shapes of the temporal sensitivity functions were the same after adaptation to a superimposed red of 678 nm as after adaptation to a blue of 470 nm, which indicates that these functions were not likely to depend on blue cone contributions. A number of less plausible optical bases for the residual flicker might be proposed, some of which we ruled out with other measurements. The role of such optical effects becomes moot, however, in view of the spectral sensitivity data.

10. A circular spot 2° in diameter and 8000 trolands was used for the flicker data. We also ran flicker studies with 10° fields and found no systematic differences in the shapes of the response curves. In the spectral sensitivity study, the circular spot was 1° in diameter and was superimposed on a 10° circular background of 8000 trolands.
11. Protanolabe and deutanolabe spectral sensitivities were measured by W. A. H. Rushton, D. S. Powell, and K. D. White [*Vision Res.* **13**, 2017 (1973)] and by T. P. Piantanida and H. G. Sperling (*ibid.*, p. 2033).
12. T. P. Piantanida, *Am. J. Optom. Physiol. Opt.* **53**, 647 (1976).
13. Supported by NIH grants EY02357 to T.P.P. and EY01128 to D.H.K. and by NIH postdoctoral fellowship EY05116 to F.S.F. This work was first reported at the 1978 Annual Meeting of the Association for Research in Vision and Ophthalmology. The abstracts appeared in *Invest. Ophthalmol.* **18** (Suppl.), 197 (1978). We thank R. M. Boynton for his help and encouragement early in the course of this work. We also thank S. L. Buck, D. Y. Teller, and especially C. S. Harris for their helpful comments on the manuscript.
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## Viral Receptors on Isolated Murine and Human Ependymal Cells

Abstract. *Viruses that infect ependyma cause ependymitis in humans and hydrocephalus in experimental animals. We report that reovirus type 1 (which induces hydrocephalus in mice) binds to the surface of isolated human and murine ciliated ependymal cells. With the use of recombinant viral clones, the binding property was mapped to the type 1 viral hemagglutinin, which also determines in vivo the affinity of reovirus type 1 for ependyma. Mumps virus, measles virus, parainfluenza type 3, and herpes simplex virus type 1 bind to murine ependyma cells, whereas reovirus type 3, herpes simplex virus type 2, and poliovirus type 1 do not.*

The binding of a viral particle to the cell surface is the first obligatory step of cell infection and depends on the interaction of the virus attachment protein and the cellular receptor. The presence of a receptor for a given virus on a particular cell membrane is a primary factor in determining the affinity of that virus for the cell and the ultimate pattern of virulence. In studies of viral receptors most investigators have used continuous cell lines, and "receptor families" have been described on HeLa cells for viruses that are unrelated but may share a common receptor (*1*). Except for binding studies with lymphocytes (*2*), the binding of viruses to isolated cells known to be the target of viral infection in vivo has not been studied directly.

Infection of ependymal cells in experimental animals by certain classes of viruses, particularly those of the myxovirus group, causes hydrocephalus; in humans, cytoplasmic inclusions of viral nucleocapsid-like material have been found in ependymal cells from the cerebrospinal fluid of patients with mumps meningitis (*3*). A definitive link between viral-induced ependymitis and congenital hydrocephalus in humans has not been proved.

Studies of reovirus type 1–induced hydrocephalus in mice have demonstrated a central role for the viral hemagglutinin. This has been shown with the use of single-segment recombinant clones between reovirus types 1 and 3. Reovirus is a segmented double-stranded RNA virus containing ten genes; clone 3.HA1 contains nine genes from type 3 and one, the S1 gene which encodes the viral hemagglutinin, from type 1; 1.HA3 is the reciprocal clone. Reovirus type 1 and clone 3.HA1 cause a nonfatal ependymal infection with no neuronal damage in newborn mice, whereas reovirus type 3 and clone 1.HA3 cause a fatal encephalitis with neuronal destruction but no ependymal cell damage (*4*). These experiments in vivo suggest that the affinities of the two reovirus serotype for ependymal or neuronal cells, and their patterns of neurovirulence, are secondary to the specific interaction of the viral hemagglutinin with a receptor on the cell surface. Thus reovirus provides an excellent model for the study of viral-receptor interactions.

