackie viruses: except for Echo 10, (Barski, 1962).

Different cell-systems were studied. HeLa cells were sensitive to polio viruses, (Scherer et al, 1953) and to Coxsackie B1 to 5, (Girardi et al, 1957). Other systems studied included: Human amnion cells, (Takemoto and Lerner, 1957; Lahelle, 1957a); human adult tonsil epithelial cells, (Evans, 1957); LLC-MK2 line of Monkey kidney cells, (Hambling and Davis, 1965); rabbit kidney, strain ERK, (Sheffield and Churcher, 1957); Hep2, (Pal et al, 1963) in comparative studies with Monkey kidney cells and HeLa cells; and human foetal diploid kidney cells compared with primary Monkey kidney cultures, (Schmidt et al, 1965a); all with varying results. The optimal system for isolation, (Lee et al, 1965), included suckling mice and several cell systems.

Fibroblastic (diploid) cultures of human embryonic lung, skin and muscle, or epithelial kidney, amnion or other tissues were used for echoviruses, (von Zeipel and Svedmyr, 1957; Kibrick et al, 1956-57; Ramos-Alvarez et al, 1960; Wenner, 1962). HeLa cells were unsuitable for primary isolation; monkey kidney continuous lines were possibly susceptible to passage-lines of echoviruses, (Hull et al, 1962).

Tissue cultures from rhesus and cynomolgus monkey kidney were the cells of choice for isolation and propagation of echoviruses, except echo 21, which was better in human amnion.

Studies were carried out on enteroviruses in different types of monkey kidney cultures, (Drouhet and Costil, 1956; Hsiung and Melnick, 1957a,b; 1958a,b; Hsiung, 1962; Kalter et al, 1962). Cultures from African monkeys, sooty mangabey (*cercopithecus fuliginosis*), white-crowned mangabey (*cercopithecus torquatus lunulatus*), Diana (*cercopithecus diana roloway*) and the Asiatic monkey, cynomolgus (*Macacus cynomolgus*) resembled those of the Indian rhesus (*Macacus mulatta*) in sensitivity.

Cultures from the African red grass monkey (*Erythrocebus patas*) were as susceptible as rhesus tissue cultures for polio viruses and about equally sensitive for Echo and Coxsackie B viruses, (Hsiung and Melnick, 1957b), being satisfactory for Echo 7 and Echo 12, but not for other echo viruses and Coxsackie A9; and as sensitive as cynomolgus monkey kidney cultures for polio viruses, (Drouhet and Costil, 1956).

Cultures of the African green (*cercopithecus aethiops sabaeus*) and tantalus (*cercopithecus aethiops tantalus*) monkeys had a high sensitivity to polio, Coxsackie and Echo viruses and a greater for Echo viruses than did rhesus cultures, (Hsiung and Melnick, 1957b) although the *cercopithecus aethiops sabaeus* was less sensitive to Echo 16. The baboon (*papio papio*), yellow baboon, drill baboon and lemur, varied in sensitivity to different viruses and could be used in the differential isolation of viruses.

Further studies, (Hsiung and Melnick, 1958b), concluded that the

difference in viral susceptibility of patas and rhesus monkey kidney cultures was a true cell effect and not due to the virus, and divided viruses into patas-positive and patas-negative groups, or Groups A and B in plaque formation, (Melnick, 1957c; Hsiung and Melnick, 1957a).

Clinical specimens with 2 or more viruses could be separated by plaquing on different cells, (Hsiung and Melnick, 1958a) or by plaquing in known antiserum. Testing of isolates could be carried out with pooled sera, (Lim and Benyesh-Melnick, 1960; Schmidt et al, 1961b). The isolate should then be retested against the single serum for confirmation.

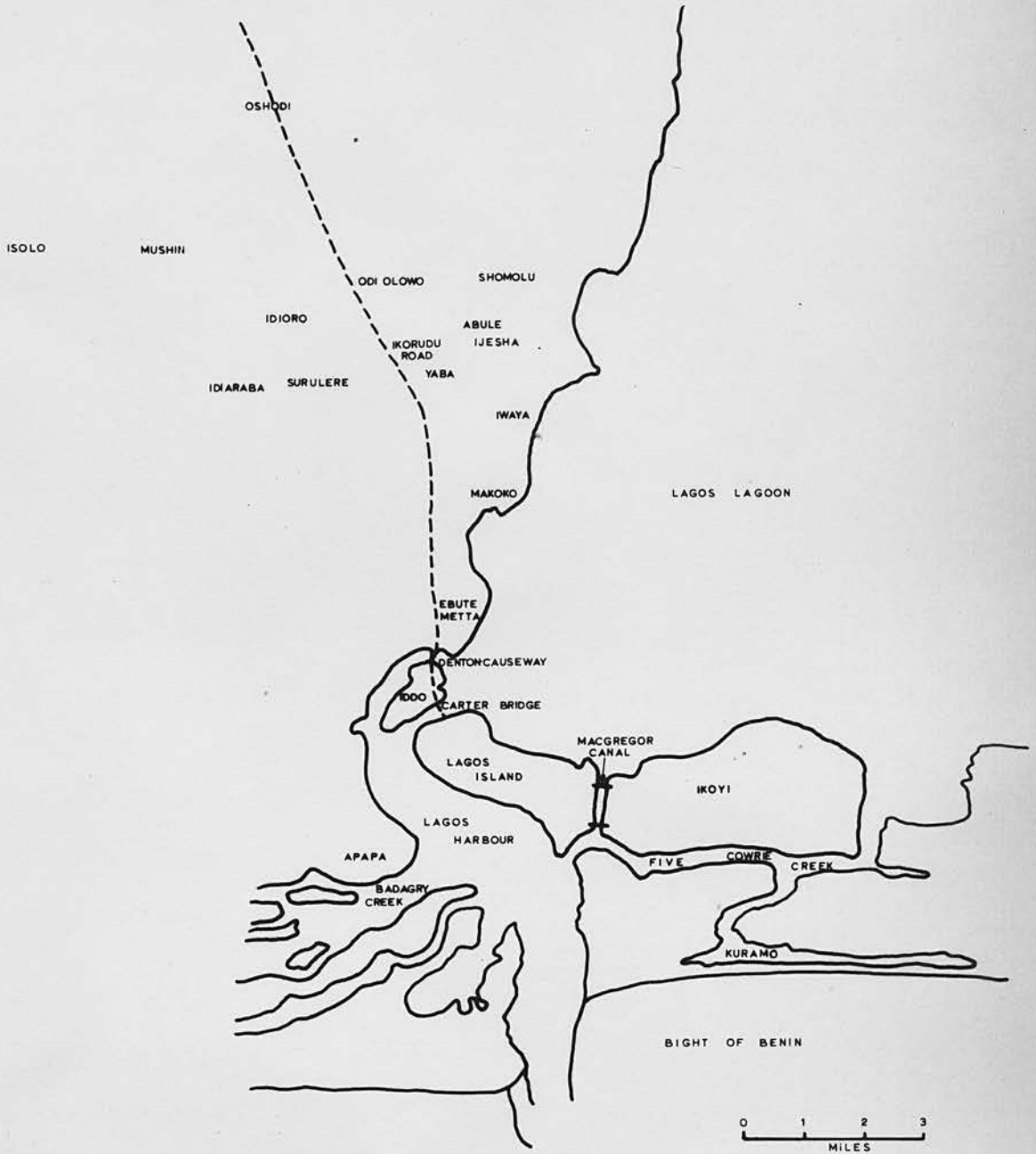
The study



Map of Lagos and environs

Map of Lagos and environs

(Adapted from Buchanan and Pugh, 1955)



The population under study

This was made up of the Nigerian Staff at the West African Council for Medical Research Laboratories, Yaba, (Lagos) and their families. This was a relatively static group of people who were most cooperative and understood the implications of the study.

Details of these families, the tribes to which they belonged, the occupations of the head of the family, residential areas, sex, family status of individuals and their ages, are given in Figure 37. 95 families (F 1 to F 95) took part.

They fell into wide "tribal" divisions, as follows -

Yoruba	-	43	families
Ibo	-	45	"
Hausa	-	2	"
Midwest	-	4	"
Cameroons	-	1	"

They consisted of complete families, as they lived in the environs of Lagos, and were not selected or sampled for the study. This led to complications in evaluating the results, but on the other hand, gave a cross-section of the population.

There was more than 1 wife in some families; this has been indicated in Figure 37.

Occupations - (Fig 37)

(1) Laboratory staff - 15 individuals.

7 were involved in the laboratory work of the present study-

Individual number 84 - Family F 12; 119 - F 23; 146 - F 29;

273 - F 53; 324 - F 64; 328 - F 66; 343 - F 71.

8 were involved in other laboratory work, mainly with arthropod-borne viruses and had no known connection with enteroviruses -

27 - F 6; 91 - F 13; 104 - F 17; 207 - F 41; 235 - F 47; 243 - F 49;  
339 - F 70; 399 - F 82.

(2) Animal attendants - 13 individuals.

6 worked with monkeys - cleaning cages, handling and feeding the animals.

1 - F 1; 98 - F 14; 99 - F 15; 175 - F 35; 232 - F 46; 329 - F 67.

7 worked in the "mouse colony" (breeding colony for experimental mice)-

43 - F 9; 126 - F 25; 165 - F 34; 235 - F 47; 260 - F 51;

301 - F 58; 311 - F 61.

(3) Others who had no direct contact with laboratory work -

Gatekeeper; Gardeners; Sickroom assistant; Storekeepers; Office staff;

Drivers; Labourers; Workshop staff (tinkers, carpenters, bricklayers,

electricians, etc.); Personal servants (Cooks and stewards to expatriate staff).

Individual families ranged from 1 to 13 persons and totalled 436 persons.

400 took part in the study; the remaining 36 were noted in Figure 37,

as they were members of the families during the study and would act

as potential reservoirs or "carriers" of viruses.

#### Age-groups

Details of individual ages are given in the list of families, (Fig 37).

Ages were taken as of November, 1962, at the beginning of the study;

some babies were born during the study.

The distribution of persons by age-group is given in Tables 1 and 2,

Figure 1.

The population was divided into 3 arbitrary age-groups.

(1) Adult - 17 years and over.

This covered husbands and wives in the families, adults living with the family as members of an "extended family", and older children, most of whom were working but continued to live within the family.

17 years was chosen as being over school age (on an average) and the approximate age of the youngest wife or mother.

The exact age of a large number of adults was difficult to ascertain.

The registration of vital statistics was compulsory in Lagos only, (Thorp, 1956), and so it was difficult in many cases, who had been born outside Lagos, to state an exact age. Most families had been resident in Lagos for 10 years or more, and most of the children had been born in the City. There were some who were born in the home area of the family.

(2) 5 to 16 years inclusive - this group covered the school-age children (on an average).

(3) under-5 years - this group covered the pre-school child. It was decided not to divide it further into smaller groups as the numbers involved were too small and increased difficulties of comparison.

The existence of more than one wife in a family and the presence of children belonging to the "extended family", resulted in the existence in some families of several children of the same age (not twins or triplets).

Sexes -

The total males and females in the study were almost equal - 199 males and 201 females. The proportions varied within the age-groups, (Tab. 27; Fig. 27a,b).

Racial and Tribal-

Although the population was, in a wide sense, racially homogeneous, the tribes have a heterogeneous ethnic background, as was described in the introductory section on Nigeria, (Johnson and Johnson, 1921; Meek, 1925; Talbot, 1926; Thorp, 1956; Burns, 1963; Niven, 1967).

In world studies, an apparent difference has been seen, associated with race: in South Africa, differences in incidence in Bantus and "white" people, (Gear et al, 1951); in Kenya, differences in those of African, Asian and European stock, (Walker, 1956; Fendall and Kake, 1958); in the USA, more infection has been seen in negroes, (Gelfand et al, 1957a, 1963), although an increase in infection in Negroes was seen in some areas after 1956, (Speers et al, 1960); in the 1945 outbreak in Mauritius, the Chinese in the population showed more infection than did the Indians, (McFarlan, 1946); and a variation in incidence in different racial groups was seen in Hawaii, (Lee, 1941; Enright, 1948).

Most likely, socio-economic factors led to the apparent "racial" differences.



Residential areas- (Map of Lagos and environs)-

The families lived in 15 adjoining areas of Lagos Island, Ebute Metta and other suburbs on the northern side of the city, within a 5-mile radius of the WACMR Laboratories, in Yaba.

The number of families in the different areas varied from 1 to 18, (Tab. 23; Fig. 23).

Some historical background of housing was described in the ~~intro-~~ ductory section on Nigeria. In most areas, water was piped to taps in the streets or compounds, (Fed. Nigeria Ann. Rep., 1957; Simmons et al, 1951). Sewage disposal was mainly of the bucket conservancy variety. In some areas, water was piped closer to individual houses and there was a water-borne sewage system to septic tanks, (Fed. Nigeria Ann. Rep., 1957; Weir, 1960; Schram, 1966).

Lagos Island - 1 family-

was one of the oldest areas historically, (Thorp, 1956), and was very crowded. Roads were mostly made-up. Water was piped to streets or compounds and sewage-disposal was by the bucket system.

Ebute Metta - 3 families-

was an old area and relatively crowded. Streets were paved and unpaved; water was piped to stands in streets and compounds; in some areas there was water-borne sewage disposal to septic tanks.

Iwaya and Makoko - 1 family in each -

were more "rural" areas lying towards the lagoon. They were relatively less crowded. Wells may be the source of some water.

WACMR, Yaba - 14 families -

this was a self-contained compound, containing laboratories, houses and flats with near-by servants' quarters, built from 1919 onwards.

The servants' quarters provided one or more room for a family. Cooking facilities were housed in a roofed-over shelter and water was piped close to this. Sanitation in some was still of the bucket variety; in others it was water-borne to septic tanks and was close to the living quarters.

In the laboratory section, showers and washing facilities were provided for the junior staff, and water-borne plumbing replaced the bucket system.

Surulere - 12 families -

this area was originally formed, at least in part, by houses built under clearance schemes from the central part of Lagos, (Nigerian Year Book, 1957). Originally spaced-out it tended to become crowded, (Marris, 1961). Roads were, on the whole, made-up. Houses were of one or two stories and, in some cases, detached, but might contain several families. Some had running water and modern plumbing.

Abule Ijesha (2), Idi Oro (3), Idi Araba (2), Mushin (16), Odi Olowo (5), Ikorodu Road (15), Shomolu (18) -

these were adjacent areas built around and spreading from the 2 main roads leading north out of the city to Ikeja Airport, Abeokuta and Ibadan.

They were made up of single and double storied buildings, with one or more rooms to a family. Some areas were crowded; water was piped to stands and there were bucket latrines for sanitation. In some there was water-borne sewage to septic tanks.

Isolo - 1 family-

this was a more isolated rural area approached by rough roads. Water may have been from wells.

Oshodi - 1 family - this was a rural area off the main road to

Ikeja Airport and Abeokuta. There was piped water but bucket disposal of sewage.

The last 2 were less crowded and had a smaller population.

Early studies, (Poore, 1893, in Blacklock, 1944) considered that sewage-disposal by burial was a better method than a water-borne system and that the latter resulted in epidemics. Later, (Casey and Aymond, 1940), the water system, sewage disposal and polio infection rate were studied together: a piped water system with no sewage disposal resulted in a higher infection rate than did a piped water system with a sewage disposal system; the situation with no piped water and no sewage disposal took an intermediary position in infection rate.

Paul considered that polio could be associated with pollution but that it was not a water-borne disease, (Paul, 1941). Later studies showed that privies and septic tanks yielded viruses which might contaminate a water-supply, (Melnick et al, 1954a,b; Horstmann et al, 1959; Paffenbarger et al, 1959; O'Connor and Morris, 1955).

#### Flies and cockroaches -

Considering climatic and other conditions, there were surprisingly few flies in the Lagos area, compared for instance with areas in the north, e.g. Kano, where the insect life was apparently more abundant-perhaps due to the presence of cattle and horses. On the other hand, cockroaches were often seen, particularly around drains and septic tanks.

Flies could harbour poliovirus, (Paul et al, 1941; Sabin and Ward, 1941c; Trask and Paul, 1943; Melnick et al, 1954a; Melnick and Dow, 1953) and Cocksackie (Melnick et al, 1949, 1954a,b; Melnick and Penner, 1952) and Echo, (Riordan et al, 1961) viruses.

This occurred especially in the presence of raw sewage and associated with open privies, (Toomey et al, 1941; Francis et al, 1953) but flies were not considered directly connected with epidemics, (Paffenbarger and Watt, 1953; Francis et al, 1953).

Cockroaches and Coxsackie viruses have been associated experimentally, (Fischer and Syverton, 1951, 1957).

Seasonal extent of the study-

The study started in November, 1962 and ended in September, 1963- i.e. the time of collection of specimens. Climatic variations were covered - both "dry" (November to March inclusive) and "rainy" (April to October inclusive) seasons being included (Maps in introduction - Nigeria).

Six seasonal periods were under study - (Tables 4, 34)

November, 1962 and December, 1962 ("dry" season)

February/March, 1963 (pre-rains)

April/May, 1963 and July, 1963 ("rainy" season)

September, 1963 (after heavy rains or, more usually, during the "small rains").

Climatic tables (rainfall and temperatures) for 1962 and 1963 (Tab.26; Fig. 26a,b) were compared with average figures (Tab.26; Fig 26c).

Neither 1962 (Fig 26a) nor 1963 (Fig 26b) was a typical year for rainfall.

1962 - The year was wetter all round. The fall was higher than normal in June and lower in September.

1963 - Instead of the normal double-peak incidence of rain, (Maps in introduction - Nigeria), the rainy season was a sustained one.

Temperatures - The overall average was normal, although June to August of 1963 was hotter than usual.

Clinical -

A daily Clinic was held for members of the Staff and their families. This was run by the author during tours of duty. Clinical details of symptoms and treatment were kept. At the end of the study the clinical records were compared with the laboratory findings to see whether any illness might be correlated with the excretion of a virus. No sera were examined and therefore a definite relationship could not be established without a rise in homologous antibody. Any relationship could only be circumstantial.



