INTRODUCTION AND AIM OF THE WORK

Several enteric viruses such as echoviruses, coxackieviruses and adenoviruses have been suggested as causes of acute diarrhoeal disease. Yet these viruses which could be isolated from stools of some patients with acute diarrhoea, by cell culture technique, could also be recovered with almost equal frequency from asymptomatic patients, particularly in the tropics (Banta vala, 1980). However, since their discovery, rotaviruses has been found to be consistantly associated with 50 to 70% of the winter associated gastroenteritis in infants and children, while Norwalk agent have been implicated in outbreaks of gastroenteritis in children and adults. In addition, there are several other viruses that also appear to be associated with acute diarrhoeal diseases. These include adenoviruses, astroviruses, caliciviruses, coronaviruses, and a group of poorly defined agents referred to as the small round viruses (Barnett, 1983).

Rotaviruses have emerged as the major etiological agents of serious diarrheal disease in infants and children under 2 years of age in practically all areas of the world where this disease has been studied etiologically,

Editorial (1977). By the end of the 3rd year of life over 90% of infants and young children had acquired rotavirus antibody a high prevalence of antibody was maintained in adulthood probably as a result of frequent reinfection with these agents (Iesudoss et al. 1978 and Yolken et al., 1978a).

It appears that older siblings or parents might be a source of rotavirus infection for young persons. The frequency of rotavirus infection in contacts also demonstrates the highly contagious nature of such infection. Tufvesson et al. (1977).

In the past few years the rotavirus has been accepted as one of the most important causes of childhood viral gastroenteritis (Steinhoff, 1980).

The aim of this work is to investigate the incidence of rotavirus in Benha city as apart of A.R.E.

HISTORY

Diarrhoea in humans has been documented since pre-hippocratic times . Discoveries made in the past century in the fields of bacteriology and parasitology resulted in elucidation of the etiology of aportion of diarrheal syndromes. However, it soon become apparent that despite the bacteriological and parasitic discoveries a significant proportion of epidemic and infantil gastroenteric could not be ascribed to any etiological agent. By exclusion it was assumed that many of these infectious gastroenteritides were due to viruses.

The human rota virus was discovered by Bishop et al. (1973) by examination of a thin section E.M. of duodenal biopsies obtained from infants and young children hospatilized with acute gastroenteritis in Australia. Subsequently it was found to be readily detectable in stool preparations by EM (Bishop et al., 1974 and Flewett et al., 1978).

In relatively short time laboratories from all over the world reported in rapid succession the presence of rotavirus in stool specimens from infants and young children with diarrheal illness.

CLASSIFICATION AND NOMENCLATURE

Bishop et al. (1973) reported that electron microscopic examination of duodenal mucosa from nine children with acute non bacterial gastroenteritis revealed virus particules in epithelial cells from six patients. The morphology of the virus particles was identical in each of the six children.

The virus belong to the orbiviruses, a group of double stranded ribonucleic acid (RNA). Shortly after,Flewett and his Co-workers (1973) detected the virus by negative staining electron microscopy of faecal extract from children with gastroenteritis. These virus particles measured 75-84 nm in diameter closely resembled reoviruses since some particules exhibited a two layered capsid. They noted also that these viruses had a sharper outline than reoviruses. Accordingly, the authors considered th at it was premature to classify the virus particles, they had observed as reovirus. Thus for some years they were referred to as reolike or orbilike viruses (Hodes, 1980).

Flewett et al. (1974a) found that the reovirus-like particles present in faeces of young children with acute gastroenteritis and the virus causing acute diarrhea in

newborn calves are indistinguish able from each other in size, shape and are serologically related to each other. Since these viruses differ morphologically and serologically both from reoviruses and orbiviruses, the workers proposed that they should be placed in a separate group and the name "rotavirus" (from the latin rota, a wheel) was suggested for them. Another term "duovirus" was used by Davidson et al. (1975) to describ the characteristic doubled-shelled capsid structure.

By the end of 1974 it became evident that rotaviruses comprise one genus of family reoviridae but they differ in morphology, in ribonucleic acid RNA of their genome and in their polypeptide composition from reoviruses and orbiviruses which make up the other genera of the reoviridiae family (Hodes, 1980).

The international committee on Taxonomy of viruses approved in (1978) that the rotaviru s group in the reoviridae to the genus rotavirus and so the family Reoviridae now comprises five genera with approved names: reovirus, orbivirus, Rotavirus, phytoreovirus and Fijivirus (Matthews, 1979).

The human rotavirus (HRV) is related to other rotaviruses that cause gastroenteritis in at least 8 species. These include the virus of epidemic diarrhea of infant mice (EDIM), the Nebraska calf diarrhea virus (NCDV), the simian rotavirus (SAII) obtained from a rectal swab of a vervet monkey, the offal (O) agent from washings of the intestine of sheep and cattle found in abatoir waste and other diarrhea producing rotaviruses including those that affect piglets, lambs, foals, young rabbits and newborn deer (Hodes, 1980).

MORPHOLOGY

Rotaviruses have a characteristic detailed structure, with a double shelled capsid layer, surrounding a 36 nm core which contains the viral nucleic acid. The inner capsid consists of radiating spoke-like capsomers and is surrounded by the outer capsid which provides the particles with a sharply defined circular outline (Flewett et al., 1974b).

Because of the similarity of the intact particle to the appearance of a wheel, viruses in this group were called "rotaviruses" (rota being the latin for a wheel) (Flewett et al., 1974b).

In the electron microscope by negative contrast, the virions appear spherical, although where the support membrane curls away from a tear, it is evident that there has been considerable flattening of the particles (Madeley, 1984). He stated that the virus is seen in 3 forms: complete, incomplete and stain-penetrated particles:

a) Complete, smooth. these have a smooth outer layer icosahedral symmetry 60-75 nm in diameter. This outer capsid was described by Stannard & Schoub (1977) as

having a honey-comb appearance on the outer surface of the particles. At the periphery it appears as a narrow layer with a smooth outer edge which often appears to be broken in pieces.

b) Incomplete, rough. These are the single-shelled particles, 50-60 nm in diameter. Stannard & Schoub (1977) described the inner capsid as an icosahedral structure with a total of 180 structural units per particle with a lattice-like arrangement. While Martin et al. (1975) proposed that the surface of the particles was composed of 32 large ring-shaped morphological capsomers. Because of the overall lattice arrangement of the capsomers, with spaces at the apices, Stannard & Schoub (1977) proposed that the icosahedron is not outlined by a rigid array of capsomers and this must allow some elasticity in the shape of the particle so that at times it becomes almost spherical.

c) Stain-penetrated particles. The particles which have been penetrated by stain appear empty and have a rounded appearance (Stannard & Schoub, 1977).

Eveloped particles of rotavirus were observed by Petrie et al. and Suzuki et al. (1984) in infected cells by thin section EM.

Tubular forms of rotaviruses have been described by many workers (Holmes et al., 1975; Kimura & Murakami, 1977; Esparza et al., 1980; Suzuki et al., 1981 & 1984 and Madeley, 1984). These have a diameter roughly similar to that of the rough, particles. The length is variable and the diameter usually varies more th an with the spherical particles.

It is not known whether the tubular forms contain RNA but since they are irregular in width they may lack the template function which could be provided by the nucleic acid. The tubular forms do not have the smooth outer layer (Madeley, 1984).

Density:

The buoyant density of rotavirus particles was estimated by Elias (1977a) who separated the particles by centrifugation to equilibrium in Cesium chloride (CsCl) gradients. He reported that complete particles have a density of approximately 1.36 g/cm^3, incomplete particles 1.38 g/cm^3, whereas

empty particles (stain-penetrated particles) have a density of approximately 1.29-1.30 g/cm^3.

Infectivity, as measured by fluorescent foci in cell culture, of the higher density (1.38 g/cm^3) was compared with that of the middle density (1.36 g/cm^3). Elias (1977a) reported that the greatest infectivity coincided with the complete particles at the density 1.36 g/cm^3.

Nucleic Acid:

The rotavirus genome is composed of 11 segments of linear double stranded RNA with a total molecular weight 10.7×10^6 (Rodger et al., 1975). This distinguishes rotaviruses from reoviruses and orbiviruses, both of which have only 10 segments of RNA in their genome (Flewett & Woode, 1978).

By polyacrylamide gel electrophoresis of stool extracts these segments may be separated readily to give a characteristic pattern. They are numbered 1 to 11 in order of descending molecular weights. This migration pattern has been used as one mean of differentiating human and animal rotaviruses and various human rotaviruses strains (Kalica et al., 1976 & 1978).

