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Surveys of Foreign Scientific and Technical Literature

SOVIET VIROLOGY

ATD Work Assignment A-67-7

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TABLE OF CONTENTS

Introduction iv

I. The Tickborne Encephalitis Complex 1

 A. Epidemiological Data 2

 B. Behavior of TBE Virus in Cell and
 Tissue Culture 4

 C. Induced Mutation of TBE Viruses 6

 D. Diagnostic Methods for TBE 6

 E. TBE Therapy

 1. Vaccines 9

 2. Challenge 9

 F. Japanese B Encephalitis 13

 G. Omsk Fever and Related Diseases 13

II. Hoof-and-Mouth Disease 25

III. Rabies 26

IV. Swine Plague 32

V. Venezuelan Equine Encephalomyelitis 33

VI. Miscellaneous Diseases 34

VII. Pox-Virus Infections 37

References 40

Appendix I. Supplementary Bibliography 70

Appendix II. Conferences on Viral Disease 82

Appendix III. Some Soviet Virological Research
 Institutes and Their Personnel 91

SOVIET VIROLOGY 1962--1965

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INTRODUCTION

This report reviews literature on Soviet virological research published between 1962 and 1965, inclusive, pertaining to the development and administration of vaccines and other therapeutic preparations designed to protect humans and animals against virus diseases. It includes data on preparation and inoculation techniques and challenge tests on laboratory animals to test the effectiveness of new vaccines. Coverage of related studies in the epidemiology, detection, diagnosis, vectors, and infection cycles of viral diseases is also included. Appendices provide additional information on virus research personnel and institutions, and on virological conferences held during those years.

I. THE TICKBORNE ENCEPHALITIS COMPLEX

Viruses of the tickborne encephalitis complex are group B arboviruses and are closely related antigenically, often behaving like distinct strains of a single viral species. The Far-Eastern form of the disease is usually more virulent than the European form [275]. Tickborne encephalitis (TBE, RSSE, Far-Eastern encephalitis, biphasic (two-wave) meningo-encephalitis, etc.) has a broad host range but is usually spread by ixodid or closely related ticks, generally *Ixodes persulcatus* and *Ixodes ricinus*.

Fleas have been implicated as vectors as well [244, 281, 111, 282]. In one study, the species composition, host range, and population dynamics of fleas in the Kalinin, Perm, and Kemerovo oblasts were studied. It was found that fleas transmitted the virus to rodents within 6 hours after their birth; this explained the relatively high antibody titers in the rodents and the low numbers of ixodid tick larvae and nymphs found in the vicinity of burrows. Transmission by fleas was confirmed experimentally [110].

Virological studies of ticks in epidemic regions are made regularly, especially during peak infective periods [110, 109, 261, 41, 233].

Hundreds of distinct strains have been isolated, including several strains that are antigenically and morphologically different from standard strains and are often highly pathogenic in tissue cultures [109, 41]. Birds, rabbits, livestock, hedgehogs, and other wild animals are also TBE vectors [164, 55]. TBE virus has been isolated from thrushes (*Oriocichla dauma*) carrying *Ixodes persulcatus* ticks [275, 248] and from hedgehogs, which maintain the virus during hibernation after being infected by tick bites or by eating contaminated food [143, 255, 74, 264].

A large-scale survey of virological and serological data on arboviruses isolated in the eastern Soviet Union revealed TBE, EEE, WEE, and Chikungunya virus in over 1000 samples from epidemic foci in Siberia, the Irkutsk region, eastern Kazakhstan, and other regions [59, 26]. Secondary examination of insect, bird, animal, and human organs and tissues from eastern

Kazakhstan yielded data on the first isolation of Casals group A viruses in the eastern USSR [12,91]. Strains K-10, K-13, and K-16 isolated from mosquitoes produced symptoms within 24 hours. Strains PT-149, PT-151, PT-161 and PT-182 isolated from the birds *Sturnus vulgaris*, *Corvus corona*, and *Larus ridibundus* had incubation periods lasting from 1--8 days [12]. Transovarian transmission of TBE virus has been demonstrated in a small percentage of certain tick species. Its potential importance is that it ensures the infection of a large number of larvae and consequently, wide dissemination among the local small-mammal population. The species displaying this trait are *Ixodes persulcatus*, *I. ricinus*, *I. hexagonis*, *Dermacentor silvarum*, *Haemaphysalis concinna*, and *H. japonica*. Experimental infection of *I. ricinus* showed that artificial means of infection could be used. Among females, 3.3% transmitted the virus when they were infected by feeding on infected mice and 21 % when the virus was injected directly into the body cavity. Although the latter method produced better results, the inoculation process was often fatal to the ticks. The strains did not lose their cytopathogenicity during transovarian passage [249].

A. Epidemiological Data On the Tickborne Encephalitis Complex

At a conference on arbovirus infections held in Moscow in September 1964, the Eleventh Scientific Session of the Institute of Poliomyelitis and Virus-Encephalitis Diseases of the Academy of Medical Sciences was devoted to discussion of tickborne encephalitis, Omsk fever, Kemerovo tick fever, and related diseases [203]. During the four days of the session, 85 papers were presented which were later incorporated into the published collected papers of the meeting. All aspects of these diseases from classification of the viruses to prophylaxis and immunization were discussed. A paper presented by A. M. Butenko and Ye. S. Sarmanova on the isolation of new virus strains in Kemerovo and Astrakhan oblasti complements the work of M. P. Chumakov et al. on the isolation of a serologically distinct member of the TBE complex which was accomplished during the joint Czech-Soviet virological expedition during the summer of 1962. This expedition made virological examinations of ixodid ticks, suspected human carriers, and healthy persons bitten by ticks in Western Siberia. Both typical and atypical strains were isolated. In one

region, 20 serologically similar strains were discovered which produced a febrile type of encephalitis in humans. The new virus strains were cytopathic in chick tissue culture in 48--72 hours, multiplied well in the yolk sacs of 7-day chick embryos, were fatal to newborn white mice but generally not to adults, and were antigenically distinct from the rest of the encephalitis complex although they produced similar symptoms. Later, 20 similar strains were isolated which were remarkably pathogenic to intracerebrally inoculated rats and hamsters, producing brief fever in Rhesus monkeys and having varied effects on tissue cultures [40]. A report of a test of a new cultural inactivated vaccine made on a million subjects was given by D. K. L'vov, and data on its production technology and quality control were given by A. V. Gagarina and I. M. Rodin. Kemerovo tick fever was discussed at a special session. Immunological shifts in humans, the capacity of the virus to agglutinate erythrocytes, morphology, and the isolation of similar viruses in other parts of the Soviet Union were also considered [203].

In typical epidemic foci, sizable percentages of captured ticks carried highly active virus [182, 232]. In some foci the outbreaks have a mosaic pattern which, some Soviet scientists suggest, depends on the variable virulence of the local strains isolated during epidemics [186]. A high percentage of wild and domestic animals and wild birds usually carry viruses, and humans in such districts usually have complement - fixing antibodies in high titers [155]. At least one case of a patient with relapsing TBE has been reported [123]. Simultaneous cases of both TBE and Omsk fever have been reported in humans and both diseases usually increases in occurrence with increases in the rodent and ectoparasite population, pointing to similarities in their mode of distribution since they are antigenically distinct [263, 59].

A study on comparative natural immunity in reptiles susceptible to TBE virus showed that in reptiles infected with TBE by various routes under different temperature conditions and by different techniques, temperature was found to be the most important single factor changing immune response. No immune response was observed in *Eremias velox* and *Agama caucasia* individuals kept at 4°C, whereas the earliest appearance of antibodies and the highest antibody titers occurred in individuals kept at 37°C [273].

B. The Behavior of TBE Virus in Cell and Tissue Culture

Czech researchers have found that in tissue cultures inoculated with two or more viruses, cell resistance involves formation of interferon-like inhibitors that suppress the invading virus in cultures already adapted to one type of virus [9, 227, 207]. This phenomenon is the basis for a method in which TBE virus can be titrated by determining cytopathic effect in tissues infected with polioviruses. In this method, the TBE viruses resisted the effects of poliovirus, and by some mechanism not yet known, but probably involving production of an interferon-like substance, inhibited the cytopathic effects of poliovirus [8]. Such an interference method can be used to evaluate the effectiveness of TBE vaccines. A method reported in 1963 by Al'shteyn et al. was economical and precise, but was more time-consuming than intracerebral inoculation of mice [11]. A plaque-assay method, on the other hand, depends on the definitive identification of plaques; any condition that interferes with plaque formation is to be avoided. Cells, therefore, must not be pretreated with interferon if plaques are to be clear. This method can also be used to obtain viral clones by repeated plaque isolation and subculturing in newborn mouse brain [140].

Many cell and organ cultures have been screened as suitable media for the evaluation of inocula and vaccines. Comparative evaluation of tissue lines by Karaseva and Semenov confirmed the suitability of chick-fibroblast or swine-embryo tissue [13, 14, 15, 92]. Titration in human-embryo fibroblasts has yielded results 50 times less sensitive than the intracerebral inoculation of mice, but within its effective range it is clearer, more precise, and reproducible, and can be used for detecting neutralizing antibody [10, 56]. Tick tissue culture is reported as the best medium for the early detection of tickborne encephalitis virus since a marked cytopathic effect is produced by small quantities of virus [189]. In growing virus in tissue cultures, agitation was shown to aid in the production of higher titer [75, 137]. Chick-embryo tissue was best for work in which gradual and continual release of virus from cells without marked cytopathic effect was desired [154]. Several authors report a "partial" cytopathic effect in lines that have been chronically infected with the virus, with a reduction in virus multiplication rate and a consistently low percentage of cell-associated virus (usually 1% intranuclear) with the rest in the culture fluid [133, 16, 19]. Some cell lines are

affected by medium composition with consequent changes in cytopathic effect [139], and all cultures of mammalian cells also had a resistance to superinfection as discussed above, although long-term culturing could not eliminate persistent infections [141].

The effects of long-term culturing on TBE viruses has been studied at the Moscow Scientific Research Institute of Viral Preparations. Culture conditions played an important role in the properties of some mutant strains, and others maintained their properties unchanged even under prolonged culturing and varying temperatures. Some strains developed the ability to destroy SMH and chick fibroblasts after adaptation [17]. The laboratory of V. V. Pogodina of the Institute of Poliomyelitis in Moscow has investigated the variable pathogenicity (virulence) of TBE and related strains for different laboratory animals under varying conditions. Various strains of TBE, Langat, Omsk fever, Louping ill, Powassan, and Kyasanur forest fever viruses were studied for both intracerebral and peripheral pathogenicity in attempts to use neurovirulence as a strain marker. They observed the following types of neurovirulence: a) high intracerebral and high peripheral--Eastern and Western TBE strains, and Omsk fever, Kyasanur, and Powassan viruses; b) low intracerebral and high peripheral--Khab-9 and Vasenkova strains (EEE); c) high intracerebral and low peripheral--Fatayev and Ix-2 (TBE); and d) low intracerebral and low peripheral--Langat strain TR-21, recommended as a vaccinal strain. Mice and hamsters were judged unsuitable for differential diagnosis of the TBE complex since they displayed uniform clinical symptoms with all strains. Lambs were ruled unsuitable because of their low susceptibility. While none of the animals tested were equally susceptible to all the strains, newborn pigs were the most suitable for differentiating strains on the basis of virulence [178, 177].

When a newly isolated TBE strain was injected into young mice and passaged, its virulence decreased. Specific resistance to challenge by the original wild-type virus was produced by injection with this attenuated strain; degree of immunity was affected by virus dose and time of immunization. The lowered virulence was connected with a decrease in the ability of the attenuated virus to multiply in extraneural tissue [107, 143, 179]. Since titration on the basis of cytopathic effect gave inconclusive results [180], the fluorescent-antibody method (FAM) was

employed in the tissue-culture studies of growth stages, of the appearance of viral antigen, and of cytopathic effects of Langat (strain TR-21) virus, a weakly virulent virus demonstrating antigen visually.

C. Induced Mutation of TBE Viruses

G. D. Zasukhina and I. A. Rapoport reported on the chemical mutagenesis of TBE viruses. General studies of the variance in hereditary characters resulting from treatment with various chemical mutagens produced mutant strains of varying virulence and morphology [286]. Changes in cytopathic effect in tissue culture were not connected with cytopathic effect in cell cultures treated with 5-bromouracil, formaldehyde, urethane, and a combined proflavin-urethane preparation [285]. In the work of Zasukhina and Rapoport, the effects of some standard mutagens on two groups of arboviruses with characteristically low and high mutation rates are compared. Strains of TBE virus with a characteristically low mutation rate and WEE strains with a higher mutation rate, better culturing properties, and a faster growth rate were treated with: 1) 5-bromouracil, 2) formaldehyde, 3) 1,4-bisdiazoacetylbutane and 4) N-nitrosomethylurea. These mutagens produced alkylation of the hereditary material; 1, 2, and 4 yielded a greater percentage of apathogenic mutants than did 3. A greater number and variety of mutations were produced by 1,4-bisdiazoacetylbutane. These results were highly significant in explaining "ecological cosmopolitanism," or ability of these viruses to survive in dozens of species of cold and warmblooded animals. These animals are the reservoirs, carriers, and victims of these viruses. A secondary aspect of the study was to determine the range of artificially induced mutant properties of these viruses with the intention of creating live vaccine strains from strains with induced mutation. Several strains were selected for further study [287].

