DISCUSSION
The pathogenicity of Coxsackie virus was first studied by Melnick and Ledinko (1949) and Melnick (1950) in Chimpanzees and Cynomologus monkeys following oral administration, although there was no apparent illness in both species. They observed the development of neutralizing antibody to the infecting strain of virus.

Subsequently, Kaplan and Melnick (1951) studied the effect of the immunologically distinct 5 strains of Coxsackie virus on newborn and adult mice following oral administration of virus by a syringe and a blunt needle. Each mouse was given 0.01 ml of virus, but in many of the instances all the amount was not consumed. However, it was evident from their observations that newborn mice contact the paralytic disease when fed with the virus $10^{-3}$ concentration. They further compared the susceptibility of newborn and adult mice and observed that the oral route was more susceptible for the newborn mice only but the concentration of virus required to infect these animals by this route was much higher than those by other routes. They did not, however, mention the percentage of mortality following the oral route, but, observed the subcutaneous route to be 10,000 times more sensitive than the oral route so far as antibody forming capacity is concerned. Their study was in line of
Von Magnus (1950) who also observed the susceptibility of newborn mice with 10 strain of mouse encephalomyelitis virus. Adult mice was, however, observed to be resistant to this virus infection but developed neutralizing antibody much more readily by the subcutaneous route than that by the oral route. However, there was variation in the development of neutralizing antibody in the surviving mice, who were given virus by oral route. They, however, observed that the adult female mice, who developed the antibody, did not transmit this antibody to their offsprings.

Sulkin et al (1951), from a wide scale study, concluded that there was difference of susceptibility of different litters of suckling mice used for primary isolation of Coxsackie viruses. They suggested that at least 2 litters including a total of 12-15 suckling mice should be used for demonstrating Coxsackie viruses in a clinical specimen. Subsequently, experimental studies were made more thoroughly in mice and lesions in susceptible animals were used as the criteria to identify Coxsackie viruses and to classify them into Group A and B. This has been proved to be reliable and reproducible (Johnsson and Lundmark, 1955; Paola et al., 1952-53; Dalldorf, 1958),
even though individual lesions were not strictly pathogenomonic, but the pattern was distinctive.

The pattern of anatomical lesions varied with the route of inoculation. Cerebral lesions were more frequent following group B virus infection, when the animals were infected intracerebrally (Gifford and Dalldorf, 1954). One type of lesion could predominate due to type and strain differences. These properties may change to further adaptation and some are known to depend on tissue used for inoculation.

The criteria for clinical presence of infection was determined in the present study by observing majority of following signs such as lethargy, separation from other litters, nodding of head, encircling to the left or right side, spastic paralysis and/or death. A number of animals survived following inoculation by any route indicated that Coxsackie virus B1 caused inapparent infection and/or transitory illness. This could be proved by histological and/or virus recovery study for various tissues.

In human neonates such type of inapparent and transitory illness due to Coxsackie virus group B had
been reported by Kibrick (1961), Brightman et al (1966)
and Hall and Miller (1969). Hence, histological study of
the surviving newborn mice infected with Coxsackie virus
group B could provide an excellent model for inapparent
infection. Transitory illness as occurs in perinatal
infection in newborn babies as has been recorded in the
nursery following recovery of virus from their excreta or
tissues (Van Greveld and De Jager, 1956; Verlinde et al.,
1956; Kibrick and Benirschke, 1956; Brown and Evans, 1967).

Dalldorf and Melnick (1965) from their
study observed that newborn mice were highly susceptible
to parenteral infection with Coxsackie virus B. The
natural portal of entry for group B Coxsackie viruses in
human is, however, the alimentary tract. On the other hand,
the experimental infection in mice can be induced by
parenteral routes and also by oral route of administration.

Loria et al (1974), with Coxsackie
virus B5, using a technique of feeding by a sterile
polyethylene tube inserted into the stomach by swallowing
reflex, claimed that there was no difference in
susceptibility in either of the oral or intraperitoneal
route and also in the mortality rate. The mortality rate
of the newborn mice infected with virus $10^6$ pfu (plaque forming unit in Hela cells) was 86\%, whereas the mortality rate by intraperitoneal route was 89\%. This indicated that per oral infection with Coxsackie virus B$_5$ could cause similar incidence of mortality as compared to the intraperitoneal route, indicating that the guts of newborn mice do not possess any effective barrier against such infection.

In the present study the susceptibility of the newborn mouse to parenteral route of infection and to oral infection with Coxsackie virus B$_1$ were again compared with the use of technique adopted by Loria et al (loc. cit.). It was observed that the susceptibility of mice to both intraperitoneal and oral routes of infection were not similar to those of Loria et al (loc. cit.). The present study showed that the percentage of mortality per oral route was 51.40\% (Table 10 and Fig. 11), whereas by intraperitoneal route it was 84.94\% (Table 8 and Fig. 11). The percentage of mortality by intracerebral and subcutaneous routes were 88.00\% and 51.76\% respectively (Tables 7, 9 and Fig. 11). It is interesting to note that the mortality rate by intracerebral and intraperitoneal routes of infection was very similar, while the incidences
in case of subcutaneous and oral routes of administration were almost equal (Fig. 11).

Although pathogenicity for the newborn mice is one of the characteristic features of Coxsackie virus infection, the present data would only confirm this and further indicate that the oral route was not as effective as in other routes. Probably, the mucosa of the intestine could have provided an effective barrier against per oral infection with Coxsackie virus group B$_1$ (Loria et al., 1974) passing through the epithelium of the gut into the circulation. Moreover, the clearance mechanism of the mucosa could also have helped to eliminate virus from gastrointestinal tract. The protective function would be attributed to the enzymatic action and morphological changes in the gut (Loria et al., 1976). Low rate of mortality by the subcutaneous route was observed similar to that by the oral route and could not be explained. On the other hand, Kaplan and Melnick (1951) observed high susceptibility of the mice by subcutaneous route. It may be pointed out that in their study strain of virus used was not very pure strain. This could explain the difference of observation made in the present study with regard to the
mortality rate. Clinical studies alone revealed that mice infected with Coxsackie virus group B develop meningoencephalitis associated with spastic paralysis. In the present studies 24 hours old newborn mice inoculated with Coxsackie virus B₁ \((10^6 \text{TCD}_{50}/\text{ml})\) by intracerebral, intraperitoneal, subcutaneous and oral routes showed clinical affection with development of spastic paralysis alone to the extent of 33.60%, 30.10%, 22.35% and 20.56% respectively (Tables 3, 4, 5, 6 and Fig. 9). It was observed that total number of mice clinically affected showing mortality, or spastic paralysis and other signs were 118 out of 125 (94.4%), 87 out of 93 (93.54%), 68 out of 85 (80.0%) and 67 out of 107 (62.61%) by intracerebral, intraperitoneal, subcutaneous and oral routes of inoculation respectively (Fig. 8). The total number of mice who died during the period of observation, was 110 out of 125 (88.0%), 79 out of 93 (84.94%), 44 out of 85 (51.76%) and 55 out of 107 (51.4%) respectively by intracerebral, intraperitoneal, subcutaneous and oral routes (Tables 7, 8, 9, 10 and Figs. 10 a, b, c, d and Fig. 11). This showed that some of the mice who were affected by various clinical symptoms, recovered clinically thus producing mild illness. The latter probably ran a short course resulting complete recovery in
all those cases. Virus could, however, be recovered from some of these mice inoculated intracerebrally from brain from the 1st to the 8th day and also from the brain of mice inoculated by intraperitoneal route from the 2nd to the 7th day (Tables 15 and 16). But histological studies (vide infra) would indicate that there could be varying types of lesions in various organs depending upon the route of inoculation in these mice, whether they died or have manifested clinical signs of disease. The latter would only indicate that the tell-tale effect of such virus infection could persist in these surviving animals.