It was hoped that all would cooperate throughout the study, but there was a "fall-off" which was to be expected considering the tedious nature of the study.

Ideally, the study would have benefitted from

(1) Cooperation with a section organising the collection of the specimens at regular intervals. As it was, the participants brought the specimens to the laboratory and the sampling was more irregular and scattered. It was originally intended to collect specimens monthly, but, following November and December, a break occurred, largely due to the onset of fasting for the Moslem "Ramadan", (Tab.4; Fig. 3, 34). As it turned out, a monthly sampling might have been too narrow a timing with a redundant overlapping of isolates; viruses being excreted in the faeces up to weeks or several months, (Horstmann, 1955a). Polio viruses have been isolated for up to 12 weeks, (Horstmann et al, 1944, 1946a,b); Coxsackie viruses have persisted for at least 1 month, (Measroch et al, 1951); Echo 7 was excreted for 24 to 25 days and up to 3½ months, (Henigst et al, 1961) and Echo 9 has shown traces after 5 to 7 weeks following the onset of illness, (Wigand and Sabin, 1962).

Studies on the incubation period of the polio viruses found it to vary between 6 to 19 days, (Aycock and Eaton, 1927; Casey, 1945; Brown et al, 1945; Casey et al, 1945); and, in Coxsackie viruses, from 1 to 14 days with a mean of 3 to 5 days, (Curnen, 1950; Findlay and Howard, 1950; Huebner et al, 1950, 1951; Warin et al, 1953).

(2) a statistical sampling of the available population to keep the groups homogeneous throughout the study. Melnick considered that sampling in such studies was important, (Melnick, 1959).

Technically, and because of the voluntary nature of the study,

this was not possible. This introduced variables which increased the complexity of evaluation.

(3) being carried out over a longer period of time to see whether excretion patterns varied (a) annually and (b) seasonally within the years for the overall virus pattern and for specific viruses. Studies were carried out in this manner, mainly in temperate regions, e.g. the large studies in the USA, (Fox et al, 1966; Gelfand et al, 1963; Spigland et al, 1966), and will be referred to later.

This was not possible technically in the present study. The plan of the study had to be fitted into the author's tours of duty.

It was hoped to make this a base or pilot study, which might be repeated, in Lagos and elsewhere in the country, in different populations. Later external circumstances ruled this out.

(4) field and laboratory work continuing at the same time for speed, so that any change in virus pattern in the community might be noted soon enough to make the information useful. This is particularly important in monitoring for polio vaccination, e.g. the surveillance programmes in the USA, (Fox et al, 1966).

(5) the carrying out of serological tests, (Fox et al, 1957). This would indicate when susceptibles became evident and increased in a population; vaccination could then be undertaken, (Melnick, 1959).

There was less cooperation from the families on this matter, particularly in the case of the children; it was felt that if the idea were insisted upon, the whole study might suffer.

Experimental

Earlier studies in the WACMR Laboratories (the author) had shown-

- (1) during a small study of faecal specimens from babies in a "well-baby" clinic at University College Hospital, Ibadan (specimens received from the Department of Professor W.R. Collis), with simultaneous inoculation of specimens into infant mice and primary monkey kidney tissue cultures, that little advantage was gained by the double method and that only an insignificant number of isolates was picked up in infant mice and not in monkey kidney cultures. Members of the Coxsackie A group (except A9) presumably would be lost.

It was decided to use monkey kidney cultures only in the present study. This was due also to technical considerations - lack of space for the handling of large numbers of mice at the same time as working on the monkey cultures.

Elsewhere, the proportion of isolation of Coxsackie A's in suckling mice and monkey kidney tissue cultures was found to be about 19:1, (Dalldorf, 1957) varying according to the number of cytopathogenic strains in circulation, which suggested it was important to use suckling mice in the study of Coxsackie viruses.

Coxsackie A9 was believed to be the only one of the group positive in monkey kidney tissue cultures, (Sickles et al, 1955), but later studies gave variable results, (Sickles et al, 1959).

and,

- (2) during testing standard strains of known enteroviruses in primary cultures of three types of locally-obtainable monkeys-

- (a) cercopithecus aethiops tantalus,

- (b) erythrocebus patas and

- (c) papio anubis,

cultures of (a) were the most sensitive to the largest number of the viruses tested; and as the tantalus monkey was the most easily obtainable, from the near-by forest terrain, it was the animal of choice.

Studies in the USA, (Hsiung and Melnick, 1957b) found kidney cultures of cercopithecus aethiops tantalus were sensitive to enteroviruses. This has been described in the introduction.

Materials and Methods



## Experimental

### Materials and methods

#### Collection of faecal specimens

Specimens were collected in disposable containers (sputum cartons), were received at the laboratory within 24 hours of excretion and stored at  $-20^{\circ}\text{C}$  for up to several weeks prior to preparation. Following removal of samples for preparation and continued storage at  $-20^{\circ}\text{C}$ , the original specimen and container were autoclaved, at 15 lbs for 30 minutes, and destroyed by incineration.

#### Preparation of specimens

Faecal specimens were homogenised by shaking with sterile glass beads in sterile Hanks' Basic Salt Solution, (Hanks and Wallace, 1949), to give a suspension of approximately 1:10, volume/volume. Penicillin (500units/ml) and streptomycin (250  $\mu\text{g}/\text{ml}$ ) were added. The suspension was centrifuged at 1500 rpm for 10 minutes at  $4^{\circ}\text{C}$  and the resultant supernatant transferred to sterile containers and left overnight at  $4^{\circ}\text{C}$ . The overnight storage at  $4^{\circ}\text{C}$  apparently allowed the penicillin and streptomycin to act, as inoculation of specimens into cultures without this step frequently led to contaminated cultures. The specimens were then stored at  $-20^{\circ}\text{C}$  to await inoculation into tissue cultures.

#### Experimental animals

Because of availability and susceptibility to enteroviruses, (Hsiung and Melnick, 1957b), primary kidney cultures of the *Cercopithecus aethiops tantalus* monkey were used for the study.

Over 200 monkeys were used and were obtained from local

"animal traders". The age of the monkeys was uncertain and was estimated at about 1 year; they were, on average, 18 inches in height.

On arrival at the laboratory, the monkeys were kept in a "Quarantine" cage for two weeks, during which time any sick animals were removed. No antibiotics were administered routinely. They were transferred to the general stock of tantalus monkeys, which was housed separately, away from the macacus rhesus and erythrocebus patas monkeys. Two to three monkeys were kept in each cage; this cut down infections.

#### Removal of kidneys

The monkey was anaesthetised by intravenous administration of veterinary Nembutal, until respiration and heartbeat ceased. This was followed by exsanguination from the neck vessels. The kidneys were removed aseptically by the abdominal approach.

When more than one animal was handled, the kidneys of each monkey were held and prepared separately, (Malherbe and Harwin, 1957).

#### Preparation of monkey kidney tissue cultures (MKTC)

The preparation of MKTC was based on standard methods, (Enders, 1952; Robbins et al, 1952; Weller et al, 1952).

Following removal of extraneous tissue, the kidneys were minced in Petri dishes to pieces of about 1-2 mm cube.

The tissue was transferred to a flask and washed x3 in sterile Hanks' Basic Salt Solution (BSS), (Hanks and Wallace, 1949).

### Trypsinisation

200 ml of 0.25% trypsin solution ('Difco' 1:250) at 37°C, (Dulbecco and Vogt, 1954), were added to the tissue and agitated on a magnetic stirrer for 20 to 25 minutes. The supernatant was decanted through sterile gauze filters into a flask, kept at 4°C in an ice-bath.

This trypsinisation procedure was carried out x5, by which time the kidney tissue was digested.

The pooled supernatants were centrifuged at 700 rpm for 10 minutes. The centrifuged cells were suspended xl in Hanks' BSS, re-centrifuged and resuspended in medium.

### Medium

2% calf serum (Difco) (inactivated at 56°C for 30 minutes)  
20% of a 2.5% lactalbumin hydrolysate solution (Melnick and Riordan, 1952)

78% Hanks' BSS

buffered to a pH of 7.3 to 7.4 with 2.8% sodium bicarbonate. The cell suspension was distributed to test tubes in a dilution containing approximately 300,000 cells/ml. Approximately 300 tubes were obtained from one monkey.

The tubes were placed horizontally in racks and left stationary for 48 hours at 37°C to settle on the glass, after which they were transferred to roller-drum assemblies and rolled, (Gey and Bang, 1939; Melnick and Riordan, 1952; Youngner et al, 1952).

The growth of the cells was checked microscopically after 1 week and the medium changed prior to inoculation. Cultures of the same age (within 24 hours) were used throughout all tests.

One laboratory (a) was used for the production of the MKTC's. Prior to tests, cultures were transferred from laboratory (a) to laboratory (b), which was geographically separate on the laboratory compound, and in which isolation and typing were carried out. No viruses, specimens or contaminated material were taken into laboratory (a). No known viruses were handled by any of the staff involved in the study during the preparation of cultures or specimens and isolation of the unknown viruses. During the typing of the isolates, control viruses were used after the isolates had been handled and sealed to prevent contamination.

All material (glassware, instruments, etc.), when used, was autoclaved at 15 lbs for 30 minutes prior to cleaning. Material used in the processing of the MKTC's, although it did not come into contact with known viruses or isolates, was autoclaved to reduce the likelihood of contamination or the infection of laboratory personnel by simian viruses.

#### Inoculation of specimens

0.1 ml of the specimen suspension was added to each of 2 tubes of MKTC containing 1.0 ml of medium.

The tubes were rolled at 37°C and examined microscopically daily for evidence of a cytopathogenic effect, (Robbins et al, 1950; Enders, 1954). The cultures were held for 8 days. They were not kept longer than 8 days, (2 to 3 weeks from the setting-up of cultures) to avoid the incubation time of extraneous viruses which might occur in the MKTC.

All specimens were given 2 independent passes from the original suspensions in different batches of MKTC. Double negatives

were considered as "negatives". Those giving doubtful results were passed serially 2 or 3 times, before being discarded. Isolates were harvested at a maximum CPE (3 to 4 plus) and were stored at  $-20^{\circ}\text{C}$  for further investigations.

#### Titration of virus isolates prior to typing

Standard methods were used.

All suspensions used in titrations and typing were first passage material.

Each specimen was titrated in MKTC using dilutions of  $10^{-1}$  to  $10^{-7}$ .

Each dilution (0.1 ml quantities) was inoculated into 5 tubes of MKTC, rolled and incubated at  $37^{\circ}\text{C}$  and examined daily for a total of 8 days.

The 50% tissue culture infective dose ( $\text{TCID}_{50}$ ) was calculated by the method of Reed and Muench, (Reed and Muench, 1938).

#### Typing of isolates

Standard methods were used.

Neutralisation tests were carried out, using antisera to Polio 1, 2, 3, Coxsackie B1, B2, B3, B4, B5, B6 and A9 and Echo 1 to 9 and 11 to 25. Antisera to Echo 26 and 27 were not available. Echo 10 and Echo 28 were omitted as not belonging to the true enterovirus class; Echo 10 being equivalent to reovirus 1, (Sabin, 1959d) and Echo 28 to a rhinovirus, (Andrewes and Pereira, 1967).

Specific viruses and antisera were obtained from Microbiological Associates, Inc., Bethesda, Maryland, U.S.A. (Table 33 ),

and were used in dilutions recommended by the manufacturer. The polio antisera were produced in rabbits using antigens prepared in HeLa cells; Coxsackie and Echo antisera were produced in rabbits using antigens prepared in Rhesus monkey kidney cultures, (Microbiological Associates, Inc.).

Standard virus, antiserum and cell controls were included in each test.

Approximately 100 TCID<sub>50</sub> of virus isolate was typed against at least 20 neutralising doses of specific antiserum.

Allowing for dilution factors, equal amounts of virus and antiserum were mixed by shaking and incubated at 37°C for 5 to 6 hours and overnight (approx. 12 to 15 hours) at 4°C.

0.1 ml of each dilution in the test was inoculated into 2 tubes of MKTC, incubated on a roller-drum at 37°C and examined microscopically daily for 8 days, for signs of neutralisation of virus. Any doubtful test were repeated.



Results of the study

Results of the study-

A total of 1476 faecal specimens was studied, (Tab 3; Fig 2). 238 viral agents (isolates) were isolated, (Tabs 7, 8, 9 and 10; Fig 5, 6, 7 and 8).

Distribution of isolates-

Of the 238 isolates, 70 (30%) were typed as enteroviruses, (Polio, Coxsackie B and Echo viruses).

168 (70%) remained untyped by the methods used, (Tab 11; Fig 9).

Of the 70 typed isolates, 37 (53%) belonged to the polio virus (Polio 1, 2, 3) group and 33 (47%) to the non-polio groups, (Coxsackie B and Echo viruses), (Tab 11; Figure 10).

The distribution of the typable and non-typable isolates, according to age-groups, sex, seasons, families and residential areas, (Tab 11 to 25, 27 to 32; Fig 9 to 37), is evaluated and discussed, along with their possible relationship to illness in the community.

To facilitate reference to the Tables and Figures, as several are referred to in more than one section of the discussion, they have been placed together in Volume 2.

Evaluation and discussion

Evaluation and discussion-

Distribution of persons and specimens-

Of the 400 persons and the 1476 specimens in the study, there was a larger number in the adult age-group than in the (5-16) group, and in each of these than in the under-5 group, (Tab 1, 2, 3)(Fig 1, 2). As a result, the three groups are not comparable in make-up and this must be considered during evaluation. More total specimens were collected in the early part of the study, (Tab 5; Fig 3).

Percentages have been calculated and given in the tables, but figures were based on numbers only as, in some cases, the numbers were too small to be significant when given as percentages.

Total distribution of isolates-

238 isolates out of a possible 1476 gave an all-over excretion rate of 16%. As the specimens were made up of a larger proportion of adults than either of the younger groups, a higher percentage might have been expected if the groups had been equal; and there may be a failure to isolate virus from specimens, especially in adults, (Lennette et al, 1959). However, the percentage is still in keeping with a developing tropical area.

In these areas incidence was mainly in the youngest children who were acquiring immunity - an "infantile tropical endemic distribution". Many studies on polio and other enteroviruses have shown this: in the Caribbean and South America, (Melnick, 1959), in Surinam, (Melnick, 1962; Melnick et al, 1962), in Egypt, (Melnick and Ågren, 1952) and in Mexico, (Ramos-Alvarez and Sabin, 1956; Ramos-Alvarez et al, 1959, 1960);

It has been seen in world-wide (Sabin, 1948, 1949b, 1951) and tropical studies, (Sabin, 1963; Fox, 1964); in Liberia, (Gelfand and Miller, 1956); and in studies in subtropical areas of the USA (South Louisiana) and South America, (Gelfand et al, 1957a,b,c; 1963), in which incidence was noted in very young children; in under-developed areas, (Gear, 1955) and in studies in Southern Africa, on polio, (Gear, 1948a,b,c; 1957a; 1958a; Gear et al, 1951) and Coxsackie viruses, (Gear, 1961/62).

Comparison of the number of isolates within each age-group, (Tab 10; Fig 8) with the number of specimens in each group, (Tab 3; Fig 2) showed that there was an increase in the excretion patterns in the (5-16) and under-5 year age-groups, and particularly in the latter.

In developing areas the infant mortality rate (IMR) was generally high and associated with an absence of polio outbreaks, due to acquisition of immunity at an early age in poor hygienic conditions, (Payne, 1954).

An improvement in these conditions, with a resultant fall in the IMR, was generally followed by outbreaks of polio, as seen in such developing tropical areas as Jamaica, (Grant and Peat, 1957b; Charles and Grant, 1962).

An increase in the endemic rate of polio in some areas was not due to the loss of exposure to viruses in early life, (Sabin et al, 1960). Hygienic conditions could remain poor, with, inspite of this, a relative loss of acquisition of immunity to polio and the other enteroviruses; perhaps due to an absence of these viruses, specifically, or of some of them.

This could lead to polio outbreaks inspite of low hygiene, for example, in the Belgian Congo and Costa Rica, where there was a high IMR with high polio rates, (Paul, 1958).

It was not always the case that a low IMR was associated with polio epidemics. In Surinam and in certain parts of the Caribbean, the IMR was falling, with no accompanying epidemics, (Melnick, 1959, 1962; Melnick et al, 1962). But neighbouring British Guiana (Guyana) had polio outbreaks of infantile distribution, in 1957 when the IMR was 70/1000 live births, (Melnick, 1959) and in 1962-63, (Witte et al, 1965; Feldman et al, 1965).

In some regions, geographic isolation - or relative isolation - led to non-immune populations and large outbreaks when infection was introduced, e.g. the historical outbreaks in St. Helena, (Bell, 1836) and Guam, (Grunwell, 1900), and, more recently, in the "virgin soil" epidemics of the Nicobar Islands, (Moses, 1948; van Loghem, 1949) and Tahiti (Rosen and Thooris, 1953).

But isolation does not necessarily lead to a non-immune situation with resultant "virgin soil" outbreaks. For example studies among the Rupununi Savannah Indians of Guyana showed polio immunity was present and as widespread as in towns, (Melnick, 1959).

Information on the IMR in Nigeria showed that a large part of the country varied between 150 and 200/1000 live births. In Katsina Province, in the north, where records ~~were~~ well kept, the IMR in 1946 was 173/1000 and, in 1960, 80/1000, (Schram, 1966).

In Lagos, the only area where vital statistics were compulsory, (Thorp, 1956), the IMR had fallen from 324/1000 in 1910 to about 70 to 80/1000 in 1960-61, (Fed.Nigeria Ann.Rep., 1957; Schram, 1966).



