Antigenic Similarity between the Virus Causing Crimean Hemorrhagic Fever and Congo Virus (33847)

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In 1967, Chumakov and associates isolated numerous strains of a single virus from blood samples collected from patients in the early period of Crimean hemorrhagic fever (CHF) (1). The etiological connection was repeatedly established by the Soviet workers; not only was the virus isolated from many patients, but also the patients showed serological conversion between the acute and convalescent phases of the disease. Owing to the close epidemiological association of the illness with *Hyalomma* ticks, observed from the earliest studies (2), the virus was considered to be arthropod-borne, an arbovirus.

Strains of the virus, immune sera and ascitic fluids prepared in experimental animals, and samples of convalescent-phase sera from recovered patients were submitted to the World Health Organization International Reference Centre for Arboviruses, Yale Arbovirus Research Unit (YARU), for additional characterization of the virus.

Materials and Methods. Viral strains. The present work was done with the Drosdov strain of CHF virus, one of the 3 strains supplied. This strain, in its mouse passage 28, was brought to the YARU laboratory as frozen mouse carcasses on solid carbon dioxide. The agent was easily reestablished; 1- or 2-day-old Swiss mice inoculated intracerebrally (ic) with either a 10^{-2} or 10^{-3} dilution of infected brain tissue appeared sick on the fourth day after inoculation, and died from 3 to 5 days later.

Three strains of Congo virus were employed: 3010 (3, 4) from the Congo; K2/61 (3, 4) from Uganda; and JD 206, isolated by Drs. C. L. Wisseman, Jr., and Fatima Begum, University of Maryland School of Medicine, from *Hyalomma* ticks collected in West Pakistan in 1963 (5). Strain JD 206 was identified as Congo virus in the YARU laboratory in 1967 by Dr. Begum, using a complement-fixing (CF) antigen and antiserum for this strain prepared elsewhere; the same CF reagents were used in the present study. Most of the other viruses included in the study are listed in the "Catalogue of Arthropod-borne Viruses" (6).

Sera. An immune serum for the Drosdov strain was prepared in this laboratory in 5- to 6-week-old mice given 2 intraperitoneal injections, 25 days apart; the injections consisted of 0.3 ml of virus in dilutions 10^{-2} and 10^{-1}, respectively. The mice were bled by cardiac puncture, under ether anesthesia, 7 days after the second injection.

Antigens. An antigen for *in vitro* tests was prepared by the sucrose–acetone method (7); it failed to agglutinate goose erythrocytes under standard conditions, but had a CF titer of 1:1024 or 1:2048 and was active by agar gel precipitin test at a dilution as high as 1:64.

Tests. A micro CF test was used, employing 1.7–2 units of complement and incubation of the first phase for 18 hr at 4°. The agar gel precipitin test was carried out in a 0.6% agarose gel in Veronal buffer, pH 8.6. The neutralization (N) test was done in 1- or 2-day-old mice inoculated ic; mixtures of undiluted serum and virus in dilutions were incubated for 2 hr at 37° in a water bath before inoculation.

Results. In an initial screening CF test, the antigen for the Drosdov strain failed to react with polyvalent immune sera or mouse ascitic fluids for arbovirus groups A, B, C, Bunyamwera, Bwamba, California, Guama, Phlebotomus Fever, and Tacaribe. It likewise did not react with monovalent immune sera for mouse polioencephalitis, ectromelia, herpes, and rabies.

In a second screening CF test, an immune

TABLE I. CF Test with the Drosdov Strain, Isolated from a Case of Crimean Hemorrhagic Fever, and Strains JD 206 and K2/61 of Congo Virus.

Antigen	Mouse serum or ascitic fluid		
	Drosdov	JD 206	K2/61
Drosdov	256/1024[a]	256/1024	16/1024
Congo, JD 206	256/256	256/256	32/128
K2/61	256/256	256/256	32/256
Normal	0	0	0

[a] Reciprocal of serum titer/reciprocal of antigen titer; 0 = no fixation at dilution 1:8 of serum and antigen, lowest used.

