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RESTRICTED REPLICATION OF THE E5"14" CLONE OF LANGAT TP21 VIRUS (FROM THE TICK-BORNE ENCEPHALITIS COMPLEX) IN CNS OF SUBADULT MICE

J. RAJČÁNI, V. MAYER, E. WEISMANOVÁ*

Institute of Virology, Slovak Academy of Sciences, 817 03 Bratislava, Czechoslovakia

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Summary. — Subadult ICR mice were infected with the low virulent Langat virus TP21 E5 strain clone "14" belonging to the tick-borne encephalitis (TBE) complex by subcutaneous (s.c.) and intracerebral (i.c.) routes. From 5 to 6 days post infection (p.i.), no virus was detected in cultured brain fragments of mice, which received 10^6 ic LD_{50} into interscapular area. Acute lethal encephalitis with lesions confined to the vicinity of the inoculation area (parietal cortex, basal ganglia, thalamus) has developed in all mice, which received $\geq$ 3 PFU of the virus by i.c. route. However, no virus was recovered from the cultured fragments of brain stem and cerebellum of these animals, although direct isolation attempts were regularly positive from brain cortex and basal ganglia. Survivors, which did not succumb to i. c. administration of $\approx$ 1 ic LD_{50} (0.3 PFU) of the attenuated Langat strain were autopsied between 53-74 days p.i. Attempts to isolate the virus from cultured fragments of brain cortex and basal ganglia remained negative despite of the presence of focal residual histological lesions in g. hippocampi in 15% of of animals examined.

Key words: *Langat virus*: *attenuated clone*; *TBE complex*; *virulence*; *immunofluorescence*; *explantation technique*

Introduction

The ability of viruses from the TBE complex — including the low virulent lines derived from them — to exhibit prolonged interactions with host cells is a matter of continuous interest (e.g. Mayer *et al.*i 1974; Mayer and Rajčáni, 1978). Existence of chronic and progressive clinical forms of TBE (Baistrukh, 1975; Asher, 1979) and efforts to characterize the markers correlating to the virulence of TBE virus (reviewed by Mayer and Mitrová, 1977) led to the development of several experimental models of persisting TBE infection

* Graduate student of the Faculty of Natural Sciences, Komenský University, Dept. of gen. and applied Virology, Bratislava, Czechoslovakia

Table 1. Lethality in mice inoculated by various routes with different doses of attenuated Langat strain

Route	Dose	Lethality	Group	Sampling days*
s.c.	10^6 PFU	0/50**		7, 10, 14, 31, 67, 70
i.c.	300 PFU	30/30		5
	30 PFU	40/40		5, 9
	3 PFU	40/40	A	5, 7, 9, 11
	0.3 PFU	28/50	B	7, 9, 11, 53, 62, 74
	0.03 PFU	0/15	C	7, 11

* Post infection.
** Number of died mice out of total infected.

(Vorobyeva *et al.*, 1980; Pogodina *et al.*, 1981). The attenuated clone E5"14" of the Langat strain TP21 is not virulent after peripheral administration to subadult mice (Mayer, 1975). A previous study reported negligible histological changes after its peripheral inoculation (Mitrová and Mayer, 1975). In the presented experiments, attempt was made to correlate the histological changes with the results of immunofluorescence (IF), direct virus isolation and tissue explantation in order to explore the presence of virus. In addition, it seemed reasonable to investigate also the ability of the attenuated strain to persist in survivors, which received a dose of approximately ≈ 1 ic LD_{50} by i.c. route.

Materials and Methods

Virus. The E5 strain of the Langat TP21 virus (Thind *et al.*, 1966) was cloned by Mayer (1975). The properties of the clone "14" were described elsewhere in detail (Mayer and Mitrová, 1977). Briefly, the attenuated clone displays the following characteristics $ic^+, sc, s^{\pm}, t, e, u^S$. It does not cause fatal encephalitis after s.c. inoculation, but after i.c. administration encephalitis develops. The stock strain was kept lyophilized at —12° C; its titer was 10^8 PFU/ml in PS cells. In the presented experiments, the E5 "14" virus was used in the 11th i.c. passage in newborn mice. The 10% brain suspension was stored at —70 °C.

