Acta virol. **28**: 232—239, 1984

PERSISTENCE OF TICK-BORNE ENCEPHALITIS VIRUS IN MONKEYS. VI. PATHOMORPHOLOGY OF CHRONIC INFECTION IN CENTRAL NERVOUS SYSTEM

M. P. FROLOVA, V. V. POGODINA

Institute of Poliomyelitis and Viral Encephalitides, U.S.S.R. Academy of Medical Sciences, 142782 Moscow, U.S.S.R.

Received December 2, 1982; revised March 16, 1983

Summary. — Histological changes were studied in the central nervous system (CNS) of 58 *Macaca rhesus* monkeys, infected intracerebrally (i.c.) and subcutaneously (s.c.) with tick-borne encephalitis (TBE) virus. Subacute degenerative process in the CNS occurred in 7 monkeys on days 15—24 post-infection (p.i.), while chronic degenerative changes were observed in 5 monkeys examined on days 45, 90, 279, 383, 789 p.i. Pathological changes in the CNS of 11 monkeys correlated with the clinical picture of subacute and chronic encephalitis. Chronic lesions were observed in the CNS of 1 monkey with asymptomic infection. The pathomorphology of the CNS lesions was characterized by progressive development of focal changes of various duration.

Key words: tick-borne encephalitis; monkeys; pathogenesis; pathomorphology; central nervous system

Introduction

The study of virus persistence in the host is closely connected with many aspects of chronic, latent and slow virus infections including pathogenesis and pathomorphology of the disease variants. Chronic froms of TBE are known for a long time to play a considerable role in human pathology. However, several aspects of their pathogenesis are still unclear. Recently, chronic encphalitis caused by TBE virus was reproduced in experimental animals namely in monkeys by a number of investigators (Komandenko *et al.*, 1972; Ilienko *et al.*, 1974, 1975; Zlotnik, 1976; Dremov, 1977; Andzhaparidze *et al.*, 1978; Asher, 1979). Our findings on the persistence of TBE virus in experimentally infected monkeys were presented in previous reports describing conditions of experiment, clinical variants of infection, and detection of virus persistence (Pogodina *et al.*, 1981*a*, *b*, *c*). We elicited a chronic process in monkeys showing stable palsy of extremities and restoration of motor functions after TBE virus infection. It was shown that TBE virus has the capacity for long-term persistence in infected animals. The present report describes the pathomorphology of subacute and chronic lesions in their CNS.

Materials and Methods

Macacca rhesus monkeys were infected by TBE virus strains Vasilchenko, Aina/1448, 41/65 and 2 mutans of strain Pan (Pan- 114 N1M and Pan-114 N1APH). Strains history, their virulence for mice and monkeys were described previously (Pogodina *et al.*, 1981a, *b*, *c*). 24 monkeys received i.c. the virus dose of 10^4—$10^{6.8}$ LD_{50}/ml; 34 monkeys were inoculated by s.c. route with the virus dose of 10^6—$10^{8.2}$ LD_{50}/ml. 30 of the latter were given cyclophosphamide (CPA) in the dose 50—100 mg/kg per animal mass one day before and one day after virus inoculation. Animals were observed for more than 2 years. The methods and results of virus isolation were described earlier (Pogodina *et al.*, 1981*a*, *b*, *c*).

The brain cortex, subcortical ganglia, thalamus, midbrain, medulla oblongata, cerebellum, the cervical and lumbar regions of the spinal cord were examined histologically. Samples were fixed in 96% alcohol, Carnoy's solution or in 10% formalin respectively and embedded into paraffin. The sections were stained with haematoxylin and eosin, with kresol-violet according to Nissl, with crystalline violet according to Kanzler. A part of the material was cut on freezing microtome, sections were impregnated by Cajal's gold sublimate for detection of astrocytes; oligodendroglia and microglia were stained according to Dubranski and Miyagawa.

Results

Histological examination was performed in 58 monkeys inoculated i.c. and s.c. with the strains Vasilchenko, Aina 1448, 41/65 and two mutants of the strain Pan of decreased neurovirulence. Clinically marked neurological symptoms occurred in all 24 animals infected by i.c. route. However, there were differences in severity and course of the disease manifested as acute, subacute and chronic encephalitis variants (Pogodina *et al.*, 1981*a*).

No clinical symptoms were observed in 34 s.c. infected monkeys; regardless whether they received CPA or not, the course of their infection was asymptomatic (Table 1).

