at least ten prey items occur in each category for all comparisons.

13. The method of analyzing both stomach and intestinal contents probably resulted in fewer of certain soft-bodied insects, such as some Homopterans, larvae, and spiders, being recognized than actually occurred, but the effect on the total distributions would be slight.
14. A. S. Rand, unpublished.
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Acetylcholinesterase: Method for Demonstration in Amacrine Cells of Rabbit Retina

Abstract. *The activity of acetylcholinesterase in the inner plexiform layer of the rabbit retina was not affected detectably by prior section of the optic nerve. After the animals were treated with diisopropyl phosphorofluoridate, acetylcholinesterase reappeared in the somata of the amacrine cells and in certain cells of the ganglion cell layer before it reappeared in the inner plexiform fibers. This confirms the normal presence of acetylcholinesterase at the former site. The possible role of acetylcholine in intraretinal transmission is considered.*

The acetylcholinesterase (AChE) of the mammalian retina has been shown by the histochemical method with copper thiocholine (*1*) to be present chiefly in two distinct bands: one extending from the innermost region of the inner nuclear layer to the bordering portion of the inner plexiform layer and the other extending from the inner zone of the inner plexiform layer to the outer portion of the ganglion cell layer. Between these two heavily stained bands the intermediate zone of the inner plexiform layer shows much lighter staining (*2*). This pattern was interpreted originally as representing the amacrine cells and their processes. However, the relatively intense staining of the rich network of fibers renders the visualization of the amacrine cell bodies extremely difficult. On the basis of essentially the same pattern of staining, other investigators have reached the same conclusion (*3*, *4*), or have attributed staining additionally or alternatively to the processes of the bipolar cells (*5*, *6*), the ganglion cells (*4*, *5*), or centrifugal fibers from the optic nerve (*7*).

When a sufficient dose (4.0 mg/kg, given intravenously) of diisopropyl phosphorofluoridate (DFP) is given to produce virtually complete, irreversible inactivation of the AChE of the ciliary ganglion of the cat, the reappearance of newly synthesized enzyme occurs in the somata of the ganglion cells before it is detectable in the surrounding axons or dendrites (*8*). This is consistent with the proposal that neuronal AChE is synthesized in the granular endoplasmic reticulum, then transported to other parts of the neuron (*9*), although quantitative evidence of this sequence is lacking (*10*). We have taken advantage of this observation in staining selectively the AChE of the amacrine-cell bodies of the rabbit retina.

We examined retinas from normal rabbits, rabbits in which the right optic nerve had been sectioned 3 or 6 months previously, and rabbits treated with DFP (4.0 mg/kg, given intravenously, followed by 10.0 mg of atropine sulfate per kilogram, given intramuscularly) ½, 5, 7, and 10 hours before enucleation. The eyes were rapidly removed, either after the animals were killed by an overdose of urethane, or, in the case of animals treated with DFP, while they were under urethane anesthesia. Frozen sections of portions of the retinas were cut at 10 μ as soon as possible; the remaining portions were fixed for 2 hours in cold 10 percent formalin solution, buffered to *p*H 7.4 and made isotonic with sucrose, and were then stored in cold, buffered sucrose solution and sectioned the following day. Immediately after the tissues had been sectioned, we stained them for AChE using the copper thiocholine method (*1*), with incubation periods of 1 to 7 hours.

The architecture of the rabbit retina is shown in Fig. 1A. Figure 1B shows the distribution of AChE in the normal retina; staining is concentrated chiefly in two bands at the borders of the inner plexiform layer; most cells in the ganglion-cell layer are stained, and there is a suggestion of staining of occasional cells along the innermost aspect of the inner nuclear layer, where the amacrine cells are located. The only difference in the retinas from rabbits in which the optic nerve had been sectioned several months previously was the absence of neuronal elements, and consequently of AChE staining, in the ganglion-cell layer (Fig. 1C). This would indicate that centrifugal fibers from the optic nerve could account at most for only a minimal portion of the AChE of the inner plexiform layer. One-half hour after treatment with DFP, there was no evidence of staining for AChE anywhere in the retina, with incubation periods up to 7 hours. In retinas from eyes removed 5 or 7 hours after treatment with DFP, the fibers of the inner plexiform layer remained unstained, but there was distinct staining of the somata of the amacrine cells and of a considerable number of cells in the ganglion-cell layer (Fig. 1D).

