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May 19, 2023

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We have completed a mandatory declassification review of the INSCOM information in accordance with Executive Order (EO) 13526. As a result of our review, information has been sanitizied that would result in an unwarranted invasion of the privacy rights of the individuals concerned. This information is exempt from the public disclosure provisions of the FOIA pursuant to Title 5 U.S. Code 552 (b)(3) and (b)(6). Exemption (b)(3) pertains to information that is exempt by statute. The applicable statute is 50 U.S.C. § 3024 (i), which protects intelligence sources and methods.

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VIROLOGY (BW RELATED) U.S.S.R. (U)

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Publication No.
FSTC-CS-03-3-68-INT
Amendment A

US ARMY MATERIEL COMMAND
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VIROLOGY (BW-RELATED)--USSR (U)

Publication No. FSTC-CS-03-3-68-INT, April 1968, is amended as follows:

Make the following pen-and-ink changes:

Page 1, para 2, line 2: Change "improvement" to "improve".

Page 3, para 4b., line 2: Change "off" to "of".

Page 10, para 4f. (2), line 2: Change "thee" to "the".

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2. (U) ~~(C)~~ Make the following pen-and-ink changes:

(U) Page v: Under LIST OF TABLES opposite the word, "Table:" Add the following title:

"0.1. Cultivation of Venezuelan Equine Encephalomyelitis Virus in Different Culture Systems ---------- 4"

(U) ~~(C)~~ Page 14, para 5b, last sentence. Change to read: "The automated continuous systems developed by Tarasov of the Military Medical Academy and by Mishin of the Institute of Virology add to this capability."

3. (U) Remove old pages and insert new pages as indicated below:

Remove pages 3, 4, 11, and 12 and insert new pages 3, 4, 4.1, 11, and 12.

4. (U) Insert new pages as follows:

Insert pages iv.1 and iv.3.

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Virology (BW Related) U.S.S.R. (U)

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PREFACE

(U)
~~(S)~~ The purpose of this study is to present virological research conducted in the U.S.S.R. that is most probably related to BW. Based on open literature and classified reports, it covers important personalities, institutions, military involvement, and limited associations with other Warsaw Pact countries.

(U) Although the cutoff date from information in this document is October 1967, major updatings have been made up to the date of final approval for printing (February 1968).

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SUBJECT MATTER	PAGE NUMBERS	DATE
Title Page	None	Original
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Summary	vii (Reverse Blank)	Original
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VIROLOGY (BW RELATED) U.S.S.R. (U)

SUMMARY

(U)
~~(S)~~ Although Soviet research and development in virology, under the Academy of Sciences, appears to be oriented solely toward the solution of public health and medical problems, there are definite indications of activity related to biological warfare (BW). The best screen for BW-related activity, and the program most emphasized by the Soviets is the search for effective vaccines to be used against pathogenic agents (many of which are potential BW agents). Behind this screen, all facets of research required for BW development can be conducted, including isolation, cultivation, strain selection (from the environment and by mutations), pathological characterization, stabilization, drying, storage, and aerosol dissemination and characterization. Scientists in a number of institutes are involved in research leading to the preparation of these viral vaccines.

(U)
~~(S)~~ The U.S.S.R. has expanded facilities, increased the number of trained personnel, and has improved the quality of research and development. A shortage of complex equipment, pure chemicals and reagents, and biological preparations still exists. Administrative politics and favoritism often tend to hinder research efforts.

(U) The fluorescent antibody staining technique (FAST) has been incorporated as a research tool in the major institutions. It is used primarily for detection and diagnosis of viral diseases and related studies.

(U)
~~(S)~~ One of the prerequisites for the production of BW viral agents is the capability for large-scale cultivation of tissue culture cells. The Soviets have demonstrated this ability to mass-produce tissue culture cells in various institutions. Much research and interest is shown in the use of human diploid cells for the preparation of desired vaccines.

(U)
~~(S)~~ Soviet research has become more basic, emphasizing the structure and biochemistry of viruses , and researchers have followed methods and techniques reported in the literature of other countries and are rapidly overcoming the lack of knowledge in basic genetics.

(U)
~~(S)~~ An interest in infectious nucleic acids of viruses has been expressed by Soviet scientists. Although the work has been directed toward practical applications related to public health, a possible application for BW offense exists, either for naked infectious material or for the tailor-making of BW agents.

(U)
~~(S)~~ There appears to be little cooperative effort between the U.S.S.R. and other Warsaw Pact countries, other than the exchange of data and personnel.

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VIROLOGY (BW RELATED) U.S.S.R. (U)

Section I. (U) ~~(S)~~ RELATION OF VIROLOGY TO BIOLOGICAL WARFARE

1. (U) ~~(S)~~ GENERAL

Research and development of virology related to biological warfare (BW) is difficult to disassociate from the study of diseases of importance to men and animals. there are areas of work in Soviet virology, however, that seem to be receiving much greater attention than is necessary to satisfy public health needs. A number of publications presented each year on the means of defense against weapons of mass destruction confirm the existence of a BW defense program. Publications by the military on techniques of detection, diagnosis, decontamination, and therapeutic measures for viral diseases give additional evidence of a BW defense program. Since methods and techniques used in "open" virology so closely parallel those necessary for BW offensive measures, virological research and development can be related to BW programs.

Section II. (U) ~~(C)~~ RESEARCH TRENDS

2. (U) ~~(C)~~ GENERAL

The U.S.S.R. has made great strides to increase facilities, train personnel, improvement equipment, and better the quality of research and development (R&D) in virology. Their efforts seem to be directed solely toward eradication of viral diseases; however, increased research on vaccines, the effect of disease agents on vaccinated and nonvaccinated animals, aerosol studies of viruses, and the mutation of viruses is directly related to BW. There has been much effort expended in practical and basic genetics to overcome the gap that existed between Soviet and Western research. The Soviets have definitely moved from the problems of isolation of viruses into more basic research, emphasizing the structure and biochemistry of viruses.

