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Soviet Search for Viruses That Cause Chronic Neurologic Diseases in the U.S.S.R.

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Viral infections of the central nervous system are usually thought to produce acute illnesses such as aseptic meningitis, encephalitis, or paralytic poliomyelitis. The one exception known to Western neurologists and virologists has been von Economo encephalitis, a disease of unknown but possibly viral etiology, where Parkinsonism develops after a latent period. Since persistence of virus in human and animal organisms after acute infection of the central nervous system has been disputed and factors precipitating overt disease have not been determined, relatively little is known about the viral pathogens which may take part in chronic forms of neurologic disease.

The recovery of filterable agents from animals with chronic diseases, such as scrapie, Visna, and Aleutian disease of mink, which are characterized by pathological changes suggesting degenerative or demyelinating diseases, has created interest in the role of slow viruses as pathogens of the central nervous systems of animals and man.

Soviet research workers have long been concerned with the relation of virus to the etiology of chronic, progressive, or relapsing diseases of the nervous system. Indeed, they were among the first to search for viruses in tissues of patients suffering from tick-borne encephalitis (formerly called Russian spring-summer encephalitis) in its chronic and progressive form. The viral etiology of other chronic or progressive diseases of the nervous system, such as amyotrophic lateral sclerosis or multiple sclerosis, is generally accepted by scientists in the U.S.S.R.

While visiting several clinical and research institutions in the U.S.S.R. in May and June of 1964 (*1*), we had an opportunity to observe Soviet research on the viral etiology of human chronic neurologic diseases, and we now offer a summary and evaluation of this research.

The authors were the members of the U.S. delegation to the U.S.S.R. on "Latent Infections, Chronic Intoxications, and Genetic Disorders of the Nervous Systems of Man and Animals" sponsored, within the framework of the U.S.S.R.–U.S.A. cultural exchange program, by the United States Public Health Service, 17 May to 12 June 1964. The names of the authors appear in alphabetical order. Dr. Brody is with the Arctic Health Research Center, Anchorage, Alaska. Dr. Hadlow is with the National Institute of Allergy and Infectious Diseases, Hamilton, Montana. Dr. Hotchin is with the New York State Department of Health at Albany. Dr. Johnson is with the Cleveland Metropolitan General Hospital, Cleveland, Ohio. Dr. Koprowski is with the Wistar Institute, Philadelphia, Pennsylvania. Dr. Kurland is with the National Institute of Neurological Diseases and Blindness, Bethesda, Maryland.

Possible Viral Etiology of Amyotrophic Lateral Sclerosis

In 1963, Zilber *et al.* reported that intracerebral inoculation of homogenates of medulla and spinal cord from patients that died of amyotrophic lateral sclerosis (ALS) induced a progressive neurologic disease in rhesus monkeys after a latent period of 1 to 3 years. Monkeys inoculated with specimens from three out of six patients developed asymmetrical muscular atrophy of the upper and lower extremities with increased tendon reflexes. The disease progressed over a period of 8 months to 3 years. Homogenates from the brain of affected monkeys and, in one case, homogenates of brains two passages after the initial inoculation, have produced similar disease in other monkeys. However, the inoculum causing disease in monkeys was nonpathogenic for mice and other laboratory animals.

Neuropathologic studies of specimens from man and monkey by Bunina *et al.* at the Neurological Institute of the Academy of Medical Sciences in Moscow indicated that inoculated monkeys had a "clear-cut" loss of ganglion cells of the anterior horns, accompanied by pronounced glial reaction, satellitosis, and neuronophagia. Oxyphilic intracytoplasmic inclusion bodies of 0.5 to 3.5 $m\mu$ were found in the nerve cells of the anterior horns. The motor cortex showed focal loss of large pyramidal and Betz cells in the third and fifth layers. There was pallor in parts of the lateral columns corresponding to the location of the pyramidal tracts in sections stained by Spielmeyer's method. The morphologic changes in monkeys were described as being similar to, but less intense than, those observed in patients, presumably because the monkeys were killed before the disease had reached an advanced stage. The Soviet scientists concluded that their experiments "proved

the possibility of reproducing ALS in *Macaca rhesus* monkeys" and that " . . . the clinical and pathologic picture was similar to that observed in human beings."

At the Gamalaya Institute of Epidemiology and Microbiology and at the Institute of Experimental Pathology in Sukhumi, we examined three inoculated monkeys showing signs of the disease described by Zilber *et al.* (*2*). The monkeys had asymmetrical atrophy and weakness of the lower extremities. No atrophy or fasciculation of the tongue, other bulbar musculature, or upper extremities was noted. Soviet workers have found involvement of the upper extremities in monkeys previously inoculated, but abnormalties of the bulbar musculature have not been observed. Brisk tendon reflexes were observed in the inoculated as well as uninoculated monkeys. Spontaneous neurologic disorders were also observed in the monkey colonies in Sukhumi, but the cause and pathology of these illnesses has not yet been systematically studied (*3*). In Sukhumi, inoculated monkeys were not isolated from one another or from uninoculated animals during the incubation period of disease.

