Thus the investigation showed for the first time that splenopentin can give an effective immunocorrective action on experimental alcohol intoxication in noninbred animals and animals predisposed to alcohol consumption. The mechanisms lying at the basis of these phenomena may be linked, first, with activation of lymphocyte proliferation [9] and inhibition of the function of suppressor T cells, which is activated by alcohol [2], and on the other hand, with its effect on the neurotransmitter systems of the brain, a disturbance of whose function lies at the basis of the pathogenesis of alcoholism [1], and may also be the cause of development of the secondary immunodeficiency state [3].

These results evidently indicate two possible situations for the potential clinical application of splenopentin: to correct disturbances of immunity characteristic of alcoholism [4, 5, 8] and to depress pathological craving for alcohol.

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EXPERIMENTAL INFECTION CAUSED BY THE ISSYK-KUL' ARBOVIRUS

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The Issyk-Kul' virus was first isolated in 1970 from the common noctule (*Nyctalus noctula*), collected in the Issyk-Kul' Depression of the Kirghiz SSR [7]. Later this virus was isolated from other species of bats, ticks, mosquitoes, and birds in Kirghizia and Tadjikistan [1, 2]. Bats are the reservoir of this virus in nature [3, 4]. Issyk-Kul' virus is pathogenic for man; it has been isolated from the blood of a febrile patient who had been collecting bats during the 5 days before his illness (in Issyk-Kul' Depression). On the 20th and 40th day after the beginning of the illness, complement-fixing antibodies were found in the patient's blood in a titer of 1:16 and 1:32 respectively. Virus-neutralizing antibodies were detected on the

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40th day. A similar disease also has been found in Dushanbe, involving a worker living uninterruptedly in the city [5, 6]. According to data obtained by Pak [5], after an incubation period of about 5 days the disease begins acutely: a rise of temperature to 39°C (or above), facial hyperemia, rigors, headache, dizziness, nausea and general weakness. Muscular pains, especially in the gastrocnemius and the spinal muscles. A dry cough, with solitary dry crepitations in the lungs. As regards the cardiovascular system moderate hypotension, bradycardia, and muffled heart sounds are observed. The liver and spleen are not enlarged. The ESR is moderately raised, The febrie period lasts about 5 days.

No data on pathogenicity of Issyk-Kul' virus for laboratory animals can be found in the literature.

The aim of this investigation was to study the pathogenic properties of Issyk-Kul' virus for green monkeys, Syrian hamsters, and albino mice, infected in different ways.

The character and degree of involvement of the organs were studied (consultant histologist Professor B. S. Gusman, M.D.) and compared with the titer of the virus in these organs and in the blood of the infected animals, and preservation of the virus also was studied after freeze—drying at different temperatures and in media of different pH.

EXPERIMENTAL METHOD

Issyk-Jul' virus (strain LEIV K-315) was isolated from a bat by intracerebral inoculation of newborn mice [8].

In this investigation the virus used had been subcultured in the brain of noninbred albino mice aged 2 weeks and weighing 5-6 g. The infectious titer in brain tissue, which was used in the experiments to inoculate the animals, reached 7.5 log LD_{50}/0.03 ml.

Animals. Albino mice weighing 5-6 g, Syrian hamsters weighing 50-60 g, and green monkeys aged 1.5-2 years.

The albino mice and hamsters were inoculated intracerebrally and subcutaneously by the usual method. Altogether 720 albino mice, 20 Syrian hamsters, and four green monkeys were used in the experiments.

Experiments on monkeys: inoculation and taking of blood were carried out under hexobarbital anesthesia. The first blood sample was taken before inoculation, the second before autopsy of the animal. Blood serum was tested by the complement fixation reaction (CFR) and the virus neutralization reaction (NR).

The titer of the virus was determined in the blood and organs taken from the animals at autopsy (brain, lungs, liver, spleen, kidneys). Pieces of the same organs were fixed simultaneously in 10% neutral formalin for histological investigation. Organs of albino mice were taken for investigation from animals which died, those from hamsters (which did not die) 14 days after the beginning of infection, and from monkeys after 50 days (period of observation).

Pieces of organs fixed in 10% neutral formalin were treated by the traditional method and embedded in paraffin wax blocks. Thin paraffin sections were stained with hematoxylin-eosin and by Nissl's method, and in certain cases, by Brachet's method.

The virus-containing mouse brain suspension was lyophilized down to 1 ml at —20°C in the course of 24 h. The same suspension was tested for preservation of the virus in it at 24°C and 56°C and in media of different pH values.

EXPERIMENTAL RESULTS

Ability of the virus to survive under different external environmental conditions was studied. At 24°C, in the course of 24 h the infectious activity of the virus was preserved, but its titer fell by 2.75 log LD_{50}/0.03 ml. After heating to 56°C for 10 min the titer of the virus fell considerably, but even after 90 min its infectious properties still remained and the titer of the virus was down to 1.75 log LD_{50}/0.03 ml. Preservation of the infectious activity of the virus was greater at pH 7.2. After lyophilization, the titer of virus surviving for 10 months reached 3.75 log LD_{50}/0.03 ml.

These results are evidence that Issyk-Kul' virus is comparatively resistant in the external medium.

