Entero-Hepatotropic Mouse Hepatitis Virus Infection in Nude Mouse

by *Per Svendsen**, *Børge Teisner*** and *Sophie Eckardt**
* Biomedical Laboratory and ** Institute of Medical Microbiology, University of Odense, DK-5230 Odense, Denmark.

Several strains of mouse hepatitis virus (MHV) are reported as the cause of fatal wasting disease in the mouse mutant nude (nu/nu). Affected animals lose weight rapidly and move stiffly. Partial paresis occurs first in the hind limbs, later in the front limbs, leading to immobility and death within one month after exposure. Post mortem examination reveals necrotic foci in the liver, characterized by necrosis of hepatocytes, acidophilic amorphous bodies and multinucleated giant cells. The presence of giant cells in the brain indicates infection by neurotropic variants of MHV (*Sebesteny & Hill* 1974, *Tamura et al.* 1986, *Ward et al.* 1977, *McKenzie et al.* 1978). One report describes infection in nude mice by an enterotropic strain of MHV, with the pathological lesions restricted to the intestinal tract. Coecum and colon are partially contracted and empty, and the mucosa show immature epithelium with a high mitotic index. Clumps of epithelial syncytia protrude into crypt lumina. Hepatic lesions are described absent except shortly after oral inoculation of virus (*Barthold et al.* 1985).

The infectious agent causing wasting disease in nude mice is usually apathogenic to normal mice (*Tamura et al.* 1977, *Hirano et al.* 1975).

In the present report an outbreak of MHV-infection in a colony of nude mice is described, as are the results of immunohistochemical staining of tissues from affected animals.

Materials and methods

Two heterozygous NC nu/+ females and one heterozygous NC nu/+ male were obtained as breeding animals in May 1982. From the offspring of these animals a breeding colony of 20 females and 5 males was produced. The animals were housed behind a barrier without contact to other animals. Feed and bedding were autoclaved (132°C for 2 min), and cages and equipment disinfected with formalin before entry. One animal technician dressed in sterile coverall and wearing headcover and gloves attended the animals. Room temperature was kept at 24°C ± 2° with a R.H. of 55 % ± 5 %. The light:dark cycle was 12:12 hours. Between 15 and 30 nu/nu mice were produced per month.

When 4 to 5 weeks old, the nu/nu offspring was brought to another animal room behind the barrier for experimental purposes, and kept there in a laminar air flow bench. Apart from the animal technician, only two persons had access to this room. Similar hygienic precautions were required. All cases of wasting disease occurred in the experimental room.

Sick animals were killed by cervical dislocation and examined post mortem. Liver, brain, spleen, kidney and intestines from a number of animals were selected for histological examination.

Tissues were fixed in 4 % buffered formaldehyde (pH 7.2), embedded in paraffin and cut in 5 µm sections. Following deparaffination, endogenous peroxidase was removed by adddition of 1 % H_2O_2 in 50 % methanol for 15 min. After 3×5 min rinse with TRIS-buffer, tissues were treated with 0.25 % trypsine in 0.02 % $CaCl_2$ $2H_2O$ for one hour at room temperature. Tissues were examined for the presence of MHV antigen by addition of MHV anti-

Table I. Tissues positive to MHV antigen in animals with positive livers.

Brain/liver	Lung/liver	Spleen/liver	Kidney/liver	Small intestine/liver	Large intestine/liver
2/4	1/2	3/3	0/1	4/4	4/4

serum (complement fixation titer 1:80, Microbiological Associates) diluted 1:60, for 16–20 hours at 4°C. After 3×5 min rinse with TRIS-buffer, rabbit anti-mouse peroxidase conjugated immunoglobulin (DAKO PATTS) diluted 1:160 was added for one hour at room temperature. Finally tissues were stained for 10 min at room temperature with a benzidine solution (13 mg benzidine in 20 ml TRIS-buffer). Immediately before use 20 µl 20 % H_2O_2 was added. After 10 min rinse in running tap water the slides were counterstained with Meyers acid hemalun for 5 min at room temperature. Each test included positive and negative tissue controls and serum controls, where MHV positive antiserum was replaced by MHV negative mouse serum in the same dilution.

Results

A total of 26 animals were affected. Six of them were found dead, the rest were killed because of severe emaciation.

