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# SEQUENCE OF MURINE CORONAVIRUS JHM INDUCED NEUROPATHOLOGICAL CHANGES IN RATS

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**Sequence of murine coronavirus JHM induced neuropathological changes in rats**

Infection of 21–25-day-old rats with the murine coronavirus JHM was followed either by an acute encephalomyelitis (AE) or subacute demyelinating encephalomyelitis (SDE). The major neuropathological finding in AE, which developed within 6–12 days p.i. consisted of necrotizing lesions distributed mainly in the grey matter of the central nervous system (CNS). SDE developed 14–30 days p.i. and affected rats revealed lesions of primary demyelination with predilection sites in the white matter. The time-course for the development of lesions, virus replication and neutralizing antiviral antibody production within the first 3 weeks p.i. were studied. Within the first 2 weeks p.i., most rats showed no clinical signs but nevertheless revealed lesions typical of AE. In parallel to these neuropathological changes infectious virus could be isolated from brain and spinal cord. However, coinciding with multiplication of neutralizing JHM antibodies 10–12 days after infection no infectious virus was recoverable from CNS material. At this time many of the clinically healthy rats showed demyelinating lesions which were located at the typical predilection sites of SDE. These observations indicated that SDE was preceded by clinically silent AE lesions.

## Introduction

Investigations of central nervous system (CNS) diseases in man and animals have shown that viruses can induce demyelination by various mechanisms (Weiner & Stohlman, 1978; ter Meulen & Hall, 1978). One possible way is the direct effect of virus replication leading to destruction of oligodendroglia cells. In addition viruses, or their products, may cause demyelination by

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direct damage to myelin membranes, since fusion protein constituents of virus envelopes can drastically alter cell membranes. Besides these direct effects, demyelination may also develop as a result of an immunopathological reaction induced by the virus infection, as circumstancial evidence suggests in Theiler's virus infection in mice (Lipton & Dal Canto, 1976). However, with the exception of oligodendroglial cell destruction by viruses, the detailed pathogenic mechanisms of virus-induced demyelination are still incompletely understood. Consequently, experimental virus infections with associated demyelinating CNS changes are of great interest for the evaluation of certain virus-cell and virus-host interactions. In this context CNS disease of rats caused by infection with the murine coronavirus strain JHM provides an important disease model for experimental analysis. Depending on the age of the animal and the biological properties of the virus inoculated either acute or predominantly subacute demyelinating encephalomyelitis are produced (Cheever *et al.*, 1949; Nagashima *et al.*, 1978, 1979; Sörensen, Percy & Dales, 1980; Sörensen *et al.*, 1982; Wege *et al.*, 1983). In the following study experiments were carried out with JHM virus in weanling rats to determine the sequence of changes which lead to demyelination in order to provide a basis for future virological and immunological studies. The data obtained indicate that the subacute demyelinating encephalomyelitis in rats is preceded by a clinically silent acute encephalitis. These acute lesions, mainly located in the grey matter, do not enlarge whereas white matter changes develop to a clinically recognizable subacute encephalomyelitis.

## Materials and methods

### *Virus*

The murine coronavirus strain JHM was kindly provided by L. Weiner, Johns Hopkins University, Baltimore, MD, USA, and passaged by intracerebral infection of suckling mice (strain BALB/c). Brain tissue of diseased animals was homogenized in Eagle's MEM to a 10% suspension (w/v) for inoculation.

### *Animals*

Specific pathogen-free weanling rats (21–25 days old) strain CHBB/Thomae were used for these experiments. The rats received a dose of $1 \times 10^4$ $TCID_{50}$/40 $\mu$l of JHM-virus, injected into the left brain hemisphere. Infected animals were observed daily for the development of clinical symptoms. Animals were anaesthetized, bled out and brain and spinal cord were removed aseptically for morphological and virological studies.

### *Histology and electron microscopy*

The rats were perfused with a solution consisting of 2% paraformaldehyde and 2·5% glutaraldehyde in 0·1 M phosphate buffer pH 7·4. For histology, coronal sections were fixed in

10% formalin and embedded in paraffin wax. Sections were stained with either haematoxylin-eosin or Klüver-Barrera stain for myelin, and by the Glees and Marsland's method for axons. The blocks for electron microscopy were postfixed in 1% osmium tetroxide, stained with 2·5% uranyl acetate and embedded in epon (Nagashima *et al.*, 1978, 1979).