Animals. Subadult ICR mice from the breeding farm Velaz (specific pathogen-free) weighing 10—12 g were used. Mice were infected with 10^6 PFU of the E5 "14" virus in 0.1 ml inoculum by s.c. route and with 5 different dilutions of the virus by i.c. route (Table 1). At intervals, the brain was removed and cut into 6 parallel slices: 1. olfactory area, 2. frontal lobe and caput n. caudati, 3. parietal lobe including thalamus and striatum, 4. occipital cortex and midbrain, 5. anterior brain stem and cerebellum, 6. posterior brain stem and cerebellum. At each interval, alternating slices coming from at least 3 brains were either fixed in cold Carnoy's solution, or minced in culture medium or used for preparation of brain suspensions.

Explantation procedure. Minced pieces of the brain cortex, basal ganglia, brain stem and cerebellum were kept in medium CMRL-1415 as previously described (Rajčáni *et al.*, 1975). The medium was exchanged on the 3rd day. Explants from the corresponding brain regions were collectec and then either quickly frozen in liquid propan-butan or used for preparation of suspensions.

Histology and immunofluorescence. The samples fixed in Carnoy's solution were quickly embedded into paraffin (Albrecht *et al.*, 1966). Parallel sections cut from the blocks were deparaffinized and stained alternatively with hematoxylin and erythrosin (H.E.) or by the indirect IF method. The quickly frozen explants were cut in cryostat (serial sections) and after acetone fixation stained by indirect IF. Serum to the Hypr strain of TBE was raised in guinea pigs. It was adsorbed to uninfected brain suspension and used in dilution 1 : 10 for staining. The conjugate SwAGp (Sevac, Prague) was diluted 1 : 5.

Table 2. Results of virus detection and the low grade histological lesions* in mice inoculated by s.c. route

Days p.i.	Interval p.i.	Virus isolation*** Direct	Explantation	IF	Hi
7	Early	0/3	0/3	0/10	3/10**
10		0/3	0/3	0/3	1/3
14		0/3	0/3	0/10	4/10
	Early total	0/9	0/9	0/23	8/23
31	Late	0/3	0/3	0/3	0/3
67—70		0/6	0/6	0/6	0/6
	Late total	0/9	0/9	0/9	0/9

IF = immunofluorescence; Hi = histology;
* For details see Figs. 1 and 2
** Number of positives out of total
*** For details see Materials and Methods.

Virus isolations. Suspensions prepared from the brain, suspensions of the cultured brain fragments as well as their culture fluids were inoculated on monolayers of chick embryo cells in tube cultures. After 3 days incubation at 37 °C, the cultures were challenged with Sindbis virus. The CPE brought about by the challenging virus was read after 2-3 days (expressed in log IFD_{50}). If the results of interference test were not clear-cut, the suspensions were inoculated i.c. into newborn mice. Selected suspensions of the cultured brain fragments were also inoculated into chick embryo cells grown on cover slips. After 48 hr, the cover slips were fixed in acetone and stained with the indirect IF method. Animals infected by i.c. route with 0.3 PFU and sacrificed 74 days p.i. received 2 injections of cyclophosphamide (CPA; VEB, Jenapharm, GDR) in a dose of 100 mg/kg body weight on days 7 and 11 before autopsy.

Results

Subcutaneous infection

Brains of subadult mice inoculated with 10^6 PFU of the E5 clone by s.c. route were removed on days 7, 10, 14, 31, 67 and 70 p.i. All animals remained healthy throughout the whole observation period (Table 1). The results of IF examinations of the brain were negative. The virus was neither isolated from the brain suspensions, nor it was recovered from the explanted tissue fragments. At histological examination of the brain negative results also prevailed. However, up to day 14 p.i., minimal lesions were found in 8 out of 23 mice examined (Table 2). These comprised of slight meningeal infiltration, cuffings of round cells surrounding some vessels and scarce mononuclear nodular infiltrates centered around vessels (Figs 1 and 2). The neurons in vicinity of the great majority of infiltrates were well-preserved. The only exception were tigrolysis and eosinophilia of a few neurons in gyrus hippocampi of one animal. The areas involved by minimal changes were the brain cortex (parietal cortex, g. hippocampi), basal ganglia and cerebellum. None out of 10 mice, which s.c. received saline only, revealed any minimal histological changes in CNS.