D. Diagnostic Methods for Tickborne Encephalitis

The fluorescent-antibody method

The principal advantages of the fluorescent-antibody method (FAM) in diagnostics are speed, accuracy, and the saving in reagents [128, 145, 156]. This method has

been employed to detect cell-bound and free TBE viral antigens in sheep-kidney cultures [129], and shows the intracellular distribution of nucleic acids and specific antigen in mouse-brain and sheep-embryo kidney tissue [220, 219]. This method has been used diagnostically and in the study of the course of experimental infections. It is easily and rapidly detectable in the central nervous system and lymph nodes soon after infection, usually within 24 hours. Viral antigens are easily demonstrable even in animals showing no clinical symptoms [83, 146].

Complement fixation reactions

Complement fixation is repeatedly cited as one of the best methods for diagnosing TBE (though the FAM is superior) [64, 54, 150, 39]. Its principal disadvantages, the length of time required to obtain definitive results (as much as 15--30 days) [64, 103] and the relatively sophisticated equipment required, make hemagglutination more acceptable [63]. A method by which dried blood on filter-paper disks is used was ruled unsuitable for the complement fixation reaction because of the large titers of anticomplementary substances in the dried blood [54]. However, this system is adaptable to the neutralization reaction [54], although in most papers on neutralization, it is carried out *in vitro* or in tissue culture [255]. The principal advantages of either fast neutralization or complement fixation is the elimination of mice. Also, since the virus multiplies faster in tissue culture than in mice, virus can be obtained, identified, and typed more rapidly [64]. Several papers report the processes of obtaining and purifying sera for complement fixation [150, 266, 58, 38].

Hemagglutination and hemagglutination-inhibition reactions

Hemagglutination and hemagglutination inhibition have been used diagnostically in large-scale serological surveys and epidemiological studies both in the field and in tissue cultures [276, 95, 126, 200, 171]. Sheep-embryo kidney cultures provide the most suitable medium for hemagglutination and for complement fixation, although use of other tissues is reported in the literature [62, 96, 223, 47, 53]. TBE hemagglutinating antigens obtained from viruses grown in various tissue cultures are highly specific, require no elaborate purification process, and are stable at 4°C. They were therefore recommended to

replace brain-derived antigens for serodiagnosis of TBE. The accumulation of virus in the culture media can be determined fairly accurately by hemagglutination and TBE virus can be detected in experimental mixed infections in tissue culture [150]. A modified hemagglutination-inhibition (HI) reaction is widely used in the Soviet Union in surveys of known and suspected vectors [201, 171, 275, 97]. One method, which enables a technician to determine the presence of TBE and Japanese encephalitis antigen without the complement-fixation reaction, involves the simultaneous performance of the HI test over a wide pH range, since the Japanese and TBE viruses agglutinate at different pH values [247]. A further modification of this test suitable for large-scale work employs paper discs and requires minute blood samples [201]. Since antigens are plentiful in the blood long before the appearance of clinical symptoms, Gaydamovich and others have recommended the HI reaction for early detection of arboviruses and suggest its use in the identification of vectors [67]. Use of this reaction to demonstrate the presence of TBE antigen in wild birds and their parasites is particularly interesting as it enables contacts of wild birds with epidemic foci to be revealed [171].

A modified precipitation reaction was chiefly used in typing and determining antigenic relationships rather than in diagnosis [224].

Neutralization

The chief benefits of neutralization methods have been found to be that clear-cut results could be obtained within 72 hours with freshly isolated strains and with convalescent sera; even sera contaminated by bacteria could be used and the tests could be carried out in ordinary culture bottles without impairing sensitivity [255, 131, 52]. Disadvantages were such external factors as season [274]; also, clinical manifestations in a given individual affected the neutralization indices so that a definite hemagglutinin-forming dose could not be determined accurately [255, 20]. This method could be used to detect infectious RNA in the course of an investigation of the properties and functions of TBE virus RNA [49].

A colorimetric test for TBE has been used experimentally with success; its only disadvantage is that definite results may be obtained only after 4--5 days. Further research on this test is underway [93].

4 4
Plaque formation has been used in the differential diagnosis of TBE because its plaques are distinct from those formed by other viruses. In one experiment, TBE virus formed large turbid plaques, Omsk fever virus formed small plaques, and Langat virus (strain TR-21) formed both large and small transparent plaques, indicating the heterogeneous composition of the inoculating strain [122]. Standardization of conditions is very important since agar inhibitors also alter plaque sizes [51]. Positive identification may be made in 3--4 days using a method reported by Logina-Parina in 1964 [121]. The infectivity of TBE infective RNA can also be shown by the plaque technique [50].

E. TBE Therapy

1. Vaccines

A 5% brain vaccine against TBE was administered to mice and guinea pigs. This vaccine was prepared from serum obtained from infected animals two or four days after infection, and was administered in two doses 28--32 days apart. When an allergic reaction appeared, the animals received normal brain suspensions or formol-inactivated vaccine as desensitizers. In guinea pigs, tissue from sick mice and from infected mice which had not yet displayed clinical symptoms produced strong anaphylactic reactions. Four-day vaccine caused only a weak response, leading to the conclusion that intermediate antigens appeared between the second and fourth day after injection [13].

A nonallergenic, formolized vaccine was obtained by Gagarina et al. by the intracerebral infection of newborn rats. Similar vaccines have been obtained by others [60, 83, 115, 260]. Vaccines adsorbed on aluminum hydroxide possessed greater immunogenicity than native vaccines [115].

Vaccines prepared from tissue cultures were generally more effective and easier to handle and store, and were areactogenic as compared to inactivated brain vaccines. In general, greater postinfection immunity was observed with the tissue-culture vaccines [43, 202, 258]. Rates of antibody accumulation and immunological shift varied during large-scale trials. High antibody titers appeared rather early [44] in some previously unvaccinated persons and after considerable delay in others, suggesting to the

researchers that three immunizations were optimal. Large-scale trials bore this out, leaving the question of the optimum intervals to be determined [259]. The immunizations last at least six months [257, 46]. In trials with human volunteers, Chumakov and his associates found that maximum immunogenicity was obtained when shots were given 1--4 days apart followed by a booster after six months, especially in tests of nonadsorbed vaccines [46].

Attenuated vaccines prepared in tissue cultures were more effective than brain vaccines in almost every case, though their effectiveness was affected by such external factors as drugs and hormones [42, 199]. It has been established that the regional lymph system limits the penetration of tickborne encephalitis virus into the bloodstream under experimental conditions [132]. Cortisone treatment of mice at the time of immunization did not improve the prophylactic effect of the vaccines; on the contrary, viremia lasted longer in animals receiving cortisone [98, 253]. X-irradiation speeded the spread of TBE virus through the regional lymphatic system except in the lungs, where a decrease in virus titers was noted [130]. Urethane-barbital preparation had much the same effect as cortisone on experimental TBE infections in mice, with viruses persisting longer and in higher titers [199]. A tissue culture vaccine effective against both eastern and western forms of the TBE complex and possibly against related diseases and which possesses almost the same virus-neutralizing capacity for all the diseases of the TBE complex was developed at the Institute of Poliomyelitis in Moscow by Zaklinskaya and coworkers [283].

Antisera and specific globulins have been found the most suitable for use in epidemic foci because of their areactogenicity and because they are easily obtainable [57, 182]. A-type immune serum against tickborne encephalitis is specific, shows no anticomplementary activity, and contains no antibodies to brain tissue; it can be used as a standard in complement-fixation and hemagglutination-inhibition tests with TBE antigen [194].

STORAGE PROPERTIES OF TBE VACCINES

Of the wet vaccines, formol-inactivated vaccines were the most stable, with a shelf life of up to 9 months. Some batches were kept in rubber-stoppered common glass bottles up to one year [35, 83, 198]. Dried vaccines kept longest, provided residual moisture did not exceed 1% [125].

Studies of the properties of viral infection in tissue culture have been made in the laboratory of J. Vilcek in Czechoslovakia. Several reports on the formation, antiviral action, and comparative properties of various interferons have been published. When mice are infected with TBE intraperitoneally or intracerebrally, high interferon levels are found in the brain of inoculated animals [269]. When cells infected with WEE, EEE, vaccinia, NDV, and homologous TBE viruses were treated with interferon from TBE-infected cells, their resistance to the other diseases increased [267]. The action of TBE-interferon and influenza-interferon was compared; results were found to be similar to those of the previous study, indicating that the specific inducing virus fails to influence the properties of the interferon formed in a given cell culture [70, 185, 268].

The following chemicals and other preparations have been more or less successfully tried as decontaminants and therapeutic compounds for virus diseases in the Soviet Union: Lactams and lactones, especially beta-propiolactone, were recommended for general disinfection use and for further study [173, 293]. Novembichin and other antibiotics were somewhat effective [28, 73]. Dichloroethylamines showed antiphage action and ethyleneimines inhibited the action of many viruses [27, 29, 288]. Naturally occurring, interferon-like inhibitors in both live animals and in tissue cultures have been reported in many papers. Most of them are enzymatic and thermolabile [147, 217, 228, 246, 250, 292].

Uniformly satisfactory results have been obtained with the administration of vaccines, drugs and therapeutic chemicals by means of aerosols [4, 5, 6, 7, 66, 77, 183, 187, 270, 271, 289, 290]. In fact, some live, attenuated vaccines are most effective when administered by aerosol in very small quantities [66].

A killed tissue vaccine prepared from TBE virus strains Yas-8 and Ix-10 was tested on animals kept in a IVK-2 chamber. Vaccinations were given at 1-, 3-, 5-, 7- and 10-day intervals via an aerosol with particles averaging 1--3 microns in diameter. This method produced a rapid rise in virus-neutralizing antibody titer and successfully protected animals challenged with the virus. The vaccine penetrated the tissue rapidly, enabling smaller doses to be used. A mixed aerosol conferred immunity to both viruses (TBE and WEE) [250].

2. Challenge of Animals Vaccinated Against Tickborne Encephalitis

There are numerous reports of challenge tests of vaccinated animals to determine the efficacy of certain methods of immunization and the properties of experimental vaccines. In a study to determine the conditions influencing the effectiveness of TBE prophylaxis in human volunteers and the relationship of the immunogenic properties of the vaccine and its immunologic effect, 548 persons, all previously unvaccinated, received doses of vaccine at 1--2 and 2--3 week intervals. They were revaccinated after 1 year. Twelve types of tissue culture vaccine and three types of brain vaccine were tested. Conclusions reached were that if the first series of shots possessed highly immunogenic properties, then a booster possessing diminished immunogenicity was effective. The reverse was not true [45].

Some of the standard vaccines, particularly the mouse-brain vaccine, are easily contaminated by bacteria and produce allergic reactions. Thus, tissue-culture monolayers, especially chick-embryo cultures, are considered a superior source for vaccines. Formalin or beta-propiolactone were used as neutralizers to make the vaccine suitable for use. Optimum inactivation conditions were determined. A satisfactory vaccine was prepared by inactivation at 4°C. (This was necessary because the 37°C temperature of some methods destroyed the immunogenicity of the virus). This vaccine had a shelf life of approximately 6 months and protected challenged mice against high concentrations of virus. In tests on humans with low or nonexistent serum-antibody titers, 66% of volunteers developed antibodies in sufficiently immunizing titers within 4 weeks after vaccination [84, 127].

Airborne infection of monkeys with TBE virus produced little CNS involvement, but other symptoms produced were like those evoked by infection from other routes [23, 114]. In two studies the infective dose for aerosol infection was found to be 10^4 -- 10^5 i.c. mouse LD₅₀. In both cases, active immunization with TBE vaccine was the best way to protect test animals against high infectious titers [23, 48]. Airborne infection is an unusual route and occurs mainly, or perhaps entirely, in the laboratory. In these studies animals were kept in a special flow chamber and aerosols were generated by a specially developed appara-

tus which generated particles with average diameters of 0.75--1.5 microns. The dose could be varied by altering the concentration of virus in the carrier fluid or by varying exposure time. A Formol vaccine offered animals better protection against aerogenic infection than against infection by other routes [48].

F. Japanese B Encephalitis

Virological surveys have shown birds to be an important factor in the dissemination of Japanese B virus. A survey of the southern Primor'ye showed the presence of hemagglutinating and complement-fixing antibodies in 200 wild birds of 36 species [166, 181]. Sheep-embryo kidney tissue culture is the most widely used medium for the evaluation of cytopathic effect in the diagnosis of the disease since no preliminary adaptation is required. The cytopathic effect is limited in adult tissue cultures [61, 165]. Attempts to culture the virus in guinea-pig tissue involve the necessity of intracranial infection of the initial animal with a suspension of infected chick tissue, and intracranial subculturing. This laborious procedure yields a preparation with unchanged antigenic properties after long passaging [197]. Noninfectious diagnostic sera and hemagglutinins for the HI test have been produced [68, 237]. Uvarov and his associates prepare concentrated hemagglutinins by precipitation in ammonium sulfate and incubation at pH 3.2--8.5 for 19--24 hr at 0--4°C [264].

