REVIEW OF LITERATURE
The Picorna Viruses include a very large number of viruses of Vertebrates. They are small, ether-sensitive, nonenveloped icosahedral viruses, which contain a single molecule of single stranded RNA and multiply in the cytoplasm in close association with membrane. The single stranded nucleic acid has a molecular weight of $2 \times 2.8 \times 10^6$ daltons and constitutes about 30% of the particle mass. The name Picorna was proposed by the Virus Subcommittee of the International Nomenclature Committee of the Society of Microbiology in Montreal in 1962. It is derived from Pico (very small) and RNA (the type of nucleic acid). The name Picorna may also recall the initial letter of the words: Poliomyelitis, Insensitivity to ether, Coxsackie, Orphan and Rhino virus (Andrews et al., 1963). Within the family there are two groups, the Enterovirus group and Entero-rhino coryza (E.R.C.) group. They are distinguished from reoviruses by size. Picorna viruses measure 20-30 nm, contain no essential lipid and are heat labile. The entero subgroup is stable at pH 3.0 while the E.R.C. subgroup is labile. All members of the group except E.R.C. viruses resist heating in the presence of divalent cations; this feature can be used to separate them from viruses of other groups (Wallis and Melnick, 1962). Picorna viruses differ from Myxo-, Papova-, Adeno- and Herpes viruses in that they are less rapidly inactivated in molar magnesium chloride solution at 50°C, than in distilled water.
The following subgroups are recognised:

A. Picorna viruses of human origin

1. Enteroviruses
   a) Polio viruses
   b) Coxsackie viruses A
   c) Coxsackie viruses B
   d) ECHO viruses

2. Rhinoviruses

3. Unclassified

B. Picorna viruses of lower animals.

The most acute infections of the central nervous system varying in their severity are Poliomyelitis, Virus-meningitis and Meningo-encephalitis. The viruses frequently associated with these illnesses include the human enterovirus—Poliomyelitis, Coxsackie, ECHO and Reo viruses (Syverton 1959, Macrae 1959, Sabin 1959). These agents are all known to be multiplying at various times in the human intestinal tract. In 1948 Dalldorf and Sickles isolated from the stools of two children living in the town of Coxsackie in New York state and suffering at the time from Poliomyelitis, a virus that induced paralysis in suckling mice and hamsters, but had no effect on the rhesus monkeys. Inoculation of virus by intracerebral, intraperitoneal and intramuscular routes causes paralysis followed by death. Severity of lesions is influenced by the age of the animal and its nutritional status, the passage, history of the virus and possibly the route and dosage of inoculation. It was
characterized by widespread degenerative lesions of the skeletal muscles with no effect on the central nervous system. Dalldorf et al., 1959 reported the antigenic difference strains cause focal rather than widespread myositis, myocardial softening, necrosis of the subcutaneous brown fat (inter scapular fatpad), necrosis of the acinar tissue in the pancreas or hepatitis (Melnick et al., 1949; Dalldorf, 1950; Pappenheimer et al., 1961; Dalldorf and Sickles, 1956). Poliomyelitis, virus meningitis and meningo-encephalitis are acute infections of the central nervous system varying in their severity. They may occur as outbreak or isolated incident. Pathogenicity for newborn mice is one of the characteristic features of Coxsackie viruses. Their relative lack of pathogenicity for weanling and adult mice and for other experimental animals accounted for their escape from recognition before this time. There are two groups A and B, separated on the basis of lesions produced in baby mice. Mice infected with Coxsackie group A strains develop a progressive flaccid paralysis and die. Acute inflammation of the voluntary striated muscles, and diffuse eosinophilic hyaline degeneration is seen on histologic examination. There are 24 immunologically distinct serotypes which have been identified so far under group A. Group A viruses are isolated by subcutaneous or intraperitoneal inoculation of mice up to 72 hours old. Older mice rapidly become insusceptible and, even if they become ill, will often recover. At postmortem, the lesions of the fatpads are usually the most prominent features, while the muscles, visceral or brain changes vary considerably.
The distinction between the two groups is not always clear. The effect on the newborn mice and the histological changes were at one stage thought to be specific for Coxsackie viruses but Foot and Mouth Disease (F.M.D.) viruses (Platt, 1956) and a number of arboviruses (Scherer 1961) can cause similar lesions. Newborn mice are susceptible also to encephalomyocarditis (E.M.C.) virus, herpes simplex virus and to some strains of Polio virus. For histological examination the carcass, after removing the skin and opening the peritoneal and thoracic cavities should be immersed in neutral formol-saline solution or in a saturated solution of corrosive sublimate to which 5% glacial acetic acid has been added. The morphological changes in mouse tissues have been described by Gifford and Dalldorf (1951) and Godman, Bunting and Melnick (1952). There are 6 immunologically distinct serotypes under group B (Dalldorf et al., 1959). Mice infected with group viruses develop tremors, weakness, and paralysis; the fatpads between the scapulae show whitish degeneration and inflammation visible through the skin, skeletal muscles are white and firm in appearance and microscopically show hyaline degeneration of the kind described by Zenker. Some strains particularly among the late types may be less virulant in mice so that illness or death does not necessarily result from inoculation. Group B viruses are preferably isolated by subcutaneous and intracerebral inoculation of mice preferably less than 24 hours old and give rise to spasticity or paralysis with delayed death. Older mice rapidly become insusceptible and even if they become ill will often recover.
At postmortem, the lesions of the fatpads are usually the most prominent features, and show acute congestion, cellular infiltration and necrosis of the lobules, the muscles, visceral or brain changes varying considerably. Extensive degeneration of the parenchymal cells with pallor of the cytoplasm and margination of the nuclear chromatin of the liver is seen, the acinar cells of the pancreas may be seen in a state of extensive dissolution. The heart often shows foci of necrosis with eosinophilic degeneration of segments of muscle fibres, loss of striation, pyknosis and fragmentation of the nuclei, associated with an acute inflammatory cell infiltration. Softening of brain with degeneration of neurons, pyknosis of the affected cells and infiltration of inflammatory cells into the affected areas and perivascular spaces is seen. The morphological changes in mouse tissues have been described by Gifford and Dalldorf (1951) and Godman, Bunting and Melnick (1952).

