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NOTES

Murine Neurotropic Retrovirus Spongiform Polioencephalomyelopathy: Acceleration of Disease by Virus Inoculum Concentration

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A 10-fold reduction in the incubation period of murine neurotropic retrovirus spongiform polioencephalomyelopathy was effected by a 1,000-fold concentration of the cloned virus inoculum.

The polioencephalomyelopathy associated with neurotropic retrovirus infection of mice has been classified as a "slow virus disease" (14). This lower motor neuron disease is of unique interest clinically as an animal model for amyotrophic lateral sclerosis (6) and histopathologically as an example of non-inflammatory spongiform degeneration caused by a conventional virus. These pathological changes are generally associated with the spongiform encephalopathy agents of scrapie, kuru, and Creutzfeldt-Jakob disease (4).

Naturally occurring ecotropic retrovirus polioencephalomyelopathy begins in mice over 1 year of age (5). Experimental intracerebral inoculation of virus leads to clinical paralysis of Swiss mice after an incubation period of 2 to 6 months (5, 14). With concentration of virus by several methods, we have found that the incubation period of paralytic disease and the development of spongiform changes can be reduced to 15 days. Thus, in slow viral diseases, pathological changes and clinical signs may be related to the quantity of infectious virus replicating in the target organ at any particular time.

Murine neurotropic retrovirus isolated from the brain of a paralyzed mouse trapped in the Lake Casitas area of California (5) was grown in persistently infected continuously subcultured SC_1 mouse embryo fibroblasts (8). This N-ecotropic neurotropic retrovirus (Cas Br)-infected SC_1 cell line, free of contaminating amphotropic retroviruses, was cloned by limiting dilution and provided to us by J. Hartley and W. P. Rowe (9). Virus infectivity was measured by the mixed cell cytopathogenicity assay (XC assay) in monolayers of SC_1 cells pretreated with 5 μg of Polybrene (Aldrich Chemical Co., Milwaukee, Wis.) per ml (15). The cell line was monitored at regular intervals in our laboratory and found free of amphotropic retrovirus by the transformed focus formation assay in a sarcoma virus-positive, leukemia virus-negative mink cell line ($MiCl_1$) and by the fluorescent focus formation assay in a sarcoma virus-negative, leukemia virus-negative mink cell line (10).

Five- to ten-liter amounts of supernatant fluids harvested over 24 to 36 h from 50 to 75% confluent virus-infected SC_1 cell cultures were clarified at 750 $\times$ g for 30 min before concentration by either ammonium sulfate precipitation (7), hollow fiber ultrafiltration with an HIP-100 filter (Amicon Corp., Lexington, Mass.) with a molecular weight cutoff of 100,000 (1), or membrane continuous flow molecular filtration with a 0.2-μm cellulose acetate filter (Millipore Corp., Bedford, Mass.) having a molecular weight cutoff of 1,000,000 (12).

Concentrates were placed on 17 to 60% (wt/vol) sucrose step gradients in 0.1% (wt/vol) bovine serum albumin-TNE (3). Gradients were centrifuged in a Sorvall SS-90 vertical rotor at 20,000 rpm for 90 min at 4°C. Virus fractions were pooled and dialyzed against 20 volumes of TNE at 4°C for 90 min before recentrifugation through a second gradient. All three methods of virus concentration yielded at least 1,000-fold concentrations of virus inocula.

Newborn Swiss and BALB/c mice (Microbiological Associates, Bethesda, Md.) free of endogenous ecotropic retrovirus were inoculated between 12 and 24 h postnatally in the right frontoparietal region of the cerebral hemisphere with 0.02 ml of freshly prepared virus suspen-

sion. Three groups of Swiss mice were inoculated with virus suspensions containing 10^7, 10^6, and 10^2 XC plaque-forming units (PFU) per ml, respectively. One group of BALB/c mice was inoculated with the 10^7-XC PFU/ml virus suspension.

Concentrated virus inocula, regardless of method, dramatically shortened the incubation period of clinical symptoms and pathological disease after intracerebral inoculation. Representative results are presented for virus inocula prepared by hollow fiber filtration (1).

