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The Pathogenesis of Pseudorabies in Mice following Peripheral Inoculation

By H. J. FIELD AND T. J. HILL
Department of Bacteriology, The Medical School, University Walk, Bristol BS8 1TD

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SUMMARY

Three-week-old mice were inoculated with pseudorabies virus by means of the left hind foot pad. Infectious virus was isolated from tissues in the sequence: foot pad, sciatic nerve and dorsal root ganglion, lower, middle, upper spinal cord, and brain. Virus was recovered in one instance only from the liver, but could not be recovered from the spleen or heart blood. The involvement of the kidneys, adrenal glands, coeliac ganglion and skin in the spread of infection was also studied. The possible role of the autonomic nervous system in the pathogenesis is suggested. In this respect, immunosympathectomy prior to inoculation reduced the incidence of infection in the adrenal glands and kidneys. Interruption of the sciatic and femoral nerves led to reduced mortality and an altered pathogenesis. A new pattern of virus isolation from the tissues was observed in mice dying at later times following section or ligation of these nerves. The electron microscope observations indicated that neurons rather than glia are of major importance in facilitating virus spread within and from the nervous system.

INTRODUCTION

The mechanisms by which neurotropic herpes viruses are transmitted in the body of the host have long been of interest to virologists, as recently reviewed by Kristensson & Olsson (1973). Since the experiments of Goodpasture & Teague (1923) using rabbits, herpes simplex virus has been a favourite member of the herpes virus family for the study of virus translocation in laboratory animals. Both herpes simplex virus and pseudorabies virus are thought to travel from a peripheral inoculation site to the central nervous system by way of the peripheral nerves (Wright, 1953; Johnson, 1964; Wildy, 1967; Kristensson, Lycke & Sjöstrand, 1971*a*; Cook & Stevens, 1973) although the exact mechanisms are disputed. Recently McCracken, McFerran & Dow (1973) presented further evidence for the importance of axoplasmic transport in calves peripherally inoculated with pseudorabies virus.

There are a few accounts of the pathogenesis of pseudorabies in small laboratory animals (Hurst, 1933; Becker, 1968; Fraser & Ramachandran, 1969). The purpose of the present study was to examine in detail the spread of virus in mice following peripheral inoculation. From this information it was hoped to evaluate the system as an *in vivo* model for the study of herpes virus interactions with neural cells, with particular reference to neural spread.

METHODS

Virus. A strain of pseudorabies virus (N1A-2) was obtained by courtesy of Dr J. B. McFerran, Belfast. The virus was originally isolated from a pig brain, and was passed twice in pig kidney and twice in VERO cells, before we received it. A further two passes in VERO cells were carried out to produce a suitable stock of virus.

Inoculation. Three-week old, random bred, Bristol mice were inoculated on the skin of the left hind foot pad with 0·025 ml of virus suspension (containing approx. $2{\cdot}5 \times 10^5$ p.f.u.), using the multi-prick method described by Wildy (1967).

Tissue samples. Mice were killed using chloroform and tissue samples were dissected out as aseptically as possible. The range of tissues sampled are shown in Table 1. In each case the whole organ was removed, except for the liver, where approximately one third was sampled. The spinal cord was removed using the method of Wildy (1967) whereby the cord is extruded by means of a syringe loaded with saline. Once removed, the spinal cord and brain were placed intact on a piece of sterile wax and divided into the various samples required. Using the Wildy technique, the dorsal root ganglia were left *in situ* and could then be dissected out separately. As far as possible, the same dorsal root ganglion (left fourth or fifth lumbar), was taken from each mouse.

Samples were stored at −20 °C in 100 mm 'replidishes' (Sterilin), each sample being placed in 1 ml of 199 medium (Wellcome Reagents Ltd.). The medium contained antibiotics, 2 % foetal calf serum, and was buffered at pH 6·9 by means of $NaHCO_3$. Infectivity was determined as soon as possible after sampling (usually within two weeks).

Infectivity determination. Samples were thawed, ground in a Griffith's tube (Jobling and Co. Ltd.) treated in a MSE sonic disintegrator for 45 to 60 s and then 0·1 ml was inoculated into each of two tubes containing VERO monolayers. The tubes were examined after 1, 2 and 3 days of incubation at 34 °C. In preliminary experiments samples failing to give positive c.p.e. after 3 days' incubation never gave positive results. The c.p.e. was assessed as follows:

no plaques	−
plaque(s) in one out of two tubes	±
average of 1 to 10 plaques	+
average of 11 to 100 plaques	++
plaques just touching	+ ++
confluent c.p.e.	++ ++
not done	.

