Reovirus Hemagglutination: Inhibition by N-Acetyl-D-glucosamine

Abstract. *Purified reoviruses types 1, 2, and 3 were specifically inhibited in their hemagglutination of human erythrocytes by 250 micromoles or more of* N-*acetyl-*D-*glucosamine but not by over 20 other sugars tested.* N-*acetyl-*D-*glucosamine inhibited reovirus hemagglutination by binding to capsid virus; it did not attach to the erythrocytes. It is possible that reovirus hemagglutination involves union between* N-*acetyl-*D-*glucosamine on the surface of the red cell and the glycoprotein of the virus coat.*

Reovirus hemagglutination of human type O erythrocytes is nonenzymatic, and involves glycoproteins on the surface of each member (*1*). The glycoprotein on the erythrocyte does not contain the sulfhydryl groups necessary for hemagglutination, while that of the virus capsomere does (*2*). This absence of sulfur suggests that the red blood cell reactant is a protein of the same character as blood group substances. Furthermore, reovirus hemagglutination may resemble the reaction between blood group substances and their antibodies, that is, specific union may require a simple oligosaccharide (*3*).

We report here that reovirus hemagglutination is specifically inhibited by the sugar *N*-acetyl-D-glucosamine. This reaction may in some ways be similar to the union between A, B, and H blood group substances and their specific antibodies, although the blood group substances are not required for reovirus hemagglutination (*4*).

Strain 988 of type 2 reovirus was grown in L-48 cells in monolayer culture and concentrated and purified by a method reported previously (*5*). The final product, after sucrose density gradient ultracentrifugation, was dialyzed overnight with Earle's balanced salt solution and some of the virus preparations were stabilized with 1 percent antibody-free fetal bovine serum. The sugars to be tested were dissolved in Earle's saline and incubated with 16 hemagglutinating units of virus for 1 hour at 23°C. A fresh suspension of human type O red blood cells was added, and the resulting mixture was shaken and allowed to settle. Virus, cell, and sugar controls accompanied each experiment (Table 1). Inhibition of reovirus hemagglutination was indicated by the presence of a distinct button of erythrocytes on the bottom of the tube after 90 to 120 minutes, when positive and negative controls were appropriate. The exact time varied somewhat according to the concentration of sugar in each system. Inhibition reactions were repeated several times.

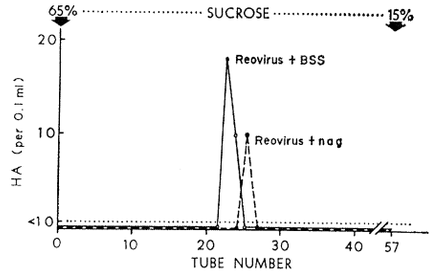

Fig. 1. Attachment of reovirus type 2, strain 988 to *N*-acetyl-D-glucosamine with inhibition of hemagglutination. Five hundred hemagglutinating units of purified reovirus were incubated for 1 hour at 23°C with 5000 μmole of *N*-acetyl-D-glucosamine in 2 ml of Earle's saline. An equal portion of virus was similarly incubated with 2 ml of Earle's saline alone. Each suspension was subsequently layered onto 15 to 65 percent sucrose gradients in Earle's saline, and centrifuged in the Spinco model-L (SW-25 rotor) ultracentrifuge for 210 minutes at 4°F. Twenty-drop fractions were collected by puncturing the bottom of the tubes and tested for HA as before. HA, Hemagglutination; BSS, balanced salt solution (Earle's saline); nag, *N*-acetyl-D-glucosamine.

Table 1. Inhibition of reovirus hemagglutination (HA) by sugars. Sixteen HA units of reovirus type 2, strain 988 in 0.1 ml of Earle's balanced salt solution were incubated for 1 hour at 23°C with 0.6 ml of various sugars in Earle's saline. Human type O red blood cells (RBC) (0.1 ml of a 1.6-percent suspension) were added, and the HA pattern was read at the end of 90 to 120 minutes.