The first case was observed in October 1982 when one mouse died with diarrhoea and enteritis. In July and August 1983 further 11 cases were recorded. The post mortem examination of these animals showed varying degrees of hepatic necrosis and atony of the intestinal tract with yellowish thin contents. In one animal the spleen was the site of acute inflammation and one animal showed nervous symptoms (circling movements). All animals left in the experimental room were killed and the room was cleaned and disinfected with formalin.

No new cases were recorded until January 1984, when one animal was killed because of nervous symptoms. No pathological processes could be detected by macroscopic examination in this animal. In March 1984 hepatitis was diagnosed in one mouse, and from August 1984 to February 1985 further 12 cases were diagnosed. The experimental room was again emptied and disinfected and the number of breeding animals reduced and replaced with animals from a germ free isolator. No new cases have since been diagnosed.

Immunohistochemical examination was performed on 14 animals. Eight of these had hepatic necroses and MHV antigen could be detected in the liver. In 6 animals both liver and intestinal tissues were examined. In all animals MHV antigen could be detected in both tissues. In 3 of these animals the spleens were examined and found positive. Brain, lung and kidney were only positive in some of the animals with MHV antigen positive liver sections (Table I). One animal, killed because of diarrhoea and emaciation, had no pathological lesions in the liver, and virus antigen was not detected in liver or intestine. The remaining five animals were killed for control purposes after February 1985. MHV antigen was not found in any of these animals.

The MHV antigen was found in apparently normal hepatocytes surrounding necrotic processes (Fig. 1A, B). No antigen appeard in the necrotic tissue itself. In the brain antigen was detected in multinucleated syncytia (Fig. 1C, D). MHV antigen was found in cell syncytia in the epithelium of all sections of the intestinal canal. Unstained syncytia are usually difficult to recognize (Fig. 1E, F and