If no plaques were visible on the first day, but appeared subsequently, the maximum score was +.

In one experiment infectious virus was detected by direct culture of tissue (ganglia and sciatic nerve). The fragments of tissue were placed in culture vessels previously seeded with a monolayer of VERO cells. The culture techniques were similar to those described for dorsal root ganglia (Hill & Field, 1973). When evidence of c.p.e. appeared in the VERO cell sheet, the presence of virus was confirmed by titrating the supernatant fluid in further VERO tube cultures as above.

Section or ligation of the sciatic and/or femoral nerve. Four-week-old mice were sedated with intra-peritoneal sodium pentobarbitone and anaesthetized by intra-venous administration of the same drug. A single skin incision (approx. 20 mm long) was made on the outer aspect of the upper limb at the level of the hip joint and parallel to the vertebral column. The nerves were exposed at the level of the upper femur by blunt dissection. Either 5 to 10 mm portions of nerve were removed, or the nerves were ligatured with fine silk (grade 0–3); the ligatures were left *in situ*. Virus was inoculated into the footpad of the operated limb one or two days after surgery.

Immunosympathectomy. A litter of nine mice were given daily subcutaneous injections of antiserum to nerve growth factor (Wellcome Reagents Ltd.) for the first five days after birth (Levi-Montalcini & Booker, 1960). These mice were challenged with virus at three weeks old, together with a normal group of similar age.

Electron Microscopy. Mice were anaesthetized with chloroform, decapitated and the tissues dissected out as rapidly as possible. Tissues were fixed in 2·5 % glutaraldehyde in cacodylate buffer at 4 °C, post-fixed in 1 % osmium tetroxide, and embedded in araldite. Sections were stained with lead citrate and uranyl acetate and examined in a Phillips EM 201 electron microscope.

RESULTS

Symptoms observed

In the first experiment, 100 mice were inoculated. The mice were divided into two batches; 50 for observation only, and 50 from which mice were removed at intervals for infectivity titrations. On the second day about half the mice showed slight paralysis of the inoculated foot and slight pruritus in the region of the left foot and lower limb. Pruritus became more marked over a period of about 6 h, but paralysis was not a prominent feature. Pruritus became so intense that the skin of the left foot, leg and flank became raw due to the animal scratching and biting. Death occurred 12 to 24 h after the appearance of symptoms, preceded by vigorous twitching and tremors. By the end of the fourth day post-inoculation all mice were affected and by the fifth day there were no survivors.

Titration of tissue samples for infectious virus

At six-hourly intervals after inoculation, three mice were killed for sampling. The various tissues that were sampled are shown in Table 1, with their approximate weights. No virus was isolated at any time from the spleen, or heart, and on one occasion only from the liver of a moribund mouse at 84 h post-inoculation. This mouse also showed high levels of virus in all other tissues except the spleen and heart. The results of virus isolations from all the tissues sampled from 6 to 96 h post-inoculation are summarized in Table 1.

It may be seen from Table 1 that virus was isolated first from the foot pad at 18 h post-inoculation and consistently from that tissue at subsequent times. At 42 h post-inoculation virus was first isolated, simultaneously from the left dorsal root ganglion, left sciatic nerve (proximal and distal) and lower spinal cord. At 54 h post-inoculation, the left (but not right) adrenal gland and kidney became positive and at 60 h post-inoculation, virus was isolated from the medulla oblongata and cerebellum. The right adrenal gland and kidney yielded virus from 66 h onwards.

It was noted that the left dorsal root ganglion regularly yielded more virus than did any other tissue (particularly in view of the relatively small size of the ganglion sample). Conversely, relatively little virus was recovered from the cerebellum and cerebrum. The adrenal gland produced similar levels of infectious virus to the kidney, though the latter sample contained about ten times as much tissue by weight. One further sample, the coeliac ganglion, was removed from a few mice only. Virus was isolated from the coeliac ganglion

Table 1. *Isolation of infectious virus from the tissues of mice inoculated in the hind foot pad*