Human rotavirus has a characteristic icosohedral structure in which the three capsomeres are arranged on each the edge of each plane Fig. (2). Using the formula [N = 10 x $(n-1)^2$ + 2] which is used for determining the capsomere number in the spherical viruses, the total capsomere number of the rough particles is calculated as 42. Subunits possibly bridge-like units seem to connect the isolated capsomeres at the corner. They persumed that they form the outer layer of the smooth particles. The absence of these intracapsomeric subunits may transform the smooth particles into rough ones. The shape and size of intracapsomeris spaces divided by the subunits resemble those of the hollow spaces in the capsomeres, consequently human rotavirus looks like a honeycomb. The inner core of rotavirus virion is enclosed in a capsid which is composed of 42 capsomeres and a hexagonal intracapsomeric spaces for each plane of the icosohedron (Kogaska et al., 1979).

Sometimes the particles when numerous were aggregated into clumps. No strands could be found connecting them to suggest that they might have been linked by globulin molecules, although in the thick layer of negative stain surrounding 60-80 nm particles, fine strands might be difficult to discern, especially in the compartiverly crudely purified material under investigation (Flewett et al., 1974b).

ANTIGENIC STRUCTURE

The rotaviruses have been classified as a separate genus with the family reoviridae (Matthews, 1979) and share many of the properties common to this family but morphologically distinguishable from other genera within the family. Rotaviruses are non enveloped viruses consisting of double shelled particles which contain 11 or 12 or even 15 segments of double stranded RNA, ranging in molecular weight from approximately $.2 \times 10^6$ to 2.2×10^6, the total molecular weight of the RNA segments was $11\text{-}12 \times 10^6$ (Todd & McNulty, 1976 and Arias et al., 1982), which can be separated by poly acrylamide gel electrophoresis (PAGE).

Each RNA segment encodes a single primary gene product (Smith & Holmes, 1981), therefore each individual segment can be thought of as an individual gene. However, it's relatively rare to obtain separate segments on PAGE, hence an individual band may represent more than one segment and they have used the term band to refer to a visible line on the gel. So it's incorrect to use segment and the term line could be misleading by implying a single component (Mata et al., 1983).

Initial studies have shown that rotaviruses possess a single structural glycoprotein which can be electrophoretically resolved on PAGE as VP7 (Rodger et al., 1975). Some further studies suggested two glycoproteins (Sabra et al., 1982b) while other studies (Arias et al., 1982) demonstrated a single entity. The two outer shell proteins are of particular interest since they play a critical role in important biological properties exhibited by the virus. Specifically the minor outer shell protein is responsible for a haemagglutinating activity (Kalica et al., 1983). The other more abundant protein has been identified as the major neutralizing antigen for calf, rhesus, monkey and simian rotaviruses (Greenberg et al., 1983 and Matsuno & Inouye, 1983).

Preliminary characterization of the protein, identified N-glycosidically linked carbohydrate moieties that appear to stabilize the virus particles (Sabra et al., 1982a and Petric et al., 1983). Tunicarmycin an inhibitor of glycosylation prevents proper assembly of outer shell proteins which ultimately hampers production of complete virus particles (Suzuki et al., 1984).

PAGE of purified preperations of simian SAII, calf and human rotaviruses showed that the 3 viruses share very similar polypeptide components although they can be distinguished from each other (Rodger et al., 1975). By periodic acid Schiff staining, major components of the outer shells of the 3 viruses have been identified as glycoproteins. This finding illustrates another similarity between rota and reoviruses which together with adenovirus represent examples of non-enveloped viruses shown to contain glycoproteins.

When the three viruses are compared on the basis of inner shell components they were indistinguishable from each other with the molecular weights of most of the corresponding components identical. Comparison of the outer shell components revealed variations occurring among the low molecular weight polypeptides, these variations gain importance when considered from a serological point of view. So Flewett et al. (1974a) suggested that though the single shelled particles of human and calf rotaviruses were serologically indistinguishable, antigenic variations were detected when the cross reactivity of double-shelled particles were examined.

Two possible explanations for the coexistence of genitically distinct rotaviruses in one animal have been proposed (Sabra et al., 1982b). The first is antigenic drift analogous to that exhibited by influenza virus (Hinshaw et al., 1978) and the second is multiple infection. PAGE of rotavirus genomes has shown that there is more than one strain of rotavirus in the community at any given time 9Spencer et al., 1983). In antigenic drift, a single host is initially infected with one strain that changes to yield a population of genetically different viruses that is expanded to the community level. Once such variants coexist in the commnity they may conceivably form reassortants. This type of event would probably give rise to further diverse genotypes of human rotavirus since rotaviruses readily recombine (reassort) in vitro under certain condition (Greenberg et al., 1981).

The serological relationships between the rotavirus associated with different species of animals and human rotavirus (Bishop et al., 1973) were discovered by Flewett et al. (1974a) and Davidson et al. (1975). They found that human antisera can neutralize calf rotavirus in cell culture using immunofluorescence techniqu e.

Kapikian et al. (1975) showed that calf virus can be used as a diagnostic antigen for studying human rotavirus infections. Serological crossing between members of the rotavirus group is mediated by the common group-specific antigens associated with the inner-capsid layer (Woode et al., 1976). However, species-specific antigens are associated with the outer capsid of rotavirus particles which are morphologically identical in different animal species (Banatvala, 1980).

Two different "serotypes" of human rotavirus were first reported by Zissis & Lambert (1978) using complement fixation and immune-electron microscopy. Also Thouless et al. (1978) distinguished serotypes by a neutralization test involving neutralization of immunofluorescent foci. This test measured the capacity of post-infection sera to neutralize the infection of (and partial replication in) cell culture by various virus strains obtained from human faeces.

Sato et al. (1982) reported that neutralization tests clearly distinguished their isolated strains: Hochi, Ito, Hosokawa and Nemoto from each other as well as from Wa (Wyatt et al., 1980) and from simian, bovine, porcine and

lapine straind of rotavirus. Their results indicated the presence of at least five serotypes of human rotavirus.

The subgroup antigens are situated in the major inner capsid as a polypeptide of relative molecular mass about 46.000. This is a product of the 6th genomic segment and two serologically distinct subgroup antigens have been detected by ELISA. Antibodies directed against these subgroup polypeptides neutralize infectivity only feebly, if at all (Bastardo et al., 1981 and Killen & Dimmock, 1982). Monoclonal antibodies specific for each of these two subgroup antigens were developed by Greenberg et al. (1983).

Serotype-specific antigens are situated in the major outer capsid polypeptide. This is a glycosylated polypeptide of relative molecular mass about 36.000. Neutralization is determind primarily by antibodies directed against the polypeptide, which in most strains, is a product of the 9th genomic segment. In some strains the segment may be in the 7th or 8th position as shown by polyacrylamide gel electrophoresis (Dyall-Smith et al., 1983 and Mason et al., 1983). Serotypes are characteristically identified by neutralization of infectivity using fluorescent focus assay (Beards et al., 1980).

To resolve the confusion in the literature, the WHO Scientific Working Group on viral diarrhoes (1984) proposed the following nomenclature for strains of human rotaviruses: 1) sugroups should be designated by Roman numerals i.e. I, II. 2) Serotypes should be designated by Arabic numerals, i.e. 1,2,3,4. Accordingly, human rotavirus Wa can be designated as subgroup II, serotype 1. Certain animal rotavirus can be classified according to these designations, for example SA-11 virus reacts as subgroup 1 serotype 2. Nebraska calf diarrhoeal virus (NCDV) reacts as subgroup I, but is distict serotypically from four human rotaviruses (WHO, 1984).

Atypical human strain, called pararotavirus was first detected by Nicolas et al. (1983). He detected only one such virus out of 350 rotavirus specimens positive by EM. The criteria employed to define such atypical strains are the same as those used with a chicken strain (McNulty et al., 1981) and a porcine one (Bohl et al., 1982). These strains contain eleven double stranded RNA segments but their electrophoretype is different to the common features observed with human rotaviruses and they do not contain the group-specific antigen, consequently, the available serological tests do not detect them. In addition this human atypical rotavirus has a distinctly different electrophoretype from equivalent of chicken and porcine strains.

Stability:

The structure of rotavirus is very stable, as human rotavirus stored at -20°C for 9 years, and another rotavirus strain of uncertain source lyophilised over 30 years previously were recognized morphologically by EM after such long storage (Albrey & Murphy, 1976).

