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Demonstration of Tissue Receptors for Viruses. (31374)

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Myxovirus particles attach to mammalian or avian cells which possess receptors suitable for their attachment. The suitability of receptors for this attachment is dependent on the species origin of the cells and on the virus strain examined. Many previous studies of virus-cell attachment have used red blood cells (RBC) since their agglutinability by virus suspensions may conveniently serve as evidence for the presence of receptor sites.

For certain purposes, however, it would be helpful if a simple and convenient method were available for demonstration of viral receptors on cells of parenchymatous organs. Since growth of cells in *in vitro* cultures may be associated with the structural alteration of their surfaces, such a method should be applicable to native tissues.

The present paper describes a method for testing cryostat sections of animal tissues for myxovirus receptors, based on a principle analogous to that of mixed agglutination(1). The latter principle has previously been applied to the demonstration of antigens on tissue sections, using immune sera and suitable RBC(2). Under these circumstances, RBC adhere to tissue with similar antigenic sites due to bridging by specific antibody. In a similar manner, the present test employs the adherence of RBC to tissues in the presence of virus suspensions as an indication of the presence of receptors common to both the tissues and RBC used.

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Materials and methods. Virus suspensions of the Victoria strain of Newcastle disease virus (NDV) and the PR-8 strain of influenza virus consisted of allantoic fluids harvested from infected chicken embryos. These materials were heated at 56°C for 30 minutes and centrifuged at low speed to remove debris. Measles virus was purchased from Microbiological Associates, Inc. as "Measles Hemagglutinating Antigen" and parainfluenza Type 1 was obtained from Flow Laboratories, Inc. as "Parainfluenza 1, CF Antigen."

Tissue sections were prepared from livers of apparently healthy individuals of various species. Following sectioning in a cryostat, the sections, approximately 10 μ thick, were fixed to coverslips by air drying and stored at −5°C until used.

RBC were obtained from blood drawn into ACD solution from a group A human donor and from healthy rhesus monkeys. Prior to use, the RBC were washed with physiologic saline and finally suspended in phosphate buffered saline, pH 7.4.

Hemadsorption tests were performed using a modification of a slide chamber method previously described(3). The wells of microculture slides were filled with the RBC suspension. After applying the coverslip with its attached tissue, the slides were placed in the inverted position at room temperature for 20 minutes. They were then reverted, coverslip up, and observed microscopically after 10 minutes (Fig. 1). Since slight hemadsorption of certain RBC to some tissues (*e.g.*,

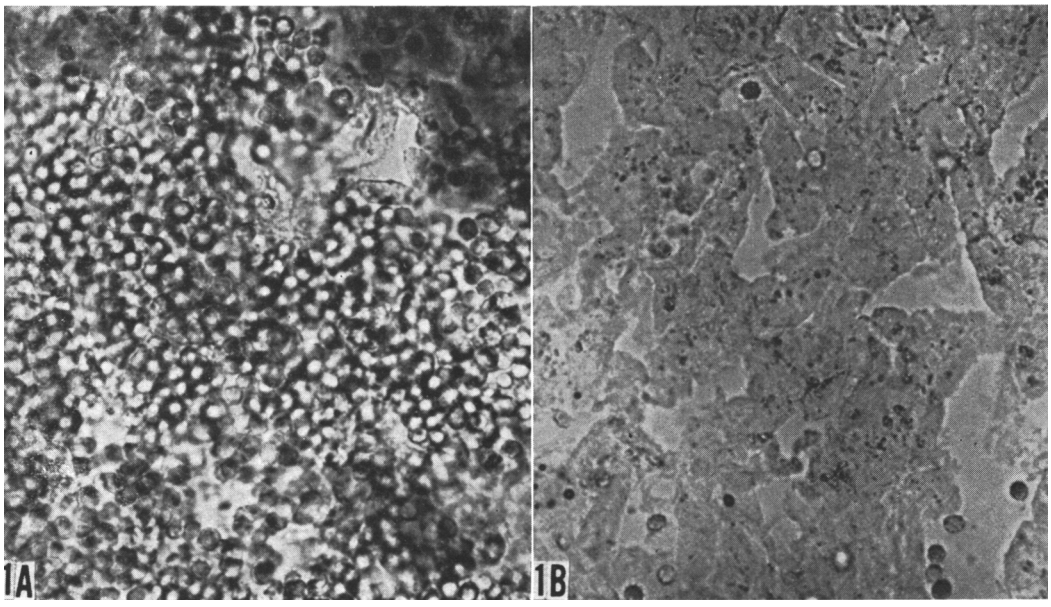

FIG. 1. Adherence of human RBC to sections of human liver. Original magnification 430×. A. Appearance when the RBC used have previously been treated with influenza PR-8. Massive adherence of sheets of RBC to the section obscure the outline of the tissue. Such tests were graded as positive (+). B. Appearance when saline-treated RBC are employed. Such tests were graded as negative (—).