Table 3. Results of the examination of brain samples in mice surviving inoculation of ≈ 0.3 PFU of the attenuated Langat virus by i.c. route

Days p.i.	Virus isolation		IF	Hi
	Direct	Explantation		
53	0/5	0/5	0/5	2/5
62	0/5	0/5	0/5	1/5
67	0/5	0/5	0/5	0/5
74 (CPA)	0/5	0/5	0/5	0/5

CPA = cyclophosphamide
For explanations see Table 2

Intracerebral inoculation

When subadult mice were infected into the thalamic area, the outcome of infection depended on the virus dose. As shown in Table 1, three different animal groups were observed: A — lethal encephalitis (viral dose ≧ 3 PFU); B — encephalitis in a part of the animals and survival of others (viral dose of ≈ 0.3 PFU); C — no disease at all (after administration of ≈ 0.03 PFU) The healthy survivors in the 2nd B group (44% of infected animals) were examined between 53—74 days p.i. Five mice autopsied on the 74th day received cyclophosphamide 7 and 11 days before autopsy.

Virus isolation from i.c. infected mice

The low virulent Langat virus was reisolated from brains of mice dying of encephalitis (group A) between day 5 to 11 p.i. Th e titre of the virus in the brain was 5.5—5.7 log IFD_{50} per g of brain tissue. Provided that the brain fragments were kept in culture, the explants coming from the brain cortex and basal ganglia yielded infectious virus. The attenuated strain was neither recovered from the cerebellum nor from the brain stem by the explantation procedure used. Animals showing clinical signs of disease were selected from the B group of mice, which received ≈ 0.3 PFU of virus. In these, the isolation results were essentially similar to those of the previous group. The titre of infectious virus in brain at direct isolation was 4.3—4.5 log IFD_{50} per g of brain tissue. However, neither IF nor isolation procedures proved the presence of virus in CNS of surviving animals autopsied between 54—74 days p.i. (Table 3). No virus was recovered from group C mice which received the lowest virus dose (0.1 ic LD_{50}) by either method used even after CPA treatment.

The virus yielding brain fragments coming from autopsy performed 11 days p.i., which were cultured for 7 days, exhibited neurons showing positive IF (Fig. 3). In contrast, no neurons revealing viral antigen(s) were seen in the explants coming from the negative brain explants.

Morphological examination of the i.c. inoculated mice

The early histological changes in CNS of group A mice were predominantly angiotoxic. Round cell (lymphocytary) infiltration was found in the meninges

and around venules. In addition to perivascular cuffings, swelling of endothelium cells and nodular mononuclear infiltrates were striking (Fig. 4). The neuronal damage (eosinophilic necrosis) and cytolysis were more abundant on days 7 and 9 p.i. (Fig. 5). Occasional necrosis of the whole vessel-wall was also seen (Fig. 6). Neurons displaying positive fluorescence at these interval were numerous in the parietal cortex, in g. hippocampi (Figs 7 and 8) in lenticulostriatum and thalamus. No viral antigen was found in the Purkyně cells of cerebellum and in neurons of brain stem. Later on (day 11), focal loss of neurons in gyrus hippocampi and parietal cortex were accompanied by proliferation of glial cells and extensive mononuclear infiltrates (Fig. 9).

In sick mice infected with the $\approx$ 0.3 PFU dose, the morphological lesions appeared a little less severe. On days 7 and 9 p.i., positive fluorescence of TBE virus antigen(s) in neurons was accompanied by changes similar to those described. In the areas of necrosis of the cortex positive patchy fluorescence of the cell debris was seen. Occasionally mononuclear pleomorphic and nodular infiltrates were found also in areas revealing slight neuronal damage (tigrolysis) only or no damage at all (Fig. 10). On day 11, despite of the presence of histological lesions, no fluorescing cells swere detected in damaged areas of the brain cortex and g. hippocampi.

Limited areas of neuronal damage (loss of neurons in g. hippocampi) accompanied by mononuclear nodular infiltrates, glial proliferation and lympho plasmocytary patchy infiltration in meninges and around a few vessels were found in 3 out of 5 semiserial sections of the brain cortex in 3 out of 20 mice autopsied later than 2 months p.i. (Table 3). Attempts to detect viral antigen(s) in the corresponding area of the brain remained unsuccessful. All morphological findings were negative in brains of mice inoculated with the extremely low ($\approx$ 0.03 PFU) virus dose.

Comparison of morphological and virological findings

As mentioned above, the outcome of i.c. infection with the attenuated Langat strain was different, depending on the virus dose used.

Lethal encephalitis (group A) developed after i.c. inoculation of the high virus dose ($\leq$ 3 PFU). All animals succumbed in this group. The histological and IF findings were positive in the parietal cortex, gyrus hippocampi, basal ganglia and thalamus. The morphological findings in the cerebellum and brain stem were negative, which was in good accordance with the negative isolation results by explantation of the corresponding brain fragments.