The morphological appearance of purified human rotavirus was unchanged after being subjected to heat (56°C for 1 hour), centrifugation (100.000 x g), high salt concentration (50% potassium tartarate), pH 3 and 10, or after treatment with chymotrypsin and papain. In contrast, however, at pH <3, human rotavirus was unstable and the outer layer of the capsid collapsed, but particles did not disintegrate. In addition, human rotavirus was extremely labile to treatment with versene/trypsin (0.125%) for two hours at 37°C, as particles were completely degraded to unrecognisable form (Palmer et al., 1977).

In another study, the double-shelled human rotavirus retained its morphologic appearance after reaction with trypsin, but was degraded to a single-shelled form by α-chymotrypsin in the presence of cesium (Rodger et al., 1977).

Infectivity is destroyed by treatment with a low concentration of chelating agents such as EDTA but it is enhanced by treating the virus with the proteolytic enzymes (pancreatin or trypsin), and this technique was useful in improving the in vitro cultivation (Jewetz et al., 1980).

Growth in cell cultures:

In early studies, attempts to propagate h uman rotavirus in cell cultures met with only limited success. Limited growth of a single strain of human rotavirus in Human Kidney (HEK) culture was accomplished by Wyatt et al. (1976). They inoculate a stool filtrate containing EM-detectable rotaviruses from a 7 month-old child into cell culture tubes a cytopathic effect developed at the fifth day of incubation in the form of a generalized cytoplasmic granularity and the cells deteriorated after 10 to 32 days. At that time the cultures were frozen and thawed and the cell fluid mixture was inoculated into fresh HEK cell cultures. the presence of virus was detected by EM (Kapikian et al., 1974), as in-- complete particles and by indirect immunoflourescence (Wyatt et al., 1974) as fine granules in the cytoplasm in less than 1% of the cells.

Trypsin was found to enhance infectivity of rotaviruses by Spendlove & Schaffer (1965). Accordingly, Theil et al. (1977) demonstrated passage of porcine rotavirus in primary procine kidney cells which was dependent only on treatment of the inocula with trypsin during adsorption. Also Babiuk et al. (1977) and Almedia et al. (1978) reported that greatly increased amounts of bovine rotavirus, detected by EM, were produced trypsin was present in the medium during the entire period of virus growth in cell cultures.

The latter authers proposed that in the presence of trypsin the course of the replicative cycle appears to be considerably shorter (48 hours) than in non-trypsin treated primary calf kidney cultures where peak yields occur at five days. Alternatively, trypsin may produce an effect on the cell surface thereby enhancing virus penetration. They concluded that the enzyme was acting in different ways at different points on the growth cycle and could be helpful in the production of enhanced amounts of human rotavirus.

The successful adaptation of a human rotavirus strain, Wa, to efficient growth in primary cultures of African green monkey kidney (AGMK) cells was first described by Wyatt et

al. (1980). Fourty two stool filtrates from paediatric patients with diarrhoeal disease, diagnosed by EM examination as rotavirus infections, were mixed with trypsin (20 ug/ml) and inoculated onto AGMK cell monolayers. After centrifuation at 1400 g for 1 hour at 37°C (Banatvala et al., 1975). The cells were re-fed with trypsin-free medium incubated for 48 hours at 37°C, fixed with cold methanol and then stained for examination by direct fluorescence microscopy using fluorescein-conjugated goat antiserum to human rotavirus. Thirty one of the faecal suspensions were positive by this technique, but the proportion of cells exhibiting antigens was low in most instances (1% or less). Only 5 specimens induced antigens in 10% or more cells, including the specimens from patients S and Wa.

To peomate the growth of the rotaviruses which induced antigens in 10% or more of the cells and hence to increase the probability of the emergence of a cell culture-adapted mutants, the authors administered orally 1-2 ml of 2% bacteria-free filtrates from patients S and Wa to 26 hour-old gnotobiotic piglets (Bohl et al., 1978). The piglets developed mild, transient diarrhoes. For subsequent passage, intestinal contents were collected from individual animals,

killed 46 hours after inoculation and examined by EM, ELISA (Brandt et al., 1979) and the fluorescence technique (Thouless et l., 1977a). Because infection of the Wa strain was slightly more extensive (10^{5-7} fluorescent foci/ml of the intestinal contents) than that seen with S strain the former was used for subsequent ten passages.

The authors then inoculated the eleventh passage material from piglets into AGMK cells to initiate serial passages of the Wa strain in cell culture. The virus was passaged 14 times in AGMK cells yielding titres ranging from 10^3 to 10^6 fluorescent foci/ml.

Wyatt et al. (1980) suggested that cultivation of type 2 human rotavirus in cell culture would facilitate a more detailed examination of its properties and permit the manipulation of its genome with the intention of developing attenuated mutants for use in the prevention of serious diarrhoeal disease in human infants.

Successful in vitro cultivation of human rotavirus, without passage in animals, was reported by Sato et al. (1981). Faecal suspensions from cases of watery diarrhoea and shown to contain rotavirus by EM were treated with

trypsin (10 ug/ml) and inoculated onto MA 104 cells grown in tubes. The cultures were then washed, re-fed with trypsin-free medium and incubated in a roller drum at 37°C. Further passages were made at intervals of 3 days with inoculated culture fluid treated with 10 ug/ml trypsin.

The authors reported that a cytopathic effect was not observed in any of the primary passage cultures, although specific fluorescence using antiserum to calf rotavirus could be shown. At the second passage a cytopathic effect was observed and became clearer at the third passage.

The cytopathic effect appeared in about 2 days and consisted of loss of cell boundaries, cell fusion, cell rounding and eventual disintegration of the cell sheets. Rotavirus particles were demonstrated by EM in culture fluid from the 5th passage. Three strains isolated in this way were designated Hochi, Ito and Toi. The authors reported that these results indicate that MA 104 cells are a good medium for primary isolation of human rotavirus. Also pretreatment of virus with trypsin and incorporation of a small amount of trypsin in the maintenance medium seem to be important for establishing new isolates in these cells.

Similarly, Kutsuzawa et al. (1982) propagated two human strains (KUN and MD) in MA-104 cells with the same method used by Wyatt et al. (1980). Meanwhile Hasegawa and Co-workers (1982) reported the successful cultivation of human rotavirus in roller tube cultures of primary cynomolgus monkey kidney (CMK) cells, and they stated that these cells were more sensitive than MA-104 cells.

Naguib and her colleagues (1984) described the comparable direct isolation of human rotavirus from Egyptian infants and young children on different types of cells using the method of Sato et al. (1981) with minor modifications: 20 ug/ml trypsin was used with the inoculated virus, and the cultures were washed and re-fed with maintenance medium containing 0-5 ug/ml trypsin after one hour. A second passage of the harvest of infected culture fluid was done and culture fluids were tested directly by ELISA for rotavirus antigens detection (Kapikian et al., 1979). They reported that the primary AGMK cells were more sensitive than primary CMK cells and MA-104 cells for supporting the growth of human rotaviruses.

IMMUNITY

Studies, performed in numerous animal model systems have demonstrated that a major component of the immune protection against rotavirus infection requires the interaction of rotavirus and specific antibody within the lumen of the small bowel, the principle site of rotavirus replication (Wyatt et al., 1978).

That is why local intestinal antibody may be more important than circulating serum antibody in protection from rotaviral illness (Black et al., 1982) as the protective effect of serum antibodies appear to be less complete for pathogens producing disease in the gastrointestinal tract mucosa 99% of children with rotavirus diarrhoea had antibodies, often in high titre before their illness. However, it's possible that the serum antibody detected by binding ELISA is not protective perse but it reflects the child's cumulative exposure to rotavirus (Kapikian et al., 1981).

Asymptomatic infections are common in infants before the age of six months, the time during which protective maternal antibody is acquired passively by new borns. Such neonatal infection does not prevent reinfection but it may

protect against the development of sever disease during reinfection (Jawetz et al., 1983).

Protection attributed to the local effect of colostrum and milk antibody in the gut lumen has been reported in piglets, sheep, lambs and calves (Wyatt et al., 1978). In addition to the presence of antirotaviral antibodies in human milk (Goldman & Smith, 1973), it also contains non-immune substances that inhibit rotavirus (Bullen, 1976). feeding of human milk to patients with active rotavirus infection can result in a decrease of viral shedding (Saulsabury et al., 1980) and the feeding human serum immunoglobulins has been shown to prevent the development of symptomatic rotaviral diarrhoea in neonates (Barnes et al., 1982).

Secretory IgA which play the main role in the intestinal resistance to rotavirus infection has been demonstrated at the level of 30 ug/ml in the serum of most normal individuals while in lactating women levels averaged five times that amount (Waldman et al., 1970).