To study the interactions between reovirus type 1 and ependymal cells in vitro, we prepared single-cell suspensions of viable ependymal cells from the central nervous system of adult mice by the technique described by Manthrope *et al.* (*5*). These preparations contain approxi-

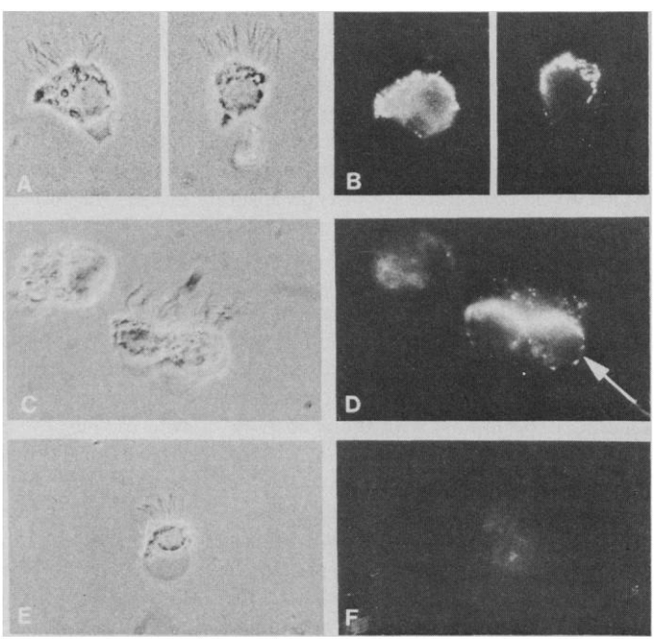


Fig. 1. The binding of reovirus to murine ependymal cells demonstrated by fluorescent staining. The cells ($2 \times 10^6$ to $3 \times 10^6$, of which 50 percent were ciliated ependymal cells) were isolated by the technique of Manthrope *et al.* (*5*). The cells ($5 \times 10^5$) were then incubated with 40 μl of purified reovirus type 1 or type 3 at a titer of $5 \times 10^9$ pfu/ml (the ratio of particles to plaque-forming units was 100:1; thus, $5 \times 10^9$ pfu/ml = $5 \times 10^{11}$ viral particles per milliliter) for 20 minutes at 4°C, after which the cells were washed three times in F12 medium containing 25 m*M* Hepes, 1 percent bovine serum albumin, and 2.5 m*M* EGTA. The viral binding was demonstrated by indirect immunofluorescence with rabbit antibody to reovirus (*7*) and with a 1/100 dilution of FITC-conjugated goat antibody to rabbit immunoglobulin (FITC-Garig, Tago Inc., California). (A and C) Unstained ependymal cells examined by phase microscopy. (B and D) The same fields as in (A) and (C) seen by fluorescence microscopy showing bright labeling of the cell after incubation with reovirus type 1. In all instances the fluorescent dots surround the cell body; in some cases the binding is also prominent in the area of the cilia. (E and F) An ependymal cell incubated with reovirus type 3 ($5 \times 10^9$ pfu/ml), stained in the same way as the cells in (B) and (D), and examined by phase (E) and fluorescence (F) microscopy. The absence of fluorescence in (F) indicates that reovirus type 3 did not bind to the cell.


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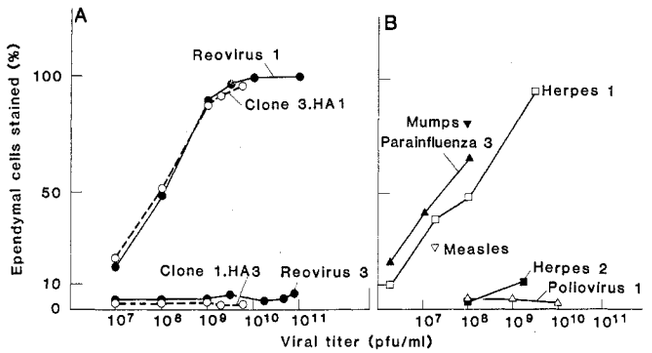