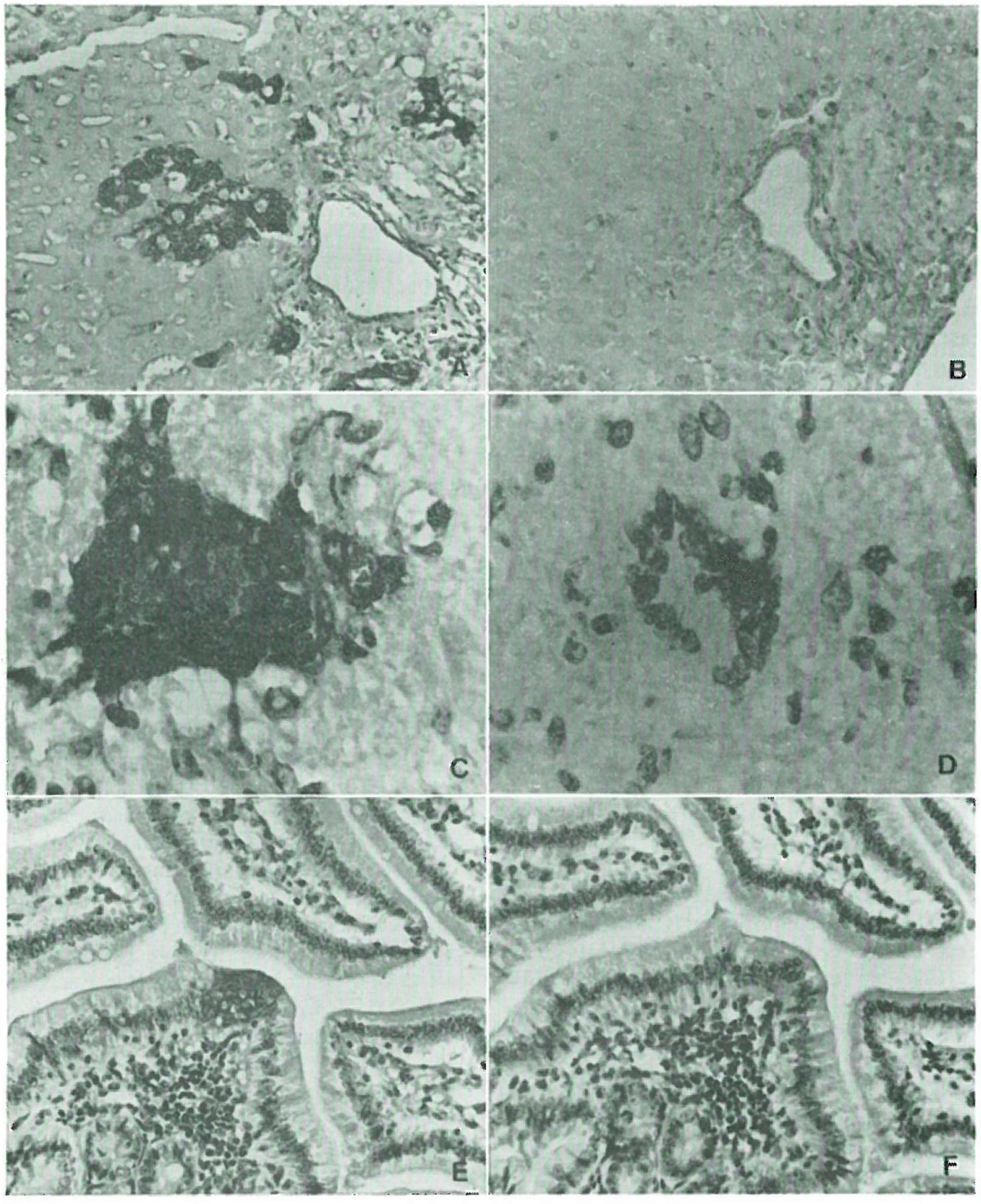

Fig. 1. Immunohistochemical staining for MHV antigen of liver (A), brain (C) and duodenum (E). Serum controls are shown in B, D and F.

