STUDIES OF MOUSE POLYOMA VIRUS INFECTION

IV. Evidence for Mucoprotein Erythrocyte Receptors in Polyoma Virus Hemagglutination

By JANET W. HARTLEY, Ph.D., WALLACE P. ROWE, M.D., ROBERT M. CHANOCK, M.D., and BASIL E. ANDREWS, M.D.

(*From the Department of Health, Education and Welfare, United States Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Infectious Diseases, Bethesda, Maryland, and the Public Health Laboratory Service, Monsall Hospital, Manchester, England*)

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Tissue culture preparations of the mouse parotid tumor agent (polyoma virus) have been shown to agglutinate erythrocytes from a variety of species (1), agglutination being prevented by pretreatment of red cells with *Vibrio comma* filtrates. The effect of crude receptor-destroying enzyme preparations suggested further studies to clarify this apparent similarity to hemagglutination by members of the myxovirus group. This report concerns the relationships of red cell receptors and hemagglutination inhibitors for polyoma virus and selected myxoviruses, serologic comparisons of polyoma virus with the myxoviruses and certain other hemagglutinating viruses, and additional information on the properties of polyoma virus hemagglutination.

Materials and Methods

Polyoma Virus.—The 3919 strain of Stewart *et al.* (2), in first mouse embryo tissue culture passage in this laboratory, was used in tests for hemagglutination-inhibition by antisera for representative members of the myxovirus group. For all other studies the LID-1 strain (3) was used. Pools of 6th to 8th mouse embryo tissue culture passage fluids, which served as the source of virus, complement-fixing antigen, and hemagglutinin, were prepared in 32 ounce prescription bottle cultures of trypsin-dispersed mouse embryo fibroblasts, grown and maintained as described previously (3). Control fluids consisted of fluid and cells from uninoculated mouse embryo cultures.

Myxoviruses.—Allantoic fluid from embryonated hens' eggs infected with influenza types A/Jap/305, B/GL/55, and B/Lee, Sendai, and mumps (Habel strain) viruses, and monkey kidney tissue culture passage of "croup associated" (CA) (Greer strain) virus were used for red cell receptor and inhibitor destruction experiments. Soluble complement-fixing antigens consisted of chorioallantoic membrane preparations of influenza A/Jap/305 and B/GL/55, Sendai, mumps, hemadsorption (HA) type II (C-39), and Newcastle disease (NDV) viruses; chick embryo extract preparation of influenza C (1233); and ether-treated monkey kidney tissue culture fluid of CA and HA type I (C-243). Preparation of soluble antigens has been described Cook *et al.* (4) and the antigens used in this study were supplied through the kindness of Dr. Cook.

Mouse Encephalomyelitis Virus.—Mouse brain suspensions of the FA and GDVII strains, and spinal cord suspension of the TO strain were kindly supplied by Dr. Karl Habel.

Antisera.—Antiserum for polyoma virus was prepared by intraperitoneal inoculation of weanling Swiss mice with 1:10 dilution of infected tissue culture fluid, using two 0.2 ml. injections 2 weeks apart. The mice were bled by decapitation 10 days after the second inoculation.

For hemagglutination-inhibition tests, myxovirus antisera included rooster serum for various influenza A viruses (Swine, PR8, FM1, FLW-1-52, FW-1-50, Hawaii 303, and Jap/305/57), influenza B viruses (1-B-1, Lee, and Va-301-55), and influenza C (1233); chicken and guinea pig serum for NDV; guinea pig serum for Sendai, CA, and mumps; and rabbit serum for HA types I and II. Myxovirus antisera used in CF tests were prepared in guinea pigs, using the same virus strains employed as antigens.

Rooster antiserum for encephalomyocarditis (EMC) virus was kindly supplied by Dr. Kenneth Takemoto.

Receptor-Destroying Enzyme (RDE).—RDE preparations consisted of unpurified filtrates of 18 hour beef-heart infusion broth cultures of *Vibrio comma*. The two preparations used titered 1:16 when tested with 8 hemagglutinating units of influenza A (PR8) or influenza B (Lee) virus.

Complement Fixation (CF) Test.—The modified Bengtson technique (5) was employed, using 2 full units of complement, 2 units of polyoma antigen, and 4 units of the myxovirus antigens.

Red Cell Suspensions.—Whole blood of various species was collected in Alsever's solution; the red cells were washed in 3 changes of pH 7.2 phosphate buffered saline and stored at 4°C. either as packed cells or as 1.0 per cent suspension in buffered saline. Guinea pig cells were used as routine for hemagglutination and hemagglutination-inhibition tests, and for cell receptor modification studies.