*Immunohistochemistry*

Indirect immunofluorescence and immunoperoxidase methods were applied to cryostat sections (thickness 8 μm) of frozen tissue by standard procedures (Sternberger & Petrali, 1977). Antiserum against JHM was prepared in mice by weekly intraperitoneal injections of purified JHM virus derived from infected Sac(−) cells (Wege *et al.*, 1981). FITC-labelled and peroxidase-conjugated anti-mouse IgG rabbit sera were purchased from Miles Biochemicals, Frankfurt, Germany. For the detection of rat IgG in the neural tissue a direct immunofluorescence technique was applied using FITC-labelled anti-rat-IgG rabbit serum (Miles Biochemicals).

*Virus isolation and determination of neutralizing antibodies*

Samples of brain and spinal cord tissue were homogenized to a 10% (w/v) suspension in Eagle's MEM. The virus was isolated by infection of monolayers of Sac(−) cells in 24 well cluster plates and titrated as described previously (Wege *et al.*, 1983). The incubation temperature was 37°C.

To measure neutralizing antibodies, mixtures of virus (100 $TCID_{50}$) and serum dilutions were incubated for 1 h at 37°C before titration on cultures of Sac(−) cells in microtitre plates (Wege *et al.*, 1981).

## Results

### *Neurovirulence of JHM virus in outbred rats*

Seventy-seven rats between 21–25 days of age, were infected intracerebrally with $1 \times 10^4$ $TCID_{50}$ of JHM virus and observed for the development of neurological signs. After an incubation period of 5–12 days fifteen animals developed a hunched posture, ruffled fur, slower movements, bizarre trembling of the neck, a clumsy gait and paralysis of the forelegs. These animals died within a few days of developing clinical signs. The neuropathological changes were typical of an acute encephalomyelitis (AE). Among the surviving sixty-two animals fifteen developed spastic hindleg paralysis, tetraplegia or sometimes hemiplegia 14–33 days p.i. These diseased rats often survived for several weeks and some recovered with complete disappearance of clinical signs. Based on neuropathological findings this disease was termed subacute demyelinating encephalomyelitis (SDE). In the following we describe the neuropathological changes typical for AE and SDE, and those found in animals without clinical signs.

### *Acute encephalomyelitis*

The major neuropathological changes consisted of inflammation and necrotic demyelinating foci which were disseminated mainly in the hippocampal

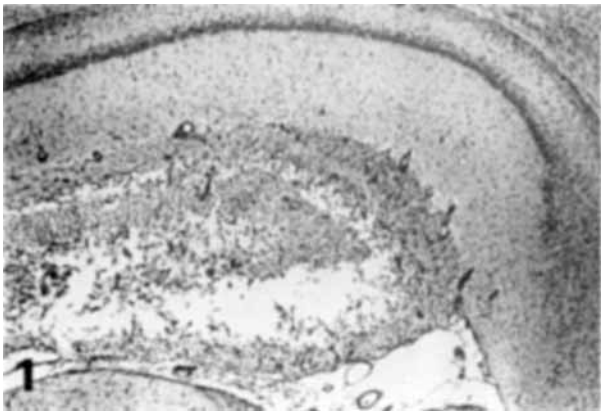

**Figure 1.** Acute encephalomyelitis, 9 days p.i. Necrosis in the hippocampal region associated with acute inflammation in the dentate gyrus and the dorsomedial part. Klüver-Barrera, ×25.