While in Moscow, we examined sections of brain and spinal cord from monkeys with the experimental disease; however, it was difficult to confirm the changes which had been reported. Paraffin-embedded blocks of spinal cord from two monkeys inoculated with the ALS material were examined by A. Hirano (Montefiore Hospital, New York City) and J. M. R. Innes (Bionetics Research Laboratories, Inc., Falls Church, Va.), and by us in the United States. No microscopic changes resembling those of ALS were found in numerous sections of the spinal cord stained by a variety of methods. Specifically, there was no demyelination of pyramidal tracts, no significant loss or change in the motor neurons, and no significant atrophy of the anterior roots. Furthermore, inflammatory changes were not seen, nor were eosinophilic cytoplasmic inclusion bodies identified in neurons.

Thus, although we have observed that inoculated animals manifest signs of motor disease, we were unable to find unequivocal pathologic changes in the central nervous systems of the animals that received the ALS material. To date, no microscopic studies of muscle or peripheral nerve are available, and no electromyographic studies have been made; therefore, neither clinical nor pathological localization of disease in monkeys appears to have been definitely established. Since all the monkeys have been inoculated with crude brain homogenates, the physical properties of a "transmissible" agent have not been determined.

However, the agent has now been made available to the National Institute of Neurological Diseases and Blindness and is currently the subject of further intensive investigations.

Possible Viral Etiology of Multiple Sclerosis

In 1944–45 Shubladze isolated a virus from a case of acute encephalitis which she and her co-workers believed to be a cause of acute and chronic encephalomyelitis and some forms of multiple sclerosis. They reported that this agent produced demyelinating lesions in mice and that it was neutralized by a number of serums obtained from patients with either disseminating encephalomyelitis or multiple sclerosis (*4*). Vaccines produced from this agent were widely used in the treatment of multiple sclerosis in the U.S.S.R. Only a few reports have appeared in Soviet literature on the success of this treatment. Subsequently, investigators have identified this virus as being related to, if not identical to, rabies virus (*5*). In view of the identification of the virus as a strain of rabies virus and the lack of controlled studies of the efficacy of the vaccine, this work was generally discounted in Western countries. However, the use of the vaccine has continued in the Soviet Union.

From additional studies in Shubladze's laboratory, other agents have been isolated from four cases of multiple sclerosis and acute disseminated encephalomyelitis which are said to be identical with the original isolate (*6*). Shubladze and her group believe that, whereas the virus is a variant of rabies virus, it can cause multiple sclerosis in humans.

Results of studies by Bychkova at the Ivanovsky Institute of the Academy of Medical Sciences in Moscow indicate that all of the virus strains are pathogenic for mice and that after either intracerebral or extraneural inoculation the animals become ataxic in 4 to 5 days and die. Pathologically, mice show a severe degree of inflammation of meninges and brain substance. Cytoplasmic eosinophilic inclusion bodies were apparent and widespread in the sections reviewed by us. Some inclusions had no internal structure (Lyssa bodies) whereas others had an internal structure typical of Negri bodies. No demyelinating lesions were seen on the sections. However, the Soviet workers had a photomicrograph showing rather diffuse demyelination.

There seems to be little doubt that the original virus strain and those subsequently isolated by Shubladze and her group are strains of rabies virus. Both a small difference in susceptibility of mice after intracerebral or extraneural inoculation and the presence of Negri bodies suggest a street strain of rabies, but the short incubation period is more consistent with characteristics of a fixed strain of rabies. However, the severity of inflammation and the alleged demyelination have not been observed in fixed- or street-rabies virus infection of mice. Even if the agent does not directly participate in the etiology of multiple sclerosis, American and Soviet workers feel that this agent should be investigated as an interesting variety of rabies virus.

Chronic Infection with Tick-Borne Encephalitis Virus

Soviet scientists began a systematic study of tick-borne encephalitides (TE) in 1937. In that year, Zilber led a Soviet scientific mission to eastern Siberia to investigate reports of numerous cases of encephalitis. Included in this group were many of the prominent Soviet virologists of today: Chumakov, Levkovich, Shubladze, and Soloviev. During the course of this study the TE virus was isolated, and the tick *Ixodes persulcatus* was identified (*7, 8*) as the vector.

Pavlovsky, in the following year, led an expedition, also to Siberia, with Smorodintsev as virologist. At this time, Pavlovsky developed his concept of the natural foci of infection and Smorodintsev produced the first mouse brain vaccine against tick-borne encephalitis.

