tained antibodies to H-2, Ia, and other cell-surface antigens (non-H-2).

Pierce *et al.* (*7*) have investigated the ability of antibodies to both H-2 and non-H-2 to inhibit in vitro primary responses to GAT_{10} (a linear polymer of glutamic acid, alanine, and tyrosine) and SRBC. They found that antiserums directed against only H-2D have no effect on the response, in agreement with our results. They also observed that antiserums which can contain antibody to Ia in addition to antibody to H-2K inhibit both IgM and IgG primary responses. An antiserum, which they assume to be a specific antibody to H-2K without any possible Ia antibodies, could in fact contain antibodies to $I\text{-}C^k$. The antiserum to H-2K that we used in our study cannot contain these antibodies (*12*). Using this antiserum we found no inhibition of the response. Pierce *et al.* (*7*) observed no effect of a specific antiserum to Ia on primary IgM responses. That serum, which lacked cytotoxicity, was obtained from early bleedings of animals that produced the antiserums that we used in our experiment. Later bleedings of the same group of animals yielded highly cytotoxic serums, which produced a marked inhibition of the primary IgM response. When we added the antiserum directly to the culture as in the experiments of Pierce *et al.*, instead of treating the cells with antiserum before starting the culture, we observed little inhibition of the secondary IgM, but the IgG response was inhibited.

We emphasize that simply attaching any antibody to the cell surface does not inhibit an immune response, as shown by antiserums directed at H-2D or H-2K specificities. Antiserums directed at I region products do alter the immune response, as would be expected if Ia antigens were either cooperative factors or the antigen receptors themselves. An important remaining question with regard to the mode of inhibition by antiserums to Ia is whether prior treatment permanently alters the capacity of the cells to respond to antigen. It would be expected that cells with antibody bound to them would lose the antibody-antigen complexes either by simply shedding the antigens, or by capping and pinocytosis. If the cells can rapidly generate new antigens, then one would predict that preliminary treatment with antiserums would alter only the kinetics of the response, by delaying initiation —that is, recognition or cooperation. In some initial experiments we have observed such an alteration of the kinetics; in 4-day-old cultures there was a marked specific suppression by antiserum to Ia, and in 5-day-old cultures there was no significant inhibition by antiserum to Ia.

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Synaptic Organization of the Amine-Containing Interplexiform Cells of the Goldfish and Cebus Monkey Retinas

Abstract. *Fluorescence microscopy has revealed a new type of amine-containing retinal neuron, the interplexiform cell, that extends processes in both plexiform layers. After intravitreal injection of 5,6-dihydroxytryptamine in goldfish and* Cebus *monkey, the processes of these cells can be identified by electron microscopy. In goldfish, the processes are pre- and postsynaptic to amacrine cells in the inner plexiform layer and presynaptic to bipolar and horizontal cells in the outer plexiform layer. Interplexiform cells thus provide an intraretinal centrifugal pathway from inner to outer plexiform layers.*

It is generally accepted that certain catecholamines act as neurotransmitters in the vertebrate central nervous system (*1*). In the retina, the fluorescence method of Falck and Hillarp (*2*) has shown that there are a small number of dopamine-containing neurons whose perikarya are found most often among the amacrine cells (*3*). At least some of these neurons appear to be of a type not previously recognized since in some animals (teleost fish and New World monkeys) they are observed to extend processes widely in both the inner and outer plexiform layers (*4*). Cells with similar morphological characteristics have recently been observed in Golgi preparations of cat, Old World monkey, and dolphin eyes and have been called interplexiform cells (*5*). The synaptic connections made by these neurons are not known.

Catecholamine-containing neurons have efficient mechanisms for the uptake and concentration of both the natural transmitters and certain analogs (*6*). Some of these analogs alter the appearance of the fine structure of the neurons taking up the drug, enabling the processes and perikarya of the amine-containing cells to be readily recognized by means of electron microscopy (*7*). We have examined the effects of a number of these drugs on the retina and report here experiments in which one such drug, 5,6-dihydroxytryptamine (5,6-DHT), was injected into the vitreous humor of goldfish (*Carassius auratus*) and *Cebus* monkey (*Cebus capucinus*) eyes. The synapses made by and onto the amine-containing cells and their processes were studied, and the synaptic organization of the amine-containing interplexiform cells was deduced. This method, which permits identification

of the synapses of neurons according to their transmitter type, appears to have considerable potential for elucidating chemical-specific synaptic pathways.