Fatal illness in newborn babies caused by Coxsackie virus group B in a maternity home in Johannesburg has been reported by Gear and Measroch (1953) and Javett et al (1956). Montgomery et al (1955) also reported the similar incidence and isolated Coxsackie B3 and B4 from outbreaks in maternity homes. Van Grevel and De Jager (1956) and Verlinde et al (1956) also confirmed that the occurrence was similar, but recovered Coxsackie virus B4 from brain and heart of newborn babies. Kibrick and Benirschke (1956) isolated B3 from spinal cord of a newborn baby died on the 7th day of birth. On examination, it
revealed diffuse myocarditis, encephalomyelitis with infiltration of cells in the meninges and the focal lesions in the cerebellum and spinal cord. As the baby died on the 7th day after birth, it was concluded that the baby had acquired the infection from the mother in utero. Brown and Evans (1967) isolated Coxsackie B₃ and B₄ virus from the heart of a fatal case of newborn babies suggesting that the congenital heart disease could be associated with maternal infection.

Coxsackie group B virus was implicated as the responsible agent in a significant number of deaths occurred in neonatal period (Montgomery, 1955; Benirschke and Pendleton, 1958; Kibrick and Benirschke, 1958; Sussman et al. 1959; Jack and Townley, 1961). It was then postulated that the infection could occur in utero by transplacental passage of virus or through direct contact with the infected individual during first few days of life.

In order to explore this aspect Soike (1967) administered Coxsackie virus group B, type 3, intraperitoneally to the mice in the last week of pregnancy to study the effect of virus on the foetuses and also on the pregnancy of these litters. Soike (loc. cit.) observed
that the virus titres in organs and tissues of pregnant mice appeared to be maximum in organs and tissues up to the 3rd day and then decreased in quantity by the 6th day. Virus completely disappeared from faeces of the pregnant mice by the 20th day. Foetuses removed from the infected mother demonstrated that the transplacental passage of virus occurred soon after inoculation, with maximal titre being reached on the 1st day of inoculation, declined from the 3rd day and disappeared from foetus by the 6th day. Virus recovered from all organs was neutralized by specific Coxsackie immune serum. Further, it was evidenced by the work of Selzer (1969) that the Coxsackie B3 and B4 could pass the placental barrier and cause abortion in mice.

In the present study, the virus was inoculated intraperitoneally on various days of gestation (8, 12, 16 days) in pregnant mice to observe actually on which day of gestation the effect of the virus on the litter size, litter weight and number was maximum. It was observed that when inoculated in mice of 8-day gestation, the mortality rate of the newborn litters in this group varied from 33.3% to 100.00% in the different individual mouse with an average of 75.6% (Table 11 and Fig. 12 a),
while there was no mortality in the control ones. The weight of litters born alive was also found to be low as compared to that in the control. The average weight of litters from virus infected pregnant mouse was 0.884 gm, whereas in the control it was 1.472 gm (Table 11 and Fig. 12 b). Number of litters born alive was on average 4.4 in the infected group against 7.5 in the control (Table 11 and Fig. 12 c).

Pregnant mice of 12-day gestation when inoculated with the virus, it was observed that the percentage of mortality in newborn litters varied from 40.0% to 75.0%, with an average of 57.2% (Table 12), which was significantly lower than those of the 8-day pregnant mice group (Table 11). The average weight of the newborn litters in the infected group was 1.011 gm while it was 1.472 gm in the control group and the average number of litters born alive was 4.9 (approximately 5) (Figs. 12 a, b, c). Pregnant mice of 16-day gestation when infected with virus, it was seen that the percentage of mortality of the newborn litters was much less in comparison to those in the 8-day or 12-day gestation groups (vide supra). The mortality percentage ranged 11.1% to 42.9% in different
individual mouse with an average 20.0%. The average weight of litters was 1.030 gm and average number of litters born alive was 5.2 (Table 13 and Figs. 12 a, b, c). The present study indicated that susceptibility of foetus was maximum to intraperitoneal inoculation of pregnant mice on the 8-day gestation rather than the last week of gestation as observed by Soike (loc. cit.).

In present study from 8-day pregnant mice inoculated Coxsackie virus B1 intraperitoneally the virus could be recovered back from the placenta of pregnant mice on the 2nd, 3rd and 4th day of infection, while Soike (1967) could recover virus upto 7th day after intraperitoneal infection. The latter would indicate that virus persists in the placenta throughout the last week of pregnancy. In the present study virus was, however, recovered from liver of foetus on the 4th, 5th and 6th day after infection. It indicated that virus passed the placental barriers and caused the infection in foetus leading to foetal mortality, growth retardation (Tables 11, 12, 13 and Figs. 12 a, b, c), intra-uterine death resulting less number of litters per gestation per mouse. It was observed that the percentage of death of
litters gradually decreased with advancement of pregnancy. 
The present observation was, thus, significantly different 
from that of Soike (loc. cit.), but similar to Lansdown 
(1975 a) (vide infra). Soike (loc. cit.) observed that the 
mortality occurred during the first 7 days of postpartum, 
but the highest incidence of mortality was observed on the 
day of birth and on the day following birth. He also 
could demonstrate virus in foetal heart and lung from 1st 
day to 3rd day, liver from 1st to 3rd day, intestine for 
four days and pancreas for 1st 2 days of infection, and 
from brain on 2nd and 3rd day of infection.

Soike (1967) further tried to observe the 
effect on breeding capacity of progeny born of Coxsackie 
virus infected mother, in an attempt to establish whether 
such virus infection could have interfered with their 
reproductive capacity of the surviving female mice resulting 
from their intrauterine infection. His data indicated no 
appreciable difference in litter size in 3 groups of mice. 
But there was increase in the mortality rate of litters in 
both the groups previously infected with Coxsackie B3 virus. 
Miranda et al (1973 a) studied the effect of Coxsackie 
virus B5 on 5-day old mice inoculated intracerebrally. They
observed reduction in litter size of the surviving infected females in their subsequent pregnancies.