Neurologists in the Soviet Union believe that chronic progressive disease of the central nervous system can follow infections with TE virus. Mental disturbances, sometimes of a progressive nature, are considered the most common sequelae of infection, but

such diseases as Parkinsonism, amyotrophic lateral sclerosis, and multiple sclerosis are also thought, by some neurologists, to follow infection (*8*). The second most common sequela of TE is a characteristic weakness and atrophy of muscles of the shoulder girdle; this, too, is believed to progress in some cases (*8*). Another clinical syndrome thought to occur primarily after TE virus infection is *epilepsia partialis continua*. In the U.S.S.R., this focal convulsive disorder is referred to as Kozhevnikov's epilepsy after the Russian neurologist who first described it in 1894 (*9*), and is more commonly seen as a sequela of TE than of any other clinical disorder; it is often considered a progressive disease and sometimes leads to death.

Investigators feel that the progressive nature of the disease is due to the persistence of the TE virus in the central nervous system, and in 1940 Chumakov recovered the TE virus in adult mice which had been injected with brain tissue removed surgically from a patient with Kozhevnikov's epilepsy (*10*). Another isolation of the virus from a patient with chronic sequelae after infection is said to have been made by Schubin in 1956. However, these two observations remain unconfirmed. At varying intervals after the onset of acute encephalitis, virus has been isolated from patients' blood. However, no systematic attempt has been made to determine the conditions under which virus can be isolated from patients with progressive disease.

Sequelae after infection with TE are not common. For example, Kozhevnikov's epilepsy is said to occur in only 1 or 2 percent of patients with diagnosed TE infection (*8*). Undoubtedly, severe sequelae can follow infection with this virus, but whether these conditions are indeed progressive and related to the persistence of virus in the patients' tissue is not yet fully established.

Vilyuisk Encephalomyelitis. Vilyuisk encephalomyelitis, a chronic neurologic disease possibly of viral etiology, is confined to a small area in Siberia inhabited by the Yakut Tribe. Explorers visiting the area north of Yakutsk between 1850 and 1900 described a strange debilitating neurologic illness among villagers along the Vilyuy River. The first clear descriptions of this illness appeared in the Soviet literature in 1926 and 1930 (*11*).

As concern about the illness increased, Soviet medical teams went to the area each summer from 1954 to 1957 to conduct clinical, virological, and epidemiological investigations. During the years of this study, fewer than 200 cases occurred. Of 290 recorded cases, 112 occurred in 50 families, and except for one case, all observed were among Yakuts (*12*). The age at onset is from 8 to 45 years, and the illness is more common among females (*13*).

Clinically, Vilyuisk encephalomyelitis occurs in two types, acute and chronic. On infrequent occasions when an acute phase occurs it may begin suddenly or after a period of depression, and it may last from several days to a month. It is characterized by mild influenza-like symptoms or by cranial nerve disturbances, extrapyramidal rigidity, tremor, and deep lethargy (*14*).

The chronic phase is sometimes characterized by increasing spasticity and dysfunction of the cranial nerve or extrapyramidal tracts. Personality changes and forgetfulness develop, progressing to severe dementia, with the patient often surviving in a demented, spastic state for 20 years or more (*15*).

The pathology of the central nervous system is that of intense panencephalomyelitis with inflammation and loss of neurons occurring at all levels. Hydrocephalus is common but demyelination does not occur.

Eleven strains of virus isolated in adult mice injected with brain cerebrospinal fluid, blood, and throat washings obtained from clinical cases (*12*) were sent to Casals at the Rockefeller Institute, who found them closely related to Mengo virus and encephalomyocarditis virus (EMC) (*16*). Possibly the isolated strains represent mouse viruses not related at all to the etiology of the disease. Nevertheless, the possibility that this virus is, indeed, the cause of the bizarre neurologic illness encountered in the Vilyuisk area cannot be excluded.

A cooperative field and laboratory study to be carried out by a team of Soviet scientists and a team from the United States Public Health Service is planned.

Encephalitis Lethargica (von Economo). Since 1932, Khodos and his groups in Irkutsk, Siberia, have described several hundred cases of a disease characterized by lethargy, hypersalivation, and diplopia leading to Parkinsonism within 2 to 3 months after onset. In some cases, oculogyric crises have also been observed. Several cases of this disease have occurred each year without particular seasonal or geographic concentration. Although the onset is sometimes preceded by trauma, the clinical pattern in most cases was remarkably similar to that of von Economo disease. No attempts have yet been made to isolate an infectious agent either in the acute or in the chronic stages of the disease (*17*).

Slow Viruses in Animals. Geneticists working with sheep and mink indicated that neither scrapie nor Aleutian disease has been observed in the U.S.S.R. Results of experimental work with mouse lymphocytic choriomeningitis conducted by Remezov (*18*) indicate that the persistent tolerant infection of mice with virus is not accompanied by late onset of overt disease.

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