Section III. (U) ~~(S)~~ INSTITUTES AND PERSONALITIES

3. (U) ~~(C)~~ GENERAL

Research and development on viral diseases has been reported from many laboratories within the U.S.S.R. The most significant research related to BW was reported by institutions discussed in par. 4, below.

4. (U) ~~(S)~~ VIROLOGICAL INSTITUTES

a. (U) ~~(S)~~ The Institute of Virology imeni Ivanovsky, Moscow. This leading Soviet research facility in virology is the World Health Organization (WHO) regional reference center for respiratory diseases other than influenza. The institute,

under the direction of Dr. Viktor M. Zhdanov and vice director Professor Olga P. Peterson, has a scientific staff composed of 28 Doctors of Medicine, 56 Bachelors of Medicine or Biological Science, and more than 200 persons with higher education in medicine, biology, chemistry, and physics; including technicians the staff totals about 700 people. Among Dr. Zhdanov's interests are mass immunization via the aerosol method or by conventional methods. Such emphasis on mass immunization may exist because he is responsible for devising measures against BW agents.

(1) The laboratory of ornithosis and aerosols continues to research the epidemiology and morphology of ornithosis virus. Workers have succeeded in devising methods for effectively detecting the disease much earlier than in the past by using a tissue diagnosticum for a skin test and an antigen for the complement fixation reaction. Experimental studies with mixed aerosol infections of ornithosis and influenza viruses showed that the animals subjected to mixed aerosols containing subcontagious doses of both viruses produced heavy mixed infections; it appeared that each virus enhanced virulence of the other. The idea of mixed infections is attractive for BW use, and a mutual enhancing effect would increase the benefits of such combinations.

(2) The laboratory of arboviruses, headed by Dr. Sofiya Gaydamovich, studies the basic biology of arboviruses--the epidemiology and ecology together with the immunological aspects. The work is becoming more basic research with greater emphasis on genetics and biochemistry of viruses. Interest is expressed in Eastern equine encephalomyelitis (EEE), Venezuelan equine encephalomyelitis (VEE), Western equine encephalomyelitis (WEE), and Chikungunya fever purportedly because of public health problems, although WEE, VEE, and Chikungunya are not native to the U.S.S.R.

(3) Gaydamovich's laboratory has investigated the preparation of vaccine against VEE in tissue culture. Vagzhanov, Gaydamovich, and Zhdanov determined that the reproduction of VEE in chick-embryo fibroblasts was accelerated by treatment with actinomycin D. N. V. Kaverin found that mouse L-fibroblast cells and human liver cells, treated with MeOH in the presence of 0.05 N HCl for 48 hours, blocked both the carboxyl and phosphate groups of the cell surface. Virus particles of VEE were absorbed, but after 1 hour over 80 percent of the virus was eluted. This suggested that phosphate groups were necessary for firm binding of VEE virus to cell surfaces. F. I. Yershov studied the replications of VEE in tissue cultures of chick embryo fibroblasts and of HeLa and SCH cells. Maximum titers were obtained in 24-48 hours at pH of 5.8 to 6.0. The hemagglutination titer and infectivity reached a maximum after 18-34 hours of cultivation and remained at a high level for 3 days. After 10 to 12 passages through chick fibroblast cells, VEE lost its hemagglutinating capacity but infectivity remained at the initial level.

(4) Gaydamovich and Vagzhanova devised a diagnostic test for VEE virus in tissue culture based on early appearance of virus hemagglutinin. A comparison of results showed that neutralization indices were higher with tissue culture than with convalescent sera. Results could be obtained within 18 hours, and the method was recommended for early diagnosis of the disease. The fluorescent antibody staining technique (FAST) is used in the study of viral reproduction and has been modified for diagnostic use. Cellular DNA synthesis is blocked by treatment with actinomyvin D, then the FAST is used to determine the site of synthesis of VEE

virus and to detect RNA precursors and viral antigen in the cytoplasm. The work constitutes a considerable effort on a virus that is not a public health problem in the U.S.S.R. and which yields valuable data for BW research and development.

(5) Scientists at the Institute of Virology have conducted mass vaccination of the population with attenuated viruses against influenza, mumps, and polio. Aerosol studies are also carried out in the IVK-2 chamber, with highly pathogenic viruses that are screened and characterized according to aerosol infectivity and stability. A. I. Gromyko adapted continuous ultramicroscopy to maintain direct visual control of conditions in the HBK-2 aerosol chamber to determine the dosimetry in infecting animals with viral aerosols. The time to achieve maximum balanced viral concentrations in the chamber was established to be shorter than that indicated by theoretical estimates. This adaptation could provide a means for determining more exact dosages, the virulence, and the decay rates of candidate BW agents.

(6) Basic research is also performed on myxoviruses, with some studies on herpes simplex, rabies, and polio. The institute cooperates with the Institute of Virology in Czechoslovakia on the structure and synthesis of myxoviruses. Selevonov studied the chemical structure of myxoviruses. P. S. Astakhova studied the mode of replications of viruses, using isotopes and actinomycin D to determine the site of replication in the cell and RNA moiety of the virus. Fluorescent antibodies and isotopes were used to study protein antigens.

(7) Yershov has studied virus growth to gain a better understanding of how a virus grows in the cell and the death of the cell from virus growth. He studied the effects that inhibitors of cell protein synthesis have on the development of resistance to viruses of cells treated with interferon. The resistance synthesis mechanism was studied by using puromycin (an antibiotic) and other similar inhibitors. He tried to use the resistance of white blood cells as a diagnostic tool for viral infections, but was unsuccessful.

