Agglutination of Bovine Erythrocytes: A General Characteristic of Reovirus Type 3.* (27679)

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It was reported recently that 3 strains of reovirus type 3 agglutinated bovine erythrocytes at 4°C, whereas 7 strains of types 1 and 2 did not(1). The present investigation was undertaken to determine whether the ability to agglutinate bovine erythrocytes is a general characteristic of reovirus type 3 strains obtained from various species. In addition, the hemagglutinating activity of all 3 types of reovirus with human erythrocytes was investigated.

Material and methods. Cell cultures. Primary cultures of rhesus monkey kidney cells in tubes were obtained from Microbiological Associates, Inc.

Viruses. Thirty-two strains of reovirus types 1, 2, and 3 were obtained from Drs. L. Rosen, A. B. Sabin, E. H. Lennette, J. B. Nelson, and A. M. Lerner, and are listed in Tables I and II. The viruses were grown in monkey kidney cells and the cell-associated and released virus harvested when cytopathic effects were maximum. The viruses were

TABLE I. Hemagglutination of Bovine and Human RBC by Reovirus Type 3 Strains.

Strain	Source	Hemagglutination titer: Bovine RBC (stabilized)	Hemagglutination titer: Human RBC (fresh)	Infectivity titer, log $TCID_{50}$/ml
Calf 151J	Calf	16	< 4	6.5
Jones*	Man	16	< 4	not done
Dearing	"	64	4	" "
Calf 808	Calf	64	< 4	7.5
Calf 376H	"	64	32	8.0
Nelson	Mouse	128	< 4	8.8
BVA6	Man	128	8	not done
Abney	"	128	8	" "

* This strain is unrelated to the reovirus prototype 2 strain D5 (Jones).

TABLE II. Hemagglutination of Bovine and Human RBC by Reovirus Type 1 and 2 Strains.

Type	Strain	Source	Hemagglutination titer: Bovine RBC (stabilized)	Hemagglutination titer: Human RBC (fresh)
I	Cocrel	Man	< 4	64
	Toluca No. 2182	"	< 4	64
	CHHE 276	"	< 4	128
	King	"	< 4	128
	Calf 806	Calf	< 4	128
	Lang	Man	< 4	256
	Sampson, A.	"	< 4	256
	Calf 104	Calf	< 4	256
	" 810	"	< 4	256
	" 802	"	< 4	512
	" 107	"	< 4	512
	CHHE 127	Man	< 4	1024
II	CHHE 318	Man	< 4	8
	D5 (Jones)	"	< 4	64
	Tahiti No. 5283	"	< 4	64
	Calf 237G	Calf	< 4	128
	Chimprhinitis 54	Chimpanzee	< 4	128
	Baden, S.	Man	< 4	128
	Calf 254A	Calf	< 4	256
	Amy	Man	< 4	256
	988	"	< 4	512
	Gaither	"	< 4	512
	Toluca No. 2442	"	< 4	2048

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used after 2 or 3 passages in this laboratory.

Diluent and medium. Phosphate buffered saline (BS) consisted of 0.85% NaCl buffered at pH 7.2 with 0.01 M phosphate buffer. Eagle's minimum essential medium (MEM)(2) without serum was used as maintenance medium for cell cultures.

Erythrocytes. Bovine erythrocytes obtained from Cappel Laboratories as a 10% stabilized suspension or from freshly killed animals were suspended in BS to a final concentration of 0.25%. Human erythrocytes obtained by venipuncture were suspended in BS to a final concentration of 0.5%. The erythrocyte suspensions were always used within 5 days.

Hemagglutination titrations. The procedure was slightly modified from that described previously(1). Serial 2-fold dilutions of virus were made in 0.5-ml volumes of BS and 0.5 ml of erythrocyte suspension added to each tube. The tubes were kept at 4°C and read after 3 hours. The titers at 2 and 4 hours were not significantly different from those determined at 3 hours. The end-point was taken to be at the dilution showing strong partial agglutination.

Infectivity titrations. Serial 10-fold dilutions of virus were made in Eagle's MEM. Tube cultures of monkey kidney cells were washed twice with Eagle's MEM, 1.9 ml of Eagle's MEM was added, the tubes were gassed with 5% CO_2 in air, and 0.1 ml of diluted virus was inoculated. The tubes were rolled at 37°C and were examined for development of viral cytopathic effects. The final reading was made on the 8th day, and the 50% infective end-point titer calculated.

The plaque procedure which employs monolayers of L cells(3) was suitable for several, but not for all of the viruses examined.

