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In Vivo and *In Vitro* Models of Demyelinating Diseases

XV. Differentiation Influences the Regulation of Coronavirus Infection in Primary Explants of Mouse CNS¹

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Mouse oligodendrocytes and astrocytes, in primary cerebral explant cultures, were infected with JHMV and MHV₃ coronaviruses. Contrary to previous findings with neural cells from the rat (S. Beushausen and S. Dales, 1985, *Virology* 141, 89-101), these agents show no discrimination in the tropism and have the ability to replicate in either type of murine glial cell. Effects of the differentiation inducer dbcAMP on levels of the myelin-specific enzyme 2':3'-cyclic nucleotide-3'-phosphohydrolase (CNPase) activity and virus replication were determined. In the mouse system there was a gradual, continuous elevation of CNPase beyond 30 days whereas in comparable rat cell cultures maximum CNPase enhancement is elicited within 21 days (F. A. McMorris, 1983, *J. Neurochem.* 41, 506-515). After dbcAMP treatment replication of both coronaviruses was profoundly suppressed in murine oligodendrocytes, consistent with our findings on JHMV replication in treated rat oligodendrocytes. By contrast the replication of JHMV and MHV₃ in dbcAMP-treated murine astrocytes was influenced only marginally. These findings provide further support for the hypothesis that susceptibility of rodents to CNS infection by coronaviruses is determined, in part, by the age-related maturation process of oligodendrocytes. © 1986 Academic Press, Inc.

INTRODUCTION

In studying the factors involved in the development of demyelinating diseases it has been demonstrated that several strains of murine coronaviruses including MHV-A59 (Lavi *et al.*, 1984), MHV₃ (Hirano *et al.*, 1981; Sorensen *et al.*, 1982), and JHMV (Cheever *et al.*, 1949; Pappenheimer, 1958; Weiner, 1973; Stohlman and Weiner, 1981; Hirano *et al.*, 1981; Sorensen *et al.*, 1982), can cause neurological disease in rodents.

Previous findings using mouse central nervous system (CNS) cultures demonstrated that both neurons and astrocytes can be infected with JHMV (Knobler *et al.*,

1981b; Dubois-Dalcq *et al.*, 1982; Collins *et al.*, 1983). Concerning oligodendrocytes of rodents, which have been implicated as the *in vivo* targets, those from the rat are indeed infectable *in vitro* by JHMV (Beushausen and Dales, 1985). Comparable *in vitro* information about murine oligodendrocytes is, however, lacking (Knobler *et al.*, 1981a, 1981b; Dubois-Dalcq *et al.*, 1982; Collins *et al.*, 1983).

Evidence at hand suggests that there are fundamental species differences in regulation of the infectious process, whereby mouse neural cells can be infected by several coronaviruses indiscriminately (Hirano *et al.*, 1981; Sorensen *et al.*, 1982; Lavi *et al.*, 1984), but oligodendrocytes and astrocytes explanted from rat brain discriminate unambiguously between JHMV and MHV₃ (Beushausen and Dales, 1985). Thus, the *in vitro* establishment of dispersed primary murine brain cultures, including oli-

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godendrocytes, would enable a direct comparison of the interaction between different coronaviruses with the cells from the CNS of the two species. Furthermore, since in such *in vitro* primary cultures a "time clock" of development follows the same sequence as that which occurs *in vivo* (McCarthy and De Vellis, 1980; Abney *et al.*, 1981; Barbarese and Pfeiffer, 1981; Bhat *et al.*, 1981; Bologna-Sondru *et al.*, 1981) such cultures may be used to better define the virus-glia cell interactions involved in the development of disease in the CNS.

MATERIALS AND METHODS

Cells and viruses. L-2 mouse fibroblasts (Rothfels *et al.*, 1959) were routinely propagated in Eagle's MEM (GIBCO, Grand Island, N.Y.) supplemented with 10% fetal bovine serum (FBS) and maintained in a 37° humid environment containing 5% CO₂.

The MHV₃ and JHMV strains of the mouse hepatitis virus (MHV) were propagated and plaque assayed on L-2 mouse fibroblasts as follows: subconfluent cultures of L-2 cells were absorbed for 1 hr at room temperature with either virus at a m.o.i. of 1 PFU per cell. Cultures were then shifted to 37° until 50% of cells had lifted off. The remainder of the monolayer was then scraped using a rubber policeman and passed through a syringe fitted with a 21-gauge needle. The resulting lysate was then sedimented at 2000 rpm for 10 min in an IEC centrifuge to remove cellular debris and the supernatant was then centrifuged at 22,000 rpm for 60 min to sediment the virus. The pelleted virus was resuspended in a small volume of MEM plus 10% FBS and stored at -70°. Infectious virus was quantitated by plaque assays, as previously described by Lucas *et al.* (1977).

The Indiana strain of vesicular stomatitis virus (VSV) was also propagated as described in Beushausen and Dales (1985).

