Mice inoculated intranasally with murine coronaviruses (mouse hepatitis viruses) were killed daily for seven days. Lung and liver sections stained by the immunoperoxidase technique indicated that with three of the four strains examined viral localisation and replication in the lung preceded that found in the liver. Thus, infection by the respiratory route may be of importance in the transmission of these viruses.

The murine coronaviruses are unusual in that, unlike the human coronaviruses strains B814, 229E and OC43 derived (Bradburne and Tyrrell 1971, Tyrrell et al 1975), and Parker's rat coronavirus (Parker et al 1970), which produce respiratory disease in man and rat respectively, no strain of mouse coronavirus has been shown to replicate in the respiratory system of mice.

It is well established that the mouse coronavirus MHV3 (mouse hepatitis virus type 3) is hepatotropic and neurotropic (Virelizier et al 1975, Le Provost et al 1975); type JHM is neurotropic (Weiner 1973, Lampert et al 1973, Goto et al 1977) and types S (Rowe et al 1963) and S/CDC (Hierholzer et al 1979) are enterotropic. With the recent advances in immunoperoxidase technique applied to paraffin sections for detecting respiratory viruses in mice (Cartthew and Sparrow 1980) it was possible to attempt to examine the possible pneumotrophic nature of four strains of murine coronavirus (obtained from the American Type Culture Collection, Rockville, Maryland).

Materials and methods

The strains of virus used were mouse hepatitis type 1, 3, A59 and JHM. Tissue culture preparations (0.25 ml titre 10⁴ TCID₅₀) of all four strains of virus grown in NCTC 1469 cells were inoculated intranasally into exogenous infected CBA-T6 mice, five to seven weeks old. A control group was also dosed with NCTC 1469 cell lysate to check for non-specific inhalation pneumonia. Mice were kept in sealed filter boxes for the duration of the experiment. One mouse from each group was killed (daily for seven days after inoculation) by cervical fracture and the lungs and livers removed and cold processed in 95 per cent ethanol by the method of Sainte Marie (1962). Immunoperoxidase staining of lung and liver sections was performed as described by Burns (1975) using mouse hepatitis virus antiserum (Microbiological Associates, Bethesda, Maryland) at a complement fixation titre of 1 in 40. Control sections were treated with non-immune mouse sera. Peroxidase conjugated antiserum to mouse IgG (Miles Research Laboratories) was used at a dilution of 1 in 100 which gave no background staining. Sections of organs were also stained with haematoxylin and eosin (H & E).

Results

Coronavirus lung pathology

H & E staining. MHV1 inoculated animals showed substantial necrosis of the alveolar septum on day 3 with interstitial infiltration and macrophage activity. This had progressed by day 4 and by days 6 and 7 there was thickening of the alveolar septum and lymphocytic infiltration. In contrast, MHV3 produced only mild oedema on days 4 to 7 after infection. MHV A59 produced a perivascular lymphocytic infiltration by day 2, which progressed to an interstitial infiltration with lymphocytes by day 7. MHV JHM also produced perivascular lymphocytic infiltration on day 2. By day 4 there was also thickening of the alveolar septum, which continued to day 7 with lymphocytic infiltration. Control mice showed some patchy oedema from day 1 to day 7.

Immunoperoxidase staining. Mice inoculated with MHV1 showed immunoperoxidase staining on day 2 after infection. Discrete areas of alveolar cells were stained and by day 3 these were confluent. On days 6 and 7 there was also staining of cells in and around an infiltrative lesion of lymphocytes. Staining of sections from mice infected with MHV3 was limited to very few cells of the alveolar septum on days 5 and 6 after infection. Lung sections from mice infected with MHV A59 showed staining of the alveolar septum from day 2 (Fig 1) to day 5, when it was strongest. MHV JHM infected mouse lung sections showed
staining as early as 24 hours after inoculation. This continued through to day 5 and again was limited to the cells of the alveolar septum. No control lung sections showed any staining by the immunoperoxidase method.

**Coronavirus liver pathology**

**H & E staining.** Mice dosed with MHV1 showed focal necrotic lesions with lymphocytic infiltration in the periportal region on day 6. On day 7 the focal necrotic lesions were also evident adjacent to hepatic veins. MHV3 infected mice showed focal necrotic lesions by day 3 after infection with accompanying lymphocytic infiltration. By days 5 and 6 the lesions had expanded so that they were confluent. Only cells around the portal tracts and hepatic veins survived. MHV A59 produced necrotic lesions by day 4 after infection. The lesions became more numerous on day 5 (Fig 2) but were regressing on day 7. MHV JHM produced small focal necrotic lesions by day 3 after infection, which progressed to confluency by day 5 after which they also regressed. No lesions were seen on control liver sections.

**Discussion**

While all strains of mouse hepatitis virus have been shown to be hepatotropic, some of them have only shown clinical signs of liver damage in the presence of intercurrent infection; for example MHV1 (Gledhill and Andrewes 1951, Niven et al 1953). A number of strains have also been shown to be enterotropic, causing diarrhoea, particularly in preweanling mice (Rowe et al 1963, Broderson et al 1976, Carthew 1977, Ishida et al 1978, Hierholzer et al 1979); MHV3 (Virelizier et al 1975; Le Provaet et al 1975) and JHM (Weiner 1973, Lampert et al 1973, Goto et al 1977) are also neurotropic.

The results of this experiment (Table 1) have shown that whereas all strains of MHV will replicate in the lung following intranasal inoculation, only MHV1, JHM and MHV A59 cause appreciable lung damage. MHV3, regarded as the most virulent strain (Le Provaet et al 1975), caused no observable pathological changes in the lung.

The pathogenesis of MHV in the lungs shows a marked contrast with two other respiratory viruses. In Sendai virus and pneumonia virus of mice (PVM) infections the primary site of virus replication is in the bronchial epithelium (Carthew and Sparrow 1980) where considerable pathological changes can be seen. With PVM, subsequent viral replication and damage is also observed in the alveolar cells. No bronchial damage was seen with the MHV strains and the virus appeared first in the alveolar cells.

MHV1, MHV A59 and JHM may be important respiratory pathogens under natural conditions and may also predispose the lung to infection with other organisms, for example, bacteria and mycoplasmas. Our findings suggest that MHV1, JHM and A59 may
be primarily pneumotropic strains of murine coronavirus which produce liver damage to a minor degree as a secondary effect.

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