region, brain stem and spinal cord. In eleven animals the hippocampus was mainly involved, with severe bilateral tissue damage (Figure 1). In such cases the number of pyramidal neurons in the dentate gyrus and dorsomedial part of the hippocampus was reduced and these lesions were associated with numerous macrophages. Perivascular infiltration consisted of polymorphonuclear cells. Mild inflammation was sometimes apparent in the entorhinal cortex. Multiple foci of various sizes occurred in the brain stem, and consisted either of necrotic or demyelinated lesions. In necrotic areas neurons, axons and myelin sheaths were completely destroyed and replaced by amorphous eosinophilic material, numerous polymorphonuclear cells and macrophages (Figure 2). In demyelinated areas, myelin sheaths were disintegrated but axons were relatively preserved. Neurons were usually degenerated or depopulated (Figure 3a,b). Perivascular inflammatory reactions were detectable in and around the lesions. Giant cell formations were rarely seen, but glial nodules were often found. The cerebellar cortex

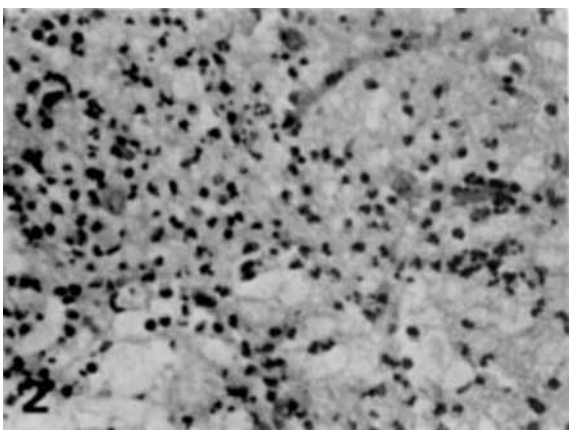

**Figure 2.** Acute encephalomyelitis, 7 days p.i. Necrotic lesion in the pons which shows numerous infiltrated polymorphonuclear cells. Haematoxylin and eosin. ×180.

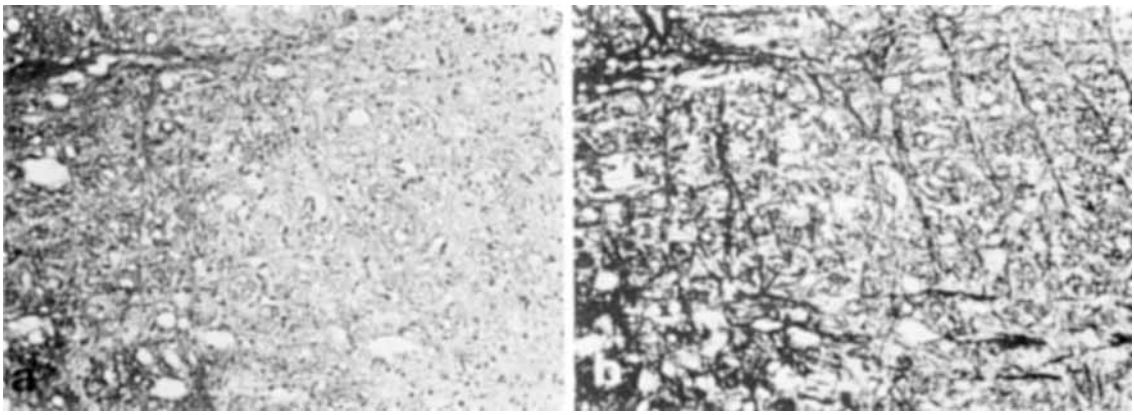

**Figure 3.** Acute encephalomyelitis, 9 days p.i. Early demyelinative lesion. **a** Loss of myelin and neurons. Stained by Klüver-Barrera. ×70. **b** Axons are well preserved. Glees & Marsland. ×70.

was only occasionally affected but inflammatory cells frequently infiltrated bilaterally the cerebellar nuclei.

In the spinal cord changes were noticed mainly in the grey matter in the form of glial nodules, neuronophagia and perivascular cuffs (Figure 4a). Neurons were depopulated to various degrees. The remaining neurons often exhibited shrinkage or vacuolar changes. Throughout the grey matter, scattered focal tissue necrosis was found which showed no preference for a particular site. The necrosis occasionally involved the total central grey matter at one level (Figure 4b). Meningitis was frequently detected adjacent to active lesions in the neural parenchyma. No pathological alterations were noticed in spinal nerve roots, liver, spleen or other organs.