Time post-inoculation (h)	Weight†	6	12	18	24	30	36	42	48‡	54	60	66	72	78	84	90	96
Foot pad	3	–*	–	++	++	++	++	+++	++	+++	++	+++	++	++	++	.	++
Left dorsal root ganglion	< 1	–	–	–	–	–	–	+	++	++	+++	+++	+++	++++	+++	.	+++
Left sciatic nerve proximal	1	–	–	–	–	–	–	±	±	±	++	++	++	++	++	.	++
Left sciatic nerve distal	1	–	–	–	–	–	–	±	±	+	+	++	++	++	++	.	++
Lower spinal cord	20	–	–	–	–	–	–	±	±	+	+	++	++	+++	+++	.	+++
Mid spinal cord	20	–	–	–	–	–	–	–	–	±	+	+++	++	+++	++++	.	+++
Upper spinal cord	20	–	–	–	–	–	–	–	–	±	±	++	++	+	++	.	++
Medulla *oblongata*	10	–	–	–	–	–	–	–	–	–	+	+	++	++	++	.	++
Cerebellum	100	–	–	–	–	–	–	–	–	–	±	+	+	++	++	.	+
Cerebrum	300	–	–	–	–	–	–	–	–	–	–	+	+	+	+	.	+
Coeliac ganglion	< 1	–	–	–	–	–	–	–	–	–	–	+	±	+	±	.	–
Left adrenal gland	2	–	–	–	–	–	–	–	–	±	±	++	++	+	+	.	++
Left kidney	100	–	–	–	–	–	–	–	–	±	±	++	+	+	+	.	±
Right adrenal gland	2	–	–	–	–	–	–	–	–	–	–	±	±	±	–	.	+
Right kidney	100	–	–	–	–	–	–	–	–	–	–	+	±	±	–	.	±
Liver	100	–	–	–	–	–	–	–	–	–	–	±	–	–	–	.	–
Heart	70	–	–	–	–	–	–	–	–	–	–	–	–	–	–	.	–
Spleen	100	–	–	–	–	–	–	–	–	–	–	–	–	–	–	.	–

* Each result represents the average of three mice; see methods section for key.
† Approximate weight of sample in mg.
‡ Symptoms first observed at 48 h.

Table 2. *Isolation of infectious virus from portions of the left sciatic nerve from mice inoculated in the left hind foot pad with pseudorabies virus*

Time post-inoculation (h) ...	24	48	60	72
Distal	–*	±	±	++
Distal mid	–	–	+	++
Proximal mid	–	±	+	+++
Proximal	–	+	++	++

* Results represent average of four mice at each time.

at 60 h post-infection (at about the time the brain became positive). Practical difficulties were encountered in handling this small tissue, but virus was irregularly isolated at times greater than 60 h after infection.

A second experiment was carried out to determine more precisely how soon after inoculation, virus could be detected in the dorsal root ganglia of the ipsilateral and contralateral sides. For this purpose, following inoculation by the usual method, mice were sampled at 12, 24, 36, and 48 h post-inoculation and four sacrolumbar ganglia were dissected from the left and right side. The groups of four ganglia were pooled and immediately placed in culture chambers containing VERO cells. Virus was first detected in the left ganglia removed at 24 h post-inoculation. By 36 h after inoculation, virus was present in both left and right ganglia.

A third experiment was carried out to investigate in more detail the appearance of infectious virus in the sciatic nerve. Three-week-old mice were inoculated as above and four mice were killed at each of the following time intervals post-inoculation: 24, 48, 60 and 72 h. The left sciatic nerve was dissected from the mice and in each case the nerve was divided into four portions of roughly equal length. These samples were stored and titrated for infectious virus as before. The results are shown in Table 2. Although the total number of samples is small, it appears that the virus at 48, 60 and 72 h was found predominantly in the proximal samples and least virus was recovered from the mid region of the nerve at these times.

In addition, in some mice at 48 to 80 h post-inoculation, the right sciatic nerve was also sampled. This was not divided up, but processed whole. Virus was regularly isolated from the contralateral nerve from 60 h post-inoculation.

As in the case of dorsal root ganglia, an attempt was made to determine the earliest time virus could be detected in the ipsilateral sciatic nerve. Again the technique of direct culture was employed. Using this method virus was occasionally detected in the left sciatic nerve at 24 h post-inoculation.

An experiment was designed to study further the progression of infectious virus in the spinal cord. In this case the spinal cord was divided longitudinally into right and left halves and each half was further divided into three equal parts. Samples were taken at the same time intervals post-inoculation (48, 60 and 72 h) with three mice killed at each time. The results are shown in Table 3 and show predominance of virus in the samples from the left (inoculated) side. This correlated with the appearance of virus first in the left and then right adrenal gland and kidney obtained in the first experiment (Table 1). There was further evidence of sequential spread from lower to upper spinal cord with a faster rate of spread in the cranial direction relative to spread from left to right.