mouse serum for the Drosdov strain was tested against antigens for the following 28 tick-borne viruses not of group B (8): Bandia, Bhanja, Chenuda, Colorado tick fever, Congo (3010), Dugbe, Farallon, Ganjam, Grand Arbaud, Hughes, Johnston Atoll, Kaisodi, Kemerovo, Lanjam, Lipovnik, Mutucare, Nyamanini, Pak Argas 461, Qalyub, Quaranfil, Sawgrass, Silverwater, Soldado, Thogoto, Tribec, Uukuniemi, Wad Medani and Wanowrie. Also included were the homologous Drosdov strain antigen and an antigen prepared with normal, uninfected mouse brain tissue. The Drosdov serum, with a homologous titer of 1:256, was tested in increasing 2-fold dilutions beginning at 1:8, and all antigens were used at dilutions 1:4, 1:8 and 1:16 except for Thogoto, which because of its anticomplementary action was used at dilutions 1:16 and higher. The Drosdov immune serum gave positive fixation only with its homologous antigen and the antigen for Congo virus; the reaction occurred with all 3 dilutions of both antigens, and the titer of the serum was 1:256 or higher with each antigen.

The total similarity, by CF test, between the Drosdov strain and the 3 available strains of Congo virus was established in further tests. Table I shows the reciprocal cross-reactions of the Drosdov isolate and Congo virus strains K2/61 and JD 206, using mouse immune sera or ascitic fluids. Table II shows the reactions of human convalescent sera with antigens for the Drosdov strain and the 3010 strain of Congo virus.

A close similarity between the Drosdov strain and Congo strain 3010 was also noted by agar gel precipitin test (Fig. 1). Complete fusion of the precipitation lines occurred when a human convalescent serum (no. 5, Table II) was placed in the center wells of the slide and the antigens, either undiluted or diluted 1:4, were placed in the peripheral wells.

The results of N tests likewise demonstrated an antigenic closeness between the Drosdov strain and Congo strain 3010 (Table III). Noteworthy in Table III is the high degree of protection against Congo strain 3010 given by a mouse immune serum prepared with the Drosdov strain and by the pool of CHF human convalescent sera.

Discussion. The experimental evidence proves that viral strain Drosdov, as submitted to this laboratory, is antigenically

TABLE II. CF Test with Convalescent-Phase Sera from CHF Patients and Antigens for the Drosdov Strain and Congo Virus Strain 3010.

Serum			Antigen	
Patient	Period of illness	Date bled and no. of months after illness	Drosdov	Congo, 3010
Serum pool	1967	1967	1:16[a]	1:8
1. Boch.	Summer 1962	July 1963, 10–12	0	0
2. Ush.	May 1963	Oct. 1963, 5	1:16	1:16
3. Fur.	Summer 1962	May 1963, 10–12	0	0
4. Dja.	May 1963	Oct. 1963, 5	1:8	1:8
5. Bash.	Summer 1962	July 1963, 10–12	1:128	1:128
6. Kril.	Summer 1962	April 1963, 7–8	1:16	1:16

[a] Results expressed as serum titer; 0 = serum negative at dilution 1:4, lowest used.

TABLE III. N Tests with the Drosdov Strain and Congo Virus.

Serum	Virus and dilutions (log_{10})											
	Drosdov						Congo, 3010					
	−2	−3	−4	−5	LD_{50}[a]	NI[a]	−2	−3	−4	−5	LD_{50}	NI
Drosdov, mouse	2[b]	0	0	0	≤1.7	≥1.9	0	0	0	0	≤1.5	≥2.5
Congo, K2/61, mouse	6	1	0	0	2.3	1.3	2	0	0	0	≤1.7	≥2.3
CHF, pool, man	0	0	0	0	≤1.5	≥2.1	0	0	0	0	≤1.5	≥2.5
Normal, mouse	8	8	1	0	3.6		8	8	4	0	4.0	

[a] LD_{50} and neutralization index (NI) expressed in dex (9) as reciprocals of log_{10}.

[b] Number of mice dead of 8 inoculated; ic test in 2-day-old mice.

closely related to, perhaps even identical to, Congo virus. In investigations with arboviruses it is, of course, advisable to bear in mind that cross-contaminations in the laboratory occur not infrequently. In the present instance, however, the dates involved, together with the fact that reagents were prepared in several different laboratories, make the possibility of a cross-contamination or mixup extremely remote. The antigens for Congo virus strains 3010 and K2/61 were prepared in 1963 in the New York Laboratories of The Rockefeller Foundation; the 6 individual convalescent sera (Table II) were collected from patients in the Soviet Union in 1963 and were brought to this laboratory in 1965, before any of the current strains had been isolated from cases of CHF.