(8) Solov'yeva investigated interferon in respect to its therapeutic and diagnostic value for tick borne encephalitis (TBE) in tissue cell culture. Treatment of reticulo-endothelial system (RES) cell cultures with interferon derived from chick embryo fibroblasts infected with Newcastle disease virus, prevented pathological effects after infection with TBE. Solov'yeva concluded that if RES cells showed no oncogenic effect the cells could be used to produce interferon for therapy. Gaydamovich has demonstrated that TBE antigens appear in the blood before clinical symptoms and suggests that the hemagglutination inhibition test be used for early diagnosis of TBE viruses.

(9) A publication by L. N. Mishin, laboratory of bioelectronics, described a system for the semiautomatic mass cultivation of tissue cells and the procedures by which cells grown in suspension might be infected. The CO_2 concentration in the culture was controlled by the addition of an automatically adjusted air-CO_2 mixture. Agitation was provided by a magnetically driven float-type blade mounted inside the vessel. This arrangement reduced contamination problems. The apparatus, operated on a semicontinuous basis for several months, maintained good culture conditions. Propagation of VEE virus was studied in suspensions of primary chick embryo fibroblast cells at concentrations from 2 to 10×10^6 cells/milliliter. The highest virus yields occurred at cell concentrations from 2 to 7×10^6

cells/milliliter with a high yield of 2.1×10^9 virus particles/milliliter at a cell concentration of 2×10^9 cells/milliliter. Virus yields from stationary cultures (1.5×10^6 cells/milliliter) and roller-bottle cultures (5×10^6 cells/milliliter) were 4×10^9 and 8×10^9 virus particles/milliliter respectively (table 0.1). Even though the titer was lower from the suspended cultures, these data indicate that suspension tissue cell culture systems could provide the means of propagating large masses of infectious viruses.

Table 0.1 (U). Cultivation of Venezuelan Equine Encephalomyelitis Virus in Different Culture Systems (U)

Culture system	Concentration (millions of cells/ml)	Yield		
		Viral particles/ml	Viral particles/cell	Average viral particle/cell
Stationary (Monolayer)	.3	4.0×10^8	1333	1748
	.5	7.0×10^8	1400	
	.8	1.3×10^9	1625	
	1.5	4.0×10^9	2633	
Roller	1.0	7.6×10^8	760	1281
	1.5	9.0×10^8	600	
	3.0	6.5×10^9	2166	
	5.0	8.0×10^9	1600	
Suspension	2.0	2.1×10^9	1050	349
	5.0	8.0×10^9	160	
	7.0	1.0×10^9	143	
	10.0	4.4×10^9	44	

(U)
b. ~~(C)~~ Institute of Poliomyelitis and Viral Encephalitides, Moscow. This institute, under the direction of Dr. Mikhail P. Chumakov, reportedly has a total staff of 870 people including 120 "high professionals", 18 professors, 60 candidates of science, and 24 postgraduate students. Work has continued on the genetics of poliomyelitis virus, in which attempts have been made to determine stable genetic markers and to map the viral genome. The main efforts, however, are concentrated on genetics and biology of TBE. Investigators are attempting to propagate this virus in tissue culture systems and are studying its pathogenesis in monkeys. The institute collaborates with the Institute of Virology, Czechoslovakia, in developing a live vaccine against TBE. Researchers also work on isolation and biological characterization of new arboviruses isolated from various regions of the U.S.S.R. Additional work at this institute is concerned with the technology of vaccine preparations; therefore, much of the work is in an area of applied research which would be equally effective in a BW program.

(1) The TBE viruses are in the group B arboviruses and all are closely related antigenically; they are endemic throughout the Soviet Union. Scientists at this institute have conducted large-scale epidemiological surveys and have mapped the principle foci of the disease. Hundreds of strains have been isolated from humans, livestock, rodents, birds, and even reptiles. The primary vector was found to be the Ixodes ticks; however, during one study in the Kalinin, Perm, and Kemerovo oblasts, near the Turkmen Antiplague Station (Ye. N. Zagniborodova), fleas were found to transmit the virus to rodents within six hours after birth. These findings were confirmed in laboratory experiments. Infecting Ixodes ticks artificially, demonstrated that ticks could be infected either by feeding on infected animals or by direct injection into the body cavity. Also, it was found that the progeny are infected through transovarian transmission. This work has strong BW implications because the combination of virus and vector constitutes a method for disseminating the disease.

(2) A. M. Butenko and Ye. S. Sarmanova isolated new strains of TBE in Kemerovo and Astrakhan oblasts from Ixodes ticks and human carriers which were serologically different from other strains of the TBE complex. The new strains produced a febrile-type encephalitis in humans, were cytopathic in chick tissue culture in 48-72 hours, multiplied well in the yolk sacs of 7-day chick embryos, and were fatal to newborn white mice. Other isolated strains showed remarkable pathogenic responses to intracerebrally inoculated rats and hamsters, causing varied effects on tissue cultures and producing a brief fever in monkeys. V. V. Pogodina investigated the variable pathogenicity of TBE and related strains in laboratory animals under varying conditions. Strains of TBE, Langat, Omsk fever, Louping ill, Powassan, and Kyasanur Forest viruses were studied for intracerebral and peripheral neurovirulence in an attempt to use neurovirulence as a strain marker. Of the strains tested, the Langat strain TB-21 had low intracerebral and peripheral neurovirulence and was recommended as a vaccine strain. Mice and hamsters were found to be unsuitable for differentiating strains of TBE because they displayed uniform clinical symptoms; lambs were unsuitable because of low susceptibility. Newborn pigs were found the most suitable, although none of the animals were equally susceptible to all strains. The Czechoslovaks found that tissue cell cultures inoculated with two or more viruses produce interferon. Cells that are adapted to TBE virus resist invasion by polio virus because of the interferon that is produced. This method is used to evaluate the effectiveness of TBE vaccines.

