These data, together with the results of the present investigation, suggest that one possible mechanism of the regulatory effect of VC on function of the retina is intensification of LPO in the latter. Since we know that the parameter most sensitive to the appearance of hydroperoxide groups in membrane lipids is ionic permeability [4], it can be tentatively suggested that a change in this parameter is reflected in the formation of the retinal electrical potential, which was observed in the present experiments.

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SOME BIOCHEMICAL AND IMMUNOLOGICAL CHANGES IN GUINEA PIGS WITH EXPERIMENTAL AMYOTROPHIC LEUKOSPONGIOSIS

V. I. Votyakov,* N. D. Kolomiets, V. P. Luchko, and A. G. Kolomiets

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Experimental investigations into the pathogenesis of slow infections of the human and animal CNS, caused by nonclassical viruses, have shown that the reticuloendothelial system plays an essential role in accumulation and dissemination of the pathogenic agents in the infected animals [13-15]. Meanwhile a distinguishing feature of these diseases is the absence of inflammatory reactions both in the CNS, in which the basic pathological changes are formed, and in other organs and systems which remain intact throughout the disease. Additionally, no immunological abnormalities have been found as yet in animals with reproduced slow infections of the CNS, including changes in cellular immunity [10, 14], with the exception of a significantly more frequent appearance of autoantibodies to nerve fiber proteins (NFP) in the blood serum of these animals [9, 19].

In recent years, in a study of the pathogenesis of slow infections and other progressive degenerative diseases of the CNS certain metabolic disturbances have been discovered [11, 12,

*Academician of the Academy of Medical Sciences of the USSR.

Department of Inhibitors of Virus Activity, Department of Slow and Chronic Virus Infections of the Central Nervous System, Belorussian Research Institute of Epidemiology and Microbiology, Ministry of Health of the Belorussian SSR, Minsk.

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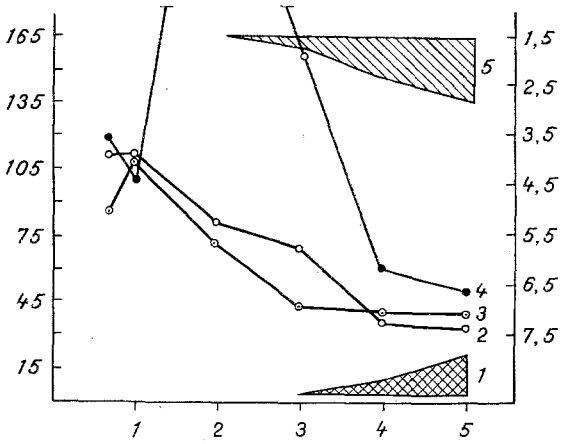

Fig. 1. Time course of accumulation of nonclassical ALS virus in tissues of the reticuloendothelial system and brain. Absissa: times of observation (in weeks); ordinate: on left — duration of incubation period (in days), on right — log LD_{50}. 1) Development of clinical picture of ALS; 2) brain; 3) spleen; 4) peripheral blood lymphocytes; 5) development of morphological features of ALS.

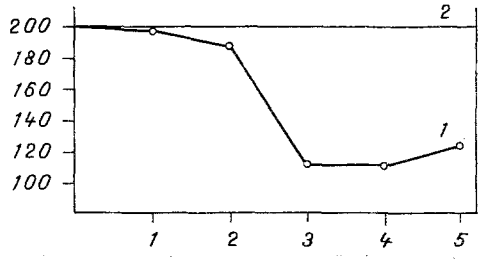

Fig. 2. Time course of change in serum complement level of animals with experimental ALS. Abscissa, times of observation (in weeks); ordinate, complement level (in units). 1) Complement level in infected animals; 2) complement level in animals of control group.

16]. For example, the serotonin concentration in the blood serum of hamsters infected with scrapie [17] was 24 times lower than in control animals.

Since persistance of the agent in the spleen and peripheral blood lymphocytes has also been established in amyotrophic leukospongiosis [20], it was decided to study certain immunological and biochemical aspects of the pathogenesis of experimental leukospongiosos in guinea pigs.

EXPERIMENTAL METHOD

Experiments were carried out on 32 guinea pigs weighing 250-300 g (16 animals in the experimental and 16 in the control groups). The animals of the experimental group received 2 injections, each of 0.25 ml of a 10% suspension of the brain of patient D., who died from amyotrophic leukospongiosis (ALS), into the retrobulbar space of both eyes. Animals of the control group received similar injections of a 100% brain suspension from a person dying from trauma. Blood was taken from the heart (under thiopental anesthesia), brain, and other organs (lungs, liver, spleen, lymph nodes, kidneys) of 3 animals from each group by total exsanguination 7, 16, 21, 28, and 35 days after infection, for virological and histological investigations, which were carried out as described previously [1-3].