Fig. 2. (A) Binding of various concentrations of reovirus type 1 and type 3 (continuous lines) or recombinant clones 3.HAI and 1.HA3 (broken line) to murine ependymal cells from adult mice. (B) Binding of various concentrations of herpes simplex virus type 1 (Kos strain), herpes simplex virus type 2 (186 strain), parainfluenza type 3, poliovirus type 1, mumps virus (Enders strain, titer expressed as hemagglutinin unit per 0.05 ml), or measles virus (Edmonston strain) to murine ependymal cells. The virus (40 μl at a given titer) was incubated with a pellet of $5 \times 10^5$ ependymal cells for 20 minutes at 4°C, after which the cells were washed three times in medium. Viral binding was demonstrated by indirect immunofluorescence with rabbit antiserums against the virus being studied and by FITC-conjugated goat antibody to rabbit immunoglobulin.

mately 50 percent ciliated ependymal cells of which some show cilia actively beating. Figure 1, A and C, shows the typical appearance of these ciliated cells under phase microscopy. In Fig. 1, B and D, the same cells were incubated with reovirus type 1 and then stained with a rabbit antibody to the virus and fluorescein isothiocyanate (FITC)–conjugated goat antibody to rabbit immunoglobulin (Ig). Ependymal cells incubated with type 3 reovirus and stained in the same way showed no fluorescence (Fig. 1, E and F).

To determine the amount of virus required to make the binding visible, we incubated a constant number of ependymal cells with different concentrations of reovirus type 1 (Fig. 2A). More than 95 percent of ciliated ependymal cells showed bound virus when the concentration of virus was between $5 \times 10^9$ and $5 \times 10^{11}$ plaque-forming units (pfu) per milliliter; at $10^8$ pfu/ml, only 50 percent of ependymal cells were positive; no positive cells were seen at less than $10^7$ pfu/ml. No binding of reovirus type 3 was observed except at concentrations above $10^{10}$ pfu/ml, when fluorescent dots were observed in the cilia appearing like trapped material in 30 percent of the cells. At no concentration of type 3 reovirus was fluorescence observed surrounding the cell body as we saw with reovirus type 1.

To establish the specificity of binding and the correlation of the binding with the affinity of the reovirus serotypes for ependymal cells in vivo, we conducted binding experiments with the single-segment recombinant clones 3.HA1 and 1.HA3 at a variety of concentrations. As shown in Fig. 2A, clone 3.HA1 binds to ependymal cells in the same way as the parental type 1, whereas the reciprocal clone, 1.HA3, does not. Thus, both the in vivo affinity of reovirus type 1 and its binding characteristics for isolated ependymal cells are determined by the viral hemagglutinin. In addition, we found that the binding of reovirus to ependymal cells is the same in newborn as in adult animals. This suggests that the age-dependant development of hydrocephalus, observed in animals (*3, 6*), is probably not due to the presence or absence of viral receptors on the ependymal cell surface.

Hydrocephalus induced by reovirus type 1 infection has been reported for mice, rats, hamsters, and primates (*6*). To determine whether reovirus type 1 had an affinity for human ependymal cells in vitro, we prepared viable ciliated ependymal cells from the brain of a 6-month-old child 8 hours after its death. The same specificity of viral binding was observed with human as with murine ependymal cells: reovirus type 1 bound to 90 percent of the ciliated cells whereas reovirus type 3 bound to only 18 percent. When human ependymal cells were incubated with the viral antibody and the FITC-labeled goat antibody without prior incubation with reovirus 12 percent of the cells showed binding.

Reoviruses rarely cause illness in humans. However, certain other groups of viruses that do cause disease in humans have been reported to show a specific affinity for ependymal cells. These and other viruses were tested for their capacity to bind to isolated murine ependymal cells. Herpes simplex virus type 1, parainfluenza type 3, and measles and mumps viruses bound to ependymal cells whereas herpes simplex type 2 and poliovirus type 1 did not (Fig. 2B). The percentage of cells binding the viruses depended on the titer of the particular viral stock and was remarkably similar to the percentages obtained with the various titers of reovirus type 1; since the ratio of the number of particles to the number of plaque-forming units for the viruses studied was in the range of 100:1 to 1000:1 (the particle to plaque-forming unit ratio is not known for measles), this suggests that irrespective of the virus, a similar minimal number of viral particles was required to demonstrate staining. For example, more than 95 percent of cells stained with herpes type 1 at a titer of $10^9$ pfu/ml, whereas between 60 and 70 percent stained with mumps virus and parainfluenza type 3 at a titer of $10^8$ pfu/ml. As a positive control for those viruses that did not bind to ependymal cells, herpes type 2 and polio type 1 at titers of $10^9$ pfu/ml were examined for binding to HeLa cells by the identical indirect immunofluorescent technique and were found to bind virtually all of the cells. Similarly, reovirus type 3 binds to a subset of murine lymphocytes (*7*) and to murine L929 cells at titers of $10^9$ pfu/ml, a titer at which it does not bind to ependymal cells.