(3) Chumakov has long strived for an aerosolizable vaccine for TBE, free from immunological cross reactions. During these studies many cell and organ cultures have been screened to find suitable media for the evaluation of inocula and vaccines. Although results were much less sensitive than the intracerebral inoculation of mice, chick fibroblasts and swine and human embryo fibroblasts were found to be more precise and reproducible, and could be used for detecting neutralizing antibody. General studies of the variance in hereditary characters caused by chemical mutagens were reported by G. D. Zasukhina and I. A. Rapoport. Mutant strains of varying virulence and morphology were produced in which the results were highly significant in explaining the ability of these viruses to survive in warm and coldblooded animals. A secondary aspect of the study was the intent to create live vaccine strains from induced mutations. Several strains were selected for further study. Nonallergenic, formalized vaccines were obtained by the intracerebral infection of newborn rats by Gagarina.

(4) Tissue cell culture vaccines were found generally more effective, were easier to handle and store, and were areactogenic as compared to inactivated brain vaccines. Tissue culture vaccines were studied against eight TBE strains for their immunogenic and antigenic properties. The vaccines were examined in assays for resistance of immunized mice and in neutralization and hemagglutination inhibition tests with sera from vaccinated volunteers. Antihemagglutinating and virus-neutralizing antibodies were formed in the vaccinated mice with equal intensity to all TBE strains. Almost the same degree of antibody production was observed for Omsk fever and Langat viruses. Less antibody formation was noted with Louping ill and Negishi viruses. Antibodies to Kyasanur and Powassan viruses were rarely demonstrable, only in low titer.

(5) Omsk fever and Kyasanur Forest disease are antigenically related to TBE and present diagnostic problems similar to those for TBE. They are often misdiagnosed as TBE, leptospirosis, hemorrhagic nephroso-nephritis (HNN), and other diseases. Tables I and II compare some characteristics of hemorrhagic nephroso-nephritis and related diseases for which HNN is often mistaken.

Table I (U). Differential Diagnostic Signs of HNN, Mosquito-Borne Japanese Encephalitis and Tick-Borne Encephalitis (U)

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 paralyses (hemimonopareses), bulbar disorders	Observed as a rule. Weak, atrophic paralyses of neck muscles and thoracic region, bulbar disorders, spastic pareses - less frequent
Cerebrospinal fluid	Usually normal 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, aneosinophilia. ESR accelerated
Urine and diuresis changes	Characteristic changes (see above)	Changes not characteristics; sometimes traces of protein	Changes not characteristic, sometimes traces of protein

Table II (U). Most Important Signs of Main Forms of Viral Endemic Hemorrhagic Fevers (scheme of M. P. Chumakov, elaborated on by A. A. Smorodintsev) (U)

Main indexes	Hemorrhagic fevers					
	HNN	Crimean	Central Asian	Omsk	Kyasanur 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 subclinical (subcl) form	Vole	?	Rabbit (subcl)	White muskrat(cl), mouse (cl)	White mouse(cl), monkeys (cl)	Guinea pig(cl), white mouse (cl)
II. Epidemiology						
Seasonality	Polyseasonal	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 of their ectoparasites	+	-	-	-	-	+
III. Clinical picture						
Incubation period (days)	11-24	2-4 (M.P. Chumakov), 7-10(E.A. Gal'-perin)	3-4	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 Turk'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	+	+	+	-	-	+

(6) Gnuni and others found that agitation during cultivation of tissue cell cultures infected with virus gave higher titers. Experiments with Sabin type III polio virus and other enteroviruses revealed that roller bottles could be used to produce massive cultures of high titer in various animal and human diploid cells. Figure 1 shows two roller bottles that contain degenerated tissue cells after a harvest of virus. From this and other experiments considerable efforts apparently are being made to produce vacciness in human diploid cells, namely, the Wistar WI-38 and the Soviet human diploid cell (HDCS) strains.

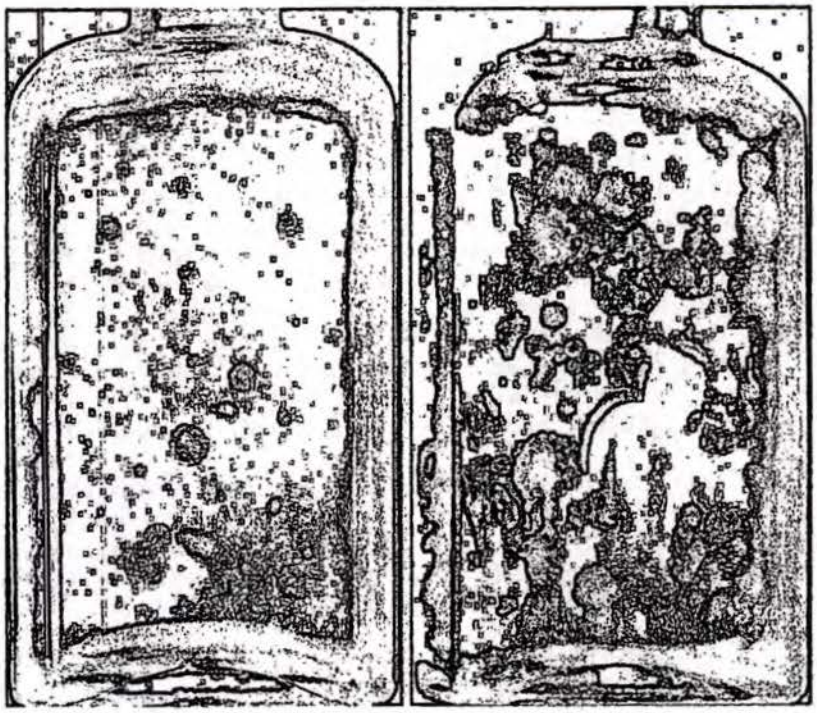


Figure 1 (U). General view of the cells after degeneration (U).