Many factors could account for a fall in the IMR - improvement in the general or specific treatment of malaria, tetanus, measles, cerebro-spinal meningitis (in the North) and bacterial gastro-intestinal conditions. And so, this fall in the IMR in the Lagos area need not necessarily be associated with an improvement in hygiene in relation to faecal-borne infections. It was noted in the introductory section on Nigeria, that the problems and controversies on "nightsoil" disposal were present in 1897, and planning in relation to sewage disposal was discussed in the early 1900's, (Schram, 1966). However, nightsoil is still disposed of as in 1902, (Weir, 1960; Fed.Nigeria Ann. Rep., 1957).

From this, we cannot assume that the lowering IMR in Lagos need necessarily be followed by outbreaks of polio; on the other hand, the continuance of low hygienic conditions need not necessarily result in immunity.

The racial and social status of the community need not mean that immunity is high. Communities in Kenya and Jamaica, "racially", and to a certain extent, socially similar, have had outbreaks on several occasions; although it is possible that the Jamaican population is more sophisticated and heterogeneous, and the Kenyan more scattered, rural and less populous than that in Lagos. The Lagos study population was tribally homogeneous, with no intermarriage, but contact with the outside world.

Historically, and in the present, the Lagos area has not been an isolated one; there has been congregation in towns for many years, especially in the western part of the country, with its tribal "town states". Less of this was seen in the eastern part of the country; there, apart from the large towns of Onitsha, Owerri, etc., communities

were smaller but closely placed - more a village "clan" system, with little space in between. There was a wider distribution of the population in the northern part of the country, made up of emirates, towns, villages and nomadic people, with a long contact with the peoples of northern Africa.

There has been a long contact with other parts of the world over the ages, with returning populations from the West Indies, Sierra Leone and Brazil and with expatriates.

And so, apparently, there was no general isolation to form a "virgin soil" territory for infection, unless in pockets in certain parts of the country, e.g. the Pagan tribes of the Plateau. But this does not necessarily mean no infection, (Melnick, 1959) and geographically isolated areas may gain immunity. And those regions which one would have considered to be well within the paths of population movements and still in a developing condition, e.g. Georgetown, Guyana, (Melnick, 1959; Witte et al, 1965; Feldman et al, 1965), seemingly can be without that immunity.

The pattern of the Lagos community cannot be taken for granted and must be elucidated.

Seasonal distribution - general -

The total numbers of specimens in each seasonal group were not directly comparable (Tab 5; Fig 3), but when each group was analysed into the arbitrary age-groups (Tab 6; Fig 4), the pattern was more comparable within each seasonal group, except in the case of July, when the number in the under-t's was greater than that in the (5-16) group.

There was a higher proportion of specimens during the months of November and December, (Tab 5; Fig 3); and a higher proportion of isolates in these months (Tab 7; Fig 5), particularly in November, where it was higher than would be expected from the larger number of specimens, the age-group pattern being roughly comparable - perhaps due to a specific outbreak.

In temperate regions, an apparent seasonal incidence was noted in the excretion of enteroviruses or resultant disease, polio cases occurring mainly from August to October, whereas, cases were seen throughout the year in tropical areas. Coxsackie viruses were found in sewage in summer and autumn, Coxsackie A being isolated in June to September in Canadian studies, (Clark et al, 1951) in Ontario, where temperatures and humidity at these times can be relatively high. They were not found widely in winter and the cold months, (Huebner et al, 1950, 1951; Cole et al, 1951; Mack et al, 1958); a seasonal distribution in temperate zones which was unexplained, (Kelly, 1953; Melnick et al, 1954a,b; Bloom et al, 1959).

Flies were infected with polio virus, after an epidemic in April to May; this was replaced by Coxsackie viruses in summer and autumn, (Melnick and Dow, 1953).

In temperate climates, the Echoviruses were found mostly in summer and autumn, although Echo 9 occurred in a February-March outbreak, (Faulkner et al, 1957). In New York studies, enteroviruses, in general, occurred mainly from August to December, (Spigland et al, 1966). Geographical variations in seasonal excretion were noted, (Melnick, 1957b; Honig et al, 1956); polio viruses varied from year to year, (Fox et al, 1956) as did the Coxsackie group, (Melnick et al, 1954a); apparently cycles of viruses occurred, (Fox et al, 1957).

In South Africa, polio virus was found in sewage in their "summer" and not in "winter", (Gear, 1948a).

In tropical regions, cases of polio were reported throughout the year, although outbreaks were noted in relation to the rainy season. In Brazil, polio cases occurred throughout the year, (Sabin, 1961/62). The epidemic incidence of polio was highest in Jamaica in the warmer months, waning in the cooler, (Grant and Pear, 1957a,b).

In Trinidad, there was an increase in virus excretion - of many types - relative to the rainy season in the summer months, (Sutton, 1965). In Hong Kong a polio 1 outbreak occurred in the summer of 1960 (rainy season) and was followed by polio 2 infection, later in the year, (Chang and Shum, 1962).

In Ceylon, an increase in the infantile incidence occurred in the season of high humidity, (De Silva, 1951). In India, polio cases were seen mostly from March to October, (McAlpine, 1945) with outbreaks in newcomers to the country. Studies in healthy people, in Bombay, showed that all ~~entero~~enteroviruses were mostly excreted in the monsoon months, (Meherhomji and Gharpure, 1961).

In Egypt, enterovirus isolations were seen all the year, (Akers

et al, 1960); 2 peaks of polio incidence occurred, in April to June and September, (El-Messih, 1960) - periods of low rainfall but high temperature. In Khartoum, a polio outbreak in 1959, showed a peak in August, (Hassan and Haseeb, 1960) - the local relatively "rainy" season.

Polio epidemics were seen in Mauritius, after a cyclone, (McFarlan, 1946) and after heavy rains, in South Africa, (Gear, 1958a); and cases in expatriates in West Africa, were seen mainly in relation to the "rains", that is, March to October, (Findlay et al, 1946).

At the other extreme, a polio epidemic occurred in the Alaskan winter of 1953/54, (Eklund and Larson, 1956) and an earlier Greenland polio epidemic took place in January, 1933, (Christensen, 1934).

The rainfall in Lagos in 1962 was higher than the average (Tab 26; Fig 26a,b,c). The greater number of isolates in November 1962, compared with those later in the study, (Tab 7; Fig 5) may be related to this rise in rainfall.

The rainfall in 1963 was a sustained one, instead of the normal double-peak.

The excretion of individual virus types at these times will be discussed in subsequent sections.

Distribution in sexes - general -

Distribution of specimens - (Tab 30; Fig 31)

The total number of specimens is greater in the males than in the females and considerably larger in the male adult group than in the female adult; but the over-all pattern in the age-groups is the same.

The distribution of specimens within the (5-16) and under-5 groups was as follows -

(5-16) group -

<u>Male</u>		<u>Age</u>		<u>Female</u>	
28		5		28	
22		6		20	
30	61%	7		32	56%
11		8		18	
13		9		36	
6		10		23	
21		11		21	
12		12		18	
9	39%	13		24	44%
8		14		3	
6		15		6	
5		16		11	

% of total specimens in (5-16) group-

Female (5-9)	32.5
" (10-16)	26.0
Male (5-9)	25.0
" (10-16)	16.5



under-5 group -

<u>Male</u>		<u>Age</u>		<u>Female</u>	
21		under-1		57	
32	50%	1		35	63%
22		2		31	
54	50%	3		44	
22		4		28	37%

% of total specimens under-5 -

Female under-3	36
Male (3-4)	22
Male (under-3)	22
Female (3-4)	20

Distribution of total isolates in sexes- (Tab 28; Fig 28)

The totals were almost equal, (118 and 120).

In the male adult group, the proportion of isolates (47 isolates/438 specs) was slightly higher than that in the female adult group, (21/281). Similarly in the 5-16 group - 34/171 per males and 36/240 per females. In the under-5 group, the females were higher than the males, 63/195 to 37/151.

Typable isolates - (Tab 29; Fig 30)

The numbers involved were small.

There was a larger proportion of females in the total although there were more total specimens in the males (Tab 30; Fig 31); but the proportion of younger children was greater in the females.

Adults -

the resultant numbers are too small for general comparison.

(5-16) group -

the totals were close and too small to compare accurately.

There were more total specimens and more at the younger end of the group in the Females, (vide supra,) although there were proportionately more in the younger end of the male group. Polio 2 and 3 were the same in the 2 groups.

Allowing for the small numbers and the above factors, there were apparently more types in the male (5-16) group than in the female. Distribution of the specimens by sexes and seasonal groups would also have a bearing on the result, but this would reduce the numbers further.

under-5 group -

there was a larger number in the female group; there was a larger number of specimens in the female under-5 group, especially in the under-3's .

There were more types in the female group - the opposite of the situation in the (5-16) group - possibly due to the distribution of specimens towards the youngest members of the age-group.

In studies in the USA, the incidence in males was found to be greater than in females, (Gelfand et al, 1963); in a 1960-63 study, infection in healthy male children was greater than in females, and isolation of virus in CNS disease was greater in males than in females in under-10 year -olds, (Froeschle et al, 1966).

More aseptic meningitis due to Echo viruses was seen in males, (Karzon, 1958). No significant difference in Coxsackie excretion was found in males and females in the 0-5 years group, although excretion was greater in the males, (Spicer, 1961; Gamble, 1962); referring to the Coxsackies, it was considered that there was not sufficient knowledge to give a distribution by sexes, (Dalldorf and Melnick, 1965).

Distribution of total isolates (typable and non-typable) by age-groups-

In the total number of isolates by age-groups, more were excreted in the under-5 age-group, (Tab 8, 10; Fig 6, 8); in the distribution of total specimens by age-group, the adult group was the largest, (Tab 3; Fig 2); and the age-group distribution pattern of specimens in the seasons was similar, (Tab 6; Fig 4).

The (5-16) age-group showed an almost equal percentage in both total specimens and isolates, (Tab 3, 8; Fig 2, 6).

In the distribution of total isolates by age-groups within the different seasonal groups, (Tab 9; Fig 7), with the exception of the December group, the under-5's excreted the largest number of isolates.

In the December seasonal group, the proportions of the age-groups differed, compared with the specimen distribution, (Tab 6; Fig 4).

In the April/May group, the pattern also varied - the (5-16) group being lower than expected.

The seasonal distribution of typable isolates by age-groups, (Tab 12; Fig 12) showed a comparable pattern in all seasonal groups, with an upwards gradation from adult to (5-16) to under-5's, allowing for extrapolation in the April-May, July and September groups. The numbers in these groups were so small that the absence in the adult and (5-16) groups was most likely not a true negative.

Allowing for this, the typable isolates appeared to form a group with a homogeneous pattern.

The seasonal distribution by age-groups in the non-typable isolates, (Tab 19; Fig 19), showed that there was a larger proportion in the adult group than in the (5-16) and under-5 groups, except in April-May, July and September. In these 3 groups, the distribution

pattern was irregular.

The non-typables apparently were a more heterogeneous group.

A more definite opinion of the make-up of the groups cannot be given without further elucidation of the composite agents, which will be undertaken in the following sections.

Distribution of typable isolates-

Of the 70 typable isolates, 37 were polio, 15 were Coxsackie B and 18 Echo viruses, (Tab.11, 16; Fig 9, 10, 11, 16).

Polio viruses-

All 3 types were excreted - Polio 1 - 2; Polio 2 - 27; and Polio 3 - 8.

Polio 1

Polio 1 occurred as 2 sporadic isolates, (Tab 11; Fig 11).

Seasons - 1 occurred in February/March seasonal group and 1 in the September group, (Tab 13; Fig 13, 33).

Age-groups - both were excreted in the under-5 age-group, (Tab 14, 15; Fig 14b, 15b).

Residential areas - the February/March isolate was from the Ikorodu Road area; that of September from Mushin, (Fig 25, 36)

Families - They occurred in 2 families - F 51 and F 62 , (Fig 35).

Sexes - both were from females, (Tab 29; Fig 30).

Polio 2

27 of this type were excreted, (Tab 11; Fig 11).

Seasons - 17 were isolated in the November group; 9 in the December and 1 in February/March, (Tab 13; Fig 13, 33). Following this the virus apparently disappeared.

Age-groups - 2 were excreted by the Adult group; 8 by the (5-16) group; and 17 by the under-5's, (Tab 14, 15; Fig 14b, 15b).

Residential areas - (Tab 25, Fig 25, 26).

1 isolate was excreted in each of Abule Ijesha, Iwaya, Idi Oro, Isolo, Makoko and WACMR, Yaba;

2 were excreted in Odi Olowo; 3 in Mushin; 4 in Shomolu;

5 in Surulere and 7 in Ikorodu Road.

Residential areas and seasons - (Fig 36)

Polio 2 was excreted in all of these areas in November; in Ikorodu Road, Mushin and Shomolu, excretion spread into December, and in Surulere a sporadic case occurred in February/March.

Families - (Fig 35)

Excretion occurred in 19 families. 15 families - F 8, 22, 29, 30, 34, 38, 42, 46, 51, 55, 71, 81, 82, 84 and 95 - excreted in November; 5 families - F 4, 31, 56, 67 and 82 - in December, and 1 - F22 - in February/March.

Sexes - (Tab 29; Fig 30)

It was excreted by Males, 4 aged (5-16) years, and 5 in the under-5 group; and by Females, 2 Adults; 4 aged (5-16) and 12 in the under-5 group.

Polio 3

This occurred as 8 isolates; in an intermediary position in number to polio 1 and polio 2, (Tab 11; Fig 11).

Seasons - (Tab 13; Fig 13, 33)

6 were excreted in February/March, and 2 in September - the same temporal distribution as Polio 1.

Age-groups - (Tab 14, 15; Fig 14b, 15b)

Polio 3 occurred in all age-groups; 1 in an adult; 4 in the (5-16) group; and 3 in the under-5's.

Residential areas - (Tab 25; Fig 25)

1 was excreted in Mushin; 6 in Surulere and 1 in WACMR, Yaba.

Residential areas and seasons - (Fig 36)

Excretion in Mushin and Surulere was in September; in Surulere it occurred also in February/March; and in WACMR in February/March.



Families - (Fig 35)

Polio 3 was isolated from 4 families - F 11, 13, 34 and 84. F 11 and F 13 excreted in September; F 34 in February and F 84 in March.

Sexes - (Tab 29; Fig 30)

It was excreted by males - 1 adult and 2 in the (5-16) group; and by females - 2 in the (5-16) group and 3 in the under-5's.

General discussion of polio isolates -

Polio 1 was excreted in very small numbers, scattered in time and place in the under-5 year age-group, suggesting a sporadic distribution in an immune population - the incidence in the under-5's being the normal acquiring of immunity by young non-immune children. No infection appeared in adults or older children, so it was possibly a recently-immune public, because so few of the under-5 age-group also were affected.

Polio 2 showed a more "epidemic" form, occurring localised in time to November/December with a sporadic case in February/March, in a number of residential areas, in all age-groups and widespread in families. Apparently there was less immunity in the community with the possibility that infection had been absent for some years or had been of a relatively sporadic nature, leaving non-immune individuals in all age-groups of the population.

The sporadic February/March case may have occurred at the tail-end of the outbreak, waning over the intervening period,

or may have been an isolated sporadic case in an individual who had not acquired immunity during the outbreak.

Polio 3 resembled polio 1 in a more sporadic incidence. It was scattered in time and place, but occurred in all age-groups, so that the population may have been relatively less immune to it than it than it was to polio 1 but more than it had been to polio 2, prior to the outbreak of the latter. Perhaps the next outbreak in the community would have been due to polio 3 - within the next few years.

As has been noted, outbreaks of polio occurred in Jamaica in 1954 and 1957; in Kenya in 1954, 1957 and 1960 and in Guyana in 1957 and 1962/63, apparently at 3-yearly intervals. Perhaps there was some general world-wide significance in this periodicity.

Coxsackie viruses- (Tab 11 to 15; Fig 10 to 15)

Coxsackie B 1, 2, 3, 4 and 5 were isolated. There was no evidence of B 6 or A 9.

As a group they were excreted from November, 1962 to July, 1963, and, apparently, were absent thereafter.

Individual viruses within the group had patterns, (Fig 33).

Cox B1 -

6 were excreted, being the largest number of the group, (Tab 11; Fig 11).

Seasons - (Tab 13; Fig 13, 33)

They were excreted sporadically from December, 1962 to July, 1963. 1 in November/December and February/March and 2 in each of April/May and July. They were absent in September.

Age-groups - (Tab 14, 15; Fig 14b, 15b)

All age-groups were represented; adults, 1; (5-16), 2; and under-5, 3.

Residential areas - (Tab 25; Fig 25, 36)

The isolate of November/December was excreted in Mushin; 1 in April/May and 2 in July, in Surulere; and 1 in February/March and 1 in April/May in WACMR, Yaba.

Families - (Fig 35)

They were excreted in 6 families.

1 in F 64 in November/December; 1 in F 10 in February/March; 2 in F 61 and 93 in May; and 2 in F 22 and 34 in July.

Sexes- (Tab 29; Fig 30)

Male excretion was 2 in the (5-16) group and 2 in the under-5's. In females, there was 1 in an adult and 1 in an under-5.

Cox B2 -

2 of this type were excreted, (Tab 11; Fig 11)

Seasons - (Tab 13; Fig 13, 33)

They were excreted in November and December.

Age-groups - ( Tab 14, 15; Fig 14b, 15b)

There was 1 in each of the (5-16) and under-5 groups.

Residential areas - (Fig 25, 36)

1, in November, was excreted in Odi Olowo; and 1, in December, in WACMR, Yaba.

Families - (Fig 35)

They were excreted in 2 families; 1 in F 20 in November; 1 in F 10 in December.

Sexes - ( Tab 29; Fig 30)

Both were excreted by females, in the (5-16)s and under-5's.

Cox B3 -

3 isolates were excreted, (Tab 11; Fig 11)

Seasons - (Tab 13; Fig 13, 33)

They were distributed sporadically in November and the following July,

Age-groups - (Tab 14, 15; Fig 14b, 15b)

They were excreted in the under-5 group only.

Residential areas-(Tab 25; Fig 25, 36)

1, in November was excreted in Surulere; 2, in July, in Shomolu and Odi Olowo.