The proportion of Swiss mice eventually developing paralytic disease in all three groups was constant at 85 to 86%, and no BALB/c mice developed the clinical or pathological disease. However, the latency of disease expression in this proportion of Swiss mice was reduced from 20 to 2 weeks with a 1,000-fold concentration of virus in the inoculum (Fig. 1).

Brains and spinal cords from animals in each group were examined individually for virus content after the animals developed hind limb paralysis. The tissues were homogenized into 10% (wt/vol) suspensions in cold Hanks balanced salt solution containing 5% heat-inactivated fetal calf serum. Homogenates were clarified at 500 × *g* for 5 min, and 10-fold dilutions were inoculated directly on SC_1 cultures for XC assay (15). The amounts of virus in the target organs—brain, brainstem, and spinal cord—were similar at the time of paralysis in the three groups of three to four individually titrated animals (Table 1). The brains and spinal cords of BALB/c mice titrated at 30 days postinfection contained 10^4 less virus than did paralyzed Swiss mice.

A further group of Swiss mice was inoculated with the 10^6-XC PFU/ml virus suspension, and two animals were perfused with 4% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.6, 735 osmolar) every 3 days for sequential pathological examination. In this group of mice, the earliest lesions were seen at 15 days postinoculation. These consisted of swollen astroglia, degenerating axons, and vacuolation in a perivascular distribution within the gray matter neuropil of the brainstem and the spinal cord (Fig. 2a and 3a). Neuronal loss, hypertrophied astroglia, and vacuolation were diffuse in the spinal cord gray matter by 30 days postinoculation, giving typical status spongiosus (Fig. 2b and 3b).

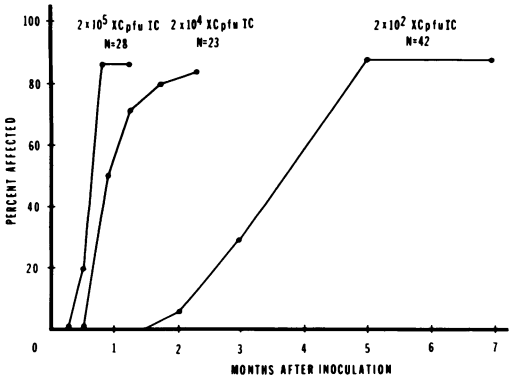

FIG. 1. *Incidence of paralytic disease after intracerebal (IC) inoculation of newborn Swiss mice with three different dosages of concentrated murine neurotropic retrovirus (Lake Casitas strain).*

TABLE 1. *Virus in brain and spinal cord*

Newborn mouse	Virus inoculum (log XC PFU/mouse)	Time of paralysis (weeks postinoculation)	Virus (log XC PFU/g of tissue ± standard deviation) in:	
			Brain/brainstem	Spinal cord
Swiss				
	5.3	3	6.2 ± 1.2	5.2 ± 0.4
	4.3	6	6.5 ± 0.3	6.6 ± 0.4
	2.3	20	5.8 ± 0.4	5.7 ± 0.7
BALB/c	5.3		1.8 ± 0.2	1.5 ± 0.3

In this study the incubation period for disease expression in the majority of mice was reduced from 150 to 15 days by a 1,000-fold concentration of virus. This phenomenon occurred also after inoculation of concentrated virus alone before rate zonal sucrose gradient sedimentation. Thus, concentration of virus, rather than removal of an inhibitor by purification, is the crucial factor. In addition, the percentage of animals eventually becoming ill was the same regardless of the amount of infectious virus in the inoculum. Furthermore, the amounts of infectious virus present in the central nervous system at the time of paralysis were similar despite the time at which paralysis occurred in affected animals.

The incubation period of viral infection in mice does not usually show such a dramatic dependency on virus inoculum concentration. For example, in measles virus encephalitis of mice a 100-fold concentration of virus reduced the incubation period from 6 to 4 days (16). The short-incubation-period model of scrapie in hamsters required a 10^7-fold concentration of infectious scrapie brain to reduce the incubation period from 150 to 60 days (11).