G. Omsk Fever and Related Diseases

Omsk fever and other renal hemorrhagic fevers present diagnostic problems similar to those surrounding the identification of various forms of the tickborne encephalitis complex. In addition, Omsk fever and Kyasanur forest disease are antigenically related to tickborne encephalitis. Hemorrhagic nephroso-nephritis (HNN) and Omsk fever are often misdiagnosed as leptospirosis, tickborne encephalitis, gastroenteritis and other diseases. The tables compare the characteristics of HNN and related diseases and abdominal conditions for which it is most often mistaken.

Table I from [216]

COMPARATIVE CLINICAL CHARACTERISTICS OF HEMORRHAGIC FEVERS			
Hemorrhagic nephritic-nephritis	Crimson hemorrhagic fever	Central Asian hemorrhagic fever	Omsk hemorrhagic fever
Predominant clinical syndrome			
Distinct hemorrhagic manifestations and a severe general intoxication at the height of the disease, acute renal damage and urinary excretion impairment, sometimes acute renal failure.	Phenomena of general intoxication with distinct hemorrhagic phenomena and relatively mild neurological lesions.	Very severe general intoxication and distinct severe damage to the gastrointestinal tract, with massive, frequently critical bleeding.	Relatively mild general intoxication and hemorrhagic diathesis. Distinct respiratory tract lesions.
Incubation period			
From 11 to 23 days. On the average 14-15 days.	From 2 to 25 days.	3-4 days. Especially short in intramural hospital infections.	2-4 days. Especially short in infections of personnel caring for infected laboratory animals.
Prodromal period			
Observed rarely. Lasting 2-3 days.	Prodromal period not manifested.	Prodromal period not manifested.	Rarely observed.
Cycles of development of the clinical picture of the disease			
Four periods of the disease: the first general febrile period.	Three periods of the disease: the first initial period.	Three periods of the disease: the first initial period.	Two periods of the disease: the first, febrile period.
Second period - hemorrhagic manifestations. Third period of maximal injuries to organs. Fourth period - reconvalescence.	Second period - height of the disease. Third period - reconvalescence.	Second period - height of the disease. Third period - reconvalescence.	Second period - apyrexia, reconvalescence.
Temperature often rises suddenly to 39-40°C and remains high until the 4th-7th day of the disease. Later resolving by lysis in 2-3 days.	Temperature rises suddenly to 39-40°C and then resolves by lysis after 7-8 days. A characteristic brief drop in temperature on the 3rd and 4th days of the disease coinciding with the beginning of the hemorrhagic manifestations.	After a severe chill the temperature rises suddenly to 39-40°C and drops somewhat on the 3rd or 4th day (with the beginning of hemorrhagic manifestations). Later it remains at a level of 37-38°C to the 7th-8th day of the disease and later returns to normal by lysis.	After a brief chill temperature rises suddenly to 39-40°C. It resolves by lysis between the 9th and 15th day of the disease. A brief decrease on the 3rd or 4th day, (beginning of hemorrhagic manifestations) is characteristic. In 5% of cases in the second or third week of the reconvalescent period there is a typical secondary temperature wave (short, not so high).

Table I continued

	Hemorrhagic nephritic-nephritis	Crimean hemorrhagic fever	Central Asian hemorrhagic fever	Omsk hemorrhagic fever
Color of skin	Typical hyperemia of the skin of the face, neck and upper half of the body from the first hours of the disease and until the convalescent period.	Typical hyperemia of the skin of the face, neck and upper half of the body from the first hours of the disease and to the convalescent period.	Hyperemia of the face not distinct.	Hyperemia of the skin of the face and the upper half of the body observed in 50% of cases from the first hours of the disease and until the convalescent period.
Color of visible mucous membranes	Typical hyperemia and injection of the vessels of the sclerae, conjunctiva and oral cavity from the first hours of the disease and until the convalescent period.	Typical hyperemia and injection of vessels of the sclerae, conjunctiva and oral cavity in the initial period and during the height of the disease.	Hyperemia of the sclerae, and of the pharynx or tonsils was rarely observed.	Hyperemia and injection of the vessels of the sclerae, pharynx and oral cavity during the febrile period.
Pulse	Moderate bradycardia during the first three periods of the disease. Relative tachycardia from the beginning of the convalescent period.	Moderate bradycardia during the period of high temperature.	Pulse rate corresponding to temperature. In severe disease courses with massive blood loss there is tachycardia.	Moderate bradycardia in the febrile period. Sometimes tachycardia.
Blood pressure	Most frequently moderate hypotension - 80-100/50-60; severe hypotension (collapse) or hypertension are rare.	Typical moderate hypotension - 90-100/50-60.	Typical distinct hypotension - 80-90/40-50. Degree of hypotension directly proportional to the degree of loss of blood.	Typical distinct arterial hypotension - 80-90/40-50 during the entire febrile period.
Hemorrhagic manifestations a) on the skin	From the 3rd-4th day there is a slight petechial rash on the skin of the lateral surfaces of the chest, abdomen, elbow, armpits; more rarely a widely distributed rash is noted. There is no roseolar rash.	From the 3rd-4th day of the disease there is a small scattered petechial rash on the skin of the anterior and lateral surfaces of the chest and abdomen. There may also be a roseolar rash during the same time and with a similar localization.	A slight petechial rash appears in the first to fifth day of the disease. It is seen mainly on the skin of the anterior surface of the body and limbs. The rash may spread over the entire body with exception of the face. Some petechiae may reach 3-4 cm in diameter.	A slight petechial scanty rash is seen only in 20% of patients, in the skin of the anterolateral surfaces of the body and limbs. Sometimes coalescent hemorrhages appear in the lumbar region.

Table I continued

	Hemorrhagic nephroic- nephritis	Crimean hemorrhagic fever	Central Asian hemor- rhagic fever	Omsk hemorrhagic fever
b) In the mucous membranes	From the 3rd-4th day of the disease there are hemorrhages in the sclerae, mucous membrane of the oral cavity, and es- pecially at the edges of the hard palate. Hemorrhages from the nose, gums and gastrointestinal tract are rare and mild.	In 50% of cases there are no hemorrhages from the nose. Hematemesis and intestinal bleeding are rare and last 3- 4 days. Copious hemorrhage occurs only in 10-15% of patients.	Typical copious hemorrhages from the nose and gums. In 50% of cases there are hemate- meses and melena, which often endan- ger the life of the patient.	Bleedings from the nose, gums, uterus, gastrointestinal tract, etc. are infrequent and mild.
Renal function	Marked decrease in diuresis (100-300 ml) sometimes to com- plete anuria in the second and third pe- riod of the disease, with development of acute renal failure. During the recovales- cent period there is a distinct and prolonged polyuria and nuchypo- natemia.	No distinct impair- ment of diuresis is observed.	No impairment in diuresis.	No impairment in diuresis.
Urine changes	Typical albuminuria during the entire pe- riod of the disease. In the second and third periods (4th-9th day) it is very high, reaching 10% and sometimes even 40- 50%. In the sedi- ment there are the following findings: early (2nd-3rd day) appearance of large number of cells from the renal epithelium with signs of degenera- tion, copious cylin- dria and hematuria of varying degree.	In the period of the height of the disease the following are typical: moderate up to 1% albumino- uria, macrohematuria and few hyaline casts.	In the period of the height of the disease there is often a mod- erate albuminuria, and in the sediment of the urine there are a few hyaline casts and fresh erythro- cytes.	Findings in the period of height of the dis- ease include: a slight (up to 1% albuminuria, renal epithelial cells, and few hyaline casts

Table I continued

	Hemorrhagic nephrotic nephritis	Crimson hemorrhagic fever	Central Asian hemorrhagic fever	Crimean hemorrhagic fever
Pain in the renal region	Regular early appearance and distinct manifestations of the Pasternak's sign in all periods of the disease, and of a typical tender point in the lumbar region on palpation	In the period of the height of the disease frequent occurrence of a positive Pasternak's sign	In the period of the height of the disease the Pasternak's sign may be observed only in few patients.	In some patients the Pasternak's sign may be weakly positive in the period of height of the disease
Blood changes (hemoglobin and erythrocytes)	Typical hemocoagulation from the first days of the disease. In the convalescent period there is some decrease in hemoglobin and in the number of erythrocytes, usually to the lower normal limits.	Hemocoagulation is typical in the first days of the disease. In the last days of the period there is a reduction in the number of erythrocytes and in the hemoglobin level. In some patients with severe bleeding a distinct anemia may develop (hemoglobin to 50-60% and erythrocytes to 3,000,000/mm ³).	In the period of the height of disease there is distinct hypochromic anemia. In some patients hemoglobin may fall to 20-25%, and the number of erythrocytes to 1,000,000/mm ³ or lower.	In the first days of the disease there may be a slight hemocoagulation. In the second half of the febrile period the hemoglobin level and erythrocyte count decreases somewhat.
b) the number of leukocytes and the leukocyte formula	Typical 1) initial leukopenia - 2,000-4,000/mm ³ in the first period (1-3 days), 2) subsequent leukocytosis - most frequently 10,000-12,000/mm ³ and sometimes a subleukemoid level of 35,000 and even 60,000/mm ³ , 3) return of the number of leukocytes to normal only in the convalescent period 4) in the last days of the disease one may even find in the presence of leukopenia neutrophils with a shift to the left (7 to 8%) and appearance of toxic cells (band forms)	Findings in the period of the height of the disease: distinct leukopenia (2,000-4,000), slight neutropenia with a moderate bandform shift, aneosinophilia and appearance of plasma cells.	Findings in the period of height of the disease: distinct leukopenia (to 1,300-2,000/mm ³), considerable neutropenia (35-45%) with a shift to the left, sometimes with juvenile cells, relative lymphocytosis and aneosinophilia.	Findings in the period of height of the disease: considerable leukopenia (3,000-5,000/mm ³), sometimes very severe (as low as 1,200-1,500), slight neutropenia with a bandform shift to the left, relative lymphocytosis, aneosinophilia, appearance of plasma cells in some cases.

Table I continued

	Hemorrhagic nephritic nephritis	Cerebral hemorrhagic fever	Central Asian hemorrhagic fever	Omsk hemorrhagic fever
	philia; b) appearance in the peripheral blood of plasma cells, frequently as high as 10% and more			
c) Number of thrombocytes	In the second and third periods of the disease there is a brief and moderate thrombopenia (up to 80,000-100,000).	In the period of the height of the disease there is a moderate thrombopenia (50,000).	In the period of the height of the disease a moderate thrombopenia (60,000), but sometimes a much more severe one (30,000).	In the period of the height of the disease, a slight thrombopenia.
d) ESR	Typical slow ESR in the first three periods and a moderate acceleration in the convalescent period (to 20-30 mm/hour).	Typical slight acceleration of ESR towards the end of the period of height of the disease (to 20-30 mm/hour).	Typical ESR to 30-50 mm/hour during the period of height of the disease.	Slight slowing down of ESR in the febrile period and acceleration during the convalescent period (to 20-30 mm/hour).
Respiratory system lesions.	Atypical changes. In the first period there is a slight diffuse bronchitis in some patients. At the end of the second period and in the third period focal pneumonia may develop as a complication in some cases.	In the period of height of the disease an acute diffuse bronchitis may develop. At the end of the period of height of the disease focal pneumonia may develop in 10% of cases.	In some patients in the period of height of the disease a diffuse acute bronchitis may develop.	From the first days of the disease there is an acute bronchitis, and in 31% of cases specific focal pneumonia is noted. At the end of the febrile period a bacterial focal pneumonia may appear as a secondary complication.
Gastrointestinal tract lesions.	From the 3rd-4th day of the disease there is nausea, persistent vomiting, less frequently hiccup, epigastric pain and tenderness on palpation. The odor from the mouth is unpleasant. Hematemesis and blood in the stools are rare. Gum bleeding is rare. In some cases in the second and third period of the disease (4th-9th day) a clinical picture of "acute abdomen" may develop (hemorrhagic).	From the first days of the disease there is a marked lack of appetite, and increasing thirst. Vomiting, often with blood, is common. There is a heavy putrid odor from the mouth. The abdomen is soft, often diffusely tender. Changes in the stools are typical. Tarry or blood-stained stools are rare.	From the first days of the disease there is lack of appetite and a putrid odor from the mouth. Gum bleedings are common. Vomiting is very frequent, sometimes almost constant. Hematemesis and repeated intestinal bleedings are common.	There is almost complete loss of appetite from the first days of the disease. Not infrequently a putrid odor from the mouth. Nausea and vomiting in 33% of cases. Soft abdomen, only slightly tender on palpation.