Previous studies with herpes and mumps viruses have shown an early predominance of virus in ependymal cells. After a herpes infection in mice, viral antigen is found first in the choroid plexus and ependymal cells. After 2 days of a mumps infection in hamsters, viral nucleocapsids can be visualized by electron microscopy in ependymal cells. Measles and parainfluenza viruses are known to cause hydrocephalus (*3*). These data are consistent with hematogenous viral spread to the brain and support the possibility that there are specific receptors for these viruses on the surface of ependymal cells. As expected, poliovirus type 1 and reovirus type 3 did not bind to ependymal cells, although the inability of herpes type 2 to bind to ependymal cells was striking. The highest percentage of ependymal cells that showed binding with herpes 2 was 15 percent, despite our conducting many experiments with different samples of antiserum and several batches of virus; as a control for each experiment we demonstrated positive staining of herpes type 2 to HeLa cells. These results suggest that there may be different receptors for herpes types 1 and 2 and are consistent with reports of such type-specific receptors on plasma membranes (*8*).

The structure on the ependymal cells to which the viruses bind remain to be identified. The same receptors might be shared by different viruses or there might be specific receptors for each different virus. Little is known about the different antigens present on the surface of ependymal cells, although two monoclonal antibodies directed against glial cell antigens have been reported to recognize

antigenic structure in ependymal cells (*9*). We have found the presence of histocompatibility antigens on ependymal cells and are also developing a panel of monoclonal antibodies against these cells. We have not found Fc receptors or Thy 1.2 on the ependymal cell surface. A better understanding of the surface antigens on ependymal cells will facilitate the study of viral receptors as determinants of the affinity of viruses for the central nervous system (CNS).

Other cells from the CNS, such as oligodendrocytes, astrocytes, or neurons, could also be used to define the various classes of viruses to which they bind specifically. Thus our data suggest a new approach to the study of viral affinity for the CNS, the role of viral receptors in the age-dependent susceptibility of the CNS to viruses, and the possibility that viral affinity for cell surface antigens may determine immune-mediated damage to specific cell types.

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### References and Notes

1. P. Boulanger and L. Philipson, in *Virus Receptors*, part 2, *Animal Viruses*, K. Lonberg-Holm and L. Philipson, Eds. (Chapman & Hall, London, 1981), pp. 122–124.
2. M. Jondal, G. Klein, M. B. A. Oldstone, V. Bokish, E. Yefenof, *Scand. J. Immunol.* **5**, 401 (1976); M. S. McGrath and I. L. Weissman, *Cell* **17**, 65 (1969).
3. R. T. Johnson and K. P. Johnson, *Exp. Mol. Pathol.* **10**, 68 (1969); R. M. Herndon, R. T. Johnson, L. E. Davis, L. R. Descalzi, *Arch. Neurol.* **30**, 475 (1974); R. T. Johnson, *J. Exp. Med.* **119**, 343 (1964); J. S. Wolinski, *Lab. Invest.* **37**, 229 (1977).
4. H. L. Weiner, D. Drayna, D. R. Averill, B. N. Fields, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5744 (1977); H. L. Weiner, M. L. Powers, B. N. Fields, *J. Infect. Dis.* **141**, 609 (1980).
5. C. M. Manthrope, G. P. Wilkin, J. E. Wilson, *Brain Res.* **134**, 407 (1977).
6. L. Kilham and G. Margolis, *Lab. Invest.* **21**, 189 (1969); A. B. Sabin, *Science* **130**, 1387 (1959); K. Hummeler, B. A. Zajac, M. Genel, P. G. Holtzapple, S. Segal, *ibid.* **168**, 859 (1970).
7. H. L. Weiner, K. A. Ault, B. N. Fields, *J. Immunol.* **124**, 2143 (1980).
8. A. Vahlne, B. Svennerholm, E. Lycke, *J. Gen. Virol.* **44**, 217 (1979).
9. M. Schachner, I. Sommer, C. Lagenaur, J. Schnitzer, paper presented at the Dalhem Conference on Neuronal-Glial Cell Interrelationships: Ontogeny, Maintenance, Injury, Repair, Berlin, West Germany, December 1980 (Verlag Chemie, Weinheim, in press).
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## Helpers: Effects of Experimental Removal on Reproductive Success