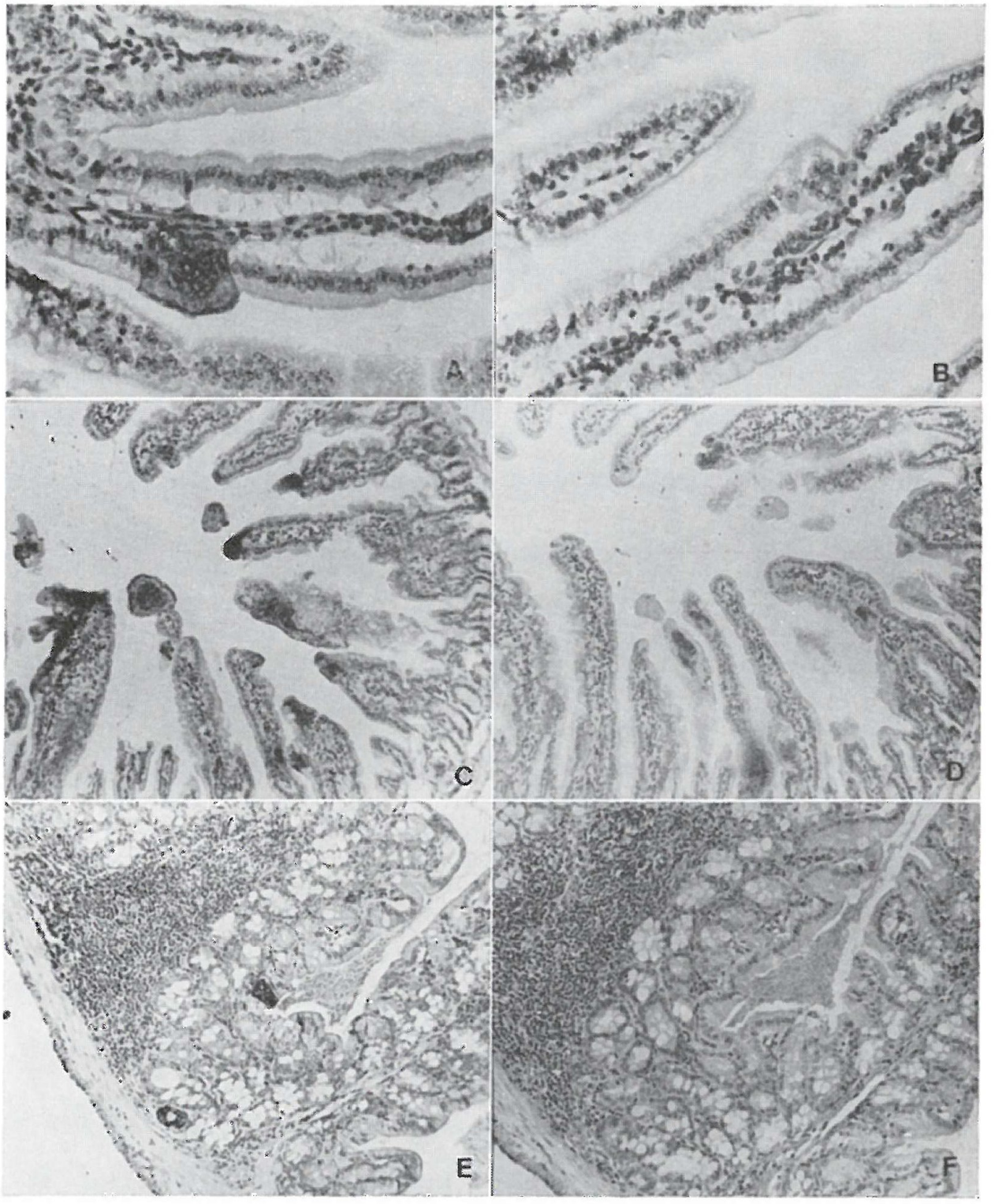

Fig. 2. Immunohistochemical staining for MHV antigen of jejunum (A), ileum (C) and colon (E). Serum controls are shown in B, D and F.

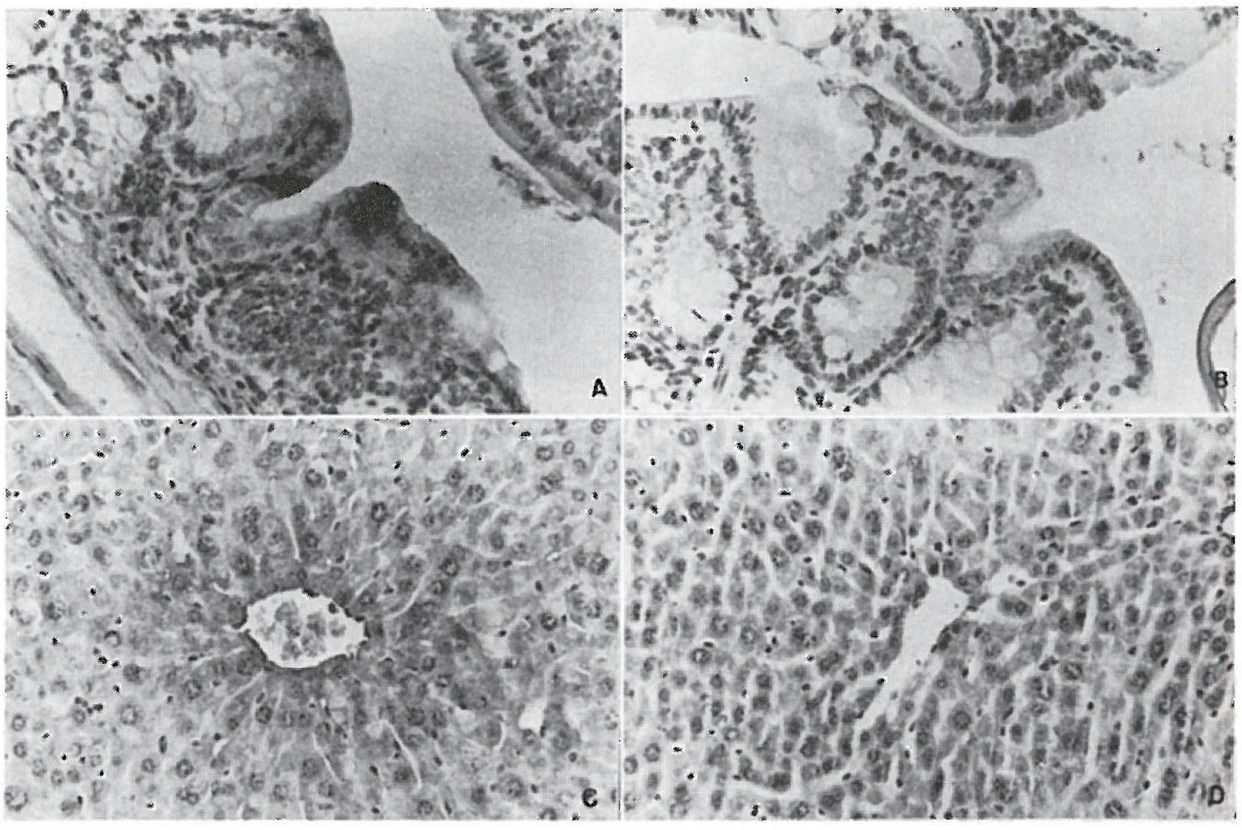

Fig. 3. Immunohistochemical staining for MHV antigen of colon (A) and liver (C). Necrotic foci characteristic of MHV-infection were absent in the liver of this animal, but hepatocytes surrounding central and portal veins show diffuse reaction to MHV antigen. Serum controls are shown in B and D.