However, antirotaviral IgG transfered to the neonate across the placenta has no protective value in the first

five days post partum (Mclean & Holmes, 1981). IgM antibody the first class of serum immunoglobulins that is produced in response to rotavirus infection, measured by direct fluorescent antibody technique, seems to be of short duration suggesting a recent rotavirus infection. It appears within the first week of illness and disappears by the sixth week after the onset of illness. Thus the lack of IgM antibodies in the serum of a child with acute gastroenteritis between the second and fifth week of illness tends to exclude rotavirus as a cause of the disease.

In contrast IgG rotavirus antibody is not present in acute phase sera, but appears only after convalescence. IgG antibody was not found untill the second week after the children became ill but it persisted in all patients (Qrstavik & Haug, 1976). So the detection of IgM in the absence of IgG is an indication of recent rotavirus infection (Yolken et al., 1978b).

CLINICAL FEATURES

Rotavirus is described from various parts of the world as an important, possibly the most important etiologic agent in acute gastroenteritis of infants and young children and a major cause of diarrhoeal disease of the young in many mammalian and avian species (Hoshino et al., 1985).

Infection with rotavirus may present either with asymtomatic infection in which there is only serological response or typical disease in which there is diarrhoea and serological response and a carrier state who harbour and execret rotavirus without generating a systemic immune response (Champsaur et al., 1984a). Consequently the recovery of rotavirus does not always indicate true infection but this will imply demonstration of rise of antibody titre to confirm rotaviral infection.

The outcome of infection depends upon age, the state of immunity and possibly the stage of maturity of gastrointestinal tract, this is demonstrated by the fact that the proportion of carriers is decreased with age whereas the proportion of asymptomatic infection and disease is increased with age (Champsaur et al., 1984b).

Rotavirus infection may be subclinical, of varying clinical severity and at time fatal disease. Adult when infected generally exhibit either no symptoms or symptoms of gastrointestinal upset for 24 hours or less (Middleton, 1977).

The incubation period for rotavirus infection in children is said to extend from 48 to 72 hours (Shepherd et al., 1975) but differed from the general observations of Davidson et al. (1975) who reported it to be less than 48 hours. Patient with rotavirus infection experienced both vomiting and dehydration significantly more often than the non-rotavirus infected patient the vomiting occur in the 1st day or two of the disease. The vomiting is sometimes projectile, but more often simple regurgitation which may persist throughout the illness. Fever is very variable, severely ill children admitted to hospital may have temperatures of 38°C to 39°C rising to 40°C if dehydration is advanced. Dehydration is isotonic in 95% of patients (Maki, 1981). Diarrhoea in the form of green loose offensive stools about 10 times per day or more usually last for 5 to 8 days and is characterized by watery stools containing mucous and no blood. The deaths occured within one to three days after onset of symptoms. The major

factors causing death were believed to be dehydration and electrolyte imbalance (Flewett et al., 1975 and Holmes et al., 1981).

Asymptomatic infection has been noted during the newborn period (Blacklow & Cuker, 1981).

Adult contact may be infected but they rarely exhibit symptoms and the virus is infrequently detected in their stools. Patients with milder forms have symptoms for 3-5 days then recover completely (Jawetz et al., 1983).

Gurwith et al. (1982) stated that respiratory symptoms frequently precede or concurrently occur with diarrhea by Rotavirus.

Human rotavirus in addition to adenovirus can be regarded as infectious agents associated with intussusception in infants and young children since 37% of the patients with intussusception tested by electron microscope and 70% of those tested by complement fixation serologic procedure showed evidence of infection with rotavirus (Konno et al., 1977).

Recently Gordon (1982) described the association of rotavirus infections and the Sompe Syndrome. The term sompe may be useful to indicate that the syndrome inovlves infection in sinus, otic, mastoid, pulmonary and enteric cavities. The author stated that air cavities leading off the upper respiratory tract can act as sumps or cesspools if they fill up with secretions, stagnating fluid or milk fluid in these cavities provides an ideal niche for bacteria or viruses like rotavirus that are found in the upper respiratory tract. Pus can then drip down into the lungs and stomach causing secondary infectious.

The study of Saulsbury et al. (1980) indicated that rotavirus may produce a chronic infection in immunodeficient children manifested by prolonged diarrhoea and faecal excretion of rotavirus for six weeks, also several complications have been associated with human rotavirus among them rectal bleeding (Delage et al., 1978) intussusception (Konno et al., 1977). Reye Syndrome and encephalitis (Salmi et al., 1978). In encephalities no virus particles were seen in cerebrospinal fluid. Although infection takes place in connection with viraemia. However viraemia caused by rotavirus has not been reported (Salmi et al., 1978). The

authors pointed out that during the rotavirus outbreak in Jurku, two adult patients developed exanthems possibly a of viraemia. The mortality rates are probably low in the developed countries but at least 30 deaths have been reported in association with human rotavirus gastroenteritis (Davidson et al., 1975 and Carlson et al., 1978).

PATHOGENESIS

Infection with human rotavirus can occur already from the 1st week of life up to about six years of age (Standfield 1978).

Rotavirus infect cells in the villi of the small intestine. They multiply in the cytoplasm of these enterocytes and damage their transport mechanisms. Damaged cells may slough into the lumen of the intestine and release large quantities of virus which appear in the stool. The diarrhea caused by rotavirus may be due to impaired sodium and glucose absorption as the damaged cells on the villi are replaced by non-absorbing immature crypt cells (Jawetz et al., 1983).

When diarrhea was induced by human rotavirus sucrase activitywas diminished and thymidine kinase activity was increased in contrast adenyl cyclase and cyclic AMP were not stimulated (Davidson et al., 1977).

Impaired D-xylose absorption was also observed (Mavromichalis et al., 1977).

Some patients had depressed disaccharidase level (Maltose, Sucrose and lactose)(Bishop et al., 1973).

EPIDEMIOLOGY

Age distribution and immunity:

Rotavirus infection is recognised as a major cause of gastroenteritis in infants and young children, but it may affect any age group (Holdaway et al., 1982).

The most commonly affected age group, are those children between 6 months and 3 years (Holmes, 1983 and Cukor et al., 1984).

Although babies under 6 months are highly susceptible to rotavirus infection, the disease is mainly asymptomatic or only minimally symptomatic. This is attributed to several factors including: maternal antibody, soluble and cellular components in breast or reduced exposure to rotavirus as their main contact is with their parents who are unlikely to excrete rotavirus (Perez Schael et al., 1984).

Whether breast feeding may affect the incidence of rotaviral infection of the neonate is still contraversial (Banatvala et al., 1978). Although it has long been known that breast-fed neonates are less likely to suffer severe

diarrhoea, and the importance of lactogenic immunity to rotavirus in infants, the actual mechanisms of passive immunity to rotaviral infections are poorly defined. It has been reported that there is no correlation between protection of newborn infants from rotaviral infection, and the presence of a variety of soluble factors in human breast milk, including rotavirus - neutralizing antibodies, non-specific antiviral antibodies, α-1-antitrypsin molecules, and nonimmunoglobulin antiviral factors (Hoshino et al., 1985).

Totterdell et al. (1980) have demonstrated that human lacteal rotavirus antibodies can cause aggregation of rotavirus in vitro and have speculated that, this aggregation may have a protective role either by preventing virus attachment to villous epithelium or by activating macrophages to take up virus.

Also, it has been proposed that the strains the rotavirus derived from asymptomatic cases of neonate may be of low virulence (Thouless et al., 1977b).

Steel and Medina in 1984 have proposed that the physological immaturity of the gut of the neoborn especially the

relatively late development of lactase coupled with the presence of trypsin inhibitors in colostrum and milk may serve as a natural defence mechanism against rotavirus illness.

The apparent lack of protection in babies over 6 months against rotavirus may be partially due to reduced total daily intake of breast milk or to the reduced amount of rotavirus antibodies in mature breast milk or to both (Kapikian et al., 1982).

The other group of workers showed that neither maternally acquired serum antibodies, nor rotavirus antibodies in breast milk correlated with protection against rotavirus infection (Totterdell et al., 1980).

Although opinions still differ as regarding beneficial effect of human milk, an attempt was made to protect hospitalized children from nosocomial rotavirus infection by adding fresh human milk to the normal diet for their age. A matched group of children receiving no human milk served as a control. Faecal samples were routinely screened for rotavirus by Rotazyme ELISA test. They found that human

milk has no effect on the frequency of nosocomial rotavirus infections but the severity of the clinical symptoms was clearly reduced (Berger et al., 1984). The protection of breast milk is not absolute, as the neonate may be contaminated either by mother's faeces at birth or by early post partum oral administration of fluids (Cushing & Anderson, 1982 and Gurwith et al., 1983).