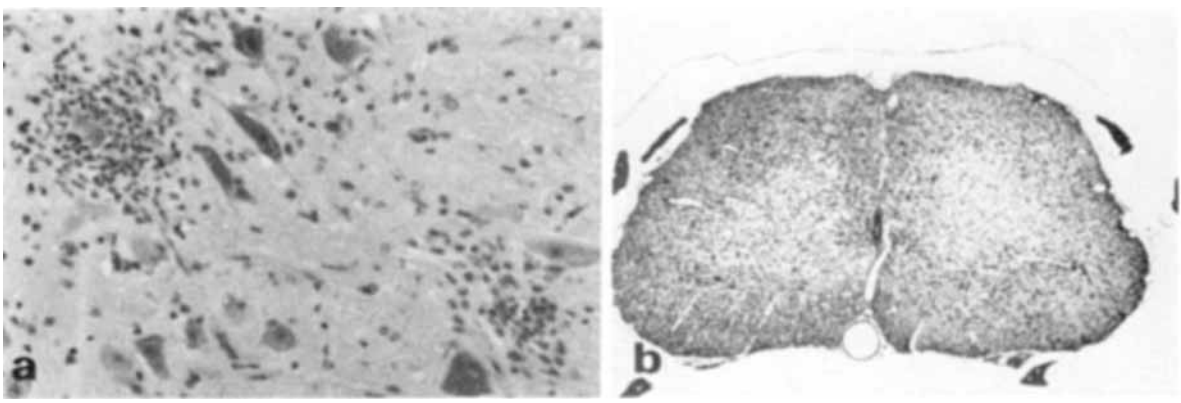

**Figure 4.** Acute encephalomyelitis, 10 days p.i. **a** Glial nodules (upper left) and neuronophagia (lower right) in the ventral horn of the spinal cord. Haematoxylin and eosin. ×120. **b** Extensive necrosis in the grey matter of the spinal cord. Haematoxylin and eosin. ×25.

Ultrastructural investigation of the necrotic areas revealed that the neural parenchyma was destroyed and infiltrated by macrophages. Macrophages and naked axons, which were quite often degenerated, were seen in demyelinated foci in the brain stem (Figure 5). Mature virus particles were detectable in oligodendrocytes of relatively early lesions (Figure 6).

Viral antigens were distributed throughout the brain and the spinal cord. In the hippocampal region and the spinal grey matter many neurons and glial cells of adjacent areas contained particularly high concentrations of virus antigen. In contrast, only glial cells carried viral antigens near to and in demyelinated foci. No autologous IgG was detected in neural tissue.

*Subacute demyelinating encephalomyelitis*

The major histological lesions consisted of well demarcated demyelinated plaques, which were disseminated in the brain stem and spinal cord (Figure 7). The optic tract, cerebral white matter, internal capsules, cerebral and cerebellar peduncles were less involved. The lesions revealed a salient predilection for the white matter unrelated to any specific tract. Neurons appeared intact in demyelinated plaques which extended to the grey matter of the pons. Perivascular cuffs consisting of macrophages, lymphocytes and plasma cells were located in and around demyelinated plaques. No infiltration by polymorphonuclear cells and no glial nodules were found.

