by SDS-PAGE (10% gels by Novex) and transferred to poly(vinylidene difluoride) (PVDF) membranes, which were then stained with Coomassie blue to confirm equal sample loading. Filters were then incubated with either antibodies PY20 and PY69 to phosphotyrosine (1:1500 dilution; ICN, Irvine, CA) or polyclonal antibodies to Shc (1:250; Transduction Labs, Lexington, KY). Detection was with horseradish peroxidase–conjugated secondary antibodies and the enhanced chemiluminescence system (ECL, Amersham).
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Unidirectional Coupling of Gap Junctions Between Neuroglia

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Gap junctions permit the passage of ions and small molecules between cells, thereby providing a basis for direct intercellular communication. In the rabbit retina, the low molecular weight dyes Lucifer yellow and biocytin passed readily from astrocytes into adjacent astrocytes, oligodendrocytes, and Müller cells. However, the dyes rarely passed from either oligodendrocytes or Müller cells into astrocytes. Unidirectional passage of dye suggests the presence of an asymmetric barrier to the movement of molecules through heterologous gap junctions and indicates the potential for a hierarchy of command between interconnected cells.

Many vertebrate and invertebrate cells are connected by "gap junctions" that provide a route for intercellular communication (*1*). Cellular coupling can be revealed by the intracellular injection of a membrane-impermeant dye of low molecular weight, such as Lucifer yellow (457 daltons) (*2*) or biocytin (373 daltons) (*3*, *4*). The assumption that molecules can pass through gap junctions equally in both directions was challenged by Flagg-Newton and Loewenstein's (*5*) experiments on cocultures of Balb/c and B fibroblasts. They reported that dye injected into B cells diffused readily into neighboring Balb/c cells but that little, if any, dye transferred from injected Balb/c cells into neighboring B cells. This remarkable finding has not been replicated, and its functional significance is unclear because the two cell lines are unlikely to encounter each other outside a petri dish. We now report that asymmetric diffusion of dye occurs between different types of neuroglia (astrocytes, oligodendrocytes, and Müller cells) in the myelinated band of the intact rabbit retina (*6*).

Retinae from adult rabbits were stained with Hoechst 38317 (*7*), and labeled periaxonal astrocytes (*8*) were injected with Lucifer yellow (*9*). We photographed 91 periaxonal astrocytes that had been injected, and in every instance dye had diffused into the majority (>90%) of stained cells within their dendritic fields (mean number of coupled cells, 70; range, 20 to 200; Fig. 1, A and B). Many of these coupled cells (50 to 70%) were neighboring periaxonal astrocytes, whereas the remainder were either oligodendrocytes (Fig. 1, E and F) or astrocytes belonging to other classes. In addition, 58 periaxonal astrocytes were injected with biocytin (*10*). They were coupled to 30 to 300 cells (mean number of coupled cells, 156; Fig. 1G), of which 50 to 70% were periaxonal astrocytes, and the remainder were either oligodendrocytes or astrocytes from other classes.

Some periaxonal astrocytes were also coupled to Müller cells (radial glia): 10 of the 91 injected with Lucifer yellow were coupled to 1 to 12 Müller cells (mean number of coupled cells, 5), whereas 40 of the 58 filled with biocytin were coupled to 1 to 20 Müller cells (mean number of coupled cells, 3). These Müller cells could be found up to 250 μm from the soma of an injected astrocyte. Because the lateral processes of rabbit Müller cells are only about 25 μm long (*11*), such labeling must be due to the diffusion of dye through gap junctions between astrocytes and Müller cells rather than to inadvertent impalement of Müller cell processes. We injected more than 100 Müller cells with Lucifer yellow or biocytin, but they were never coupled to other Müller cells or to astrocytes.