(U)
c. ~~(S)~~ The Institute of Virus Preparations, Moscow

(1) The major mission of this institute is the production of human virus vaccines, virus diagnostic reagents, and tissue cell culture reagents. Much emphasis has been placed on the mechanisms involved in the role of nucleic acids. Dr. S. S. Unanov has replaced Dr. O. G. Andzhaparidze as head of the MRIVP. Andzhaparidze is now Deputy Minister of Health and is responsible for all virus vaccine production in the Soviet Union. He still maintains a laboratory at MRIVP and directs a research group wrking on TBE.

(2) The MRIVP produces the following products:

(a) Rabies vaccine - Two types, one prepared in sheep brain and one in embryo rat brain

(b) Smallpox vaccine

(c) Measles vaccine - A strain of measles virus developed by A. A. Smorodintsev and produced on primary guinea pig kidney cells

(d) Influenza vaccine

(e) Tick-borne encephalitis vaccine

(f) Adenovirus, Echo and Coxsackie A antigens, and antisera for research purposes

(g) Tissue culture media (199, Eagle's Medium, trypsin, balanced salt solution, and serum).

(3) Many of the new viral vaccine developments use the Soviets human diploid cell strains (HDCS) or Wistar WI-38. Four lots of Edmonston and one lot of Beckenham measles virus were grown in WI-38 cells. The vaccines were administered to about 100 children by subcutaneous inoculations of 10 to 300 TCD50 (Tissue Culture Dose), along with an injection of gamma globulin. Immunological responses after 1 month indicated that 93 percent of the children developed seroconversions. Only those children who received 10 to 50 TCD50 showed no conversion. The Beckman strain produced the least reactions. As a result of these studies using human diploid WI-38 strain, a large pool of vaccine was to be prepared for a mass field trial involving several hundred-thousand children.

(4) An inactivated virus vaccine for TBE that was grown either in HDCS or WI-38 cells has been reported by Andzhaparidze. An attenuated TBE vaccine which does not produce a cytopathogenic effect also was developed. Large-scale clinical trials were to be started with both types of vaccine. Clinical trials of vaccines are made through the Ministry of Health.

(5) The interference between six strains of TBE and one strain of WEE was studied by Andzhaparidze. Cell culture of chick fibroblast cells, human diploid (strain L-46), A-1, SCH, KEM, HEp-2, and HeLa cells were used. Interference decreased in the following order: chick fibroblast, HEp-2, L-46, KEM, A-1, SCH, and HeLa and varied with the strain of TBE. No correlation was found to exist between the degree of interference and the pathogenicity of TBE strains on the peripheral infection of mice. The properties of Pan and $1x^{-10}$ strains of TBE, and louping ill strain 1-40 were studied after 70 to 80 passages on swine embryo kidney cells or 120 passages on chick embryos. The neuropathogenic characteristics for white mice were unchanged. Pan, $1x^{-10}$, and 1-40 viruses produced a cytopathogenic effect on chick fibroblasts after culturing on swine embryo kidney cells, but not after culturing on chick embryos. None of the variants had a cytopathogenic effect on L or Hep-2 cells. The initial culture and resulting variants of the Pan and 1-40 strains formed plaques in SCH cells.

(6) In experiments testing old and new strains of TBE in human diploid cells, B. F. Semenov found that all strains readily grew in diploid cells and did not produce a cytopathogenic effect. After long passage in diploid cells the Pan strain acquired the ability to destroy cells. Its cytopathogenic activity in swine embryo kidney cells increased tenfold to one hundredfold and the pathogenicity for mice that were inoculated intracerebrally or peripherally was reduced.

(7) The Institute of Viral Preparations has the most active program for infectious nucleic acid research. Gendon has published articles concerning the genetic stability of Sabin strains of polio virus. He has used the conventional methodology of the photodynamic effect of proflavine and formaldehyde to induce mutants in the study of genetic markers. His experiments with infectious nucleic acids, extracted by the cold phenol technique from Sabin strains of polio, showed that infectious ribonucleic acids (IRNA) were not pathogenic for monkeys. Virulent IRNA in animals, however, cause earlier infections and have a greater effect on the central nervous system than the intact virus. Using hydroxylamine, which in-

activates virus by attacking components of its DNA and RNA, Gendon prepared inactivated antigens of polio, hoof-and-mouth disease, Newcastle disease, WEE, Echo 7, Echo 12, and influenza A2. These antigens had high hemagglutinating activity and high complement fixing activity.

(8) B. S. Diskina has studied the synthesis of polio and TBE viruses and phage in cell-free systems. Results of the studies have shown apparent increases in virus components, but there was no conclusive evidence of in vitro synthesis. IRNA was also shown to be more heat resistant than the whole virus, and mice were shown to be sensitive to intracerebral, subcutaneous, and intraperitoneal administration but not to oral administration of IRNA.

(9) The R&D conducted at this institute is closely allied to the R&D required for BW agents. Vaccine production capabilities could easily be converted and used to produce BW agents.

d. (S) Institute of Epidemiology and Microbiology imeni Gamaleya, Moscow.

(1) This Institute is the largest research and instructional center in the U.S.S.R. on problems of medical microbiology, immunology, and epidemiology. Directed by Professor O. V. Baroyan, the institute has 53 research laboratories and a production department. About 250 professional people are on the staff, which numbers close to 2000. Development of associated and live vaccines is of special interest.