Encephalitis occurred after administration of $\approx$ 0.3 PFU of the attenuated Langat virus in approximately 56% of animals (group B). The histological and IF findings were less severe and direct isolation experiments revealed by 1 log lower virus titres than in the previous group. No viral antigen was seen from day 11 p.i. in CNS of mice of this group. Despite of the presence of focal residual lesions in g. hippocampi, the explantation procedure failed to detect any persisting or covert virus in the brain of survivors by 53—74 days p.i.

All morphological findings and virus isolation attempts were negative in mice which received the lowest virus dose (0.03 PFU).

Discussion

The highly attenuated E5 "14" clone of naturally low virulent Langat virus does not cause lethal encephalitis in subadult mice after s.c. inoculation. Mitrová and Mayer (1975) described very mild and low grade changes in serial sections of mouse brains after peripheral inoculation of this clone scarce capillary endothelium swelling, very few perivascular infiltrates, occasional leptomeningeal patches, and slight focal damage to very few neurons. In this study slight infiltrates around vessels were found in 8 out of 23 brain samples between 5—14 days p.i. Mayer and Mitrová (1977) concluded that the E5 "14" virus had not reached the CNS of adult mice in a way stimulating progressive glial proliferation. This notion can be supported by our negative IF findings in the brain and by negative virus isolations either from the brain homogenates or from the explanted brain fragments. The significance of the low grade histological changes is unclear. The attenuated Langat clone causes a transient viraemia only with trace levels of virus in the blood resulting from an extremely limited replication at the extraneural sites (Albrecht, 1968; Mayer, 1975). This limitation, however, is host and age dependent. The attenuated clone replicates — although moderately — in different organs and tissues of s.c. inoculated suckling mice (Mayer *et al.*, 1974). In suckling and weanling mice (up to 21 days after birth), the naturally occurring low virulent Skalica strain of TBE virus causes encephalitis by s.c. inoculation although it is apathogenic for 2-month-old mice (Rajčáni and Gresíková, 1982). The low neuroinvasivity of the attenuated clone in subadult mice could be associated with a higher efficiency of macrophage clearance and with a lower permissiveness of supporting tissues for virus growth. The clearance of the virus mediated by macrophage is more efficient against attenuated alfaviruses than against virulent ones (Jahrling, 1977). The attenuated clone Hypr HK_{28} "2" also failed to penetrate the olfactory nerve endings after intranasal inoculation (Mayer and Rajčáni, 1968). The possibility, that a low virulent virus is less effectively adsorbed to nerve endings should be considered as well.

The limited spread of the attenuated clones within the CNS is an important property indicating decreased virulence. Neurons distant from the inoculation site (Purkyně cells, motoneurons in the brain nuclei) revealed occasional slight changes (eosinophilia, triangulation), but they did not show typical damage, seen in the brain cortex and nucleus hippocampi (oedema, tigrolysis, eosinophilic necrosis, cytolysis). No inflammatory lesions were seen in cerebellum of i.c. infected mice, which had received the attenuated strain into thalamus. The TBE virus antigen was neither found in nor was isolated from sections of brain stem and cerebellum. In contrast, severe lesions were seen in the parietal cortex and basal ganglia of the same animals. These lesions were of grade 3 according to Arya (1977) and probably corresponded to grade II and occasionally to grade III lesions as advised by Mitrová (1977). The limited spread of the attenuated virus within the CNS may be due to a restricted spread of virus in axons. The low invasivity of the attenuated Hypr HK_{28} "2" strain after intranasal inoculation mentioned above, could also be mediated by a similar mechanism. Thus, the

supposed rapid clearance of the attenuated virus by macrophages, its lower affinity to nerve endings and its limited spread in axons may reflect the change(s) of its surface (i.e. properties of glycoproteins) possibly relevant to the u^s and *e* markers.

Vorobyeva (1980) described the different behaviour in Syrian hamsters of the attenuated strains Yelantsev (from the TBE virus complex) and Langat TP21 (revealing limited but definite replication in nonneural tissues of mice after peripheral inoculation). She found, that her Langat TP21 strain rarely caused viraemia after s.c. inoculation in immunosuppressed animals, while the Yelantsev strain did so in addition to its replication in several organs, including brain. Vorobyeva also succeeded in reisolation of a strain designated A205 from the brain culture of a hamster 65 days after infection with the Langat TP21 virus. The reisolated virus A205 had properties intermediate between the original Langat and Yelantsev strains. The differences in the behaviour of various attenuated TBE strains may reflect the difficulties in obtaining and maintenance of genetically homogeneous virion population due to its natural variability (Mayer and Mitrová, 1977). According to Kańtoch (1978) the attenuated poliovirus vaccine must not contain more than 1 PFU of virulent virus in 10^6 PFU of vaccine. To maintain the high degree of biological homogeneity of an cloned attenuated virus is a very tedious task, at present still unsolved; even a long year storage in lyophilized state may change the ratio of virulent to attenuated particles in favour of the former, which seems to be more resistant (Mayer, 1980).