rhesus RBC to rat liver) occurs in the absence of viral particles, it was necessary to include appropriate saline-treated controls in each test and to grade tests as positive only if strikingly increased adherence of RBC was observed in the presence of virus. Such tests were arbitrarily scored as "+." It was occasionally noted that RBC adherence to tissue sections was slightly increased in the presence of virus. Such tests were graded "±," and were of uncertain significance.

Results. 1. Virus-induced hemadsorption to tissue sections (Table I). RBC were treated at 4°C for 20 minutes with undiluted virus suspensions, washed twice and finally resuspended to a concentration of 0.5%. Human RBC treated in this manner with NDV, influenza, or parainfluenza virus gave positive tests with tissue sections of all species tested with the exception of mouse tissues to which NDV-treated RBC failed to adhere. Treatment of human RBC with measles virus did not increase their adherence to any tissues tested. In contrast, rhesus RBC gave strongly positive tests only when sensitized with measles virus and then only when tested against tissues of man, monkey or chick embryo, but not of rat and mouse.

In some experiments, tissue sections were treated with virus suspensions by overlaying for 20 minutes at 4°C and then they were washed. When such tissues were tested against untreated RBC, results were comparable to those obtained using untreated tissue sections and virus-coated RBC. In performing the test in this manner, it was noted that a 1%

TABLE I. Adsorption of Virus-Treated RBC to Liver Sections.

	Human RBC treated with:				Rhesus RBC treated with:			
Tissue	NDV	PR-8	Measles	Parainfluenza	NDV	PR-8	Measles	Parainfluenza
Human	+	+	—	+	—	—	+	—
Rhesus	+	+	—	+	±	±	+	—
Rat	+	+	—	+	—	—	±	—
Mouse	—	+	—	+	—	—	—	—
Chick embryo	+	+	—	±	±	±	+	—

TABLE II. Hemadsorption by Tissue Sections. Effect of treatment of human RBC and human liver sections with RDE and NDV in various combinations.

Treatment of tissue	Treatment of RBC	Hemadsorption
Saline	Saline	—
RDE	"	—
Saline	RDE	—
NDV	Saline	+
"	RDE	—
RDE, then NDV	Saline	—
Saline	NDV	+
RDE	"	—
Saline	RDE, then NDV	—

suspension of RBC gave more clear-cut results than the 0.5% suspension used in tests with virus-coated RBC.

2. *Effect of receptor-destroying enzyme.* The effect of this enzyme (RDE) was investigated in order to ascertain that the observed adherence of RBC to tissue sections represented specific bridging of one cell type to the other by the viruses used. Human liver sections and human RBC were treated for 30 minutes at 37°C with an RDE preparation† diluted 1:20 in buffered saline, pH 7.4, with added Ca^{++} and Mg^{++}. The efficacy of this treatment in destroying RBC receptors was proven by observing that RBC so treated were not agglutinable even by concentrated NDV suspensions. Other sections and RBC were similarly treated with saline. After washing, some of the RDE- and saline-treated materials were then incubated with NDV. A summary of the results obtained with various combinations of RDE, NDV or saline treatments is displayed in Table II. It can be seen that positive tests resulted only when the virus was present and neither tissue nor RBC had been treated with RDE.

3. *Effect of other treatments.* In order to study the effects of other forms of treatment on tissue receptors, sections of human liver were treated at 37°C with a 1% solution of sodium periodate for 1 hour, or with trypsin at a 2.5% concentration for 15 minutes. After washing, the sections were tested with NDV-treated human RBC. It was found that periodate treatment of the sections completely abolished their ability to bind NDV-treated RBC. On the other hand, trypsin-treated sections bound these RBC to the same extent as saline-treated controls.