Hemagglutination and Hemagglutination-Inhibition (HI) Tests.—For hemagglutination tests, 0.2 ml. of 0.4 per cent guinea pig red cells was added to 0.2 ml. of virus dilution. The tubes were shaken and the cells allowed to settle at 4°C. Readings were made in the cold by the pattern method. Titers were expressed as the highest dilution producing a partial pattern (approximately 50 per cent agglutination).

HI tests employed 0.2 ml. serum dilution and 0.2 ml. of virus diluted to contain 8 to 16 hemagglutinating units. After incubation of the mixtures for 30 minutes at room temperature, 0.1 ml. of 1.0 per cent guinea pig red cells was added, and the tubes were incubated at 4°C. and read as above. Titers were expressed as the highest initial serum dilution giving a distinct button.

Sera were heated as routine at 56°C. for 30 minutes before testing. RDE treatment of serum consisted of mixing equal volumes of undiluted unheated serum and undiluted RDE, or 1 volume of 1:10 serum dilution and 1/10 volume undiluted RDE, incubating for 18 hours at 37°C., and heating at 56°C. for 30 minutes.

All dilutions were made in phosphate buffered saline, pH 7.2.

Treatment of Red Cells with RDE.—0.2 ml. samples of 0.4 per cent guinea pig red cells were treated with an equal volume of serial twofold dilutions of RDE in calcium acetate saline (6). After incubation at room temperature for 90 minutes, the supernatant fluid was removed and replaced with 8 units of hemagglutinin contained in 0.4 ml. The erythrocytes were then resuspended and allowed to sediment at 4°C.

Preparation of Virus-Treated Red Cells.—Guinea pig red cells were added in a final concentration of 0.5 per cent to undiluted allantoic fluid preparations of influenza A and B viruses, and to undiluted polyoma virus tissue culture fluid, and the suspensions incubated in a 37°C. water bath for 18 hours. Since polyoma-treated red cells usually agglutinated spontaneously even after thorough washing in saline, they were washed once with a 1:800

dilution of polyoma mouse antiserum, containing 8 HI units, in an attempt to stabilize the suspensions. In certain experiments, influenza A and B virus-treated red cells were washed initially in polyoma antiserum or homologous immune serum. The cells were then washed 3 times in phosphate buffered saline and resuspended to a concentration of 0.4 per cent.

Electrophoresis.—Measurement of electrophoretic mobility was carried out on the following guinea pig red cell preparations: (*a*) untreated cells; (*b*) cells exposed to control mouse embryo tissue culture fluid; (*c*) cells exposed to polyoma virus-infected tissue culture fluid having a hemagglutination titer of 1:2560 per 0.2 ml.; and (*d*) cells exposed to RDE. Polyoma virus and control fluid treatment consisted of mixing 0.25 ml. packed red cells and 7.5 ml. fluid, holding at 4°C. for 1 hour, followed by incubation at 37°C. for 18 hours. For RDE treatment, 0.25 ml. packed cells was added to 7.5 ml. of RDE dilution consisting of 2 parts RDE to 1 part calcium acetate saline, and the mixture incubated at 37°C. for 2 hours. The cells were washed thoroughly and resuspended to 1.0 per cent in phosphate buffered saline. Virus-treated and 1 aliquot of control fluid-treated cells were washed once in polyoma virus mouse antiserum, diluted 1:800.

Electrophoresis studies were carried out in a microelectrophoresis apparatus designed by Dr. John W. Hornibrook and used through his courtesy; the internal measurements of the electrophoresis chamber were 12 x 36 x 0.6 mm. The chamber was coated with 1.0 per cent gelatin in distilled water before testing each sample. The time in seconds for an erythrocyte to travel an arbitrary distance, as measured by an ocular micrometer, was determined for each lot of treated cells. Four to 7 readings were made for each group of control cells, and at least 8 determinations for virus- and RDE-treated cells.