A very often discussed problem is the property of attenuated TBE virus strains to persist in the CNS of experimental animals. The persistence of flaviviruses may be accompanied by vacuolizing lesions of neurons and diffuse proliferation of astroglia resembling to spongiform encephalopathy (Zlotnik and Grant, 1975). In chronic encephalitis of man caused by Japanese encephalitis virus, proliferation of microglial cells is a prominent feature. The interpretation of focal lesions of neurons and inflammatory changes found between 53—74 days p. i. in survivors who received 0.3 PFU is difficult, because of the absence of positive IF findings and the negative virus isolation results. The persisting attenuated virus, probably present in a non-productive form, might have altered the metabolism of affected neurons leading to progressive necrobiosis of these cells. Interesting findings were described in the brains of 4-week-old mice infected with a defective Newcastle disease virus (strain Cg), which given intracerebrally caused encephalopathy without positive IF staining, little or no recoverable virus and occasional neuroglial proliferation (Barks *et al.*, 1976). In the limits of our methods there was no residual infectious virus in the CNS of mice surviving the administration of 1 LD_{50} by i.c. route, however, this should be considered with high caution. Our attempts to isolate the virus from culture fluid of the explanted brain fragments or from fragments themselves might not have been relevant to the nature of virus persistenec. A final answer could be given by hybridization in situ using highly labelled complementary RNA.

References

Albrecht, P. (1968): Pathogenesis of neurotropic arbovirus infections. *Current Topics in Microbiol. Immun.* **43**, 45—91.

Albrecht, P., Mrenová, M., and Karelová, E. (1966): Paraffin embedding techniques for immunofluorescent demonstration of neurotropic viruses. *Acta virol.* **10**, 155—160.

Arya, S. S. (1979): The behaviour of Sabin attenuated poliovirus strains in the central nervous system of rhesus monkeys. *J. biol. Standard.* 8, 175—281.

Asher, D. M. (1977): Persistent tick-borne encephalitis infection in mna and monkeys in relation to chornic neurological disease, pp. 179—195. In E. Kurstak (Ed.): *Arctic- and Tropical Viruses.* Proc. Int. Symp., Mont Gabriel, Canada, 1977. Academic Press, New York, 1979.

Baistrukh, M. N. (1975): Clinical characteristics of chronic tick-borne encephalitis in Primorsk district (in Russian). *Vop. med. Virus.* **1978**, 381—382.

Burks, J. S., Narayan, O. M., McFarland, H. F., and Johnson, R. O. (1976): Acute encephalopathy caused by defective virus infection. *Neurology* **10**, 585—588.

Jahrling, P. B., Hilmans, D. E., and Heard, C. G. (1977): Vascular clearance of Venezuelan equine encephalomyelitis viruses as a correlate to virulence for rhesus monkey. Arch. Virol. **88**, 161—164.

Kantoch, M. (1978): Markers and vaccines. *Adv. Virus. Res.* 11, 259—325.

Mayer, V. (1974): A live vaccine against tick-borne encephalitis: integrated studies. I. Basic properties and behaviour of the E5 "14" clone (Langat virus). *Acta virol.* **19**, 209—218.

Mayer, V. (1980): Experimentally segregated attenuated tickborne encephalitis virus: altered behaviour after prolonged storage at subzero temperatures. *Acta virol.* **12**, 194—206.

Mayer, V., and Rajčáni, J. (1968): Studies on the virulence of tick-borne encephalitis virus. IX. Intranasal infection of *Macaca mulatta* monkeys with genetically defined virus clones. *Acta virol.* **11**, 403—413.

Mayer, V., and Mitrová, E. (1977): Low virulent mutants of flaviviruses. *Biol. Práce (Bratislava)* **13** (6), 153 pp.

Mayer, V., Rajčáni, J., and Leššo, J. (1974): Dissimilar nature of two highly attenuated virus clones from the tick-borne encephalitis complex. *Acta virol.* **18**, 41—46.