Oligodendrocytes were identified by their characteristic morphology (*12*) (Fig. 1, C, D, F, and H) and by their absence of immunoreactivity to glial fibrillary acidic protein. Approximately half of the stained nuclei at the margin of the myelinated band were oligodendrocytes. Of the 92 oligodendrocytes injected with Lucifer yellow, 90 exhibited no dye diffusion into adjacent cells (Fig. 1, C and D). The remaining 2 oligodendrocytes were surrounded by 8 to 10 lightly labeled oligodendrocytes and Müller cells. Similarly, 49 of the 70 oligodendrocytes injected with biocytin were not coupled. The remaining 21 oligodendrocytes were surrounded by lightly labeled oligodendrocytes and astrocytes (mean number of coupled cells, 49; range, 1 to 150; Fig. 1H).

Our results show that, when periaxonal astrocytes are injected with Lucifer yellow or biocytin, most of the nearby oligodendrocytes and some Müller cells fill with dye. By contrast, when oligodendrocytes are injected, few (if any) of the nearby astrocytes show coupling. Furthermore, when Müller cells are injected, nearby astrocytes are never labeled with dye (Fig. 2).

It is conceivable that the unidirectional spread of dye is attributable simply to damage to the oligodendrocytes (and Müller cells) caused by the injection procedure and consequent closing of their gap junctions. To investigate this possibility, oligodendrocytes were injected with either Lucifer yellow or biocytin, and nearby astrocytes were immediately injected with the other dye (*13*). Of the 23 successful fills, 21 oligodendrocytes were double-labeled. In these 21 cases, dye had passed from astrocytes into oligodendrocytes after the oligodendrocytes

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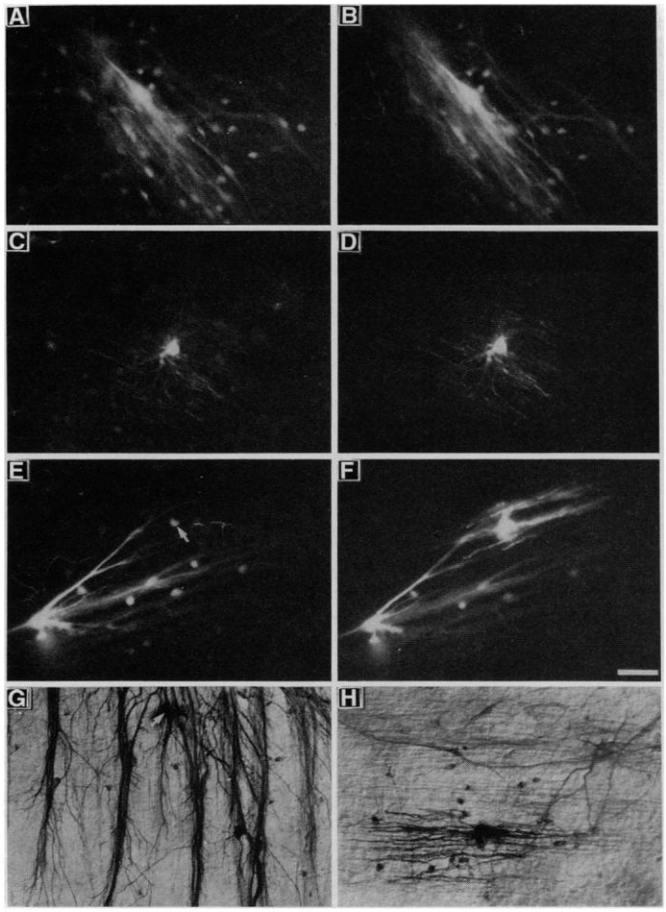

Fig. 1. Nuclei labeled with Hoechst 38317 in the nerve fiber layer were photographed under violet excitation (400 nm), injected with Lucifer yellow, and rephotographed under blue-violet excitation (436 nm), a wavelength that excites Lucifer yellow strongly but the Hoechst dye only weakly. The photomicrographs were compared to determine the total number of Hoechst-labeled cells that were dye-coupled to each injected cell. We restricted our investigation to the edge of the myelinated band, peripheral to the blood vessels, because neuroglia in more central locations were too densely packed to allow accurate analysis. (**A** and **B**) Periaxonal astrocyte filled with Lucifer yellow and viewed under violet excitation (A) and blue-violet excitation (B). (**C** and **D**) Oligodendrocyte filled with Lucifer yellow and viewed under violet excitation (C) and blue-violet excitation (D). (**E** and **F**) Periaxonal astrocyte filled with Lucifer yellow and viewed under blue-violet light (E). A dye-coupled soma (arrow) was subsequently injected. Dye did not spread from this oligodendrocyte (F) into any other cells. (**G**) A periaxonal astrocyte (arrow) filled with biocytin. (**H**) A weakly coupled oligodendrocyte that has been filled with biocytin. Bar, 50 μm.

