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Neutralizing Antibodies Against Simian Viruses SV_5 and SV_{20} in Human Sera.* (29482)

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Since 1955 there have been recovered from tissues and excreta of chimpanzees and monkeys approximately 100 viruses(1,2,3): the rhesus monkey has been the source of about 50 of these(2). With the exception of herpesvirus simiae (B virus)(4), SV_{40}(5,6), and the chimpanzee coryza agent (RS virus)(7,8), little effort has been made to determine whether other viruses of lower primate origin are infectious for man. This paper reports and interprets findings in an examination of sera obtained from people in 2 different ecologic settings (New Guinea and the United States) for presence of neutralizing antibodies against 2 simian viruses: parainfluenza virus SV_5 and adenovirus SV_{20}.

Materials and methods. Viruses: Viruses employed were SV_5 virus, strain Norris, and SV_{20} virus, strain Chin (A57). Both viruses were recovered as contaminants in primary cultures of rhesus monkey kidney cells, the former in the laboratories of the Division of Biologics Standards, and the latter in the laboratories of Dr. Tom Chin (U.S.P.H.S., Kansas City). Seed viruses were prepared by inoculating rhesus monkey kidney cells and fluids infected with SV_5 or SV_{20} into cultures of primary vervet monkey kidney cells for SV_5 and into cultures of BS-C-1 cells for SV_{20}. Maintenance medium used to support the viability of host cells during viral growth consisted of serum-free 199 medium. After 4 to 7 days incubation infected cells and fluids were harvested, centrifuged at low speeds to remove cellular debris and the resultant supernatant fluids representing virus seeds were stored in convenient amounts at —60°C.

Sera: Blood samples were drawn from 267 persons from 1 to 60 years of age. New Guinea sera were obtained in March, 1963, by Dr. D. C. Gajdusek from residents of Sabron Village, West New Guinea. The New Guinea donors had never received vaccine of any kind and it is reported that they had never had contact with a primate other than man. The U. S. sera were obtained in the summer of 1960 from military recruits and dependents prior to and, in some instances after administration of adenovirus vaccine. These sera were given to us by Dr. E. L. Buescher. For certain tests use was made of sera obtained in 1956-57 from patients with clinically diagnosed infectious hepatitis in United Nations military hospitals in Japan, Korea, and Okinawa. All sera were stored frozen at —20°C.

Virus neutralization tests. To 0.3 ml volumes of serial 2-fold dilutions of serum (in-

* This study was made possible through the generous cooperation of the following persons: Drs. T. O. Y. Chin, E. L. Buescher, R. M. Chanock and D. C. Gajdusek.

TABLE I. Results of Tests for Neutralizing Substances in Human Sera Against SV_5 and SV_{20}.

Source of sera†	No. positive*/No. tested SV_5	SV_{20}
West New Guinea (Sabron Village)	0/148	103/140
U. S., military dependents	2/39	4/24
U. S., military recruits	2/10	4/18

* Serum diluted 1:4 inhibited hemadsorbing activity of SV_5 and cytopathogenic effect of SV_{20} in tests with 100 $TCID_{50}$ of virus.

† Sera obtained through courtesy of Drs. D. C. Gajdusek and E. L. Buescher.

activated at 56°C for 30 minutes) were added an equal volume of seed virus diluted to contain 250 hemadsorbing units of SV_5 virus or 250 cytopathogenic doses of SV_{20} virus. Following incubation at room temperature for one hour, 2 tubes of appropriate cell cultures (vervet monkey kidney cells for SV_5 and BS-C-1 cells for SV_{20}) were each inoculated with 0.25 ml amounts of each mixture. The cultures were observed for 5 or 6 days and the neutralization titer of the test serum was considered to be the highest initial dilution which completely inhibited the hemadsorption activity of SV_5 virus or the cytopathogenicity of SV_{20} virus. In certain tests performed to determine presence or absence of virus neutralizing substances in test serum only a single dilution of serum was used.