The present study was in the line of Miranda et al. (1973 b) in the 5-day old mice to observe the effect of virus in the form of on the rebreeding of the progeny derived from Coxsackie B$_1$ virus infected mice with regard to number of litters born of the surviving female mice for any anatomical abnormality and/or size and weight of litters per gestation. However, an appreciable difference in weight and size of the litters born of mice who survived Coxsackie B$_1$ infection in utero as compared to those from the control. The average number of litters per female mouse born of post-infected group showed a decrease (Tables 11, 12, 13 and Figs. 12 a, b, c). All these suggested that previous virus infection of mother, while in utero could have left some residual effect on the reproductive capacity either in the form of increased mortality and/or low in weight and size of the litters. This could only occur with possible persistence of latent or active infection of B$_1$ virus in the reproductive organs of female mouse born of Coxsackie B$_1$ infected female mouse.
These studies, therefore, could partly explain the possibility of congenital defect in man following intra-uterine infection by Coxsackie virus (Cotalano and Sever, 1971; Davies, 1972; Tordury and Tordury, 1972). Although in these instances the foetal deformities may possibly due to virus exerting a direct pathological effect on the foetal tissue in utero as was observed in this experimental study (vide infra) or a reduction in the state of health of mother could be a contributory factor (Edwards, 1972; Cold and Wardman, 1971, 1972). The latter was studied by Soike (1967), Lansdown and Cold (1974) and Lansdown (1975 a, b) and also in the present study.

Although Soike (1967) found no change on the mortality or litter size if infected early in pregnancy, Lansdown and Cold (1974) observed that there was increase in the rate of foetal mortality and reduction in the weight of litters if the mice were infected at about mid-pregnancy. This was also confirmed in the present study. The cause of mortality, foetal death and retarded foetal development were attributed in case of Coxsackie B3 virus infection due to reduction on the state
of maternal health, which was, however, not clinically apparent. Recovery of virus in the present study from the foetal liver indicated that the viral infection of the foetus also occurred. Lansdown (1975 a) also observed increased foetal wastage when the virus was injected 4 or 6 day of gestation than 12-day gestation. Foetal weight in the infected was less than that in the control. This was also confirmed in the present study. He attributed this to exocrine pancreatic affection. The maternal illness following infection was attributed to pancreatic affection of B_3 virus resulting defect on breakdown of dietary protein and digestion of complex dietary protein and were consequently subject to nutritional deficiency (Goldstein, 1968). Blaxter (1968), however, postulated that in pregnant mice liver plays a vital role in synthesizing and storing additional labile protein for the maintenance of normal foetal growth. It is reasonable from this that a disturbance in this tissue could result in foetal death or retardation of foetal growth. The affection of pancreas is minimal histologically although virus could be recovered from this organ for 2 - 5 days.

Burch et al (1973) observed direct relationship in the nonpregnant mice infected with B_1 virus
In order to investigate this contributory role of liver affected by virus on the pregnant mice at different day of gestation, the present study was, however, extended to three other parameters, e.g., (i) Liver/Body weight (L/B) ratio, (ii) protein content of liver of pregnant mice, and (iii) alkaline phosphatase level of the infected pregnant mice. The former two were done to assess the gross or biochemical alterations and the third was to find out one of the functional parameters of the liver.

On estimating the liver/body weight (L/B) ratio of the pregnant mice infected at different days of gestation (8, 12, 16 day) groups with virus intraperitoneally, it was observed that in 8-day gestation group the L/B ratio on the 4 day post-infection period was 5.676 while that in the control was 6.12, which was significantly low. On the 6 and 8 day post-infection in the same group of animal the L/B ratio decreased significantly (P < 0.001) (Table 18). On 12-day gestation group the L/B ratio on the 4th day of post-infection period was 5.992 while that in the control was 6.35 (0.2 < P < 0.3). This was not significant.

...
8th day of post-infection the ratio in the infected group L/B ratio were 5.542 and 4.678 respectively, while those in the control were 6.57 and 6.16 respectively. These were highly significant with P value P < 0.001 (Table 18). On 16-day gestation group on 4th day of post-infection period just before parturation the L/B ratio in the infected group was 5.574 while that in the control group it was 6.31. This was highly significant (P value 0.001 < P < 0.01) (Table 18).

The protein content of liver of mothers from the infected group decreased significantly P < 0.001 (Table 21) from those of the control group at different intervals of post-infection period.

It was, thus, evident from the above data that there was definite decrease in liver weight and its protein content when the pregnant mice were inoculated with Coxsackie virus B₁ intraperitoneally. The foetal growth retardation could, therefore, have been due to protein deficiency in the maternal liver. This agrees with the observations of Lansdown and Cold (1974) stating that there was the foetal growth retardation due to viral infection by Coxsackie virus B₃ of the liver and this was due to
deficiency of protein in the diet. Lansdown (1975 b) extending their earlier observation (Lansdown and Cold, 1974) had shown that if the diets of Coxsackie virus B₃ infected pregnant mice were supplemented with casein hydrolysate, foetal growth and plasma protein were not significantly affected. Lansdown (1975 b) stated that the virus induced pancreatic exocrine insufficiency leading to maternal protein deficiency. He observed changes in the pancreas primarily, while no changes in the liver tissue. Therefore, he felt that protein changes in the liver could be secondary. Lansdown (1975 b) did not recovered virus from the pancreas or liver, hence it is difficult to agree with his conclusion that the histological affection of pancreas observed by him was primarily due to virus infection. Soike (1967) could recover virus from pancreas of the pregnant mother only for two days post-infection.

But indirect evidences of such effects on the liver were brought out by Cold and Ramsden (1973) from an indirect biochemical serum level from a low ratio of serum albumin to alpha-feto protein in small foetuses born of or by Lansdown (1975 b) from low serum protein in
Coxsackie B<sub>3</sub> infected mice. This could be from a direct pathological effect of the virus on the foetal liver where these proteins are elaborated, or it could be the result of some other mechanism affecting indirectly on the maturation of the foetuses (Lansdown and Coid, 1973). The present study was also undertaken to find out the extent of hepatic functional alteration induced in the pregnant mice on different day of gestation (8, 12, 16 day) by estimating the serum alkaline phosphatase level. There was significant increase of the alkaline phosphatase level from 4 day of post-inoculation when compared with that from the control group. There was gradual increase of alkaline phosphatase as the days advanced in all of the 8, 12, 16 day of gestation groups of mice (Table 26). On statistical analysis, these increases were highly significant (P < 0.001). Lansdown and Ellaby (1974) observed increase of elevated alkaline phosphatase in liver of infected pregnant mice 2 days after infection. This was the only change identified consequent with presence of virus in the tissue. Chaturvedi et al (1972) studied the histochemical alteration by Coxsackie virus B<sub>3</sub> in monkey kidney tissue culture cells and reported that there was an increase in the alkaline phosphatase granules with the time of infection. The
continued increase in the lysosomal enzymes may be responsible for bringing about cytopathogenic changes in the infected cells. This was confirmed electron microscopic study (Fig. 4) in the present study where there large number of lysosomal globules. The other way by which the foetal growth could be retarded is the affection of the heart i.e., myocarditis in pregnant mice following intraperitoneal inoculation. This was studied in different groups of gestation (8, 12, 16 day). It was observed that creatine phosphokinase level gradually increased in the 8th, 12th day gestation group and from 4-day post-inoculation to 8-day post-inoculation period similar to that of alkaline phosphatase. The difference between the control and the infected group was highly significant in all the groups indicating thereby that heart was affected in addition to the liver, since creatine phosphokinase level is a very sensitive indicator of myocardial damage. As such this would also indicate that functionally heart would be much more weaker as the degree of damage and release of creatine phosphokinase level in serum increases since amount of creatine phosphokinase level in serum is regarded to be proportional
to the myocardial damage. If this be so, this could also be a contributory factor in foetal nutrition and may explain the retarded growth and/or reduced fecundity of in the infected pregnant mice.