(2) A Department for Genetics, headed by V. D. Timakov, investigated the genetic structure of bacteriophages, some animal viruses, and the infectiousness of nucleic acids isolated from various phages containing RNA and DNA.

(3) The Immunology Department, headed by the late Lev Zilber, experimented with Rous sarcoma virus in rats, mice, and monkeys, and studied the specific antigens present in sarcoma. FAST methods were used to determine antigenic components by Dr. Natasha Engelgardt.

(4) The Morphology Department under Professor Dr. A. A. Avakyan employed electron microscopy and FAST to study reproduction of viruses in the cell. Although Avakyan's theory that pox-type viruses were reproduced as independent organisms instead of being built up from subunits within the cell was not substantiated, his experiments with ultrathin sections under the electron microscope and acridine orange staining showed improved techniques.

(5) The Department of Virology studied the natural immunity, cytopathology, and biochemistry of nucleic acids. Solov'yev used a modified hemagglutination inhibition test to demonstrate the presence of the TBE antigen in wild birds. V. V. Kucheruk and A. A. Pchelkina examined the viremia and dynamics of complement fixation (CF) antibodies in hedgehogs infected with TBE. They found that hedgehogs were highly susceptible to TBE when given very small subcutaneous doses. Doses of 0.1 LD50 produced long and intensive viremia of the two-wave type. The disease reached a peak on the 4th to 6th and 12th to 15th day after infection. Complement fixing antibodies appeared in the blood by the 10th day, peaked on the 30th day, and were nearly gone by the 124th day.

(6) Scientists also demonstrated the important role of phagocytosis, the significance of route of infection, and the degree of antibody synthesis in cells of lymphatic organs. The intravenous and intranasal routes of inoculation were the most effective. Vaccines that were prepared by clonal mutation in tissue cell cultures were also found to be immunogenic.

(7) The natural foci of epidemic hemorrhagic fever and TBE were studied in parts of Khabarovskiy Kray by Vereta and Yur-Yeva. Reports of both diseases in humans frequently coincided with an increase in the rodent population and their ectoparasites. The lack of serological relationships between the viruses was demonstrated by the hemagglutination inhibition test. Coincidentally, V. G. Petrov reported a method of artificially rearing ticks in the laboratory; however, ticks that were artificially infected with viruses provided an excellent system for disseminating BW agents. After removal from the breeding chamber, the ticks were stored at 4° to 6° C. in special containers for as long as 212 days.

(8) Ilyashenko studied the chemical and physical characteristics of infectious nucleic acid (INA). The role of cytosine photohydration reaction products and the participation of thymine dimers in the ultraviolet inactivation of infectious Φ-X-174 phage DNA and its replicative form (RF) were evaluated. DNA cytosine photohydration products (5-hydro-6-hydroxycytosine) do not enter into UV-inactivation of DNA phage. Sensitivity of UV was correlated with DNA thymine level for complementary chains of phages RF. Such correlation was not demonstrated when different phages were compared. An alternate hypothesis to the "repair" theory of Setlow and Carrier was proposed to explain nonlethal structural damage to DNA. Their "information correction" theory proposes that, while the initial DNA strand remains injured throughout the replication process, it is possible that an undamaged corrected DNA strand is formed by a process analogous to the correction of transmission errors in systems containing redundant information in communications theory.

(U)
f. ~~(S)~~ Institute of Experimental Medicine, Leningrad.

(1) This institute occupies several buildings in the northeast section of Leningrad. The Virology Department, which is headed by A.. A. Smorodintsev, has one division working on respiratory diseases and another working on arboviruses. Smorodintsev continues to search for an influenza vaccine that is effective, genetically stable, and live. The virus loses virulence and immunological properties during passage in chick embryos. The live vaccine used in the U.S.S.R. is no more potent than the inactivated vaccines used in the United States. Reportedly, the military are intranasally vaccinated against influenza, with the vaccine probably administered by drops, since the Soviets have not yet found an aerosolizable influenza vaccine. Considerable work is now being done on measles and mumps viruses--vaccines for measles and mumps have been prepared and successfully used.

(2) Researchers in the arbovirus department have prepared vaccines for Russian-spring-summer-encephalitis (RSSE). In 1964, the Ministry stopped Smorodintsev from preparing vaccines in monkey kidney cells because the cells contained viruses. The laboratory found that by passing the Langat strain of RSSE through guinea pig tissue cells, the infectivity was increased enough to produce good antibody response. Several thousand people have been successfully vaccinated with this RSSE vaccine.

(3) N. Ye. Garev used the agar diffusion precipitation reaction for serological identification of TBE viruses. The reaction was highly specific, easy to use, and produced results in 24 to 48 hours.

(4) Smorodintsev cited a number of difficulties that have hindered virology R&D in the U.S.S.R. A shortage of complex equipment necessary for modern research exists; biological preparations (insulin, erythromycin, new forms of penicillin, and pure chemicals) and many experimental vaccines are difficult to obtain; and administrative politics are detrimental to research programs. Approval of the Ministry of Health is necessary to test vaccines. If a vaccine has good propaganda or political value, clinical trials can begin within a few days, otherwise, action on routine requests is uncertain. Recent information indicates that Smorodintsev now heads the Institute of Influenza in Leningrad. Little is known about this new organization, but it was reportedly established expressly for the study of influenza and other respiratory diseases.
(U)

g. ~~(C)~~ Institute of Molecular Biology, Moscow.

(1) In 1958, the Institute of Radiation and Physical-Chemical Biology was established by the Academy of Sciences; then, in 1962, the name was changed to the Institute of Molecular Biology. Research in genetics was misdirected and partially ignored by Soviet scientists before 1956. Few personnel were trained in basic genetics, and facilities and equipment were inadequate for conducting the sophisticated research required. To get started in the field, the Soviets bent their efforts toward the study of basic genetics to provide the necessary background and skill to carry out modern research. True to form, they mustered available resources, avidly digested foreign literature and began the struggle to catch up in the field.