Melnick et al. (1949) reported that Coxsackie B virus is incriminated as the cause of aseptic meningitis, epidemic pleurodynia (Bornholm disease), pharyngitis, illness with fever. It is clear that both group A and B are important pathogens of man. Huebner et al. (1951) reported that Coxsackie A virus causes herpangina in man. Coxsackie Virus A type 5 and 6 have been associated with fever, lymphadenitis, while hand, foot and mouth disease is caused by A type 16 (Rabinson et al., 1958). A virus from blood and faeces of the patients with a morbilliform
rash along with features of scarlet fever and rubella, papular rash. Coxsackie A viruses are often isolated from patients with paralytic poliomyelitis so frequently that Polio virus and Coxsackie A is seemed to act synergically. In many cases A9, A23 (better known as ECHO virus 9) have been isolated from patients with fever, rash, meningoencephalitis in an extensive epidemics in Europe and North America.

The enteroviruses may produce disease in the central nervous system, gastro-intestinal tract and respiratory passages and although they all cause aseptic meningitis certain types have been predominantly associated with gastro-intestinal or respiratory symptomatology, including the development of severe interstitial pneumonitis and death. Coxsackie A virus infections are most frequent in infancy causing gastroenteritis with diarrhoea, vomiting, tracheitis, bronchitis, pneumonitis (Archetti and Bortolozzi, 1954). Melnick et al. (1949) reported that Coxsackie B viruses are also associated with meningoencephalitis, paralysis, pancreatitis, myocarditis, hepatitis both in children and adult. It causes often fatal illness in newborn children, the prominent pathological findings are extensive myocarditis, hepatitis with focal lesions in abdominal organs and central nervous system. Gear and Measroch (1953), Javett et al. (1956) reported that same severe and often fatal illness in newborn babies in an outbreak in maternity home in Johannesburg in October, November, 1952. Montgomery et al. (1955) reported
the isolation of Coxsackie B₄ and B₂ from an outbreak in a maternity home in Rhodesia in 1954. Postmortem examination showed focal myocarditis, marked congestion of the lungs, liver, kidneys and suprarenals. Van Crevelo and De Jager (1956) and Verlinde et al. (1956) from Holland also confirmed that Coxsackie B viruses play a fatal role in newborn babies and recovered Coxsackie B₄ virus from brain and heart muscle.

Kibrick and Benirschke (1956) reported the isolation of Coxsackie B₃ virus from the spinal cord of a newborn infant, who died on the 7th day of birth. Postmortem examination revealed a diffuse myocarditis, encephalomyelitis with infiltration of cells in meninges and focal lesions in the cerebellum and spinal cord. The baby was alright at birth but developed fever, cough after several hours and ultimately died on the 7th day. It was concluded that this infection had been acquired in utero from the mother.

Suckling and Vogelpoel (1958), Simenoff and Kys (1958), Naude et al. (1958) and Kipps et al. (1958) reported the involvement of Coxsackie virus group B in two outbreaks involving 9 babies in a maternity home at Capetown. There is evidence suggesting that congenital heart disease may be associated with maternal infections by Coxsackie B₃ and B₄ viruses (Brown and Evans, 1967). Coxsackie B₃ and B₄ passes the placental barrier and causes abortions in mice (Selzer, 1969). It causes myocarditis and pericarditis.
with B₃ and B₅ (Gear, 1958). In maternity home the infection might have been introduced by expectant mother or by nursing staff or acquire infection from other babies in a common nursery. Burch and Giles (1971) also confirmed the role of this virus and emphasized the need for further study causing chronic heart disease, endocarditis, myocarditis resulting from rheumatic fever.

The group B Coxsackie viruses have peculiar trophism for the brown fat tissues. The lipids play a role in the growth and pathogenesis of neurotrophic viruses. Coxsackie B viruses are also neurotropic, hence part played by different lipids present in brain and brown fat is of prime importance.

Encephalitis due to viruses are reported in Jamshedpur, India (Khan, 1954; Chari and Swamy, 1955; Pandit, 1955). Gupta et al. (1959) and Paul et al. (1959) reported isolation of Polio 1, 2, 3; Coxsackie B₃, B₅; ECHO 11 from cases in an epidemic in Lucknow. Sarkar et al. (1966) reported isolation of Coxsackie A₄ virus from two cases of encephalitis in Calcutta. Madhavan and Sharma (1969) isolated ECHO 7 from clinically diagnosed cases of encephalitis in Pondicherry. Isolation of ECHO 7 virus is of interest, since this virus has been mainly known to cause mild aseptic meningitis (Curman, 1964). Chandrasekhar et al. (1975)
reported the fatal virus myocarditis taking the life of an 18 year old girl is caused by Coxsackie virus $B_2$. They could not isolate virus from blood, throat swabs or stool specimens collected on different occasions but found a high titre 1 in 128 against Coxsackie virus $B_2$ in serum-virus neutralisation test. Madhavan et al. (1973) reported isolation of ECHO 9 from two fatal cases of encephalitis. Fletcher and Brennen (1957) and Weinstein (1957) reported pericarditis due to Coxsackie B viruses in adults. Madhavan et al. (1974) stated the aetiological role of Coxsackie virus B in myocarditis in Pondicherry. Most outbreaks of Coxsackie B myocarditis have occurred in the warm spring, summer and autumn months when Coxsackie viruses as well as Polio viruses infections are most prevalent.
<table>
<thead>
<tr>
<th>Property</th>
<th>Picornaviridae</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Virus particles</strong></td>
<td></td>
</tr>
<tr>
<td>Morphology — symmetrical</td>
<td></td>
</tr>
<tr>
<td>Sedimentation value</td>
<td>140 - 160 S</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>$8.5 \times 10^6$</td>
</tr>
<tr>
<td>R.N.A.</td>
<td></td>
</tr>
<tr>
<td>Sedimentation value</td>
<td>33 - 35 S</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>$2.5 \times 10^6$</td>
</tr>
<tr>
<td>Polypeptides</td>
<td>4 major, 1 minor</td>
</tr>
<tr>
<td>Replication</td>
<td>Translation into large protein precursor, which is subsequently cleaved (monocistronic-like messenger).</td>
</tr>
</tbody>
</table>