Table I continued

	Hemorrhagic nephrotic-nephritis	Crimmean hemorrhagic fever	Central Asian hemorrhagic fever	Omsk hemorrhagic fever
	into the stomach and intestine with the mesentery, pancreas and perirenal tissue)			
Liver and spleen	At the height of the disease the liver in 50% of cases may be palpated and is moderately enlarged. The spleen is rarely enlarged.	There is slight enlargement of the liver and spleen during the period of height of the disease, approximately in 33% of patients.	In the period of height of the disease the liver is usually considerably enlarged (palpated at 4 cm below the costal margin).	In the period of height of the disease the liver is slightly enlarged and tender to palpation. Enlargement of the spleen not usual.
Nervous system lesions	Changes regularly. From the first days there is a severe headache in all patients. In 27.5% of cases from the beginning of the third period there is a marked state of stupor, mild meningeal symptoms, and weak pathological reflexes (Babinski and others). CSF is usually unaltered, the spinal fluid pressure is increased. One may observe distinct phenomena of serous meningitis. Rarely does one see a clinical picture of meningo-encephalitis with distinct impairment of consciousness - stupor, delirium, psychomotor excitation and sometimes coma.	From the first days there is headache, drowsiness, and sometimes impairment of consciousness, delirium and hallucinations, and there may be brief meningeal signs and signs of impairment of cerebrocranial innervation asymmetry of the face, deviation of the tongue, irregular pupils and pathological reflexes (Babinski and others). CSF is usually not altered.	From the first days there is headache, drowsiness and apathy. Rarely there may be a brief loss of consciousness and in severe cases a comatose condition. Meningeal phenomena and disorders of tendon reflexes are rare and not distinct.	From the first days of the disease the following signs appear: headache, limb pains. Manifestations of organic nervous system lesions (pathological reflexes and meningeal signs) are rare, brief and not distinct. In the Bukovinian variant of hemorrhagic fever the injury to the nervous system is more distinct. The following signs are very frequent: brief psychomotor excitation, anisoreflexia, appearance of pathological reflexes. In one third of the cases there are meningeal signs.

Table I continued

	Hemorrhagic nephrosi- nephritis	Crimson hemorrhagic fever	Central Asian hemor- rhagic fever	Crisis hemorrhagic fever
Metabolic disorders	At the height of the disease (second and third period) the following are typical: some hypoproteinemia, distinct azotemia, frequently high (up to 200-400 mg % of residual nitrogen and 400-500 mg % of urea), acidosis and decrease of alkali reserves of the blood to below 50 volume % (sometimes as low as 20 volume %), moderate chloropenia, distinct hypocholesteremia, pathologically high and persistent sugar curve.	Metabolic disorders are rare. At the height of the disease and during the first day of the reconvalescent period there may, sometimes be moderate azotemia.	At the height of the disease there is a moderate hypoproteinemia (5-6 g %), mainly due to decrease in globulins and fibrinogen. During this time there is a regular tendency to a moderate hypoglycemia. A considerable decrease in the ascorbic acid in the blood is typical.	Changes in metabolism not regular. At the height of the disease one observes only a moderate hypoproteinemia and some times slight azotemia.
Complications	One rarely observes massive hemorrhages in the kidney (and tear of the kidney) and in the suprarenal parenchyma, with development of a clinical picture of "acute abdomen". Hemorrhages in the mesentery and the wall of the intestines with development of an ileus syndrome; hemorrhages into the stomach and other internal organs; acute insufficiency of the kidneys and development of a clinical picture of azotemic uremia, focal pneumonia, purulent parotitis and submaxillitis.	Massive bleedings (gastro-intestinal, pulmonary) very rare. Focal pneumonia observed in 10-16% of cases. In some patients parotitis, epidelimitis and peritonitis develop.	Massive bleedings from the gastro-intestinal tract are very frequent and are a danger to life. In some cases a deep focal pneumonia develops. One very rarely observes inflammation of the salivary glands.	Focal pneumonia both early (viral) and late (bacterial) are very frequent. Otitis, parotitis and pyelitis develop rarely.

Table II from [216]

Differential diagnostic signs of HNN and "acute abdomen"		
Signs and combinations of signs	Clinical characteristics	
	HNN	"acute abdomen"
Features of abdominal pain	Abdominal pain appears on 3-5th day	Sudden onset of pain - the first clinical sign of onset of pathological condition (gastrointestinal perforations, acute peritonitis)
a) origin		
b) intensity	Usually mild pains, never intractable ("knife stroke")	Very acute pains ("knifing") frequent
c) localization	Most frequently localized in epigastric region but may also be diffuse	Most often in epigastric region, but may also appear elsewhere
d) combination with vomiting		Pains usually precede vomiting
e) combination with fever	Abdominal pains usually preceded by typical high temperature reaction	Pains usually begin while temperature still normal, temperature later rises
Combination of abdominal pains with other pain	Abdominal pains usually combined with pains in the lumbar region	Lumbar pains only when a gastroduodenal ulcer penetrates into the pancreas, in nephrolithiasis colic, or retroperitoneal appendicitis
Urine and diuresis changes	Characteristic	Not characteristic, occur rarely
Blood changes	Characteristic (see other tables)	At the onset of pain attack blood may be normal, with neutrophil leukocytosis developing later
Combination of pain syndrome with other clinical signs	Abdominal pain combined with various symptoms characteristic of HNN (hemorrhagic rash, renal injury etc.)	Abdominal pains dominate clinical picture, often typical clinical manifestations of acute peritonitis also appear within the first 12-24 hours

Table III from [216]

Differential diagnostic signs of HNN, mosquito-borne Japanese encephalitis and tick-borne encephalitis

Signs	HNN	Mosquito-borne Japanese (autumn) encephalitis	Tick-borne encephalitis (spring-summer)
Period of maximum morbidity	Maximum morbidity in autumn	August-September	April-July
Epidemic features	No traces of tick bites	No traces of tick bites	Traces of tick bite are of diagnostic value
Consciousness	Persists in most patients	Most often impairment, often coma	Various degrees of consciousness impairment
Meningeal signs	Rarely observed, approx. in 5% of cases	Usually observed, distinct	Usually observed, distinct
Encephalitis, pathological reflexes	Rarely observed, approx. in 5-10% of cases, usually disappears rapidly	Observed as a rule, tonic convulsions characteristic, central paralysis (hemiparesis), bulbar disorders	Observed as a rule. Weak, ataxic paralysis of neck muscles and thoracic region, bulbar disorders, spastic pareses - less frequent
Cerebrospinal fluid	Usually not observed. Approx. in 5% of cases moderate rise in protein, pleocytosis and very rarely bloody liquor	Changes always observed. During first days a rise in cell numbers, later - an increase in protein (up to 2%), distinct cytosis (up to 200 cells per 1 mm ³)	Clear liquor, increased pressure and protein (up to 2%). Globulin reactions positive, pleocytosis with prevalence of lymphocytes
Blood changes	See above (other tables)	Moderate leukocytosis with a neutrophil shift to the left, ESR accelerated	Moderate leukocytosis, neutrophilia, eosinophilia. ESR accelerated
Urine and diuresis changes	Characteristic changes (see above)	Changes not characteristic, sometimes traces of protein	Changes not characteristic, sometimes traces of protein

Table IV from [216]

The most important signs of the main forms of viral endemic hemorrhagic fevers (scheme of M.P. Chumakov, elaborated on by A.A. Smorodintsev)

Main indexes	Hemorrhagic fevers					
	HNN	Crimean	Central Asian	Omsk	Kyassaur Forest disease	Argentinian
I. Properties of the virus						
Cultivation outside the human body	-	-	-	+	+	+
Experimental infection in white mice	-	-	-	+	+	+
Experimental infection (febrile reaction) in monkeys	-	+	-	+	+	+
Antigenic relationship to tick-borne encephalitis virus	-	-	-	+	+	-
Susceptible animals with clinical (cl) or sub-clinical (subcl) form	Vole	?	Rabbit (subcl)	White muskrat (cl), mouse (cl)	White mouse (cl), monkeys (cl)	Guinea pig (cl), white mouse (cl)
II. Epidemiology						
Seasonality	Poly-seasonal	Spring-summer	Spring-summer	Spring-summer	Summer-autumn	Autumn-winter
Role of Ixodid ticks as carriers and vectors of the disease in nature	-	+	+	+	+	-
Participation of Gamasoid mites in the circulation of the virus in mice-like rodents	+	-	-	-	-	+
Transmission of the virus to man by rodents or their ectoparasites	+	-	-	-	-	+
III. Clinical picture						
Incubation period (days)	11-24	2-4 (M.P. Chumakov), 7-10 (E.A. Gal'perin)	3-6	3-7	4-8	9-14
Diphasic temperature curve	-	+	-	-	+	-
Diphasic fevers	-	-	-	+	+	-
Nasal and uterine bleeding	+	+	+	+	+	-
Gastrointestinal bleeding	±	+	++	+	-	+
Severe renal pathology	++	-	-	-	-	+
Hemorrhagic rash	+	+	++	-	-	-
Condition deteriorating after decrease in temperature	++	-	-	-	-	-
Leukocytosis and increase in number of Tolk's cells	+	-	-	-	-	-
Leukopenia, shift of blood formula to the left	-	+	+	+	+	+
Lethality (%)	3-5	3-8	10-30	0.5-3	4.6	18
IV. Main preventive measures						
Vaccination	-	-	-	+	+	+
Deratization and disinfection	+	+	+	-	-	+

Most cases occur in the fall and early winter (October and November) and blood in the urine has been reported in all cases [18, 278]. Chicken erythrocytes, the aldolase reaction, and special complement-fixing antigens have been tested as diagnostic media. L. A. Vereta and T. P. Yur'yeva reported on the demonstration of complement-fixing antigen in Omsk-fever patients when convalescent serum was the antibody source. The antigen was detectable up to the twelfth day after infection and could be demonstrated both *in vitro* and in infected tissue culture from reactions produced by yellow fever and several simian viruses [265]. Since the lytic reaction was clearly demonstrable in only 57--60% of test cases, it was recommended as an auxiliary test rather than for differential diagnosis [87, 144]. Results of the aldolase reaction proved entirely satisfactory for the differential diagnosis of HNN [204]. Since viremia was present in animals with an experimental infection for 23 days, it was concluded that apparently healthy animals or convalescing animals were the principal vectors of the disease in an epidemic area [284]. Omsk-fever virus produces an extensive cytopathic effect in susceptible swine-embryo kidney cultures; RNA-containing inclusions and viral antigen are easily demonstrated by the FAM [94].

Serotherapy methods were compared by Rodin et al. and were found to be only moderately effective [190].

In general, diagnosis of HNN is difficult as the clinical symptoms are so similar to those of other diseases and because, when the disease is suspected, confirmatory diagnosis is difficult and time consuming. Smorodintsev [216] suggests that, in view of these difficulties, the patient should be questioned extensively as soon as possible so that his contact with vectors may be established or disproved. Hemorrhagic fevers are naturally focal diseases with foci in the Rostov, Bulgarian, Western Siberian, Khabarovsk, Moscow, and Amur regions [18, 30, 88, 172, 277, 278]. High humidity and the proximity of the population to swampy areas affects the mortality rate [18]. Mild cases may be undetected or misdiagnosed, while acute cases may be fatal [278]. The red field mouse, rats, other mice, voles, and muskrats have been carriers in various epidemics and an increase in the forest-mouse population seems to herald outbreaks. Because of the diagnostic and therapeutic difficulties, extensive prevention involving frequent land clearing and vigorous antirodent programs is practiced [88, 277, 278].

II. HOOF-AND-MOUTH DISEASE

Hoof-and-mouth virus enters the bloodstream rapidly, penetrates organs, and disrupts normal hematopoiesis. Changes in virulence were found to be linked to changes in penetration properties and multiplication capacity. Viruses whose virulence has been attenuated by passaging penetrate the blood and organs less easily and multiply with difficulty [78, 272].

Investigations have shown that various tissue cultures are suitable for the cultivation of virus for diagnostic and vaccinal use. Bovine epithelium [148, 243], swine kidney, and newborn rabbit tissues [102, 206, 235, 245] have all proven satisfactory for these uses. Foot-and-mouth virus has a complex immunological structure and therefore, many diagnostic tests for the disease can be used [195]; these include complement fixation [155, 215], virus neutralization [242], and diffusion precipitation in agar [165]. Skomorokhov [215] recommends complement fixation, which can be used to evaluate vaccine potency.

Techniques for the preparation of virus-containing tissue which the Soviets have found effective are the use of Ascangel [24], chloroform [102, 272], freon [272], and the mincing of freeze-dried rather than wet tissues [116, 161]. A combined treatment with freon, n-butanol, and chloroform removed 98--99% of tissue proteins without lowering the original virus titer [272]. Adding the purified virus to fat-free milk and spray drying at 30-35°C is a convenient drying method. When stored, the virus had a shelf life of 1 year at 17--25°C when its residual moisture content was 6--9%. No change in storage properties was observed when the moisture content was reduced to 1.3--4.4%. This extra-dry preparation could then be stored at 37°C for 60 days without loss of activity [71, 161].

Attenuated and dry hoof-and-mouth vaccines have been successful in large-scale use. A dry adsorbed vaccine which is 99% effective had been tested on 3.5 million sheep as of 1963. An attenuated vaccine which protects up to 9 months is also in use [215]; the dry vaccines usually immunized for 2-6 months [117]. A dry vaccine prepared from strain L-III was tested on cattle. Its preliminary success and low allergenicity have recommended it for wider use [176].

III. RABIES, AUJESZKY'S DISEASE, AND POLAR MADNESS

Rabies and "polar madness" or "rage" (Tundra rabies) of arctic animals, especially foxes, and Aujeszky's disease or "pseudorabies" are viral diseases that affect wild animals primarily. Humans are susceptible to rabies and possibly to rage virus but not to Aujeszky's disease virus [254]. The table presents criteria for distinguishing rabies from Aujeszky's disease.