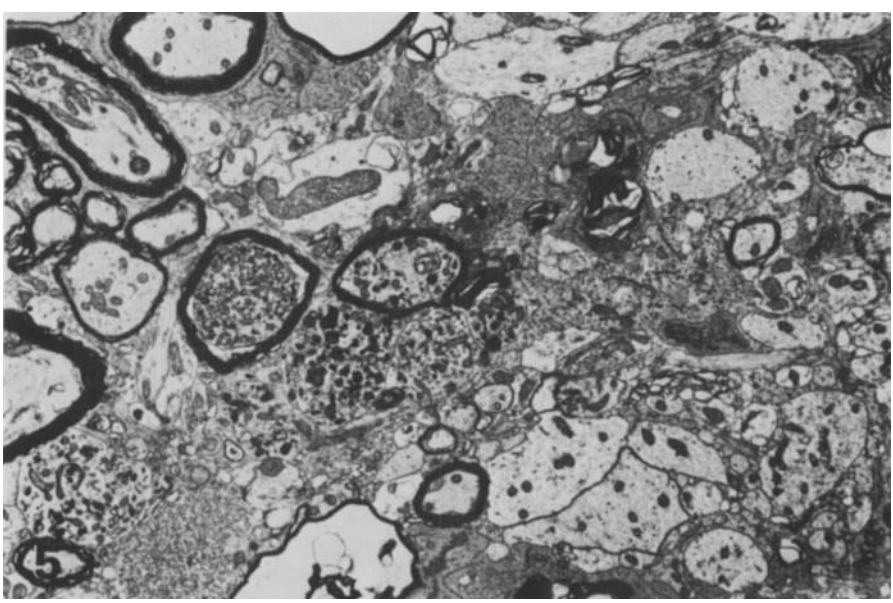

**Figure 5.** Acute encephalomyelitis, 10 days p.i. Electron micrograph of a rather demyelinative lesion in the pons. Naked axons are visible, some degenerated axons are filled with microorganelles and dense bodies. Uranyl acetate. ×5200.

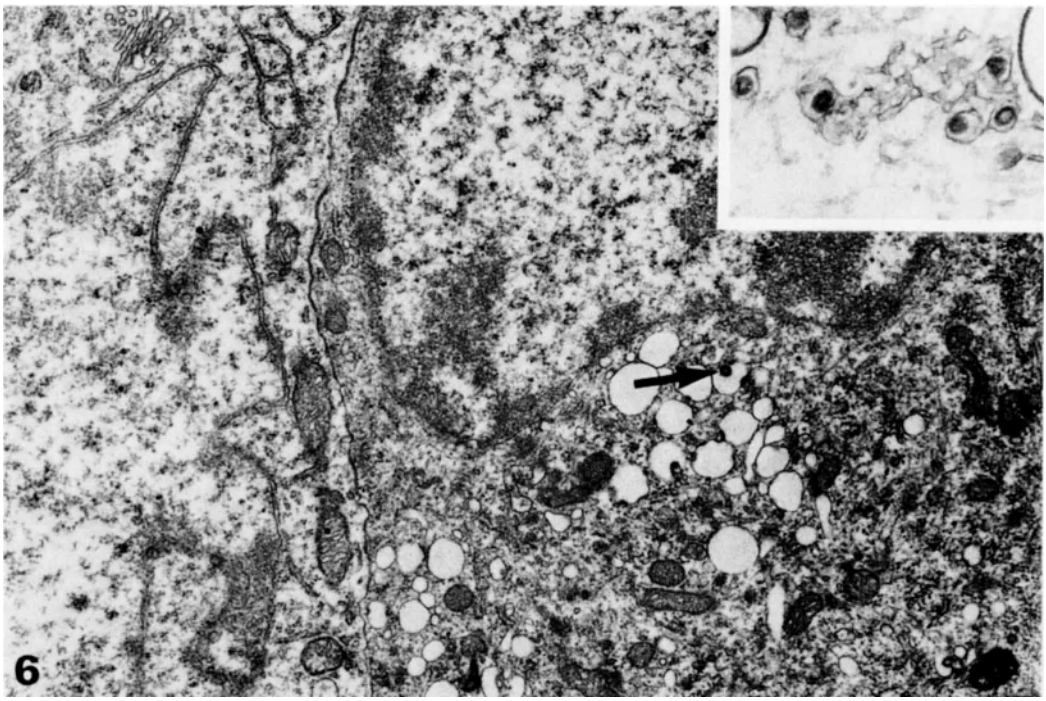


**Figure 6.** Acute encephalomyelitis, 7 days p.i. Virus particles budding into cytoplasmatic vacuoles of a satellite oligodendrocyte in the pons (←). Neurons show no contributory changes (left side). Uranyl acetate. ×14 200. Inset shows budding virus particles in a glial cell from the same lesion (Uranyl acetate. ×42 600).

Residual changes of AE were detectable in the majority of animals, as well as the typical lesions of SDE (Figure 8). Such old necrotic lesions of the brain cortex often showed cavitation.

