xenobiotics which, if they are carcinogenic, could act directly in the colon or, after reabsorption, in other tissues. The significance of the microbial production of carcinogens in the colon has been discussed (*13*).

The presence of this pathway for the metabolism of mercapturate-forming xenobiotics shows that a complete study of the metabolic fate of a compound should include a determination of the biliary secretion of its metabolites and study of its metabolism in germfree animals. The study in the germfree animal would define the role of the flora in the metabolism of the compound and would provide the ultimate model for determination of the effect that antibiotic feeding or therapy could have on the metabolic fate of xenobiotics. These studies could be of practical importance where antibiotics are added to animal feed and to patients on antibiotic therapy. Rats treated with antibiotics show measurable germfree characteristics for prolonged periods after antibiotic administration (*14*).

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Acetylcholine Synthesis by Displaced Amacrine Cells

Abstract. *The ganglion cell layer of the rabbit retina contains neurons that synthesize acetylcholine. To identify these neurons, the ganglion cells were labeled by retrograde transport of a fluorescent dye, and the acetylcholine-synthesizing cells of the same retinas were labeled by exposing the tissue to tritiated choline. Autoradiographs inspected by fluorescence microscopy showed that tritiated acetylcholine and the dye accumulated in different cells. Optic nerves of other animals were sectioned, causing degeneration of many neurons of the ganglion cell layer. This loss affected neither the retina's overall rate of acetylcholine synthesis nor the number of acetylcholine-containing cells in the ganglion cell layer. The acetylcholine-synthesizing neurons thus appear to be displaced amacrine cells.*

Acetylcholine is almost certainly a neurotransmitter used in mammalian retinas. The enzymes of its metabolism are present in substantial amounts; it is synthesized by the tissue and released in response to photic stimulation; and, when it is applied to the retina, many ganglion cells are excited. The retinal pathways in which acetylcholine is involved are selected ones. Ganglion cells of some functional classes are stimulated by acetylcholine and depressed by cholinergic antagonists, while ganglion cells with other functional characteristics are unaffected (*1*). The narrow role of acetylcholine in the retina's physiology is matched anatomically by its restriction to a small subset of retinal neurons. These cells are sparsely scattered along both margins of the inner plexiform layer, and they appear to have processes that are confined to two thin planes in the neuropil of that layer (*2*, *3*).

The acetylcholine-synthesizing cells of the inner nuclear layer have the soma size and position of amacrine cells. In the rabbit they make up about 5 percent of the cells that line the inner margin of the layer. The acetylcholine-synthesizing cells of the ganglion cell layer are approximately equal in absolute number to those of the inner nuclear layer, but in the ganglion cell layer they make up more than 20 percent of the total cell population. These neurons must be either ganglion cells or displaced amacrines—neurons that cannot be securely distinguished by ordinary histological methods (*4*).

The acetylcholine-synthesizing neurons of the ganglion cell layer, because they are so numerous, would at first appear to be ganglion cells. On the other hand, there are indications that the number of amacrine cells located in the ganglion cell layer may be larger than was once thought. We carried out two experiments designed to resolve the ambiguity. (i) We labeled the ganglion cells by retrograde transport of a marker dye and examined the localization of acetylcholine in the same retinas by autoradiography. (ii) We sectioned the optic nerve and, after ganglion cell degeneration, examined the retina's acetylcholine synthesis and its cellular localization.

In order to identify the ganglion cells, we needed a marker that is retrogradely transported and is compatible with the dry process used for the localization of acetylcholine. This precluded the use of horseradish peroxidase because the histochemical reaction used to visualize the enzyme would extract acetylcholine from the tissue. Instead, we used a mixture of fluorescent compounds introduced for pathway tracing by Kuypers *et al*. (*5*). These dyes retain fluorescence in the presence of the chemicals used in our autoradiographic method. In addition, they accumulate in the nucleus of the cell and provide a focal region that is easily visible in sections of 2 to 4 μm.

The dorsal thalamus of New Zealand rabbits was exposed by removal, through suction, of overlying brain structures. A slit was made across the total width of the lateral geniculate body, extending slightly ventral and medial to it. The cut thus severed optic tract fibers destined for tectal regions as well as many fibers in the geniculate. A piece of Gelfoam that had been soaked in a solution of 1.3 percent 4,6-diamidino-2-phenylindole (Serva), 1.3 percent primuline (Lachat), and 3 percent lysolecithin (Sigma) (*6*) was inserted into the slit. After 4 days the contralateral retina was removed from the eye and incubated in vitro (*7*). After a 10-minute preliminary incubation in control medium, the retina was transferred to medium containing 0.3 μM [*methyl*-^{3}H]choline (84 Ci/mmole; New England Nuclear), incubated for 15 minutes, and then rinsed for 10 minutes in control medium. High-voltage electrophoresis (*8*) of acid extracts from dye-labeled retinas, homogenized after such incubations, demonstrated that they contain approximately 9.5×10^{-14} mole of [^{3}H]acetylcholine per milligram (wet weight). After incubation, the retina was divided in half. As a check of the number of ganglion

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cells labeled, one half was floated onto a gelatin-coated slide and fixed in place for 1 hour with 4 percent Formalin. It was then dehydrated in an ethanol series and placed under a cover slip with Krystalon. Small sections of the other half were placed on aluminum foil and quick-frozen in propane at liquid nitrogen temperature. These samples were slowly freeze-dried, lightly fixed with osmium vapor, embedded in Spurr's resin, sectioned at 2 or 4 μm on a dry knife, and pressed onto previously dried NTB-2 emulsion for autoradiography (*3*). After 2 to 6 weeks, the autoradiographs were developed and the sections were placed under a cover slip with water or glycerol and inspected in a fluorescence microscope.