Families - (Fig 35)

They were excreted in 3 families-

1, in F 13 in November; 1, in F 71 and 1 in F 77 in July.

Sexes - (Tab 29; Fig 30)

1 was excreted by a male, under-5; and 2 by females, also under-5.

Cox B4-

There was only 1 isolate of this type, (Tab 11; Fig 11)

Seasons - ( Tab 13; Fig 13, 33)

It occurred in July, 1963.

Residential area - (Tab 25; Fig 25, 36)

It was excreted in Mushin,

Families - ( Fig 35)

It occurred in 1 family - F 51;

Sexes - (Tab 29; Fig 30)

in a female, in the under-5 group.

Cox B5 -

3 of this type were isolated, (Tab 11; Fig 11)

Seasons - (Tab 13; Fig 13, 33)

1 was isolated in May and 2 in July.

Age-groups- (Tab 14, 15; Fig 14b, 15b)

1 occurred in the (5-16) group and 2 in the under-5 group.

Residential areas - (Tab 25; Fig 25, 36)

1 was excreted in Ebute Metta, in May; and 2 in Surulere, in July.

Families - (Fig 35)

They were excreted by 2 families; 1 in F2, in May; and 2 in F 25, in July.

Sexes - (Tab 29; Fig 30)

2 were excreted by males, in the (5-16) and under-5 groups; and 1 by a female, in the under-5 group.

General discussion of Coxsackie B isolates -

The numbers were small, especially when divided into composite types.

As a group, excretion spread from late November, 1962 to July of 1963, with an increase in July, at the height of the "rains", (Tab 26; Fig 26), and an apparent absence in the September group. In temperate regions, the incidence of Coxsackie viruses in summer and autumn, (Huebner et al, 1950, 1951; Cole et al, 1951; Mack et al, 1958) and variation within a year, (Melnick et al, 1954a) have been noted.

Within the group, Coxsackie B1 had a wide distribution in time, residential areas and families, and all age-groups were represented. This appeared to be an agent for which the immunity in the population was not high. A scatter of the community gained infection and resultant immunity, rather than the non-immune youngest children only - as might be found in an immune population.

The virus may have been of low invasive power, otherwise it would have occurred in a more localised outbreak in a non-immune community. Or, the occurrence of the polio 2 outbreak may have kept the virus in abeyance, as a result of their mutual interference, (Dalldorf, 1960; 1955; Gurnen et al, 1949; Dalldorf and Albrecht, 1955).

Its presence in this form was of interest, comparing it with records of the appearance of B 1 infection in other parts of the world.

It occurred, in South Africa, after an apparent absence until 1960, (Gear, 1961/62). In the USA studies of Gelfand and others, (Gelfand et al, 1963) there was an absence of B 1 in 1960-61; and absence was noted in Louisiana, prior to 1959. This could result in a possible lack of immunity and the potential threat of an epidemic outbreak.

Coxsackie B2 occurred as 2 isolates, localised in time in the lowest age-groups. Allowing for the small numbers - this might suggest less immunity to this type. Their occurrence in November and December and apparent disappearance thereafter, could suggest an outbreak; but this was unlikely as a polio 2 outbreak occurred at this time, (Dalldorf, 1960).



It is more likely a sporadic acquiring of immunity in 2 non-immune younger children, in an already immune community.

Coxsackie B3's 3 isolates were scattered in time and place and occurred in the under-5 age-group. This appeared the normal acquiring of infection in the youngest children in an immune community.

In 1962, in the USA, there was an increase in B3, previously absent, (Gelfand et al, 1963).

Coxsackie B4, occurring as 1 isolate in the season of maximum Coxsackie B excretion, and in the under-5 group, suggested the normal acquisition of immunity in an immune community. Excretion possibly stopped in a community with the onset of immunity and the development of homotypic antibody, (Brown and Ainslie, 1951; Brown, 1955).

The 3 isolates of Coxsackie B5 had an intermediate excretion pattern, in time, sporadic isolates occurring over 2 months, and in the age-groups, (5-16) and under-5, perhaps suggesting less immunity.

Comparison of polio and Coxsackie excretion patterns in the seasons, (Fig 33; Tab 26, Fig 26), showed a disappearance of polios during the "rains" and an increase of Coxsackie B's. Coxsackie B viruses were relatively low when polio viruses were being excreted in November and December 1962 and February/March, 1963, but increased during the period of absence of polio viruses - April/May and July. When the polios recurred in September, 1963, the Coxsackies were absent - or the development of immunity to the Coxsackies resulted in their disappearance allowing the polios to reappear.

This may have been due to climatic factors or to the interference-relationship between polios and Coxsackie B's mentioned above.

Echo viruses - (Tab 11 to 15; Fig 10 to 15)

4 types were isolated - Echo 1, 7, 9 and 11.

As a group, they were excreted sporadically throughout the study, (Fig 33), but as individual types there were patterns.

Echo 1 -

7 were isolated.

Seasons - (Tab 13; Fig 13)

1 was excreted in the November group; 4 in the November/December group and 2 in the February/March group.

Age-groups - (Tab 14, 15; Fig 14b, 15b)

They occurred in all age-groups; 1 in the adult group; 3 in the (5-16) group; and 3 in the under-5's.

Residential areas - (Tab 25; Fig 25, 36)

They were found in 4 residential areas; 3, in November, in Shomolu; 1 in both November and December in Surulere; 1 in February in WACMR, Yaba; and 1 in March in Odi Olowo.

Families - (Fig 35)

They were excreted in 5 families; 4, in F 34 and 40 in November; 1, in F 54 in December; 1, in F 10 in February; and 1, in F 77 in March.

Sexes - (Tab 29; Fig 30)

In males, 1 was excreted in each of the age-groups; in females, 2 were excreted in each of the (5-16) and under-5 groups.

Echo 7 -

There were 6 isolates of this type, (Tab 11; Fig 11)

Seasons - (Tab 13; Fig 13, 33)

They were distributed sporadically from December, 1962 to September, 1963; 1 in each of November, March, May and September and 2 in July.

Age-groups - (Tab 14, 15; Fig 14b, 15b)

They were excreted in the (5-16) and under-5 groups; 1 in the former and 5 in the latter.

Residential areas - (Tab 25; Fig 25, 36)

They were found in 4 residential areas; 1 in July in Idi Araba; 1 in November and 1 in July, in Ikorodu Road; 1 in March and 1 in September, in Surulere; and 1 in May, in WACMR, Yaba.

Families - (Fig 35)

They were excreted in 6 families ; 1 in F 8, in November; 1 in F 13, in March; 1 in F 24, in May; 2 in F1 and F 4, in July; and 1 in F 34, in September.

Sexes - (Tab 29; Fig 30)

In males, 1 was excreted in the (5-16) group and 1 in the under-5 group; in females, 4 were excreted in the under-5 group.

Echo 9 -

There were 2 isolates in this type, (Tab 11; Fig 11)

Seasons - (Tab 13; Fig 13, 33)

Both were found in November, 1962.

Age-groups - (Tab 14, 15; Fig 14b, 15b)

They were isolated in the (5-16) and under-5 groups.

Residential areas - (Fig 25, 36)

The isolates were in Surulere and Shomolu.

Families - (Fig 35)

They were excreted by 2 families, F 25 and F 69.

Sexes - (Tab 29; Fig 30)

In males, 1 occurred in the (5-16) group and in females, 1 occurred in the under-5 group.

Echo 11-

There were 3 isolates, (Tab 11; Fig 11)

Seasons - (Tab 13; Fig 13, 33)

Their distribution was sporadic, one each in November, February/  
March and July.

Age-groups - (Tab 14, 15; Fig 14b, 15b)

They occurred in the youngest age-group.

Residential areas - (Fig 25, 36)

They were found in 3 residential areas; 1, in November in Shomolu;  
1, in February in Surulere; and 1, in July in Ikorodu Road.

Families - (Fig 35)

They were excreted by 3 families; 1, F 82 in November; 1, F 53  
in February; and 1, F 6 in July.

Sexes - (Tab 29; Fig 30)

They were all excreted by males under 5 years of age.

General discussion of Echo viruses -

As a group, they were excreted sporadically throughout the study  
period, (Fig 33), but mostly in November and December. Within the group-

Echo 1 consisted of 5 isolates in November and December and 2 in  
February/March, spread in residential areas, families and in age-groups.

They may have constituted an outbreak in November and December - at  
the time of the polio 2 outbreak - with an apparent disappearance

from the community thereafter, except for 2 sporadic isolates early in 1963. Perhaps the population was now immune following the outbreak. The pattern was similar to that seen in polio 2.

Echo 7 occurred sporadically from late November, 1962 to September, 1963, overlapping and following after the infection with Echo 1. It was relatively wide-spread in residential areas and families and occurred in the (5-16) and under-5 age-groups, suggesting a less-immune population, although isolates were mainly (5 out of 6) in the under-5's. With the widespread distribution in time, this is more likely to be a normal acquisition of immunity in the youngest children of an immune population.

Echo 9 occurred as 2 localised isolates very early in November and did not recur. The occurrence in the (5-16) and under-5 groups could suggest less immunity in the community to this virus and it's subsequent absence might suggest that this was the end of an outbreak, with a resultant immune population and no further isolates.

Echo 9 was seen in many outbreaks (as described in the introductory section) and was rarely found in healthy children, (Sabin, 1960).

In 1962, in studies in the USA, Echo 9 was another virus found after a previous absence, (Gelfand et al, 1963). Perhaps this was a world-wide situation.

Echo 11 occurred sporadically, scattered in time, with all isolates in the under-5 group, suggesting the natural acquisition of immunity in the youngest children in an immune population.

There was no interference between polio and Echo viruses.

The viruses of the three groups will be discussed more fully in relation to families, individuals and residential areas.

Multiple excretion of typable isolates - (Tab 2a; Fig 21a)

25 families excreted 1 typable virus. 8 families (F 4, 71, 77, 82, 84, 22, 25, 8) excreted 2 different types - each type could be excreted by more than 1 person. 3 families ( F 10, 13, 51) excreted 3 types; and 1 family (F 34) excreted 5 types.

Many factors could be involved, including the distribution of specimens, the size of the family and the presence of young children.

42 persons ( 5 adults; 14 (5-16); 23 (under-5) ) excreted 1 type.  
11 persons ( 2 (5-16); 8 (under-5) ) excreted 2 different types.  
2 persons ( 1 (5-16); 1 (under-5) ) excreted 3 different types.

In those excreting 2 types, in the (5-16) age-group, the ages were 10 and 6 years. In the under-5's, 4 were under-1, 2 were 2 years, and the others 3 and 4 years of age.

In those excreting 3 types, the individual in the under-5 group was aged 1 year, and the individual in the (5-16) group was a 6-year-old in the same family, F 34.

The multiple excretion would appear to relate to the youngest members of the community.



Distribution of non-typable isolates - General -

168 non-typable agents were isolated, (Tab 11; Fig 9), in all seasonal groups, (Tab 17; Fig 17, 33); 48 in November; 33 in December; 15 in February/March; 29 in April/May; 24 in July and 19 in September, (Fig 20a,b).

The pattern was similar to that of the seasonal distribution of total isolates, (Tab 7; Fig 5), but less similar to that of the seasonal distribution of typable isolates (Tab 12; Fig 12).

They were excreted in all age-groups; 64 in adults; 48 in the (5-16) group; and 56 in the under-5's, (Tab 18; Fig 18).

The pattern of the distribution of non-typable isolates in age-groups (Tab 18; Fig 18) did not resemble closely the pattern of the specimen distribution by age-groups (Tab 3; Fig 2) nor that of the isolates by age-groups, (Tab 8; Fig 6).

Seasonal distribution-

Within each seasonal group the following distribution patterns will be compared -

- (1) Non-typable isolates by age-groups and seasons, (Tab 19; Fig 19)
- (2) Total isolates by age-groups and seasons, (Tab 9; Fig 7)
- (3) Typable isolates by age-groups and seasons (Tab 12; Fig 12)
- (4) Total specimens by age-groups and seasons, (Tab 6; Fig 4).

<u>Seasons</u>		<u>Total</u>	<u>Distribution in age-groups</u>
November	1. non-typable isolates	48	almost equal distribution in age-groups - (17/16/15)
	2. total isolates	71	most in the under-5's - (19/24/28)
	3. typable isolates	23	most in the under-5's - (2/8/13)
	4. total specimens	333	most in Adults - (170/94/69)
December	1. non-typable	33	most in adults and (5-16) - (16/13/6)
	2. total isolates	49	most in adult and (5-16) - (18/18/13)
	3. typable isolates	16	most in under-5's - (2/5/9)
	4. total specimens	352	most in adults - (172/102/78)
February/ March	1. non-typables	15	more even in groups, but numbers are small - (6/5/4)
	2. total isols.	28	most in under-5's - (7/9/12)
	3. typable isols.	13	most in under-5's - (1/4/8)
	4. total specimens	235	most in adults - (113/68/54)
April/ May	1. non-typables	29	(5-16) group much smaller - (13/2/14)
	2. total isols.	33	(5-16) group low - (13/2/18)
	3. typable isols.	4	small number in under-5's only - (-/-/4)
	4. total specimens	191	most in adults - (96/49/46)
July	1. non-typables	24	almost equal- small numbers - (9/7/8)
	2. total isols.	34	most in under-5's - (9/10/15)
	3. typable isols.	10	in (5-16) and under-5's only - (-/3/7)
	4. total specimens	198	most in adults - (89/52/57)
September	1. non-typables	19	most in under-5's - (3/5/11)
	2. total isolates	23	most in under-5's (3/6/14)
	3. typable isols.	4	in under-5's only - (-/-/4)
	4. total specimens	167	most in adults - (79/46/42)

The pattern of the non-typables was not as consistent as that of the typable isolates. This may have been due to less homogeneous components.

Seasonal distribution - based on Fig. 33 -

In the November, November/December and February/March groups, the non-typable isolates appeared to follow the excretion pattern of total isolates, especially the polios. In April/May, no polios were excreted but the non-typable pattern was still raised, although it did not follow the patterns of the Coxsackies and Echos.

In July/August, the pattern was again raised, when there were no polios, but Coxsackies were present and on the increase. In September, they again followed the isoate pattern, when polios and Echos were excreted but no Coxsackies.

Again, they appear to form a heterogeneous group.

Distribution in residential areas - (Tab 23; Fig 23, 36)

They occurred in all areas.

1 in each of Iwaya and Lagos Island; 2 in each of Abule Ijesha, Ebute Metta Isolo and Makoko; 3 in Oshodi; 4 in Idi Araba, Idi Oro, and Od Olowo; 19 in WACMR, Yaba; 23 in Shomolu; 26 in Ikorodu Road; 37 in Mushin and 38 in Surulere.

Residential areas and seasons (Fig 36)

In Idi Araba, there was a sporadic incidence of 3 isolates in July; in Ebute Metta, sporadic isolates of 1 each, in April and July; in Ikorodu Road, they occurred sporadically in all seasons - possibly with an increase in December, April/May and September; in Shomolu, they occurred in all seasons except April/May, with an increase in November; in Surulere, they occurred in all seasons, with more in November and April/May; in Mushin, in all seasons, but especially

in November, December and September; in WACMR, Yaba, they occurred in all seasons, except September; in Iwaya and Lagos Island, as 1 isolate each in December; in Odi Olowo, they appeared as scattered isolates in November, May and September; in Oshodi, scattered in November and May; in Isolo, in May and July; in Makoko, in November and May; in Idi Oro, scattered in November, December and May; and in AbuleIjesha, 1 isolate in November.

Again, a very varied distribution, which must be compared with the total specimens in the areas, (Tab 23; Fig 23) and the age-group distribution of persons in the areas, (Tab 24; Fig 24); and referred to the distribution of specimens by residential areas, by sexes and age-groups, (Tab 31; Fig 32) and the distribution of persons in the areas by sexes and ages, (Tab.32).

In Iwaya and Lagos Island, where 1 non-typable was isolated, the numbers involved were small - 2 and 1 isolates. In the latter, 1 male adult only was involved. There were more persons (Iwaya - (2/4/2) Lagos Island - (1/-/-))and specimens (Iwaya - (3/8/3) and Lagos Island (6/-/-) in the adult and (5-16) groups.

In Abule Ijesha, Ebute Metta, Isolo and Makoko, where 2 non-typables were isolated, the numbers involved were small - 3 isolates in each case. There were more persons and specimens in the older age-groups-

	Persons	Specimens
Abule Ijesha	- (3/1/2)	(13/5/4)
Ebute Metta	- (5/1/1)	(18/1/5)
Isolo	- (2/1/1)	(12/5/4)
Makoko	- (2/1/1)	(8/3/1)

In Oshodi, where 3 non-typables were isolated, there were 3 isolates only; more persons were in the oldest age-groups, (2/3/2); and more specimens also, (7/5/6).

In Idi Araba, Idi Oro and Odi Olowo, where 4 non-typables were found, isolates again were small - 5, 5 and 9. There were more persons and specimens in the oldest age-groups-

	Persons	specimens
Idi Araba	(4/4/-)	(18/20/-)
Idi Oro	(7/1/3)	(22/1/8)
Odi Olowo	(13/6/7)	(29/10/15)

In WACMR, Yaba, 19 non-typables were excreted. Isolates totalled 26; and persons were mainly in the oldest age-groups (21/8/11). Specimens were in the oldest age-groups, (71/29/39); 85 in males and 54 in females - with distribution by age in sexes - Male (49/19/17) and Female (22/10/22).

In Shomolu, 23 non-typables were excreted. Isolated totalled 33; and persons were mainly in the oldest age-groups, (39/28/16); and specimens were in the oldest groups - (140/78/43). 140 were in males and 121 in females, with age-distribution of (85/32/23) and (55/46/20) respectively.

In Ikorodu Road, 26 non-typables were isolated, from a total of 37 isolates. Persons were mainly in the oldest groups (30/19/14) and specimens also (106/54/55). 120 were in males and 95 in females, with age-distribution of (69/18/33) and (37/36/22).