Table V
Differential diagnostic criteria for Rabies and Aujeszky's disease

DIAGNOSTIC SIGN	RABIES	AUJESZKY'S DISEASE
Reaction of virus to drying	weakens	quite resistant
Location of virus in the body	nerves	blood and lymph
Infectivity of saliva	always	not in the absence of nasal discharge
Infectivity of blood	sometimes	yes
Behavior of virus on being heated to 60°	killed in 5-10 min	killed in 30-50 min
Babes-Negri bodies	present	absent - acidophilic inclusions seen in spinal ganglia of cattle
Incubation period	days to weeks	always short
Prodromal period	several days	absent, rapid onset of disease
Aggressiveness in the affected animal	marked	absent
Scratching	rarely	as in swine
Drooping of lower jaw	often	seldom
Quick death	seldom	often
Susceptibility of humans	yes	no
Oral infection	no	yes
Virus in the parenchymal organs	no	yes
Swallowing inedible objects	yes	no

Table V continued

Means of spreading the disease	biting	by contact and in food
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Taken from: Orlov, F. M., Comp. Virusnyye bolezni zhivotnykh (Virus diseases of animals) Moskva, Sel'khozizdat, 1963, 163-164.

"Rage" has been observed in wild and domesticated polar foxes [89, 90, 104, 105, 106, 235, 236], wolves, dogs, deer, bear, elk, and lemmings [235]. It is believed that the mortality rate is over 90% and that lemmings form a natural reservoir of this disease and spread it to foxes during their migrations [235]. These foxes probably infect domesticated dogs. Immunological and serological tests show that this virus is similar to but distinct from rabies and all other neurotropic viruses [89, 90, 104, 105, 235]. Specific antibodies to "rage" are found in the blood of foxes surviving an asymptomatic form of the disease [89]. No Babes-Negri bodies were observed in any case, although unidentified intracellular inclusions were found in fox neural tissue taken from experimentally infected animals [106].

One human victim has been reported [90]. In the Nov.--Dec. 1961 outbreak in the Yamal region, a child was bitten by a fox. The child died 25 days after being bitten, despite treatment with antirabies serum. Post-mortem examination of both the human and fox tissue revealed no Babes-Negri bodies; "rage" was therefore given as the cause of death. Serological methods employing complement-fixation and virus neutralization show that this virus is related to Aujeszky's virus and infectious equine encephalomyelitis virus as well as to rabies virus [235, 236].

No data on any diagnostic methods faster or more accurate than those mentioned have been discovered in the literature. Unconfirmed reports of the effectiveness of antirabies gammaglobulin in neutralizing this virus have been published; claims that preparations made from this virus will prevent rabies have also appeared [235]. Rabies vaccine will protect foxes against "rage" virus but no cure is known for the disease once the symptoms, similar to those of rabies, appear. Therefore, prevention of contact between wild and domesticated animals is the only effective

means known for checking the spread of the disease [235]. Animals whose winter rations contain added minerals possess slightly more resistance than animals fed a standard diet [235]. Wild and domestic rodents and their ectoparasites are considered the principal reservoirs and vectors of pseudorabies or Aujeszky's disease, and fleas and ticks are the actual mechanical vectors [254]. The carrier state can last as long as 100-130 days in fleas and the disease can run its full course in 2 days in livestock, dogs and cats [254]. This shorter incubation period is one of the signs that differentiates this disease from rabies (see Table V). The disease is sporadic, usually occurs in the fall, and generally kills young animals [254]. Bioassay, histology, and diagnosis by clinical symptoms are being replaced as diagnostic methods by the more rapid immune reactions, diffusion-precipitation tests and, most recently, the fluorescent-antibody method (FAM) [32, 101, 170, 222]. The diffusion-precipitation-in-agar reaction is rapid, inexpensive, and simple to perform. Its suitability for general use is subject to proof of a lack of additional nonspecific reactions [22]. To date, the FAM is the fastest and most accurate method; the appearance of viral antigen can be detected 2 hours after infection of a chick-embryo tissue culture [3]. The types of vaccines in use are: a specific gammaglobulin which is obtained by alcohol precipitation at low temperature [124], to which killed virus is added, a chick-embryo tissue-culture vaccine in which the virulence of the virus is decreased by long passaging [213, 242], a similar vaccine prepared from virus passaged on mouse tissue [242], hyperimmune serum, brain homogenate [254], and a vaccine prepared in Czechoslovakia from a weakly virulent, freshly isolated strain [214]. The last vaccine, still experimental, was announced in 1964. The virus was suggested as a possible vaccinal strain; if it is, the need for long passaging [87] to obtain sufficiently attenuated virus would be eliminated. Challenging vaccinated laboratory animals with the wild-type virus showed the efficacy of all the vaccines tested. In addition, specific gammaglobulin alone protected newborn pigs with an immunity that lasted for three weeks [124, 4]. Passaged virus was used in large-scale tests and was the most effective among thousands tested. Only a few animals died [213]. "SUCH-1," the viral strain isolated in Czechoslovakia, protected test animals as well as a passaged strain [214].

Examination of rabies vectors covered rodents.

including bats, and emphasized canines. Stray dogs are the principal vector in civilized areas. Wild foxes are considered the principal reservoir of the disease, spreading it to dogs. A survey has shown that pigs and birds also carry the disease [157]. Strains of rabies virus isolated from wolves and dogs are the most virulent and the least virulent strains come from humans, simians, and livestock [157]. Immunofluorescence and the FAM are used to determine specific antigens for differential diagnosis of rabies. These methods are both faster and more accurate than diagnosis by clinical symptoms, histology, and bioassay [218]. The FAM permits visual localization of antigen and cytoplasmic inclusion formation in living cells [196], and when the dye is bound to hyperimmune donkey serum, it is the most useful method for differential diagnosis of rabies [120, 192, 221].

Since some of the most effective rabies vaccines are difficult to prepare and maintain and have dangerous side effects, both tissue cultures and various viral strains are being screened in the search for suitable replacements for antirabies gammaglobulin and brain-tissue vaccines. A study of rabies virus variants showed that after long passaging in tissue culture the original wild-type strain acquires a shorter incubation period before causing symptoms and requires a lower D₅₀ (intracerebral) for infection of rabbits, but is completely apathogenic in rabbits when administered subcutaneously [158]. Variants of standard strains have been obtained; some of them are more pathogenic than the wildtype, producing symptoms in 1--2 days but with no discernable Babes-Negri bodies, while others cause atypical clinical forms of the disease. Especially interesting are strains isolated from African dogs suffering from a rabies-like disease related to Arctic "polar madness" or "rage," strains from cattle suffering from a disease described as "plague," and miscellaneous atypical strains from human adults [158]. When several variants were tested on different animals' tissues it was shown that homologous immune sera were the most accurate for diagnostic purposes [192]. These results have prompted research into different virus strains and substrate tissues for vaccine preparation.

Since passage in a given animal brain-tissue culture increases the virulence of the virus for that particular species and lessens it for other species, and since route of administration alters the clinical course of the

disease [153], it is likely that a variety of tissue-culture vaccines will become standard for these reasons and because cost and production technology will be simplified.

A specific gammaglobulin obtained from vaccinated humans was slightly effective but was inferior to horse-serum globulin [76].

Rabies virus has been cultured successfully on chick-embryo fibroblasts and an effective vaccine has been prepared from this culture [197]. Allergic reactions are one of the principal disadvantages of rabies vaccines in current use and several papers were published between 1962 and 1965 on experimental vaccines with reduced allergenic and anaphylactic properties [31, 160, 210, 239]. Partial proteolytic hydrolysis of crude globulins during production reduces production volumes with increased vaccine yield [160]. Vaccine prepared from newborn rat and rabbit brain causes fewer allergic reactions than vaccine prepared from adult rat brains [31, 256]. Donkeys have been suggested as a source of antirabies gamma globulin since immune serum can be obtained from them more rapidly, their serum causes fewer reactions in use, and the animals themselves are easier to maintain and manage than horses [192].

Challenge tests of various vaccines and vaccination schemes have been reported in the literature. Production of active-passive immunity in laboratory animals has been achieved by the administration of a combined dose of antirabies gamma-globulin and 0.5% vaccine. Interference was eliminated by doses of gammaglobulin 4 days before starting the vaccination program [163]. The injection of cortisone interfered with the immune reaction, allowing a marked increase in virus multiplication [212]. Other experiments gave similar results [162, 210, 211]. The injection of standardized Fermi (formol) vaccine with or without gamma-globulin produced a plasmacytic reaction in test animals, intensified RNA synthesis, and increased antibody titer in the resistant animals [211]. There was a direct relationship between the serum antibody titer and degree of resistance in the animal which, in turn depended on the potency of the vaccine [162, 210, 211].

At a conference on the effectiveness and safety of nonallergenic rabies vaccines held on the 18th and 19th of March 1965 at the Ivanovskiy Institute of Virology of the Soviet Academy of Medical Sciences and sponsored by a com-

mission of the Ministry of Public Health, all participants agreed that nonallergenic vaccines should replace the standard Fermi vaccine [210]. This special commission was established in 1962 especially to investigate the properties of new preparations and to engage in large-scale tests of them. Both wet and dried vaccines were tested by various laboratories and agencies. Members of the commission were: R. M. Shen, Institute of Virology; O. G. Andzhaparidze, head of the State Control Institute for Medical and Biological Preparations, Ministry of Public Health; S. S. Unanov, director of the Moscow Viral Preparations Research Institute; M. A. Selimov, head of the Rabies Laboratory at the Institute of Poliomyelitis and Viral Infections, AMN SSSR; M. A. Khiyenenson, Moscow Municipal Sanitary-Epidemiological Station; A. S. Makaryan, senior inspector of the Ministry of Public Health RSFSR; A. A. Mtvarelidze, Tbilisi Sanitary-Epidemiological Station, and B. A. Kantorovich, senior scientist of the Institute of Virology. V. M. Zhdanov, director of the Institute of Virology, was in charge of the organization of the work [210]. The commission began its work in January 1963, and made the following recommendations to the Ministry of Public Health SSSR in 1965: 1) immediate introduction of the nonallergenic vaccines into medical practice; 2) broader use of the dry nonallergenic vaccine; 3) the establishment of a similar commission to carry out more detailed studies of such vaccination programs [210].

IV. SWINE PLAGUE

Swine plague causes extensive damage to the circulatory system as shown by extensive septicemia in infected animals. Secondary infections such as paratyphoid, bacterial hemorrhagic septicemia, diplococcal infections, and listeriosis are the principal dangers of this disease. Differential diagnosis is complicated by the lack of really reliable laboratory tests. Likhachev states in *Virus Diseases of Animals* that the only definite means of differential diagnosis are analysis of clinical data, results of bacteriological studies, the epidemiological characteristics of the local outbreak, and the final isolation of the agent and production of the disease in animals [118]. Most vaccines are derived from infected tissue cultures and are concentrated by treatment with freon and subsequent precipitation in acetone [34]. Virus passaged in tissue cultures (usually swine embryo) differs in virulence from the original virus. Several passaged strains retained their virulence but were not immunogenic. However, these strains were used to produce hyperimmune sera and subsequently to inoculate other tissue cultures from which an avirulent dry vaccine was prepared for administration by aerosol. This aerogenic vaccine required seven days to become effective and protected 100% of test animals for two months [33, 152, 153, 251].

V. VENEZUELAN EQUINE ENCEPHALOMYELITIS

A fluorescent antibody test and a specific hemagglutination test based on recent discoveries have been suggested as possible diagnostic methods for Venezuelan equine encephalomyelitis (VEE). In an investigation of virus reproductive dynamics in 1965, Gaydamovich and Vagzhanova discovered that a positive hemagglutination reaction can be obtained in tissue culture during the beginning of the logarithmic growth phase (6--7 hours after infection), and that there is a "threshold of infectivity" below which a positive hemadsorption reaction is not obtainable. They suggested that the hemagglutinating properties of the culture fluid would be useful for the early diagnosis of the disease and for studies of viral growth dynamics [279].

A diagnostic test for VEE based on the early appearance of VEE virus hemagglutinin in tissue cultures was devised by Gaydamovich and Vagzhanova. A comparison of results showed that neutralization indices were higher with tissue cultures than with convalescent sera and that the indices were 10,000 times lower when the cytopathic effect in mice was determined than when neutralization was performed with immune rabbit sera. Results could be obtained within 18 hours, and the method was recommended for early diagnosis of the disease [69]. The fluorescent antibody method (FAM) is also used in the study of viral reproduction and it has been modified for diagnostic use. Actinomycin D selectively halts cellular, but not viral, biosynthesis [280]; the acceleration of viral reproduction in tissue treated with the antibiotic has been reported [291]. Once cellular DNA synthesis has ceased, the FAM is employed to determine the synthesis site of VEE virus. RNA precursors and viral antigen can be detected visually in the cytoplasm by this method [280]. In the second method, when viral reproduction in tissue culture is accelerated by actinomycin-D, hemmagglutination tests can be used earlier than when the suspected culture is untreated [291].

Chemical inhibition of viral attachment to the cell membrane is reported by N. V. Kaverin. In his study, mouse fibroblasts and human liver cells in culture were treated with uranyl acetate which inhibited phosphate-group activity of the cell membrane. VEE virus attached to the cells but was easily removable, suggesting that phosphate groups are necessary for the firm binding of VEE virus to cell surfaces [100].

VI. MISCELLANEOUS DISEASES OF LIVESTOCK AND HUMANS

Insects and contaminated food and water are the principal sources of vesicular stomatitis, a disease which infects cattle, swine, and humans (the latter under laboratory conditions). Some of the principal mechanical vectors are: *Stomoxys calcitrans*, *Liberus* spp., *Crysops* spp., and miscellaneous flying insects. Complement-fixation reactions are commonly used for differential diagnosis. Surviving animals are immune for one year after recovery. No specific therapy was reported in the literature studied and the most common treatment is symptomatic [132].