(2) Under the leadership of Dr. V. A. Engelgardt, the staff comprises the most competent group of biochemists in the U.S.S.R. About 70 scientists and 150 technicians are on the staff. The Department of Ribonucleic Acid Structure, headed by Professor A. A. Bayev, has worked on the primary structure and the sequence of nucleotides of tyrosine transfer RNA. The Virology Department (Georgiyev) investigated nucleic acid metabolism of cells that were infected with adenoviruses. Several categories of RNA that were extracted from animal cells by phenol procedures were isolated and characterized. The Morphology Department, headed by Mrs. Tikhonenko, used the negative staining method for electron microscopy to study the structure of T-even bacteriophages.

(3) Viruses are nucleoproteins that consist of protein and DNA or RNA. The nucleic acid is the infectious element of the virus and carries the genetic information. The study of the properties of viral nucleic acids and their role in transmitting infections has been actively pursued by molecular virologists. Knowledge of the nature of viruses and their interrelationship with cells is important for developing vaccines against viral diseases and for preparing infective viruses with variable characteristics, or their nucleic acids, for BW use.

(4) A method for sequential extraction of isolated cell nuclei by dilute salt solution at pH of 8.0 and 7.8 to 8.0 was reported by Samarino. He found that RNA was sensitive to RNase in homogeneous particles, was resistant to DNase, and was resistant to changes in the magnesium ion concentration. The particles were

considered to be transfer-messenger RNA. M. K. Kukhanova and N. V. Kaverin studied the mechanism of protein synthesis in cells that were infected with Newcastle disease virus by using C^{14} to label amino acids in cell protein to determine hemagglutinin production. A decrease of C^{14} amino acids in total cell protein began 5 hours after infection and accompanied the synthesis of virus hemagglutinin. A period after infection was concluded where the rate of cellular protein synthesis was reduced in the presence of intact polyribosomes. A part of the polyribosomes might be linked to the virus RNA since hemagglutinin starts to form at that stage and at the stage where polyribosomes still function, showing that the regulatory mechanism of protein synthesis acts on the genetic level. The protein regulatory mechanism might relate to depressed protein synthesis in virus infection as well as to cellular protein synthesis regulation.

(5) Investigations were made by E. S. Zalmanzon and L. S. Labareva on the incorporation of P^{32} and C^{14} adenine into fractions of RNA of the cytoplasm and nuclei from FL cells with type 1 poliovirus. Results showed that in the presence of actinomycin D the synthesis of all RNA fractions was inhibited within 60 to 90 minutes after infection. From 2 1/2 to 4 hours after inoculation, actinomycin D inhibited 98 to 99 percent of the synthesis of nuclear RNA not extractable by cold phenol from infected and uninfected cells. The synthesis of cytoplasmic RNA was inhibited by 45 to 62 percent in infected cells and by 73 to 99 percent in uninfected cells. Cytoplasmic RNA that was extractable by cold phenol was inhibited more in uninfected than in infected cells. Since 99 to 100 percent of infectious poliovirus was detected in cytoplasmic fractions, the conclusion was that synthesis of polioviruses RNA takes place in the cytoplasm without participation of the nucleus.

(6) Analysis of available reports indicates that Soviet molecular biologists are hindered by the lack of sophisticated equipment, the shortage of highly purified chemicals and reagents, and the lack of synthetic media. Despite these shortcomings, many Soviet researchers are accomplishing high-caliber research with the tools that are available. Most of their work has copied free world techniques and has provided the necessary experience to begin original research. Although Soviet scientists are still lagging behind in most facets of molecular biology, they are rapidly closing the gap.

(U)

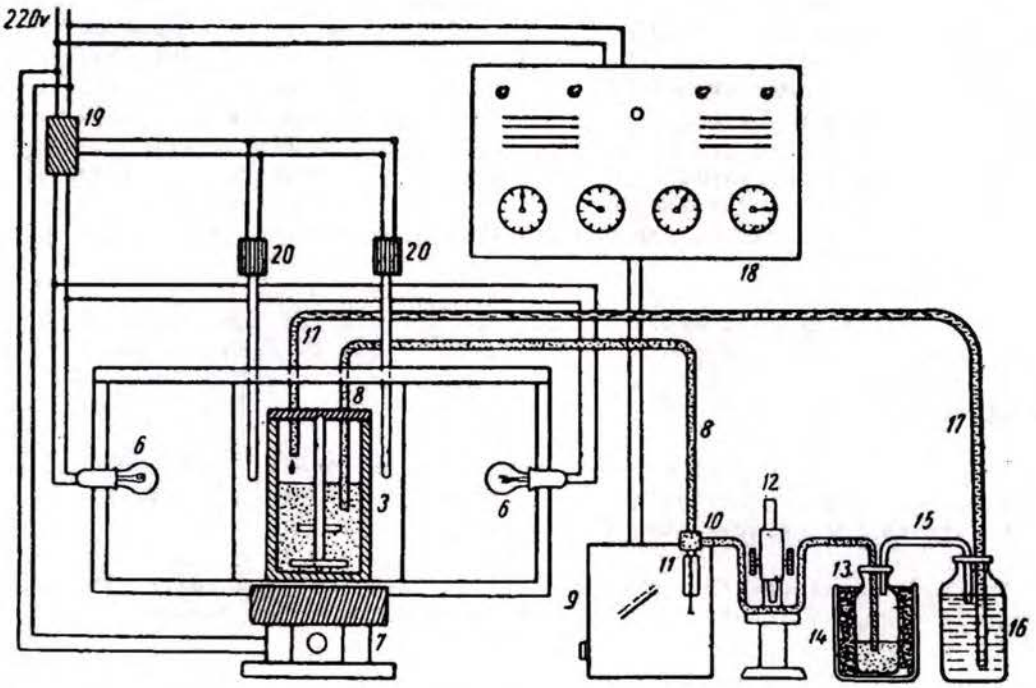
h. ~~(C)~~ The Military Medical Academy imeni Kirov, Leningrad.