TABLE 1. Detection of Antibodies to NFP in Blood Serum of Guinea Pigs

Time of testing, weeks	Experiment		Control	
	Number of animals with positive reaction/number of animals tested	Range of titers	Number of animals with positive reaction/number of animals tested	Titers
1	0/5	—	1/4	1:16
2	0/3	—	0/5	—
3	2/3	1:32—1:128	0/5	—
4	3/4	1:16—1:128	0/5	—
5	3/3	1:32—1:128	1/5	1:8

TABLE 2. LDH Activity and Pyruvate and Lactate Concentrations in Blood Serum of Guinea Pigs Infected with Nonclassical ALS Virus (M ± m)

Parameter studied	Control animals (n = 8)	Time of testing animals after infection, weeks				
		(n=3)	(n=3)	(n=3)	(n=4)	(n=3)
LDH, U/liter	620,8±10,9	610,0±16,5	583,0±20,6	486,0±62,9*	437,8±81,2*	429,0±36,0*
Pyruvate, mg %	1,26±0,03	1,23±0,07	1,3±0,0	1,9±0,0*	2,4±0,0*	3,2±0,0*
Lactate, mg %	29,87±1,89	28,83±1,05	67,80±3,47*	75,26±0,71*	72,63±2,29*	74,57±3,19*

Legend. *P < 0.05 compared with control.

To detect antibodies to NFP in the blood serum and to determine their level the indirect fluorescent antibodies method was used. Tests were carried out on longitudinal sections through the rat spinal cord [19]. The serum complement concentration was determined by the standard method [5]. Peripheral blood lymphocytes were isolated in a Verografin—Ficoll gradient. The blast transformation test on lymphocytes and the rosette formation test were carried out by the method described previously [14].

Lactate dehydrogenase (LDH) activity and concentrations of pyruvate and lactate were determined in the blood plasma [8, 18].

The significance of differences between parameters of guinea pigs infected with ALS and the control animals was determined by a biometric method [7].

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that, with the method of infection used, the nonclassical ALS virus was discovered in the highest titer in the early stages after infection (1-2 weeks) in the spleen. In the late stages (4-5 weeks) it was found at virtually the same level both in the spleen and peripheral blood lymphocytes and also in different parts of the CNS. During this period of development of the disease secondary "massive release" of the agent of ALS into the blood stream was evidently observed from sites of reproduction and (or) accumulation (spleen, CNS). This hypothesis was confirmed by data on the discovery of an increase in the concentration of the nonclassical ALS virus in tissues of various internal organs of the animal during this period of observation. Incidentally, in the visceral organs of the infected animals no pathological changes were visible under the light microscope.

The earliest histological signs of development of the pathological process were found in animals of the experimental group at the end of the second week of observation, mainly in the anterior horns of the thoracic portion of the spinal cord, and they were characterized by the development of degeneration in the cytoplasm of the neurons. Later the severity of the pathological changes steadily increased and the process spread to other parts of the spinal cord and brain, and by the 5th week of the experiment it was characterized by the basic features of ALS, present in high intensity in the guinea pigs, as described by the writers previously [4, 20]. By this time of observation the animals also had developed a clinical picture characteristic of ALS [1, 2, 4]. The development of severe damage to the CNS and manifestation of the clinical picture of the disease correlated to a certain degree with an increase in the concentration of the agents of ALS in the tissues, and also with changes in certain immunological and biochemical parameters. For instance, starting with the 2nd week

after infection, a significant decrease in the complement concentration was observed in the blood serum of animals of the experimental group (Fig. 2). Furthermore, 3 weeks after infection heterologous antibodies to NFP appeared in a titer of 1:16-1:128 (Table 1). Meanwhile, throughout the period of observation no changes were found in the relative numbers of B and T lymphocytes in the peripheral blood of the animals, and no differences in proliferation of the lymphocytes in response to phytohemagglutinin.

Biochemical tests of the blood serum revealed certain enzyme changes, indicating that definite metabolic disturbances were taking place in the infected animals. It will be clear from Table 2 that as early as two weeks after infection, there was a significant increase in the lactate concentration in the animals' blood serum, followed one week later by an increase in the pyruvate concentration, whereas LDH activity was lowered. The differences discovered in serum levels of lactate and pyruvate and of LDH activity in animals of the experimental and control groups are evidence of a change in the character of oxidative metabolism in the sick animals. These data showing an increase in the intensity of oxidative metabolism in the infected animals starting from the 2nd-3rd week after infection are in agreement with the parameters of ALS development given above and they correlate well with the change in body weight of the sick animals, which was particularly marked in the terminal stages of the disease.

We know that in ALS a disturbance of respiration of the spinal type is observed. It can be tentatively suggested that the increase in the intensity of glycolysis is a compensatory-adaptive reaction of the animal to hypoxia. The compensatory intensification of glycolysis may perhaps be the result of mobilization of the blood function for supplying oxygen and intermediates to the tissues. At the same time, considering data showing the character of oxidative metabolism is under the control of the nervous system [6], degeneration and death of motoneurons observed in ALS are evidently the pathogenetic mechanism responsible for the changes discovered.

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