In the Soviet Union, infectious equine encephalomyelitis is spread by ticks, flies, and mosquitos. The mortality rate is often 100% and Babes-Negri bodies are frequently seen in brain tissue of dead animals. Several papers have been published investigating its connection with rabies [206]. The two most common diagnostic methods are bioassay and serodiagnosis (complement-fixation). Although therapeutic use of immune serum has been successful, the resultant protection is only transitory; this method has been replaced by vaccination with a chick-embryo vaccine (used on at least 45,000 horses as of 1963) or by an attenuated live vaccine obtained from rabbit brain [206].

Infectious equine anemia is spread in a variety of ways, generally from infected to uninfected horses via contaminated food or by *Diptera*. Transmission by intestinal parasites is also suspected. After recovery, an animal can carry the infection up to 6 years. Differential diagnosis is difficult since there are several diseases with similar symptoms, and chemical diagnostic methods are often unreliable. Diagnosis is mainly based on observation of symptoms and on the fact that outbreaks usually occur in late summer--early fall. Formol and aluminum hydroxide vaccines are often unsatisfactory; quarantine is thus the only really effective preventive measure [191].

Japanese infectious equine encephalomyelitis, a disease usually called "Japanese-B" encephalitis when observed in humans, also displays seasonality. It is most common near swampy areas during the summer, when it is spread by infected horses. Transovarial transmission has been demonstrated in the following ticks which are the principal vectors during the winter months: *Dermacentor pictus*, *D. Nutt III*, *Ixodes ricinus*, *Rhip. cephalus turanicus*, *R. bursa*, *Rhiphcephalus sanguineus*, by *Ixodes sinensis*.

H. In ..., [208]. Complement-fixation with specific antigen is the most common diagnostic method. Recovery confers immunity in animals. Administration of formol vaccine (in three shots) each year before the start of the epidemic season, is effective as a vaccination program, although insect eradication is considered to be the most effective control measure [208].

Contagious pustulant dermatitis of sheep and goats (contagious ecthyma) has been reported in humans, including volunteers for experimental infection and individuals infected by a diseased animal. The disease affects mainly young sheep and goats, is spread by contaminated food and water, and has an incubation period of 8--10 days. It may be diagnosed by clinical observation, by the agglutination, precipitation, and complement-fixation reactions, by neutralization, and by bioassay. A dried vaccine, administered in saline, has been developed which protects against experimental and natural infections. Sheep are immune following recovery from natural infection. In the limited studies involving humans, little or no immunity existed after recovery and the vaccine gave limited protection [234].

Cattle are most susceptible to Rift Valley fever and are infected by insects which carry the virus from its rodent reservoirs to its principal hosts. Humans usually acquire the infection from infected cattle, and the mosquitoes *Aedes caballus* and *Culex theileri*. Other vectors are the fly *Eretmapodites chrysogaster* and the *Rhipicephalus* spp. tick [231]. Standard serological reactions are used diagnostically and several experimental live-virus vaccines have been tested [231].

Livestock, particularly cattle and their wild relatives, are the most susceptible to rinderpest. The domestic animals acquire it from contact with wild carriers which include rodents, wild birds, insects, and pigs, as well as dogs and cats. The suslik *Citellus mongolicus* is the principal reservoir and carrier [21]. There are numerous papers reporting the results of studies on the serological relationships of this virus to various simian and swine-plague viruses. In the Soviet Union, complement-fixation employing specific hyperimmune serum or viral antigen preparations is used for early diagnosis. The diffuse precipitation-in-agar reaction is commonly used for differential diagnosis [21].

Most current research published deals with studies of various viral vaccines. A formol-aluminum hydroxide vaccine has been reported to be more effective than the tissue vaccines used previously, and a live-virus vaccine (attenuated) produces an immune response within 24 hours [21]. The use of ultrasound does not inactivate lapinized vaccines unless application is prolonged (60--120 min); small doses are useful in separating the lapinized virus from the tissue culture [167]. A dry vaccine prepared from stabilized Nakamura-III (L-III) strain proved as effective as the standard formol vaccine; however, its peak immunization titer required 20 days to appear [241].

VII. POX-VIRUS INFECTIONS AND THE DEVELOPMENT OF EFFECTIVE VACCINES

Pox viruses grown in tissue culture have been employed in vaccine research and in studies of the synthesis and intracellular localization of DNA. The reactivation of heat-killed viruses of this group by closely related strains has been studied and the results of one such study are shown in Table 6.

Table VI from [33].
Reactivation between variola, vaccinia and ectromelia viruses

Inactivated virus	Reactivating virus	Control	Tested in		
			rabbits (scarification)	chick embryos (chorio-allantois)	mice (intra-plantarily)
Vaccinia (neurovaccinia)	Variola	Vaccinia, inactivated	NV	NV + VA	—
		Vaccinia, active	0	0	—
		Variola, active	NV	NV	—
			0	VA	—
Vaccinia (neurovaccinia)	Alastrim	Vaccinia, inactivated	NV	NV + AL	—
		Vaccinia, active	0	0	—
		Alastrim, active	NV	NV	—
			0	AL	—
Ectromelia	Alastrim	Ectromelia, inactivated	—	EC + AL	EC
		Ectromelia, active	—	0	0
		Alastrim, active	—	EC	EC
			—	AL	0
Alastrim	Ectromelia	Alastrim, inactivated	—	AL + EC	—
		Alastrim, active	—	0	—
		Ectromelia, active	—	AL	—
			—	EC	—
Ectromelia	Vaccinia	Ectromelia, inactivated	—	EC + OV	EC
		Ectromelia, active	—	0	0
		Vaccinia, active	—	EC	EC
			—	NV	0
Vaccinia (neurovaccinia)	Ectromelia	Vaccinia, inactivated	NV	NV + EC	—
		Vaccinia, active	0	0	—
		Ectromelia, active	NV	NV	—
			0	EC	—

NV — lesions characteristic of vaccinia (neurovaccinia strain)
 VA — lesions characteristic of variola
 AL — lesions characteristic of alastrim
 EC — lesions characteristic of ectromelia
 0 — no lesions.

In this study [36], the authors demonstrated reactivation using vaccinia, varicella, alastrim, and ectromelia viruses. Results published in later papers indicated that reactivation of a heat-killed virus depends on the integrity of its DNA and part of its protein component [37], and that the factor causing deproteinization of viral nucleocapsids is coded in the cell genome rather than that of the virus and that effects of this factor are observable within 2--2 1/2 hours after infection [151]. Reactivation does not occur in cell cultures lacking this factor. Autoradiography and the FAM (fluorescent antibody method) have been used extensively in the study of cell-virus interaction and for differential diagnosis [80, 149], and autoradiography has been employed in studies of early viral DNA and antigen synthesis [175]. The FAM detects specific viral antigens in tissue cultures within 6 hours and has been used to distinguish vaccinia, variola, alastrim, varicella, cowpox, *Herpes zoster*, and *Herpes simplex* viruses. The chief advantages of the FAM are its specificity and simplicity [80]. Using cytopathic effect in tissue culture as part of the diagnostic procedure was complicated in one case by lack of temperature control; the importance of controlled, optimum temperatures during culture incubation was stressed as cytopathic effects often were not observed at temperatures higher or lower than optimum [79]. Because of their sensitivity, standard tissue-culture methods were generally recommended for confirming diagnosis and for diagnosis of asymptomatic carriers [133]. The two variants of vaccinia virus most commonly used in the preparation of vaccines have been called "red" and "white" because of the pockmarks they produce on chick chorioallantoic membranes. Differentiation is therefore done visually and confirmed by the differing hemagglutination activity of the two variants [133].

Antiviral compounds such as 6-azauracil riboside and urethane delay mortality in mice infected with vaccinia virus intranasally and intracerebrally, but do not lower mortality rate [86].

Tissue cultures are used to evaluate the activity of strains being screened and the potency of viral preparations, and to select mutants for further screening [81]. The plaque-forming properties of vaccinia virus in chick tissue culture have rendered it useful for a rapid, accurate, and economical procedure for determining vaccine potency. The method employs test-tube cultures and gives easily interpreted results in 48 hours [81].

Clonal mutant vaccines have been prepared in tissue culture and these vaccines have successfully immunized test animals and children [227], although they possess atypical properties in comparison to the parent strains and the heterogeneous virus population from which they were isolated [135]. Heterogeneous vaccines displayed properties dependent upon the properties of their most common component [135]. The interference of inactivated virus with the multiplication of live virus in tissue culture has been traced to an as-yet-unidentified factor located in or on the inactivated virus. One of the principal factors governing the effect of the inactivated virus on live virus multiplication is the time between the injection of each type [174].

The immune mechanism involves plasmacytic response. In a series of papers [136, 225, 226, 229] the authors demonstrate the important role of phagocytosis, the significance of the site of inoculation and the inoculum dose (intravenous and intranasal are the most effective routes and intranasal is most economical inoculation method), and the degree of antibody synthesis in immunologically competent cells of the lymphatic organs. Antibody titers are highest after intravenous inoculation. The duration of immunity depends on the degree of plasmacytic response over a period of time and not necessarily on the initial degree of response [225]. Data from an experiment performed in 1962 confirm the above conclusions. In this experiment, animals irradiated with sublethal doses of gamma radiation were immunized with a dry live-virus vaccine 6--10 days after irradiation. In most cases the radiation slowed the development but not the degree of immunity. These results differ from those obtained with killed vaccines and from those obtained in experiments in which previously immunized animals were exposed to ionizing radiation [56].

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APPENDIX I.
SUPPLEMENTARY BIBLIOGRAPHY OF ARTICLES AND BOOKS CONCERN-
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APPENDIX II.
REPORTS OF CONFERENCES ON VIRAL DISEASES HELD BETWEEN 1962
AND 1965 AND THEIR PARTICIPANTS

1. Baroyan, O. V. Summary report of the Presidium of the Academy of Medical Sciences, USSR. IN: Akademiya meditsinskikh nauk SSSR. Vestnik, no. 4, 1964, 6.
2. Belikov, G. P. Local application of antibiotics. Subject discussed at the Conference on the Use of Antibiotics held in Moscow 1963. Antibiotiki, no. 5, 1964, 471.
3. Belikov, G. P. Conference of the Presidium of the I. I. Mechnikov All-Union Scientific Medical Society of Epidemiologists Microbiologists and Infectious Diseases Specialists held 8 October 1965. IN: Akademiya nauk Kazakhskoy SSR. Vestnik, no. 9, 1966, 150.
4. Bychkova, M. V. Seventh Joint Scientific Session of the Poliomyelitis and Viral Encephalitis Institute of the Soviet Academy of Medical Sciences and the Byelorussian Institute of Epidemiology Microbiology and Hygiene devoted to a discussion of the problem of tickborne encephalitis. IN: Akademiya meditsinskikh nauk SSSR. Vestnik, no. 1, 1964, 89.

Participants: Chumakov, M. P., Gilmanova, G. Kh., Gorchakovskaya, N. N., Karpov, S. P., Karpovic Panov, A. G., Pivanova, G. P., Robinson, I. A., Rzhakhova, O. Ye., Sarmanova, Ye. S., Serjeyeva, G. I. Shalunova, N. V., Smorodintsev, A. A., Stepanova, L. G., Vereta, L. A., Votyakov, V. I., Zasukhina, G. D.

5. Kats, L. N. Problems of biology and medicine discussed at the Thirteenth All-Union Conference on Luminescence held in Kharkov from 25 June to 1 July 1964. IN: Akademiya nauk SSSR. Izvestiya. Seriya biologicheskaya, no. 2, 1965, 319.
6. Kats, L. N. The Sixth Session of the Interdepartmental Scientific Methodological Commission on Anthrax Control held in Moscow 12--14 March 1964. Zhurnal mikrobiologii, epidemiologii i immunobiologii, no. 2, 1965, 156.

7. Veber, L. G. Proceedings of the Second Scientific Practices Conference devoted to the activities of sanitary epidemiologic centers of Sverdlovsk and its environs. Gigiyena i sanitariya, no. 7, 1964, 124-125.
8. Yerkhov, I. S. Joint Scientific Session on Vascular and infectious Diseases of the Nervous System held 9--12 December 1964 in Sverdlovsk. Zhurnal nevropatologii i psikiyatrit, v. 66, no. 5, 1966, 791-792.
9. Fourteenth All-Union Conference of Epidemiologists and Microbiologists and infectious Disease Specialists held in Moscow from 29 June to 4 July 1964. Zhurnal mikrobiologii, epidemiologii i immunobiologii, no. 12, 1964, 3.

Participants: Alisov, P. A., Belikov, G. P., Sokolov, M. I., Timakov, V. D., Vashkov, V. I., Yelkin, I. I.
10. Ninth Scientific Session of the Poliomyelitis Institute. Voprosy Virusologii, no. 1, 1965, 121-126.
11. Seventeenth Scientific Session of the Institute of Virology im. D. I. Ivanovskiy of the Academy of Medical Sciences, USSR. Voprosy virusologii, no. 1, 1965, 119-121.
12. Zeytlenok, N. A. Poliomyelitis and other enterovirus diseases. IN: Akademiya meditsinskikh nauk SSSR. Vestnik, no. 3, 1964, 94-96.