In Mushin, 37 non-typables were excreted, with 44 total isolates. Persons were mostly in the oldest age-groups - (38/24/25) and also specimens - (135/83/93). 137 were from males and 174 from females; and in age-groups by sexes - (71/33/33) and (64/50/60).

In Surulere, 38 non-typables were excreted, with total isolates of 61. Persons were mainly in the oldest groups - (35/30/16) and specimens were also in the oldest - (131/109/70). 159 were in males and 151 in females; and in age-groups by sexes - (80/47/32) and (51/62/38).

Distribution in families - (Fig 35, 37)

They occurred in 65 families. Negatives were F 3, 14-16, 18-20, 28, 38, 39, 41, 43, 44, 46, 49, 55, 56, 64, 65, 67, 75, 79, 80, 83, 88-92 and 94. Detailed distribution will be discussed in later sections.

Distribution by sexes - (Tab 29; Fig 29)

The total non-typable isolates were 168, of which 90 were in males and 78 in females; with age-group distribution - (45/22/23) and (18/27/33). The pattern of distribution in females in age-groups was similar to the pattern of typable isolates, (Tab 29; Fig 30) with most isolates in the youngest age-group. The pattern in the males differed.

For comparison-

Typable isolates in age-groups (Tab 29; Fig 30)

Total - 70      Male - 28; female - 42  
by age-groups -      Male (2/12/14)      Female (3/9/30)

Specimens in sexes and age-groups (Tab 30; Fig 31)

Total - 1476      Male - 760; female - 716  
by age-groups -      Male (438/171/151)      Female (281/240/195)

Total isolates by sexes and age-groups (Tab 28; Fig 28)

Total - 238      Male - 118; female - 120  
by age-groups -      Male (47/34/37)      Female (21/36/63)



Multiple excretion - (Tab 21; Fig 22)

108 persons - in age-groups, (47/28/33) excreted 1 non-typable.

22 persons - " ( 7/8/7) excreted 2 non-typables.

5 persons - " (1/1/3) excreted 3 non-typables.

Comparing this with distribution in typable isolates, (Tab 20; Fig 21b), excretion of multiple typable isolates apparently followed the age pattern. In the case of the non-typables the pattern was irregular.

Discussion on non-typables-

From the above, no common factors have evolved to point to an identity for the non-typable isolates. Rather, it has appeared that they form a heterogeneous group.

Other viruses have been excreted and isolated in similar studies, (e.g. Gelfand et al, 1957a) and were described in the introduction.

They might be covered by the following-

(1) Viruses of the Polio, Coxsackie and Echo groups which did not react with specific antisera because of actual or technical differences.

(a) mixtures of viruses, including typable isolates. They could be separated by plaqueing on different cell cultures, (Hsiung and Melnick, 1958a) or by plaquing in known antiserum.

(b) prime strains of viruses exist-

Within the Coxsackie A group, mixtures of types and "prime" variants have been found, (Melnick, 1953; Wigand and Sabin, 1962b).

Some members of the Coxsackie B group do not cross with prototype antisera, (Davis and Melnick, 1958; Wigand and Sabin, 1962; Melnick, 1958), and break through in tube-cultures after a few days and require plaquing techniques for typing, (Davis and Melnick, 1958).

Some Echo viruses exist in prime forms, e.g. Echo 4, (Barron and Karzon, 1961; Yohn and Hammon, 1960) and Echo 6, (Karzon et al, 1959), and Echos 1, 3, 5 and 9, (Melnick, 1957c).

(c) low titre enteroviruses - some viruses are found in low titres and give rise to technical difficulties in typing. Some of the non-typables only had a TCID<sub>50</sub> of  $10^{-3}$ .

(2) Some enteroviruses were not tested for, Echo 26, 27, etc. for which no antisera were available at the time. Cytopathogenic forms of Coxsackie A (not A9) were not tested for, (Sickles et al, 1955, 1959; Dalldorf, 1957).

(3) The non-typables might be new serotypes - particularly in the case of the Echo viruses, (Comm. on Ent., 1957, 1962).

(4) They may belong to non-enteroviruses which passed through the intestine - e.g. reoviruses, adenoviruses, influenza etc (Gelfand et al, 1957a). These have been described in the introductory section.

(5) It was thought that simian viruses might give rise to confusion. These have been briefly described in the introduction.

In attempts to avoid picking-up these viruses, small batches of kidney cultures were prepared at a time, from 1 or 2 monkeys only.

The cultures were kept for the minimum time to avoid those viruses which had long incubation periods.

The non-typables did not appear in batches of cultures although certain simian viruses have been known to affect some tubes only.

It was thought that the presence of extraneous viruses, even if they did not produce a cytopathogenic effect, might interfere with the growth of isolates. Studies with certain simian viruses, (Hsiung et al, 1966), have shown that they do not block infection with other viruses, including polio virus.

It was found that Echo 1 and Echo 8 were similar, (Comm. on Ent., 1962). In this study neutralisation tests on isolates were carried out with antisera to Echo 1 and Echo 8. Apparently, there was no cross-reaction. Anamnestic responses also occurred among Echos, with other enteroviruses, (von Zeipel and Svedmyr, 1957; Hammon et al, 1958; Neva and Malone, 1959; Halonen et al, 1959; Schmidt et al, 1962; Bell et al, 1964) and with prime strains, (Melnick, 1965).

More detailed discussion of isolates -

Temporal distribution of typable isolates in Families (Fig 35, 37)

<u>Family</u>	<u>Date</u>	<u>Type</u>	<u>age</u>
F 1	July 12	E7	7
F 2	May 10	B5	1
F 4	Dec 1	P2	5
	3	P2	6/12
	Jul 18	E7	3
F 6	Jul 10	E 11	1
F 8	Nov 1	P2	9
	5	P2	under-1
	29	E7	under-1
F 10	Dec 1	B2	under-1
	Feb 22	E1	under-1
	23	B1	5
F 11	Sep 19	P3	under-1
F 13	Nov 3	B3	under-1
	Mar 7	E7	under-1
	Sep 14	P3	7
F 20	Nov 12	B2	8
F 22	Nov 2	P2	2
	Feb 27	P2	4
	Jul 13	B1	2
F 24	May 6	E7	1
F 25	Nov 1	E9	6
	Jul 13	B5	3, 6
F 29	Nov 1	P2	Adult (F)
F 30	Nov 1	P2	9
F 31	Dec 1	P2	6

<u>Family</u>	<u>Date</u>	<u>Type</u>	<u>Age</u>
F 34	Nov 1	P2	6, 4, 1
		E1	10
	Feb 27	P3	1,6,7,10
	28	P3	4
	Jul 10	B1	6
	Sep 11	E7	1
F 38	Nov 17	P2	1
F 40	Nov 29	E1	7,5,1
F 42	Nov 2	P2	3
F 46	Nov 2	P2	5
F 51	Nov 29	P2	8,2
	Jul 13	B4	under-1
	Sep 19	P1	2
F 53	Feb 22	E 11	3
F 54	Dec 4	E 1	Adult (M)
F 55	Nov 1	P2	4
F 56	Dec 4	P2	1
F 61	May 16	B1	2
F 62	Mar 21	P1	under-1
F 64	Nov 29	B1	Adult (F)
F 67	Dec 10	P2	3
F 69	Nov 7	E 9	3
F 71	Nov 29	P2	3
	Jul 16	B3	3
F 77	Mar 12	E 1	under-1
	Jul 13	B3	under-1
F 81	Nov 1	P2	Adult (F)
F 82	Nov 3	P2	under-1
	8	E 11	2
	Dec 1	P2	under-1
F 84	Nov 5	P2	1
	Mar 4	P3	Adult (M)
F 93	May 6	B1	3
F 95	Nov 8	P2	7

Different types occurring in families were spaced-out in time, except in -

F 8 - where P2 was excreted early in November and E 7, 24 days later in the same child. In the same family, P2 was excreted in 2 children, aged under-1 and 9; early in November. Either child could have brought the infection into the family to pass to other members, (Fox et al, 1955); perhaps in this case the 9-year-old school child was the index case.

Intra-familial spread was seen to be rapid, (Siegel and Greenberg, 1953; Zintek, 1947), with 7-14 days between infected individuals, (Pearson et al, 1945; Schabel et al, 1948; Littell and Smith, 1955). In the case of Coxsackie viruses, the interval was about 5 days, (Johnsson, 1954).

F 10- E 1 and B1 were both excreted in February, in 2 young children.

F 34- P2 and E 1 were excreted in November.

F 82- P2 and E 11 were found in very young children, in early November.

The under-1 year-old was still excreting P2 almost a month later.

Perhaps this prevented acquisition of infection of E 1 from the 2 year-old, due to normal interference between the 2 viruses.

No specific group interference has been noted between polios and Echos or between the latter and Coxsackie B's.

When polio and Coxsackie B viruses were excreted in the same family they were separated by time, (Curnen et al, 1949; Dalldorf, 1955, 1960), for example -

F 13 - B 3 was excreted in November, 1962 and P 3 the following September.



F 22 - P2 was excreted in November, 1962 and February, 1963; B1 was later seen, in July, 1963, in the child excreting P2 the previous November.

F 34 - P2 was excreted in November by several children, and P3 in February, again by several children; B1 occurred in July, in one of the children who had earlier excreted both P2 and P3.

Perhaps the B1 in the 2 children in July (F 22 and F 34) was unable to infect these children in the previous November, although it was in circulation (F 64) at that time, because these children were then excreting P2.

F 51 - P2 occurred in November and P1 in the following September; B4 was excreted in July, 1963.

F 71 - P2 was excreted in November and B3 in the following July, in the same child. B3 was in circulation (F 13) in November but perhaps it was blocked from infecting the child because of the P2 already present.

#### Infection in youngest children-

Most of the typable isolates were excreted by the youngest children, (Gard, 1960) <sup>(Olin and Wesslen, 1957; Dalldorf and Melnick, 1965)</sup>; and when multiple cases occurred in families most occurred in the youngest members, e.g. F 34 (Fig 37). This had been noted, (Casey et al, 1945; Wenner, 1962), earlier.

It was thought that, frequently, multiple infections were related to the number of persons in the family, (Siegel and Greenberg, 1953; Fox et al, 1956; Wenner, 1962), although others believed that there was no distinct association with family size, (Gelfand et al, 1957a).

In the present study, it was noted that, even if there were a number of children in a family, the absence of young children reduced the likelihood of the infection being passed on.

In F2 - Coxsackie B5 occurred in a 1 year-old. The only others in the family were a 15 year-old and 2 adults and the infection did not pass on.

In F 4 - there were 2 adults and 3 children of 5, 3 and 6/12, (Fig 37), and infection and types were multiple, including infection in the mother.

Distribution in the adults-

	<u>specimens</u>	<u>typable</u>	<u>non-typable</u>
Laboratory staff	69	-	4
Animal attendants	70	-	7
Others	218	-	25
Personal servants	38	1	2
Wives (mothers)	270	3	19

Horizontal spread was noted in children followed by vertical spread to older members of the family, (Gelfand, 1961). It was believed that spread from mothers was possibly greater than that from fathers, (Kalwij et al, 1959); and illnesses occurred with increasing frequency from fathers to mothers to children over-5 to children under-5, (Fox et al, 1966).

In this study, in F 29, P2 infection was seen in the mother, with nothing in the younger children. Perhaps infection had occurred in them earlier. In F 30, the mother excreted a non-typable isolate and a child of 9 excreted P2. The non-typable might have also been P2.

1 father, in F 84, excreted P3. The young child in the family was negative at the time.

There was no apparent increase in excretion of viruses in those whose occupations might have made them susceptible to infection,

e.g. laboratory staff and animal attendants, who might have been infected with simian viruses.

Personal servants - In studies in the Philippines, (Hammon et al, 1957a) and Kenya, (Walker, 1956), the importance was noted of family servants and their families in the spread of infection to expatriates. In the present study, few servants excreted viruses; a large number did not have families living with them.

Families who excreted no viruses-

11 families (F 14, 19, 28, 41, 80, 88 - 92 and 94) contained only 1 adult (Male) and few specimens.

3 contained only 2 adults and no children - F 15, 65, 79.

Others -

F 3 - 3 adults and 1 child of 15 years.

F16 - 2 adults and 1 child, aged 6 years.

F18 - 2 adults and 1 child, aged 16.

F39 - 2 adults and 1 child of 15.

F43 - 2 adults and 1 child of 1 year, but no specimens from the mother and child.

F44 - 2 adults and 3 children, aged 8, 6 and 4, but with specimens from the father only.

F49 - 2 adults and 6 children, aged 12, 12, 10, 10, 3 and 1, but with few and scattered specimens.

F75 - 3 adults and 6 children, aged 13, 8, 8, 4, 4, and 3, with few specimens.

F83 - 2 adults and 1 child, under-1, but few specimens were received.

Families excreting non-typable isolates with typable isolates-

<u>Family</u>	<u>Adults</u>	<u>Children</u>	<u>Age</u>
Excreting 1 non-typable -			
F 2	2	2	15, 1
F 8	2	3	14, 9, under-1
F 95	2	6	7, 6, 5, 5, 4, 2
F 38	2	3	12, 3, 1
F 61	2	5	11, 10, 6, 4, 2
F 81	3	2	3, 1
F 82	3	7	16, 16, 9, 7, 2, 2, under-1
F 93	2	2	3, under-1
Excreting 2 non-typables-			
F 29	2	2	9, 3
F 30	2	2	9, under-1
F 40	2	7	13,12,9,7,5,3,1
F 51	3	7	15, 11, 11, 8, 4, 2, under-1
F 62	2	4	7,4,3,under-1
F 69	2	4	14,11,6,3
F 77	5	7	10,9,7,6,2,1,under-1
F 84	2	1	1
Excreting 3 non-typables-			
F1	3	4	13,12,7,5
F 22	2	3	4,2,under-1
F 25	2	4	13, 10, 6, 3
F 53	2	4	13,7,4,3
F 6	2	6	15,10,3,1,1,1 (triplets)
F 24	2	3	16,3,1
F 31	3	5	16,9,6,6,6
F 42	2	4	8,7,5,3
F 10	2	8	13,11,7,5,5,3,under-1,under-1 twins.
F 11	3	10	11,8,6,4,4,(twing),2; 2,2,(twins), under-1, under-1, (twins).
F 13	2	5	13,7,5,3,under-1

3 non-typables (continued)

F 54	6 adults	4 children	14,10,2,1
F 71	2	4	8,5,3,under-1

7 non-typables-

F 4	2	3	5,3,6/12
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10 non-typables-

F 34	2	8	14,12,10,9,7,6,4,1
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(The non-typables are not necessarily different)

There were no particular factors in the excretion patterns to point to the identity of the non-typable isolates.

Some families excreted non-typable isolates (in some cases, multiple),  
without typable isolates-

<u>Family</u>	<u>Adults</u>	<u>Children</u>	<u>Age</u>
1 non-typable-			
F 17	1		
F 32	1		
F 48	1		
F 68	1		
F 60	2		
F 66	2		
F 86	2		
F 23	2	1	12
F 33	2	1	under-1
F 37	2	1	under-1
F 52	2	2	1,1 (twins)
F 59	2	1	3
F 70	2	4	9,5,3,1
F 74	2	2	4,3
F 5	3	3	9,7,5
F 45	3	2	3,1
F 72	3	3	8,3,under-1
F 12	5	2	13,11
F 50	5	6	16,15,13,11,7,4
2 non-typables-			
F 21	1		
F 57	1		
F 73	1		
F 87	1		
F 7	2	2	5,2
F 76	2	2	9,5
F 63	4	3	10,8,8
3 non typables-			
F 26	2	5	13,12,5,2,1
F 27	3	3	10, 7,5
F 35	3	1	8
F 78	3	2	14,12



4 non-typables-

F 58	2	1	under-1
F 85	2	4	7,5,2,under-1

6 non-typables-

F 9	2	4	9,3,3,(twins), under-1
F 47-	3	4	11,7,6,1

8 non-typables-

F 36	4	3	11,7,4
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There were possibly fewer children in the above families than in those excreting non-typables with typable isolates.

Distribution of non-typable isolates in families - (Fig 35, 37)

<u>Family</u>	<u>Isol.</u>	<u>Date</u>	<u>Age</u>
F1	1	Nov 1	13
	1	Nov 30	Adult (M)
	2	July 12	Adults (M,F) - <u>E 7</u> in 7 year-old on July 12.
F 2	1	July 11	Adult (M)
F 4	1	Nov 1	6/12
	1	Nov 5	Adult (F) - <u>P2</u> in children of (mother) 6/12 and 5 on Dec 1,3.
	1	Apr 30	Adult (F)(mother)
	1	May 2	3
	1	May 6	6/12
	1	Sep 12	6/12
	1	Sep 13	5
	F 5	1	Jul 13
F 6	1	Feb 27	1
	1	Apr 30	1*
	2	Sep 11	1, 1*
			(* denotes different child of same age)
F 7	1	Feb 27	Adult (M)
	1	Mar 2	Adult (F)
F 8	1	Dec 1	Adult (M)- <u>P2</u> in under-1 and 9, on Nov 1,5; <u>E7</u> in under-1 on Nov 29.
F 9	2	Nov 2	9, under-1
	1	Dec 3	3
	1	Jul 10	under-1
	1	Sep 18	under-1
	1	Sep 19	3

<u>Family</u>	<u>Isol.</u>	<u>Date</u>	<u>Age</u>	
F 10	1	Nov 30	5*	
	1	Dec 10	7	<u>B 2</u> in under-1 on Dec 1.
	1	Feb 23	11	
	1	Feb 26	5	<u>B1</u> in 5* on Feb 23; <u>E1</u> in under-1 on Feb 22.
	1	Jul 12	5*	
F 11	1	Nov 2	under-1	
	1	May 13	4	
	1	May 30	6	
	1	Jul 17	under-1*	
	1	Sep 19	6	<u>P3</u> in under-1* on Sep 19.
F 95	1	Dec 13	4	<u>P2</u> in 7 year-old on Nov 8.
F 12	1	Apr 30	Adult (M) (child)	
F 13	1	Dec 4	Adult (M)	<u>B3</u> in under-1 on Nov 3.
	1	Mar 7	3	<u>E7</u> in under-1 on Mar 7.
	1	May 7	under-1	
	2	Sep 13	3, under-1	<u>P3</u> in 7 year-old on Sep 14.
F 17	1	Dec 5	Adult (M)	
F 21	1	May 2	Adult (M)	
	1	Jul 13	Adult (M)	
F 22	1	Nov 6	under-1	<u>P2</u> in 2 year-old on Nov 2.
	2	May 6	2, Adult (M)	- perhaps infection from child or vice versa, (Fox et al, 1955).