Sugar	Quantity (μmole)	HA	
		Sugar and RBC*	Sugar, reovirus and RBC*
D-Glucose	300	−	+
D-Galactose	600	−	+
D-Mannose	300	−	+
Galactosamine HCl	600	−	+
N-Acetyl-D-galactosamine	600	−	+
N-Acetyl-D-glucosamine	400	−	−
L-Fucose	600	−	+
Sialic acid	30	+	+
Stachyose	400	+	+
Gentiobiose	400	+	+
Methyl-β-D-galactoside†	28	−	+
Galactinol†	17	−	+
4-*O*-β-D-Galactopyranosyl-*N*-acetyl-D-glucosamine†	9	−	+
Fructose	400	−	+
D-Ribose	400	−	+
Raffinose	800	+	+
D-Arabinose	400	+	+
D-Lactose (hydrate)	400	+	+
D-Maltose (hydrate)	400	+	+
D-Melibiose (hydrate)	400	+	+
Methyl-α-L-fucopyranoside‡	32	−	+
Methyl-β-L-fucopyranoside‡	33	−	+
Methyl-α-D-galactoside‡	40	−	+
Methyl-β-D-galactoside‡	311	−	+

* Results are those obtained with and without the addition of fetal bovine serum. (+), Hemagglutination; (−), no hemagglutination. † Supplied by E. A. Kabat. ‡ Supplied by G. E. Springer.

Type 2 reovirus hemagglutination was specifically inhibited by quantities of 250 to 400 μmole of *N*-acetyl-D-glucosamine. None of the tests with 24 other sugars showed any inhibition. The inhibition reaction was noted in virus preparations stabilized with fetal bovine serum and also in other preparations in which this serum was absent (Table 1). Similar experiments with type 1 (Lang) and type 3 (Abney) reoviruses showed that they were also unable to hemagglutinate human red blood cells after incubation with *N*-acetyl-D-glucosamine.

In order to determine whether inhibition of reovirus hemagglutination by *N*-acetyl-D-glucosamine occurred through the sugar's attachment to the virus, the red blood cells, or to both, we performed the following experiments.

1) One milliliter of citrated packed type O red blood cells was incubated at 23°C for 1 hour with 5000 μmole of *N*-acetyl-D-glucosamine in 5 ml of Earle's saline. The cells were centrifuged at 600*g* for 10 minutes and washed three times in Earle's saline to remove any of the sugar not firmly attached to the red cells. These "treated" packed eryth-

rocytes were tested with 16 hemagglutinating units of type 2 reovirus in the usual hemagglutination titration but no inhibition of hemagglutination was observed. *N*-acetyl-D-glucosamine did not attach to the red blood cells in the inhibition reaction.

2) The converse experiment was performed to test the attachment of *N*-acetyl-D-glucosamine to the virus. Five hundred hemagglutinating units of reovirus type 2, strain 988 were incubated in 2 ml of Earle's saline containing 5000 μmole of *N*-acetyl-D-glucosamine for 1 hour at 23°C. An equal portion of the virus preparation was incubated in Earle's saline alone as a control. Each was layered separately onto 15 to 65 percent sucrose gradients, and centrifuged at the same time in the Spinco model-L (SW-25 rotor) ultracentrifuge at 24,000 rev/min for 210 minutes at 4°F.

Fractions were collected in 20-drop portions, and each portion was tested for hemagglutination at a dilution of 1 to 10 (Fig. 1). The amount of reovirus recovered in the sample treated with *N*-acetyl-D-glucosamine was about half of that in the control. Moreover, the treated virus banded in a slightly less dense region of the gradient in several duplicate runs. *N*-Acetyl-D-glucosamine, therefore, attaches directly to the reovirus, but not to the red blood cells, in inhibiting hemagglutination.