Ultrastructural investigation demonstrated axons without myelin sheaths and macrophages containing myelin debris. In early demyelinated plaques which contained oligodendrocytes mature virus particles were only rarely found. No autologous IgG was demonstrable in demyelinated plaques. Occasionally plasma cells which carried rat IgG were seen around blood vessels.

*Sequence of neuropathological changes after JHM virus infection*

The observation that animals with SDE had residual old lesions of AE (Figure 8) suggested a biphasic pattern for the development of SDE. To prove this hypothesis seventy-five rats were infected with JHM virus and animals without clinical signs were killed on day 3, 6, 9, 12, 15, 18, 21 and 24 p.i. respectively. Samples were taken from each rat for virus isolation and histological examination. Furthermore, the titre of neutralizing antiviral

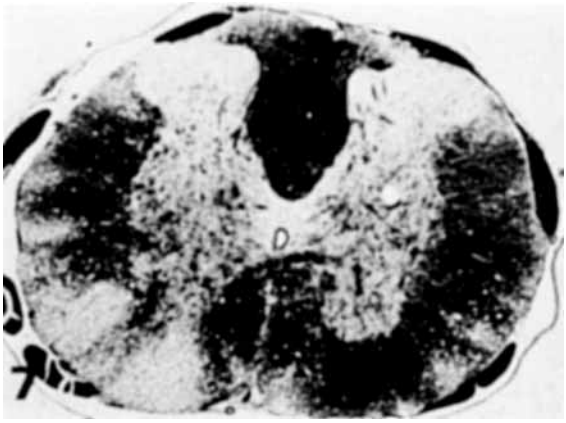

**Figure 7.** Subacute demyelinating encephalomyelitis, 24 days p.i. Demyelinating plaques disseminated in the white matter of the spinal cord. Klüver-Barrera. ×22.

antibodies in the serum was determined. The results of these experiments are summarized in the Table 1. Almost all animals dissected within 6–12 days p.i. revealed typical changes of AE. Within 6 days p.i., JHM virus antigen-containing cells were found in brain and spinal cord, in areas which did not show definite inflammatory changes (Figure 9a and b). The histological changes characteristic of SDE were detected later than 12 days p.i. (Figure 10). This clinically silent SDE was often associated with relatively old necrotic lesions, which were regarded as residual AE lesions. Infectious virus was isolated from brain and spinal cord during the first 12 days after infection. Later, coinciding with a rise in neutralizing antibodies, virus could no longer be isolated despite the presence of viral antigen in the lesions. Those animals which developed a clinically apparent disease were exceptions. In these cases virus could be isolated from brain tissue. It is likely, therefore, that in animals with silent lesions the host immune response influences the virus cell interaction and inhibited rapid virus spread.

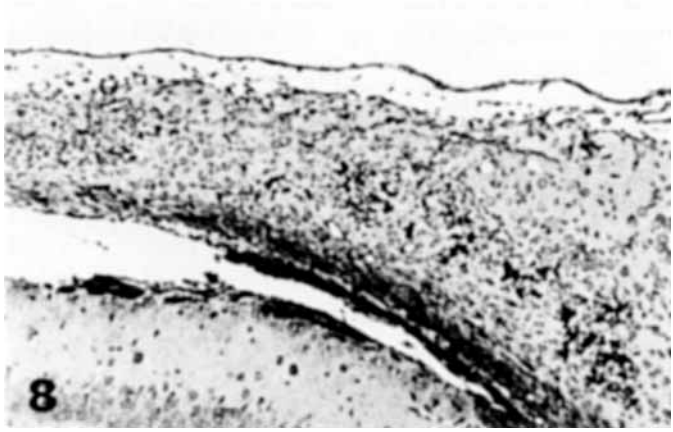


**Figure 8.** Old lesion of clinically silent AE (6 weeks p.i.) in brain cortex (parietal lobe) in SDE animals. Note shrinkage of cortex (left), extensive calcium deposition and loss of neurons. The periventricular white matter is relatively well preserved. Klüver-Barrera. ×112.