(1) The Military laboratories openly conduct research and development on BW defense. Many papers on BW defensive measures, including research on detection, identification, decontamination, and therapeutic applications are published by military scientists. Viral agents are produced at these laboratories, suggesting a possible interest in offensive BW. The research laboratories are tightly controlled and guarded--even visitors from the Warsaw Pact countries are not given access to laboratories that are conducting BW defense.

(2) The military has shown great interest in detection of viruses by using FAST to rapidly diagnose diseases. G. A. Bashnokov has reported fluorescent antibody detection of tick-borne and Japanese encephalitis virus. Applied to various types of cells infected with TBE or Jap B, the virus was detected in 18 to 48 hours, depending on the type of cells used. The fact that virus was detected in the medium after 24 hours makes FAST a fairly rapid method.

(3) V. V. Skvortsov stressed the importance of detecting viruses in aerosols and water. The main methods of detection discussed for viruses were the conventional techniques--filtration followed by animal inoculation, serological techniques, and the use of fluorescent antibodies. A report on decontamination showed that E. coli could be used as an indicator for the decontamination of adenoviruses in water. The time for deactivating a mixture of E. coli and adenoviruses with chlorine was essentially the same.

(4) Plastics were screened for use in the growth of tissue cultures. Large plastic containers were found to be suitable for the mass production of cells in suspension cultures. V. N. Tarasov incorporated a plastic reactor in a modified hemostat (Figure 2) to mass produce tissue cells and viruses. The system was provided with semiautomatic devices to control pH, temperature, regulated feeding, and a receptacle to collect the cells. Development of this device indicates that Soviet interest in mass-production of viruses exists and that such systems would provide an excellent means for producing viruses for BW purposes.



(1) portable thermostat (not shown); (3) plastic reactor; (6) heaters; (7) magnetic agitator; (8) polyvinyl chloride discharge tube; (9) pouring machine or peristaltic pump; (10) valve adapter; (11) injector; (12) device for microscopic inspection of growing cells; (13) receiving flask; (14) container for ice; (15) connecting pipe; (16) flask with extra nutrient medium; (17) polyvinyl chloride feeding tube; (18) electronic device for regulating the feeding of fresh nutrient medium into instrument reactor; (19) thermorelay; (20) contact thermometer.

Figure 2 (U). Diagram of design of a simple hemostat for continuous cultivation of tissue cell cultures (author's modification) (U).

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Section IV. (U) ~~(S)~~ BIOLOGICAL WARFARE OFFENSE

5. (U) ~~(S)~~ VIROLOGY RELATED TO BIOLOGICAL WARFARE

a. No clear-cut, direct evidence exists that the U.S.S.R. has an active offensive BW program. On the surface, all of the Soviet work appears to be directed toward public health and medical needs; however, greater emphasis seems to be directed into areas other than those necessary for public health measures. Soviet R&D on TBE and hemorrhagic fevers continues year after year and is reported from many laboratories, including military groups. During a visit to the United States, Dr. Smorodintsev expressed the opinion that the amount of research being carried out with TBE was far out of proportion to the harm that the disease does to the general public. The experience accumulated from years of research on the preparation of vaccines, assessment of vaccines in animals and comparisons with noninfected animals, methods of cultivation in tissue cell culture, and studies of infections in the natural environment leaves no doubt that the Soviets could produce these viruses for BW purposes. Scientists have reported successful research on the artificial infection of ticks in the laboratory and found that the diseases are transferred to the progeny by transovarian passage. The tick-carrier has been reared artificially in the laboratory and stored at temperatures of 4° to 6° C for long periods of time. These results clearly indicate that the capability exists for the development of a vector-virus for a BW agent system.

b. The development of tissue cell culture systems for the production of viruses enhances the Soviet capability to produce viral BW agents. The automated continuous systems developed by Tarasov of the Military Medical Academy ~~adds to this capability.~~ and by Mishin of the Institute of Virology add to this capability.

c. Research on the mutation of viruses, studies on viral RNA and DNA, and the infectious nucleic acids receives much attention. This work could easily be directed toward tailoring organisms for BW use.

d. The vast knowledge that has been gained from virus research gives the Soviet Union the capability to produce pathogenic viruses for BW use.

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13. ABSTRACT

This report gives a summary of available literature on research and development of virology in the Soviet Union and its possible relation to BW. ~~(C)~~(U)

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Virus						
Viral disease						
Viral antigen						
Viral genetics						
Viral RNA						
Viral nucleic acid						
Virus cultivation						
Viral immunization						
Viral vaccine						
Tissue culture vaccine						
Phage						
Viral aerosol						
Virus-vector						
BW agent						
Fluorescent antibody staining technique (FAST)						

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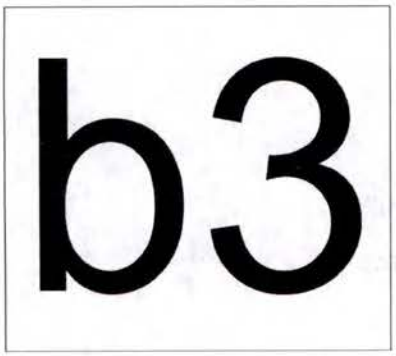

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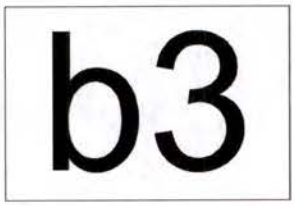

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