Study on the pathogenic lesions in suckling mice by certain viruses isolated from cases of so-called poliomyelitis and pleurodynia was recorded by Pappenheimer et al (1950). They inoculated the virus into suckling mice and 5-day old mice by intraperitoneal and intracerebral route and sacrificed at 5 days, 7 days, 9 days, 14 days and 18 days after inoculation. Animals were sacrificed when they were found to be moribund and studied the histology of various tissues in such cases. Five strains of virus were used in their experiment. The overall pathological changes were indistinguishable whether the virus was given by intracerebral or intraperitoneal route, but cerebral lesions were, however, more pronounced when the route of inoculation was intracerebral. The cerebral lesion consisted of necrobiosis, sparse polymorphonuclear infiltration. In the lung they only mentioned nonspecific lesions secondary probably due to cardiac insufficiency. The observed lesions in the adipose tissue which was
essentially of colliquative necrosis with coarse
eosinophilic clumps and polymorphonuclear leucocytes,
followed by replacement fibrosis. Liver did not reveal
any specific lesions, while pancreas showed changes
varying from acute interstitial oedema with cellular
infiltration of polymorphonuclears and histiocytes, leading
to almost complete destruction of all the acinar cells.
They observed no lesions in skeletal muscle with any of
the virus strains. Lesion in the brain was most severe
with Conn. 5 strain whereas lesions in heart and adipose
tissue with powers strain were more severe. They observed
in heart acute inflammatory reaction characterized by
polymorphonuclear leucocytes, lymphocytes, and large
mononuclear cells. There was patchy areas of necrosis,
loss of cross striation of muscle fibres with eosinophilic
areas only. In a single mouse infected intracerebrally,
they observed large areas of myocardial degeneration with
calcification after 6 days. Melnick and Godman (1951)
confined their study to a single strain of virus, i.e.,
Conn. 5 strain obtained from brain suspension of mice.
Dose of virus was 0.02 ml of $10^{-6}$ of brain suspension.
They limited their study to two groups of animals
inoculated at 1 day, and 4 to 5 days of age. In two groups of mice one of 1-day old and other 4-5 day old mice, paralysis was observed after 5-8 days of infection. Histologically, striated muscle showed degeneration swelling and necrosis. Mice were sacrificed for histopathological examination at different intervals upto 18 days. Melnick and Godman (1951) observed that Coxsackie virus infection in newborn mice caused wide spread degenerated lesions of the striated muscle and generalised fat necrosis, encephalitis, hepatitis and pancreatitis in the suckling mice. This observation was quite different from those of Pappenheimer et al (loc. cit.), who did not observe any lesion in the striated muscle, whereas the lesions in lung, heart, adipose tissue and brain were more or less same.

Gifford and Dalldorf (1951) first observed that the mice, infected with group B strain of virus, younger than 10 days of age, developed spastic paralysis following intracerebral inoculation. Mice inoculated by intraperitoneal route developed more severe muscle changes than those inoculated by intracerebral route. Following intracerebral inoculation they observed liquefaction of cerebral hemispheres, congestion or pallor of fat between the scapulae.
These animals survived as long as 10 days and some also recovered. In the cerebrum they observed widespread liquefaction with infiltration of a few polymorphonuclear leukocytes. Damage was focal and degeneration was limited to small areas. In the fatpad area they observed only ghost outline of cells mixed up with inflammatory cells and connective tissue elements. They also observed relatively large diffuse irregular areas of muscle degeneration consisting of fragmentation and loss of muscle fibres with infiltration of polymorphonuclear leukocytes and oedema. Liver showed hepatitis of varying degrees with severe parenchymal degeneration. They stretched the importance of striated muscle lesion along with central nervous system and fatpad as characteristic feature of Coxsackie virus B infection. It may be noted that Pappenheimer et al (loc. cit.) did not observe any skeletal lesion.

Godman et al (1952) observed lesions in the suckling mice manifested by lesions in heart and central nervous system. In the heart, affected muscle fibres appeared to be homogenous, condensed, hyalinised and without any nuclei. The fibres were separated by very
scanty oedema fluid along with infiltration of mononuclear cells. In the brain there were marked generalised hyperaemia, lymphocyte infiltration and dense large areas of necrosis with sparse infiltration of polymorphonuclear leukocytes. Lesions of liver and pancreas were described as necrotic, but there was no mention regarding the type of cellular infiltrate and morphology of necrosis.

Grodums and Dempster (1959 a) made a study about the influence of age factor on the susceptibility of white mice to infection with standard strain of Coxsackie virus B3 in order to ascertain the susceptibility of brain, heart and brown fat tissue. From a study of a sufficient large group of animals, inoculating intracerebrally with known standard mouse adapted B3 virus strain on inbred strain of albino mice of varying age 1 to 182 days for histopathological and virological procedures. The animals were sacrificed 7 days after inoculation. Death within 48 hours were considered to be as nonspecific and were not subjected to study. One half of each group after the 7th day was kept for histopathological study and reminder half for virological study. Brain lesions were found in mice inoculated before 12 days of age but heart lesions were
severest in animals inoculated between 12 to 23 days of age. They observed pathological response in brown fat tissue of mice between newborn to 12 days old, consisting of extensive necrosis of fat lobules in the periphery with slight inflammatory response, while the central part showed a mixture of normal cells with decrease of lipid globules and granulation tissue. Frequency and severity of myocardial lesion caused by Coxsackie B3 infection in mice appeared to be similar when mice of 4 to 12 day old were inoculated by intracerebral or subcutaneous route. Attention was then drawn to the fact that pathological responses in brown fat tissue were different in suckling, weanling and adult mice and so also in their heart and brain. In their subsequent study these authors (Grodums and Dempster, 1959 b) produced myocarditis in both young and adult mice by inoculating a suspension of B3 strain TCP<sub>0</sub> of 10<sup>-5.25</sup> per 0.1 ml. Newborn mice were found to be not susceptible to myocarditis as were 12-day old mice. Susceptibility increased with an increase of age upto 23 days and thereafter there was a decline. Virus concentration 10<sup>-4.5</sup> was observed to be as effective as 10<sup>-2</sup> in adult mice so far as myocarditis is concerned.
They further observed that other factors - route of inoculation, sex, virus concentration, nature of inoculating material, did not greatly affect the outcome. On the other hand, both the strain of animal and the age at which it was inoculated, appeared to be significant factors in pathogenic lesions. Importance of these findings lies in the fact that myocarditis due to Coxsackie virus could be produced regularly in young and adult mice without other extrinsic factors. This was the first host-virus model which enabled us to imitate to study Coxsackie virus - human relationship as observed by various authors (Van Cleveland and De Jager, 1956; Javett et al., 1956; Fletcher and Brennan, 1957; Kagan and Bernkopf, 1957; Kibrick and Benirschke, 1956 and 1958; Hosier and Newton, 1958; Movitt et al., 1958; McLean et al., 1958; Naude et al., 1958; Simenhoff and Uys, 1958; Suckling and Vogelpoel, 1958; Rapmund et al., 1959; Roberts et al., 1959).