All strains under study caused acute encephalitis. Severe inflammatory and degenerative changes of meningoencephalitis in typical localisation were detected in the CNS of 13 monkeys at histological examination. The exsudative reaction in the brain was marked. A great number of cuffings around vessels consisted of lymphocytes, plasmocytes, mononuclear cells and a considerable amount of polymorphonuclear leukocytes. In addition, diffuse mononuclear infiltrates were seen in the gray matter. In neurons early degenerative and necrotic changes were found. Neuronal destruction was accom-

Table 1. Survey of clinico-morphological examination of monkeys

Virus administration	Number of monkeys examined	Character of encephalitis		
		acute	subacute	chronic
i.c.	24/24	13*	7*	4*
s.c.	0/34	0	0	1**
Total	24/58	13	7	5

Numerator — number of monkeys examined morphologically;
denominator — number of monkeys with clinical manifestations of infection.
* Monkeys infected with the TBE virus strains (as indicated in the text) not receiving CPA.
** Monkey infected with strain Aina/1448 receiving CPA.

panied with neuronophagy, loss of neurons, diffuse and local proliferation of micro- and oligodendroglia cells.

Long-term observation of the experimentally infected monkeys and their histological examination at various intervals p.i. allowed to follow the dynamics of morphological changes in the CNS. Subacute inflammatory process was seen in 5 monkeys inoculated i.c. with the mutants of Pan strain, and in 2 animals infected with the Vasilchenko strain. As described (Pogodina *et al.*, 1981*a*), subacute variant of TBE in monkeys was characterized by prolonged incubation period (8—11 days) and a little prolonged course leading to death on days 15—24. Pathological lesions in the CNS in these monkeys were the following: a slow development of inflammatory changes with stepwise involvement of those areas of brain and spinal cord, which may be injured by TBE; the infiltrates and exsudative changes were less marked.

The chronic encephalitis in monkeys was characterized by a prolonged incubation period, progressive development of neurologic symptoms and occasionally by a partial repair of locomotion. Chronic pathological changes were seen in the CNS of monkeys which had been infected with the strain Vasilchenko and the mutants of the Pan strain and were examined on days 45, 90 383, 783 p.i. The pathomorphology was characterized by progressive development of lesions alike to a long-lasting inflammatory process, with infiltrative vascular reaction, neuronal changes of various degree and glial proliferation. Focal lesions in the CNS in chronic TBE were of various duration in contrast to subacute TBE, where the pathologic process revealed a rather short course. Freshly formed nodules of neuronophagy and perivascular infiltration at the sites of considerable neuronal loss were seen along with old reparative processes like development of glial scars and proliferation of microglia.

Regardless to the general diffuseness of the CNS lesions, certain selection in localization of the inflammatory reaction and necrotic changes was seen in the brain parenchyma. Large foci of lesions with severe damage and loss of neurons were observed in the brain cortex, thalamic nuclei, substantia nigra, midbrain, cerebellar nuclei and cerebellar cortex, in anterior horns of the cervical and lumbar parts of the spinal cord. Foci of nodular infiltration and perivascular cuffings were present, consisting of lympohistiocytic elements and plasmatic cells (Fig. 1). Polymorphonuclear leukocytes were rarely detected in the nodules of neuronophagy. In all parts of the brain distinct changes of neurons (vacuolization, pycnosis) pericellular and perivascular oedema were observed. Prolonged degenerative process was found in monkeys, examined on days 45, 90, 383 p.i. Fresh foci of neuron destruction, active neuronophagy, abundant perivascular and diffuse infiltration of the gray substance documented the developing encephalitis (Figs 2, 3). The lesions in cerebellar cortex and spinal cord showed features of longer duration than in the other parts of the CNS, as evidenced by the absence of leukocyte infiltration and substituting glial reaction. Active foci were observed on the background of other changes, areas of neuron loss, marked astrocyte reaction and developing substitution processes (Fig. 4). The gradual increase in severity and extent

of lesions in brain and spinal cord was due to summation of the foci of various age.