Ovomucin Inhibitor.—The method of ovomucin preparation was modified from that described by Gottschalk and Lind (7). Egg white was diluted in approximately 3 volumes of distilled water and the resulting precipitate washed 3 times in 0.5 per cent NaCl and resuspended in 2 volumes 10 per cent NaCl. The mixture was held overnight at 4°C., centrifuged, and the sediment dispersed in 20 volumes distilled water. The precipitate was washed 3 times and suspended in an equal volume of distilled water; 10 per cent NaCl was added to a final salt concentration of 0.5 per cent and the mixture homogenized in a Waring blendor for 30 minutes. The supernatant fluid obtained after centrifugation at 2000 R.P.M. for 10 minutes was dialyzed for 60 hours against 2 changes of 1.8 per cent NaCl, and for 36 hours against 3 changes of 1.0 per cent NaCl, and stored at 4°C.

Treatment of ovomucin with virus consisted of adding 1 volume of ovomucin to 2 volumes of undiluted infected allantoic fluid or tissue culture fluid, and incubating at 37°C. for 18 hours. To inactivate the hemagglutinin of the treating virus, ovomucin-myxovirus and ovomucin-polyoma virus mixtures were heated for 30 minutes at 65° and 70°C., respectively. Control ovomucin preparations consisted of 1:3 dilutions of ovomucin in saline and in control mouse embryo tissue culture fluid, incubated at 37°C. for 18 hours, and heated at 70°C. for 30 minutes.

To determine residual inhibitor in virus-treated ovomucin, serial 2-fold dilutions of the treated ovomucin samples were prepared and 0.2 ml. of test virus diluted to give a final concentration of 3 to 4 units of hemagglutinin was added to 0.2 ml. of each ovomucin dilution. After 2 hours at 4°C., 0.1 ml. of 1.0 per cent guinea pig red cells was added to each tube; the tubes were placed at 4°C. and read at intervals until complete settling occurred. Controls consisted of titrations of each test virus and determination of the inhibitor titer of saline-treated and control fluid-treated ovomucin against each test virus.

RESULTS

Hemagglutination of Various Species Erythrocytes.—Tissue culture preparations of polyoma virus agglutinated red cells from a variety of mammalian and

avian species. Comparable hemagglutinin titers were obtained with erythrocytes from humans (type O), guinea pigs, mice, *rhesus* and *cynomolgous* monkeys, and chickens; sheep and cat red cells were agglutinated only by low dilutions. Marked variation was noted in the susceptibility of red cells obtained from different white rats. No agglutination of horse, cow, grivet or patas monkey, chimpanzee, pigeon, alligator, or hamster erythrocytes was obtained. However, Eddy (1) has obtained high hemagglutinin titers with hamster red cells.

Guinea pig red cells were used as routine because of the consistently high hemagglutination titers obtained and the lack of variation among individual animals.

Receptor Modification by RDE.—Treatment of guinea pig red cells with RDE rendered them inagglutinable by polyoma virus. Prevention of polyoma virus hemagglutination occurred through 1:64 dilution of RDE in a test in which simultaneous titration of the RDE prevented hemagglutination by influenza A (PR8) to a titer of 1:16.

Receptor Modification by Virus.—In view of the possible similarity of red cell receptors for polyoma virus and members of the myxovirus group as indicated by the action of RDE, it was of interest to test for enzymatic activity of polyoma virus on its cell receptors, and for reciprocal receptor destruction by polyoma and myxoviruses.

Polyoma virus hemagglutinin readily adsorbed to guinea pig red cells at 4°C., and eluted at 37°C. with varying efficiency. Supernatant fluids from mixtures of guinea pig red cells and polyoma virus-infected tissue culture fluid, held at 4°C. for 1 hour, were reduced in hemagglutination titer to 1:10 or less, from a starting titer of 1:2560 or greater. When the cells were resuspended in tissue culture maintenance medium and held at 37°C. for 30 minutes to 2½ hours, 50 to 100 per cent of the hemagglutinin was recovered in the fluid phase. However, cells thus exposed remained completely agglutinable on subsequent treatment with fresh virus. The results of titrations of polyoma virus using red cells treated at 37°C. for 18 hours with polyoma virus and influenza virus types A and B are given in Table I. The procedure described in Materials and Methods was followed, with the exception that in test 1, the mixtures of cells plus treating virus were held at 4°C. for 1½ hours before incubation at 37°C. Pretreatment of red cells with influenza A and B viruses markedly reduced their agglutinability by polyoma virus. Receptor destruction was prevented by pre-incubation of the treating viruses with homologous immune serum, indicating that receptor modification was a result of viral enzymatic activity, and was not due to some enzyme present in the allantoic fluid. Cells exposed to polyoma virus showed no detectable exhaustion of receptors for any of the viruses tested. One preparation of polyoma-treated cells (test 1) appeared to be somewhat more sensitive to agglutination than were control cells.