Primary murine brain cultures. Primary brain cultures were prepared with slight modifications as described by Beushausen and Dales (1985). Briefly, twenty 1- to 2-day-old mouse pups were decapitated, the meninges were dissected away, and the cerebral hemispheres were removed and

placed in ice-cold BME₁₀ consisting of basal minimum essential medium (BME, GIBCO), supplemented with 10% heat inactivated FBS, sodium bicarbonate (1 g/liter), 0.6% dextrose, and garamycin (10 µg/ml, Schering Pharmaceuticals, Kenilworth, N.J.). The isolated hemispheres were washed three times with 10 ml of cold BME₁₀ to displace any leftover meninges and contaminating red blood cells (RBC), then were triturated through a 10-ml pipet thereby producing a homogenous suspension of free cells. The larger aggregates and debris were removed by filtration through a Nitex 130-µm mesh and the filtrate containing monodispersed cells was centrifuged 5 min at 750 rpm to pellet the cells. The pellets of cells derived from 40 hemispheres were resuspended in 10 vol of BME₁₀, plated into 175-cm² culture flasks (Nunc, 156502), and maintained at 37° in a humidified atmosphere containing 5% CO₂.

To obtain cultures enriched in either oligodendrocytes or astrocytes, minor modifications to those of Beushausen and Dales (1985), were made, based on the techniques of McCarthy and de Vellis (1980), as adapted by McMorris (1983). These primary cultures were propagated for 2 weeks, with medium changes every 2-3 days, and used to obtain oligodendrocyte-enriched cultures as follows. The BME₁₀ medium was replaced with 30 ml of warm phosphate-buffered saline (PBS), pH 7.4. The flasks were then shaken vigorously by hand to release the less adherent oligodendrocytes, the amount of cell release being monitored under phase-contrast optics. The free cells were centrifuged at 750 rpm for 5 min and cell pellets were resuspended in BME₁₀, the volume being adjusted to give the desired cell concentration, usually about 3 × 10⁵ cells/ml. After dispensing into 24-well plates the cells were allowed to become attached for 24 hr before use.

Cultures enriched in astrocytes were produced using tightly adherent cells remaining in flasks from which oligodendrocytes had been removed. The adherent cells were released with 0.25% trypsin in PBS, and the cell suspension in BME₁₀ was then centrifuged at 750 rpm for 5 min. Cell pellets were resuspended in a volume of BME₁₀

adjusted to give a suspension of 3×10^5 cells/ml and the astrocytes were dispensed into 24-well plates. Cells used for immunofluorescence were grown on 12-mm glass coverslips (Chance, Propper Ltd., Smethwick, Warley, England, No. 1). Mouse strains CD.1 and DBA/2 were purchased from Jackson Laboratory, Bar Harbor, Maine.

Infection conditions for primary cultures. Cultures in 24-well plates were absorbed with 0.2 ml virus suspension at a m.o.i. of 1 at 37° for 1 hr. Following adsorption virus was removed and cultures washed three times with BME₁₀ and then incubated with 1 ml per well of medium at 37°.

Preparation of antisera and immune labeling of cells. The techniques used for the production of antisera and the immune labeling of cells have been previously reported (Beushausen and Dales, 1985). Briefly, antibodies specific against (>) galactosyl cerebroside (>GC) were prepared from rabbit antiserum by passing the serum sequentially through columns of protein A-Sepharose, to select out the IgG, then through BSA-Sepharose to remove contaminating BSA antibodies. The avidity of >GC IgG was tested by radioimmunoassay (RIA) of Holmgren *et al.* (1980), as adapted for use with GC by Raine *et al.* (1981). Rabbit polyclonal sera to bovine glial fibrillary acidic protein (GFAP) was purchased from Cedarlane, Hornby, Ontario, and to myelin basic protein (MBP) was obtained through the courtesy of Dr. Zobeeda Hosein of this Department.

Either living cells or acetone-fixed cultures on coverslips were reacted sequentially with specific antibodies for 30 to 60 min and then with fluorochrome-labeled antibody conjugates to mouse or rabbit immunoglobulin for 30 to 60 min, as described in Dales and Oldstone (1982). Polyclonal goat > mouse fluorescein (G > mu FITC), goat > rabbit rhodamine (G > ra Rho) and rabbit > bovine rhodamine (Ra > bo Rho) conjugates were purchased from Cappel Laboratories, Inc., Downingtown, Pennsylvania.

Immune lysis of infected cultures. Primary cultures of oligodendrocytes or astrocytes were infected and subjected to

immune lysis using >GC antibodies and rabbit Lo Tox complement (Cedarlane, Hornby, Ontario), as previously described (Beushausen and Dales, 1985).