From 4 to 12 hours after intravitreal injection of 5,6-DHT dissolved in 0.9 percent saline—5 to 20 nmole into goldfish or 100 to 400 nmole into monkey—eyes were excised, and pieces of the posterior part were fixed in 2 percent OsO_4 buffered in barbital acetate, or 2.5 percent glutaraldehyde buffered in sodium cacodylate followed by 2 percent OsO_4 buffered in barbital acetate (*8*, *9*). After dehydration, the tissue was embedded in Epon, cut into thin sections, and stained with uranyl acetate and lead citrate (*9*). Other pieces of the eye were freeze-dried and treated with formaldehyde gas for fluorescence microscopy of catecholamines and indoleamines according to the method of Falck and Hillarp (*2*). Appropriate controls were obtained by processing normal retinas in parallel with drug-treated retinas and, for the fluorescence microscopy, by omitting the formaldehyde treatment for some tissue pieces.

By fluorescence microscopy, the amine-containing neurons of the drug-treated retinas of both goldfish and *Cebus* showed the strong yellowish fluorescence typical of 5,6-DHT which contrasts with the greenish fluorescence

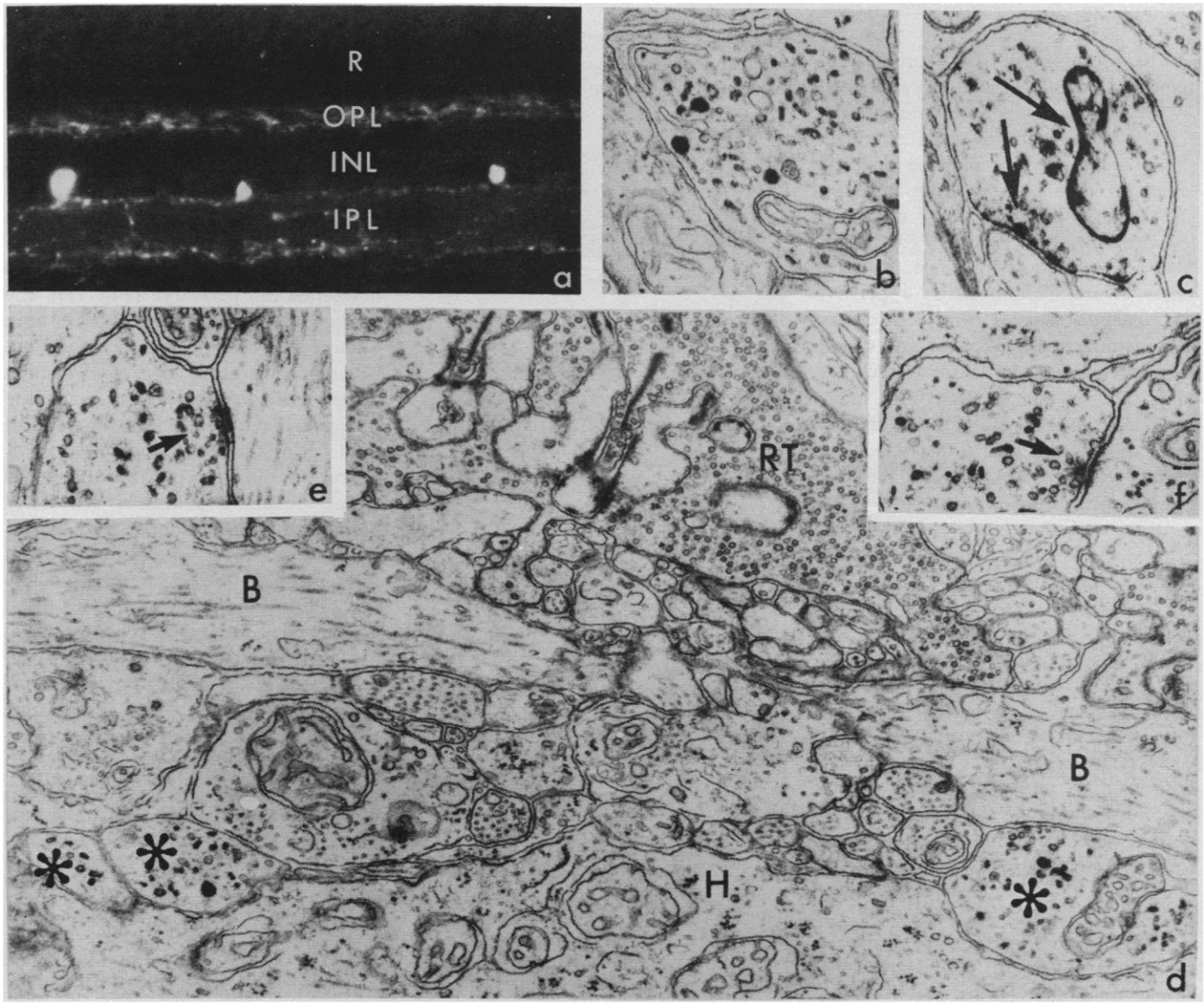

Fig. 1. Micrographs from eyes injected intravitreally with 5,6-dihydroxytryptamine creatine sulfate dissolved in 0.9 percent saline. All the electron micrographs (b to f) are from retinas fixed in buffered OsO_4. (a) Fluorescence micrograph of the amine-accumulating cells of the goldfish retina. The perikarya of these cells are found in the proximal half of the inner nuclear layer (*INL*); their processes extend in both the inner (*IPL*) and outer (*OPL*) plexiform layers; *R*, receptors (× 325). (b) An amine-containing process in the outer plexiform layer of goldfish showing pleomorphic synaptic vesicles, many of which demonstrate electron-opaque cores (× 33,000). (c) A process in the outer plexiform layer of the *Cebus* monkey showing early degenerative changes induced by the drug. Both the plasma and mitochondrial membranes (arrows) show focal accumulation of electron-opaque material (× 33,000). (d) A low-power micrograph of the outer plexiform layer of goldfish showing the typical position and distribution of amine-containing processes (stars); *H*, horizontal cell perikaryon; *B*, bipolar