The results of electrophoresis experiments are given in Table II. No difference

TABLE I

Tests for Reciprocal Erythrocyte Receptor Destruction by Polyoma Virus and Influenza A and B Viruses

Treatment of erythrocytes			Hemagglutination titer of standard virus pools against treated cells (reciprocal)				
Virus	Units of hemagglutinin per ml. of virus-RBC mixture	Initial wash of treated cells	Polyoma virus			Influenza virus	
						A (Jap/305)	B (GL)
			Test 1	Test 2	Test 3	Test 1	Test 3
Polyoma	3200	Polyoma mouse serum (8 HI units)	640	320	320	≥64	128
Influenza A (Jap/305/57)	160	Control mouse serum (1:800) or saline	10	20		NT*	
		Polyoma mouse serum (8 HI units)	10			8	
"	40	Influenza A rooster serum (4 HI units)			20		16
" + A antiserum‡	<5	Saline			320		128
Influenza B (GL/55)	160	Control mouse serum (1:800) or saline		<20			
		Polyoma mouse serum (8 HI units)		<20			
"	640	Influenza B rooster serum (4 HI units)			<20		<8
" + A antiserum‡	320	Saline			<20		<8
" + B antiserum§	<5	Saline			320		64
None		Control mouse serum (1:800)	320	320	320	32	128
		Polyoma mouse serum (8 HI units)	320			32	

* No test; cells agglutinated spontaneously.

‡ Two volumes treating virus pre-incubated 37°C. 1 hour with 1 volume influenza A (Jap/305/57) rooster antiserum (RDE-treated, heated 60°C. 30 minutes), with HI titer of 1:320 against 4 units A (Jap/305) and <1:40 against 4 units of B (GL).

§ Two volumes treating virus pre-incubated 37°C. 1 hour with 1 volume influenza B (GL/55) rooster antiserum (RDE-treated, heated 60°C. 30 minutes), with HI titer of 1:640 against 4 units of B (GL).

in mobility of polyoma virus-treated red cells, as compared to untreated or control fluid-treated cells, was detected by a procedure which indicated an approximate 4-fold decrease in mobility of RDE-treated cells.

Serum Inhibitors.—In standard HI tests employing 8 to 16 units of hemagglutinin, RDE-sensitive inhibitor of polyoma virus hemagglutination was found in low titer (1:40) in occasional sera from uninoculated mice or from those receiving saline or control tissue culture fluids. In tests in which mouse sera were screened at dilutions of 1:100 and 1:200, RDE-sensitive inhibitor has been encountered rarely. Thus, of 478 sera from uninoculated mice of a variety of inbred and non-inbred strains in infection-free colonies (8), only 2 demonstrated RDE-sensitive inhibitor, both in titers of 1:100. Among 791 Swiss mice receiving control inoculations, one was found to have RDE-sensitive inhibitor, titering 1:200. As shown in Table III, the titer of non-specific serum inhibitor was inversely proportional to the test dose of hemagglutinin. In contrast, only a 4- to 8-fold decrease in inhibitor titer of polyoma-immune mouse serum, either heat- or RDE-treated, was detected with a 64-fold increase in hemagglutinin. RDE treatment of polyoma-immune mouse, hamster, or rabbit serum had no significant effect on HI titers as determined in the standard test.

TABLE II

Electrophoretic Mobility of Polyoma Virus-Treated Red Cells, as Compared to Control and RDE-Treated Cells

Cell treatment	Initial wash of treated cells	Electrophoretic mobility*
None	Saline	31.0 ± 1.2
Control tissue culture fluid	Saline	31.1 ± 0.5
	Polyoma MS‡	30.3 ± 0.7
Polyoma virus	Polyoma MS	30.2 ± 0.8
RDE	Saline	133.7 ± 10.0

* Expressed as time in seconds required for a red cell to travel an arbitrary distance across an ocular micrometer.

‡ Polyoma-immune mouse serum diluted 1:800 in saline (8 HI units).

RDE-sensitive inhibitor was found at a dilution of 1:160 in 2 rabbits receiving control tissue culture fluid inoculations. No inhibitor was detected in pooled chicken, calf, or sheep serum, in tests of 1:20 dilution of unheated serum against 8 to 16 units of virus.

Because of the generally low inhibitor titers, no attempt was made to carry out comparative tests for reciprocal destruction of serum inhibitors by polyoma virus and members of the myxovirus group.