The pathogenicity of Coxsackie A and B viruses are given in a tabular form below (Tobin, 1953).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimum age of mice for inoculation.</td>
<td>48 hours</td>
<td>24 hours</td>
</tr>
<tr>
<td>Incubation period in suckling mice.</td>
<td>2 - 6 days</td>
<td>4 - 12 days</td>
</tr>
<tr>
<td>Type of paralysis</td>
<td>Flaccid</td>
<td>Spastic</td>
</tr>
<tr>
<td>Myositis in suckling mice.</td>
<td>Generalised</td>
<td>Focal or absent</td>
</tr>
<tr>
<td>Panniculitis in suckling mice.</td>
<td>Absent</td>
<td>Marked</td>
</tr>
<tr>
<td>Encephalitis in suckling mice.</td>
<td>Absent</td>
<td>Often present</td>
</tr>
<tr>
<td>Pancreatitis in weaned mice</td>
<td>Absent</td>
<td>Often present</td>
</tr>
<tr>
<td>Growth in primate tissue culture</td>
<td>Exceptional</td>
<td>Usually positive</td>
</tr>
</tbody>
</table>
**Infections associated with Coxsackie and ECHO viruses in man**

<table>
<thead>
<tr>
<th>Clinical reaction</th>
<th>Coxsackie viruses Group A</th>
<th>Group B</th>
<th>ECHO viruses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild paralysis</td>
<td>4, 7, 9*</td>
<td>2, 3, 4, 5*</td>
<td>4, 9, 11, 16*</td>
</tr>
<tr>
<td>Meningitis and Meningoencephalitis</td>
<td>2, 3, 5, 7, 9*, 10, 23*</td>
<td>1, 2, 3, 4*</td>
<td>2*, 3, 4*, 5*</td>
</tr>
<tr>
<td>Febrile illness</td>
<td>5, 9</td>
<td>1*, 2, 3* , 4, 5*</td>
<td>4*, 9*, 16*, 13*</td>
</tr>
<tr>
<td>Herpangina</td>
<td>2, 4, 5, 6, 8, 10*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vesicular stomatitis with exanthem (Hand F.M.D.)</td>
<td>5, 16*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bornholm disease</td>
<td>-</td>
<td>1, 2, 3, 4*</td>
<td>-</td>
</tr>
<tr>
<td>Diarrhoea**</td>
<td>-</td>
<td>-</td>
<td>2, 7, 8, 11, 12, 14, 13</td>
</tr>
<tr>
<td>Myocarditis and pericarditis</td>
<td>-</td>
<td>2, 3, 4*</td>
<td>-</td>
</tr>
<tr>
<td>Respiratory infections</td>
<td>-</td>
<td>-</td>
<td>8, 11, 20, 28</td>
</tr>
</tbody>
</table>

* Some times with an erythematous maculopapular rash.

** Reo viruses associated.
Diseases associated with Enteroviruses:

**Polio viruses:**
- Paralysis (complete to slight muscular weakness), aseptic meningitis, undifferentiated febrile illness, particularly during the summer.

**Coxsackie viruses**
- Group A:
  - Herpangina (types 2, 4, 5, 6, 8, 10),
  - undifferentiated febrile illness, particularly during summer.
  - Aseptic meningitis (types A7, A9)
- Group B:
  - Aseptic meningitis.
  - Pleurodynia (Bornholm disease).
  - Undifferentiated febrile illness with pharyngitis.
  - Myocarditis or encephalomyocarditis during neonatal period and early childhood.
  - Mild paralysis (?) or encephalitis.

**ECHO Viruses:**
- Aseptic meningitis (types 2, 3, 4, 5, 9, 14, 16, 21).
- Febrile illness with rash (types 4, 9, 16).
- Boston exanthem (type 16).
- Undifferentiated febrile illness, particularly during the summer.
- Mild paralysis (?) (type 6, 9) or encephalitis (type 9).
- Summer diarrhoea of infants and children (type 18).
Epidemiology:

Coxsackie viruses are recovered much more frequently during the summer and early fall. Also it has been seen that most outbreaks of Coxsackie B myocarditis have occurred in the warm spring, summer and autumn months when Coxsackie viruses as well as Polio viruses infections are most prevalent. Also children develop neutralising and complement fixing antibodies during the summer, indicating infection by these agents during this period; such children have much higher incidence rates for acute, febrile minor illness during the summer than children who fail to develop Coxsackie virus antibodies. Viruses of the Coxsackie group have been encountered in all parts of the world. Isolations have been made mainly from sewage, pharyngeal swabblings, human faeces and flies. The detection of the antibodies in serum collected from individuals in different parts of the world suggest the wide distribution of the virus. All the susceptible persons in a family acquire the infection once the virus is introduced in the family members, but all do not develop clinically apparent disease. In a family about 30% of infected persons develop faucial lesion in case of herpangina, others may show a mild febrile illness without the throat lesions. Viruses are usually recovered from the alimentary tracts of children of those are living under the
poor socio-economic conditions. Coxsackie virus B₁, B₃ and B₅ have been isolated from nose, throat, rectal swabs of dogs (Lundgren et al., 1968). As the name implies, enteroviruses are found primarily as inhabitants of the intestinal tract, particularly of young hosts of those living under poor socio-economic conditions. They commonly cause no illness, but may spread from the gut and cause destructive lesions in the central nervous system. It is seen that when the enteroviruses are restricted to the gut, there is absence of any clinical manifestation. From the gut the virus enter the blood stream and then invade other tissues resulting fever, vesicle formation, conjunctivitis, myelgia, myocarditis, meningitis and paralysis due to destruction of anterior horn cells. In the case of the symptoms of diarrhoea a question is raised regarding the aetiological significance of various viruses that are being recovered from infants and children with diarrhoeal diseases. Polio viruses and cytopathogenic Coxsackie viruses were recovered with the same frequency from the diarrhoeal and non-diarrhoeal cases, hence it is impossible to be sure that they were responsible for the illness observed in the children from whom they were isolated. The reasons may be that all these viruses produce a spectrum of clinical manifestation of minor illness to more severe diseases with
varying degrees of involvement of central nervous system, diarrhoeal illness occurred concurrently with an acute infection as shown by the development of neutralizing antibodies during convalescence. The group A types are commonly related to febrile illness in young individuals during summer months characterized by headache, stiffness of neck, muscle soreness and vesicular pharyngitis with small serous blisters on the pharynx. Group B types are aetiologically related to epidemic pleurodynia (Bornholm disease), characterized clinically by sudden onset of fever and severe pain in chest (devil's grip), with accompanying signs of meningitis, meningoencephalitis, myocarditis in infants. Liver enlargement, cyanosis, circulatory collapse with rapid and also weak pulse.

As regards the vectors in respect of transmission of disease arthropods may play a minor role, but mechanically faecal contaminated food and water plays a role in transmission of the disease. It has been reported that enteroviruses were efficiently adsorbed to coxfilters from tap water at pH level 7.6. (Sobsey et al., 1973).

A wave of Coxsackie B3 in 1952 was followed by a wave of B4 in 1954; there was a wave of B3 in 1956-57, and again in 1959. During 10 years 1950 - 1959 Coxsackie virus B1 was not isolated and first isolation was made in 1960-61. (Fig. 1).
Fig. 1
Size of the virus and Antigenic structure:

Coxsackie viruses measure 24-30 nm in diameter, of icosahedral in size and have the structure of the other Picorna viruses. Coxsackie viruses Group A have 24 serotypes, and Group B have 6 serotypes, which can be distinguished by neutralisation test in baby mice or tissue culture (Rosen et al., 1970). Cross reactions among B types have been studied (Wenner et al., 1965).

Electron microscopic examination of Coxsackie viruses A (Mattern and Du Buy, 1956) and B (Morgan et al., 1959) revealed approximately spherical naked virions with uniform diameter estimated between 20 and 30 nm, and a nucleoid 6 to 20 nm across. Variation in size has been reported by Jamison and Mayor (1966) Coxsackie B2 (mean diameter 21.0 nm).

The morphogenesis of Coxsackie virus (Stuart and Fogh, 1967) takes place mainly in the cytoplasm although nuclear and nucleolar participation in viral synthesis has been suggested. Virions are often seen in association with small cytoplasmic vesicles or with fibrillar structures. Crystalline arrays of virions are often seen. Virus particles are synthesized in membrane
bound complex and assembled in cytoplasm. Virus is released by a lytic "burst".

It contains infectious single stranded linear R.N.A. having sedimentation coefficient 33-35 S, subject to variation according to ionic strength. The R.N.A. content is about 30%. Analysis of protein composition of Coxsackie viruses (Kiehn and Holland, 1970) reveals 3 major structural polypeptides with molecular weight ranging from 24,000 to 35,000. One minor structural polypeptide and several non-structural components, the largest being a precursor cleaved into the structural polypeptides on assembly into virions (Jacobson and Baltimore, 1970). The protein percentage weight of virion is about 70%. All 6 B serotypes share a common complement fixing antigen while certain of 24 A serotypes are antigenically related to one another. Some strains of Coxsackie B viruses have been found to be more difficult to neutralize by antisera than other strains and this property varied on passage (Wigand, 1960; Wigand and Sabin, 1958). These strains contained, in varying proportion, virus particles which differed in sensitivity to specific antibody. Choppin and Egger (1962) described the isolation and characterization of two kinds of Coxsackie B₄ virus particle which differ in antibody sensitivity and other biological properties, including inhibition sensitivity plaque morphology and growth rate.
Physico-chemical characters:

The viruses both group A and B are similar in physico-chemical characters. Coxsackie viruses are resistant to lipid solvents, survive well at -70° C, not easily preserved by lyophilization, as most of the infectivity is lost through this procedure, are stable between pH 2.3 and 9.4 for 1 day or between pH 4.0 and 8.0 for 7 days (Robinson, 1950), are resistant to 5% lysol, 70% ethanol, 1% Roccal, but are inactivated by 0.1 N HCl or 0.3% formaldehyde (Kaplan and Melnick, 1952), by 2(1-hydroxybenzyl)-benzimidazole (HBB) (Eggers and Tamm, 1961), and guanidine (Rightsel et al., 1961). Both these substances act by inhibiting the production of RNA polymerase (Baltimore et al., 1963) and of viral RNA and coat protein (Eggers and Tamm, 1962; Crowther and Melnick, 1961). Mattern, in 1962 reported that Coxsackie virus appear to have greater proportion of guanine and lesser amount of adenine than Polio virus.
Cultivation of virus:

The Coxsackie virus grows well in monkey kidney tissue culture, producing a typical cytopathogenic effect (C.P.E.). Cytological biosynthetic alterations associated with the growth of enteroviruses have been reviewed by Godman (1960). Virus is adsorbed to lipoprotein cell receptors present only in susceptible cells (Holland and McLaren, 1961). Soon after infection the synthesis of cellular nucleic acids and proteins are inhibited. Virus replication is independent of cellular DNA function (Simon, 1961; Reich et al., 1961). Replication of Coxsackie virus (Mattern and Chi, 1962) is entirely cytoplasmic. Synthesis of early and late proteins leads to the formation of "procapsids" containing large polypeptides, which are cleaved during the process of assembly with RNA (Jacobson and Baltimore, 1968). Some strains of group A grow in human amnion cells. Chimpanzees and Cynomolgus monkeys can be infected subclinically, virus appears in the blood and throat for short periods and is excreted in the faeces for 2 to 5 weeks. Type A14 produces poliomyelitis-like lesions in adult mice and in monkeys, but upon inoculation into suckling mice this type produces only myositis. Type A7 strains produce paralysis and severe CNS lesions in monkeys. Cytopathological changes
are seen in cells when a cytocidal virus infect the susceptible host or culture. Cellular resistance to infection may be overcome by using infectious RNA rather than virus (Holland et al., 1959). Mechanisms of viral penetration and uncoating are unknown. The morphological lesions caused by different viruses varies (Pereira, 1961; Walker, 1960) and on basis of these differences viruses are also classified (Barski, 1962). Enders in 1954 termed these virus induced changes in cells as virus cytopathogenic effect (C.P.E.). The changes in cells induced by virus include: (i) loss of chromatin in the nucleus and appearance of eosinophilic cytoplasmic mass, (ii) distortion and displacement of the nucleus, (iii) wrinkling of the nuclear membrane, (iv) increased vacuolization in the cytoplasm, (v) swelling and rounding of cells, and (vi) finally complete destruction of cells.

Experimental studies in vivo have shown that some enteroviruses multiply in hosts in which they are not found in nature; e.g., Polio viruses replicate in monkeys, Coxsackie viruses replicate in newborn mice and chimpanzees and to a lesser degree in monkeys; Cardio viruses replicate in mice and rats. In vitro studies have revealed that the enteroviruses tend to be specific for the cells from their natural host or from animals closely related to their natural host. Some exceptions are known, e.g., Coxsackie virus B5 replicates in pig kidney cells.
Animal susceptibility:

The newborn mice is susceptible to the Coxsackie viruses of both the groups A and B. Optimum age for mice inoculation is 24 hours old for group B and 48 hours old for group A viruses. Suckling mice below 24 hours old is ideal for producing infection by various routes, i.e., subcutaneously, intraperitoneally, oral route, intracerebrally but preference is given for the latter route, i.e., intracerebral route. Coxsackie B causes spastic paralysis and tremors. It causes focal necrosis of muscle, myocarditis, hepatitis, parotitis, pancreatitis. Microscopically, the most prominent necrosis lesion is seen in interscapular brown fatpad. Gravid mice are more susceptible than normal. Acute cell necrosis is seen in brain as well as in other tissues. There is widespread degeneration, infiltration of inflammatory cells in muscle (Lepine et al., 1952), in heart, brain, liver and pancreas.

The mechanism underlying the susceptibility of the experimental mice as regards their age with certain viral agents is still obscure and many factors which are interrelated may be involved (Siegel, 1952; Overman, 1954; Dalldorf and Gifford, 1954; Dalldorf, 1955; McLaren and Sanders, 1959; Grodums and Dempster, 1959; Kunin et al., 1961). Among many of the interrelated factors endocrine
activity has got a direct relationship to the gradual increase of resistance in maturing animals. The enhanced susceptibility is due to increased concentration of adrenocortical hormone in the milk during postpartum period (Dalldorf and Gifford, 1954; Teodoru and Shwartzman, 1954; Kass and Finland, 1958; Campbell, 1960; Greenberg and Morgan, 1961). Mortalities of newborn mice were increased in litters from infected mothers and the increased fatalities continues for 7 days after infection. The increased susceptibility of the newborn mouse could be due to increased adrenal cortical function both in the mother and in the newborn mouse. The colostrum of the puerperal mother is known to contain an increased concentration of adrenal cortical hormones.

Kilbourne and Horsfall (1951) have shown that cortisone produces lethal effects in Coxsackie virus infections of adult mice. Montgomery et al. (1955) reported that Coxsackie viruses group B have been implicated as the responsible agents in a significant number of infant deaths occurring during the neonatal period (Benirschke and Pendleton, 1958; Jack and Townlay, 1961; Kibrick and Benirschke, 1958; Sussman et al., 1959). By transplacental passage of virus infection may occur in
uterus (Benirschke and Pendleton, 1958; Kibrick and Benirschke, 1958) or through direct contact with the infected individual in the first few days of life (Moosy and Gear, 1960; Sussman et al., 1969). The situation in the pregnant mother and in the newborn infant particularly is more severe. The increased susceptibility of the gravid females during last week of pregnancy disappears immediately after parturition (Dalldorf and Gifford, 1954). The newborns get their infection from the mother, if the latter gets the infection during the last months of pregnancy. The infants may die due to severe generalised infection within 3 to 10 days of life. Histopathological observations revealed encephalitis and myocarditis and virus has been recovered from brain, heart, spinal cord (Delany and Fukunaga, 1958; Benirschke and Pendleton, 1958; Jack and Townley, 1961; Kibrick and Benirschke, 1958; Mc Lean et al., 1961; Sussman et al., 1959). Older mice become progressively less susceptible. The cortico-steroids may enhance the susceptibility of older mice to infection of the pancreas.