Especially severe and polymorhic changes of different age were seen in a monkey, sacrificed on day 383 p.i. with the Vasilchenko strain, revealing stable plegia of the left anterior extremity. The highly virulent TBE strain (Malenko *et al.*, 1982) was isolated from the spinal cord of this monkey. The cerebellum and the cervical section in the right spinal cord were the most injured (Fig. 5). Of interest was that in cerebellum as nowhere else the focal lesions showed various age, obviously because they had been developing since the most early period of disease. Whole lobules of the cerebellar cortex showed as honeycomb-like structure due to the loss of a great number of Purkinje cells (Figs 6, 7); the spongy degeneration extended to the granular layer, where reduction of granular cells was found along with marked proliferation of Bergmann's glia and astrocytes. Thick fibrillar network was formed in the molecular layer by branching of their hypertophic processes (Fig. 8). The remaining Purkinje cells showed distinct degeneration and atrophy. Fresh inflammatory foci with perivascular infiltration. neuronophagy, focal proliferation of micro- and oligodendroglia were detected along with old, in a certain way reparative changes in the cortex, and especially in the cerebellar nuclei. Separate focal round cell infiltrates were also observed in meninges. The character of morphological changes testified the long-term progressive course of pathological process in the CNS suggesting that the disease of monkeys described above was a chronic form of experimental TBE.

It should be noted that in the course of TBE the chronic process in CNS became less diffuse by time and stabilized in certain areas. Chronic changes were seen also in the monkey examined at recovery on day 783 p.i. with the Vasilchenko strain. Meninges were thickened, there were areas of loss of Purkinje cells in the cerebellum and of motoneurons in the spinal cord and a marked reparative reaction. Fresh foci of inflammation were not detected. No virus antigen was discovered by immunofluorescence in the cerebellum of this monkey. The infectious virus was recovered from the cerebellum, subcortical ganglia and internal organs by explantation method only (Pogodina *et al.*, 1981*b*). The virus isolated from the spleen was more virulent for young white mice weighing 5—6 g (virus dose $10^{4.6}$ LD_{50}/g) than that from the CNS (Levina, personal report).

Of great interest are the chronic pathological changes in the CNS of CPA-treated monkey infected s.c. with the strain Aina/1448. This monkey was observed for 279 days, but no clinical symptoms were noted. The absence of clinical manifestations can be explained by small focal changes observed in CNS similar to those seen in monkeys with chronic form of TBE. No specific inflammatory changes in the CNS were observed in other s.c. infected monkeys. Degenerative changes, destruction and loss of certain neurons were seen in the cortex of hemispheres, in the nuclei of brain stem, in the cerebellum and anterior horns of the spinal cord in addition to focal proliferation of astroglia and its degeneration. The changes observed in the CNS at late interval after s.c. inoculation were not seen in all CPA-treated animals.

No clear morphological differences were detected in the CNS of monkeys infected with TBE virus either CPA-treated or untreated.

Summing up, TBE virus persisted in all animals displaying chronic encephalitis. In addition, persistence of the virus and TBE virus antigen were detected in 12 monkeys infected s.c.

Discussion

The present study is a part of complex investigation on experimental TBE in monkeys. The demonstration of TBE virus persistence in monkeys (Pogodina *et al.*, 1981*a*) and the pathomorphological results presented in this report confirming the chronic, progressive development of encephalitis in these monkeys are of principle significance for understanding of TBE pathogenesis. The pattern and dynamics of morphologic changes reflected the peculiarities of the chronic forms of TBE representing a basis for their clinical manifestations. Subacute inflammatory degenerative process in the CNS was detected in 7 monkeys and chronic in 5; in addition, 11 monkeys were infected i.c. with the Vasilchenko strain and with attenuated variant of Pan strain of TBE virus; 1 monkey was infected s.c. with the strain Aina 1448 and treated with CPA. Monkeys were examined on days 15—24, 45, 90, 279, 383 and 783 p.i. Subacute and chronic encephalitis developed in monkeys infected by i.c. route. Asymptomic lesions were discovered in the CNS of monkey infected s.c. with the strain Aina/1448.

The pathomorphologic picture of chronic TBE in monkeys is highly variable as its clinical manifestations in monkeys (Komandenko *et al.*, 1972; Ilienko *et al.*, 1974) and man (Shapoval, 1976). The development of chronic TBE variants, its progressive course has been connected with several factors, the role of which is insufficiently known at present. The properties of virus, its capacity to persist, the genetic and immunologic status of organism, autoimmune reactions should be considered (Nathanson and Cole, 1970; Ilienko *et al.*, 1974, 1975; Panov *et al.*, 1975; Panov and Komandenko, 1975; Zhdanov and Gavrilov, 1975; Umansky, 1975). Analysis of autopsies and of experimental material showed that the pathomorphological specificity of chronic progressive CNS infection is manifested already in acute stage of its development (Robinson and Sergeeva, 1939; Frolova, 1962, 1964; Robinson and Frolova, 1964).

