HERPES SIMPLEX VIRUS AND PARALYSIS OF ANIMALS

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THE ability of herpes simplex virus to invade the central nervous system following peripheral inoculation of mice or rabbits, resulting in encephalitis, has long been known, although the route taken by the virus has been somewhat obscure. The work presented in this paper was stimulated by preliminary observations of paralysis, not followed by clinical encephalitis, in rabbits being hyperimmunized by intramuscular inoculations of the MS strain of herpes simplex, isolated by Gudnadottir from the brain of a patient with multiple sclerosis. The virus has been shown (Plummer, 1964) to be serologically distinguishable from other strains of herpes simplex. In view of the origin of this virus, its serological distinctness and the paralysis obtained in the rabbits, more detailed studies were done of its effect in adult rabbits and mice ; similar experiments were done with an " ordinary " strain of herpes simplex.

MATERIALS AND METHODS

Viruses.—The L2 strain of herpes simplex virus was isolated in Russia from common cold sores (Shubladze, Maevskaya, Ananev and Volkova, 1960). Its tissue culture passage level is not known. The MS strain of herpes simplex virus was isolated by Dr. M. Gudnadottir of the University of Iceland from the central nervous system of a case of multiple sclerosis ; it has been passed thirteen times in rabbit kidney tissue cultures. The two viruses are members of the herpes group as indicated by particle structure and by their cytopathic effect in rabbit kidney cultures. They are serologically distinguishable, the L2 strain being similar to the " ordinary " strains of simplex (Plummer, 1964). The stocks of L2 and MS viruses were stored at $-70°$ and had titres of $10^{7.0}$ and $10^{5.6}$ TCD_{50}/1 ml. respectively. They had been grown in human embryonic lung tissue cultures, maintained on Eagle's basal medium with 2 per cent calf serum. The culture fluid was harvested and clarified at 2000 *g* for 15 min.

Rabbits and mice.—Young adults were used. They were in no way pre-treated or sensitized before use. Adult animals were injected with one or other of the virus strains, either into the posterior femoral muscle of the left back leg or the muscle of the upper part of the left front leg—0·2 ml. of virus into a rabbit and 0·05 ml. into a mouse. The MS virus had a titre of $10^{5.6}$ TCD_{50}/1 ml. and the L2 strain $10^{7.0}$ TCD_{50}/1 ml. Equal numbers of control animals were inoculated with tissue culture fluid.

RESULTS

Inoculation of rabbits and mice with MS and L2 virus strains

The results are summarized in Tables I and II. None of the control animals developed paralysis. As can be seen, about 70 per cent of rabbits receiving MS virus developed paralysis, whereas none of those inoculated with L2 virus showed

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abnormalities. All but one of the paralysed rabbits survived. The one that did not survive had the virus in the front leg ; it had developed paralysis of that limb 10 days after inoculation and died of encephalitis 6 days later. Of mice inoculated with either MS or L2 virus 19–30 per cent developed paralysis of the appropriate limb. In all those receiving MS virus it proceeded to a fatal encephalomyelitis ; 38 per cent of those paralysed by the L2 strain, however, survived with permanent paralysis of the limb, and no ensuing encephalomyelitis.

TABLE I.—*Results of Inoculation of MS and L2 Strains of Herpes virus hominis into the Back Left or Front Left Leg of Rabbits*

	MS strain		L2 strain	
	Back leg	Front leg	Back leg	Front leg
Animals paralysed.	11/16	6/8	0/14	0/5
Per cent	69	75	0	0
Paralysed animals surviving	11/11	5/6	Not applicable	
Per cent	100	83		

The numbers and percentages developing paralysis of the inoculated limb are given, as are the proportions of paralysed animals surviving (*i.e.* not developing encephalomyelitis).

TABLE II.—*Results of Inoculation of MS and L2 Strains of Herpes virus hominis Into the Back Left or Front Left Leg of Mice*

	MS strain		L2 strain	
	Back leg	Front leg	Back leg	Front leg
Animals paralysed.	24/80	9/46	21/90	Not done
Per cent	30	19	23	
Paralysed animals surviving	0/24	0/9	8/21	
Per cent	0	0	38	

The numbers and percentages developing paralysis of the inoculated limb are given, as are the proportions of paralysed animals surviving (*i.e.* not developing encephalomyelitis).

MS virus was also inoculated intradermally into the shaved skin on the femoral part of the left back leg of 8 rabbits. Four of them developed paralysis of the left back leg. None of them died, but they did remain paralysed.