<u>Family</u>	<u>Isol</u>	<u>Date</u>	<u>Age</u>	
F 23	1	Nov 1	Adult (M)	
F 24	2	Nov 1	Adult (M), 3	
	1	Nov 30	Adult (M)	perhaps still excreting from Nov 1.
	1	Feb 22	1	
F 25	1	Nov 1	3	<u>E9</u> on Nov 1 in 6 year-old.
	1	Nov 30	6	excreting <u>E9</u> on Nov 1.
	1	Apr 30	3	
F 26	2	Nov 6	2, 5	
	1	May 8	1	
F 27	3	Dec 5	Adult (M)(father) Adult (M) (child) 7	
F 29	1	May 13	3	
	1	Jul 12	9	
F 30	1	Nov 3	Adult (F) (mother)	<u>P2</u> in 9 year-old on Nov 1.
	1	May 11	Adult (M)	
F 31	2	Nov 3	6, 16	<u>P2</u> in 6 year-old on Dec 1.
	1	May 11	Adult (F)(mother)	
	1	Sep 17	6*	
F 32	1	Apr 30	Adult (M)	
F 33	1	Nov 2	Adult (M)	
F 34	2	Nov 1	9, 12	<u>P2</u> in 6, 4, 1 on Nov 1. <u>E1</u> in 10 on Nov 1.
	3	Nov 29	9, 10, 14	<u>E1</u> in 10 on Nov 1.
	1	May 1	1	
	3	Jul 10	10,4,1	
	1	Jul 11	14	<u>B1</u> in 6 year-old on Jul 10.

<u>Family</u>	<u>Isol</u>	<u>Date</u>	<u>Age</u>	
F 35	1	Nov 6	Adult (M)	(father)
	1	Dec 4	Adult (M)	"
	1	May 1	Adult (M)	"
F 36	3	Nov 1	4,7,11	
	2	Dec 8	4,7	
	1	Dec 10	Adult (M)	(father)
	1	Dec 12	Adult (F)	(mother)
	1	Dec 13	Adult (M)	(child)
F 37	1	Sep 14	Adult (M)	
F 38	1	Nov 2	Adult (M)	<u>P2</u> in 1 year-old, on Nov 17.
F 40	1	Nov 2	Adult (M)	<u>E1</u> in 5, 7, 1 on Nov 29.
	1	Jul 10	Adult (F)	(mother)
F 42	3	Jul 11	Adult (F)	(mother) 7, 8
	1	Sep 14	5	
F 45	1	Jul 24	1	
F 47	1	Nov 30	Adult (M)	(child)
	1	Dec 1	7	
	1	Feb 23	Adult (M)	(father)
	1	Mar 1	7	
	2	May 8	Adult (F)	(mother), 1
F 48	1	Dec 4	Adult (M)	
F 50	1	Dec 6	15	
F 51	1	Feb 27	15	
	1	Sep 19	4	<u>P1</u> in 2 year-old on Sep 19.
F 52	1	Sep 12	1	
F 53	3	Nov 1	7, 4, 3	

<u>Family</u>	<u>Isol</u>	<u>Date</u>	<u>Age</u>	
F 54	1	Nov 6	10	
	1	Dec 1	Adult (F)	<u>E1</u> in Adult (M) (child)
	1	Dec 4	10	on Dec 4.
	2	May 8	10, 1	
F 57	1	Nov 29	Adult (M)	
	1	May 1	Adult (M)	
F 58	1	Nov 2	under-1	
	1	Dec 13	under-1	
	1	Mar 1	Adult (f)(mother)	
	1	Sep 14	under-1	
F 59	1	Nov 15	3	
F 60	1	Nov 1	Adult (M)	
F 61	1	May 15	Adult (M)(father)-	<u>B1</u> in 2 year-old on May 16.
F 62	1	Apr 30	3	
	1	Jul 26	under-1	
F 63	1	Nov 1	Adult (M)(child)	
	1	May 6	Adult (M)(father)	
F 66	1	Dec 3	Adult (M)	
F 69	1	Nov 1	Adult (M)	
F 69	2	Nov 1	14, 11	<u>E9</u> in 3 year-old on Nov 7.
F 70	1	Dec 3	9	
F 71	1	Nov 29	5	<u>P2</u> in 3 year-old on Nov 29.
	2	Feb 28	Adult (F)(mother) 8	
	1	Jul 16	8	<u>B3</u> in 3 year-old on Jul 16.
	1	Sep 14	3	
F 72	1	Nov 1	8	
F 73	1	Nov 2	Adult (M)	
	1	Sep 16	Adult (M)	
F 74	1	Nov 1	3	



<u>Family</u>	<u>Isol</u>	<u>Date</u>	<u>Age</u>
F 76	2	Sep 14	Adult (F)(mother), 9
F 77	1	Nov 7	2
	1	May 7	Adult (M)(father)
F 78	2	Nov 1	Adult (F)(mother), Adult (M)(child)
	1	Nov 2	14
F 81	1	Nov 1	Adult (M)(child) <u>P2</u> in Adult (mother) and 3 year-old on Nov 1.
F 82	1	Mar 2	under-1
F 84	2	Jul 18	Adult (F)(mother), 1
F 85	2	Nov 2	7, under-1
	1	Nov 3	Adult (F)(mother)
	1	May 1	2
F 86	1	Nov 1	Adult (F)
F 87	1	Feb 28	Adult (M)
	1	Jul 13	Adult (M)
F 93	1	Jul 11	under-1

From the above, by proximity to other members of the family excreting typable viruses, some of the non-typables may be one, or a mixture of more than one, of the following -

Polio 2, 3; Coxsackie B 1, B 2, B3; or Echo 1, 7 or 9.

It has been seen that spread in the family is greater than that to the community, (McCarroll et al, 1955; Clemmer et al, 1966; Brown et al, 1954; Gelfand et al, 1957c; Siegel et al, 1955).

This intra-familial spread is seen in this study, including possible infection from children to parents, e.g. in F 22.

Distribution of typable isolates in residential areas (Fig 36)

<u>Area</u>	<u>Date</u>	<u>Type</u>	<u>Family</u>	
Idi Araba	Jul 12	E7	F1	
Ebute Metta	May 10	B5	F2	
Ikoro Road	Nov 1	P2	F8	
	2	P2	F42	
	5	P2	F8	
	29	E7	F8	
	Dec 1	P2	F4	
	3	P2	F4	
	4	P2	F56	
	10	P2	F 67	
			P2 and E7 in the same area at the same time.	
		Mar 21	P1	F62
	Jul 10	E11	F6	
	18	E7	F4	
Shomolu	Nov 2	P2	F46	
	3	P2	F82	
	7	E9	F69	
	8	E11	F82	
	29	P2	F71	
			E1	F40
		Dec 1	P2	F82
	Jul 16	B3	F71	
		- P2, E1, E9, E11 in the same area at the same time.		
Surulere	Nov 1	P2	F34	
		E1	F34	
		E9	F25	
	2	P2	F22	
	3	B3	F13	
	Dec 4	E1	F54	
		- P2, B3, E1, E9 in same area at same time, but in different families.		

<u>Area</u>	<u>Date</u>	<u>Type</u>	<u>Family</u>
Surulere (continued)	Feb 22	E11	F22
	27	P2	F22
		P3	F34
	28	P3	F34
	Mar 7	E7	F13
			P2, P3, E7, E11 in same area at same time.
	May 16	B1	F61
	Jul 10	B1	F22
	13	B1	F34
		B5	F25
Sep 11	E7	F34	
14	P3	F13 - P3 and E7 in same area at same time.	
Mushin	Nov 29	P2	F51
		B1	F64
	Dec 1	P2	F31 - P2 and B1 in same area at same time but in different families.
	Jul 13	B4	F51
	Sep 19	P1	F51
		P3	F11
WACMR, Yaba	Nov 5	P2	F84
	Dec 1	B2	F10 - P2 and B2 in same area at same time but in different families.
	Feb 22	E1	F10
	23	B1	F10
	Mar 4	P3	F84 - P3, B1 and E1 in the same area at the same time; but the polio and Coxsackie B viruses were in different families.
	May 6	B1	F93
		E7	F24

<u>Area</u>	<u>Date</u>	<u>Type</u>	<u>Family</u>
Iwaya	Nov 8	P2	F95
Odi Olowo	Nov 1	P2	F81
	12	B2	F20 - P2 and B2 in same area at the same time, but in different families.
	Mar 12	E1	F77
	Jul 13	B3	F77
Isolo	Nov 1	P2	F29
Makoko	Nov 1	P2	F30
Idi Oro	Nov 1	P2	F55
Abule Ijesha	Nov 17	P2	F38

Lagos Island and Oshodi had no typable isolates.

One type only was excreted in 6 areas. In Idi Araba and Ebute Metta, there were isolates of Echo 7 and Coxsackie B5, respectively.

Polio 2 was excreted, during a general outbreak in Iwaya, Isolo, Makoko, Idi Oro and Abule Ijesha; the limited isolates probably being due to the population and specimen make-up of the areas at the different times- as has been discussed.

Where multiple type excretion occurred, this was relatively spaced out -

Ikorodu Road- in November, there was excretion of polio 2, in an outbreak, and Echo 7, as a sporadic "case". In December, the polio 2 continued. A sporadic isolate of polio 1 occurred in March, followed by Echos 7 and 11 in July.

No Coxsackie B viruses were typed in this area, perhaps owing to the heavy P2 infection, (Melnick, 1950; Dalldorf, 1951, 1955, 1960).

The non-typables will be discussed in a later section.

Shomolu - Polio 2 was excreted, during the outbreak, in November and into December, with sporadic Echos 1, 9 and 11. Following this, there were no typable isolates until July, 1963, when Coxsackie B3 was found. The polio 2 and B3 excretion was well separated in time, (Dalldorf, 1960).

Surulere- in November, polio 2 was excreted, apparently early in the month. Coxsackie B3 also occurred, in a family not excreting polio 2. Echo 9 was seen early in November; Echo 1 occurred early in November and again in early December - suggesting that it might have been passing in the neighbourhood in the intervening time.

In February, polio 2 again occurred, as 1 isolate; Polio 3 took the form of a small family outbreak ; Echo 11 again occurred as a sporadic case. In March, Echo 7 occurred sporadically.

Polio 2 and Echo 9 both occurred in November in Surulere and in Shomolu. It has been noted that polio 2 and Echo 9 may have a synergistic action on the central nervous system, (Pette et al, 1961) and have been isolated together in a clinical case, (Verlinde et al, 1961).

In May, the only typable isolate was Coxsackie B, with non-typable isolates. In July, B1 was again isolated, apparently with an increased spread, as also B5. By September, the excretion was taken over by polio 3 and Echo 7, both of which had occurred a few months earlier.

Mushin- Polio 2 was isolated at the end of November and beginning of December. B1 also occurred late in November, in a different family. These were ~~preceded~~ and followed by a large number of non-typables. Apart from the non-typables, there was only a sporadic isolate of B4 the following July and polio 1 and 3 in September.

WACMR, Yaba - polio 2 was excreted early in November, with non-typables, followed by B2 early in December, in different families. Late in February, Echo 1 and Coxsackie B1 were found in the same family, and, early in March, polio 3, in a different family.

Early in May, B1 was again excreted, and Echo 7, again in different families. Perhaps the B1 had been circulating in the area from late February to early May.

Odi Olowo- Polio 2 occurred early in November, followed by a sporadic isolate of B2, in the middle of the month, in different families. In March, Echo 1 only was excreted, as a sporadic isolate; and one isolate of B3 in mid-July.

The type patterns varied in the different areas, possibly related to the make-up and immunity of the families; distribution in a community being selective, (Francis, 1952b).



Detailed discussion of typable isolates and their distribution in residential areas- (Fig 33, 35, 36, 37)

Polio 1-

<u>Area</u>	<u>Date</u>	<u>Age</u>	<u>Family</u>
<u>Ikorodu Road</u>	March 21	under-1	F 62
<u>Mushin</u>	Sep 19	2	F 51

These isolates, scattered in time and place, and in very young children, still appear to be the normal acquisition of immunity in a population, whose own immunity may have been gained within the past 2 to 3 years. On the other hand, the isolate in the Ikorodu Road area is followed by a number of non-typable isolates. If these include polio 1, the pattern may change. They will be discussed later.

The isolate in Mushin, (Fig 36) occurred with non-typables and at the end of the study. Further cases may have followed and suggested a less immune community. Its occurrence may have been blocked by the polio 2 outbreak and the presence of Coxsackie B viruses, increasing from May onwards, (Fig 33).

Polio 2-

<u>Area</u>	<u>Date</u>	<u>Age</u>	<u>Family</u>
<u>Ikorodu Road</u>	Nov 1	9	F 8
	2	3	F 42
	5	under-1	F 8
	Dec 1	5	F4
	3	6/12	F 4
	4	1	F 56
	10	3	F 67

<u>Area</u>	<u>Date</u>	<u>Age</u>	<u>Family</u>
<u>Shomolu</u>	Nov 2	5	F 46
	3	under-1	F 82
	29	3	F 71
	Dec 1	under-1	F82
<u>Surulere</u>	Nov 1	4	F 34
		1	F 34
		6	F 34
	2	2	F 22
	Feb 27	4	F 22
<u>Mushin</u>	Nov 29	2	F 51
		8	F 51
	Dec 1	6	F 31
<u>WACMR, Yaba</u>	Nov 5	1	F 84
<u>Iwaya</u>	Nov 8	7	F 95
<u>Odi Olowo</u>	Nov 1	Adult (F)	F 81
		3	F 81
<u>Isolo</u>	Nov 1	Adult (F)	F 29
<u>Makoko</u>	Nov 1	9	F 30
<u>Idi Oro</u>	Nov 1	4	F 55
<u>Abule Ijesha</u>	Nov 17	1	F 38

All areas, except Mushin, were excreting polio 2 early in November. The isolates in Mushin were preceded by a number of non-typables which might have included polio 2. By the time the study began, the outbreak was at its height, which makes it impossible to trace a path of infection from one area to another. Early studies in the USA, (Casey, 1945), put the "speed" of radial spread of infection as approximately 10 to 11 days, for the distance of 1 mile.

The only area where excretion was maintained was Surulere, where an isolate occurred in February. It had occurred in another young

child of the same family (F 22) in November, and may have been passing unnoticed in the interim period.

Occurrence in individuals in age-groups-(Fig 37)

Adults- (1) Mother in F 81 - with a child of 3, also excreting P2. There was another child, aged 1, in the family, and an "adult" child; it is possible that the mother had avoided previous infection with this virus until this time.

(2) Mother in F 29 - with children of 3 and 9 (negatives) in the family; and living in the more "rural" area of Isolo, has not acquired an immunity previously. The date of excretion was Nov 1, and it is possible that the children may have excreted the virus earlier and ceased to excrete with the onset of immunity, (Brown and Ainslie, 1951; Brown, 1955), and been missed in the study.

(5-16) group- a total of 8 excreted, the 2 oldest being 9 year of age.

(1) 9 year-old - in F 8 - with child of under-1 also excreting P2. Others in the family were adults or aged 14 (negatives) and may have become immune and ceased to excrete, or may have acquired immunity some years earlier. As infection is more likely to pass from the younger to the older individuals, the latter state is the more likely.

(2) 9 year-old - in F 30 - the only other child in the family was not born at the time of the outbreak, (ind. no. 453). The mother excreted a non-typable, possibly polio 2.

(3) 8 year-old - in F 51 - with 2 year-old excreting P2. Others in the family (negatives) were adults and aged 4, 11 and 15. The baby (no. 447) was not born at the time of the specimen.

(4) 7 year-old <sup>in F 95</sup> -/isolated case - younger children in the family but no isolates. The isolate was on Nov 8; perhaps the others had already acquired immunity.

(5) 6 year-old - in F 34 - with children of 1 and 4 excreting P2. A child of 10 excreted Echo 1 on Nov 1, the date of the above isolates; perhaps this child was already immune to polio 2 from a previous outbreak; and 2 children, aged 9 and 12, excreted non-typables on the same date. Perhaps these were polio 2, or Echo 1, or mixtures. Or, if immunity to polio 2 begins around 10 years-of-age, the 9 year-old may <sup>have</sup> excreted polio 2 and the 12 year-old, Echo 1.

(6) 6 year-old - in F 31 - this isolate was excreted on Dec 1. This child excreted a non-typable on Nov 3; perhaps this was also P2. Others in the family, adults, and children of 6, 9 and 16, were negatives.

(7) 5 year-old - in F 4 - with a child of 6/12 also excreting P2; the infection could have spread either way, (Fox et al, 1955). Adults and a 3 year-old in the family were negative.

(8) 5 year-old - in F 46 - was the only child in the family. The parents were negative.

under-5 group -

(1) 4 year -old - in F 22 - excreted in February following excretion in November by 2 year-old; a child, under-1 year, excreted a non-typable on Nov 6, so it is just possible that the virus circulated in the family long enough to be isolated, without a break, in February, allowing for the length of time polio viruses have been found in faeces. This was described earlier.