The terminal nonreducing ends of the antigenic determinant of blood group substances A, B, and H are *N*-acetyl galactosamine, galactose, and α-linked fucose residues (*3*). It cannot be stated definitely that the reovirus–erythrocyte union occurs through the attachment of the protein of the virus capsomeres to the *N*-acetyl-D-glucosamine which is present in ample amounts on the surface of the red blood cells (*2*). This is indeed a cardinal possibility, but the inhibition of reovirus hemagglutination by *N*-acetyl-D-glucosamine could be a steric effect as well (*6*).

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6. Recent experiments in this laboratory have indicated that *N*-acetyl-D-glucosamine does not inhibit reovirus infectivity titers in rhesus kidney tube cultures.
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Messenger RNA Utilization during Development of Chick Embryo Lens

Abstract. *A marked increase in synthesis or availability of messenger RNA which can accommodate eight to ten ribosomes was observed in the chick embryo lens between 11 and 12 days of development. The increase is seen in a shift from polyribosomes consisting of predominantly two to five single ribosomes to polyribosomes of predominantly eight to ten ribosomes in lens cells. Short-lived (half-life, 3 hours) and long-lived (half-life, > 30 hours) messenger RNA's coexist in whole lenses on the 14th day of development.*

A change in the kinds of messenger RNA's which occur during differentiation of a specialized tissue should be detectable by a change in the polyribosome distribution of its cells, particularly if the tissue begins to synthesize a large amount of a few species of message. Humphreys, Penman, and Bell (*1*) have reported that during feather development a marked increase in the number of polysomes containing four and especially of those containing five and six ribosomes occurs between 13 and 14 days of incubation. Long-lived messenger RNA's were associated with these ribosomal complexes.

A more remarkable change, described below, in the distribution of polyribosomes occurs during development of the lens in the chick embryo. We also present further evidence for coexistence of short and long-lived messenger RNA's in the crystalline lens. It has been shown (*2*) that some lens proteins are made on polyribosomes which have a long half-life.

As late as the 10th day of incubation, the lens polyribosome profile displayed in a sucrose density gradient (Fig. 1) is similar to that seen in 3- or 5-day whole embryos (*2*), in which polyribosomes containing two to five ribosomes predominate. By the 11th day a slight shoulder appears in the profile, indicating a new class of larger polyribosomes. These larger polyribosomes increase in number during the next 24 hours until the profile is dominated by a sharp high peak in the region of eight- to ten-member polyribosomes. This peak also characterizes the 13-, 14-, and 15-day profiles. Polyribosomal protein synthesis was observed in all lenses after a 2-minute exposure to radioactive algal protein hydrolyzate. The lens polyribosomes

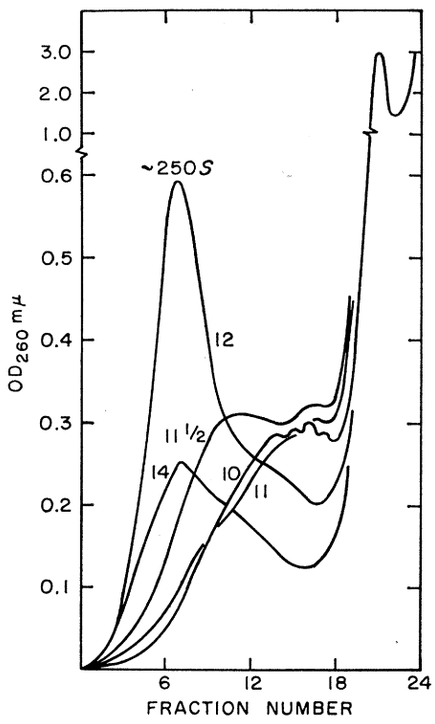

Fig. 1. Sucrose density gradient centrifugation of lens polyribosomes of 10- to 14-day chick embryos. One milliliter of homogenate (*2*) was layered on a 15- to 30-percent sucrose gradient (25 ml), centrifuged for 2 hours at 24,000 rev/min (SW-25 spinco rotor). The direction of sedimentation was to the left. The large peak at tube 21 represents single ribosomes. The peak at about 250*S* represents polyribosomes containing eight to ten ribosomes. Equivalent amounts of lens tissues were used in each experiment.