SUMMARY AND CONCLUSION
1. Coxsackie B1 virus was negatively stained with 1% phosphotungstie acid, and with the electron microscope revealed that the virus was of icosahedral in shape. The size of the virus was measured by shadow casting. The size of the virus was 26-30 nm.

2. The Coxsackie virus is highly cytocidal one. Ultrastructure studies of the monkey kidney primary tissue culture cells infected with virus in vitro at intervals of 24, 48 and 72 hours of post-infection period revealed that there was gradual to complete destruction of cells with the time. The cells showed disorganisation of endoplasmic reticulum with increase of lysosomal granules.

3. Mice of 24 hours old were inoculated with the virus parenterally and orally. The total number of mice showed spastic paralysis were respectively 42/125 (33.60%), 28/93 (30.10%), 19/85 (22.35%) and 22/107 (20.56%). The total number of mice affected clinically revealed by spastic paralysis, lethargy, separation from other litters, nodding of head, encircling to the left or right side, paralysis and/or death were 118 out of 125 (94.4%) by intracerebral route, 87 out of 93 (93.54%) by the intraperitoneal route, 68 out of 85 (80.0%) by the
subcutaneous route, and 67 out of 107 (62.61%) by oral route of inoculation. The overall mortality was 110 out of 125 (88.0%) by intracerebral route, 79 out of 93 (84.94%) by intraperitoneal route, 44 out of 85 (51.76%) by subcutaneous route and 55 out of 107 (51.40%) by oral route of administration of virus. The mortality rate of mice was high on the 4th, 5th and 6th day of post-infection period by different routes after inoculation of virus.

4. Virus could be recovered from 24 hour old mice inoculated intracerebrally from heart from 1st to 7th day, liver from 2nd to 6th day, lung from 2nd to 4th day, brain from 1st to 8th day, kidney from 4th day only, spleen from 3rd to 5th day, fatpad from 1st to 7th day, pancreas from 2nd to 5th day, muscle from 3rd and 4th day after infection.

5. Virus was also recovered from other groups inoculated intraperitoneally of 24 hour old mice from heart 2nd to 6th day, liver from 2nd to 7th day, lung from 3rd to 5th day, brain from 2nd to 7th day, kidney from 3rd and 4th day, spleen from 3rd and 4th day, fatpad from 1st to 6th day, pancreas from 2nd to 5th day and muscle from 3rd and 4th day after infection.
6. Histopathological study of various tissues from neonatal mice following intracerebral or intraperitoneal inoculation revealed more or less similar lesions except in degree depending on the route of inoculation. The following tissues were studied (i) brain, (ii) heart, (iii) liver, (iv) skeletal muscle, (v) lung, (vi) fatpad and (vii) pancreas.

(i) There was evidence of inflammation with necrosis and other degenerative changes as early as the 2nd day increasing in degree till the 6th day. Subsequently, there were gradual regression of lesion.

(ii) The lesion in the heart consisted of focal areas of interstitial oedema on the 2nd day and subsequently there was increase of cellular infiltrate around the areas of necrosis and loss of striation till the 6th day. There was almost complete regression of lesion by the 20th day without any fibrosis.

(iii) In the liver there was focal areas of degenerative changes on the 4th day onwards but regressed to normal by the 20th day without any residual fibrosis.
(iv) Skeletal muscle showed histological affection from the 6th day and consisted of interstitial oedema, loss of striation and accumulation of inflammatory cells. By the 20th day there was complete resolution of inflammatory infiltrate.

(v) In the lungs there was evidence of interstitial pneumonia, which was evident on the 4th day onwards, but there was no residual lesion at the end.

(vi) In the fatpad there was lesions consisting of necrosis, inflammatory infiltrate and focal disorganisation of the adipose tissue. This lesion healed up by the end of observation.

(vii) In the pancreas the lesion was very minimum with affection on acinar and interstitial tissue.