Treatment of primary cultures with dibutyryl cyclic AMP and assay of 2':3' cyclic nucleotide-3'-phosphohydrolase. Cultures in 24-well plates were incubated in 1 ml BME₁₀ in the absence or presence of N⁶,O²-dibutyryl adenosine 3':5'-cyclic monophosphate (dbcAMP, Sigma), at a final concentration of 1 mM. The medium was changed every 2 days. The methods used for determination of the enzyme 2':3'-cyclic nucleotide-3'-phosphohydrolase (CNPase) were those developed by Prohaska *et al.* (1973), as modified by McMorris (1983). Protein was determined according to Lowry *et al.* (1951), using bovine serum albumin (BSA) as the standard.

RESULTS

Characterization of the Cell Types in Primary Explants from Mouse Brain

Primary brain cultures, termed mixed cultures, examined under phase-contrast optics contained predominantly two morphological cell types, organized in stratified layers. The bottom layer was occupied by large, flat, tightly adherent cells typical of astrocytes. The upper layer contained more widely dispersed cells possessing smaller cell bodies of greater phase density and extensive processes, morphologically like oligodendrocytes of the rat (McCarthy and de Vellis, 1980; Pfeiffer *et al.*, 1981; Beushausen and Dales, 1985). Due to the differential adhesiveness of these cell types the less adherent cells, at the surface, could be removed by manual shaking so as to provide relatively pure oligodendrocytic cultures (Fig. 1). Also present occasionally in low number, within the mixed cultures, were cells with large cell bodies and asymmetric processes, usually consisting of a single long process on one side and several smaller extensions on the opposite side of the cell body, reminiscent of neuronal morphology.

More definitive identification of the cell types involved was undertaken by means

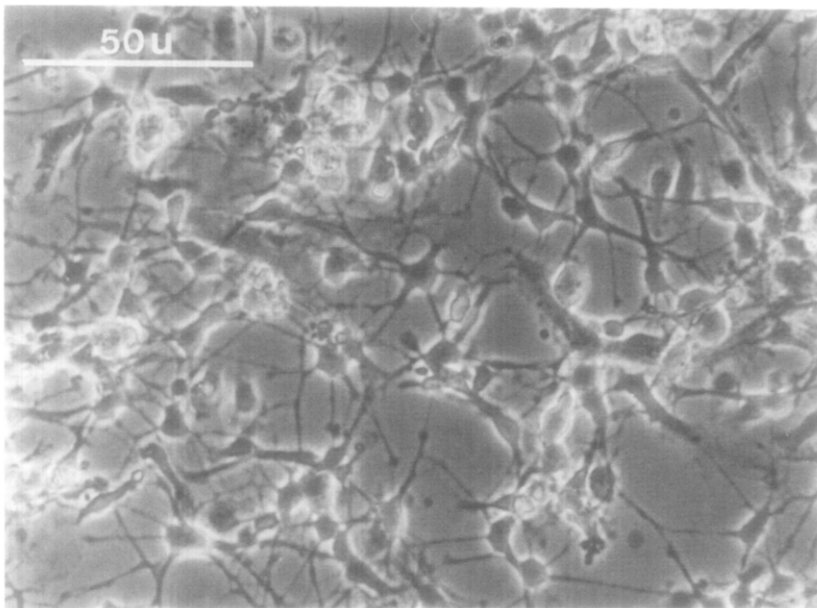


FIG. 1. Culture enriched in oligodendrocytes of CD.1 mice, viewed under phase-contrast 24 hr after plating, following release from a mixed culture ($\times 600$).

of indirect immunofluorescence, using specific antibodies to differentiation marker antigens. Antibodies to MBP were used for identifying oligodendrocytes and to GFAP for astrocytes. Judging by the fraction of MBP positive cells, between 80 and 90% of the cells present in enriched cultures were oligodendrocytes. In the astrocytic cultures over 99% of cells were GFAP positive.

Identification of Cell Types Expressing Viral Antigen

Although with neural cells from the rat there is a coronavirus strain-related specificity, whereby oligodendrocytes support the replication of JHMV and astrocytes of MHV₃, published studies with murine CNS cultures (Dubois-Dalcq *et al.*, 1982), suggest that exclusive tropisms of this type may not prevail in the mouse. To test this idea using a better defined system, we examined replication of JHMV and MHV₃ in dispersed cultures from mouse brain, containing predominantly either oligodendrocytes or astrocytes. For this purpose the cells were grown on glass coverslips and infected with either JHMV or MHV₃ at a

m.o.i. of 1. The infection was allowed to proceed at 37° for 30 hr before processing for immunofluorescence.

In the case of oligodendrocytic cultures, dual indirect tagging using Rho and FITC and Mab against JHMV nucleocapsid antigen (kindly supplied by Dr. M. Buchmeier, Scripps Clinic and Research Foundation, La Jolla, Calif.) as well as polyclonal antibodies to MBP, revealed that oligodendrocytes became infected with both JHMV and MHV₃, as illustrated in Figs. 2A-F. Likewise dual labeling with >GFAP antibodies and > nucleocapsid Mab showed that astrocytes were infectable with JHMV (Figs. 3A, B) and MHV₃ (data not shown).