Normal adult mice tolerate infections with group B Coxsackie viruses but in mice subjected to sustained
post-weaning under nutrition (marasmus), the B3 viruses produce severe disease including persistence of infective virus in the heart, spleen, liver and pancreas. Lymphoid tissues are markedly atrophic in marasmic animals. Transfer of lymphoid cells from normal mice immunized against the virus provides virus infected marasmic mice with significant protection against the severe sequelae. These observations support the hypothesis that lymphocyte-mediated defense mechanisms may play an important role in normal recovery from primary viral infections. Group A type 7 and 14 viruses caused mild paralysis on inoculation into monkeys (Habel and Loomis, 1957; Dalldorf, 1957).
Role of different routes of inoculation in newborn mice:

There is marked susceptibility of the newborn mouse to parenteral and oral infection with Coxsackie virus group B\textsubscript{1}. Loria et al. (1974) reported that the newborn mice are as susceptible to these viruses by oral route as by the parenteral route. Both human neonates and newborn mice generally show similar responses to per oral infection of Coxsackie viruses group B. Hence, newborn mice infected by different routes could provide a model for study of pathogenesis in the human neonate. The natural portal of entry for group B Coxsackie viruses in human is the alimentary tract. Kaplan and Melnick (1951) however reported that parenteral route was about 10,000 times more sensitive than oral route. They further indicated that this failure was due to lack of administration of full dose of virus by oral route. Loria et al. (1974) adopted a more sensitive technique for delivery of the inoculum, administered through a sterile polyethylene tube (3.5 cm in length) into the stomach with the help of swallowing reflex, and observed that there was no differences in susceptibility of mice by two different routes intraperitoneal (I/P) and oral route. This indicated that the gut of the newborn mouse did not provide an effective barrier against per oral infection.
Host macromolecular synthesis of protein infected with Picorna virus:

The host macromolecular synthesis is altered causing the inhibition of host RNA, DNA and protein synthesis (Martin and Kerr, 1968; Reizman and Spear, 1969; Bablanian, 1972; Ackermann, 1959; Ackermann et al., 1959; Salzman et al., 1959). The morphological changes by the cytocidal virus in cell cultures have been studied extensively both by Phase and Electron microscopy (Barski et al., 1955; Dunnebacks, 1956; Dales et al., 1965; Amako and Dales, 1967; Dales and Franklin, 1962; Skinner et al., 1968; Plagemann et al., 1970). The cell killing property at the end lies with inhibition of host RNA, DNA and protein synthesis at the time of replication (Martin and Work, 1961; Holland, 1963, 1964) and accumulation of viral proteins (Bablanian et al., 1965). The ultraviolet light inactivated virus according to Gauntt and Lockart, 1966; Bablanian, 1972 or isolated viral capsids (Wolff, 1965) failed to cause any alteration in morphological lesions. Partially U-V inactivated virus does not cause this inhibition (Franklin and Baltimore, 1962; Penman and Summers, 1965; Bablanian, 1972). Inhibition of DNA synthesis occurred later than that of host RNA and protein synthesis (Baltimore and Franklin, 1962; Franklin and Baltimore, 1962; Scholtissek et al., 1962; Ackermann et al., 1966).
The host RNA synthesis in all species is inhibited (Fenwick, 1963; Homma and Graham, 1963). The inhibition of ribosomal RNA synthesis occurred before that of messenger RNA (Darnell et al., 1967; Cantreras et al., 1973). Cantreras et al. (1973) reported that there is temporary stimulation of ribosomal RNA synthesis. Martin et al. (1961) and Franklin and Baltimore (1962) reported that infection did not cause a net changes in the amount of RNA in cells. Polysomes of cells which is associated with the inhibition of host protein synthesis disaggregate rapidly soon after infection (Penman et al., 1963; Summers et al., 1965; Joklik and Mergan, 1966; Dalgarno et al., 1967). Penman et al. (1963) reported that during the late stage of infection a larger polysome was formed, which synthesized immunologically identifiable virus protein (Scharff et al., 1963).

Several biochemical and morphological studies demonstrate that Picorna virus RNA synthesis occurs in cytoplasmic areas, such as "small bodies", "virus synthesizing bodies" or the smooth ER fraction etc. (Becker, Penman and Darnell, 1963; Caliguiri and
Tamm, 1968; 1970a, 1970b; Crocker, Pfendt and Spendlove, 1964; Dales, Eggers, Tamm and Palade, 1975; Girard, Baltimore and Darnell, 1967; Penman, Becker and Darnell, 1964). Other authors suggest that the nucleus may play a part in the picorna virus replication (Anzai and Ozaki, 1969; Bienz, Bienz-Isler, Egger, Weiss and Loeffler, 1970; Fliikke, Christiansen and Lahelle, 1965; Harrison, Murphy and Gary, 1971; Levy, 1961; Mattern and Daniel, 1965). But the biochemical role of the nucleus in the synthesis of viral macromolecules is not well defined. Furthermore, results from previous investigations seem to fluctuate with experimental conditions.

There is a depression of host cell RNA synthesis shortly after infection (Baltimore and Franklin, 1962; Fenwick, 1963; Franklin and Baltimore, 1962; Franklin and Rosner, 1962; Holland and Peterson, 1964; Martin and Work, 1961; Plageman and Swim, 1966).
Clinical findings:

The incubation period of Coxsackie virus infection ranges from 2-9 days. The clinical manifestations of infection with various Coxsackie viruses are diverse and may present as distinct disease entities.