Hemagglutination-inhibition tests were carried out with SV_{20} virus and albino rat erythrocytes employing the technique described by Rosen(9). Titers are expressed as the highest initial dilution of serum completely inhibiting the hemagglutinating activity of 8 units of virus.

Preparation of SV_{20} hyperimmune serum was accomplished by injecting into each of the foot pads and into each of 5 different subcutaneous sites of rabbits 0.2 ml of undiluted SV_{20} seed virus containing 50,000 $TCID_{50}$/ml and bleeding the inoculated animals to obtain serum 28 days later.

Results. Incidence of SV_5 and SV_{20} inhibitors in human serum. Table I summarizes results of tests employing a single dilution (1:4) of serum to detect presence of SV_5 and SV_{20} inhibitors in the sera of people in New Guinea and in the U. S. None of 148 New Guinea sera examined contained inhibitors against SV_5 virus, but 4 of 49 U. S. sera did. In tests with SV_{20} virus 103 of 140 New Guinea sera and 8 of 42 United States sera were positive for presence of virus inhibitor.

Physical and chemical properties of SV_{20} inhibitor in human serum. Since it was known that some human sera contain nonspecific, heat labile inhibitors to a variety of viruses (10-12), tests were made to determine the effect of certain physical and chemical agents on the virus neutralizing substance present in human serum which inhibited cytopathogenicity of SV_{20} virus. The heat labile inhibitor to SV_5 virus in human serum has already been shown to possess properties of antibody (13-15). Data presented in Table II show that SV_{20} inhibitor present in each of 4 human sera was resistant to exposure to 56°C for 30 minutes while exposure to 60°C for the same period reduced the inhibitory effect. Increasing the temperature to 65°C completely inactivated the SV_{20} inhibitor. Additional studies summarized in Table III showed that SV_{20} inhibitor was not inactivated by trypsin (16) or by receptor destroying enzyme(17). Data in Table IV show that in tests employing sera separated into albumen and globulin fractions, most of the SV_{20} inhibitor was

TABLE II. Effect of Heat on SV_{20} Inhibitor in Human Serum.

Treatment	Serum obtained from donor 1	2	3	4
None	16*	16	16	16
56°C/30 min	8	16	16	16
60°C/30 min	8	8	8	8
65°C/30 min	<8	<8	<8	<8

* Reciprocal of neutralizing antibody titer against 8 $TCID_{50}$ of virus.

TABLE III. Effect of Trypsin and RDE Treatment of SV_{20} Inhibitor in Human Serum.

Treatment	Serum obtained from donor 1	2	3	4
None	16*	16	16	16
1% Trypsin at 56°C/30 min	8	8	8	8
RDE (1:1000)	16	16	16	16

* Reciprocal of neutralizing antibody titer against 8 $TCID_{50}$ of virus.

TABLE IV. Inhibition of SV_{20} by Human Serum Fractions.

Treatment	Serum obtained from donor 1	2	3	4
Whole serum	16*	16	16	16
Albumen†	<2	4	<2	4
Globulin†	8	8	16	8

* Reciprocal of neutralizing antibody titer against 8 $TCID_{50}$ of virus.
† Obtained by precipitation with 1/2 saturated $(NH_4)_2SO_4$ at 4°C for 12 hr.

TABLE V. SV_{20} Neutralizing Substances in Persons of Different Ages in New Guinea and in the United States.

Age group (yr)	New Guinea* No. post†/No. tested†		United States‡ No. post†/No. tested†	
<2-2	6/9	(67%)	0/4	
3-6	17/24	(71%)	0/8	
7-9	17/23	(74%)	2/5	
10-14	18/27	(67%)	1/2	
15-18	9/11	(82%)	1/5	
18	36/46	(78%)	4/18	
Totals	103/140	(74%)	8/42	(19%)

* Sabron Village.
† Serum diluted 1:4 inhibited CPE of SV_{20} in tests with 100 $TCID_{50}$ of virus.
‡ Military dependents and recruits.

present in the globulin fraction. These characteristics of the SV_{20} inhibitor are consistent with the properties of antibody.