Experiments and results. Agglutination of bovine and human erythrocytes by reovirus type 3 strains. Table I shows the results obtained with 8 reovirus type 3 strains; they all agglutinated stabilized bovine erythrocytes. Those strains which had a relatively low infectivity titer also gave relatively low hemagglutination titers; higher infectivity titers were correlated with higher hemagglutination titers. One additional virus preparation, designated Calf 814 strain, contained only $10^{5.4}$ $TCID_{50}$/ml and failed to agglutinate bovine erythrocytes, probably because of too low virus content. It should be stressed that with reovirus type 3, Dearing strain variant, 1 HAU (bovine erythrocytes) corresponded to 6.2×10^6 PFU (L cells)(1).

Of the 8 reovirus type 3 strains which agglutinated bovine erythrocytes, only 4 agglutinated human erythrocytes, and with these, the titers were lower with human than with bovine erythrocytes. Furthermore, the Nelson strain which had an infectivity titer of $10^{8.8}$ $TCID_{50}$/ml failed to agglutinate human erythrocytes, whereas the Calf 376H strain, with an infectivity titer of $10^{8.0}$, agglutinated human erythrocytes to a titer of 32. Thus, the ability of reovirus type 3 strains to agglutinate human erythrocytes appears to be an inconstant property of these viruses; in contrast, ability to agglutinate bovine erythrocytes is a constant property.

It should be emphasized that strain Jones of reovirus type 3 is unrelated to the reovirus type 2 prototype strain D5 (Jones).

Agglutination of bovine and human erythrocytes by reovirus types 1 and 2. None of the 23 strains of reovirus types 1 and 2 agglutinated bovine erythrocytes although adequate amounts of each virus were present to agglutinate human erythrocytes (Table II).

Comparison of hemagglutination patterns and titers obtained with stabilized and freshly drawn bovine erythrocytes. Because the hemagglutination patterns produced by reovirus 3 with stabilized bovine erythrocytes rarely consisted of cup-shaped sheets of agglutinated cells, the patterns and titers obtained with stabilized suspensions and fresh erythrocytes were compared. When freshly drawn cells were used, the hemagglutination titers were 2- to 16-fold higher than when cells from a stabilized suspension were used (Table III). Stable, cup-shaped sheets of agglutinated erythrocytes regularly formed when the freshly drawn cells were used.

Susceptibility of reovirus 3 receptor on human erythrocytes to V. cholerae *extract.* Because the receptors on bovine erythrocytes for reovirus 3 are destroyed by *V. cholerae*

TABLE III. Hemagglutination of Fresh or Stabilized Bovine RBC by Reovirus Type 3 Strains.

Strain	Hemagglutination titer: Fresh RBC	Stabilized RBC	Ratio of titers, fresh/stabilized bovine RBC
Calf 151J	32	16	2
" 808	32	16	2
Dearing	256	64	4
Jones	128	16	8
Nelson	64	8	8
Calf 376H	256	16	16

filtrate(1), and because the receptors on human erythrocytes for all types of reovirus are reportedly resistant to the action of RDE (4), the interaction of several strains of reovirus 3 with human erythrocytes was investigated. Treatment of human erythrocytes for 1 hour at 37° with a *V. cholerae* filtrate had no effect on the weak agglutination of human erythrocytes by Dearing, Dearing variant, BVA6, and Abney strains, or on the stronger agglutination by calf 376H strain. Such treatment rendered bovine erythrocytes inagglutinable by reovirus 3.

Discussion. The present evidence indicates that the ability to agglutinate bovine erythrocytes is a general characteristic of type 3 reovirus, and that reovirus types 1 and 2 lack this characteristic. In the interaction of type 3 reovirus with bovine and sheep erythrocytes, neuraminic acid-containing receptors of the erythrocytes are involved(1). Furthermore, such receptor substances are also involved in the interaction of reovirus 3 with ovomucin and serum inhibitors. In contrast, there is evidence that reovirus 1 and 2 do not react with neuraminic acid-containing receptors(4,5,6,7). It appears also that such receptors do not play an important role in the agglutination of human erythrocytes by reovirus 3. With all 3 types, intact sulfhydryl groups on the virus, or disulfide linkages are involved in the hemagglutinating activity as well as infectivity(1,8,9).

Summary. 1. Reovirus type 3 strains, originally isolated from different animal species, all agglutinate bovine erythrocytes; only some agglutinate human erythrocytes. 2. None of the strains of reovirus types 1 and 2 agglutinate bovine erythrocytes; they all agglutinate human erythrocytes.

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Accelerated Development of Malignant Lymphomas in AKR Mice Injected at Birth with 7, 12-Dimethylbenz(a)Anthracene.* (27680)

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AK strain mice have a high spontaneous incidence of malignant lymphoma(1). A viral component has been demonstrated in these lymphomas, which can be transmitted by cell-free extracts to newborn C_3H mice (2), and virus-like particles have been observed with the electron microscope in lymphoid organs of this strain(3,4,5). The development of malignant lymphomas can be accelerated by injection of a cell-free filtrate of AK mouse spontaneous lymphoma into newborn AK mice(6,7).

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