Rabin et al (1964) reinvestigated the development of myocarditis and its relationship to virus multiplication by means of electronmicroscopic, immunofluorescent and virus assay study. They inoculated Coxsackie B3 (Nancy strain) of $10^5$ pfu in 0.05 ml intraperitoneally in Swiss white mice (Webster strain) between
12 and 20 days of age. It was observed that on the 3rd to 4th day after inoculation the myocardial fibres appeared fragmentated, swollen and composed of strands and spherical aggregates which was intensive eosinophilic. Nucleus showed pyknosis, karyorrhexis. They observed earlier infection or damage in the form of inflammatory infiltrates consisting of histiocytes, plasma cells, lymphocytes. It is interesting to note that they observed histological evidence of myocarditis to the extent of 90% between the 3rd to 7th day, while mortality was more between the 5th to 9th day. They observed maximum myocardial damage on the 7th day, and, thereafter, the lesions showed progressive scarring and calcification. They did not observe any lesion in the brain. In addition they observed focal necrosis in pancreas, skeletal muscle and liver.

Viral hepatitis resulting from Coxsackie B virus infection has been reported in all patients, who were infants and children (Kibrick and Benirschke, 1956; Hosier and Newton, 1958; Fechner, Smith and Middlekamp, 1963). The pathological changes in the liver consisted of mild fatty metamorphosis to severe focal parenchymal necrosis. Virus was isolated from faeces and from various tissues,
such as heart, brain, spinal cord, liver, kidney, spleen and pancreas, but Coxsackie virus crystals could never be demonstrated in infected tissues.

Experimentally, Pappenheimer et al (1950) and Godman et al (1952) mentioned hepatitis following intracerebral and intraperitoneal injection of Coxsackie virus in neonatal mice and suckling mice (*vide supra*). Minkowitz and Berkovich (1970) reported Coxsackie B1 viral hepatitis in adult mice in which the severity of hepatic lesions was related to the sex of the animals with male predilection. However, they did not make a thorough histological study of the hepatic lesions produced by the virus. Burch et al (1973) extended their earlier study (Tsui, Burch and Harb, 1972) with Coxsackie B1 virus in 3 groups of mice of different ages of (i) 1 to 2 day old newborn mice, (ii) 14-day old mice and (iii) five weeks old mice. They inoculated intraperitoneally with 0.1 ml to 0.2 ml of the Coxsackie virus B1 having a titre of $10^{-4}$ TCID$_{50}$ and killed these groups of mice respectively between 1 to 3 days, 1 to 4 days and 1 to 8 days after inoculation. Histologically, the degenerative and necrotic lesion was observed and consisted of focal cell
necrosis, ballooning of the parenchymal cells with karyolytic nuclei, dense eosinophilic round bodies in the cytoplasm with or without pyknotic nuclei in the livers of the newborn and in 14 day old mice after 24 hours following inoculation, but not in 5 weeks old young mice. Electron microscopically, they failed to locate any virus crystal in the parenchymal tissue. They, however, observed extensive hepatic damage. This appears to be quite different from the effect of \(B_3\) virus infection on the liver of the pregnant mice (Lansdown and Gold, 1974). Lansdown and Ellaby (1974) injected tissue culture virus of \(10^7/\text{ml of TCID}_{50}\) intraperitoneally resulted a very slight histological changes 4 days after injection, but only after 6 days of infection there was periportal cell vacuolation, central veins were dilated and deposit of neutral fat in the cells was pronounced. They felt that the changes in the liver of the pregnant animal were secondary to pancreatitis, which they could demonstrate histologically. They were unable to support the view of Burch et al (1973) that direct relationship exists between the infection and the hepatic damage.

The present data from 2 groups of neonatal mice were studied when Coxsackie \(B_1\) virus was injected
intracerebrally and intraperitoneally. Histological study on the brain, heart, liver, skeletal muscle, lung, fatpad and pancreas were studied from the 2 groups. These animals were killed at various intervals from the 2nd day onwards till the 20th day. There were evidence of inflammation in the brain with degenerative changes as early as 2 day, when the route of inoculation was intracerebral. On the other hand, in the intraperitoneal route of inoculation the degenerative changes were not seen till 6th day. Subsequently, type of necrosis, inflammatory infiltrate and extent of inflammation were more or less same (Figs. 17-21 and 45). In a single case granulomata lesion was observed on the 20th day in mice following intraperitoneal injection. Pappenheimer et al. (1950) failed to differentiate between the histological lesions in the brain irrespective of the route of inoculation. Only there was more pronounced cerebral lesions when the route of inoculation was intracerebral. The present histological study revealed the presence of lymphocytes and mononuclear cells and was, thus, different from their study where polymorphonuclear neutrophils were observed to be the chief cells of the infiltrates. Grodums and Dempster (1959 a) observed hydrocephalus but
never mentioned the type of cellular infiltrate or necrosis. Interestingly, they did not find any lesion in the brain upto 12 day of age even when inoculated by intracerebral route. Godman et al (1952), however, observed lesions in brain similar to that observed in the present study. There was presence of granulomata lesions in one mouse on the 20th day following intraperitoneal infection but localising granulomata lesion were seen in other mice also injected intracerebrally in the later period of the present study. This would only indicate that those animals - suckling mice who could survive from the onslaught of initial viremia injected intracerebral route, would attempt to localise the lesion as a part of general immunological response to infection.