Survey of human sera for SV_{20} antibody. Results of virus neutralization tests to determine the occurrence of SV_{20} virus neutralizing antibody in the sera of people in different age groups in New Guinea and the United States are given in Table V. It is evident from these data that a number of people examined in this study possessed SV_{20} virus neutralizing antibody. This antibody was encountered in ⅔ or more of the individuals in all age groups in New Guinea, and was present in about ⅕ of a small group of persons examined in the U. S. The age group distribution of positive U. S. sera, however, was different from that found for positive New Guinea sera. In the small number of U. S. sera examined, SV_{20} antibody was found in at least 1 serum obtained from persons in each of the age groups above 7 years, but was not encountered in children 6 years of age or younger. The relative infrequency in occurrence of SV_{20} antibody in the small U. S. group studied might not be indicative of the frequency of occurrence of this antibody in the U. S. population: in another U. S. group of 20 persons 12 were found to possess SV_{20} neutralizing antibody (see Table VII).

Titer distribution of SV_{20} virus neutralizing antibody in New Guinea sera. Table VI summarizes by age group comparative levels of serum antibody against SV_{20} virus in New Guinea sera. No significant differences were noted in distribution of antibody titers by age groups. This finding suggests either persistence of a very stable antibody or repeated antigenic contact. Additionally, it is seen from the tabular data that the range of titers of the entire group of 103 positive sera was distributed normally with 97 of the specimens falling in the range of 1:10 to 1:40.

Examination of sera obtained before and after vaccination with adenovirus vaccine. Donors of New Guinea sera employed in this work had not received vaccine of any kind. Accordingly, vaccines could not be the source of the SV_{20} virus neutralizing antibody present in their sera. The possibility was considered, however, that the genesis of the neutralizing antibody directed against SV_5 and SV_{20}

TABLE VI. Titer Distribution of SV_{20} Neutralizing Substances in Sera of New Guinea Natives According to Age.

Age group (yr)	No. in group	No. of sera showing titer of <10	10	20	40	80	160	>160
<2-2	9	3	3	2	0	1	0	0
3-6	24	7	8	4	3	0	0	2
7-9	23	6	7	6	4	0	0	0
10-14	27	9	8	5	3	1	0	1
15-18	11	2	6	3	0	0	0	0
>18	46	10	14	16	5	1	0	0
Totals	140	37	46	36	15	3	0	3

TABLE VII. Tests for SV_5 and SV_{20} Neutralizing Substances in Sera of Adenovirus Vaccine Recipients.

Source of serum		No. pos/No. tested SV_5	No. pos/No. tested SV_{20}
Man (2 vaccine lots)	Before injection of adenovirus vaccine	2/10	12/20
	After " " " "	2/10	12/20
Guinea pigs (3 vaccine lots)	Before injection of adenovirus vaccine	0/7	0/7
	After " " " "	0/7	0/7

All men and guinea pigs whose sera were included in tests responded to vaccination with development of adenovirus neutralizing antibodies directed against each of the 3 adenoviruses (types 3, 4 and 7) included in vaccine in titers ranging from 1:10 to 1:320.

TABLE VIII. Neutralizing Antibody Response in Man to Trivalent Adenovirus (Types 3, 4 and 7) Vaccine.