In comparison with enteroviral infections, TBE is characterized by a gradual, non-synchronous involvement of neurons by relatively slow development of their damage and of the inflammatory reactions and a lesser pronounced local cellular protective reaction in the CNS (Robinson and Frolova, 1964). The early pathology of astroglia in the pathogenesis of the chronic TBE is significant (Zlotnik, 1968; Robinson, 1975). Destruction and early proliferative reaction of astrocytes are caused by wide tropism of TBE virus, its capacity to attack not only neural cells, but also other structural elements of the brain as confirmed by immunofluorescence and electron microscopy (Albrecht, 1960; Boulton and Webb, 1971; Frolova *et al.*, 1975).

The decrease of phagocytic function of vascular histiocytes and microglia was detected during the chronic stage of TBE (Magasanik and Robinson, 1966).

The present examinations showed that the same TBE virus strain can cause different forms of infection (acute, subacute, chronic). The experimental development in monkeys of slow progressive encephalitis was followed up and its dynamics and pathomorphological peculiarities were determined. The latter explain to a certain degree the origin of progressive TBE variants in man. That is why monkeys are useful for investigation of pathogenesis of the chronic TBE. The results of our study contributed to the data in the literature on morphology of the chronic TBE in monkeys (Komandenko *et al.*, 1972; Ilienko *et al.*, 1974; Zlotnik *et al.*, 1976; Dremov, 1977; Andzaparidze *et al.*, 1978; Asher, 1979). The findings confirmed again the necrosis of neurons, their destruction being the result of direct effect of TBE virus as evidenced by immunofluorescence by long-term persistence of the virus in the host and possibility of 1st isolation at late intervals until day 383—783 p.i. On the whole, the prolonged development of the pathological process in the CNS is caused not only by direct effect of the virus on neurons, but also nonspecific factors common for other neuroinfections should be taken into account.

The chronic variants of TBE represent the possible manifestations of virus interaction with the host, development of which is connected with persistence of the infectious agent. Virological findings speak for the pathogenetic role of virus persistence in promoting the pathological changes in the CNS poiting out the possibility that degenerative changes of neurons are the distant consequence of TBE virus infection.

From our experiments in monkeys and Syrian hamsters infected s.c. with TBE virus and treated with CPA, follows that immunosuppression does not play the same role in different stages of chronic infection (Pogodina *et al.*, 1981*a*; Fokina *et al.*, 1982; Frolova *et al.*, 1982) and that immunosuppression was no prerequisite for its development. No significant influence was observed on frequency of persistent infection in monkeys receiving CPA at days —1, +1 (Pogodina *et al.*, 1981*a*) in a dose of 50 mg/kg body weight. At the same time the course of persistent infection revealed some peculiarities in immunosuppressed monkeys. Several changes in localization of persisting TBE virus were detected in CPA-treated monkeys early p.i. (Fokina *et al.*, 1982). Pathomorphological picture of chronic TBE developed after s.c. infection only in CPA-treated monkeys, but not in those animals which had not received CPA. Immunosuppression had clear activation effect in the case when CPA was introduced at late time of TBE virus persistence (Frolova *et al.*, 1982). In Syrian hamsters receiving CPA twice on day 170 p.i. we saw the following: virus reproduction increased, clinically marked disease with lethal result developed in separate animals, the severity and distribution of the changes in the CNS increased as compared with the group of animals, which did not receive CPA. As follows from this observation, the influence of CPA immunosuppression may activate the persisting TBE virus or may cause an initial progression of chronic TBE.

References

Albrecht, P. (1960): Pathogenesis of experimental infection with tick-borne encephalitis virus, pp. 247—259. In *Proc. Symp. Biol. Viruses Tick-borne Encephalitis Complex* (Smolenice, October 11—14).

Andzhaparidze, O. G., Rozina, E. E., Bogomolova, N. N., and Boriskin, Yu. S. (1978): Morphological characteristics of the infection of animals with tick-borne encephalitis virus persisting for a long time in cell cultures. *Acta virol.* **22**, 218—224.

Asher, D. M. (1979): Persistant tick-borne encephalitis infection in man and monkeys: relation to chronic neurologic disease, pp. 179—195. In *Arctic and Tropical Arboviruses; Proc. 2nd Intern. Symp. on Arctic Arboviruses*, Mont Gabriel, Canada. Academic Press, New York.