- (2) 4 year-old - in F 34 - with other members of the family as described in (5-16) no. (5).
- (3) 4 year-old - in F 55 - adults and children, 2 and under-1, were negative. This isolate was on Nov 1; perhaps the other children had already acquired immunity.
- (4) 3 year-old - in F 42 - adults and other children, 5, 7 and 8, were negative; possibly already immune during the outbreak.
- (5) 3 year-old - in F 67 - only child in the family. The parents were negative.
- (6) 3 year-old - in F 71 - Adults and 8 year-old were negative. But a 5 year-old excreted a non-typable on the same day as the isolate. The 8 year-old and the mother later excreted non-typables on Feb 28. As in (1) above, these may have been polio 2. The baby, (no. 456) was not born at the time.
- (7) 3 year-old - in F 81 - the mother excreted polio 2- see Adult (1) above.
- (8) 2 year-old - in F 22 - as discussed in (1) above.
- (9) 2 year-old - in F 51 - 8 year-old excreted polio 2, as in (5-16) no (3), above.
- (10) 1 year-old - in F 34 - as described in (2) above.
- (11) 1 year-old - in F 38 - mother and 2 children, 3 and 12, were negative. The father, excreted a non-typable on Nov 2. This <sup>Polio 2</sup>/isolate was excreted on Nov 17. As infection generally passes to the adult, and the specimens from the other young children were before the isolate and negative, (Fig 35), it <sup>is</sup> not so likely that the father was excreting polio 2. On the other hand, the 1 year-old may have been excreting for some time prior to the specimen and passed infection to the father.

- (12) 1 year-old - in F 56 - adults and children, 7 and 10, negative.
- (13) 1 year-old - in F 84 - parents were negative.
- (14) under-1 - in F 4 - this child excreted a non-typable on Nov 1, possibly polio 2, and still excreting on Dec 3. Others in the family were described in (5-16) no (7) above.
- (15) under-1 - in F 8 - excreted with a 9 year-old, see (5-16) no (1) above.
- (16) under-1 - in F 82 - this child excreted polio 2 on Nov 3 and Dec 1. Adults and children, 16, 9 and 7 years-old, were negative; but a child of 2 excreted Echo 11 on Nov 8.

From the above, infection in an adult or older child was associated with excretion in a younger child in most cases.

The 2 infections in the mothers may have been isolated "cases" owing to low immunity in these 2 individuals, rather than to lack of immunity in adults in the community in general. Infection appeared to spare those of around 10 year of age and above. For example, in F 34, a child of 10 was infected with Echo 1, when polio 2 was abundant in the family and affected younger children. This could suggest that polio 2 may have been absent from the community, to any great extent, for up to 9 to 10 years, leaving a relatively non-immune population younger than this.

Areas apparently negative for polio 2 -

Idi Araba - 2 families only

(1) F 1 - an adult (M) excreted a nontypable isolate on Nov 30 and a child of 13 excreted a non-typable on Nov 1. These may have been polio 2, in which case the age-level affected is raised; they will be discussed later.



(2) F 80 - contained 1 male adult only and all specimens were negative.

Ebute Metta- 3 families -

(1) F 2 - 1 child of 1 and one of 15 only.

(2) F 32 - 1 adult only.

(3) F 79- 2 adults only.

Lagos Island - 1 adult only, <sup>in F 17</sup> who excreted a non-typable on Dec 5.

This could have been polio 2, but unlikely from the argument above.

Oshodi - F 26 - 2 children, aged 2 and 5, excreted non-typables on Nov 6. These could have been polio 2.

Polio 3 -

<u>Area</u>	<u>Date</u>	<u>Age</u>	<u>Family</u>
<u>Surulere</u>	Feb 27	6,7,10	F 34
		1	F 34
	28	4	F 34
	Sep 14	7	F 13
<u>Mushin</u>	Sep 19	under-1	F 11
<u>WACMR, Yaba</u>	March 4	adult	F 84

Polio 3 did not occur in the community until late in February, with the outbreak of 5 "cases" in F 34, in Surulere. This was followed by an apparent disappearance in the area until September. However, in the intervening period, in the area, there were a number of non-typables in April and May and the increase in Coxsackie excretion in July. This was a similar pattern to the disappearance of polio 1 from Ikorodu Road in March to its reappearance in September in a different area, Mushin. 1 isolated "case" <sup>of Polio 3</sup> occurred in early March, in contiguous Yaba.

Occurrence in individuals in age-groups - (Fig 37)

Adult - Father in F 84 - (possibly aged 55-60) - mother and child of 1, negative, although they may have been positive earlier and now acquired immunity and ceased to excrete virus. The individual's immunity may have been low; this child was the first child in the family for almost 10 years. Previous child died about 8-9 years before when under-1. (The individual was the author's steward).

(5-16) group -

(1, 2, 3) - 10, 7 and 6 year-olds in F 34 - children of 4 and 1 also excreted polio 3 at the same time, in February. Parents and children of 12 and 9 were negative.

(4) - 7 year-old - in F 13 - children of 3 and under-1 excreted non-typables at the same time, in September. Parents and children of 13 and 5 were negative at the time of the specimens.

under-5 group-

- (1) 4 year-old - in F 34 - see (5-16) nos. (1,2,3) above.
- (2) 1 year-old - in F 34 - see (5-16) nos. (1,2,3) above.
- (3) under-1 - in F 11 - child of 6 excreted non-typable at same time; possibly polio 3. Others in large family negative at time. Perhaps infection was just beginning in family, area and community.

From the above, the isolate in the adult could be due to lack of immunity in the individual, rather than lack of immunity in adults in the community in general.

In the younger groups, isolates occurred in those under 11 years in 2 families only, in what were obviously family outbreaks at this stage. Whether this was due to lack of immunity in these families as individual groups within the community or could be reflected

in the community at large, was impossible to state . If the study had continued for another year, the pattern would have shown up.

Assuming that it can be extended into the community, it would appear that polio 3 immunity was low in those aged somewhere between 7 and 10 years of age. This assumed also that excretion of the virus meant lack of immunity and absence of excretion, immunity.

Carrying this into a comparison of polio 1, 2 and 3, there appeared to be lack of immunity to polio 1, say under 2 to 3 years of age; to polio 2, under 10 years of age, and to polio 3, under 7-plus years of age. As a polio 2 outbreak occurred in 1962 resulting in a community immune to this virus, and polio 1 seemed to have been a relatively recent infecting agent, polio 3 might be looked upon as the next of the group to cause an outbreak.

This would be confirmed by serological studies.

Coxsackie B-

B1-

<u>Area</u>	<u>Date</u>	<u>Age</u>	<u>Family</u>
<u>Surulere</u>	May 16	2	F 61
	July 10	2	F 22
	13	6	F 34
<u>Mushin</u>	Nov 29	Adult (F)	F 64
<u>WACMR, Yaba</u>	Feb 23	5	F 10
	May 6	3	F 93

B2-

<u>WACMR, Yaba</u>	Dec 1	under-1	F 10
<u>Odi Olowo</u>	Nov 12	8	F 20

B3-

<u>Shomolu</u>	Jul 16	3	F 71
<u>Surulere</u>	Nov 3	under-1	F 13
<u>Odi Olowo</u>	Jul 13	under-1	F 77

B4-

<u>Mushin</u>	Jul 13	under-1	F 51
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B5-

<u>Ebute Metta</u>	May 10	1	F 2
<u>Surulere</u>	Jul 13	3, 6	F 25

From the above, and allowing for the small numbers, the isolates of B 1, scattered in place, time and age-groups, might suggest a low immunity in the population. B2 occurred in November and December only, thereafter disappearing, as though an outbreak had just occurred. B3, 4 and 5 appeared as sporadic isolates. The distribution in ages will be studied.

Occurrence in individuals in age-groups - (Fig 37)

Cox Bl

Adult - Wife in F 64 - on Nov 29. No children in the family, and the individual had recently arrived in Lagos from her home country in the east. No other viruses were isolated in the family.

(5-16) group-

(1) 6 year-old - in F 34, on Jul 10 - other children, aged 14, 10, 4 and 1, excreted non-typables on Jul 10 and 11. These may have been Bl.

(2) 5 year-old - in F 10, on Feb 23 - other children, aged 11, 5, excreted non-typables on Feb 23 and 26. And 1 child, of under-1, excreted Echo 1 on Feb 22. Perhaps the non-typables were Bl or El, or a mixture.

under-5 group -

- (1) 3 year-old - in F 93, on May 6 - isolated case in the family.
- (2) 2 year-old - in F 22, on Jul 10 - isolated case in the family.
- (3) 2 year-old - in F 61, on May 16 - Adult (M) (father) excreted non-typable on May 15. This could have been Bl.

The adult isolate, in F 64, may have been a non-immune person coming from a distant area, and not a true reflection of the community under study. Otherwise, the age scatter was between 2 and 6 years. Allowing for possibles in the non-typables, this might be spread to between 14 and 1 year of age, with an additional adult isolate.

The distribution in 3 areas and 6 families, over a period of at least 8 months, had the pattern of a sporadic infection; however the age-incidence suggested a less-immune community. As already mentioned Bl occurred, after an absence, in certain areas of the world around the early 1960's, (Gear, 1961/62; Gelfand et al, 1963).

Cox B2-

(5-16) group - 8 year-old, in F 20/<sup>Nov 12</sup>- isolated excretion.

under-5 group - under-1 year old, in F 10, on Dec 1. In the family, children of 5 and 7 excreted non-typables on Nov 30 and Dec 10.

These could have been B2.

The numbers involved were very small. Following these localised isolates the type did not recur. Allowing for the non-typables being included in the group, this may have been the end of an outbreak, which occurred before the beginning of the study and prior to the polio 2 outbreak; perhaps at the time of year in 1962 comparable to the season in 1963, in which the Coxsackie group showed an increase - i.e. in July. The non-recurrence might suggest a non-immune community.

On the other hand, the isolates may have been sporadic "cases", the type being blocked by the onset of polio 2 infection in the community, before it could gain any real access to the population.

The former is probably the more likely.

Cox B3-

under-5 group -

(1) 3 year-old - in F 71, on Jul 16. Another child, aged 8, in the family excreted a non-typable on Jul 16; possibly B3.

(2) under-1 year old - in F 13, on Nov 3. Adult (M) (father) excreted a non-typable on Dec 4 - possibly B3.

(3) under-1 year-old - in F 77, on Jul 13. An isolated case.

If the typed isolates are taken into consideration, only the youngest children were involved, suggesting an immune community with infection in the non-immune youngest section. If the non-



typables are noted, older individuals would be included in the group, and suggest a less-immune population.

Cox B4 -

under-5 group - under-1 year-old - in F 51, on Jul 13. An isolated case.

Generalising on 1 isolate, this appears to be the acquisition of immunity of a very young child in a community with considerable immunity. The isolate occurred at a time when polio viruses were absent, and so, if other non-immune people had existed, there would have been no reason for non-passage of the virus.

Cox B5-

(5-16) group - 6 year-old - in F 25, on July 13; in the same family-  
under-5 group - (1) 3 year-old - in F 25, on July 13. Adults and older children, of 13 and 10, were negative.

(2) 1 year-old - in F2 on May 10. An isolated case in a family; Adults and child of 15 years, were negative.

Again, small numbers are involved for generalisation. The older child excreting, was in the same family as a younger one, rather than in the community. Otherwise, very young children only were involved at a time when infection could have spread in non-immunes.

Echoviruses-

Echo 1-

<u>Area</u>	<u>Date</u>	<u>Age</u>	<u>Family</u>
<u>Shomolu</u>	Nov 29	7,5	F 40
		1	F 40
<u>Surulere</u>	<del>Nov 10</del>	10	F 34
	Dec 4	Adult	F 54
<u>WACMR, Yaba</u>	Feb 22	under-1	F 10
<u>Odi Olowo</u>	Mar 12	under-1	F 77

Echo 7-

<u>Idi Araba</u>	Jul 12	7	F1
<u>Ikorodu Road</u>	Nov 29	under-1	F 8
	Jul 18	3	F 4
<u>Surulere</u>	Mar 7	under-1	F 13
	Sep 11	1	F 34
<u>WACMR, Yaba</u>	May 6	1	F 24

Echo 9 -

<u>Shomolu</u>	Nov 7	3	F 69
<u>Surulere</u>	Nov 1	6	F 25

Echo 11-

<u>Ikorodu Road</u>	Jul 10	1	F 6
<u>Shomolu</u>	Nov 8	2	F 82
<u>Surulere</u>	Feb 22	3	F 22

From the above, and allowing for the small numbers involved - the Echo 1 isolates may have been the end of an outbreak towards the end of 1962, followed by sporadic cases in the lowest ages - non-immune now acquiring their immunity; a pattern similar to that of polio 2. Echo 7 affected mainly those in the lowest age-group, suggesting a relatively immune population. Echo 9 may have been the end

of an outbreak, as no isolates occurred again within the time of the study; a pattern possibly resembling that of Cox B2. Echo 11 occurred in the youngest children and scattered in time, and may have been the normal acquisition of immunity in an immune community.

They will be discussed in ages.

Occurrence in individuals in ages - (Fig 37)

Echo 1-

Adult - in F 54, on Dec 4. Adult (F) (wife) and child, aged 10, excreted non-typables on Dec 1 and 4. These may have been Echo 1, or polio 2, also being excreted at this time, or a mixture.

(5-16) group -

(1) 10 year-old - in F 34, on Nov 1. Others excreted Polio 2 or non-typables. Those excreting non-typable were aged 9 and 12; these isolates may have been Echo 1, polio 2 or a mixture. Similarly, on Nov 29, children of 14, 10 and 9, excreted non-typables, which may have had the same identities.

(2) 7 year-old - in F 40, on Nov 29, with

(3) 5 year-old - in F 40, on Nov 29,

and,

under-5 group-

(1) 1 year old - in F 40, on Nov 29.

(2) under-1 year-old - in F 10, on Feb 22. Two children, aged 11 and 5, excreted non-typables on Feb 23 and 26. A 5 year-old excreted B1 on Feb 23. The non-typables may have been Echo 1 of B1 or a mixture of these.

(3) under-1 year-old - in F 77 on Mar 12. Isolated case.

With or without the inclusion of the non-typables, all age-groups were affected. With the exception of 2 isolates in under-1's, all occurred in November and December. One of the under-1's (F 10) excreted Cox B2 in December, so this may be the reason for its not being affected during the peak of the outbreak. There was no further outbreak of the type during the study, immunity having been acquired by the community.

Echo 7-

(5-16) year group- 7 year-old in F 1, on Jul 12. Adults (father and wife) excreted 2 non-typables on Jul 12. Possibly Echo 7. Other children, 5, 12 and 13, negative.

under-5 group-

- (1) 3 year-old - in F 4, on Jul 18. Isolated case.
- (2) under-1 year-old - in F 8, on Nov 29. Adult (M) (father) excreted a non-typable on Dec 1 - possibly Echo 7.
- (3) under-1 year-old- in F 13, on Mar 7. A child of 3 excreted a non-typable on Mar 7. Possibly Echo 7.
- (4) 1 year-old - in F 34, on Sep 11. Isolated case.
- (5) 1 year-old - in F 24, on May 6. Isolated case.

The incidence of this type was widespread in place and time. It occurred mainly in the very young, suggesting a relatively immune community. If non-typables were taken into consideration, adults would be included in the group, but, as both were in the same family, this could have been isolated non-immunity in an individual family rather than in the population in general.

Echo 9-

(5-16) group - 6 year-old - in F 25, on Nov 1. A child of 3 excreted a non-typable on Nov 1 and on Nov 30. Possibly Echo 9 or polio 2 or a mixture.

under-5 group - 3 year-old - in F 69, on Nov 7. 2 children, of 14 and 11, excreted non-typables on Nov 1. Possibly Echo 9, or a mixture with polio 2.

This type occurred in the 2 youngest age-groups, and allowing for non-typables, scattered from 3 to 14 years of age. It was isolated in time to early in November, at the beginning of the study, and this, with its subsequent disappearance from the community, might point to highly immune population resulting from an outbreak.

Echo 11-

- under-5 group - (1) 3 year-old - in F 53, on Feb 22. Isolated case.  
(2) 2 year-old - in F 82, on Nov 8.  
(3) 1 year-old - in F 6, on Jul 10. Isolated case.

Echo 11 was scattered in time and place, affecting the lowest ages. This would suggest an immune population with acquisition of immunity sporadically in the youngest non-immunes.

From the above, Echo 1 and Echo 9 may have occurred in outbreaks, Echo 9 followed by Echo 1. This resulted in an immune population and relative disappearance of the types. Echos 7 and 11 either found more immunity in the community and only appeared sporadically in young non-immunes, or, the sporadic form may be an inherent quality of the viruses - perhaps a lack of invasiveness.

Distribution of non-typables in residential areas - (Fig 36, 37)

<u>Area</u>	<u>No.</u>	<u>Date</u>	<u>Family</u>
<u>Idi Araba</u>	1	Nov 1	F 1
	1	Nov 30	F1
	2	Jul 12	F 1

Echo 7 was excreted in F 1 in July. Otherwise no other typables in this area.

<u>Ebute Metta</u>	1	Apr 30	F 32
	1	Jul 11	F 2

Cox B5 was excreted in F 2 on May 10, but no other typables in the area. The non-typables could have been B5, by family and community association.

<u>Ikorodu Road</u>	1	Nov 1	F 4	
	1	Nov 5	F 4	<u>P2</u> on Dec 1, 3 in F 4.
	1	Nov 29	F 57	
	1	Nov 30	F 47	
	2	Dec 1	F 8, 47	
	1	Dec 4	F 48	
	3	Dec 5	F 27	<u>P2</u> and <u>E7</u> in F8, on Nov 1, 5 and 29.
	1	Feb 23	F 47	
	1	Feb 27	F 6	
	1	Mar 1	F 47	<u>P1</u> in F 62 on Mar 21.
	3	Apr 30	F 4, 6, 62	
	1	May 1	F 57	
	1	May 2	F 4	
	1	May 6	F 4	
	2	May 8	F 47	No typables in families or area.
	3	Jul 11	F 42	
	1	Jul 26	F 62	<u>E7</u> in F4; <u>E11</u> in F 6 in the area at time.