A chance observation of virus in a sample of skin taken from a point distant to the inoculation site in a moribund mouse led to a further experiment to investigate the extent of

Table 3. *Isolation of infectious virus from various parts of the spinal cord from mice inoculated in the left hind foot pad with pseudorabies virus*

	48		60		72	
Time post-inoculation (h) ...	Left	Right	Left	Right	Left	Right
Lower spinal cord	−*	−	++	±	++ ++	+ ++
Mid spinal cord	−	−	+	±	++ ++	++
Upper spinal cord	−	−	±	+	++ ++	+

* Results represent average of two mice at each time.

Table 4. *Isolation of infectious virus from the skin following inoculation of the left hind foot pad with pseudorabies virus*

Time post-inoculation (h) ...		6	12	18	24	30	36	42	48	54	60	66	72
Tissue* sample	(1)	−†	−	−	±	.	++	++	+++	++	+++	++	+
	(2)	−	−	−	−	.	−	±	±	±	±	±	++
	(3)	.	.	.	.	.	−	.	−	±	±	±	±
	(4)	.	.	.	.	.	.	.	−	−	−	−	++
	(5)	.	.	.	.	.	.	.	−	−	−	−	±
	(6)	.	.	.	.	.	.	.	−	+	±	±	+

* Sample (1) skin from left hind foot pad; (2) skin from outer aspect of limb midway between hock and knee; (3) skin from midway between knee and hip; (4) skin from similar site to (3) on inner aspect of limb; (5) skin from abdomen, midway between last rib and upper thigh; (6) small piece of lower spinal cord.

† Results represent the average of two mice at each time.

the spread of infection to the skin. In this experiment, 30 mice were inoculated by the usual method and exhibited normal pathogenesis. Mice were killed at six-hourly intervals post-inoculation, with two mice at each time interval. Samples of skin were removed from the following sites for infectivity estimation: (1) from the left hind foot pad, (2) from the outer aspect of the limb midway between hock and knee joints, (3) midway between knee and hip, (4) from an equivalent site to (3), but from the inner aspect of the thigh, and (5) from the abdomen midway between the last rib and upper thigh. Samples were also taken at later stages (48 to 80 h) from sites equivalent to (2) and (3) on the right side. Further to the skin samples, a small piece of lower spinal cord was also removed (smaller than in previous experiments). Except for the footpad sample, which was smaller, each piece of skin was roughly circular and about 0·5 cm in diam. The results of the infectivity titrations are shown in Table 4. Virus was first detected in the skin of the foot pad at 24 h post-inoculation, then in the skin of the lower leg at 36 h post-inoculation. Virus was isolated from the inner thigh from 54 h onwards. The skin in the remaining areas did not yield virus until 72 h post-inoculation and of the two mice sampled at this time one was just dead and the other moribund. On the right side both skin sites yielded virus consistently from 72 h onwards.

Nerve section or ligation

A number of experiments were performed involving either section or ligation of the left sciatic nerve and/or femoral nerve, followed by challenge with pseudorabies virus. Four-week-old mice were used since anaesthesia could be more easily controlled than in the three-week-old mice used in the previous experiment. However, from preliminary results obtained

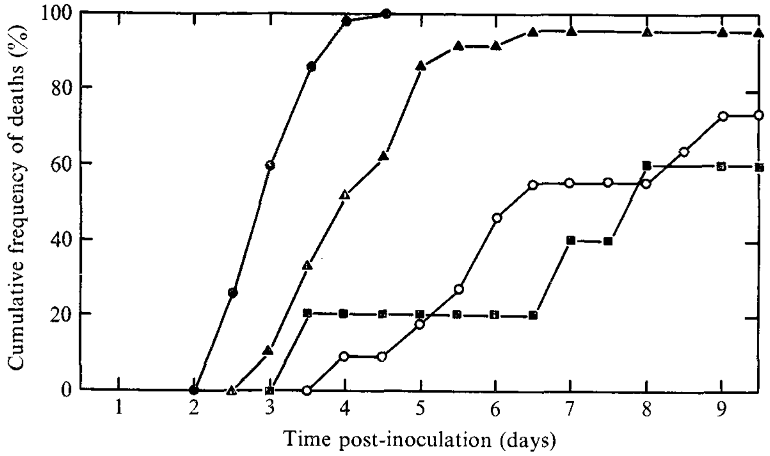

Fig. 1. The percentage cumulative frequency of deaths in mice infected with pseudorabies virus following nerve section or ligation. ●—●, infected, unoperated controls (43 mice; 4 experiments); ▲—▲, left sciatic nerve sectioned (21 mice; 3 experiments); ■—■, left sciatic and femoral nerve ligatures (5 mice); ○—○, left sciatic and femoral nerve sectioned (11 mice).

with four-week mice, it appeared that they were equally susceptible to peripheral inoculation with pseudorabies virus, showing 100 % mortality and a similar pathogenesis to that previously described.