To better quantitate the percentage of cells that are positive for virus antigen, independent cultures of oligodendrocytes and astrocytes from CD.1 mouse brain were inoculated at a m.o.i. of 1 PFU/cell and monitored on the 1st and 3rd days by immunofluorescence, as above. The results, summarized in Table 1, show that by Day 1 the percentage of oligodendrocytes infected with JHMV was 52, and with MHV₃, 65. By the 3rd day the frequency of infection for both viruses increased to approximately

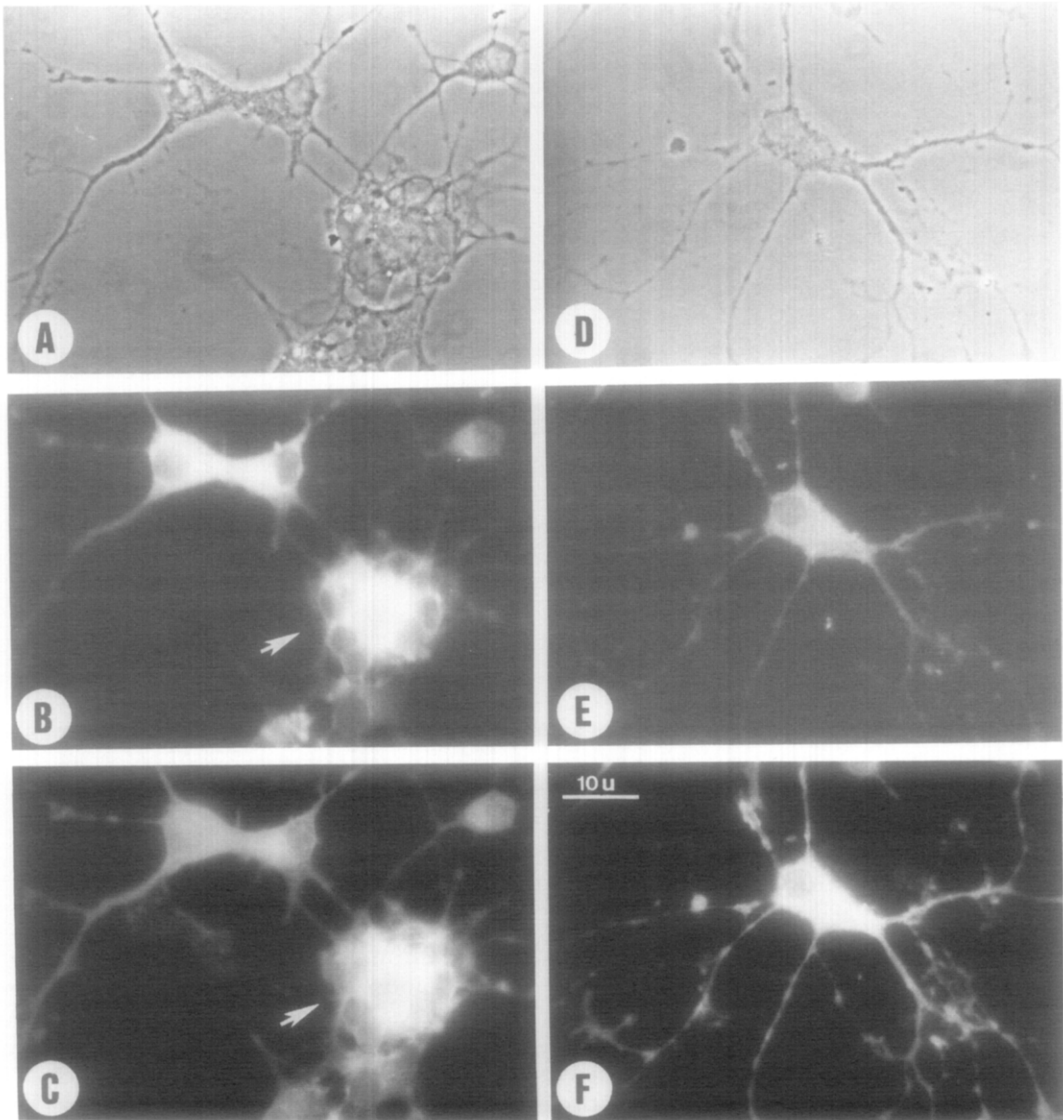


FIG. 2. Cultures, as in Fig. 1, were infected with coronaviruses for 30 hr prior to fixation and staining. (A-C) Infected with JHMV. (D-F) Infected with MHV₃. (A, D) Viewed by phase-contrast. (B, C, E, F) Examined under uv illumination. (B, E) Reacted with Mab > JHMV nucleocapsid and G > μ FITC. (C, F) Reacted with polyclonal > MBP antiserum and G > μ Rho. Note the extensive process formation of the oligodendrocytes. In (B, C) arrows point toward oligodendrocytes formed into a syncytium following infection ($\times 990$).