Infection with a Coxsackie virus is suggested by the clinical manifestations of herpangina, pleurodynia, aseptic meningitis, summer minor illness of non-bacterial origin or neonatal disease, particularly myocarditis. Virus is present in the central nervous system and heart muscle of fatal cases in the newborn. Coxsackie viruses may be responsible for myocardopathies in adults more frequently than has been recognised.

Herpangina:

This disease is caused by certain group A viruses, it is characterized by an abrupt onset of fever and sore throat. There may be anorexia, dysphagia, vomiting and abdominal pain. The pharynx is usually hyperemic and characteristic discrete vesicular lesions may be seen on the anterior pillars of the fauces and less frequently on the palate, uvula, tonsils, or tongue. The illness is self limited and most frequent in small children.
Summer minor illness:

Coxsackie viruses are often isolated from patients with acute febrile illness of short duration and without distinctive features. Occurring during summer or fall. Information is lacking on the frequency of oropharyngeal lesions in these patients, whose illness in other respects resemble herpangina.

Pleurodynia (Epidemic, myalgia, Bornholm disease):

This disease is caused by certain group B viruses. Fever and chest pain are almost invariably present together; they are usually abrupt in onset, although they may be preceded by malaise, headache, anorexia and other vague prodromal symptoms. The chest pain may be located on either side or substermally, is intensified by movement and may last from 2 days to almost 2 weeks. Abdominal pain occurs in approximately half the cases and in children may be the chief complaint. The illness is self-limited and recovery is complete although relapses are common.

Aseptic meningitis and mild paresis:

This type of syndrome is caused by all types of group B; certain Coxsackie A types (A7, A9) also have been clearly implicated in several epidemics. Fever,
Malaise, headache, nausea and abdominal pain are common early symptoms. Signs of meningeal irritation with stiffness of the neck or back and vomiting may appear 1-2 days later. The disease sometimes progresses to mild muscle weakness, which is often confused clinically with paralytic poliomyelitis. Patients almost always recover completely from non-polio virus paresis with no residual disability. Examination of the cerebrospinal fluid early in the acute phase of the illness reveals an increase in the number of leucocytes, which in most instances does not exceed 100/cu mm. The percentage of polymorphonuclear cells ranges from 10-50. Encephalitis is the commonest manifestations of involvement of the nervous system by any of the enteroviruses. Meningoencephalitis has been found in fatal infections of the newborn due to Coxsackie B3 (Kibrick and Benirschke, 1956). Aseptic meningitis with or without encephalitis (Curnen, 1960; Mc Allister, 1960; Melnick and Sabin, 1959; Faulkner and Ozere, 1960) has been reported.

In India, Coxsackie B3 and B5 have been isolated from faeces of meningitis, encephalitis cases in Lucknow (Gupta and Paul, 1958). Coxsackie B viruses have been more commonly implicated with the production of
aseptic meningitis or meningoencephalitis than Coxsackie A viruses. Coxsackie A9 was isolated from cases of meningoencephalitis (Cramblett et al., 1964) and meningitis (Lerner et al., 1960) and involvement of central nervous system by Coxsackie A4 is also not unknown.

Colds:

It has become apparent that the common cold may be caused by many different viruses. A number of enteroviruses have been associated with common colds, among these are Coxsackie viruses A10, A21, A24, and B3.

Hand, Foot and Mouth disease:

This disease has been associated particularly with Coxsackie virus A16 but A4, A5, A9 and A10 have also been implicated. Virus may be recovered not only from the stool and pharyngeal secretions but also from vesicular fluid. Outbreaks have occurred in Newzealand, England, Canada and in several states of the U.S.A.
Neonatal disease:

Neonatal disease caused by Coxsackie group B viruses may be more common than has generally been recognised. The clinical syndrome may consist merely of lethargy, feeding difficulty and vomiting with or without fever. In several cases myocarditis or pericarditis with or without severe generalised disease can occur within the first 8 days of life, it may be preceded by a brief episode of diarrhoea and anorexia. Cardiac and respiratory embarrassment are indicated by tachycardia, dyspnoea, cyanosis, and changes in the electrocardiogram. The clinical course may be rapidly fatal or the patient may progress to complete recovery. The disease may sometimes be acquired transplacentally. Myocarditis has also been caused by some of the group A Coxsackie viruses.

Myocardopathy:

Coxsackie virus B infections are increasingly recognised as causative agents of a significant proportion of primary myocardial disease in adults as well as children. In some series upto 30% of persons
infected with Coxsackie virus B₅ developed cardiac abnormalities. In one study of 22 cases of adult heart disease suspected of being due to viral infection, 19 of the patients had evidence of infections with Coxsackie viruses of group B and in follow-up studies 12 of the patients were found to have developed chronic heart disease. In recent years, Coxsackie viruses of group A and ECHO viruses have also been implicated in cardiac disorders. Evidence for a high degree of association of the virus with disease has been obtained, usually at autopsy, by demonstration of virus localized in the myocardium, endocardium, and pericardial fluid; the presence and specificity of the virus at the sites of pathologic change have been demonstrated by a variety of methods, including immunofluorescence, peroxidase-labeled antibody, or ferritin-labeled antibody. It has been estimated that about 5% of all symptomatic Coxsackie virus infections induce heart disease. The virus may affect the endocardium, pericardium, myocardium or all three. Acute myocardopathies have been shown to be caused by Coxsackie virus A₄, A₁₄, B₁₋₅, and also by ECHO virus types 9 and 22. On the basis of less complete evidence, Coxsackie viruses A₁, A₂, A₅, A₈, A₉ have also been associated with these illness, as have ECHO viruses of
types 1, 4, 6, 14, 19, 25 and 30 as well. Following the acute phase of the illness, a significant proportion of patients develop serious heart disease. In man, Coxsackie virus B₄ may be associated with incompetence of the aortic or the mitral valve (or both) and Coxsackie viruses B₂, B₄ and B₅ with chronic myocardopathies. Coxsackie virus B₁ may be associated with constrictive pericarditis. Monkeys infected with Coxsackie virus B₄ develop pancarditis, with a pathologic picture strikingly similar to that of rheumatic heart disease.