A group of four mice were sham-operated; that is, subjected to all the procedures of anaesthesia and surgery with the exception of ligation or removing a segment of nerve. Another small group were neurectomized in the usual way, but inoculated in the contralateral foot. Following challenge with pseudorabies virus all these mice showed an identical response to the control, unoperated, group.

Mice with neurectomy or nerve ligation were challenged two or three days after surgery by inoculation with pseudorabies virus in the footpad on the same side; the symptoms and deaths were recorded. Fig. 1 shows the percentage cumulative frequency of deaths obtained. The curves for the controls and sciatic nerve section, represent the combined results of four and three experiments, respectively. It may be seen from Fig. 1 that section of the sciatic nerve alone did not protect the mice from challenge with virus, but these mice showed a consistent delay of about 12 h in the onset of death. Section of the femoral nerve alone (not shown) appeared to have no effect on the course of the disease.

Section or ligation of both nerves clearly reduced the mortality rate to about 70 %. Mice which died following double neurectomy did so between four and nine days post-infection, compared to two or three days in the control groups. Furthermore, whilst some of these mice showed the usual pruritus others showed no evidence of pruritus before death.

Tissues were sampled from six of the inoculated mice which died after double nerve section. The resulting virus isolations are shown in Table 5. Mice *A* and *B* died on the fourth and fifth day, respectively, post-inoculation; neither showed pruritus. As shown in the table, virus was isolated from a wide range of tissues. In both cases high levels of virus were recovered from the spleen, and in the case of mouse *B*, also the heart and liver. Mice *C*, *D*, and *E* (see Table 5) died at days 5, 5½, and 8½ post-inoculation. All these mice showed the usual symptoms, except that the onset of disease was delayed beyond the normal incubation period of two to three days. Virus was again isolated from the spleen in mice *C* and *E* and also from the heart of *C*. Mouse *F*, remained apparently normal and was killed at 9½ days

Table 5. *Isolation of infectious virus from pseudorabies-infected mice, following section or ligation of the sciatic and femoral nerves*

Mouse ...	*A*	*B*	*C*	*D*	*E*	*F*
Days post-infection ...	4	5	5	5½	8½	9½
Sample						
Footpad	.	+++	+++	+++	−	−
Sciatic nerve	.	+	−	−	−	.
Left dorsal root ganglion	.	.	+	+++	++	−
Mid spinal cord	+	++	+	+	++	−
Cerebrum	+	+	+	+	+	−
Cerebellum	.	.	++	+	−	−
Left Adrenal gland	+++	−	++	++	++	.
Left kidney	±	+	+	++	−	−
Lung	+	−	+	.	.	−
Heart	−	+	+	−	−	−
Spleen	+++	+++	+	−	+	−
Liver	−	±	−	−	−	−

Mouse (*A*) Rapid death, no pruritus. (*B*) Rapid death, no pruritus. (*C*) Normal course of symptoms. (*D*) Normal course of symptoms. (*E*) Normal course of symptoms. (*F*) No symptoms.

post-inoculation. No virus was isolated from any of the tissues tested, including the footpad which had previously been inoculated.

Immunosympathectomy

The group of immunosympathectomized mice was challenged when three weeks old. These mice appeared normal in other respects and, following peripheral inoculation with pseudorabies virus, the normal pathogenesis was observed. However, there was some evidence of a slight delay (approx. 6 h) in the onset of symptoms. There was a more clear cut reduction in the recovery of virus from the left adrenal glands of the immunosympathectomized mice at a late stage of the disease (66 to 78 h after infection), as shown in Table 6. There also appeared to be a reduction in the frequency and level of virus recovery from the left and right kidneys.