The lesion in the heart, in the present longitudinal study, in suckling mice when inoculated intracerebrally or intraperitoneally, revealed more or less similar histological feature consisting of focal areas of interstitial oedema as early as the 2nd day. Subsequently, there was increase in cellular collection consisting lymphocytes and mononuclear cells. By the 6th day areas of necrosis with loss of striation were seen. But there
was no fibroblastic proliferation although there were areas of focal necrosis and cellular infiltrate even on the 10th day. When the mice was injected intraperitoneally, the inoculated group showed little more persistence of inflammatory infiltrate as long as the 20th day, without any replacement of fibrosis, but Rabin et al (1964) observed myocardial damage with B3 virus in mice between 12-20 days of age injected intraperitoneally. They observed similar histological picture maximum between the 3rd to 7th day of postinoculation. Thereafter lesions showed progressive scarring and calcification. The present study failed to corroborate their last part of this study, i.e., presence of scarring and calcification. In the earlier study, in a single mice injected intracerebrally, Pappenheimer et al (1950) observed large areas of myocardial degeneration with calcification after the 6th day. The virus injected at that time was not a pure strain. It is only from the study of Grodums and Dempster (1959 a, b) and, subsequently, when pure Coxsackie B3 strain of virus was available for study the histological lesion in the heart, was described in details. This followed the discovery that the group B viruses were
reported to be associated with foetal myocarditis of newborn babies (Javett et al., 1956; Kibrick and Benirschke, 1956; 1958; Van Creveld and De Jager, 1956; Fletcher and Brennan, 1957; 1958; Kagan and Bernkopf, 1957; Delaney and Fukunaga, 1958; Hosier and Newton, 1958; Movitt et al., 1958; McLean et al., 1958; Naude et al., 1958; Simenhoff and Uys, 1958; Suckling and Vogelpoel, 1958; Raymund et al., 1959; Roberts et al., 1959). Heart lesions in the experimental Coxsackie virus infection received comparatively little attention in the past. Dalldorf (1952) commented on the paucity of lesions in the heart, based on the observations of Gifford and Dalldorf (1961) who when examining the suckling mice infected with different strains of B virus, observed cardiac lesions in only 3 of 51 of mice subjected to careful examination. These workers used Conn. 5 strain of virus. Verlinde et al (1956) reported that myocarditis was a constant manifestation in suckling mice infected with Coxsackie B4 in tissue suspensions from the heart muscle of infants who died from histologically proved myocarditis. However, no actual figures of incidence were quoted. Earlier review of Dalldorf (1949) observed that animals
more than 12 days old were resistant to infection. He also noted that resistance of mature mice was not due to peripheral barriers that interfere with certain other viral infection, older mice were equally resistant to intracerebral inoculation of B viruses. Grodums and Dempster's (1959 a, b) observations were contrary to this. They observed susceptibility of Coxsackie B3 virus in suckling mice and also in mice between age 12-23 days and found that the latter group, i.e., 12-23 days old, showed more frequent involvement of myocardium than those in the neonatal group. Although there was marked clinical signs of disease consisting of paralysis, ataxia, tremors and spasm in the animals upto 9 day old. From the heart the recovery of virus could be done by them as late as the 7th day and till the 6th day period when inoculated intracerebrally and intraperitoneally respectively.

In the present study lesions in liver consisted of focal degenerative changes but was not observed earlier than the 4th day of post-infection day following intracerebral inoculation, but following intraperitoneal route such focal lesion was observed to be early. Main histological evidence of inflammation
consisted of mononuclear cells, lymphocytes similar to those of Burch et al (1973). Burch et al (loc. cit.) could not demonstrate viral crystals by electron microscopy but they found lesions similar to those observed in the present study. Their mode of inoculation was intraperitoneal and Coxsackie B
1 virus 0.1 - 0.2 ml 10^{-4} TCID_{50}/ml was injected, but more or less similar dose of virus was employed in the present study. Virus, however, could be recovered from liver upto 7 days by intraperitoneal route, while upto 6 days in case of intracerebral route in the present study (Tables 15 and 16) thereby indicating the presence of virus in the tissue and could possibly be responsible for the hepatic lesions. Pathological changes consisting of necrosis, cellular infiltrate indicated a causal relationship between the lesions in liver and Coxsackie virus B
1 infection. This was not possible for Burch et al (1973) even by electron microscopy study. The present experiment, therefore, indicated that Coxsackie virus in neonatal mice when inoculated intracerebrally or intraperitoneally could produce moderate to severe hepatic lesions depending on the presence of virus as evident by recovery study. In the end of the study, i.e., 20th day,
in the mice which survived, there was no evidence of cellular degeneration and necrosis indicating that liver recover to normal without any evidence of residual fibrosis. Burch et al (1973) did not investigate longitudinal aspect since all their mice were killed by the 8th day after inoculation, but they made a plea to extend their study for a long period of time to observe the fate of hepatic lesion.

Skeletal muscles were affected from the clinical evidence of spastic paralysis in the present study. Histological evidence was not revealed till the 6th day when there was interstitial oedema, loss of cross striation of muscle with centering of the nuclei, accumulation of histiocytes and lymphocytes in the interstitial tissue. Pappenheimer et al (1950) did not observe any skeletal muscle involvement. It was Helnick and Godman (1951) who first observed spastic paralysis in between 5-8 days of infection with histological evidence of swelling, necrosis and degeneration of muscle fibres and was responsible for differentiating Coxsackie group A and B virus. Grodums and Dempster (1959 a, b) did not mention any affection of skeletal muscle, when studied the
experimentally Coxsackie B₃ infection in suckling mice.
In the present study there is complete resolution of
inflammatory infiltrate by the 20th day. This would only
indicate that transient myositis was due to presence of
virus in these muscles. Recovery of virus from skeletal
muscle was possible between the 2nd and 3rd day in both
the groups inoculated intracerebrally or intraperitoneally
indicating that virus could be isolated from the tissue
at a time before the maximum histological lesion was
observed.

The presence of lesion in lung in the
present study consisted of interstitial inflammation of
the alveoli which was evident on the 4th day onwards in
case of intracerebral route (Figs. 34 to 37) and in case
of intraperitoneal route (Figs. 53 and 54). Earlier
observations of Pappenheimer et al (1950) felt that lung
was involved secondarily probably due to cardiac
insufficiency. No mention was made regarding lesions in
the lung by subsequent authors. Recovery of the virus in
the present study from the lung during the 1st to 4th days
in case of intracerebral and for 5 days in case of
intraperitoneal route indicated that the virus was there
during these periods. It is, therefore, quite reasonable that a causal relationship between Coxsackie virus and the lesions in the lung could be arrived at. Subsequently, virus could not be recovered, which only indicated that virus was not there. This would indicate that natural resistance of the host, in these cases of neonatal mice, helped them to recover the transient interstitial pneumonia, since at the end of the experiment in the surviving animals, there was no evidence of persistence of inflammation. These lesions were not similar to chronic passive venous congestion in the lungs due to cardiac failure as suggested by Pappenheimer et al (1950).