		Reciprocal of neutralizing antibody titer against				
		Human adenoviruses			Simian viruses	
Person	Serum obtained	Type 3	Type 4	Type 7	SV_5	SV_{20}
1	Before vaccination	0*	0	0	0	0
	After "	10	20	40	0	0
2	Before vaccination	80	0	0	0	0
	After "	>320	10	40	0	0
3	Before vaccination	10	0	0	0	0
	After "	80	20	40	0	0

* 0 = <10, lowest serum dilution tested.

in U. S. sera might be associated with use of vaccines of monkey kidney origin. In an attempt to obtain information on this point, we examined in neutralization tests with SV_5 and SV_{20} viruses the sera obtained from 20 people before and after vaccination with 2 different lots of formalin inactivated trivalent (types 3, 4 and 7) adenovirus vaccine. Results given in Table VII show that neither of the 2 lots of vaccine used increased the number of positive sera in tests with either SV_5 and SV_{20} viruses. Further, it is seen that the negative findings in man were substantiated by findings in guinea pigs. None of 7 guinea pigs developed either SV_5 or SV_{20} virus neutralizing antibodies following vaccination with one or the other of 3 lots of formalin inactivated trivalent adenovirus vaccines. Moreover, results presented in Table VIII show that while each of 3 persons vaccinated with a commercial adenovirus vaccine containing types 3, 4 and 7 developed neutralizing antibodies to the adenovirus types contained in the vaccine, none of the 3 made antibody which neutralized either of the simian viruses. These results suggest that none of the 6 lots of vaccine included in the current tests contained SV_5 or SV_{20} antigens in levels sufficient to elicit detectable amounts of neutralizing antibody in our test subjects.

Serologic relationship of monkey adenovirus SV_{20} to human adenoviruses. SV_{20} virus possesses all the properties required for its inclusion in the adenovirus group(2). In addition, it produces partial agglutination patterns with albino rat red blood cells, a biologic marker it shares with several human adenovirus types (Table IX)(9). However,

TABLE IX. Adenovirus Types of Human Origin Which Failed to React in Hemagglutination Inhibition Tests with SV_{20} Hyperimmune Rabbit Serum.

Adenovirus type	Biologic marker group*
1, 2, 4, 5, 6	1
8, 9, 10, 13, 15, 17, 19, 22, 23, 24, 26, 27, 29, 30	2
3, 7a, 11, 14, 16, 20, 21, 25, 28	3

* Biologic markers characterizing members are partial and complete agglutination patterns of rat erythrocytes (groups 1 and 2, respectively) and complete agglutination pattern of rhesus monkey erythrocytes (group 3) (see Ref. 9).

to demonstrate this property with the Chin strain, it was necessary to employ undiluted infected cell culture fluids. Evidence that SV_{20} virus is distinct from any of 28 adenoviruses belonging to each of the 3 biologic groups established by Rosen(9) was obtained in hemagglutination-inhibition tests with SV_{20} virus hyperimmune rabbit serum which contained high levels of specific complement fixing and virus neutralizing (titers 1:160 and 1:320 respectively) antibodies. This hyperimmune serum failed to inhibit the hemagglutinating activity of any of the adenoviruses listed in Table IX. The reciprocal tests, *i.e.* examination of hyperimmune sera prepared against human adenovirus types in tests with SV_{20} virus were not done. Repeated attempts to prepare SV_{20} antigen with a titer greater than 1:1 were not successful.

Discussion. Our interests in monkey parainfluenza virus SV_5 and monkey adenovirus SV_{20} are centered around their potentialities as infectious agents of man. Isolation of SV_5 virus in human embryo cell cultures from the blood of a patient with clinically diagnosed infectious hepatitis† (14) and its recovery in embryonated hens' eggs inoculated with nasal washings of a volunteer participating in "common cold" studies(15) provide direct information suggesting infectivity of SV_5 virus in man. To this evidence are now added our serologic data. It might be postulated that the genesis of the SV_5 virus neutralizing antibody encountered in the serum of man in the present work is infection with a myxovirus of human origin sharing SV_5 virus antigens. Results of Chanock's work(13), however, would seem to indicate that this is not likely. Chanock has shown that SV_5 virus shares with other parainfluenza viruses group complement fixing antigens, but SV_5 virus in neutralization tests is clearly distinct from all known myxoviruses(13). Failure to detect SV_5 neutralizing antibody in New Guinea sera and its infrequent occurrence in U. S. sera might be accounted for by use of a relative insensitive hemagglutination inhibition test in which approximately 100 hemagglutinating units of virus and a 1:4 dilution of serum were employed. Use of the more sensitive plaque reduction technique might have increased the number of positive sera.