**Table 1.** Kinetics of virus growth, development of neutralizing antibodies and clinically silent encephalomyelitis in the course of JHM virus infection in weanling rats

| *Days p.i.*[1] | *Neuropathological changes/number of dissected animals* | *Type of changes*[2] *AE* | *AE+SDE* | *SDE* | *Infectious virus*[3] *TCID50/g tissue* *Brain* | *Spinal cord* | *Neutralizing antibody titre* |
|---|---|---|---|---|---|---|---|
| | | | *1* | | | | |
| 3 | 1/4 | 1* | — | — | $1{\cdot}4\times10^{3}\pm0{\cdot}8$ | $2{\cdot}8\times10^{1}\pm1{\cdot}1$ | <1:8 |
| 6 | 3/4 | 3 | — | — | $1{\cdot}1\times10^{3}\pm1{\cdot}1$ | $1{\cdot}8\times10^{3}\pm2{\cdot}1$ | <1:8 |
| 9 | 3/4 | 3 | — | — | $4{\cdot}8\times10^{1}\pm4{\cdot}3$ | $1{\cdot}2\times10^{3}\pm1{\cdot}9$ | 1:8 |
| 12 | 4/4 | 3 | 1 | — | — | $4\times10^{1}\pm0{\cdot}8$ | 1:11 |
| 15 | 1/4 | — | 1 | — | — | — | 1:18 |
| 18 | 3/4 | 1 | 2 | — | — | — | 1:26 |
| 21 | 3/4 | — | 1 | 2 | — | — | 1:32 |
| 24 | 4/4 | — | 2 | 2 | — | — | 1:32 |

[1]The rats were infected (i.c.) at an age of 21–25 day with a dose $1\times10^{4}$ TCID50 of JHM-virus. [2]AE = Acute encephalomyelitis. SDE = subacute demyelinating encephalomyelitis. AE+SDE = changes of both types were observed in the same CNS. [3]Isolation from brain and spinal cord tissue was done separately for each animal.
*Number of animals with specific neuropathological changes.

## Discussion

These experiments demonstrate that the murine coronavirus JHM, derived by passage in mouse brain, is capable of inducing acute and subacute encephalomyelitis in rats. Both disease processes can be differentiated by particular neuropathological features and by the incubation time. It has been suggested that the wild JHM strain consists of a heterogeneous virus population with different virological properties causing either acute or subacute demyelinating encephalomyelitis (Wege *et al.*, 1983). So far attempts to separate these populations have failed since tissue culture adaptation of wild JHM virus resulted always in virus which induced acute encephalomyelitis. Only after chemical mutagenesis of the tissue culture-grown JHM virus were mutants with altered virulence obtained (Wege *et al.*, 1983). These less virulent JHM virus particles could be present in the wild population but grew poorly under tissue culture conditions and could not, therefore, be isolated from brain tissue of diseased rats.

In addition, host mechanisms evidently play an important part in the outcome of the virus infection. Thus, SDE is preceded by clinically silent AE which is arrested and changed into a subacute form at a time when an antiviral immune response is first detectable. These antibodies may not only neutralize extracellular virus, but also influence viral growth. It has been shown in tissue culture experiments with different virus groups that a lytic viral infection can be changed to a persistent one in the presence of antiviral