Electron microscope observations

Morphological evidence of virus infection was readily found in the sciatic nerve and lumbar dorsal root ganglia from mice showing severe pruritus. In these tissues marked differences were noted between the response of neurons and glia to virus infection. Whilst the neurons of the dorsal root ganglia showed signs of normal virus replication, the glia of the dorsal root ganglia and sciatic nerve, either appeared uninfected, or showed signs of abortive infection. In particular, whilst some glia showed intra-nuclear capsids (a few with dense cores) and occasionally naked intractyoplasmic capsids, there was no evidence of capsid envelopment (Fig. 2). The overall picture in the dorsal root ganglion was one of small foci of infected neurons surrounded by large numbers of normal neurons and satellite cells. In most cases the satellite cells associated with infected neurons showed the signs of abortive infection already described. Infected satellite cells were never seen in association with uninfected neurons.

In the sciatic nerve (samples came mainly from the mid region) enveloped and naked virus particles were observed intra-axonally in unmyelinated axons. The associated Schwann cells either appeared uninfected or showed the signs of infection already described. Similar accumulations of intra-axonal particles were seen in the dorsal root ganglia but also within

Table 6. *Presence of virus in adrenal glands and kidneys following immunosympathectomy*

	Mouse number																		
	Controls									Immunosympathectomized									
Tissue*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Left adrenal	+++	+	+	+	+	++	+	+++	++	++	−	−	−	+	−	−	−	−	±
Right adrenal	−	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−
Left kidney	+	+	+	+	+	++	++	+	+	+	+	±	±	+	±	−	−	+	+
Right kidney	−	−	+	±	+	±	−	±	±	+	−	−	−	+	−	−	−	−	−

* Tissues were removed either just after or a few hours before death, between 66 and 78 h post-inoculation.

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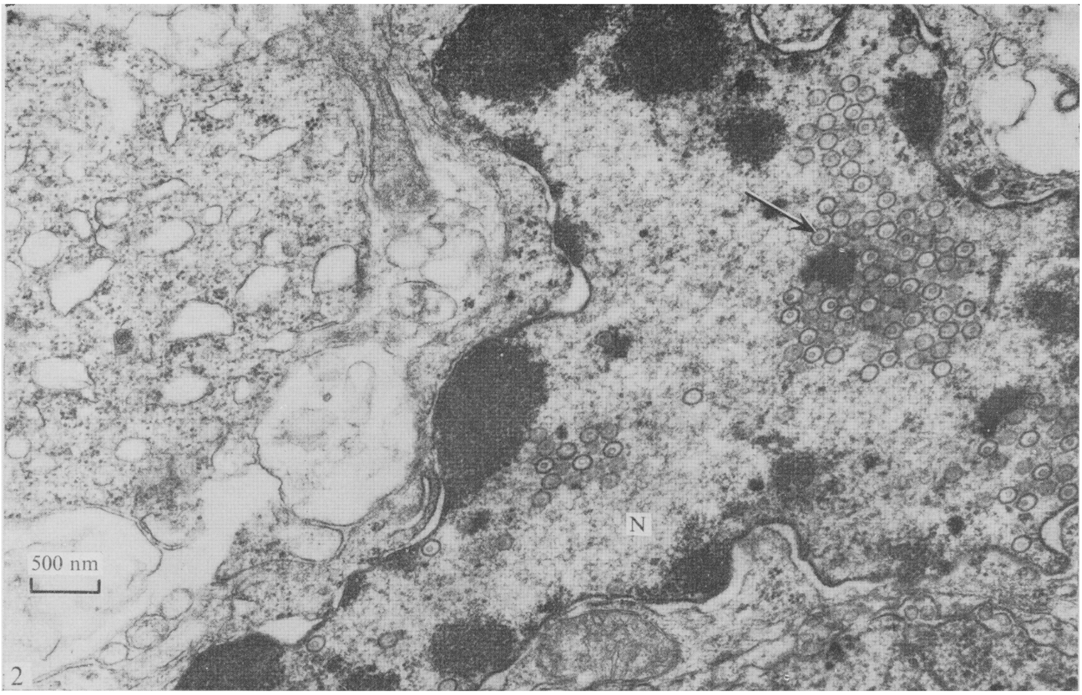