Albino mice infected intracerebrally died on the 6th day. Histological investigation of the organs revealed meningoencephalitis in the brain. The pia mater was edematous and abundantly infiltrated. Cortical neurons were dystrophic. Mild diffuse gliosis was present with solitary nodules of gliosis in the deep layers of the cortex. In the white matter there were abundant perivascular foci of infiltration composed of glial cells and glial nodules. Focal interstitial pneumonia was present in the lungs. Hepatitis with dystrophic changes in the hepatocytes and foci of lymphoid infiltration were present in the liver. The kidneys showed nephritis, and the spleen presented a picture of inflammation.

After subcutaneous infection mice died on the 7th day. The following lesions were observed in the organs. Mild edema and congestion of the vessels in the brain; severe congestion of vessels of the pia mater, and solitary nodules of gliosis in the white matter Marked dilatation of the vessels was present in the lungs, with hemorrhages into the alveoli in some places Edema of the alveolar septa was accompanied by mild thickening of these structures due to perivascular infiltration. Dystrophic changes were present in the bronchial epithelium, together with areas of metaplasia. Severe congestion, accompanied in some places by hemorrhages, were present in the liver, with marked dystrophic changes in the hepatocytes and swelling of the Kupffer cells. Severe circulatory disturbances were present in the kidneys, with dystrophic changes in the epithelium of the convoluted tubules. The spleen showed severe congestion. Thus death of mice infected intracerebrally and subcutaneously took place as a result of damage to the CNS and inflammatory and dystrophic changes in the internal organs (the virus was isolated from the blood and organs).

Infection of hamsters intracerebrally and subcutaneously did not cause their death, so that after 14 days the animals were killed and examined. In the organs of hamsters infected intracerebrally, histological investigations revealed the following lesions: meningoencephalitis in the brain, focal interstitial pneumonia in the lungs. Venous stasis was present in all the internal organs.

In hamsters infected subcutaneously, mild meningoencephalitis was present in the brain. Focal bronchopneumonia was found in the lungs and glomerulonephritis in the kidneys. The spleen showed an immunomorphological reaction (the virus was isolated from the blood and organs).

Green monkeys infected intracerebrally and into the right atrium did not die. Clinically manifest illnesses were not observed, with the exception of reduction of activity and loss of appetite for the first 3 or 4 days. At autopsy only congestion of the vessels of the brain and lungs was noted.

In monkeys infected intracerebrally, histological study of the organs revealed the following changes: marked meningoencephalitis, with congestion of the vessels of the pia mater and brain substance in the brain. Severe pericapillary and pericellular edema was present. In all parts of the brain there was diffuse gliosis and also numerous nodules of gliosis which could be unconnected with the vessels. The vascular endothelium was swollen. The neurons were severely dystrophic, with pycnosis tigrolysis, and conversion into ghost cells. Intensive lymphoid infiltration of the edematous meninges could be seen.

In the lungs thrombosis of the vessels was accompanied by extensive areas of hemorrhage, and edema of the alveolar septa.

The heart showed interstitial myocarditis, with marked dystrophic changes and there was an extensive focus of lymphoid infiltration of interstitial tissue. Marked perivascular edema and perivascular lymphoid infiltration could be observed.

Circulatory and dystrophic changes were present in the liver.

Glomerulonephritis was present in the kidneys, with severe dystrophic changes in the epithelium of the convoluted tubules, in whose lumen erythrocytes accumulated.

An immunomorphological reaction could be seen in the spleen.

Lesions affecting all organs also were found in green monkeys infected in the right atrium.

Marked meningoencephalitis was present in the brain with severe circulatory disturbances, and foci of stasis could be seen with hemolysis of erythrocytes in the dilated vessels of the pia mater and capillaries. Severe dystrophic changes affected the neurons, with abundant evidence of neuronophagy. Extensive diffuse gliosis was present in the region of the gray and white matter of the brain. Severe edema of the meninges and perivascular edema of the brain substance could be observed. The endothelium of the blood vessels was swollen. Circulatory disturbances and an immunomorphological reaction, with thrombosis of blood vessels and hemorrhages were present in the lungs. There was also edema of the alveolar septa and foci of atelectasis. Circulatory disturbances and dystrophic changes in the hepatocytes were present in the liver, accompanied by hepatitis. The kidneys showed glomerulonephritis, accompanied by severe congestion of the blood vessels and hemorrhage into the lumen of the convoluted tubules and the glomeruli. The epithelium of the convoluted and straight tubules showed severe dystrophic changes.

Interstitial myocarditis was present in the heart. There were many foci of lymphoid infiltration of the interstitial tissues. The muscle fibers showed dystrophic changes and there was edema and diffuse infiltration of the epicardium.

The spleen was reactive, with greatly widened pale centers of their follicles, with numerous mitotically dividing cells. The sinuses were filled with desquamated cells.

The virus was isolated from the blood of a monkey infected in the atrium, and also from the blood and organs of all the experimental monkeys. In addition, specific virus-neutralizing antibodies were present in the blood of all the animals, with the highest index (4.75 log) in a monkey infected intracerebrally.

As the experiments showed, the study of the viremia, the character of the distribution and severity of the lesions in the organs, and also the affinity for the tissues determine the pathogenic properties of this virus. Issyk-Kul' virus possesses pantropic properties and induces a generalized infection in experimental animals, irrespective of the mode of infection.

The specific nature of the lesions found in monkeys and other animals in the brain lungs, liver, kidneys, and myocardium is confirmed by the presence of the virus in these organs and also the presence of specific antibodies in the monkeys' blood.

Issyk-Kul' virus causes a symptom-free infection in green monkeys. Humoral antibodies circulating in the blood stream of these monkeys did not rid then of the virus or of development of lesions in the organs, which could be detected after 50 days.

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