had already been injected, and therefore we can rule out the possibility that the unidirectional coupling is a methodological artifact.

Another trivial explanation of our results is that oligodendrocytes in intact rabbit retina may establish only a small number of gap junctions with each other but are nonetheless coupled in an extensive network. Consequently, dye passing from an injected oligodendrocyte into that network may be diluted below detectable levels. To investigate this possibility, we placed scalpel lesions at the edge of the medullary rays to isolate small groups of oligodendrocytes ($n < 10$) from the rest of the glial network. Seven oligodendrocytes isolated in this way were injected with Lucifer yellow, but none showed any indication of coupling. Thus, our results cannot be explained in terms of asymmetric dye dilution.

Loewenstein (*14*) noted that a condition needed for asymmetric dye diffusion is that different classes of channels must be present on either side of the gap junction. Consistent with this hypothesis, astrocyte gap junctions contain the protein connexin 43, whereas oligodendrocyte gap junctions contain connexin 32 (*15*). Furthermore, connexin 43 immunoreactivity is restricted to the astrocyte side of gap junctions between astrocytes and oligodendrocytes (*16*).

A second condition is that the permeability of the different classes of channels must differ. Loewenstein proposed that asymmetric diffusion of dye between cells is due to "an asymmetric free-energy barrier

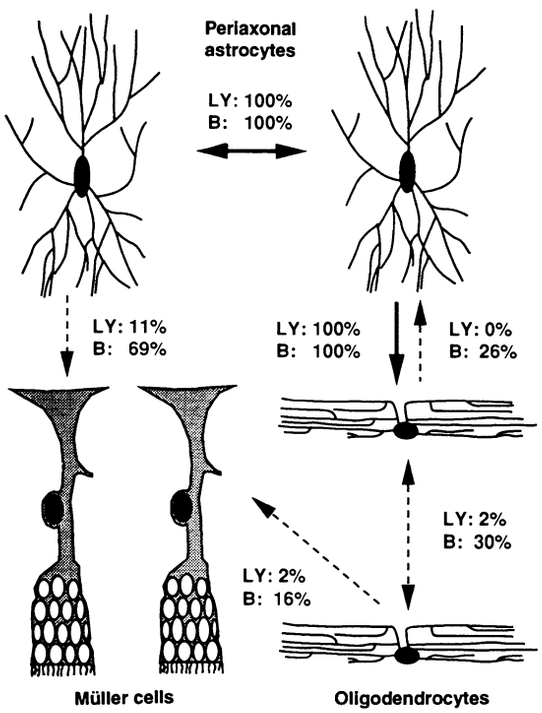

Fig. 2. Summary of the dye diffusion we have observed among three types of retinal glia. The solid arrows represent coupling that occurs consistently, and the dashed arrows represent coupling that is sporadic. The proportions of injected cells that displayed coupling with Lucifer yellow (LY) or biocytin (B) are indicated.