While the available evidence is substantial that SV_5 or a closely related virus infects man, there is a striking lack of information on the clinical response to the infection. Recovery of this virus from the blood of a patient with clinically diagnosed infectious hepatitis and from the nasal washings of another patient with severe upper respiratory illness constitutes the only recorded instances of recovery of SV_5 from man. The present study adds no information to establish an etiologic association between SV_5 virus and respiratory illness in man. Evidence is presented, however, to suggest that SV_5 virus was not of etiologic importance in any of 50 cases of infectious hepatitis occurring in 1956-1957 in United Nations troops in the Far East.

The significance of presence of SV_{20} virus neutralizing substances in human serum is not clear, but it is deserving of clarification. If it is assumed that SV_{20} neutralizing substance present in the serum of man represents an antibody resulting from infection, then it is reasonable to assume that infection with the eliciting agent is a common occurrence in New Guinea and a less common, but still frequent, occurrence in the U. S. The idea must be entertained that formation of the neutralizing substance in human serum which reacts with SV_{20} virus could have been elicited by infection with an adenovirus other

† Hsiung and her co-workers reported the recovery of SV_5 virus from the blood of a patient who contracted hepatitis during an outbreak of this disease in Naples(14) and demonstrated SV_5 virus antibody rises in the course of illness in other hepatitis cases involved in the Naples outbreak(14). Accordingly, we examined paired sera obtained from 50 cases of infectious hepatitis contracted by U. S. troops in several geographic areas in the Far East for presence of neutralizing antibody to SV_5 virus. Paired sera of 41 of the 50 hepatitis patients in tests employing a 1:4 dilution of serum were found to be free of SV_5 antibody, while paired sera from the remaining 9 patients contained equal levels of SV_5 antibody titers ranging from 1:8 to 1:32 in the acute and convalescent specimens. On the basis of these data it is reasonable to assume that SV_5 virus was not of etiologic importance in the hepatitis cases examined in this study.

than SV_{20}. The data obtained in hemagglutination inhibition tests with SV_{20} virus hyperimmune rabbit serum make it appear, however, that SV_{20} is distinct from any of the 28 adenoviruses of human origin against which it was tested. It might be profitable to look for a primary infection occurring commonly in children in New Guinea and less frequently in children in the United States which is accompanied by development of SV_{20} virus neutralizing antibody. In addition, search might also be made of a recurrent infection occurring in adults in presence of SV_{20} antibody.

Summary. Sera from 267 infants, children, and adults living in 2 markedly different ecologic settings, New Guinea and the United States, were examined for presence of neutralizing antibodies to monkey myxovirus SV_5 and monkey adenovirus SV_{20}. SV_{20} neutralizing antibody in titers of 1:10 to 1:160 were present in New Guinea in 70% of ½- to 5-year olds; in 72% of 6- to 18-year olds; and in 78% of adults. This antibody was absent in the U. S. in individuals in the ½- to 5-year old age group, but was present in titers of 1:10 to 1:80 in 33% of 6- to 18-year olds and in 22% of adults. None of the New Guinea sera and only 8% of the U. S. sera possessed SV_5 neutralizing antibody. These data suggest the possibility that SV_5 and SV_{20}, or agents antigenically related to them, might be infectious for man.

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Effect of Glucose Infusion on the Individual Plasma Free Amino Acids in Man.* (29483)

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There is a recognized relationship between the concentration of plasma free amino acids

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