Abstract. *Experimental removal of helpers from a communal bird population decreased the reproductive success of the reduced units. By controlling for variables that are potentially correlated with both reproductive success and unit size, this experiment establishes that helpers contribute significantly to their indirect fitness.*

Helping behavior may be defined as the performance of parent-like behavior toward young that are not offspring of the helper (*1*), usually a nonbreeder. Thus helpers offer the closest parallel among vertebrates to the "sterile" workers of the social insects. Consequently, helping is useful in field tests of inclusive fitness theory (*2*) in vertebrates because of its potential contribution to indirect fitness (*3*), an effect often invoked in theories of the evolution of helping behavior (*4*). Previous studies of this contribution in populations of communal birds and mammals have relied on the positive correlation of reproductive success with numbers of helpers in situations where the helpers and recipient are usually closely related (*5*).

It has seemed likely that helpers caused increased reproductive success in their social units, since their help was often conspicuously visible. Nevertheless, competing hypotheses have not been rejected. A positive correlation between reproductive success and unit size in singular breeding species (*6*) could also arise from positive correlations of reproductive success and unit size with other variables. A factor not adequately controlled in previous studies is the quality of the territory. Since helpers in many species are the offspring of the same parents in a previous breeding season, if certain territories usually enable greater reproductive success than others, then a positive correlation between reproductive success and unit size could arise from this relation alone, with no contribution from the helpers (*7*). Similar results could arise if older parents were consistently more successful than younger ones.

Another objection to the interpretation that helpers increase reproductive success was raised by Zahavi (*8*) after observing agonistic behavior within social units of babblers. He claimed that "nonbreeding birds may do more harm than help to the reproduction of the breeding pair." Zahavi did not find a positive correlation between reproductive success and unit size in his data, although on reexamination it was found by others (*9*). In this and other studies in which the anticipated positive correlation was not significant, the small sample sizes made it difficult to show a significant relation of any kind (*10*).

To determine whether the correlation between reproductive success and unit size was caused by the number of helpers or by other variables correlated with both reproductive success and unit size, we removed helpers, in an experiment performed in the field under natural conditions. Twenty social units of the gray-crowned babbler (*Pomatostomus temporalis*) of approximately the same original size (six, seven, or eight birds) were separated into groups of nine experimental units and 11 control units, matched as nearly as possible for original size. Experimental units were reduced to three birds, the original breeding pair and one yearling helper chosen at random; the other group of units served as controls (*11*). Because the removals were performed after the first brood of young, the dependent variable used as an index of reproductive success was the total number of young that were reared to the point of leaving the nest from second and subsequent broods—that is, fledglings from all broods after the first (F2). In this design, other variables that might be correlated with the original size of the unit, such as vegetation, other components of territorial quality, and age or ability of parents, would not be affected by the removals and should average the same in the two groups (*12*).

Comparison of experimental units with natural control units of the same original size, by controlling for variables correlated with original size, allows an independent estimate of the effect of helpers on reproductive success. Control units had an average of 2.4 fledglings from the second and subsequent broods, more than twice as many as the experimental units, which had an average of 0.8 (*13*). This result allows us to reject the hypothesis that the difference in reproductive success between control and experimental groups is the result of factors correlated with the original size.

Comparison of experimental units with natural units of two and three members, by controlling number of helpers, allows an estimate of the effects of other factors correlated with the original size. The reproductive success of experimental units was very close to that of natural


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