Histological study of fatpad revealed lesions similar to those by Grodums and Dempster (1959 a) in their group I mice, but difference was that there was some which showed granulomatous lesion, such lesion was present in one case in the present study. Moreover, virus could be recovered from the fatpad from 1st to 7th day by intracerebral route and the 6th day by intraperitoneal route. Lesions in fatpad was described in details by Grodums and Dempster (1959 a). They observed severe damage to brown fat tissue in weanling stage but they noted that
the susceptibility of brown fat was variable and quite different in other groups. These varied types of response in brown fat with different age group were although gradual but quite distinct. Typical peripheral necrosis of brown fat was seen in the suckling and the weanlings but entirely absent in the weaned and adult mice. This could be due to maturity or changes in the size of the intercellular droplets since the droplet size may be of prime importance in the assessment of cell activity (Deane, 1958). It is natural then to wonder whether the physiological or pathological activity of the brown fat as an organ effects the susceptibility to virus infection. It is tempting to suggest that a relationship could exist between the histological changes in the brown fat and maturity of the tissue rather than the dietary and endocrinal change.

Pancreatic lesion in the present study was observed to be minimum as compared to other studies (vide supra). It is but natural to think the strain variation in mice may be responsible since pancreatropic affection was observed by other observers (Pappenheimer et al., 1950) and, therefore, indicate the species
variability could only explain this, although the virus could be recovered from pancreas for 2 to 5 days it was not possible to observe the severe lesions as described by other workers (Pappenheimer et al., 1950). The lesions observed to be a mild one and could not be correlated to the recovery of the virus from the pancreatic tissue. The loss of adaptibility of strain used in the study to pancreatic tissue could be another way to explain the low degree of pancreatic lesion apart from the susceptibility of the strain of mice used in the present study.

Miranda et al. (1973a) were successful in regularly developing myocarditis in 5 to 9 days old mice following intracerebral inoculation of $2 \times 10^{-4} \text{TCD}_{50}$ of Coxsackie virus B$_5$ in 0.02 ml. In the control group, they injected normal saline solution in order to evaluate the role of intracerebral trauma in such cases. The infected mice along with the controls were killed at various intervals upto 270 days after inoculation. Each group was divided into 2 subgroups, one subgroup was used for virological work and other for histopathology. Heart, liver, lung, kidney of the infected group were also studied.
Heart was dissected out and weighed. They observed in their longitudinal study that body weight remained low for 200 days after infection in both sexes. There was significant increase in the heart/body weight ratio in these animals. This could be possibly due to (i) reduction in the weight of the body due to infection and (ii) increase in the weight of heart due to dilatation. This dilatation could help accumulation of blood in the heart, since the heart was weighed intact after dissection with blood in its chambers. Histological appearance of heart revealed the following lesions: very occasional granulomata, numerous focal necrosis and some cellular reaction between the 2nd to 9th day. On the 12th day there was healing phase with occasional residual small granuloma. By the 15th day necrotic phase ended, several small healing granulomata were seen. There were few areas of previous large necrosis visible, with occasional small fibrous scar present. Recovery of virus could be done from heart in these cases up to 6 days after inoculation. Thereafter there was no virus isolation. Their results were similar to those of Wilson et al (1969).
Miranda et al (1973 b) observed mild histological lesion in five days' old mice. This study was quite different from the observations of Grodums and Dempster (1959 a, b), who found severe lesions in the hearts in 12-23 day old mice only with Coxsackie B3 virus. Pappenheimer et al (1950), Godman et al (1952) however, observed lesions in the heart of the suckling mice following intracerebral inoculation of Coxsackie virus. Subsequently, Grodums and Dempster (1962) showed that with the Coxsackie B virus there were various patterns of pathogenic lesions: the B3 strain caused most severe cardiac lesions in the weanling mice, whilst the B1, B2 and B4 strains were mildly cardiotropic in the suckling mice but had a reduced effect on the weanlings. Rubin et al (1964) observed myocarditis in 12-20 days old mice with Coxsackie B3 virus. They also observed progressive scarring and calcification with B3 virus in heart. The observations of Miranda et al (1973 b) showed focal scarring in certain cases. This indicated that the myocardial lesion with scarring was observed by them when they have used Coxsackie B3 or B5 virus on different days (Rabin et al., 1964; Miranda et al., 1973 b).
Endocardial lesion including valvulitis have been produced in cynomolgus monkeys infected with Coxsackie B$_4$ virus (Low et al., 1960). This study was extended by Burch et al. (1966) in mice, when they inoculated intraperitoneally Coxsackie B$_4$ virus in mice of varying age between 2-21 days. Histopathological evidence showed of valvular endocarditis was found in 55% of cases. The involvement of valves were in the following order of frequency tricuspid, mitral, aortic, and pulmonic. Thus, the importance of cardiotropism and endocardium in particular was brought out by this study. This has a great bearing in the implication of aortic disorders following Coxsackie B group of virus infection in young adults in man. In man Coxsackie B$_4$ has been associated with aortic incompetence and mitral valve disease or both. Isolated case reports from India (Madhavan et al., 1974; Chandrasekhar et al., 1975) indicated relationship of this infection to different heart lesions.

In order to evaluate the above data the present work was undertaken to induce experimental myocarditis in 5-day old mice with Coxsackie B$_1$ virus.
infection. In the preliminary study it was observed that myocarditis could be produced in them following intracerebral inoculation of B1 virus. Hence the study was done initially in the line of Miranda et al (1973 a, b) and was then extended to other parameters.

In the early stage heart/body weight ratio was observed to be increased significantly on the 8th and 10th day. This sudden increase in the heart weight was attributed to an increase in the water content of the heart and histologically to the evidence of diffuse myocarditis and interstitial oedema (Figs. 57 to 63). This increase in water content could be a part of the associated inflammation. The inflammatory infiltrates mainly consisted of lymphocytes and histocytes with degenerative changes of muscle fibres characterized by loss of cross striation increased eosinophilia in the cytoplasm (Figs. 57 to 63), and separation of muscle fibres due to marked oedema. In the present study the heart was opened up and blood was wiped out as far as possible. Hence the heart weight due to contained blood could be avoided. From the present study it is possible to attribute the increase in the weight of the heart to
increase in water content rather than to an increased amount of blood within the heart, brought about by dilatation, as suggested by Miranda et al (loc. cit.).