Studies have shown with the experimental animals that the virulence of acute viral myocardiopathies are greatly increased by hydrocortisone therapy, vigorous exercise, alcohol consumption, pregnancy and undernutrition and is seen greater in males than in females. Observations with the human illness suggest that these factors may similarly increase the severity of the disease in human beings. Serious fatal myocarditis due to Coxsackie B viruses occur in newborn infants (Gear, 1958). Congenital heart disease may be associated with maternal infections by Coxsackie B viruses especially B₃ and B₄ (Brown and Evans, 1967). These two types pass the placental barriers and cause many abortions in mice (Selzer, 1969).
Viral pancreatitis in experimental animals and man:

Gamble et al. (1969) and Gamble and Taylor (1969) reported from a series of serological studies that Coxsackie virus B1 infection may lead to an abrupt onset of diabetes mellitus. Craighead and Mc Lane (1968) stated that Picorna-virus encephalomyocarditis virus have got the capacity to produce lesions in islets of Langerhans of pancreas and cause a disease, like diabetes mellitus, in mice. It is assumed that a viral infection in man may cause damage to the insulin secreting beta cells of the islets of Langerhans. Pappenheimer et al. (1950, 1951) reported that suckling mice infected with Coxsackie virus B1 shows necrotizing lesions of the exocrine pancreas suggesting that virus multiplies in Pancreas. This work was supported by Vizoso and Sanders (1964); Godman et al. (1952); Pappenheimer (1952); Melnick and Godman (1961); Woodruff (1970); and Craighead (1972). The acinar tissue of infected mice showed severe atrophy, necrosis, diffuse interstitial oedema, infiltration of histocytes and mononuclear inflammatory cells. Kibrick and Benirschke (1956) reported focal inflammatory cells infiltrations in pancreas from an infant dying with
meningoencephalitis and myocarditis and recovered Coxsackie virus B3. They have also recovered Coxsackie B4 virus from two neonatally acquired cases, showing focal necrosis, inflammation of the pancreas. Fechner et al. (1963) also recovered the same virus Coxsackie B4 from the haemorrhagic pancreatic tissue of a child.

**Effect of virus on pregnant mice**

Lansdown and Cold (1974) reported that pregnant mice infected with Coxsackie virus B3 shows growth retardation and foetal wastage and there is maternal pancreatitis and hepatitis. The cause and mechanism of foetal growth impairment is still not clear, but according to Blaxter (1968) liver plays a key role for the proper maintenance of foetal growth. Hence, it is thought that infection in liver leads to foetal death and/or retardation of foetal growth. Burch et al. (1973) reported the histological changes in liver of pregnant mice infected with Coxsackie virus B3 and could not detect virus in the tissue by electron microscopy. Soike (1967) did not report any histological changes in the livers of pregnant mice infected with Coxsackie virus B3 but isolated virus from livers of these infected pregnant mice. Lansdown and Ellaby (1974) demonstrated
the development of histological and histochemical changes in the livers of pregnant mice infected with Coxsackie virus B\textsubscript{3}. They reported that there is sequential changes in liver cell morphology in mice infected with Coxsackie virus B\textsubscript{3} using enzymic markers for early cell damage. There is increase of alkaline phosphatase activity 2 days after infection suggesting that virus affects the liver and causes hepatitis, ultimately reduces the health condition of pregnant mice resulting impairment of foetal growth and foetal death. Histological changes of liver showed cytoplasmic vacuolation, dilatation of periportal cells, nuclear pyknosis and deposition of neutral fat in periportal areas. After 4 days of inoculation of virus there is slight changes in histological picture, i.e., cytoplasmic vacuolation was minimal with slight increase in neutral fat deposit in periportal areas. After 6 days of inoculation there is nuclear pyknosis with a loss of cytoplasmic eosinophilia. The possible explanation given by Lansdown and Ellaby (1974) that the virus causes foetal death and foetal growth retardation in two ways; (i) directly affecting the liver, causing hepatitis which is manifested with an increase of alkaline phosphatase activity, subsequently
as a reduction in succinic dehydrogenase (S.D.H.) and glucose-6-phosphatase (G-6-P), increased lysosomal activity as indicated by the marker enzyme acid phosphatase and histological changes; and by (ii) producing pancreatitis with advanced acinar atrophy (Pappenheimer et al., 1950; Tsui et al., 1972; Harrison et al., 1972; Soike, 1967) followed by hepatitis. But there was no conclusive evidence exists that the virus replicated in the liver (Minkowitz and Berkovich, 1970; Burch et al., 1973). It is possible that pancreatitis with acinar atrophy consequently causing hepatitis can lead to nutritional protein deficiency (Goldstein, 1968), suppression of protein synthesis, histological changes in the liver cell (Emwonus and Sreenby, 1971), ultimately resulting to foetal growth retardation and occasionally intrauterine death (Emwonus and Glover, 1973). Infection may occur in uterus by transplacental passage of the virus (Benirschke and Pendleton, 1958; Kibbrick and Benirschke, 1958) or through direct contact with the infected individual in the first few days of life (Moosy and Gear, 1960; Sussman et al., 1959).