7 (a). Pregnant mice at different day of gestation (8, 12, 16 days) were inoculated virus intraperitoneally. The mortality rate of the newborn litters at 8-day gestation group pregnant mice varied from 33.3% to 100.0% in different individual mouse, average being 75.6%; at 12-day gestation it was 40.0% to 75.0%, average 57.2%; in 16-day gestation it was 11.1% to 42.9%, average being 20.0%.
7 (b). The average litter weight born from the 8-day gestation infected pregnant mice was 0.884 gm, from 12 day gestation infected group was 1.011 gm, from 16-day gestation group was 1.030 gm.

7 (c). The average number of litters born alive from virus infected 8-day gestation pregnant mice was 4.4 in comparison with the control group it was 7.5, which was about half the number of the control ones from 12-day gestation group it was 4.9 (approx. 5), and from 16-day gestation group it was 5.2 on an average.

7 (d). Virus was recovered from placenta of 8-day gestation pregnant mice on 2nd, 3rd and 4th day of infection and 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 and intra-uterine death resulting to less number of litters per gestation per mouse.

7 (e). The liver weight/body weight (L/B) ratio of infected pregnant mice at different days of gestation (8, 12, 16 days) showed the ratio decreased
significantly in all the groups with that of the control group. The protein content of the liver in the infected mice of different days of gestation showed a significant decrease of liver protein with $P < 0.001$ when compared with that of the control group.

7 (f). The alkaline phosphatase level of the infected mice of gestation group (vide supra), was found to have a gradual but significant increase ($P < 0.001$) of alkaline phosphatase as the days of pregnancy advanced in all when compared with that of the control.

7 (g). The creatine phosphokinase (CPK) level was also observed to be increased significantly as compared with the control group. The release of CPK level in serum indicated the myocardial damage. This indicated that the efficiency of heart was also affected. This could be a contributory factor in physiological disfunction of the heart. All these factors could be contributory to the foetal mortality, retarded growth, and/or reduced fecundity of pregnant mice.

8 (a). The study of 5-day old mice inoculated intracerebrally showed that the virus affected
The female reproductive organs (vide supra). The litters born from these infected female mice did not show any anatomical abnormality, but average number of litters born per gestation was 5 in number, whereas in the control it was 7.5. Average weight of litters was 1.239 gm, whereas in the control group it was 1.472 gm.

8(b). Experimentally myocarditis was induced by inoculating intracerebrally the virus in 5-day old mice. The heart weight/body weight (H/B) ratio was found to be increased significantly on the 8th and 10th day and subsequently there was no such difference. It was also observed that body weight of infected mice was low. This sudden increase was attributed biochemically from the increase in the water content of the heart and histologically to the evidence of diffuse myocarditis, interstitial edema and inflammatory infiltrates. Histologically the lesions were evident in the heart on 4th day and became florid by the 6th day and there was decline in the inflammatory lesions. The study of the protein content of the heart indicated that there was significant depression of protein content from 6th to 10th day of post-infection period, which indicated that the virus inhibits the protein synthesis,
resulting from inhibition of host macromolecular synthesis of protein. The RNA and DNA content of infected heart muscle showed significantly low throughout the experiment (P < 0.001). The virus was recovered from the infected heart up to 9th day of infection. Histological study revealed definite myocardial changes up to 10th day of infection and by the 20th day the heart muscle appeared to be normal, did not leave any permanent mark in the form of scarring or any abnormality. The study of hydroxyproline level was found to be increased significantly in the infected animal heart tissue almost throughout the experiment (P < 0.001). The study of the amino acid pattern of heart tissue indicated the derangement of amino acid pattern due to virus infection. 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 also. 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.
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 8 and 10 day after infection. Methionine was not detected in number of samples including the control, probably may be due to oxidation during protein hydrolysis or lack of sulphur containing of amino acid in virus infection.

From the above study it can be concluded that the Coxsackie B1 virus infection, however transient it may be, leaves a permanent mark on the host animal, which is evident either from retarded growth or alteration in the protein metabolism in the form of low protein, decrease of RNA and DNA content with alteration of host amino acid in the heart muscle of the 5-day old mice.