The present longitudinal histological study indicated that there was no scarring of the heart in any of the animals until the 20th day up to which time the histology of the heart appeared to be normal. Miranda et al (1973 a) with Coxsackie virus B\textsubscript{5} and Rabin et al (1964) with B\textsubscript{3} observed scarring and even calcification but Miranda et al (1973 a) observed relatively mild myocarditis as in the present study. Besides the increase in the water content, the increase in the heart weight was not due to an increase in protein content or in DNA or RNA contents, as a result of the Coxsackie virus B\textsubscript{1} infection. There was relative decrease in the body weight of the animals (Fig. 14 b). This could be a factor in the increase of heart/body weight ratio on the 8th and 10th day of observation. Histologically, the lesions were evident in the heart on the 4th day although there was a florid lesion only by the 6th day. Then there was definite decline in the inflammatory lesions (Figs. 56 to 63). It was thought
that these cellular infiltrates could have contributed to the increase in cell mass, hence estimations of protein, RNA and DNA contents were undertaken. The study of the protein content of the heart (Table 20) indicated that there was significant decrease of protein content from the 6th to 10th day of post-infection period and subsequently. This would indicate that there could be decrease in protein synthesis from this virus infection in the growing animals. This was in agreement with observations, in general, of an effect of Picorna virus infection on the host macromolecular synthesis of protein in tissue culture (Ackermann et al., 1959; and Salzman et al., 1959; Martin and Kerr, 1968; Reisman and Spear, 1969; Bablanian et al., 1972). It was observed by Ackermann et al. (1958); Baltimore and Franklin (1962); Franklin and Baltimore (1962); Scholtissak et al. (1962) that inhibition of DNA synthesis occurred later than that of host RNA and protein synthesis. This has also been studied extensively in cell culture by phase and electron microscopy (Barski et al., 1955; Dunnebache, 1956; Dales and Franklin, 1962; Dales et al., 1965; Amako and Dales, 1967; Skinner et al., 1968; Flagemann et al., 1970).
But this phenomenon has not been studied in the experimental animal tissue. It was, therefore, of relevance that the observations made in cell culture could also be true in the case of experimental animals associated with viral myocarditis.

In the present work sequential synthesis was not possible to study in the experimental animals, but it was possible to estimate quantitatively RNA and DNA content of infected heart muscle in virus infected 5-day old mice inoculated intracerebrally. It was observed that both RNA and DNA contents of the heart muscle was significantly low throughout, although there were cellular infiltrates during 4th day to 10th day. It may be noted that virus could not be recovered after the 9th day from the heart muscle. Moreover, although histologically, by the 20th day, the muscle appeared to be normal, both total protein, RNA and DNA contents of heart muscles remained significantly low throughout. This indicated that there was profound depression in the mechanism of protein synthesis resulting in low RNA and DNA and protein content of the heart muscle of the infected animals.

In tissue culture, inoculated with the virus, electron microscopical observations (Figs. 4, 5, 6 and 7)
showed increase in lysosomes and disorganisation of endoplasmic reticulum. If similar changes do occur in the infected heart tissue, it can be presumed that although viral infection of the heart did not produce any microscopic imprint in the form of scarring or calcification, biochemically this might have caused a great rearrangement of protein formation in the heart muscle at the cell organelle level, which led to significant decrease in the protein, RNA and DNA contents, at least in the experimental animals. But water content was only altered between 8th to 10th day. This could be due partly to manifestation of accumulation of interstitial fluid from inflammation.

This depression in protein synthesis in growing animals could explain their low weight (Fig. 14 b) in the present study. Such mechanism could explain the observations of Miranda et al (1978), who observed such lasting effect on the mice. Although experimentally, Coxsackie B 5 is of low virulence in mice, the animals required as long as six months to recover their weight to the levels of the controls from the effect of transient inflammation of heart. This could explain to a certain
extent the fatality that occurs from myocarditis not only in children but also in adults.

The present study was extended to find out whether in the absence of any light microscopic evidence, as has been stressed by Miranda et al (1973) and also Rabin et al (1964), there was biochemical lesion in such cases.

Hydroxyproline is one of the characteristic aminoacid present in collagen measured in heart tissue. It was, therefore, interesting to observe that the level of the hydroxyproline under these conditions increased significantly in the infected animal heart tissue throughout, in the absence of histological evidence of fibrosis or scarring in the heart muscle. Moreover, there was no fibroblastic proliferation throughout, which could be attributed to such increase of hydroxyproline nor there was any scarring even on the 20th day. But this significant increase was observed from the 4th day onwards, when histologically there was very little inflammation. This might indicate that the virus infection affected tissues other than heart muscle resulting in release of bound or free hydroxyproline from the milieu interior of
the body in circulation. This could also be a manifestation of altered amino acid metabolism in the tissue concerned from virus infection. Moreover, the low RNA and DNA content of heart muscle could be a manifestation of depression of cell replication in general. This could be corroborated from the absence of fibroblastic proliferation. Both of these factors taken together would indicate that increase of hydroxyproline could not be due to mobilisation of hydroxyproline, but could be a manifestation of alteration of amino acid metabolism of the virus infected tissue.

Amino acid analysis of the heart tissue at different days after the virus infection was, therefore, undertaken (Table 25). Immediately after infection there was a decrease in 8 amino acids such as threonine, serine, glutamic acid, glycine, alanine, isoleucine, leucine and tyrosine and an increase in 7 amino acids, such as, lysine, histidine, arginine, aspartic acid, proline, valine, and phenylalanine and hydroxyproline. Nine amino acids, such as, lysine, histidine, aspartic acid, threonine, serine, glycine, glutamic acid, proline, and tyrosine decreased to a minimum level on the 8th to 10th day after viral infection when the histological evidence of inflammation of heart
was florid. Alanine reached minimum value on the 2nd day, phenylalanine on the 4th day and leucine on the 6th day after infection. Arginine, valine and isoleucine reached their minimum on the 16th day. Eleven of the amino acids, such as, lysine, histidine, aspartic acid, threonine, glutamic acid, proline, glycine, alanine, leucine, tyrosine and phenylalanine showed an increase on the 16th day after reaching their minimum between the 8 and the 10 days after infection. Methionine was not detected in a number of samples probably due to oxidation during protein hydrolysis or there was marked depression of this amino acid which is involved in cell replication. Lansdown (1975 b) observed that casein hydrolysate supplement in the diet of pregnant animals infected with B3 virus markedly reduced the foetal wastings. This could indirectly explain the importance of protein, particularly sulphur containing amino acids to be helpful in replacement therapy in virus infected tissue.

The study of the amino acid pattern of the heart tissue further indicated indirectly that the rearrangement of protein synthesis does occur in virus infected tissue and thereby corroborated our earlier contention that after entering into the cell the Coxsackie
virus 

multiply within the cells, alter the cell function
leading to alteration of proper functioning of the cell
including cell replication. This is manifestated grossly
by transient increase in the heart weight from increase in
the water content. Histologically transient myocarditis was
observed as evidenced by increase in the interstitial oedema,
series of degenerative changes of muscle fibres characterized
by cross striation, changes in the nuclei but complete
recovery at the end.

It, thus, appears Coxsackie B\textsubscript{1} infection
produces mild lesion in the heart of 5-day old mice, similar
to that of B\textsubscript{5} virus myocarditis produced by Miranda et al
(1973 a). This could be explained by the fact that viral
infection leaves a permanent mark on the host animal which
could not be evident otherwise. It is from the present
study alone it is possible to explain the reason of the
retarded growth observed by Miranda et al (1973 a). From
the biochemical parameters it was possible to bring into
sharp focus a marked alteration in the protein metabolism
of cells in the mice from B\textsubscript{1} viral infection leading to a
decrease in protein content, depression of RNA and DNA
contents and alteration of host amino acids.