82%. Similar results were obtained with astrocytes, although the percentage of infected cells was less. Virus-positive astrocytes ranged from 20 to 33% on the 1st day and from 22 to 42% on the 3rd day for MHV₃ and JHMV, respectively. Thus it appears that spread of infection and cell kill-

ing occur somewhat more rapidly with oligodendrocytes than with astrocytes.

Species and Neural Cell Type Related Differences in Coronavirus Replication

Quantitative data concerning virus production complementary to observations

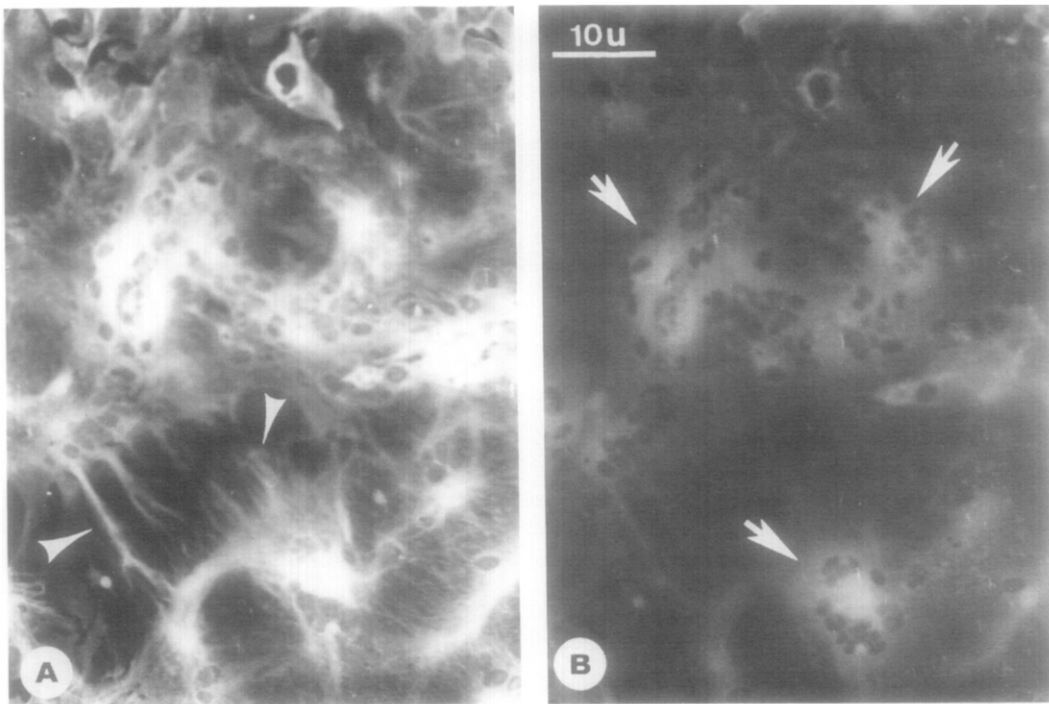


FIG. 3. The appearance under uv optics of an astrocyte culture from CD.1 mice, fixed 54 hr after plating and 30 hr following infection with JHMV. In (A) reacted with polyclonal γ GFAP antiserum and Ra $>$ bo Rho. Note the massive bundles of filaments containing GFAP antigen (arrowheads). In (B) reacted with Mab γ JHMV nucleocapsid antigen and G γ mu FITC. Multinucleated cells are identified by arrows ($\times 1400$).

from immunofluorescence were obtained by infecting murine cultures enriched in oligodendrocytes or astrocytes. The results obtained, summarized in Table 2, showed that both JHMV and MHV₃ could replicate with about equal facility in oligodendrocytes and astrocytes explanted from brains of CD.1 mice. To establish this fact care had to be exercised to ensure that the cells employed were at approximately equal, uniform density. The profound decrease in yields of PFU from these neural cultures, between the 1st and 3rd days was almost certainly the consequence of cytopathic effects, which became evident within 48 hr after infection.

Neural cells from rat brain were, by contrast, discriminating hosts for replication of JHMV and MHV₃, as evident in Table 2, confirming our previous findings that JHMV was tropic for oligodendrocytes and

MHV₃ for astrocytes of this species (Beushausen and Dales, 1985). Therefore, a clear-cut species difference in control of coronavirus replication has been demonstrated.

Immune Lysis of Oligodendrocytes

To further determine the uniformity of oligodendrocytic cultures, cells producing coronavirus were subjected to complement-mediated immune lysis and tested for yields of PFU. It is evident from the data in Table 3 that only cultures enriched for oligodendrocytes almost ceased producing JHMV after exposure to both GC antibodies and complement, but were essentially unaffected by either γ GC antibodies or complement, when added separately. Virus production by astrocyte cultures was unaffected by addition of combined GC antibodies and complement (Table 3).