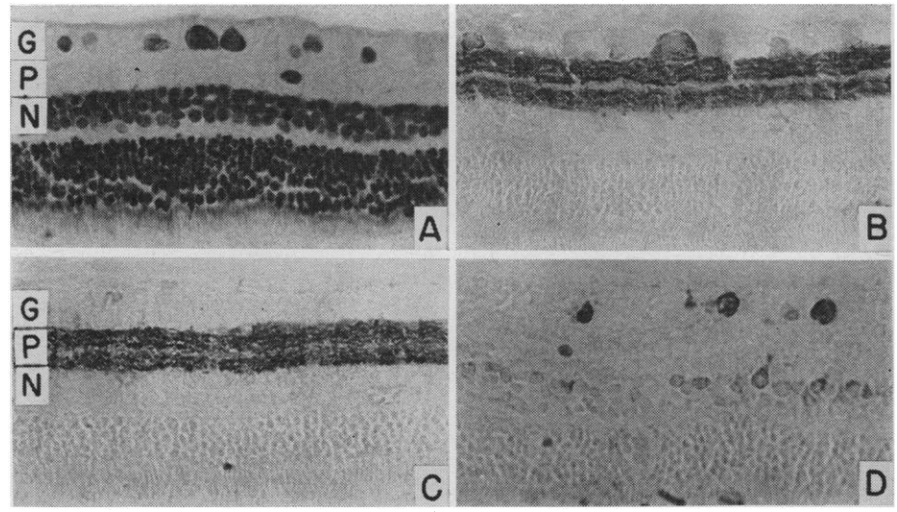

Fig. 1. (A) Normal rabbit retina; cresyl-violet stain. (B) Normal rabbit retina; section incubated 1 hour with acetylthiocholine for localization of AChE. (C) Rabbit retina 6 months after sectioning of optic nerve; stained for AChE as in B. (D) Normal rabbit retina from eye removed 7 hours after 4 mg of DFP per kilogram was given intravenously. The section was incubated 5 hours with acetylthiocholine for localization of AChE. *G*, Ganglion-cell layer; *P*, inner plexiform layer; *N*, inner nuclear layer (× 290).

At 10 hours, there was, in addition, light staining of nerve fibers in the inner plexiform layer. Thus, it appears likely that the amacrine cells are the major source of the AChE of the inner plexiform layer of the normal rabbit retina.

The appearance of AChE in the majority of the neurons of the ganglion-cell layer of the rabbit, in contrast to the pattern in the cat where extremely few ganglion cells contain detectable AChE (*2*), was unexpected, since the optic nerve in those species studied contains extremely low concentrations of AChE (*11*) and of choline acetylase (*12*).

The presence of AChE in the amacrine cells of the retina has an interesting analogy in the organ of Corti, where AChE is confined to the terminals of the olivo-cochlear bundle (*13*). The amacrine cells are involved in lateral interactions in the retina, which may, like the olivo-cochlear bundle (*14*), exert an inhibitory effect on the transmission of peripheral sensory impulses to the central nervous system (*15*). In spite of their AChE content, the olivo-cochlear fibers are apparently not cholinergic, since their action is not blocked by characteristic cholinergic blocking agents but is highly sensitive to blockade by strychnine (*16*). On the basis of the ability of strychnine to prevent the release of acetylcholine from cholinergic fibers, it has been proposed that acetylcholine is a mediator in the release of an unidentified, hyperpolarizing transmitter by the olivo-cochlear fibers (*17*). Similarly, the production by strychnine of rhythmic retinal discharges (*18*), and its blockade of post-excitatory inhibition in the retina (*19*) may be caused by interference with an acetylcholine-mediated release of an inhibitory transmitter by the amacrine cells.

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Crayfish Muscle Fiber: Ionic Requirements for Depolarizing Synaptic Electrogenesis

Abstract. *Presence of sodium in the bathing medium is not essential for the electrically excitable depolarizing electrogenesis of crayfish muscle fibers, production of action potentials being dependent on calcium. The depolarizing electrogenesis of the excitatory synaptic membrane component does require sodium, however, and this ion cannot be replaced by lithium as it can in spike electrogenesis of many cells. Ionophoretic applications of glutamate, which in the presence of sodium depolarize the cell by activating the excitatory synaptic membrane, are without effect in the absence of sodium. Not only is there no depolarization, but the membrane conductance also remains unchanged. Thus, in the absence of inward movement of sodium across the synaptic membrane there is also no outward movement of potassium. Accordingly, it seems that increased conductance for potassium is not an independent process in the synaptic membrane, whereas it is independent of sodium activation in spike electrogenesis. Chloride activation is independent, however; increase in conductance and the electrogenesis of the inhibitory synaptic component are not affected by the absence of sodium. Implications of these findings regarding the structure of differently excitable membrane components are discussed.*

Axons and muscle fibers can generate action potentials (spikes) after all the Na of the bathing medium has been replaced with Li (*1*). However, excitatory postsynaptic potentials (EPSP's) in cat superior cervical ganglion (*2*) and *Tenebrio* muscle fibers (*3*) are blocked when Li replaces all the Na. Furthermore, the generator potential evoked by light in photosensitive neurons of *Limulus* when Na is present is abolished by substitution of Li (*4*).

Generator potentials and EPSP's are both variants of depolarizing electrogenesis in electrically inexcitable membrane components (*5*). The above-mentioned findings suggested the possibility of further conclusions regarding the ionic channels that must be opened during the synaptic activity. Because of its theoretical importance, we investigated the matter in several parallel studies. Our work on crayfish neuromuscular junctions is the most extensive, but other findings will be mentioned.

Neuromuscular preparations of crayfish (*Procambarus* and *Orconectes*) respond to ionophoretic applications of glutamate with large depolarizations caused by activation of the excitatory synaptic membrane (*6*). They also exhibit inhibitory electrogenesis caused by a different ionic mechanism (*7*). Thus, the effects of Li could be tested on two bioelectric generators. In most experiments, recordings with intracellular microelectrodes were made from individual fibers of various muscles of the walking legs and the abdominal extensors. In some, however, single fibers were prepared as described by Girardier *et al.* (*8*). The results ob-