Similar experiments, using periodate and trypsin-treated human RBC- and NDV-treated human liver sections showed no decrease in hemadsorption as compared to saline-treated controls. However, the treatments did not succeed in rendering the RBC non-agglutinable by NDV in the test tube.

Preliminary experiments on the effect of infection with myxoviruses on tissue receptors demonstrable by the described technique were performed. Twelve-day-old embryonated eggs were inoculated by the allantoic route, each with 0.1 ml of a 1:100 dilution of NDV-containing allantoic fluid. After incubating 2 days at 37°C, the embryos had died with gross hemorrhagic lesions and had developed hemagglutinins for human RBC in their allantoic fluids. Their livers were sectioned and the sections were preserved at —5°C for 4 weeks. After this interval, virus-induced hemadsorption of untreated human RBC could no longer be demonstrated. At this time, the sections were tested with NDV-coated human RBC and no hemadsorption was observed in any trial. Sections from uninfected control embryos sacrificed at the same time and stored under identical conditions gave strongly positive tests with the virus-coated RBC.

Discussion. The use of immune sera to bind RBC to the surfaces of various cells by specific bridging between antigens was reported by Coombs and Bedford(1). A slide chamber method for applying this phenomenon to the investigation of antigens in tissue sections was reported by Tönder *et al*(2). The feasibility of using this principle, with substitution of a hemagglutinating virus for antibody, to demonstrate receptor sites was suggested by the demonstration that hemagglutination by viruses depends on specific bridging of one RBC to another *via* attachment to receptors(4).

The present experiments show that hemagglutinating myxoviruses cause adherence of some RBC to tissue sections derived from certain species. The adherence appears to be

† Vibrio comma filtrate, Microbioligical Associates, Inc.

mediated by bridging of cells which possess common receptors, based on the following observations:

First, the phenomenon is not universal with respect to species. RBC species inagglutinable by a virus cannot be conditioned by that virus to adhere to tissue sections. For example, measles virus, which agglutinates only rhesus RBC, does not cause adherence of human RBC to tissue sections. On the other hand, the other myxoviruses tested which do not agglutinate rhesus RBC do not cause these RBC to adhere to tissue sections. Even when agglutinable RBC are used, they do not adhere to all tissues tested but instead show selectivity with regard to species. In this connection, it is interesting to note that measles virus, which is capable of infecting only primate species and the chick embryo (5), caused rhesus RBC to adhere to tissues of these animals but not to those of the mouse or rat.

Secondly, the adherence of RBC to tissues in the presence of agglutinating virus can be abolished by pre-treatment with RDE. This occurs regardless of whether the tissues or the RBC are thus treated, and without regard to whether the RDE-treated material is the one which is then treated with virus or the one which is then tested against its virus-coated partner.

Thirdly, the lack of adherence of virus-treated RBC to tissues pre-treated with periodate tends to confirm that the phenomenon is due to the presence of specific virus receptors on the tested tissues, rather than to a virus-induced nonspecific increase in RBC adhesiveness. Similarly, the absence of adherence seen when tissues of NDV-infected embryos are tested with NDV-treated RBC argues for this, since receptor material apparently disappears from embryos infected with myxoviruses(6).

The described procedure, by revealing the presence or absence of receptors of native tissues, may prove useful in the investigation of mechanisms of species-determined resistance to infection by viruses. In the light of evidence showing that artificially-induced receptor destruction protects chick embryos and mice against infection with influenza viruses (7,8), it may be postulated that certain species owe their immunity against some of the viruses to the lack of tissue receptors suitable for these viruses.

In addition, the ability to display receptor content of various organs may be of use in investigating problems of organotropism of various viral strains.

Summary. By using a principle analogous to that of mixed agglutination, virus-treated RBC may be caused to adhere to tissue sections in a slide chamber test. The adherence depends on the presence of virus receptor sites on both tissue and RBC; thus the occurrence of hemadsorption serves as a useful indicator for the presence of receptor sites in the tissue tested. Treatment of tissue or RBC with RDE prevents the occurrence of hemadsorption, as does treatment of the tissue with periodate. In the chick embryo, infection with NDV seemed to eliminate tissue receptors for this virus. The technique should prove applicable to studies of species susceptibility to viral infection and to studies of organotropism of viruses.

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