The time taken for the development of the paralysis after inoculation of MS or L2 was in all cases between 6 and 13 days. The paralysis in the rabbits receiving MS virus appeared to be spastic, the limb being drawn up close to the body. However, it was difficult to diagnose spastic paralysis with certainty in a small laboratory animal. The limbs were not dragged or flaccid. No pain was evident, nor was loss of sensitivity as judged with a needle. Electromyographic examination of a rabbit with severe paralysis of the left back leg showed no involvement of the lower motor neurone. In paralysed mice the left back leg was extended behind the body. It was difficult to determine whether this was spastic or flaccid paralysis. Paralysis of the front limb seemed to be of a spastic nature. The degree of paralysis in the surviving animals varied. In some cases it was

sufficiently severe to cause the animal to roll over onto its side when attempting to walk. Most of the paralysed animals were kept for 3 months after which they were killed.

Histology

The central nervous systems of 3 rabbits with left back legs paralysed by MS virus were examined histologically. Two of the animals had been inoculated 18 days previously, and had been paralysed for 8 days, and the third animal had been inoculated 2 months previously and paralysed for 7 weeks.

In all 3 rabbits there was severe inflammation of the lumbar dorsal nerve roots and the sensory fibres of the lumbar nerves of the left side only. Some demyelination was evident due to death of the axons. No involvement of the lower motor neurones or ventral nerve roots was seen. Inflammation of a few of the thoracic nerve roots was noted. Once again only those on the left side were involved. No plaques of demyelination such as are characteristic of multiple sclerosis were seen in the spinal cord.

DISCUSSION

The two strains of herpes simplex have been shown to differ in their neurovirulence. The MS virus caused paralysis in about 70 per cent of the rabbits inoculated with it. In marked contrast was the complete absence of paralysis in rabbits receiving L2 virus, though its titre, as estimated in tissue culture, was twenty-fold higher than that of MS virus. The greater neurovirulence of the MS strain was also illustrated in the death by encephalomyelitis of all the mice developing paralysis after its inoculation, whereas 38 per cent of the mice paralysed by L2 virus survived. It cannot at present be suggested that this difference can be correlated with their antigenic difference until more strains are studied, because it may reflect simply the tissue culture passage level of the strains.

Observation of the living rabbits indicated a spastic type of paralysis. The electromyograph on one rabbit confirmed that the lower motor neurone still functioned. However, histopathology showed only destruction of the sensory nerve roots with no obvious involvement of the upper or lower motor neurone. This indicates that the paralysis was due to the destruction of the sensory nerves. Other changes in the central nervous system, such as plaques of demyelination, were not seen, though they cannot be completely excluded. It is interesting that, with one exception the paralysis in the rabbits did not progress to clinical encephalomyelitis, which is rather in contrast to the findings of other workers (*e.g.* Goodpasture, 1925), who emphasized encephalitis as the final outcome of experimental infection of animals.

One of the purposes of this work was to investigate the possible role of MS virus in multiple sclerosis. However, no plaques of demyelination were found in the central nervous system of paralysed rabbits. Ross, Lenman and Rutter (1965) found no evidence of a particular correlation between complement fixing herpes simplex antibody and multiple sclerosis. The only virus complement fixing antibody with which they found a correlation of possible significance was that of varicella-zoster. It is noteworthy that the MS virus damaged the sensory nerve roots, making a striking similarity to the activities of zoster. No serological comparison has yet been made between MS virus and varicella-zoster virus.

Parallel titrations of the neutralizing antibodies to MS virus and L2 virus in sera from 10 multiple sclerosis patients have in fact been done, though not described in this paper ; only 8 of them had antibody, and in each case the titre of neutralizing antibody against MS was much lower than against L2 virus.

Schmidt and Rasmussen (1960) clearly showed that a chronic latent infection by herpes simplex virus could be created in rabbits. Herpes encephalitis could be precipitated by a variety of stimuli many months later. It seems likely that the MS virus would persist in the rabbits. It will be of interest in the future to determine if this can be reactivated and what form the reactivation takes.

SUMMARY

The serologically distinct strains of herpes simplex, MS (from a case of multiple sclerosis) and L2, were inoculated intramuscularly into the legs of rabbits and mice. About 70 per cent of the rabbits receiving MS virus developed paralysis of the inoculated limbs ; none of the rabbits receiving L2 virus was paralysed. All but one of the paralysed rabbits survived and remained permanently paralysed ; the one exception died of encephalitis. Histopathology revealed destruction, or partial destruction, of the appropriate sensory nerve roots, which probably caused the spastic-type paralysis observed in the rabbits.

Of mice receiving either MS or L2 viruses, 20–30 per cent developed paralysis of the inoculated limb. All the paralysed animals receiving MS virus progressed to encephalomyelitis, whereas 38 per cent of those paralysed by L2 virus survived permanently paralysed.

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