Incidence rates were highest during the second year of life when administration of contaminated weaning foods was at its peak. Mata et al. (1983) proposed that, by the end of 3 years of life the incidence of rotavirus infection diminshes which is suggestive of acquired immunity which may be life long. Reinfection with rotavirus can occur with second and subsequent episodes resulting in mild or subclinical infection owing to the presence of antibodies or because infection in non-immune older children are subclinical (Bishop et al., 1983).

Hospital based studies indicate that the median age of patients with diarrhoea due to rotavirus differ among various ethenic subpopulations in the same geographic area a result which suggests that environmental and/or genetic

factors influence the age of onset of rotavirus associated illness (Engleberg et al., 1982).

Season:

Ever since discovered and subsequently associated with infantile gastroenteritis, rotaviruses have exhibited a seasonal prevelance in temperate countries. It occurs almost exclusively during the cold winter months in Washington, Toronto, Canada, London, England, The West Midlands of England, Yamagata, Japan, Melbourne, Australia, Salisbury, Rhodesia (Paul et al., 1982), withonly a few sporadic cases in summer months (Holdaway et al., 1982). the clinical epidemiological pattern described for patients with rotavirus infection corresponds very closely with what was described by Zahorsky (1929) as winter vomiting disease (Rodriguez et al., 1977).

Also Engleberg in 1982, found that in temperate climate the rotavirus diarrhoea season usually begins in November or December, Peaks in January or February and ends in April.

However, this fact is true for temperate climates but for tropical countries with less dramatic changes in climatic

conditions, it was found that rotaviruses are year round pathogens (Soenarto et al., 1981).

A cold environmental temperature was the most important factor in rotavirus infectivity (Paul et al., 1982).

Humidity:

A survey was done to study the influence of humidity on rotavirus prevelance in Nigerian infants and young children with gastroenteritis during a five months period, they found that the rate of rotavirus detection varied inversely with relative humidity and is highest in winter when the humidity is lower. Low relative humidity (49-78%) is apparently the most important environmental factor for rotavirus survival and spread. There is not such a distinct relationship with temperature or vapour pressure (Paul et al., 1982).

Socioeconomic level and preexisting nutritional status:

In a longitudinal study of acute diarrhoea it was found that the socioeconomic level and preexisting nutritional state did not influence the rate of rotavirus execretion as determined by the ability of the parents to pay hospital

casts (Soenarto et al., 1981). however, incontrast to this finding, a similar survey in South India was done where the incidence of rotavirus infection increased in children with low socioeconomic level (Maiya et al., 1977).

Early exposure to the virus is universal in spite of strikingly different sanitary conditions in different countries (Linhares et al., 1981).

Sex:

Males are more commonly affected by rotavirus than females.

This was reported by Shepherd et al. (1975) who studied hospitalised infants suffering from gastrointestinal symptoms due to infections with human rotavirus identified by EM.

In contrast Elias (1977b) showed no statistical difference in incidence off rotavirus between males (100%) and females (85.7%) within the same age 1-3 years.

Also, Rodriguez et al. 91977) found that there was no significant difference in male to female ratios.

Course:

Since repeated infections with several serotypes and subgroup antigenic variants, the overall prevelance incidence ratio was near unity and most rotavirus episodes did not last for more than two weeks i.e. an acute process not evolving into chronicity (Mata et al., 1983).

Transmission:

Faecal or oral and air-born mechanisms are the suspected modes of transmission of rotaviruses and both are favored by crowding and the low levels of education, personal hygiene and environmental sanitation (Mata et al., 1983). Although it has been postulated that rotavirus infection occurs in young infants because the virus is brought into the home by older siblings (Gurwith et al., 1981) were unable to find a difference in the rate of rotavirus infection among infants with or without siblings. It's likely that the rotavirus infection is so prevalent that only brief exposure to other children is required or that there is enough asymptomatic infection of parents to account for transmission to infants. Nosocomial transmission of rotavirus is a serious problem and it may be indirectly from patient to another on the hands of hospital personnel.

Actual infection or colonization of nursey personnel was apparently not a major factor in transmission (Ryder et al., 1977). In nurseries, it's most likely that the virus enters the nursery through a visting child or the rare adult exercretor and then passes readily from neonate to another, the result is persisting endemic infection as susceptible babies enter the nursery (Murphy et al., 1977). Transmission of infection from adults to their offspring may occur as there is evidence suggesting that, calves are infected from adult population (Woode et al., 1976).

Reinfection:

The little data that exists on the protection afforded by humeral rotaviral antibodies against naturally acquired rotavirus infection are conflicting (Ryder et al., 1985). Bishop et al. (1983) did not find any evidence of protection against subsequent reinfection which was usually asymptomatic but demonstrated that neonatal rotavirus infection does not confer immunity against reinfection, but it does protect young children against the development of clinically severe disease during reinfection. Ryder et al. (1985) findings extend those of Bishop et al. and suggest that the protection against development of clinically severe disease

during reinfection is long lasting. Mata et al. (1983), in Guatemala found no evidence of clinical immunity occurring after infection.

PREVENTION

Rotavirus infection is very difficult to control as during the diarrhoeal phase in man, very large number (up to 10^{10}/g) of rotavirus particles are execreted in faeces which can remain infectious for prolonged periods, up to seven months of storage at room temperature (Flewett & Woode, 1978). Consequently it could readily become a source of infection for other susceptible individuals in the immediate surroundings. Moreover, it has been demonstrated that asymptomatic cases of rotavirus infection also excerete the virus in their faeces (Chrysti et al., 1978).

Ryder et al. 91985) reported that there are two basic mechanisms that prevent diarrhoeal disease in developing world:

Environmental sanitation and engineering to ensure adequate supplies of potable water and immunization of susceptible population.

As local immunity is much more important for protection against rotavirus, hence priority should be given to a live vaccine. In future, human rotaviruses (HRV) adapted

for tissue culture or reassortants of human and animal rotavirus may be sufficiently attenuated to be used in manufacturing of candidate vaccines (Zissis & Lambert, 1980).

Another approach to the production of rotavirus vaccine for man could be the use of an attenuated heterologous rotavirus such as calf rotavirus which share a common group antigen with human rotavirus to induce cross protection against human rotavirus (Woode et al., 1976).

RIT 4237 live attenuated bovine rotavirus used for protection of infants against rotavirus diarrhoea is derived from the Lincoln isolate of NCDV which is a strain of bovine rotavirus antigenically related to human rotavirus strains of subgroup I (Kapikian et al., 1981).

RIT 4237 strain has a long passage history in tissue culture and can be easily produced in large quantities in primary monkey kidney cells which are a suitable substrate for human vaccine production (Mebus et al., 1971).

The safety of a single oral dose of RIT 4237 in terms of absence of diarrhoea, other gastrointestinal symptoms and

of virus execretion in stools and its immunogenicity in susceptible human beings i.e. its ability to induce an antibody response were established.

Kapikian et al. (1981) found that the sero-conversion rate induced by live attenuated RIT vaccine strain can be increased by giving this oral vaccine after milk to neutralize gastric acidity as this strain is stable at pH 4 but becomes labile at a lower pH. also this gastric acidity could be neutralized by antacids e.g. $NaHCO_3$ but this seems much less predictable than milk feeding in developing countries.

Passive immunization via maternal colostrum and milk is now the most commonly practiced method for rotavirus vaccination in cattle (Snodgrass et al., 1977).

A live attenuated calf rotavirus vaccine is available commercially. It's administered orally to calves as soon as possible after birth (Mebus et al., 1971).

Breast feeding: is one of the preventive measures for rotavirus diarrhoea particularly in developing countries as recommended by the WHO as a control strategy. Several

studies have shown that rotavirus infection occurs less frequently among breast fed babies and that infected infants execrete less virus particles if they are breast fed (Banatvala et al., 1978).

Prevention of nosocomial infection:

Albey & Murphy (1976) reported that rotaviruses are well known as an important cause of nosocomial outbreaks of acute gastroenteritis infants and children (Marie et al., 1982).

However, very rare is known about the modes of transmission of rotavirus in hospital environment. From the available evidence it appears that transfer of the virus is brought about mainly by the contaminated hands of hospital personnel. Further support for this came where rotaviruses were detected in hand-washings from nearly 79% of the attendants of patients with rotavirus diarrhoea. Proper disinfection of hands and contaminated objects would form therefore an integral part of any measures directed towards the effective control and prevention of nosocomial outbreaks of rotavirus gastroenteritis (Ryder et al., 1977).