<u>Area</u>	<u>No.</u>	<u>Date</u>	<u>Family</u>
<u>Ikorodu Road</u>	2	Sep 11	F 6
(continued)	1	Sep 12	F 4
	1	Sep 13	F 4
	1	Sep 14	F 42

No typables in families or area at time.

In this area, the non-typables might be Polio 2 or Echo 7 by family association and Polio 2, Echo 7, polio 1, or Echo 11 by community association.

<u>Shomolu</u>	4	Nov 1	F 69, 78
	4	Nov 2	F 33,40,58,78
	1	Nov 29	F 71
	1	Dec 3	F 66
	1	Dec 13	F 58
			<u>P2</u> in F 46, 71, 82, on Nov 2,3,29; Dec 1.
			<u>E1</u> in F 40 on Nov 29.
			<u>E9</u> in F 69 on Nov 7.
			<u>E11</u> in F 82 on Nov 8.
	2	Feb 28	F 71
	1	Mar 1	F 58
	1	Mar 2	F 82
			No typables in families or area at this time.
	1	Jul 10	F 40
	1	Jul 13	F 5
	1	Jul 16	F 71
	1	Jul 24	F 45
			<u>B3</u> in F 71 on Jul 16.
	4	Sep 14	F 58, 71, 76
			No typables in families or area at time.

Some of the non-typables could have been Polio 2, Echo 1, Echo 9 or Cox B3 by family association; or Polio 2, Echo 1, 9 or 11, or Cox B3 by community association.

<u>Area</u>	<u>No.</u>	<u>Date</u>	<u>Family</u>
<u>Surulere</u>	8	Nov 1	F 25, 34, 53, 63
	2	Nov 6	F 22, 54
			<u>P2</u> in F22, 34 on Nov 1,2.
			<u>B3</u> in F 13 on Nov 3.
			<u>E1</u> in F 34 on Nov 1.
			<u>E9</u> in F 25 on Nov 1.
	2	Nov 29	F 34
	1	Nov 30	F 25
	1	Dec 1	F 54
	2	Dec 4	F 13, 54
	1	Dec 6	F 50
			<u>E1</u> in F 54 on Dec 4.
	1	Feb 27	F 7
	1	Mar 2	F 7
	1	Mar 7	F 13
			<u>P2</u> in F 22 on Feb 27
			<u>P3</u> in F 34 on Feb 27,28
			<u>E7</u> in F 13 on Mar 7
			<u>E11</u> in F 53 on Feb 22
	2	Apr 30	F 12, 25
	1	May 1	F 34
	1	May 2	F 21
	3	May 6	F 22, 63
	1	May 7	F 13
	2	May 8	F 54
	1	May 15	F 61
			<u>B1</u> in F 61 on May 16
	3	Jul 10	F 34
	1	Jul 11	F 34
	1	Jul 13	F 21
			<u>B1</u> in F22 on Jul 13 and in F34 on Jul 10.
			<u>B5</u> in F 25 on Jul 13.
	2	Sep 13	F 13
			<u>P3</u> in F 13 on Sep 14.
			<u>E7</u> in F 34 on Sep 11.

Family association with polio 2, Echo 1 and 9 and community association with polio 2, Cox B3, Echo 1 and 9 were possible in the area.

<u>area</u>	<u>Nos.</u>	<u>Date</u>	<u>Family</u>
<u>Mushin</u>	5	Nov 1	F 23, 36, 72
	5	Nov 2	F9,11,73,74
	2	Nov 3	F 31
	1	Nov 15	F 59 No typables in area.
	2	Dec 3	F 9, 70
	2	Dec 8	F 36
	1	Dec 10	F 36
	1	Dec 12	F 36
	1	Dec 13	F 36 <u>P2</u> in F31 on Dec 1 in F 51 on Nov 29. <u>B1</u> in F 64 on Nov 29.
	1	Feb 27	F 51 No typables in area.
	1	May 11	F 31
	1	May 13	F 11
	1	May 30	F 11 No typables in area.
	1	Jul 10	F9
	1	Jul 17	F 11 <u>B4</u> in F 51 on Jul 13.
	1	Sep 14	F 37
	1	Sep 16	F 73
	1	Sep 17	F 31
	1	Sep 18	F 9
	3	Sep 19	F9, 11, 51 <u>P1</u> in F51 on Sep 19. <u>P3</u> in F11 on Sep 19.

There could have been family association with Polio 1 and 3; and community association with Polio 1, 2, and 3, Cox B1 and 4.

<u>WACMR, Yaba</u>	3	Nov 1	F 24, 86
	2	Nov 2	F85
	1	Nov 3	F 85
	2	Nov 30	F 10, 24 <u>P2</u> in F84 on Nov 5. <u>B2</u> in F10 on Dec 1.

<u>Area</u>	<u>No.</u>	<u>Date</u>	<u>Family</u>	
<u>WACMR, Yaba</u>	1	Feb 22	F 24	
(continued)	1	Feb 23	F 10	
	1	Feb 26	F 10	
	1	Feb 28	F 87	<u>P3</u> in F 84 on Mar 4. <u>B1</u> in F 10 on Feb 23 <u>E1</u> in F 10 on Feb 22
	1	May 1	F 85	<u>B1</u> in F93 on May 6 <u>E7</u> in F 24 on May 6.
	1	Jul 11	F 93	
	1	Jul 12	F 10	
	1	Jul 13	F 87	
	2	Jul 18	F 84	No typables in area.

There could have been family association with B2, B1 and Echo 1; and community association with polio 2, Cox B2, polio 3, Cox B1 and Echos 1 and 7.

<u>Iwaya</u>	1	Dec 13	F 95	<u>P2</u> in F 95 on Nov 8.
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There was possible family association with polio 2.

<u>Lagos Island</u>	1	Dec 5	F 17	No typables in area.
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<u>Odi Olowo</u>	1	Nov 1	F 81	
	1	Nov 7	F 77	<u>P2</u> in F 81 on Nov 1. <u>B2</u> in F 20 on Nov 12.
	1	May 7	F 77	No typables in area.
	1	Sept 12	F 52	No typables in area.

There could have been family association with Polio 2 and community association with polio 2 and Cox B2, at certain times.

<u>Oshodi</u>	2	Nov 6	F 26	
	1	May 8	F 26	No typables in area.

<u>Isolo</u>	1	May 13	F 29	
	1	Jul 12	F 29	No typables in area.

<u>Area</u>	<u>No.</u>	<u>Date</u>	<u>Family</u>	
<u>Idi Oro</u>	1	Nov 1	F 60	
	1	Nov 6	F 35	
	1	Dec 4	F 35	<u>P2</u> in F 55 on Nov 1.
	1	May 1	F 35	No typables in area at this time.

There could have been community spread of polio 2 in November/December.

<u>Makoko</u>	1	Nov 3	F 30	<u>P2</u> in F 30 in Nov 1.
	1	May 11	F 30	

There could have been family spread of polio 2 in November.

<u>Abule Ijesha</u>	1	Nov 1	F 68	
	1	Nov 2	F 38	<u>P2</u> in F 38 on Nov 17

There could have been family spread and community spread of polio 2 in November.

From the above, certain non-typables could have been covered by contact in a family or between families in the area. It has been noted earlier that spread within the family was greater than in the community in general, (Gelfand et al, 1957c; Clemmer et al, 1966; McCarroll et al, 1955). It has been seen that the chance of transmission of paralytic infection was 70 times as great in a family as in the general public, and of non-paralytic infection, 100 times as great, (Siegel et al, 1955).

Earlier community studies showed that polio spread in a rural area was local and sporadic, (Pearson and Rendtorff, 1945a), in a small town, occurred in families and close associates, (Pearson and Rendtorff, 1945b), and in larger urban populations, was in close contacts and also unknown contacts, (Pearson et al, 1945). In this study, each area could form a small community, but together they formed a large urban community. Family infections have been seen in infections of Echo 7, (Kleinman et al, 1962a) and Echo 9, (Nihoul et al, 1957).

Possible correlation of isolates with illness-

The only way to ascertain a true relationship between virus and infection is to show a rise in specific antibody between pre- and post- infection specimens of serum. This was not possible in this study. Any relationship is circumstantial.

Case 1 - Family - F 4 Ind. no. 18 Area - Ikorodu Road.

Age - Adult (F) mother.

Season - November date of specimen - Nov 5.

Date reported sick - Oct 18

Clinical notes - Temp. - 99° - chest pain.

Isolate - non-typable.

In the family (1) a child aged 6/12 excreted a non-typable, on Nov 1, and on Dec 3, the same child was excreting polio 2.

(2) another child, aged 5, excreted polio 2 on Dec 1.

Possible association with polio 2.

Case 2 - Family - F 95 Ind. no. 81 Area - Iwaya

Age - 7 - male.

Season - November Date of specimen - Nov 8

Date reported sick - Oct 17

Clinical notes - Temp.- 102°

Isolate - P2

Case 3 - Family - F 34 Ind.no. 174 Area - Surulere

Age - 1 - female

Season - November date of specimen - Nov 1

Date reported sick - Oct 23

Clinical notes - Temp.- 100° - vomiting, diarrhoea.

Isolate - Polio 2



Case 4 - Family - F 34      Ind.no. 167      Area - Surulere

Age - 6 - male

Season - February/March      date of specimen - Feb 27

Dates reported sick - Feb 9 and 28

Clinical notes - diarrhoea - no fever.

Isolate - Polio 3

Case 5 - Family - F 42      Ind.no. 217      Area - Ikorodu Road

Age - 8 - male

Season - July      date of specimen - July 11

Dat of reporting sick - June 29

Clinical notes - sore throat - no fever.

Isolate - non-typable.

In the family, the mother and a child, aged 7, excreted non-typables on July 11. Ikorodu Road area - Echo 7 and Echo 11 were excreted at this time, (Fig 36).

Case 6 - Family - F 47      Ind.no. 241      Area Ikorodu Road.

Age - 1 - female.

Season - April/May      date of specimen - May 8

Date of reporting sick - May 1

Clinical notes - Diarrhoea - no fever

Isolate - non-typable.

In the family, the mother excreted a non-typable on May 8.

In the area, there were no typables isolated at this time.

In other areas, Cox B1, B5 and Echo 7 were excreted.

Case 7- Family - F 72 Ind.no. 351 Area - Mushin

Age - 8 - female

Season - November date of specimen - Nov 1

Date reported sick - Oct 22

Clinical notes - Temp. 101<sup>0</sup> - vomiting.

Isolate - non-typable.

No isolates occurred in the family at this time.

Polio 2 and Cox B1 were excreted in the area at the time.

From the above, Polio 2, polio 3, Cox B1 and B5, and Echo 7 and Echo 11 may have had some association with illness in the population under study; either by isolation from the individual or by association with infection in the family, the residential area or the general community.

All the viruses isolated during the study have been found with various illnesses and this has been described in the introduction. Of those mentioned above, Polio 3, Cox B1 and B5, and Echo 11 were considered more likely to give rise to CNS disease than polio 2, and Echo 7, (Froeschle et al, 1966).

Echo 11 has been found with respiratory-enteric/<sup>conditions,</sup> (Philipson and Wesslen, 1958; Philipson, 1958a,b,c; Buckland et al, 1959; Klein et al, 1960) and respiratory infections, clinically, (Philipson, 1958a,c; Philipson and Wesslen, 1958) and experimentally, (Philipson, 1958b; Lippi et al, 1962b). Case no. 5, with the tenuous relationship,<sup>with Echo 11,</sup> complained of sore throat.

Assuming that there is a connection between the virus and illness this suggests that the agents are not passing through the community unnoticed but are producing overt illness.

Vaccination -

The presence of non-immune children in the (5-16) age-group, and the possible link with clinical symptoms, suggest that the epidemiological pattern may be changing. This is backed by the apparent occurrence of polio infection in outbreaks rather than as sporadic cases throughout the year.

There are 2 alternatives-

- (1) To carry out a vaccination programme.

The age level of non-immune individuals to the polio types should be confirmed by serological studies, (Sabin, 1961-62; Sabin, 1963).

Tri-valent vaccination should then be carried out

- (a) in at least 80-85% of susceptibles, in age-groups according to the immunity pattern of the area;

- (b) in a very short period, e.g. 4 days;

- (c) followed up by continued vaccination of those under 6 months of age, otherwise a very dangerous situation may result, with an accumulation of young non-immune children, (Sabin et al, 1960).

The social pattern of the community must be considered. Although the study population was relatively settled in Lagos, every year many families spent their leaves, of up to 1 month or more, in their "tribal" home areas, where living conditions could be very different to those in Lagos. This situation does not arise so frequently in countries in temperate-climate regions, where living standards are more homogeneous. It could again be dangerous to produce an artificially-immunised population who might be exposed to wild viruses in another locality, unless this artificial immunity can be maintained.

The presence of Coxsackie and Echo viruses, potential disease-producers, in some cases in the form of outbreaks, suggests that the relative "eradication" of the polio viruses from the community might not be the answer to the problem of illness, or potential illness, in that community.

(2) It might be preferable to monitor infection and immunity in the population, by virus isolation in healthy individuals and clinical cases, and by serological studies. If evidence occurred of an increase in polio infection, or frank cases, to a potentially epidemic level, immediate vaccination could be carried out with a monovalent vaccine of a type not that of the outbreak. This has been effective in several areas, (Hale et al, 1959, 1961; Sabin, 1962; Witte et al, 1965; Feldman et al, 1965).

Should vaccination be carried out as a programme, the optimum time would be important. Assuming that the pattern seen in this study is repeated annually, with polio infection lasting from the autumn of one year into the early spring of the following, and being closely followed by an increased incidence of Coxsackie viruses in, say, late May to August, the optimum time to avoid interference, is apparently from mid-March to early May, (Fig 33).

Again, rapidly carried out monitoring of the flora in the community could help to fix an exact time.

Relationship of viruses with disease -

The relationship between the enteroviruses and illness was described in the introductory section.

Polio 1, 2 and 3, Coxsackie B 1, 2, 3, 4 and 5 and Echo 1, 7, 9 and 11 were isolated during the study.

The relationship of the polios to diseases is a well-known fact. The Coxsackie B group has also been implicated clinically, for example- Coxsackie B1, (Gear, 1961-62); B2, (Jack and Townley, 1961); B3, (Disney et al, 1953; Kibrick and Benirschke, 1956; Kendall, 1960); B4, (Ginevri and Felici, 1959; Jack and Townley, 1961; Fechner et al, 1963); and B5, (Walker and Togo, 1963; Cherry et al, 1963a).

Echo viruses were also associated with illness, especially in young children, (Drouhet, 1960; Wenner, 1962); for example- Echo 1, (Matumoto, 1963); Echo 7, (Kleinman et al, 1962a); Echo 9, (Sabin, 1960), and Echo 11, (Steigman and Lipton, 1960; Melnick, 1965).

Conclusions-

(1) The enteroviruses, as a group, occurred throughout the year, mostly in the youngest members of the community.

(2) Within this, the polio, Coxsackie B and Echo viruses had excretion patterns of their own -

(a) The polio viruses appeared to occur in outbreaks, lasting from the autumn of one year to the early spring of the following. Whether these outbreaks were annual or occurred at longer intervals could not be ascertained from the length of the present study.

Polio 1 appeared to have the highest immunity level in the community, perhaps having occurred as an outbreak approximately 2 years before the study.

Polio 2 occurred in the form of an outbreak, whose peak may have been at the beginning of the study.

Polio 3 took an intermediate position with sporadic cases in a relatively immune population; and may be the virus of the next polio outbreak.

There may be a relationship in the pattern to the 3-yearly polio outbreaks noted elsewhere in the world.

(b) Coxsackie B also showed a variation in pattern in the different types excreted-

B1 - occurred as a spread-out "outbreak" suggesting relatively low immunity in the community. Its non-occurrence as a localised outbreak may have been due to some inherent quality of the virus or to the presence of other factors, e.g. the interference of the polio 2 outbreak.



B2 - was noted at the beginning of the study, possibly as the end of an outbreak prior to that of polio 2, occurring during the summer and early autumn, in relation to the rainy season, and at the season of Coxsackie B increase noted in 1963. It resulted in the appearance of an immune community.

B3 - appeared in a more sporadic form, possibly due to more immunity in the population, than there was to B 1 and 2.

B4 - occurred in sporadic form, suggesting an outbreak of this virus relatively recently - possibly intermediate to B2 and B3.

B5 - occurred in sporadic form but with less immunity than B4, from age distribution patterns (allowing for the small numbers involved); perhaps in a position intermediate to B3 and B4.

As a group, interference with the polio viruses, as a group also, was evident.

(c) Echo viruses- also occurred in patterns within the group but did not show any interference with viruses of the Coxsackie or polio groups.

Echo 1 - was seen in the form of an outbreak occurring with the polio 2 outbreak, thereafter apparently resulting in an immune population.

Echo 7 - occurred sporadically in the youngest members of the community suggesting a relatively high immunity in the community.

Echo 9 - the pattern of excretion suggested the end of an outbreak, prior to that of polio 2, and possibly prior to that of Coxsackie B 2, but with overlapping. This was no interference between the Coxsackie and Echo viruses.

Echo 11 - occurred sporadically, suggesting a relatively high community immunity.

Non-typable isolates- a possible relationship for a number of these was found by proximity to typable isolates in families, residential areas or the community in general. Others remained untyped.

(2) There was no obvious relationship of excretion pattern to tribe, occupation or sex, which could not be accounted for by population distribution of specimens; nor was there a discrimination in residential areas.

(3) A circumstantial relationship was found between some of the excreted viruses - polio 2 and 3, Coxsackie B 1 and 5, Echo 7 and 11- and illness in the community.

(4) It was considered that under present circumstances and considering the practical aspects of a very large-scale vaccination programme and its continued maintenance, in conjunction with the priorities of other medical conditions in the area, that vaccination should not be carried out, unless in selected groups in the community, whose immunity might be lower, e.g. those of higher socio-economic level. Rather, that priority should be given to monitoring the situation in the study area, and in other areas in the country, with provision for vaccination, if necessary; and, that, to complete the studies, monitoring for members of the Coxsackie A group should be included.

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