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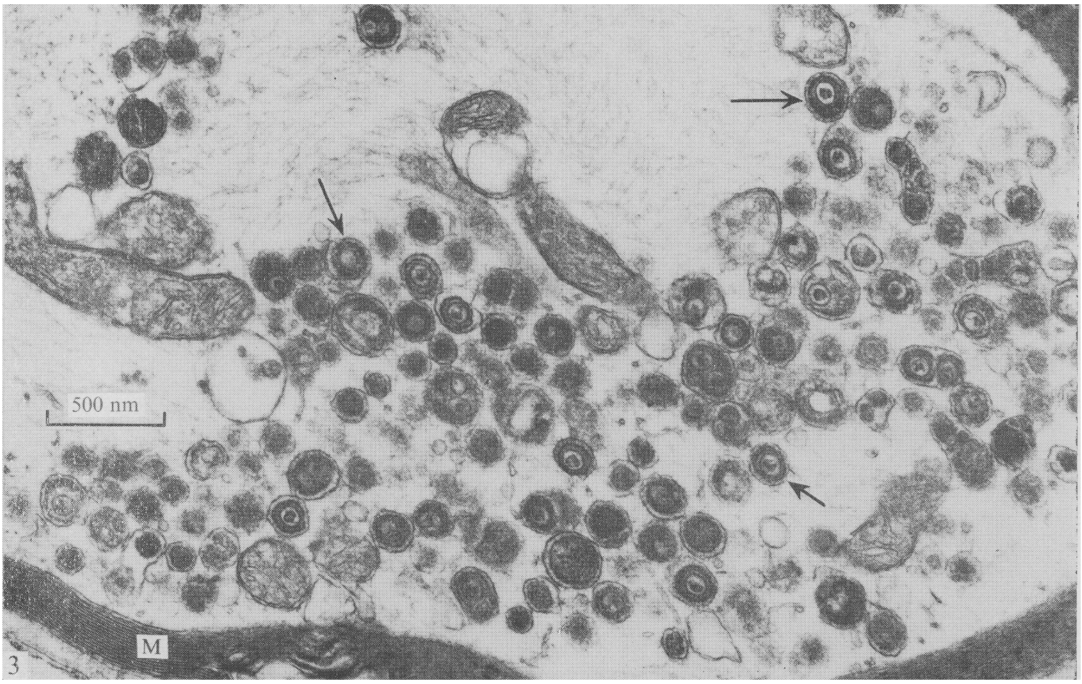

Fig. 2. Satellite cell from dorsal root ganglion. Aggregates of naked virus particles (arrow) in the nucleus (N).

Fig. 3. Myelinated axon from dorsal root ganglion. Many enveloped virus particles (arrows) within vesicles in the axoplasm. Myelin (M).

myelinated axons (Fig. 3). Evidence of infection was never observed in myelinating Schwann cells of the ganglia or sciatic nerve.

Small foci of infected cells were also found in the medulla of the left adrenal gland from moribund mice examined at 72 and 80 h post-inoculation.

Whilst in some mice at later stages of the disease, infectious virus was detected in the kidneys and coeliac ganglia, intensive searching of thin sections of these tissues failed to reveal morphological evidence of virus infection.

DISCUSSION

The results of the present study confirm and extend the observations of previous workers on the pathogenesis of pseudorabies in peripherally inoculated pigs (McFerran & Dow, 1965); rats and mice (Fraser & Ramachandran, 1969); rabbits (Becker, 1968) and calves (McCracken *et al.* 1973). With respect to the spread of virus from the inoculation site, it seems that as in the calf, rat, mouse and pig (references above), viraemia rarely occurs following peripheral inoculation. However, our results suggest that viraemia is more likely to occur in the mouse when the disease is prolonged beyond the normal three to four days by nerve section or ligation (see Table 5).

The results suggest that the initial spread is limited to the peripheral and central nervous system. However, the mode of transport within the nervous system is still uncertain. As argued by McCracken *et al.* (1973) for pseudorabies infection in the calf, it seems unlikely that such rapid spread could be mediated by progressive cell to cell infection along the nerve.

If the spread of virus centripetally along the sciatic nerve is by axoplasmic transport, then the present results indicate a rate of movement of about 42 mm/day assuming a length of 4·2 cm for the sciatic nerve and ignoring the possible requirement for preliminary virus replication at the inoculation site. This figure is similar to that indicated by the results of Wildy (1967); Kristensson *et al.* (1971 *a*) and Cook & Stevens (1973) for the neural spread of herpes simplex virus in young mice. However, these rates are much lower than that suggested by the results of McCracken *et al.* (1973) for the neural spread of pseudorabies in calves (approx. 240 mm/day) and the estimated rate of Kristensson, Olsson & Sjöstrand (1971 *b*) for the retrograde axonal transport of proteins in the rabbit hypoglossal nerve (approx. 120 mm/day). The present lack of information on retrograde axonal transport does not allow any critical appraisal of the validity of these differences in rates. Furthermore, such inferences on the rate of virus transport are greatly influenced by the extent of virus replication at the site of inoculation before entry into the nerve.