TREATMENT

The treatment is supportive, to correct the loss of water and electrolyte which may lead to dehydration, acidosiss shock and death. Management consists of replacement of fluids and restoration of electrolyte balance either intravenously or orally (Jawetz et al., 1983).

World health organisation recommended the use of an oral glucose electrolyte solution consisting of sodium 90 potassium 20, chloride 80, bicarbonate 30, and glucose 111 m.mol/L. This solution has been used successfully for treatment of children with cholera and other acute diarrheal diseases (Editorial, 1975).

Reports from Costa-Rica (Nalin et al., 1978) and Bangaladesh (Sack et al., 1978) indicated that this oral solution is effective for rehydration in human rotavirus gastroenteritis.

Rotavirus diarrhea is accompanied by a decrease in disaccharidase secretion by the injured epithelial alls lactase, maltose andsucrase production are nearly always diminished and for this reason liquids that contain

disaccharides should be avoided u ntil several days after clinical improvement has occurred (Hodes, 1980), however, Sack et al. (1978) showed that patients with rotavirus diarrhea who are considerably dehydrated can be rehydrated and maintained on oral electrolyte solution containing either sucrose or glucose.

There is no specific antiviral theraby available for human rotavirus infection. As all diarrheal disease, fluid replacement remain the most important aspect of therapy for human rotavirus gastroenteritis (Steinhoff, 1980).

DIAGNOSIS

1. Electron Microscopy (EM):

EM examination of faeces has been the original diagnostic technique and remains the standard by which newer methods are judged (Steinhoff, 1980).

EM can provide an accurate rapid diagnosis of rotavirus gastroenteritis, but it's not widely available and require that the specimens be examined at a time, making it difficult to assay a large No. of specimens in a practical time (Kapikian et al., 1976).

2. Immunoelectron microscopy (IEM):

Kapikian et al. (1972, 1973) have utilized the IEM for detection of virus particles in faeces. In this technique convalescent serum from a patient was employed as a source of specific antiviral antibodies in order to enable the recognition of viral agent. Flewett et al. (1974b) reported that this sera cause agglutination of both human and calf viruses, clumps of 4-40 or more particles could be seen at low magnification, while at higher magnifications, strands of globulin could be

seen linking the virions together.

Barndt et al. (1981) reported that IEM especially in rectal swab specimens but with most stool specimens IEM takes hours longer than direct EM to provide the same diagnostic result.

3. **Complete Fixation Test (CFT):**

Spence et al. (1975) reported the use of stool suspensions as an antigen in complement fixation and counter immune electrophoresis with a hyperimmune rabbit antiserum prepared against Nebraska calf diarrhoea virus. In CF they are titrated against a constant dilution of antiserum. These methods have the following advantages:

a) The diagnosis can be made during the acute phase of illness.

b) The collection of 2 blood samples from the infant is not essential for diagnosis.

c) An EM is not needed for diagnosis.

However, anticomplementary activity is the greatest handicap in C.F.T. and it's less sensitive than E.M. A

modified C.F.T. was described by Clementi et al. (1981) in which in order to reduce anticomplementary activity.

Zissis et al. (1978) compared both electron microscopy and complement fixation tests, they also compared the results of the three techniques: Electron microscopy, Immune electron microscopy and complement fixation. In the first comparative study, 196 stool specimens were collected from children with acute gastroenteritis who came to pediatric clinic, faecal suspensions were prepared and examined by electron microscope and complement fixation test, they found that 149 clarified faecal suspensions were found negative by both techniques, 44 were found positive by E.M., and 47 were positive by C.F., later the three specimens that were initially detected only by C.F. were confirmed as positive by E.M. It was believed that C.F. could advantageously replace E.M.

In the second comparative study, 20 further stool specimens were collected and examined by E.M. I.E.M., an C.F. the workers wanted to see if it was possible to increase the sensitivity of detection of rotavirus by using I.E.M., contrary to expectation I.E.M. was not more sensitive than the other two methods, five of twenty stools

examined yielded rotavirus as was also demonstrated by the other method of these methods (EM, IEM, CF) C.F. test was prefered because of its high specificity and because the preparation of the antigen is much simpler. Further CF has a distinct advantage in that it can measure the amount of the virus excreted in the stool.

In the complement fixation technique antiserum from three sources may be used to detect rotavirus antigen:

a) Rabbit antiserum but it must be used with caution since the anticomplementary titre is close to the antibody titre.

b) Calf convalescent serum: it was suitable but the antigen titres obtained were to four times lower than with human serum.

c) Sera of human origin: This was found to give better results (Zissis et al., 1978).

Simples tests like immuno-electroosmophoresis (I.E.O.P.), fluorescent antibody techniques (F.A.) and optimized enzyme-linked immunosorbent assay (E.L.I.S.A.) have therefore gained wide application for diagnosis

of rotavirus infections in human and bovin patients (Holmes, 1979).

4. Counter immunoelectrophoresis (CIEP):

In 1975, Spence et al. reported its use for detection of HRV antigen in stools. A suspension of patient's faeces was used as an antigen and the antiserum was obtained from rabbits that had been hyper-immunized with NCDV. In 1976, Middleton et al., gave a detalied description of a CIEOP test for detection of HRV Ag in faecal specimens. It was found that CIEOP is sensitive, reliable and valuable test for routine laboratory diagnosis and for epidemiological studies because large No. of specimens can be processed by this method (Spence et al. 1977).

5. Enzyme immuno-assay:

Principle: an enzyme label as peroxidase or alkaline phosphatase can be linked to antigen or antibody molecules the presence of an enzyme linked molecules is detected by means of the enzyme substrate and can be measured spectrophotometrically. Either labelled antigen or antibody can be attached to insoluble support as plastic

agglutination plates. After the material has attached, excess has been washed away, enzyme linked antigen or antibody is added with the test substrate. The antigen or antibody whichever is being measured in test solution competes with added labelled reagent (antigen or antibody) for material attached to plastic plates. Amount of enzyme labelled reagent can be estimated by addition of enzyme substrate. The product of reaction between enzyme and substrate is finally determined spectrophotometrically producing colour changes (Weir, 1983). The author demonstrated that ELISA provides an accurate and rapid mean for diagnosis of HRV infection and found it to be both as sensitive and as efficand as RIA and EM for detection of HRV. Brandt et al. (1981) found that ELISA utilizes reagents which are both stable and non radioactive and is ideal for heandling large no of specimens. Further more as the test can be read by the nacked eye or a simple colorimeter, there is no need for sophisticated equipment.

Yolken & Leister (1982) devised rapid quantitative multiple determinant EIA system for the detection of human rotavirus in stool specimens. The multiple determinant technique is based on the fact that viral antigens are often multivalent and thus can attach to antigens at

distinct binding sites.

Yolken et al. (1980) and Yolken & Leister (1981), found that two available antibodies to rotavirus reacted at an antigenically distinct sites as determined by competition studies. Therefore, they used one of these antibodies to coat the solid ph ase and the other as the enzyme labelled conjugate in a multiple determinant assay. This technique provides a practical means for the accurate detection of rotavirus antigen in stool specimens in less than 40 min. which is an interval sufficiently short to allow for decisions concerning the management of patients in the acute phase of gastroenteritis also detect execretion of rotavirus in a patient before admission of the patient in closed environment such as the hospital ward.

A four layer solid phase enzyme-immunoassay of (EIA) with antisera against Nebraska calf diarrhoea virus (NCDV) as immunoreagents was developed to detect human rotavirus antigens from stool specimens of patients with acute rotavirus gastroenteritis. Polystyrone beads were used as the solid phase, guinea-pig and rabbit anti-NCDV Ig as the catching and secondary antibody and peroxidase-conjugated swine anti-rabbit Ig as the indicator antibody. A comparison

of the developed NCDV-EIA with an identical EIA using antisera against human rotavirus (HRV-EIA) instead of nCDV antisera was made. A complete agreement was obtained between the two methods provided that the appropriate confirmatory tests were included. The developed NCDV-EIA was as sensitive and specific for rotavirus as the HRV-EIA, and it allowed the detection of both established rotavirus types 1 and 2 from stools with equal sensitivity (Sarkkinen, 1981).

The most widely and commercially available test for rotavirus and the only one with which there is wide published experience is the Rotazyme enzyme immunoassay (Abbot laboratories, North Chicago III). It has been found to be equal to or slightly less sensitive than EM for detecting rotavirus (Hovi et al., 1982 and Rubenstein & Miller, 1982).