TABLE 1

FREQUENCY OF CORONAVIRUS ANTIGEN-POSITIVE CELLS WITHIN NEURAL CULTURES DETERMINED BY IMMUNOFLOUORESCENCE

Virus strain	Days after inoculation	Nuclei per syncytium (average and standard deviation)	Percentage of antigen-positive cells and standard deviation
Oligodendrocytes			
JHMV	1	4.8 ± 1.02	52.0 ± 4.93
JHMV	3	8.2 ± 1.96	82.4 ± 7.83
MHV ₃	1	5.7 ± 0.97	65.4 ± 7.41
MHV ₃	3	6.6 ± 1.25	82.8 ± 8.04
Astrocytes			
JHMV	1	5.6 ± 3.37	32.8 ± 3.66
JHMV	3	5.4 ± 1.80	41.6 ± 11.67
MHV ₃	1	6.0 ± 1.69	20.0 ± 4.56
MHV ₃	3	6.6 ± 1.80	22.4 ± 5.01

Note. The total number of nuclei counted in random fields of oligodendrocytes ranged from 783 to 1245. Similarly, in astrocyte cultures the total number of nuclei ranged from 477 to 632.

Relationship between Induction of Oligodendrocyte-Associated Enzyme 2':3'-Cyclic Nucleotide-3'-phosphohydrolase (CNPase) and Virus Replication

CNPase activity is associated with myelin-forming cells of the nervous system. In rat oligodendrocytes treated with

dbcAMP the specific activity of this enzyme is greatly enhanced (McMorris, 1983), while replication of JHMV is arrested (Beushausen and Dales, 1985). This inducer, however, has limited influence on production of MHV₃ in rat astrocytes following treatment with the drug for 2 days. Since murine oligodendrocytes support the replication of both JHMV and MHV₃, it was necessary to determine whether, upon induction of differentiation, these cells ceased to replicate both coronaviruses.

The time-related induction of CNPase activity was examined using mixed primary neural cultures treated continually with 1 mM dbcAMP. Enzyme was assayed in duplicate samples of controls and treated cells at various intervals for a period of 36 days. It is evident from Fig. 4 that in untreated cells CNPase activity increased about threefold within 6 days and remained constant thereafter. By contrast, in the presence of the inducer CNPase was at a much elevated value and continuously increased for over 30 days, up to the time sampling was terminated. Thus, in murine cultures containing oligodendrocytes dbcAMP enhanced many-fold CNPase activity, in the manner observed with rat material. However, with rat oligodendrocytes the maximum induction occurs 15-21 days after treatment (Sprinkle *et al.*, 1978; McMorris, 1983; Beushausen and Dales, 1985), whereas with murine cells the increase most probably continued beyond 30 days. Incidentally, murine cultures en-

TABLE 2

SPECIES-RELATED DIFFERENCES IN THE REPLICATION OF CORONAVIRUSES IN NEURAL CULTURES

Culture derived from	Days after inoculation	Titer × 10 ² PFU/ml ^a			
		Oligodendrocytes ^b		Astrocytes ^b	
		JHMV	MHV ₃	JHMV	MHV ₃
CD.1 mice	1	4500 (8)	7000 (6)	3900 (8)	6200 (6)
CD.1 mice	3	1400 (6)	190 (2)	550 (4)	260 (4)
Wistar-Furth rats	1	12 (4)	0 (4)	0 (4)	34 (2)
Wistar-Furth rats	3	20 (4)	0 (4)	0 (4)	15 (2)

^a The values are averages from the number of experiments shown in parentheses.

^b Cell density was approximately 3 × 10⁵ cells per well.

TABLE 3
EFFECT OF IMMUNE LYSIS ON THE REPLICATION OF JHMV IN CD.1 MOUSE
ASTROCYTE AND OLIGODENDROCYTE CULTURES

Treatment of culture	Virus produced $\times 10^2$ PFU/ml			
	Astrocytes ^a		Oligodendrocytes ^a	
	Before treatment ^b	After treatment ^b	Before treatment ^b	After treatment ^b
Anti-GC antibody only	780	7400 ^c	7.5	4
Complement only	790	5600 ^c	16	15
Anti-GC antibody + complement	1300	5900 ^c	10	0.5

^a Cell density was approximately 1×10^5 cells per well. It should be noted, as previously reported (Beushausen and Dales, 1985), that when oligodendrocytes are present at low density of 10^5 cells per well or less, JHMV replication is inefficient. This may explain the discrepancy in titers from astrocytes and oligodendrocytes.

^b Fluid from cultures was sampled 24 hr prior to and 24 hr after treatment to determine released PFU.

^c Note the large increase in titer during the 24 hr infection of astrocyte cultures.