cell dendrites; *RT*, receptor terminal (× 23,000). (e) Amine-containing process in the goldfish outer plexiform layer forming a synapse (arrow) onto a bipolar cell dendrite. This section is serial to that shown in (d). The process making the synapse is the same as the one on the right side of (d) (× 39,000). (f) Amine-containing process making a synapse (arrow) onto a horizontal cell perikaryon in the outer plexiform layer of the goldfish (× 39,000).

of the adrenergic axons of the choroid. Normally, retinal amine-containing neurons also have this greenish color because of their dopamine content, but after injection the dopamine was largely replaced by the 5,6-DHT. There was usually a very slight, diffusely distributed yellowish fluorescence of all other retinal parts, especially the Müller cells, but this weak fluorescence did not interfere with the identification of the amine-accumulating neurons (Fig. 1a). There appeared to be no retinal neurons with green fluorescence remaining in the drug-treated retinas (*10*).

The processes of the neurons containing the 5,6-DHT can be recognized by electron microscopy by various criteria, which depend to some extent on the amount of drug injected and the length of time between drug injection and fixation of the retina. (i) With low drug dosages (5 nmole, goldfish or 100 nmole, *Cebus*) or short exposure times before fixation (2 to 4 hours), the synaptic vesicles of the processes demonstrate electron-opaque cores, and the vesicles frequently appear tubular (Fig. 1, b, d, and f). (ii) With intermediate dosages (10 or 200 nmole) and exposure times (4 to 8 hours), the plasma membrane of many of the processes, most often at the site of a synapse, and the mitochondrial membranes show focal accumulations of electron-opaque material (Fig. 1c). (iii) With high dosages (20 or 400 nmole) and long exposure times (7 to 12 hours), many processes become generally electron-opaque and show changes typical of degenerating and dying processes. In all specimens, processes showing any one of the above criteria may be seen. The synapses made by and onto the processes and perikarya of the amine-containing neurons can be most satisfactorily identified when low or intermediate doses and exposure times are used.