In 1963, the Eighth Scientific Session of the Institute of Poliomyelitis and Virus Encephalitis of the Academy of Medical Sciences, USSR, was held in Moscow. Four hundred and thirty-nine scientists from 45 cities in 14 Soviet republics, 21 scientists from seven Communist countries, and two American virologists participated in the session.

The conference dealt with problems of eliminating poliomyelitis, poliomyelitis-like diseases, non-poliomyelitic enteroviruses, latent oncogenic viruses, the improvement of live poliomyelitis vaccine, the nature of viruses, and their relationships with the cell. Twenty-six reports and talks were given on the study of the nature of diseases

observed after mass immunization similar to the mild forms of poliomyelitis and expressed in the form of a brief paresis of the lower extremity, not uncommonly recorded under the clinical diagnosis of "poliomyelitis" or "myelitis." The Session indicated the need for investigators to concentrate their attention on determining the nature of the flaccid pareses of undetermined etiology from the viewpoint of the possible role of Coxsackie A viruses, adenoviruses, new, still unknown viruses, as well as agents or factors which are not viruses. Serological studies with Coxsackie A virus strains adapted to tissue cultures, with antigens of other enteroviruses as well as adenoviruses, should be carried out on a broader scale and, depending on the results of the investigation of paired sera, methods of isolating the viruses in suckling white mice should be used.

The Session recommended that the Ministry of Health, USSR, record and take into consideration the poliomyelitis-like cases as "flaccid paresis of undetermined etiology." It is essential that in the case of these diseases as well as in cases suspicious of poliomyelitis, clarification of the diagnosis with a final recording of the diagnosis be made by commissions of authoritative specialists consisting of neuropathologists, specialists on infectious diseases, virologists, and epidemiologists. The Session also considered it necessary to organize qualified virological and serological studies in every case of a disease suspicious of poliomyelitis as well as of all cases of flaccid paresis of undetermined etiology.

The Session indicated the expediency of 1) determining the list of laboratories which can perform the necessary and adequately qualified virological and serological studies for poliomyelitis, the ECHO viruses, and Coxsackie viruses; 2) dividing the territory of the USSR into regions, assigning them to the nearest virological laboratories according to the list mentioned, and in the absence of such laboratories, assigning them locally to the laboratories of large central institutes; 3) giving aid to the laboratories in the matter of equipment, and the creation of the required conditions for carrying out the work on the necessary scale.

At the Session, 48 reports were presented on the problem of nonpoliomyelitis enteroviruses and the diseases which they cause. A study was made of different types of interaction of enteroviruses with one another and in combination with infectious diseases in human beings and animals, and processes of variation of Coxsackie A viruses on their adaptation to different tissue cultures.

Seventeen reports presented the results of a complex study of latent tumor viruses, chiefly the OV40 virus (simian vacuolating), which can be found in virus vaccines. Frequent infection of *Macacus rhesus* monkeys with the OV40 virus was determined and frequent infection of monkeys of other species was also found when they were kept together with *Macacus rhesus*.

Twenty-six reports and talks were given over to the improvement of live poliomyelitis vaccine, and 24 reports on new data were presented and discussed on the nature of the viruses and their interrelations with the cells. The other reports were on problems such as the study of distinctive inhibitors of virus activity, formed in cultures of transplantable strains of cells free of viruses. These reports aroused great interest.

The Conference noted the great importance of theoretical studies presented in the field of genetics and physiology of viruses and experimental chemotherapy and recommended the further development of work along these lines, particularly on the study of the intracellular processes of virus multiplication and the biosynthesis of their components.

13. Levkovich, Ye. N. Seventh Joint Scientific Session of the Institute of Poliomyelitis and Virus Encephalitis of the Academy of Medical Sciences, USSR, and the Belorussian Institute of Epidemiology, Microbiology and Hygiene on tickborne encephalitis and other arbovirus diseases. IN: *Academiya meditsinskikh nauk SSSR. Vestnik*, no. 1, 1964, 89-90.

In 1963 the Seventh Joint Scientific Session of the Poliomyelitis and Viral Encephalitis Institute of Epidemiology, Microbiology, and Hygiene sponsored a discussion of the problem of tickborne encephalitis

and other arbovirus diseases which was held in Minsk. Representatives of scientific institutions of 28 cities in the USSR as well as scientists from Czechoslovakia, a total of 181 participants, took part in the Session. One hundred and twenty-four reports were given on problems of differentiation and classification of viruses of the tickborne encephalitis group, new methods of studying arbovirus diseases and problems of epidemic-control measures, as well as data on the distribution of other arboviruses on the territory of the Soviet Union.

Reports of the following Soviet scientists were presented: M. P. Chumakov, L. G. Karpovich, Ye. S. Sarmanova, G. I. Sergeyeva, M. V. Bychkova, V. O. Tapupere; and from Bratislava, Czechoslovakian scientists Ye. O. Libkova, V. Mayer, R. Rzegacek, O. Kozukh, and E. Eriek concerning the isolation of virus strains different from the viruses of the tickborne encephalitis isolated in Kemerovskaya oblast' from *Ixodes persulcatus* ticks from sick persons with symptoms of an undetermined febrile illness. It was determined that these strains are distinctly different from the viruses of the tickborne encephalitis group with respect to their behavior in tissue cultures, their range of pathogenicity for animals, and in their antigenic structure. The fact that the strains isolated were arboviruses was substantiated. On a comparative study of these strains with different serological groups of other arboviruses, the characteristics of the isolated virus, which the authors called the "Kemerovskiy virus," were demonstrated. The finding of antibodies to this virus in the blood sera of human beings and animals in the region where the virus was isolated makes it possible to suppose that it has a possible role in human pathology and that it circulates in nature.

In reports of Ye. N. Levkovich, V. I. Votyakova, V. V. Pogodina, O. Ye. Rzhakhova, I. A. Robinzon, and others, the possibility was shown of isolating, as individual species, the viruses of Far Eastern and Central European encephalitides, Scottish encephalitis, Povassan, Omsk hemorrhagic fever, Kyasanur forest disease, and the Malayan virus (Langat).

On problems of the selection of attenuated strains of tickborne encephalitis, a number of reports and communications were given by L. G. Stepanova, G. D. Zasukhina, V. I. Mayer, Ye. O. Libikova, P. I. Al'brekht, and M. K. Tyushnyakova. Data were presented on obtaining strains of the tickborne encephalitis virus with attenuated nervous system virulence. A lively discussion revolved about the criteria characterizing attenuation of strains and possibilities of utilizing the latter as a living vaccine. V. I. Il'yenko and A. A. Smorodintsev reported preliminary data on the testing of the immunogenic characteristics and side-effects produced by the live vaccine against tickborne encephalitis, prepared from the Malayan virus (Langat).

In the reports of V. I. Il'yenko, A. D. Al'tshteyn, N. V. Shalunova, A. G. Karpovich, Ye. N. Levkovich, and others, on the topic, "New Methods of Studying tickborne encephalitis," the studies dealt with the phenomenon of interference, the use of cultures and tissue, the hemagglutination-inhibition test, and others. It was shown that the interference phenomenon can be used for the demonstration in tissue culture of viruses which do not exert a cytopathogenic effect, for the titration of viruses, and for the determination of antibodies in blood sera. The accumulation of interferon in the tissue culture was noted.

A series of reports was given on the utilization of the cytopathogenic effect of the virus, immunofluorescence, the diffusion-in-gel test, plaque formation, and the hemagglutination-inhibition test by V. I. Votyakov, G. I. Sergeyeva, P. K. Al'brekht, Han Shih-Tse, P. S. Karaseva, N. V. Loginova, R. G. Desyatskaya, G. P. Pivanova, S. P. Karpov, L. A. Vereta, L. G. Karpovich, O. Ye. Rzhakhova, and others. Achievements in the field of applying tissue cultures to the study of tickborne encephalitis were presented. New serological methods, particularly the hemagglutination-inhibition test, have become extensively used in the practice of laboratory, clinical, and epidemiological research. The improvement of antigens for the hemagglutination-inhibition test as well as methods for eliminating nonspecific inhibition from them have been suggested.

A. A. Smorodintsev and S. A. A. Ananyan reported the use of the hemagglutination-inhibition test for the serological demonstration of arbovirus diseases, which made it possible to detect the existence of previously unknown foci of arthropod-borne diseases of group A, C, and Buniamwer (after Casals) in the territory of the USSR.

A comprehensive study was made of the mechanism of the circulation of the virus during the period of activity of ixodid ticks and outside of the period of the biocenotic relationships of the tickborne encephalitis virus by zoologists, parasitologists, and virologists L. P. Nikiforov, Yu. A. Morozov, V. A. Bouko, L. A. Vereta, and others. Successful work was accomplished in controlling tickborne encephalitis through the utilization of the characteristic features of landscape types of foci for epizootological and epidemiological prognoses. Data on the study of the natural foci of tickborne encephalitis in various geographic regions of the Soviet Union were obtained for Belorussia, the Ukraine, the Urals, Siberia, the Far East, Tatarskaya ASSR, Udmurtskaya ASSR, and others by V. I. Votyakov, L. V. Gromashevskiy, G. I. Netskiy, L. G. Tatarinova, G. Kh. Gil'manova, A. V. Mishin, V. I. Chabovskiy, and others.

The clinical aspect of the problem of tickborne encephalitis and the tendency toward tickborne encephalitis evolution as a clinical phenomenon was presented in a review report by A. G. Panov, as well as in reports of A. I. Shapoval, K. G. Umanskiy, R. M. Gurariy, and others. The differentiation of Eastern and Western variants of tickborne encephalitis, reflecting the clinical characteristics and characteristics of the course of the disease, was considered sufficiently substantiated. It was recommended that the route of infection - arthropod-borne or alimentary - be formulated in the diagnosis. Attention was called to the need for seeking out new, more effective methods and means of therapy.

In a report by D. K. L'vov and others, data on the extensive testing of cultured, inactivated vaccine against tickborne encephalitis were presented,

Epidemiological observations and serological experiments have shown that it produces no side effects and that it is highly effective immunologically.

APPENDIX III.
SOME SOVIET VIROLOGICAL RESEARCH INSTITUTES AND THEIR
PERSONNEL

Introduction

The numerous Soviet institutions involved in virological research range from small stations or field hospitals dealing with specific problems to institutes covering the entire field. The latter perform the important research and publish most of the scientific materials on the subject. All-Union institutes also carry out studies directed toward policymaking and serve as advisory bodies to the Ministry of Health, USSR; the main virological institute in each Soviet Republic performs a similar function. Usually the central institute does research work and coordinates the studies of sanitation stations, public-health centers, and field hospitals. Among the leading institutes in Moscow are the D. I. Ivanovskiy Institute of Virology; Academy of Medical Sciences, USSR; the Institute of Poliomyelitis and Viral Encephalitis, Academy of Medical Sciences, USSR; the Institute of Epidemiology and Microbiology, Ministry of Health, RSFSR; and the Scientific Research Institute of Viral Preparations. Institutes in Leningrad include the Institute of Experimental Medicine, Academy of Medical Sciences, USSR, and the Medical Institute of Sanitation-Hygiene; the Institute of Medicine and the Institute for the Advanced Training of Physicians are located in Kiev.

The following is a list of institutions which are engaged in virological or closely related research. Affiliated scientists are listed under each institution, along with any available information on their professional qualifications, positions, and honors conferred upon them.

APPENDIX III.
SOME SOVIET VIROLOGICAL RESEARCH INSTITUTES AND THEIR
PERSONNEL

All-Union Scientific Research Chemical-Pharmaceutical
Institute im. G. K. Ordzhonikidze

Belikov, G. P.	Katunina, V. T.
Daniyelyan, N. M.	Pershin, G. N.

All-Union Scientific Research Institute of Railroad Hy-
giene, Ministry of Railroads, USSR

Ametirova, M. N.	Kozina, M. P.
Dubrovina, B. G.	Kulinkova, M. K.
Fayershteyn, S. G.	Parkhomenko, I. M.
Kazakovtseva, Ye. D.	Sokolova, N. N.
Koral'nik, B. P.	

All-Union Scientific Research Institute of Veterinary
Virology and Microbiology Ministry of Agriculture,
USSR

Polosov, V. M.	Sergeyev, V. A.
Nikitin, Ye. Ye.	Syurin, V. N.
Pozdnyakov, A. A.	Vladimirov, A. G.

Belorussian Scientific Research Institute of Epidemiology
and Microbiology

Dir: Votyakov, Veniamin Tosifovich (specialty: virology). Doctor of Medical Sciences; Professor; Director of the Belorussian Scientific Research Institute of Epidemiology and Microbiology. Nominated for corresponding membership in the Academy of Medical Sciences, USSR by the academic councils of the Belorussian Institute of Epidemiology and Microbiology; the Belorussian Society of Epidemiologists, Microbiologists, and Infectious Disease Specialists; the Ministry of Health, Belorussian SSR, and the All-Union Scientific Medical Society of Epidemiologists, Microbiologists, and Infectious Disease Specialists im. I. I. Mechnikov.

Protas, T. T.

Central Asian Scientific Research Antiplague Institute,
Alma-Ata

Shashayev, M. A.

Central Institute for Advanced Training of Physicians of
the Academy of Medical Sciences, USSR

Dir: M. D. Kovrigina

Bektimirov, M. A.