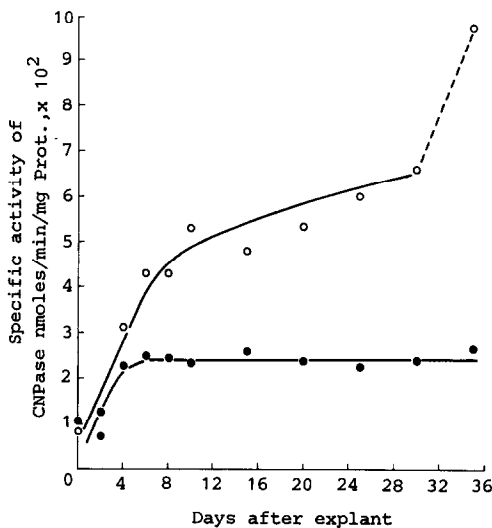


FIG. 4. Time course for induction of CNPase activity in primary, dispersed neural cultures from DBA/2 mice. Treatment with dbcAMP and the initial sampling were undertaken at the time of explantation. Controls, ●; dbcAMP treated, ○. Two independent cultures were used to assay CNPase activity on the days indicated. However, since data were not obtained on the 35th day in two other, comparable time-course experiments, the reliability of this value is uncertain and for this reason data for the 30th and 35th day are connected by a dashed line. Since the specific activity of CNPase was calculated on the basis of protein content in mixed cultures the actual specific activity in oligodendrocytes must have been greater.

riched for astrocytes, like their rat counterpart, are devoid of CNPase activity, so that the data from mixed cultures in Fig. 4 pertain only to oligodendrocytes.

The influence of differentiation on replication of the two coronavirus strains was tested by treating cultures enriched for astrocytes or oligodendrocytes with dbcAMP for various periods, as indicated in Table 4. It is evident from the results summarized in this Table that exposure of astrocytes to dbcAMP for 9 or 14 days affected coronavirus replication to a variable degree, reducing JHMV either not at all or to about 20% of the controls, while MHV₃ yields were diminished to about 20 and 8%, respectively. By contrast either zero or almost no virus of either strain was generated by oligodendrocytes exposed to dbcAMP for 11, 15, or 20 days. Controls, using VSV showed that prolonged treatment of oligodendrocytes with dbcAMP had no effect on replication of this agent. Although the murine oligodendrocytes treated for 9 days or longer with dbcAMP were not checked for expression of coronavirus antigens, surveys of rat oligodendrocytes revealed that treatment for 48 hr abolished production of JHMV antigens. Thus, in agreement with data from the rat model, coronavirus replication is blocked

TABLE 4
EFFECT OF TREATMENT WITH dbcAMP ON VIRAL REPLICATION IN CD.1 MOUSE
ASTROCYTES AND OLIGODENDROCYTES

Days after treatment commenced	JHMV $\times 10^2$ PFU/ml ^a		MHV ₃ $\times 10^2$ PFU/ml ^a	
	Control	1 mM dbcAMP	Control	1 mM dbcAMP
	Astrocytes ^b			
9	100	100	65	13
14	420	83	530	40
	Oligodendrocytes ^b			
11	167	0.5	81	0.5
15	250	0	45	0
20	270	1	61	0.5
11 VSV ^c	300 ^d	450 ^d		

^a Twenty-four hours before the days indicated in the first column cultures were infected and then sampled 24 hr later for PFU in the culture supernatant. The discrepancy between virus titers from oligodendrocyte cultures shown in Tables 2, 3, and 4 is most probably due to the much less efficient replication of virus in cultures of low density, containing 10^5 or fewer cells per well, as noted by Beushausen and Dales (1985).

^b Cells were allowed to attach for 24 hr following isolation before commencing treatment with 1 mM dbcAMP. The dbcAMP-containing or control medium was replaced every other day throughout the duration of the experiment. Cell density at the time of plating was approximately 1×10^5 cells per well.

^c Oligodendrocyte culture infected with VSV.

^d The data shown in this table were derived from duplicate cultures and are representative of three reproducible experiments.

almost entirely in murine oligodendrocytes induced to differentiate *in vitro*, but is affected to a lesser degree in dbcAMP-treated astrocytes.

DISCUSSION

The present and other recent studies (Bologna-Sandru *et al.*, 1981; Suzumura *et al.*, 1984) show that it is possible to establish dispersed neural cultures containing oligodendrocytes from cerebral hemispheres of mice. As is the case with the rat (McCarthy and de Vellis, 1980; Pfeiffer *et al.*, 1981; Beushausen and Dales, 1985), the murine cells become organized into stratified layers consisting of tightly adherent astrocytes at the bottom and loosely attached oligodendrocytes on top. The differential adhesiveness enabled us to obtain cultures that are almost entirely astrocytes or highly enriched in oligodendrocytes. Challenge with the neurotropic coronavi-

rus JHMV and viscerotropic MHV₃ showed that both strains can be replicated with equal facility in murine astrocytes and oligodendrocytes. This finding contrasts entirely with our data from rat cells which show that tropism of JHMV is exclusive for oligodendrocytes and MHV₃ for astrocytes (Beushausen and Dales, 1985). Thus, species-related distinction between the *in vitro* infectability of specific cell types from the CNS has been demonstrated.