In both inner and outer plexiform layers, the synapses formed by the amine-containing neurons are all of the conventional type (*11*), with synaptic vesicles clustered along the presumed presynaptic membrane (Fig. 1, e and f). Both pre- and postsynaptic membranes usually show some increased electron density. The synaptic cleft is typically 200 to 300 Å in width and often is bridged by a few electron dense filaments. The synaptic organization of the amine-containing interplexiform cells was analyzed mainly in the goldfish, but enough attention was given to the *Cebus* retina to show that the amine-containing interplexiform cells in that retina have the same general synaptic organization. We note where differences between these species have been observed.

In the inner plexiform layer synapses made by the amine-containing neuronal processes are most often presynaptic to amacrine cell processes, but occasionally they contact bipolar cell terminals as well (*12*). Several times an amine-containing process was observed to form the first synapse of a serial synaptic arrangement (*11, 12*). In goldfish, the amine-containing processes were observed to be postsynaptic only to processes that made conventional synapses and that consequently are most likely to be amacrine cell processes (*8, 11*). Efferent fibers are known to make conventional synapses in the inner plexiform layer of some species (*13*). They are relatively rare, however, and we have no evidence that any of the synapses onto the amine-containing processes were from efferent fibers. In *Cebus*, there is also the suggestion of some bipolar input to the amine-containing neurons, but the evidence for such junctions is still equivocal. No convincing contacts of the amine-containing cells or their processes with ganglion cell perikarya and their dendrites have been seen despite a careful search.

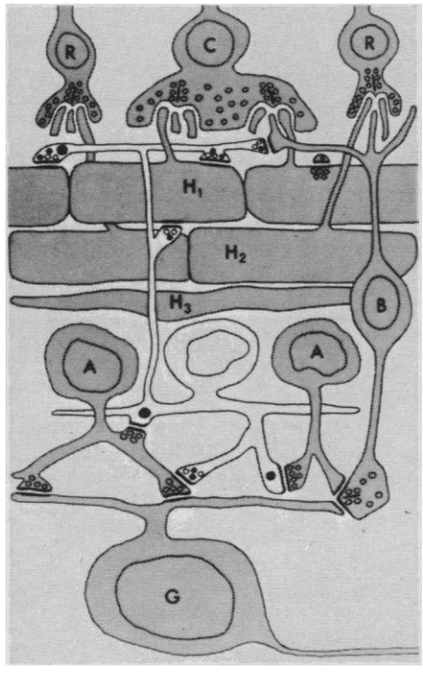

Fig. 2. A summary diagram of the synaptic connections of amine-containing interplexiform cells of the goldfish retina. The input to these neurons is in the inner plexiform layer via conventional synapses of amacrine cells (*A*). The interplexiform cell processes have been observed to make conventional synapses onto amacrine cell processes in the inner plexiform layer, but never do they contact the ganglion cells (*G*) or their dendrites. In the outer plexiform layer, the processes of the interplexiform cells make synapses on the perikarya of the external horizontal cells (H_1) and onto bipolar cell (*B*) dendrites. The interplexiform cell processes have never been observed as postsynaptic elements in the outer plexiform layer at either rod (*R*) or cone (*C*) receptor terminal synapses or at the occasional external horizontal cell synapse. No synapses have yet been seen between interplexiform cell processes and elements in the intermediate (H_2) or internal (H_3) horizontal cell layers.

In the outer plexiform layer, the amine-containing interplexiform cell processes were observed to make synaptic contacts on bipolar cell dendrites (Fig. 1e), on horizontal cell processes (*14*), and in goldfish on the external horizontal cell perikarya (Fig. 1f). The amine-containing processes have been observed close to cone pedicles, but never was there any sign of a junction between them. The amine-containing processes have never been observed at the level of the rod spherules.