Rotazyme based on the method of Yolken et al. (1977b) plastic beads coated with guinea pig rotavirus antibody were supplied. An aliquot of patient faeces suspended in a diluent buffer was incubated with the bead. After removal of the unreacted specimen by washing the bead was reacted with virus antibody conjugated to horse radish peroxidase. The unbound material was again removed by washing. Hydrogen

peroxidase is added as a substrate, together with the chromogen, O phenyl endiamine 2 HCl. The absorbance of the product of the reaction is measured spectrophotometrially and compared with that of specimens containing (1) rotavirus and (2) rotavirus negative control. This test is a sensitive as other methods and is much simpler and more rapid than EM.

6. **Latex agglutination (LA):**

Latex agglutination tests have been recently introduced to diagnose rotavirus in faecal samples. They have several advantages over EIA (enzyme immunoassays) as it can be performed by unskilled personnel, rapid, completed within 30 minutes and relatively cheap (Sambourg et al., 1985). The principle as described by Sanekata et al. (1981): latex particles were coated with anti-calf rotavirus Ig. The antibody coated particles were specifically agglutinated by both calf rotavirus and HRV and the agglutination was evident macroscopically within a minute.

7. Immunofluorescence (IF):

Clementi et al. (1981) have employed counter current immunoelectrophoresis (CIEOP). Banatvala et al. in 1975 described a method using indirect immunofluoresence for detection of rotavirus antigen in faeces which is a useful method for laboratories which don't have access to an EM. They were examined using a feitz "Dialux" microscope with iodine quartz illumination.

Positive cells shows with the brillient intracytoplasmic fluorescence characteristic for rotavirus antigen. The results obtained by indirect I.F. added of the knowledge of the intestinal lesions produced by the virus. It was found that rotavirus antigen is located only in the cytoplasm of epithelial cells of villi. No identifiable rotavirus was detected in the cells of lamina propria. Rotavirus was difficult to detect in mucos obtained more than four days after onset of symptoms. It's also suggested that rotaviral replication in the human gut is confined to the columner epithelium of the doudenum and upper jejenum (Middleton et al., 1974). I.F. reactions also indicate that virus particles from different patients are antigenically similar since rota-

virus particles fluoresced when incubated with heterologous sera.

8. **Ultrasensitive enzymatic radioimmunoassay (USERIA):**
Described by Harris et al. (1979).

Each specimen must be fractionated by column chromatography and a scintillation counter is needed. However these methods have not been widely accepted first because none of them seems suitable for routine use, secondly a sensitivity 100 times that of ELISA is unnecessary in an assay for detection of virus usually present in great amount in faeces (Grauballe et al., 1981).

9. **Cell culture:**

Woode et al. (1974) were able to diagnose rotavirus infection in calves by innoculating faeces on to a calf-kidney (CK) monolayer cultures followed by immunofluorescent staining (FA) to demonstrate growth of virus.

Wyatt et al. (1976) succeeded in propagating the human virus in organ cultures of human embryonic small bowel and could then passage the virus 14 times on human embryo-kidney (HEK) monolayer cultures.

Another cell culture of monkey cells (LLC-MK2) was also used the highest proportion of infected cells was obtained with LLC-MK2. Primary HEK and primary CK cultures showed fewer infected cells. When one strongly positive sample was titrated in LLC-MK2 and secondary HEK cells by the micromethod, the titre was found to be 10 times higher in LLC-MK2 cells. the effect of any differences among the virus strains tested are the various kidneys used for cell culture preparation is not yet known and will require further investigation. Infected multinucleated cells were sometimes seen in the LLC-NK2 and secondary HEK culture faecal samples rich in virus particles often produced fluorescence in few cells, even without the aid of centrifugation, but when the specimen was centrifuged on to the cultures the number of ifnected cells increases more than a thousand fold (Bryden et al., 1977).

Possibly the concentration of specimens before inoculation will further increase the sensitivity of the test, as it does for EM (Flewett et al., 1974b).

During study apiece of small intestine from a fatal case of gastroenteritis in a one year old boy was submitted for investigation, it was emulsified and examined for

rotavirus positive results were obtained by EM, but not by FA, the virus particles however looked as if they were coated with immunoglobulin, this was confirmed after treating the emulsified specimen with an equal volume of glycine-HCl buffer, pH 2.8 for one hour at room temperature, it give positive FA staining in LIC-KK2 cell. Naturally occuring antibody in faeces that may prevent virus from infecting cell cultures must be regarded as a potential cause of false negative results when testing by FA technique. An advantage of EM is that it can often detect other viruses, the LIC-MK2 cell line is very easy to grow and to handle and appears to be the cell of choice for rotavirus (Bryden et al., 1977).

- Toyokokutsuzawa et al. (1982) found that one strain of human rotavirus subgroup I (KUV) and one strain of subgroup 2 (MO) were isolated with the MA-104 cell line, a fetal rhesus monkey kidney cell line.

- Many attempts to propagate human rotavirus in vitro have faild. Wyatt et al. (1980) suggested that human rotavirus was cultivated in primary cultures of African monkey kidney cells after 11 passage in new born piglets.

- Recently Sato et al. (1981) succeeded in the direct cultivation of human rotavirus from faecal specimens by using cultures of MA-104 cells together with pre-treatment of the specimens with trypsin and incorporation of small amountof trypsin into the maintenance medium.

Hasegawa et al. (1982) have indicated that primary monkey kidney cells were more sensitive than MA 104 cells for propagation of HRV. Rotaviruses were propagated more efficiently in primary cells (both African green and cynomologous monkey kidney) than in the continuous monkey kidney cell line (CVI or MA-104 cells) when tested at the same time and under the same conditions (Wyatt et al., 1983).

Although growth of HRV directly from faecal specimen was not consistently observed in any of the continuous cell lines, these viruses could be adapted for consistent growth in these cells by passage through primary AGMK cells. A single passage through the primary cells partially adapted the viruses for continuous cell lines (MA-104 cells) whereas two passages appeared to allow full adaptation.

It was also found that although rotaviruses in faecal specimens were propagated more efficiently in primary cells than in continuous lines, the average infectious titre of these specimens in MA 104 cells, was nearly identical to the infetious titre determined by viral growth in primary AGMK cells. Birch et al. (1983). This implies that equivalent numbers of viruses in faecal specimens were able to infect the primary cells and continuous cell lines, but that, in certain instances, infection of the cell lines was abortive and did not lead to the production of infectious progeny viruses. Urasawa et al. (1981) passage through primary cells appeared to somehow overcome this abortive response. The mechanism of adaptation is not known, possibly, either it may that, inhibitors which present in the faecal material will limit rotavirus growth in the continuous cell lines or the progeny viruses grown in primary cells are somhow different from virus particles in the stool preparation. Passage through primary AGMK cells also increased the average infectivity of virus particles in MA 104 cells (Ward et al., 1984).

Growth requirements of rotavirus:

Although rotaviruses are widely distributed and considered highly contagious in many places as the field,

mouse breeding establishment or hospital, their adaptation to laboratory cultivation has so far proved to be extremly difficult. This paradox has impressed many investigators and led them to consider what are the special requirements for rotavirus growth.

1. **Lactase**:

Sato et al. (1981) proposed that, in vivo rotaviruses bind to lactase present on the brush border membrane of the small intestinal epithelial cells, and that uncoating occurs during transfer of the particles into the cell. The difficulties encountered in primary isolation and adaptation of rotaviruses could thus be due to the absence of lactase on the surface of cells most commonly used. It may even be possible to convert insusceptible cells to rotavirus susceptibility via adsorption of exogenous lactase (or B galactosidase) on their plasma membranes.

2. **Trypsin**:

Another important requirement for rotavirus growth is the presence of trypsin. In has been widely reported that it's essential to pretreat the virus with trypsin

(proteolytic enzyme), incorporate it in maintenance medium as it enhances rotavirus replication (Estes et al., 1981).

3. **Foetal bovine serum:**

The third requirement for rotavirus growth is the foetal bovine serum (FBS). A low percentage of FBS 0.5-2% is usually included in the culture medium of virus infected cultures and in viral plaque assays to maintain cellular integrity untill the cells are lysed by the virus. However FBS which contain trypsin inhibitors can not be included in the culture medium during the propagation of rotaviruses because of the strict requirements for trypsin (Twist et al., 1984).

Cytopathic effect were identified by immunofluorescence and electron microscopy. It was always detected between 48-72 hours after inoculating 0.2 ml supernatent of CPE positive cells into fresh cells, isolated viruses were passaged 10 times more in cell culture. Cytopathic effect consisted of obscure cell boundary, cell fusion, cell rounding and eventual disintegration of cell sheets.