Boulton, P. S., and Webb, H. E. (1971): An electron microscope of Langat virus encephalitis in mice. *Brain*, **94**, 411—418.

Dremov, D. (1977): Comparative pathomorphological study of neurovirulence of attenuated variants of tick-borne virus encephalitis on monkeys (in Russian), pp. 95—112. *Nauchnye Osnovy Proizvodstva virusnykh i bakteriynykh Preparatov*, Tomsk.

Fokina, G. I., Malenko, G. V., Levina, L. S., Koreshkova, G. V., Rzhakhova, O. E., Mamonenko, L. L., Pogodina, V. V., and Frolova, M. P. (1982): Persistence of tick-borne encephalitis virus in monkeys. V. Virus localization after subcutaneous inoculations. *Acta virol.* **26**, 369—375.

Frolova, M. P. (1962): Materials on experimental morphological study of tick-borne encephalitis in experiment on monkeys (in Russian), pp. 38—39. In *Kleshchevoi Entsefalit i drugie arbovirusnye Infektsii*, Moskva-Minsk.

Frolova, M. P. (1964): Pathomorphology of experimental tick-borne encephalitis in monkeys caused by virus strains obtained in eastern areas of the Soviet Union (in Russian), pp. 40—42. In: *Kleshchevoi Entsefalit, Kemerovskaya kleshchevaya Likhoradka, gemorragicheskie Likhoradki i drugie arbovirusnye Infektsii*. Moskva.

Frolova, M. P., Tikhomirova, T. I., and Shestopalova, N. M. (1975): Some problems of pathogenesis of tick-borne encephalitis (in Russian), pp. 368—369. In M. P. Chumakov (Ed.): *Voprosy meditsinskoi Virusologii*. Moskva.

Frolova, T. V., Pogodina, V. V., Larina, G. I., Frolova, M. P. and Karmysheva, V. Ya. (1982): The activating effect of cyclophosphamide at late stages of tick-borne virus encephalitis persistence (in Russian). Vop. *Virus.* **27**, 578—585.

Ilienko, V. I., Platonov, V. G., Komandenko, V. G., Prozorova, I. N., and Panov, A. T. (1974): Pathogenetic study on chronic forms of tick-borne encephalitis. *Acta virol.* **18**, 341—347.

Ilienko, V. I., Platonov, V. G., and Komandenko, N. I. (1975): Biological variants of tick-borne virus encephalitis causing chronic forms of infection (in Russian), pp. 296—397. In M. P. Chumakov (Ed.): *Voprosy meditsinskoi Virusologii*, Moskva.

Komandenko, N. I., Ilienko, V. I., Platonov, V. G., and Panov, A. G. (1972): Clinics and several questions of pathogenesis of progredient forms of experimental tick-borne encephalitis (in Russian). *Nevropat. i Psichiat.* **72** (7), 1000—1007.

Magasanik, S. S., and Robinson, I. A. (1966): Pathogenesis of progredient forms of tick-borne encephalitis (in Russian), pp. 38—47. In *Voprosy Psikhiatrii i Nevropatologii* **12**, Leningrad.

Malenko, G. V., Fokina, G. I., Levina, L. S., Mamonenko, L. E., Rzhakhova, O. E., Pogodina, V. V., and Frolova, M. P. (1982): Persistence of tick-borne encephalitis virus in monkeys. IV. Virus localization after intracerebral inoculation. *Acta virol.* **26**, 362—368.

Nathanson, N., and Cole, G. A. (1970): Immunosuppression and experimental virus infection of the nervous system. *Adv. Virus Res.* **16**, 397—448.

Panov, A. G., Ilienko, V. I., and Komandenko, N. I. (1975): Correlation of morphological changes and distribution of virus in the nervous system in chronic tick-borne enecephalitis of monkeys (in Russian), pp. 393—394. In M. P. Chumakov (Ed.): *Voprosy meditsinskoi Virusologii*, Moskva.

Panov, A. G., and Komandenko, N. I. (1975): The question of pathogenesis of progredient forms of tick-borne encephalitis (in Russian), pp. 394—395. In M. P. Chumakov (Ed.): *Voprosy meditsinskoi Virusologii*, Moskva.