Mitrová, E. (1977): Semiquentitative grading scale for evaluation fo the inflammatory and degenerative changes in the CNS of mice. Neurohistological characterization of the attenuated E5"14" clone. *Proc. Morph. Session 50th Anniversary Histol. Embr. Inst. Med. Fac. Comenius Comenius University*, Bratislava, pp. 60—78.

Mitrová, E., and Mayer, V. (1975): A live vaccine against tick-borne encephalitis: integrated studies. II. Histopathology of mice peripherally immunized with E5 "14" virus and challenged with virulent virus. *Acta virol.* **19**, 219—228.

Pogodina, V. V., Frolova, M. P., Malenko, G. V., Fokina, G. I., Levina, L. S., Mamonenko, L. L., Koreshkova, G. V., and Ralf, N. M. (1981): Peristence of tick-borne encephalitis virus in monkeys. I. Features of experimental infection. *Acta virol.* 18, 337—343.

Rajčáni, J., and Grešíková, M. (1982): Pathogenicity of the Skalca strain (from the tick-borne encephalitis virus complex) for white mice. *Acta virol.* **26**, 264—269.

Rajčáni, J., Čiampor, F., and Sabó, A. (1975): Experimental latent herpesvirus infection in rabbits, mice and hamsters: ultrastructure of the virus activation in explanted gasseric ganglia. *Acta virol.* **19**, 19—28.

Thind, I. S., and Price, W. H. (1966): A chick embryo attenuated strain (TP21E5) of Langat virus. I. Virulence of the virus for mice and monkeys. *Am. J. Epidem.* **84**, 193—212.

Vorobyeva, M. S. (1980): Studies of the virus isolated from the brain of Syrian hamsters persistently infected with an attenuated strain of tick-borne encephalitis virus complex, pp. 155—164. In J. Rajčáni, J. Szántó and G. Hunter (Eds): *Int. Symp. on Chronic Virus Infections*, Veda, Bratislava, 1980.

Zlotnik, I., and Grant, D. P. (1975): The occurrence of vacuolated neurons in the brains of ters affected with subacute sclerosing encephalitis following measles or Langat virus infection. *Brit, J. exp. Path.* **80**, 72—76.

Explanation of Micrographs (Plates LVII-LIX):

Fig. 1. Cerebellum of subadult mice infected with 10^6 PFU of the attenuated Langat strain by s.c. route by day 14 p.i. Patchy mononuclear infiltration in the meninges, slight nodular pleomorphic infiltrate in the molecular layer and cuffings around venule Magn. × 70, H. E.

Fig. 2. Thalamus in subadult mice 14 days after s.c. infection with the attenuated clone. Detail of a nodular mononuclear infiltrate centered over a capillary and perivascular cuffing around a venule. Magn. × 400, H. E.

Fig. 3. Specific fluorescence in neurons of an explanted brain fragment from the cortex of a mouse infected with ≈ 0.3 PFU of the Langat E5 "14" strain into the thalamus and autopsied 11 days p.i. Viral antigen in neurons, indirect IF method, × 180.

Fig. 4. Abundant perivascular cuffings and nodular infiltrates consisting of mononuclear cells. The slightly damaged neurons look unchanged at this magnification. Day 5 p.i. ≈ 30 PFU given by i.c. route, H. E., × 140.

Fig. 5. Cytolysis of neurons, oedema and mononuclear infiltration in the brain cortex; 9 days p.i. 30 PFU given by i.c. route, × 140, H.E.

Fig. 6. (insert): A necrotic venule surrounded by mononuclear cells in the deep layer of parietal ccortex. × 400, H.E.

Fig. 7. Positive fluorescence in the cytoplasm of neurons in g. hippocampi 7 days p. i. with ≈ 3 PFU of the virus given by i.c. route; indirect IF, × 120 (paraffin embedding).

Fig. 8. (insert): A group of neurons from Fig. 6, showing positive IF, × 450.

Fig. 9. Loss of neurons in g. hippocampi proliferation of glial cells and mononuclear infiltration 11 days p.i. ≈ 3 PFU given by i.c. route, × 140, H.E.

Fig. 10. Perivascular round cell infiltration, nodular and diffuse mononuclear pleomorphic infiltrate, very mild changes in neurons; 7 days p.i. with ≈ 0.3 PFU of the attenuated strain given by i.c. route, × 300, H.E.

Fig. 10. Perivascular round cell infiltration, nodular and diffuse mononuclear pleomorphic infiltrate, very mild changes in neurons; 7 days p.i. with ≈ 0.3 PFU of the attenuated strain given by i.c. route, × 300, H.E.