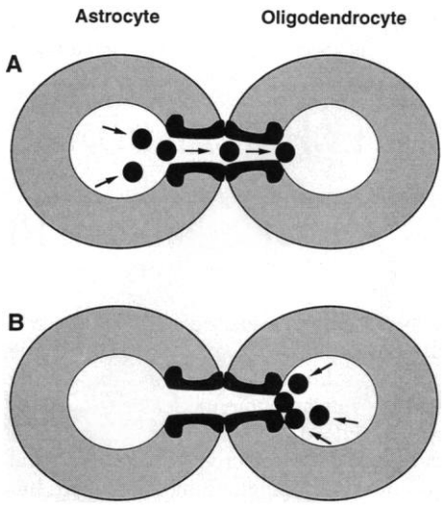

Fig. 3. Model of the unidirectional diffusion of dye between coupled oligodendrocytes and astrocytes, based on differences in connexon pore diameter. Like a fish in a fish trap, dye molecules (black circles) can pass from an astrocyte to an oligodendrocyte (**A**) but not back in the other direction (**B**). Connexons are shaded black and the direction of dye flow is indicated by arrows.

for permeant movement in the channel that is higher in one direction than in the other" (*14*, p. 860). Loewenstein suggested that the asymmetry could be provided by electrostatic interactions between the permeant and the channel, interactions through hydrogen bonding, or rapid conformational changes in the channel. An additional possibility, we suggest, is that the asymmetry is attributable to differences in pore diameter at either side of a heterologous gap junction (Fig. 3). Molecules of an appropriate size may be able to enter the junction through the larger pore and then pass through to the other side. However, similar molecules would be unlikely to pass in the opposite direction because they would be blocked by the smaller pore. This variation of Loewenstein's model implies that ions and molecules smaller than a certain size will be able to pass freely in both directions. It also predicts that gap junctions composed of the protein connexin 43 (astrocytes) have a larger pore diameter, and hence a higher unitary conductance, than those composed of connexin 32 (oligodendrocytes). Presumably, gap junctions in Müller cells have an even smaller pore diameter.

Our findings have two implications. First, they support the idea that gap junctions composed of a particular connexin protein have a characteristic channel diameter and permeability. It follows from this that the exchange of certain trophic and signal molecules may be limited to gap junctions composed of particular connexins. Second, and more important, as the bidirectional exchange of molecules through gap junctions is thought to provide a basis for intercellular communication (*1*), the unidirectional transfer of molecules provides the potential for a hierarchy of command.

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TECHNICAL COMMENTS

Protection from HIV Infection or AIDS?

One of the major goals of AIDS research is the development of an efficacious vaccine providing broad, long-lasting protection against human immunodeficiency virus–type 1 (HIV-1) infection. In the past, for induction of protective immunity, viral vaccine development aimed to elicit humoral immunity (that is, to produce strong neutralizing antibodies) that would protect one from infection. In contrast, cellular immunity was in principle thought to be associated with recovery from viral disease and convalescence. In a provocative Perspective, Jonas Salk and his colleagues hypothesize that a protective vaccine against HIV-1 infection should induce cellular rather than humoral immunity (*1*). Not only do they propose that cell-mediated immunity (CMI) can protect one from HIV-1 infection, but they also suggest that antibody responses are associated with increased *susceptibility* to such infection. This hypothesis is based on two distinct sets of data on the immunology of HIV-1 infection, in part published and in part presented during the IXth International Conference on AIDS held in Berlin in June 1993.

First, Clerici *et al.* (*2*) found that a large percentage of individuals exposed to HIV-1 who tested negative for the virus, but a small percentage of unexposed or low-risk subjects, showed evidence of HIV-1–specific CMI. Peripheral blood mononuclear cells from the former group released measurable amounts of interleukin-2 (IL-2) (which prompts lymphocyte proliferation) when stimulated with HIV-1 envelope peptides. From six persons who were studied longitudinally, two eventually became seropositive for HIV-1 antibodies. In subsequent studies, larger groups were included, and cells from up to 50% of the individuals responded with IL-2 production in the HIV-1 peptide assay (*3*). Furthermore, macaque monkeys inoculated with low doses of simian immunodeficiency virus (SIV) frequently had CMI responses that were detectable up to 64 weeks after inoculation, yet they did not produce SIV-specific antibodies or show evidence of infection (*4*). In contrast, all but one of the animals that received higher doses of virus became infected, tested seropositive, and showed no CMI response against the SIV peptides 64 weeks after infection. Salk *et al.* conclude from