In addition to the established strains of Congo virus mentioned—3010 and K2/61 from man in the Congo and Uganda, and JD 206 from ticks in West Pakistan—7 strains have been isolated recently (1966–67) in Nigeria by Dr. O. R. Causey, a Rockefeller Foundation staff member assigned to the Virus Research Laboratory, University of Ibadan (10). These 7 strains, recovered from ticks, cattle, *Culicoides* and an African hedgehog (*Atelerix*), were forwarded by Dr. Causey to YARU for identification and were found to be indistinguishable from Congo virus in CF test. Given the combined picture of the widespread geographic distribution of Congo virus, including the similar virus represented by the Drosdov strain, its severe pathogenic potential for man as reported from various areas of the Soviet Union and from Uganda (3, 4, 11) and the variety of sources from which it has been obtained in nature, a close watch on this agent is clearly warranted.

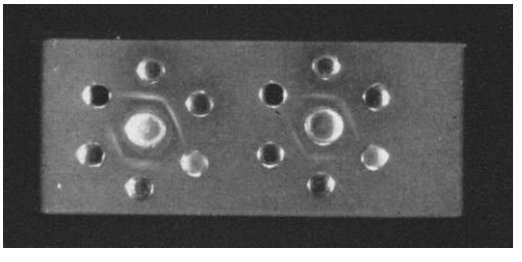

FIG. 1. Agar gel precipitin test with a CHF human convalescent serum and antigens for the Drosdov strain and Congo virus strain 3010: center wells contain serum (from patient no. 5, Table II) undiluted, *left*, and diluted 1:4, *right*. The 6 peripheral wells, reading clockwise from the top, contain antigens as follows: Drosdov, undiluted; Congo 3010, undiluted; normal brain, undiluted; Drosdov, diluted 1:4; Congo 3010, diluted 1:4; empty space.

Summary. A viral strain isolated in the USSR from a patient suffering from Crimean hemorrhagic fever and reported to be etiologically related to the illness, has been shown to be antigenically indistinguishable from Congo virus.

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Creatine Entry into Skeletal Muscle of Normal and of Dystrophic Mice* (33848)

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Since there is no evidence for creatine synthesis in skeletal muscle or for creatine degradation other than by slow, nonenzymatic formation of creatinine, one of three hypotheses should explain the low creatine content of skeletal muscle of dystrophic mice (1–3). Entry of creatine into muscle might be inhibited, loss of creatine from muscle might be accelerated, or the two defects might coexist. Relevant to choosing the correct hypothesis are the following two observations from studies of creatine kinetics *in vivo*: Dystrophic mice have higher than normal specific activity of muscle creatine 30 min after creatine-^{14}C injection, and they have a shortened turnover time of body creatine (1). These observations are consistent with the hypothesis of accelerated loss of creatine as the cause of the low creatine content of dystrophic muscle.

Additional evidence is needed, however, because interpretation of data from studies *in vivo* requires a large number of assumptions (4), despite the apparent simplicity of creatine metabolism. Therefore, the present studies of creatine entry into isolated extensor digitorum longus muscles were undertaken. In agreement with the interpretation of creatine kinetics *in vivo*, we found greater than normal entry of creatine into dystropic muscles. In addition, we found the creatine entry process in mouse muscle to differ from that of rat muscle (5, 6) by having a major nonsaturable component.

Materials and Methods. One- to 2-month-old, dystrophic mice of Bar Harbor Strain 129 and matching normal mice of the same strain, sex, and age were obtained from the Roscoe B. Jackson Memorial Laboratory. Normal and dystrophic mice were always treated similarly and studied concurrently. Some of the normal mice were heterozygous for the dystrophic trait, but they were indistinguishable from homozygous normal mice in these studies. After being fed a complete, purified diet (1) for a minimum of 1 week, the mice were decapitated, and their extensor digitorum longus muscles were removed and placed in vessels containing media for incubation. Approximately 3 min were required to obtain each muscle.

To study entry, 0.1–0.2 μCi of creatine-1-^{14}C (sp act, 2.62 or 5.08 mCi/mmole)[2] per

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