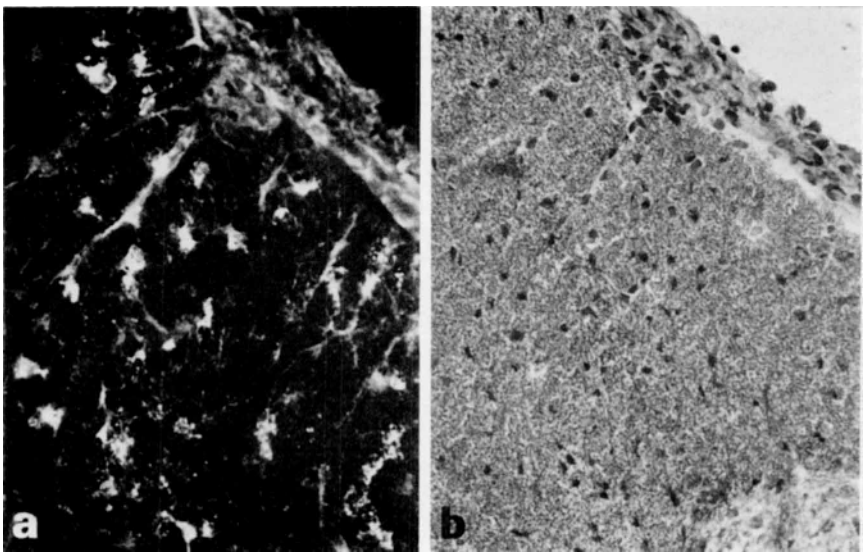


**Figure 9.** Clinically healthy rat, 6 days p.i. **a** JHM virus antigen containing cells are detectable in the subpial area of the white matter. FITC-method. ×68. **b** Same area as **a**, haematoxylin and eosin staining. No definite inflammation except for slight meningitis is visible in neuronal tissue. ×68.

antibodies (reviewed by Rima & Martin, 1976). Antibodies inhibit not only the spread of virus and infection of neighbouring cells, but also the expression of viral antigen. In cell lines persistently infected with measles virus it has been shown that antibodies against measles virus act at the cell membrane, modulate the expression of viral antigen and block the development of cytopathic effects (Joseph & Oldstone, 1975). Similarly such effects may occur

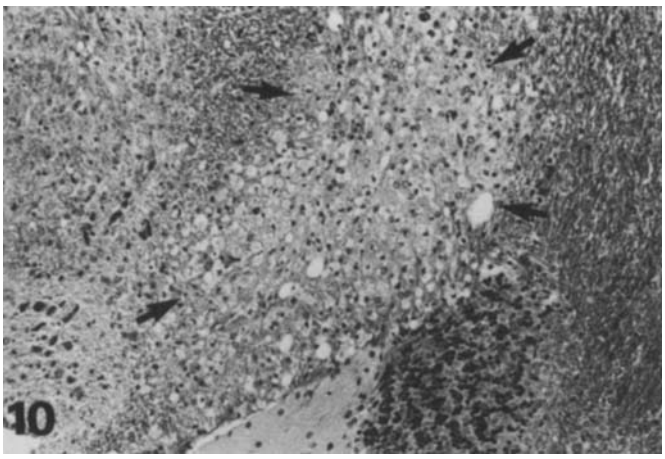

**Figure 10.** Small demyelinating plaque (←) in clinically healthy rat (21 days p.i.) in the cerebellar white matter. Infiltrated phagocytes are found within the plaque. Stained with haematoxylin and eosin and luxol fast blue. ×140.

in rat brains infected with JHM virus and help to change an acute lytic infection to a subacute one. this interpretation is supported by the observation that infectious virus can no longer be isolated from CNS tissue after JHM antibodies have appeared. Virus can only be recovered from rats with clinically recognizable SDE. The immune response in these animals may not be adequate enough to suppress growth of virus.

The lesions of AE are located mainly in the grey matter, whereas subsequent SDE leads to white matter changes. Therefore the target cells for JHM infection may have changed during the course of infection. In AE both neurons and glial cells are highly susceptible to JHM infection. However, this susceptibility probably alters with brain maturation or selection of JHM virus mutants. The latter possibility is supported by studies with ts-mutants of JHM virus in mice. In contrast to wild type JHM virus, ts-mutants selectively infect oligodendroglial cells (Knobler *et al.*, 1981; Knobler, Lampert & Oldstone, 1982). Thus the high frequency of demyelination caused by mutants of JHM virus in mice and rats may be correlated with an altered cellular tropism of such mutants in contrast to the wild JHM virus strain.

The mechanism by which JHM virus persists in brain cells is presently unknown. Tissue culture experiments have shown that coronavirus can easily establish persistency with the production of infectious virus which reveals altered biological properties (Stohlman, Sakaguchi & Weiner, 1979; H. Baybutt *et al.*, unpublished observation). Similar virus-cell interactions may occur during growth in brain tissue. AE is probably caused by the destruction of the target cells. In SDE a more complex virus-host interaction occurs by which an early lytic infection is converted to persistence under the influence of the immune system.

## Acknowledgements

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