Ovomucin Inhibitor.—An ovomucin preparation with inhibitory activity for myxovirus hemagglutination was found to contain high titer inhibitor for polyoma virus hemagglutinin, permitting cross-tests of inhibitor destruction. The results of a representative cross-test are given in Table IV; these findings were confirmed in several additional tests. Ovomucin inhibitor titers for polyoma virus were markedly reduced by pretreatment of the ovomucin with influenza A virus and significant reduction was noted with influenza B and CA viruses. No reduction in ovomucin inhibitor titer for any of the viruses tested was detected after treatment of ovomucin with polyoma virus. The test presented in the table appears to be somewhat insensitive for detecting weak enzymatic activity, since no destruction of mumps or Sendai virus inhibitor by

TABLE III

Effect of RDE and Dose of Hemagglutinin on HI Titer of Control and Polyoma-Immune Mouse Serum

Pooled mouse serum	Treatment of serum	Reciprocal of serum HI titer against increasing hemagglutinin doses						
		1U	2U	4U	8U	16U	32U	64U
Control*	56°C., 30 min.	160	40	<20	<20	<20		
	RDE, 56°C., 30 min.	40	20	<20	<20	<20		
Polyoma-immune*	56°C., 30 min.	3200	1600	1600	1600	800	800	800
	RDE, 56°C., 30 min.	3200	1600	1600	1600	800	800	400

* Mice received 2 intraperitoneal inoculations with 0.2 ml. of 1:10 dilution of control tissue culture fluid or polyoma infectious tissue culture fluid, respectively.

the homologous viruses was obtained. However, in an additional test using the same reagents an 8-fold reduction in Sendai inhibitor and a 2-fold reduction, of borderline significance, in mumps inhibitor were detected. In this test polyoma virus showed no evidence of enzymatic activity.

Serologic Tests.—No inhibition of polyoma virus hemagglutination occurred in tests with antisera prepared against representative strains of types A, B, and C influenza viruses, or against Sendai, CA, mumps, NDV, and types I and II HA viruses, in tests performed with 8 hemagglutinating units of polyoma virus and serum dilutions representing at least 8 HI units of homologous antibody.

CF tests using 2 units of polyoma antigen and 8 units of antiserum for the various myxoviruses were completely negative. In addition, no reaction was obtained with 8 units of polyoma mouse antiserum and 4 units of soluble myxovirus CF antigens.

Relation to Other Hemagglutinating Viruses Found in Rodents.—Since the hemagglutination properties of certain other viruses found in rodents have

attributes in common with polyoma virus it was of interest to confirm the distinctness of polyoma virus from these agents.

Encephalomyocarditis (EMC) virus agglutinates sheep red cells in the cold, and the hemagglutinin can be eluted on warming, but with no loss in agglutinability of the cells (9). Also, it has been reported that treatment with RDE renders red cells inagglutinable (10) and prevents adsorption and multiplication of the virus in HeLa cell cultures (11). Rooster antiserum against EMC virus, having an homologous neutralization titer of 1:3125, was treated with RDE;

TABLE IV

Cross-Tests of Destruction of Ovomucin Inhibitor Using Polyoma Virus and Selected Myxoviruses

Ovomucin treatment		Reciprocal of ovomucin inhibitor titer when tested against 3 to 4 units of:						
		Influenza						
Virus	Units of hemagglutinin per ml. ovomucin	A	B (GL)*	B (Lee)*	CA	Sendai	Mumps‡	Polyoma
Influenza A (Jap/305)	160	30						<15
Influenza B (GL)	320		<15	<15				240
CA	160				<15			480
Sendai	1280					120		1920
Mumps	160						480	1920
Polyoma	6400	480	240	1920	30	120	480	1920
Control tissue culture fluid		480	240	1920	30	240	480	1920
Saline		480	240	1920	15	120	480	1920

* Test virus heated at 56°C. for 30 minutes.

‡ Eight units of test virus.

this serum in a dilution of 1:40 was negative in HI tests against 8 units of polyoma hemagglutinin.