Pogodina, V. V., Frolova, M. P., Malenko, G. V., Fokina, D. I., Levina, L. S., Mamonenko, L. L., Koreshkova, G. V., and Ralf, N. M. (1981*a*): Persistence of tick-borne encephalitis virus in monkeys. I. Features of experimental infection. *Acta virol.* **25**, 337—343.

Pogodina, V. V., Malenko, G. V., Fokina, G. I., Levina, L. S., Koreshkova, G. V., Rzhakhova, O. E., Bochkova, N. G., and Mamonenko, L. L. (1981*b*): Persistence of tick-borne encephalitis virus in monkeys. II. Effectiveness of methods used for virus detection. *Acta virol.* **25**, 344—351.

Pogodina, V. V., Levina, L. S., Fokina, G. I., Koreshkova, G. V., Malenko, G. V., Bochkova, N. G., and Rzhakhova, O. E. (1981c): Persistence of tick-borne encephalitis virus in monkeys. III. Phenotypes of the persisting virus. *Acta virol.* **25**, 352—360.

Robinson, I. A. (1975): General questions of pathogenesis and pathologic anatomy of virus lesions of nervous system (in Russian), pp. 5—64. In M. B. Tsuker (Ed.): *Meningity i Entsefality u Detei*. Meditsina, Moskva.

Robinson, I. A., and Sergeeva, U. S. (1939): Partologoanatomic changes in the nervous system at tick-borne encephalitis (in Russian). *Arkh. biol. Nauk* **56** (2), 71—82.

Robinson, I. A., and Frolova, M. P. (1964): Some questions of pathologic anatomy and pathogenesis of tick-borne encephalitides (in Russian), pp. 36—38. *Kleshchevoi Entsefalit, Kemerovskaya kleshchevaya Likhoradka, gemorragicheskie Likhoradki i drugie arbovirusnye Infektsii*, Moskva.

Shapoval, A. M. (1976): *Khronicheskie Formy kleshchevogo Entsefalita*, Meditsina, Leningrad.

Umansky, K. G. (1975): Pathogenesis and therapy of progredient forms of tick-borne encephalitis (in Russian), pp. 400—401. In M. P. Chumakov (Ed.): *Voprosy meditsinskoi Virusologii*, Moskva.

Zhdanov, V. M., and Gavrilov, V. I. (1975): To the problem of the study of molecular basis of chronic virus infections (in Russian), pp. 562—564. In M. P. Chumakov (Ed.): *Voprosy meditsinskoi Virusologii*, Moskva.

Zlotnik, I. (1968): The reaction of astrocytes to acute virus infection of the central nervous system. *Brit. J. exp. Path.* **49**, 555—564.

Zlotnik, I., Grant, D. P., and Carter, J. B. (1976): Experimental infection of monkeys with viruses of the tick-borne encephalitis complex: Degenerative cerebellar lesions following inapparent forms of the disease or recovery from clinical encephalitis. Brit. *J. exp. Path.* **57**, 200—210.

Explanation of Micrographs (Plates XXXVIII—XLI):

Changes in CNS of monkeys infected i.c. with TBE virus, Vasilchenko strain; Figs 1, 2, 7, 8—90 days p.i.; Figs 3, 4, 5, 6 —383 days p.i.

Fig. 1. Neuronal loss, perivascular cuffings, lymphoid and histiocyte infiltration in the cortex of hemispheres. Nissl stain, × 200.

Fig. 2. Infiltrative focus in the thalamus; destruction of neurons, vascular infiltration, glial proliferation. Nissl stain, × 200.

Fig. 3. Active focus in substantia nigra showing neuronophagy, diffuse and perivascular infiltration, glial proliferation. Nissl stain, × 200.

Fig. 4. Focal astrocyte reaction in the cortex of hemispheres. Cajal's method, × 200.

Fig. 5. Anterior horn of the cervical section of spinal cord. Loss of neurons, diffuse glial proliferation, perivascular infiltrate. Nissl stain, × 200.

Fig. 6. Purkinje cell loss in the cerebellar cortex. Bergmann's glia shows proliferation. Nissl stain, × 200.

Fig. 7. Cerebellum, massive loss of Purkinje cells. In their place and in the molecular layer hyperplasia and hypertrophy of glial cells, formation of nodular infiltrates, abrupt destruction of the granular layer. Focal infiltration on meninges. Nissl stain, × 100.

Fig. 8. Loss of Purkinje cells, astrocyte proliferation and hypertrophy in the cerebellar cortex. Cajal's method, × 100.