the view that cooling leads to inhibition of metabolic processes in the tissue and to a decrease in their oxygen consumption (and for that reason the anoxic stimulus is less important) [14] is oversimplified and requires analysis and detailed scrutiny.

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EFFECT OF VITAMIN E AND THYMALIN ON THE DEVELOPMENT OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

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It was reported previously that during the development of experimental allergic encephalomyelitis (EAE) lipid peroxidation (LPO) is activated in the blood and brain of animals immunized with an encephalitogenic emulsion [9]. At the same time it has been shown that there is a marked deficiency of the T-lymphocyte population and their functional activity is depressed in EAE and multiple sclerosis [3, 5, 11, 12, 14].

In this investigation, to establish the pathogenetic role of intensification of LPO in EAE, an attempt was made to reproduce the neuroallergic process in noninbred albino rats, which are resistant to EAE, against the background of avitaminosis E, i.e., when the antioxidant balance in the body is disturbed, and to demonstrate whether it is possible to correct the pathological process by acting on the T-cell stage of immunity. For this purpose, in experiments on guinea pigs, which are sensitive to EAE, immunization with encephalitogenic material was carried out after administration of vitamin E and the polypeptide thymus preparation — thymalin. There is evidence in the literature that the number of T suppressor cells

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TABLE 1. Concentration of LPO Products in the Brain of Rats Immunized with Encephalitogenic Emulsion (M ± m)

Experimental conditions	Diene conjugates, μmoles/g tissue	MDA, nmoles/g tissue
Control	1,8±0,06	26,8±0,8
Avitaminosis E + EAE	14,9±0,4	98,8±2,8
p	<0,001	<0,001
p_1	<0,001	<0,001
Avitaminosis E + thymalin	4,6±0,3	33,2±0,3
With no signs of EAE p	<0,01	<0,05

Legend. p) Significance of differences from control, p_1) the same, from group of animals with no signs of EAE.

is increased and their functional activity enhanced under the influence of thymalin [6], and also data on an increase in the number of T lymphocytes and, in particular, of "active" T lymphocytes, during the treatment of patients with multiple sclerosis with thymalin [3].

EXPERIMENTAL METHOD

Experiments were carried out on 43 noninbred male albino rats weighing 150-180 g and on 40 guinea pigs weighing 250-300 g. For 100 days the rats were kept on a diet without vitamin E, and 20% of their daily ration consisted of lard. The rats were then immunized. The encephalitogenic emulsion consisted of Freund's complete adjuvant (40 mg of a killed culture of BCG in 1 ml) and a 50% heterologous brain homogenate in physiological saline. The emulsion was injected into the hind footpads in a dose of 0.1 ml. Three days before immunization, 15 rats were given an intramuscular injection of 250 μg thymalin, prepared as a freeze-dried powder at the "Lenmyasomolprom" Medical Preparations Factory by the method in [7]. The control group consisted of 15 intact rats immunized with the encephalitogenic material. On the 7th day after the beginning of immunization neurocytotoxins were determined in the animals' blood [1]. The brain concentration of diene conjugates [8] and of malonic dialdehyde (MDA) [2] was determined in rats with EAE and in animals which remained resistant to injection of the encephalitogenic material. In the experiments on guinea pigs, 7 days before immunization with the encephalitogenic emulsion and during the 20 days after immunization, 10 animals were immunized daily by intramuscular injection of 100 mg/kg of vitamin E (group 1). Three days before immunization, each animal of this group was given an intramuscular injection of 250 μg thymalin. In group 2 the guinea pigs received an injection of thymalin (250 μg) alone 3 days before immunization with the encephalitogenic material. The guinea pigs of group 3 received vitamin E alone by the same scheme as those of group 1. Intact guinea pigs immunized with the encephalitogenic emulsion were used as the control.

For morphological identification of the presence of EAE, histological and electron-microscopic investigations were made of the lumbar region of the spinal cord from seven rats with clinical manifestations of the process.