With EM, numerous virus particles with a typical rota virus morphology were seen in supernatent fluids from CPE positive monolayers. Also, the viruses isolated were identified by indirect immunofluorescence with anti-human rotavirus serum, all cultures which shared CPE, also showed intense granular cytoplasmic fluorescence (Sato et al., 1981).

METHODOLOGY

Materials:

71 stool specimens of watery diarrhoea cases were collected from patients attending rehydration centers and from in patient in pediatric department treated from dehydration caused by gastroenteritis in Benha University in the period from May till September 1986 and also 24 control cases collected during September from healthy infants all cases were chosen below 2 years age. From these 71 cases there were 43 males and 27 females and from 24 control cases there were 10 males and 8 females also type of feeding, clinical data and residence of each case were collected these samples were examined in Hoechst Company in Septemper 1986 on Behring ELISA processor for the presence of rotavirus antigen according to method of (Hovi et al., 1982 and Rubenstein & Miller, 1982).

Method:

Solid phase Enzyme immunoassay (ELISA) for the qualitative and quantitative Determination of Rotavirus Antigen.

A. Equipment required:

test tubes 100.

Centrifuge.

Micropipettors 500 uL, 100 ul, 50 ul with disposal tips.

Incubator 37°C.

Multichannel photometer (Behring ELISA processor).

B. Reagent for test:

- Enzygnost Rotavirus (Ag), test plate, consisting of 6 strips in special holder. Each strip has 2x8 reaction wells, coated with SA 11 antiserum from the rabbit (SA 11 = simian rotavirus).

- Rotavirus antigen for the complement fixation test and for Enzygnost Rotavirus serves as a positive antigen control, titre is 1:1280. The virus grown in cell cultures and inactivated after purification.

- Anti-Rotavirus/AP conjugate for Enzygnost Rotavirus (Ag) is produced by coupling alkaline phosphatase (AP) to bovine anti-NCDV antibodies (NCDV = bovine rotavirus).
  working dilution 1:35.

- Supplementary reagents for enzygnost:

  a) Substrate tablets AP for preparation of substrate solution.

  b) Substrate buffer AP

  c) Dilution buffer AP ready for use for feces samples and and enzyme conjugates AP.

  d) washing solution (concentrate).

  e) stopping solution AP (2N NaOH).

Preparatory work:

- Test plate: Remove plate from pack and allow to stand for about 5 minutes at +20 to +25°C.

- Rotavirus antigen: Dissolve in 1 ml of distilled water then dilute the solution with dilution buffer AP in such manner that a log 2 steps under given titre here titre = 1 : 1280, so the antigen dilution to be tested is 1 : 640 (according to dilution schema) i.e.after 1:80 dilution.
  Select dilution schema A with one dilution step.
  Steps of dilution:
  0.05 ml antigen + 4.0 ml dilution buffer AP = 1. : 80
  0.1 ml antigen 1/80 + 0.7 ml dilution buffer AP = 1 : 640
  1 : 640 dilution is the antigen dilution tested.

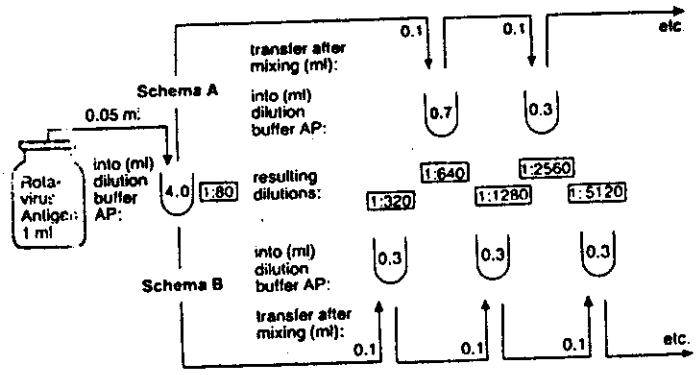

Fig. 1: Dilution schemata for Rotavirus Antigen outside the strip.
The desirable end-dilution depends on the Enzygnost titer of the Rotavirus Antigen.
Example calculation: given titer: 1:5120; antigen dilution to be tested: 1:2560, i.e. after the 1:80 dilution select Dilution Schema A with two dilution steps.

- Anti Rotavirus Ap conjugate for Enzygnost Rotavirus (Ag) working dilution 1:35 i.e. 1 ml + 34 ml dilution buffer.

- Substrate solution: Dissolve 2 substrate tablets AP in 10 ml of substrate buffer AP.

- Washing solution AP buffer was warmed before use in tepid water in order to redissolve any crestals that may be present, and then dilute 1:20 (1 part by volume of concentrate and 19 part by volume of sterile distilled water) pH 7 to 7.2. The amount prepared is 40 parts by volume concentrate + 760 part by volume of steril distilled water.

- Sample of patient feces: dilute approximately 1:5 with dilution buffer AP to form a good suspension i.e. 1 ml fecal sample and 4 ml dilution buffer AP. i.e. ½ ml fecal sample and 2 ml dilution buffer AP. and then centrifuge

at approx. 3000 rpm for 10 min. use the supernatant in the test.

- The control negative faecal sample should be prepared in the same manner as the patient sample.

- All reagents must be brought to +20 to +25°C before use

Procedure:

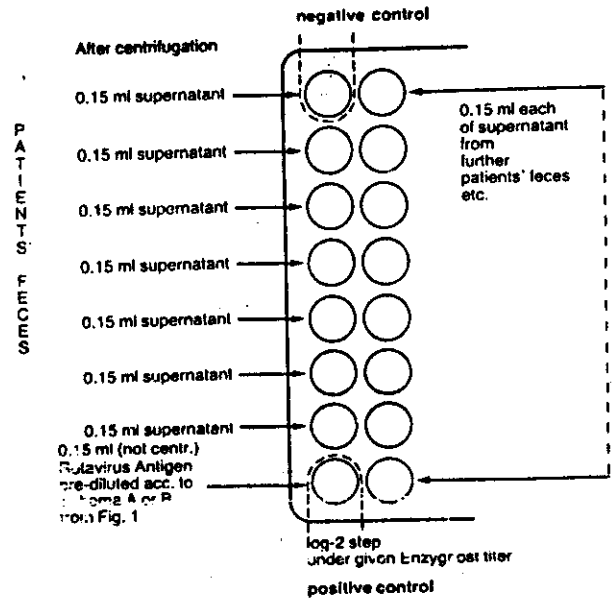

Fig. 2: Pipetting of the samples to be tested into the reaction wells of the strip.

1. First introduce 0.15 ml of the centrifugation supernatant from the negative control sample into a well. The negative control must occupy the position A_1 of the plate to allow for the automatic evaluation of results. Then complete the row 1 by negative control sample but in H_1 put

the antigen 0.15 ml. Then pipette 0.15 ml each of the centrifugation supernatents from the samples to be tested into reaction wells. 24 control samples and 71 samples from diarrhea are examined.

2. Incubate the test plate for 2 hours (± 5 min.) at 37°C in a moisture chamber. The plate should not be placed on a surface which is a good conductor of heat (metal, moist paper) an empty holder is suitable.

3. Suction off the feces dilution and pipette at least 0.2 ml of diluted washing solution into each well after about 1 to 2 min., suction off repeat this washing process twice allowing the washing solution to stand for the given period prevents non-specificity of tests this step is done in Behring ELISA processor and washing is done 4 times.

4. Add 0.05 ml of the diluted enzyme conjugate solution to each well and incubate the plate for 1 hour (± 5 min) in a moisture chamber at 37°C as described under 2 also this done in the same processor and incubation in the incubator.

5. Suction off the enzyme conjugate and wash the test plate as described under 3. Add 0.1 ml of substrate solution to each well (this step done in Behring ELISA processor) and incubate for 45 min. (± 5 min.) at +20 to +25°C or 30 min. (± 1 min.) in a moisture chamber at +37°C the last is done (in incubator).

6. At the end of described period stop reaction by addition of 0.05 ml of stopping solution AP (2N NaOH)

7. Evaluate the yellow green colour reaction within one hour after putting in dark place at +20 to 25°C..... after that put in Behring ELISA processor to take result quantitative on sheet.

Evaluation:

The individual test samples may be evaluated visually or photometrically.

a) Visual: It advisable to read in indirect day light against a white background. Any sample which manifests a yellowish-green colour in comparison with the negative faece sample is to be evaluated as positive.

b) Photometric: Measurement directly in the test plate

wavelength of 405 nm against 0.1 ml substrate solution plus 0.05 ml of stopping solution AP as reference solution diluted 1 : 20 or undiluted respectively.