The species-related differences between the *in vitro* mouse and rat models can be correlated with the observed *in vivo* patterns of CNS neurological disease. These patterns are regulated by the age at the time of challenge, route of inoculation, and genetic constitution of the rodent host and virus strain employed, as shown by previous work in our (Sorensen *et al.*, 1980, 1982, 1984) and other laboratories (Nagashima *et al.*, 1978a, 1978b, 1979; LePrevost *et al.*, 1975; Stohlman and Weiner, 1981; Knobler *et al.*, 1981b; Lavi *et al.*, 1984;

Weiner, 1973; Stohlman and Frelinger, 1978; Herndon *et al.*, 1975; Levy-Leblond *et al.*, 1979; and for other citations see Sorensen *et al.*, 1982). Thus in rats JHMV, but not MHV₃ can produce either an acute encephalomyelitis, if inoculated intracerebrally (ic) into very young animals, or a progressive paralytic disease with demyelinating lesions, if the agent is administered ic prior to weaning (Sorensen *et al.*, 1982). In the mouse, however, both JHMV and MHV₃ induce neurological disease and, depending on the genetic endowment, this species may remain susceptible to coronavirus even beyond 1 year of age (Lampert *et al.*, 1973; Weiner, 1973; Herndon *et al.*, 1975; Hirano *et al.*, 1981; Stohlman and Weiner, 1981; Sorensen *et al.*, 1982). Therefore, specificities of coronavirus tropism manifested in tissue culture of neural cells appear to reflect accurately the events occurring within the CNS of the animal.

As previously demonstrated with material from the rat, addition of dbcAMP to murine oligodendrocytes induced in them an accelerated process of differentiation, manifested as elevation in CNPase activity (Sprinkle *et al.*, 1978; McMorris, 1983; Beushausen and Dales, 1985), which is diagnostic of myelin production. As found with rat cells, the process of murine oligodendrocyte differentiation is closely correlated with absence of virus production, in this case involving both JHMV and MHV₃. By contrast, dbcAMP treatment has much less influence on replication of these coronavirus in mouse astrocytes, again consistent with the previous finding that this inducer has limited effect on production of MHV₃ in rat astrocytes (Beushausen and Dales, 1985). The observed reduction in virus yield from cultures of astrocytes treated with dbcAMP might be due to a slower effect on astrocyte differentiation thereby allowing production of coronaviruses. Thus the current and previous observations indicate that infectability of the CNS of rodents is, at least in part, related to the maturation and differentiation of oligodendrocytes and, perhaps, astrocytes.

It should be mentioned that within 2 days after dbcAMP treatment JHMV pro-

duction in rat oligodendrocytes ceases whereas at least 5 days of exposure to the inducer are required to inhibit MHV₃ or JHMV replication in mouse oligodendrocytes (data not shown). This finding might be related to differences in age between rats and mice when myelination is complete and also to the time interval when the animals remain susceptible to CNS disease caused by coronaviruses. In mice the prolonged susceptibility to disease caused by infection is in line with an extended time period during which CNPase induction and myelination occur (Kurihara *et al.*, 1970; Morell *et al.*, 1972; Barbarese *et al.*, 1978) and is consistent with the long time required to obtain maximum CNPase induction *in vitro*, as shown in Fig. 4. By contrast, the unusually sensitive (Sorensen *et al.*, 1980), Wistar-Furth rats remain susceptible for only about 15-21 days, to disease caused by JHMV, which is coincident with the completion of myelination and CNPase induction *in vivo* (Sprinkle *et al.*, 1978) and *in vitro* (Abney *et al.*, 1981; Barbarese and Pfeiffer, 1981; McMorris, 1983). These findings focus further on the possible relevance of age-related oligodendrocyte maturation in the CNS and susceptibility to disease within the CNS caused by coronaviruses.

It should be kept in mind that the infectability of oligodendrocytes by these agents cannot account entirely for the CNS diseases observed, since the ability of coronaviruses to replicate in neurons and other cell types has been abundantly documented (Nagashima *et al.*, 1978; Knobler *et al.*, 1981a, 1981b; Dubois-Dalcq *et al.*, 1982; Collins *et al.*, 1983; Sorensen *et al.*, 1984; Beushausen and Dales, 1985). Our recent investigations using cDNA probes have, in fact, shown by *in situ* hybridization that latent JHMV can be maintained for prolonged periods in the cytoplasm of neurons within specific areas of the rat brain, particularly in the hippocampus (Sorensen and Dales, 1985), suggesting that neurons may function as repositories of latent and persistent infections. The relative importance of oligodendrocytes versus the other neural cell types in the disease process must await future elucidation.

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