The electron microscope observations on the involvement of different cell types (neurons and glia) in pseudorabies infection in the mouse, are similar to those obtained by other workers with pseudorabies in the calf (McCracken *et al.* 1973); rabbit (Becker, 1968); and with herpes simplex virus in the mouse (Dillard, Cheetham & Moses, 1972; Cook & Stevens, 1973) and *in vitro* ganglion cultures (Hill & Field, 1973). The defective replication in glial cells suggests that these cells may play a relatively minor role in facilitating spread of virus within the nervous system. It is of interest to note that a similar lack of involvement of glial cells has been reported for the neural spread of rabies and rabies-like viruses (Murphy *et al.* 1973). The majority of the rapid neural spread demonstrated by the present study may occur via neurons and their widespread axonal and dendritic connexions. However, the possible role of a fluid medium such as the peri-neural or endoneural fluids cannot be entirely eliminated (Kristensson & Olsson, 1973).

The limitation of morphological evidence of infection to the nonmyelinating Schwann cells in the sciatic nerve has also been observed for herpes simplex virus in the mouse by Dillard *et al.* (1972) and Cook & Stevens (1973). It is difficult at present to assess whether this represents a true difference in the response of the two types of Schwann cell to virus infection or whether the myelin provides a barrier which prevents infection of myelinating cells.

The results of the ligation and nerve section experiments were consistent with the hypothesis of neural spread as the most important means of transmission of pseudorabies in the peripherally infected mouse. It was found that the femoral nerve as well as the sciatic was significant in virus spread, and this may be relevant when considering the use of the mouse-footpad model with other agents such as herpes simplex virus (Kristensson *et al.* 1971 *a*) or tetanus toxin (Dean & Evans, 1963).

Mice which died later than two or three days post-inoculation, despite section or ligation of both nerves, appeared to fall into two categories: (i) those in which pruritus was absent and virus present in many tissues, including heart, liver, and spleen; (ii) those in which the pattern of symptoms and virus isolation was more typical. It seems likely that the former category results from viraemic spread of infection to the central nervous system. However, we suggest that in the mice showing pruritus, there may have been lateral spread of infection in the dermal or epidermal tissues, into a region with intact nerve supply. Mice which survived, presumably did so because their immunological mechanisms controlled the local infection before either viraemic or neural spread occurred.

The presence of virus in the adrenal glands, kidneys and autonomic ganglia following peripheral inoculation with pseudorabies virus has been reported in rats and mice (Fraser & Ramachandran, 1969); calves (McCracken *et al.* 1973) and pigs (McFerran & Dow, 1965). Similar observations in mice infected with herpes simplex virus were reported by Dillard *et al.* (1972). This was also a notable feature of the present study in mice at later stages of infection (after 50 h post-inoculation). A further feature of the present study was the detection of infectious virus in the skin at sites distant from the original point of inoculation at late stages of the disease.

We propose that the likely mode of spread to the kidneys' adrenal glands and probably skin of the upper limb and abdomen, is by centrifugal movement of virus along axons of infected neurons in the spinal cord, dorsal root ganglion and autonomic ganglia. The detection of infectious virus at late stages in the sciatic nerve on the contralateral side also supports this proposal. Large masses of intra-axonal virus particles were regularly seen (Fig. 3) further suggesting that virus may spread readily along axons to infect extra-neural tissues. Such accumulations of intra-axonal virus particles have been reported previously for herpes simplex virus in mice (Hill, Field & Roome, 1972; Cook & Stevens, 1973) and for pseudorabies in the calf (McCracken *et al.* 1973).

Further evidence for the involvement of the sympathetic nervous system in spread of virus to the adrenal glands and kidneys, came from the immunosympathectomy experiment. However, in interpreting these results, it should be remembered that immunosympathectomy does not completely eliminate the sympathetic nervous system (Klingman & Klingman, 1972). Even so, the results do suggest that depletion of the sympathetic system is accompanied by a significant reduction in the spread of virus to the kidneys and adrenal glands. Furthermore, in the case of the adrenal gland, the evidence from electron microscopy, of the limitation of infection to the medullary cells, is highly suggestive of neural spread since these cells are directly innervated by preganglionic fibres from the spinal cord. The lack of evidence of virus in coeliac ganglia and kidney by electron microscopy probably reflects the extremely focal infection in these organs. Certainly the results suggest that the role of the sympathetic nervous system in the pathogenesis of neurotropic virus infections requires further consideration.

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