The GDVII strain of mouse encephalomyelitis virus agglutinates human red cells in the cold, and elutes on warming, the cells remaining agglutinable (12). Mouse protection tests were performed in which polyoma-immune mice were challenged with the TO, FA, and GDVII strains. Weanling Swiss mice were immunized with high titer polyoma virus-infected tissue culture fluid by giving 2 intraperitoneal inoculations of undiluted fluid 1 week apart. Twenty days after the second injection equal numbers of polyoma-immunized and uninoculated control mice were challenged with each strain of virus as a 10 per cent brain or cord suspension; challenge with FA and GDVII strains consisted of intracerebral inoculation of 0.04 ml. volumes; TO-challenged mice received 0.02 ml. intraspinally. Results are given in Table V; all positive mice succumbed with typical paralytic symptoms.

Lung extracts from mice or hamsters infected with pneumonia virus of mice (PVM) contain an agglutinin for mouse and hamster red cells (13, 14). In the infected lung, the virus is firmly combined with a tissue component which prevents hemagglutination (15). Dissociation of hemagglutinin from inhibitor occurs on heating to 70°C., the property of hemagglutination being much more stable than infectivity; such heating is also required to release the virus from agglutinated red cells (14). Reagents were not available for serologic comparisons of polyoma virus and PVM. However, polyoma virus hemagglutinin and infectivity are resistant to heating at 60°C. for 30 minutes but are destroyed or very much reduced after 30 minutes at 70°C. (16); there are also marked differences in pathogenicity and in the species of erythrocytes susceptible to agglutination.

TABLE V

Mouse Protection Test with Polyoma-Immune Mice and Mouse Encephalomyelitis Virus

Challenge virus*	Route of inoculation	Polyoma-immune mice	Uninoculated mice
TO	Intraspinal	9/9 (6–11)‡	9/10 (6–11)
FA	Intracerebral	8/8 (4–5)	8/8 (4–5)
GDVII	"	6/6 (4–5)	6/6 (4)

* Strains of mouse encephalomyelitis virus.

‡ Numerator = number succumbing with paralysis and death; denominator = number inoculated; figures in parentheses indicate range of incubation period in days.

DISCUSSION

The following considerations suggest that the red cell receptors involved in polyoma virus hemagglutination are very similar to the mucoprotein red cell substrates of myxoviruses:—

1. Red cells exposed to the action of RDE or to the enzymatic activity of influenza viruses types A and B were inagglutinable by polyoma virus.

2. RDE-sensitive inhibitor active against polyoma virus hemagglutination was found in certain normal sera.

3. An ovomucin preparation inhibited polyoma virus hemagglutination, and this inhibitor was susceptible to destructive activity by certain members of the myxovirus group.

However, no enzymatic activity for polyoma virus was demonstrated, since polyoma virus produced no detectable modification in red cell agglutinability or ovomucin inhibition of polyoma or myxoviruses. Also, no alteration in electrophoretic mobility of polyoma-treated red cells was detected. An apparent inhibitor of hemagglutination, which is sensitive to heat and RDE, has been detected in cell-free saline eluates obtained after treatment of red cells with polyoma virus (17). It is possible that this inhibitor represents receptor sub-

stance and its presence may indicate an otherwise undetectable modification of the red cell surface. Repeated treatment of red cells with fresh virus might result in some detectable receptor modification.

Although polyoma virus and myxoviruses apparently attach to the same erythrocyte receptors, there is adequate evidence that polyoma virus is not a member of the myxovirus group. The temperature instability, ether sensitivity, and 80 to 150 mμ size range considered characteristic of the myxovirus group are not associated with polyoma virus. Polyoma virus withstands prolonged storage at 4°C. and heating at 60°C. for 30 minutes without decrease in infectivity titer (16), is ether-resistant (18), and has a particle size of approximately 40 to 60 mμ diameter (19).

RDE-removable red cell receptors have been described for two other viruses otherwise apparently different biologically from members of the myxovirus group, namely, EMC virus and the rat virus (RV) of Kilham (20). Studies of destruction of cell receptors for these viruses by influenza virus action have not been reported. Both are serologically distinct from polyoma virus.

SUMMARY

Treatment of guinea pig erythrocytes with types A and B influenza viruses rendered them inagglutinable by polyoma virus; also, the inhibitory effect of ovomucin on polyoma virus hemagglutination was destroyed by pretreatment of the ovomucin with various myxoviruses. These results indicate that polyoma virus and myxovirus erythrocyte receptor sites are identical. However, no destruction by polyoma virus of its own or of myxovirus receptors or inhibitors was detected.

No serologic relationship was detected between polyoma virus and members of the myxovirus group; differences in size and stability further indicate their distinctness.

No evidence was found of biologic or serologic relationship of polyoma virus with encephalomyocarditis virus or mouse encephalomyelitis virus.

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