Gutikina, A. V.

Matyukova, Yu. N.

Sumarokov, A. A.

Sarayeva, N. T.

Sokkar, T. M.

Varoslavskaya, N. V.

Central Scientific Research Institute of Epidemiology,
Ministry of Health, USSR

Pille, E. R.

Control Institute for Medical and Biological Preparations
Im. L. A. Tarasevich.

Al'tshteyn, A. D.

Kravchenko, A. T.

Donetsk Province Sanitary Epidemiological Station

Anishchenko, G. A.

Gorki Scientific Research Institute of Epidemiology and
Microbiology

Dir: I. N. Blokhina

Nogicheva, M. A.

Institute for Advanced Training of Physicians. Kiev.

Zhalko-Titarenko, V. P.

Institute of Epidemiology and Microbiology. Kiev.

Dyadichev, N. R.

Institute of Epidemiology and Microbiology im. N. F.
Gamalei of the Academy of Medical Sciences, USSR

Al'tshteyn, A. D.

Avakyan, A. A.

Fedorov, Yu. B.

Kats, L. N.

Kaulen, D. P.

Ketiladze, Ye. S.

Kirillova, F. M.

Mayorova, G. G.

Mirol'yubova, L. V.

Pavlova, I. B.

Solov'yev, Valientin Dmitriyevich (specialty: virology). Corresponding Member of the Academy of Medical Sciences, USSR; Doctor of Medical Sciences; Professor; State Prize Winner; Head of the Section of Virology, Institute of Epidemiology and Microbiology im. N. F. Gamalei of the Academy of Medical Sciences, USSR; Head of the Chair of Virology, Central Institute for Advanced Training of Physicians. Nominated for active membership in the Academy of Medical Sciences, USSR, by the academic councils of the Institute of Epidemiology im. N. F. Gamalei of the Academy of Medical Sciences, USSR, Central Institute for Advanced Training of Physicians of the Academy of Medical Sciences, USSR, Institute of Poliomyelitis and Viral Encephalitis of the Academy of Medical Sciences, USSR, Institute of Virology im. D. I. Ivanovskiy of the Academy of Medical Sciences, USSR, and the Board of the All-Russian Society of Epidemiologists, Microbiologists, and Infectious Disease Specialists.

Institute of Experimental and Clinical Oncology, Academy
of Medical Sciences, USSR

Dir: A. I. Serebnov

Mazurenko, Nikolay Petrovich (specialty: virology)
Doctor of Medical Sciences; Head of the Laboratory
for Pathology of Leukoses, Institute of Experimental
and Clinical Oncology of the Academy of Medical
Sciences, USSR. Nominated for corresponding mem-
bership in the Academy of Medical Sciences, USSR,
by the academic councils of the Institute of Exper-

imental and Clinical Oncology of the Academy of Medical Sciences, USSR and the Board of the All-Union Scientific Medical Society of Epidemiologists, Microbiologists, and Infectious Disease Specialists im. I. I. Mechnikov.

Institute of Medical Parasitology and Tropical Medicine,
Ministry of Health, USSR

Naumov, R. L.

Pospelova-Shtorm, M. V.

Institute of Medical Radiology of the Academy of Medical Sciences, USSR

Dir: G. A. Zedgenidze

Cherkasov, V. F. - Manager of Scientific Methods
Department

Chermyakhovskaya, A. K.

Filatov, P. P.

Yelashov, Yu. G.

Institute of Zoology and Parasitology, Academy of Sciences,
Uzbek SSR

Lakhanov, Zh. L.

Institute of Poliomyelitis and Viral Encephalitis of the
Academy of Medical Sciences, USSR

Dir: Chumakov, M. P.

Agol, V. I.

Al'tshteyn, A. D.

Avakyan, A. A.

Bannova, G. G.

Bychkova, M. V.

Gnuni, I. M.

Gagarina, A. V.

Gavrilovskaya, I. N.

Gol'dfarb, L. G.

Iyks, S. R.

Kalinina, L. I.

Karmysheva, V. Ya.

Karpovich, L. G.

Khanina, M. K.

Khan Shi-tsze

Levina, L. S.

Levkovich, Ye. N.

L'vov, D. K.

Malygina, I. G.

Pistsov, N. G.

Pogodina, V. V.

Rodin, I. M.

Sarmanova, Ye. S.
Selimov, M. A.
Sergeyev, N. N.
Shalunova, N. V.
Shirman, G. D.

Shoshiyev, L. N.
Svet-Moldavskaya, I. A.
Svet-Moldavskiy, G. Ya.
Tsilinskiy, Ya. Ya.
Vil'ner, L. M.

Volorshilova, Marina Konstantinovna (specialty: virology) Doctor of Medical Sciences; Professor; Head of the Section of Poliomyelitis and Enteroviral Infections, Institute of Poliomyelitis and Viral Encephalitis of the Academy of Medical Sciences, USSR. Nominated for corresponding membership in the Academy of Medical Sciences, USSR, by the Council of the Institute of Poliomyelitis and Viral Encephalitis of the Academy of Medical Sciences, USSR.

Yan Zhu su

Zaklinskaya, V. A.

Institute of Virology, Czechoslovak Academy of Sciences.
Bratislava.

Albrecht, P.
Blaskovic, D.
Brauner, I.
Jakubik, J.
Jasinska, St.
Lesso, J.

Link, F.
Mayer, V.
Rada, B.
Sadecky, E.
Skoda, R.

Institute of Virology im. D. I. Ivanovskiy of the Academy
of Medical Sciences, USSR

Dir: V. M. Zhdanov - Corresponding Member of the
Academy of Medical Sciences, USSR

Bekleshova, A. Yu.
Beletskiy, V. D.
Berezina, O. N.
Bukrinskaya, A. G.
Burducheva, O.
Buzinov, I. A.
Bychkova, Ye. N.
Chervonskiy, V. I.
Danilov, A. I.
Dmitriyeva, R. A.
Dormidontov, R. V.
Dreyzin, R. S.
Feklisova, L. V.

Gaydamovich, S. Ya.
Gitel'man, A. K.
Gorshunova, L. P.
Gromykov, A. I.
Kantorovich, R. A.
Kareva, M. P.
Kaverin, N. V.
Ketiladze, Ye. S.
Klimenko, S. M.
Klisenko, G. A.
Konvalov, G. V.
Kosyakov, P. N.
Kozlova, I. A.

Kozlyakova, A. I.
Kurbanov, I. A.
Li Yuy
Lipkind, L. A.
Markaryan, A. G.
Medvedeva, G. I.
Mekler, L. B.
Mel'nikova, L. A.
Moroz, A. G.
Naumova, V. K.
Obukhovskaya, N. M.

Parfanovich, M. I.
Peterson, O. P.
Popova, O. M.
Posevaya, T. A.
Priymyagi, L. S.
Razulashova, E. B.
Rovnova, Z. I.
Ryutova, V. P.
Sergeyenko, A. D.
Shen, R. K.

Shubladze, Antonina Konstantinovna (specialty: virology) Doctor of Medical Sciences; Professor; State Prize Winner; Head of the Laboratory of Comparative Virology, Institute of Virology im. D. I. Ivanovskiy of the Academy of Medical Sciences, USSR. Nominated for corresponding membership in the Academy of Medical Sciences, USSR, by the academic councils of the Institute of Virology im. D. I. Ivanovskiy of the Academy of Medical Sciences, USSR, of the Institute of Epidemiology and Microbiology im. N. F. Gamaley of the Academy of Medical Sciences, USSR, and by the Board of the All-Union Scientific Medical Society of Epidemiologists, Microbiologists, and Infectious Disease Specialists im. I. I. Mechnikov.

Shukeyr, A. A. Sivoshinskiy, D. S.
Sokolov, Mikhail Ignat'yevich (specialty: virology) Doctor of Medical Sciences; Professor; Head of the Section for Flu Prevention; Head of the Laboratory of Viral Genetics, Institute of Virology im. D. I. Ivanovskiy of the Academy of Medical Sciences, USSR. Nominated for corresponding membership in the Academy of Medical Sciences, USSR, by the academic councils of the Institute of Virology im. D. I. Ivanovskiy of the Academy of Medical Sciences, USSR, of the Kazakh Institute of Epidemiology, Microbiology, and Hygiene, and by the Boards of the All-Union and Kazakh Scientific Societies of Epidemiologists, Microbiologists, and Infectious Disease Specialists. Also nominated by P. G. Sergiyev, V. D. Timakov, P. F. Zdrodovskiy, and A. A. Smorodintsev (Active Member of the Academy of Medical Sciences, USSR, and Kh. Zh. Zhumatov (Corresponding Member of the Academy of Medical Sciences, USSR).

Spitsyna, L. N.
Stakhanova, V. K.
Terskikh, I. I.

Titova, N. G.
Tsareva, A. A.
Tsi Tyan'-mac

Vagzhanova, V. A.
Vanag, K. A.
Vasilenko, V. A.
Vlodavets, V. V.
Vorkunova, G. K.

Yankevich, O. D.
Yershov, F. I.
Zairov, G. K.
Zakstel'skaya, L. Ya.
Zhantyueva, Ye. M.

Kaluga Province Division of Public Health

Kobzar', M. S.

Yavrumov, V. A.

Khabraovsk Antiplague Station

Kalmykova, A. D.
Kraminskaya, N. N.

Vasilenko, O. G.

Khabarovsk Scientific Research Institute of Epidemiology
and Microbiology

Vereta, L. A.

Yur'yeva, T. P.

Kiev Institute of Medicine

Dir: V. D. Bratus'

Suchkov, B. P.

Krasnodarsk Institute of Medicine

Babkin, P. S.
Lopatin, A. N.

L'vov, D. K.

Kuybyshev Institute of Epidemiology, Microbiology, and
Hygiene

Bukovskaya, S. N.

Leningrad Institute of Experimental Medicine of the Aca-
demy of Medical Sciences, USSR

Smorodintsev, A. A. - Head Department of Virology

Leningrad Sanitary Hygiene Medical Institute

Borisov, L. B.

Yakovleva, G. S.

L'vov Institute of Epidemiology, Microbiology, and Hygiene

Dir: A. I. Stolmakova

Urin, A. I.

Kishko, Ya. G.

Ioncv, L. I.

Lipkin, M. Ye.

Kolotilova, L. V.

Moscow Order of Lenin Medical Institute im. I. M. Sechenov

Yashkul', V. K.

Yelkin, I. I.

Moscow Provincial Sanitary Epidemiological Station

Spotarenko, S. S.

Moscow Scientific Research Institute of Viral Preparations

Dir: O. G. Andzhaparidze

Bogonolova, N. N.

Milushin, V. N.

Desyatskova, R. G.

Neustroyev, V. D.

Dosser, Y. M.

Rapoport, R. I.

Gendon, Yu. Z.

Rozina, N. Ye.

Genkina, F. B.

Semenov, V. F.

Gurvich, E. B.

Stepanova, L. G.

Karaseva, P. S.

Shutov, A. V.

Kokovikhina, K. I.

Unanov, S. S.

Kolosov, V. I.

Varshaver, N. B.

Levchnko, V. D.

Yermakova, M. N.

Mal'tseva, N. N.

Zalkind, S. Ya.

Marennikova, S. S.

Odessa Scientific Research Institute of Epidemiology and
Microbiology im. I. I. Mechnikov

Dir: Kh. Zh. Zhumatov

Feoktistov, A. Z.

Tokar', R. G.

Kondrashova, Z. N.

Omsk Scientific Research Institute of Naturally Focal
Infections, Ministry of Health, RSFSR

Fedorova, T. N.
Korsh, P. V.
Ravdonikas, O. V.

Yegorova, L. S.
Zakorkina, T. N.

Order of Lenin Military Medical Academy im. S. M. Kirov

Bashmakov, G. A.

Tarasov, V. N.

Perm Scientific Research Institute of Vaccines and Sera

Minayeva, V. K.
Pshenichnov, A. V.

Starodubtseva, G. I.

Primor'e Territory Antiplague Station

Sotnikova, A. N.

Riga Republic Sanitary Epidemiological Station

Gromova, Z. V.
Marder, B. B.

Marder, V. L.

Rostov Sanitary Epidemiological Station

Perelatov, V. D.

Rostov Scientific Research Institute of Medical Parasitology

Komarova, O. N.

Kvashnina, A. S.

Scientific Research Institute of Epidemiology, Microbiology and Hygiene. Kazan.

Gil'manova, G. Kh.
Lapshina, G. N.

Livanova, I. A.
Tuisheva, R. M.

Scientific Research Institute of Epidemiology and Microbiology of the Ministry of Health, RSFSR

Chernos, V. I.
Genkina, F. D.

Gondon, Yu. Z.

Scientific Research Institute of Vaccines and Sera im. I. I. Mechnikov

Kobrinskiy, G. D.
Mikitenko, A. A.

Yumasheva, M. A.

Turkmenian Antiplague Station, Institute of Zoology and Parasitology, Academy of Sciences, Turkmen SSR

Bel'skaya, G. S.

Zagniborodova, Ye. H.

Ukrainian Scientific Research Dermatovenereological Institute

Bogdanova, M. G.
Kislyakova, L. N.

Limarenko, M. I.
Tseraidis, G. S.

Vladivostok Institute of Epidemiology, Microbiology and Hygiene

Sonov, G. P.

Soldatov, G. H.