Other data have suggested that the incubation period of retrovirus disease may show relatively greater dependence on inoculum dosage. Induction of disease in (BALB/c × NZB) F_1 mice by Scripps leukemia virus shows a dependence on the inoculum dose similar to, though less dramatic than, that reported in this study (2). In the present study the contents of virus in the brain, brainstem, and spinal cord of paralyzed

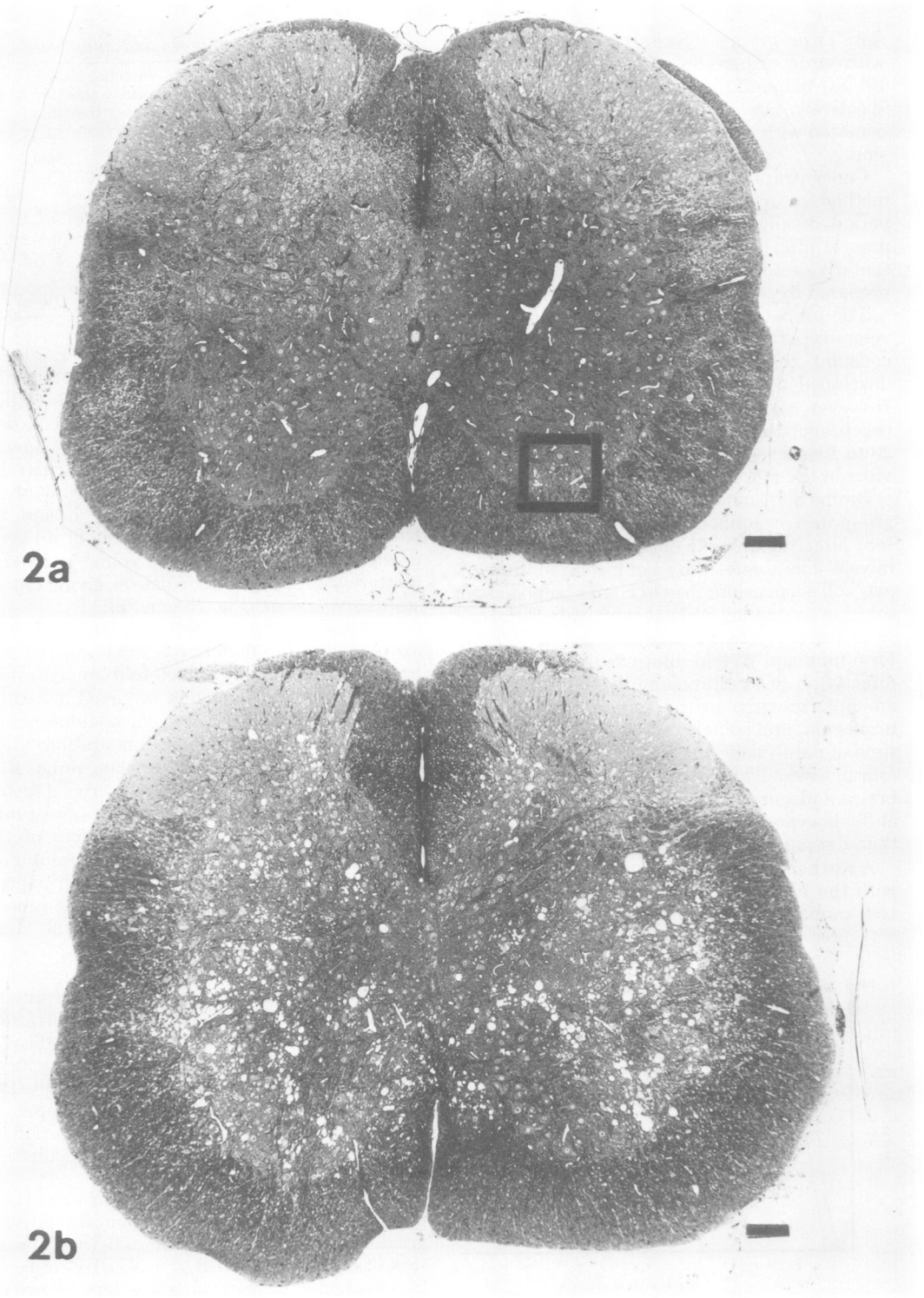

FIG. 2. *Toluidine blue-stained 1-μm plastic sections of lumbar spinal cords. (a) Fifteen-day-old mouse: isolated swollen cells in the anterior horn (box, see Fig. 3a). (b) Thirty-day-old mouse: the entire gray matter shows degeneration of both neurons and glia with marked vacuolation in the gray matter neuropil. Bar, 100 μm.*