EXPERIMENTAL RESULTS

After the rats had been kept for 100 days on the diet they began to show signs of avitaminosis E: loss of body weight, apathy, jaundice, and falling out of the hair. Of 13 animals immunized with the encephalitogenic material against the background of avitaminosis E (group 1) nine developed EAE (on the 16th-22nd day after the beginning of immunization), which was manifested as the appearance of pareses and paralyses of the limbs and sphincters. In the group of rats which received thymalin before immunization (group 2) signs of EAE were observed in only two of fifteen animals. All rats of the control group remained resistant to injection of the encephalitogenic emulsion.

Morphological investigation of the brain showed the presence of typical allergic encephalomyelitis with perivascular infiltration of the cerebral vessels, meninges, and subarachnoid

TABLE 2. Effect of Vitamin E and Thymalin on Development of EAE in Guinea Pigs

Experimental conditions	Number of animals				
	in experiment	with paralyses	which died	which did not develop the disease	incubation period, days
Vitamin E + thymalin	10	3	1	7	16—21
Vitamin E	10	10	10	—	11—14
Thymalin	10	10	10	—	10—13
Control	10	10	10	—	11—14

spaces. Electron microscopic investigations showed vacuolation of the cytoplasm and widening of the perinuclear space in the oligodendrocytes. The cytoplasm of the macrophages contained fragments of myelin sheaths. Demyelination was apparent as unwinding of the myelin lamellae and complete exposure of the axis cylinders.

Investigation of neurocytotoxins in the blood serum on the 7th day after the beginning of immunization with the encephalitogenic material showed that they were present in the blood of the rats of group 1 but absent in the control animals and those of group 2. These results agree with data obtained previously in experiments on dogs. The presence of neurocytotoxins was demonstrated starting from the 7th day after the beginning of immunization in the blood of dogs which subsequently developed EAE, and conversely, neurocytotoxins were not detected in the blood of animals that remained resistant to injection of the encephalitogenic material at the same time of immunization [1].

The results of the study of the concentrations of diene conjugates and MDA in the brain tissue of the control and experimental rats are given in Table 1. The concentration of diene conjugates in the animals of group 1 was 8.2 times higher than in the control group and 3.2 times higher than in the rats of group 2. The MDA concentration in the rats of group 1 was 3.7 times higher than the control values and 2.9 times higher than in the animals of group 2.

The results thus confirm the important role of activation of LPO in the development of EAE.

Avitaminosis E is currently placed in the group of free-radical diseases [4, 13], which share a common pathogenetic factor, namely an increased intensity of peroxidation of membrane phospholipids, with a consequent disturbance of membrane functions. At the same time, we know that keeping rats on a diet deficient in vitamin E leads to depression of T-lymphocyte function [10].

These results suggest that the breakdown of resistance to EAE in noninbred albino rats was due both to a fall in the level of oxidative defense of the membranes and inhibition of function of T-suppressor cells. Injection of thymalin, evidently by activating suppressor T-cells, prevents the appearance of neurocytotoxic antibodies in the rats of group 2 and also prevents the development of EAE and activation of LPO processes in the brain accompanying it.

This hypothesis is confirmed by the results of the experiments on guinea pigs. Injection of vitamin E together with thymalin prevented the development of EAE in seven of the 10 animals (Table 2). Two guinea pigs developed EAE, but with a mild course, followed by recovery of all the motor functions. Only in one case did EAE develop and follow an acute course, terminating in the animal's death on the 17th day after the beginning of immunization with the encephalitogenic emulsion. The incubation period in this case was lengthened to 16-21 days, by contrast with the control, in which the incubation period of development of EAE was 11-14 days. All animals of the control group died within 3-4 days of developing the first clinical symptoms. No prophylactic effect was observed in groups of animals immunized with encephalitogenic material, receiving each preparation separately. The process developed and followed an acute course, and all the animals died within 1 week of the beginning of the disease. The incubation period in these groups of animals did not differ from the control.

The study of the character of the effect of vitamin E and thymalin on the development of EAE is interesting not only to explain the pathogenesis of neuroallergic lesions of the nervous system, but also in connection with the possible use of these preparations to correct neuroimmune processes in clinical medicine.

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