Fig. 2 E, F). In some cases, however, the syncytia protrude from the surface of the villi (Fig. 2 A, B and Fig. 2 C, D).
In one animal with typical lesions in the colon, necrotic lesions were absent in the liver. A weak immunohistochemical staining, however, was detected in hepatocytes surrounding the portal and central veins (Fig. 3).

Discussion

Since the disease was confined to the experimental room and never occurred in the breeding room, it is most likely that virus was introduced either by a leakage into the experimental room or by staff or equipment in contact with the animals during the experimental procedure. The disease was apparently reintroduced 5 months after the first outbreak was eradicated.
It is remarkable that virus was present both in the intestinal epithelium and in the hepatocytes. This observation differs from all previous reports, in which the pathological changes are confined either to liver and brain (*Sebesteny & Hill* 1974, *Ward et al.* 1977, *Tamura et al.* 1976, *Tamura et al. 1977*, *McKenzie et al.* 1978), to the liver alone (*Hirano et al.* 1975, *Karasek & Rudolph* 1983) or primarily to the intestinal tract (*Barthold et al.* 1985). The present virus seems to infect primarily the intestinal epithelium and from here reach the liver via the portal vein (Fig. 3). The immunological reaction to MHV infection is poor in nude mice (*Tamura et al.* 1977) ,with a low antibody response. The

specific diagnosis is therefore based on the detection of virus or viral antigens in tissues of affected animals. The immunohistochemical technique described here offers a simple diagnostic method for MHV-infection.

Sammendrag

Der beskrives et udbrud af muse hepatitis virus (MHV) infektion hos nøgne (nu/nu) mus. Virus organtropisme er undersøgt ved hjælp af en immunhistokemisk teknik. Sygdommen adskiller sig fra tidligere beskrevne tilfælde ved virus tilstedeværelse i både tarmepithel og leverceller. Det primære infektionssted formodes at være tarmepithelet, hvorfra der sker en hæmatogen spredning til lever og milt. Hos enkelte dyr er også lunge- og hjernevæv inficeret med MHV.

Abstrakti / K. Pelkonen

Yhteenveto

Artikkelissa kuvataan hiiren maksatulehdusviruksen (MHV) aiheuttaman taudin puhkeaminen nude-hiirikoloniassa (nu/nu). Viruksen elinhakuisuutta tutkittiin immunohistokemiallisella tekniikalla. Sairous eroaa aiemmin kuvatuista tapauksista siinä, että virusta löytyy sekä suoliston epiteelistä että maksasoluista. Primääriseksi infektiokohdaksi arvellaan suoliston epiteeliä, josta leviäminen tapahtuu veriteitse maksaan ja pernaan. Yksittäisissä eläimissä MHV-virusta tavattiin myös keuhko- ja aivokudoksesta.

References

Barthold, S. W., A. L. Smith & M. L. Povar: Enterotropic mouse hepatitis virus infection in nude mice. *Lab. Anim. Sci.* 1985, *35*, 613—618.

Hirano, N., T. Tamura, F. Taguchi, K. Ueda & K. Fujiwara: Isolation of low-virulent mouse hepatitis virus from nude mice with wasting syndrome and hepatitis. *Japan. J. Exp. Med.* 1975, *45*, 429—432.

Karasek, E. & M. Rudolph: Electron microscopic demonstration of mouse hepatitis virus in nu/nu mice. *Z. Versuchstierk.* 1983, *25*, 114—115.

McKenzie, R. A., R. J. Parker & F. W. Eaves: Suspected viral hepatitis of nude mice in Australia. *Lab. Anim.* 1978, *12*, 27—28.

Sebesteny, A. & A. C. Hill: Hepatitis and brain lesions due to mouse hepatitis virus accompanied by wasting in nude mice. *Lab. Anim.* 1974, *8*, 317—326.

Tamura, T., K. Ueda, N. Hirano & K. Fujiwara: Persistent infection with mouse hepatitis virus of low virulence in nude mice. *Microbiol. Immunol.* 1977, *21*, 683—691.

Ward, J. M., M. J. Collins Jr. & J. C. Parker: Naturally occurring mouse hepatitis virus infection in the nude mouse. *Lab. Anim. Sci.* 1977, *27*, 372—376.