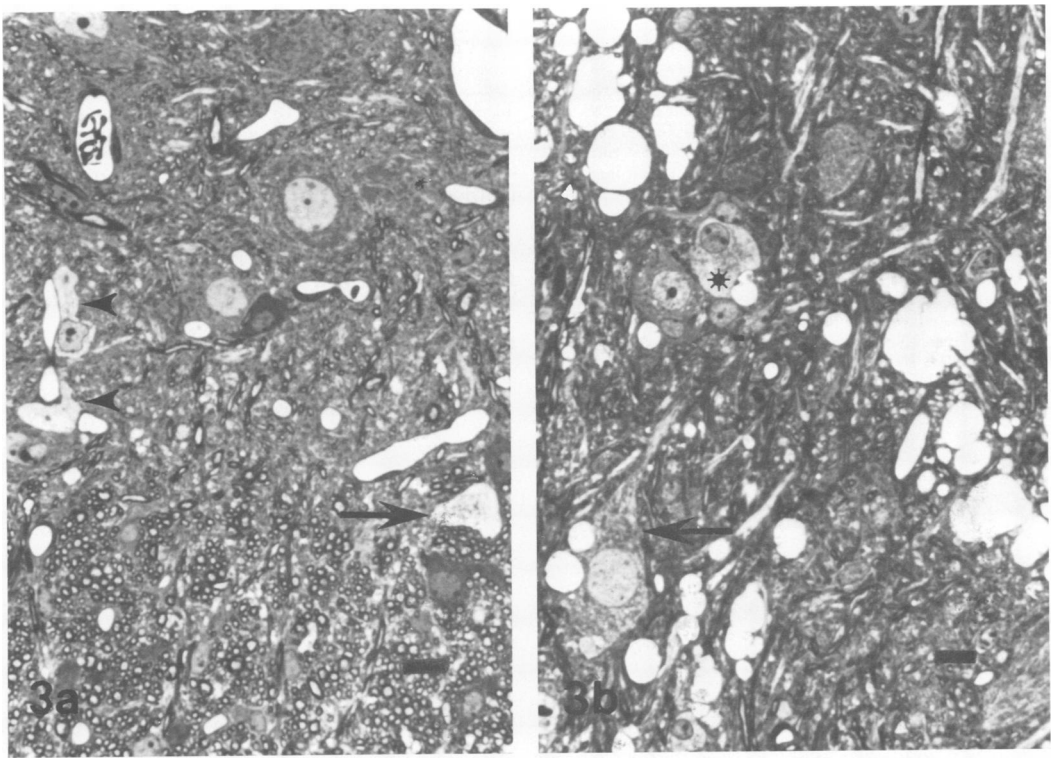

FIG. 3. *(a) Detail of Fig. 2a: two degenerating cells in juxtaposition to blood vessels (arrowheads); a third, unidentified cell in the white matter is also degenerating (arrow). (b) Detail of Fig. 2b: extensive vacuolization throughout the neuropil; vacuoles are present in a degenerating neuron (arrow), distinct from a reactive astrocyte (*). Bar, 10 μm.*

mice are nearly identical regardless of the time of occurrence of paralysis. Thus, the incubation period of the disease may be directly related to the time required to reach a critical level of virus replication in the target organs.

In visna of sheep, another slow retrovirus disease, recent studies have shown that pathological lesions could be accelerated by concentration of the inoculum virus (13; O. Narayan, unpublished data). Furthermore, lesions could be induced in less susceptible breeds by such inocula. However, in the present study increased inoculum dosage did not breach the Fv-1 genetic restriction of BALB/c mice to this N-ecotropic virus (5).

Acceleration of experimental disease by concentrated virus inocula will facilitate pathogenesis studies of this retrovirus-induced spongiform polioencephalomyelopathy. Such studies may also shed light on the pathogenesis of the spongiform encephalopathies associated with the unconventional agents of scrapie, kuru, and Creutzfeldt-Jakob disease (4).

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