Since in the outer plexiform layer the amine-containing processes are never postsynaptic, all the input into the interplexiform cells appears to be from the inner plexiform layer. Occasionally in goldfish, the horizontal cells have been observed to make synapses (*15*), but these contacts have all been on processes not showing evidence of amine accumulation. The postsynaptic processes at these junctions do often contain synaptic vesicles, but their origin is uncertain (*15*). Finally, fine, radially oriented, amine-containing processes (up to 0.5 μm) have been observed in the inner nuclear layer, apparently connecting the amine-containing processes of the inner plexiform layer with those of the outer plexiform layer.

Our observations in goldfish are summarized in Fig. 2. The amine-containing interplexiform cells have their perikarya among the amacrine cells. They send processes into both the inner and outer plexiform layers. All the input into these neurons in goldfish appears to be from amacrine cells. The amine-containing processes make synapses mainly onto amacrine cell processes in the inner plexiform layer and onto bipolar cell dendrites and hori-

zontal cells and their processes in the outer plexiform layer.

As can be ascertained from Fig. 2, the amine-containing interplexiform cells provide a centrifugal pathway from the inner to the outer plexiform layers of the retina. Such a retinal pathway has not been generally accepted in recent years (*16*). The abundance of processes of the amine-containing interplexiform cells both in the outer and inner plexiform layers of goldfish (Fig. 1a) suggests that these cells may be of more than marginal importance for retinal function. Golgi impregnations have shown that interplexiform cells are found in the retinas of several species (*5*), although they may not contain amines demonstrable by fluorescence microscopy. It must be inferred, therefore, that interplexiform cells are a general feature of vertebrate retinas and that they may employ different neurotransmitters.

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Mechanism of Axonal Transport: A Proposed Role for Calcium Ions

Abstract. *In vitro axonal transport of tritiated protein decreased 40 to 60 percent when neuronal cell bodies were incubated in calcium-free medium, but was not affected when only nerve trunks were exposed to calcium-free conditions. In addition, calcium-45 was transported along axons at a rate similar to that of rapidly transported tritiated protein. These data are interpreted to suggest that calcium ions are involved in the initiation of axonal transport and in the coupling of transported proteins to the transport system.*

Neurons are characterized by numerous cytoplasmic projections—axons and dendrites—that frequently extend for relatively large distances from their cell bodies. A major portion of the macromolecules and organelles required by distant regions of the neuron is supplied by a system known as axonal (or dendritic) transport, which actively moves these materials from their site of synthesis in the perikaryon to their sites of structural and metabolic utilization (*1, 2*). While the mechanisms of axonal transport are not clearly understood, broad outlines of the system have emerged: different materials are transported from as well as toward the cell body at markedly differing rates (*1, 2*); once macromolecules have been synthesized and "loaded" onto the transport system the cell body is no longer required for their continued transport, but the system is dependent on a local supply of metabolic energy (*3*); and the intra-axonal longitudinal networks of microtubules or neurofilaments may be involved in the transport system (*4*).

Models of axonal transport (*5*) have been based on the mechanochemical coupling system of muscle contraction, in which linkage of the proteins actin and myosin is mediated by calcium ions (*6*). Our previous studies (*7*) have indicated that calcium ions are required for axonal transport of proteins in vitro. Calcium-free conditions (that reduced endogenous calcium concentrations to 25 percent of normal) resulted in a decrease of approximately 60 percent in the amount of protein transported along axons. This effect was not due to depression of uptake of the precursor amino acid into the cell bodies nor to a selective action on synthesis or degradation of proteins. We now report that the calcium requirement appears localized in the cell body, and that $^{45}Ca^{2+}$ actively moves along axons at a rate similar to that of rapidly transported proteins. These observations more directly suggest a role for calcium in the mechanism of axonal transport.

Axonal transport was followed along the peripheral axons of dorsal root ganglion neurons from the bullfrog, *Rana catesbeiana*. The sciatic nerves, eighth and ninth spinal nerves, spinal roots, and dorsal root ganglia were dissected together with the spinal cord. In