The dye was readily transported by retinal ganglion cells. When we compared the number of fluorescent cells in the tissue that had been mounted flat with the total cell densities measured in Nissl-stained material, we found that 60 to 75 percent of the cells in the ganglion cell layer showed bright fluorescence. Ganglion cells of all sizes appeared to be labeled. [In some of the flat-mounts very faint labeling of a second group of retinal cells could also be discerned, but this secondary labeling did not survive infiltration and embedding (*9*).] The bright fluorescence of the ganglion cells was visible, though attenuated, in the 2- to 4-μm sections that were autoradiographed. We inspected more than 200 such sections, from five retinas, and found that the two labels did not coexist; the cells that fluoresced formed a population separate from those that synthesize acetylcholine (Fig. 1).

In the second experiment, the right optic nerve was sectioned just behind its exit from the optic foramen. After 6 to 8 months, the right and left retinas were removed and incubated in the presence of [^{3}H]choline, under conditions similar to those described above. At the end of incubation, the retinas were divided into three strips by cuts oriented perpendicular to the visual streak. (The streak is a narrow region of high ganglion cell density that extends across much of the width of the retina. Since the region of retina that has an ipsilateral central projection is lost when the globe is hemisected during our isolation procedure, essentially comparable areas of retina are sampled this way.) The central piece, which contained the optic nerve head, was floated onto a gelatin-coated slide, fixed with Formalin, and stained with cresyl violet acetate in order to assess the amount of degeneration. One of the other pieces was homogenized and its radioactive choline metabolites were established by high-voltage electrophoresis. The third section was quick-frozen and processed for autoradiography.

Degeneration of cells in the ganglion cell layer was evaluated by counting cells in the flat-mounted, Nissl-stained tissue samples. Counts of the total cells present in the ganglion cell layer were made at 1-mm intervals above and below the visual streak along a 2-cm section. They revealed that, in the retinas whose optic nerves had been cut, the total number of cells present in the ganglion cell layer was reduced by 55 to 70 percent. Despite the loss of well over half of the cells of the ganglion cell layer, the rate of synthesis of [^{3}H]acetylcholine in the retinas whose optic nerves had been cut was identical to that seen in normal retinas. This finding was confirmed by autoradiographs: the number of acetylcholine-containing cells and the distribution of acetylcholine in the inner plexiform layer were identical in eyes that had been operated on and in controls (Fig. 2).

Both the transport and the degeneration experiments seem to indicate that the acetylcholine-synthesizing cells of the ganglion cell layer are not ganglion cells. This conclusion is supported by the failure of previous attempts to demonstrate cholinergic actions of mammalian optic nerve terminals (*10*). The implication is that the acetylcholine-synthesizing cells are displaced amacrines; this would mean that displaced amacrines form more than 20 percent of the cells of the ganglion cell layer. Other evidence has been accumulating to suggest that the number of displaced amacrines in mammalian retinas is, in fact, quite large. Perry's (*11*) studies in which the Golgi method was used in the rat have shown that many cells in the ganglion cell layer lack axons and have dendritic branching patterns similar to those of

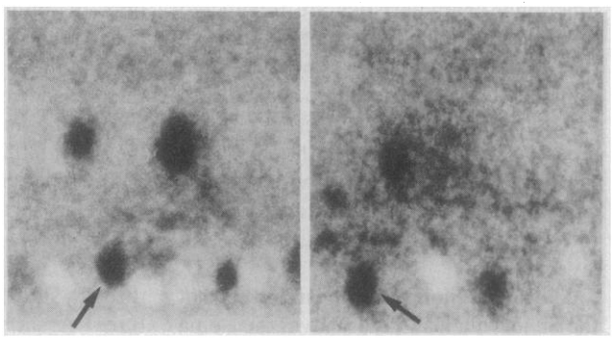

Fig. 1. Fluorescence micrographs of autoradiographed sections from two different retinas. The sections were labeled with [^{3}H]acetylcholine by exposure to [^{3}H]choline with fluorescent dye applied to the optic tract. The cells that accumulated the dye form a different population from those over which silver grains, representing [^{3}H]acetylcholine, accumulated (arrows) (*16*). Transmitted ultraviolet illumination (Leitz exciting filters UG-1 and BG-38, barrier filter K430). Focus was on the fluorescent cells; exposure was 3 minutes (magnification × 800).

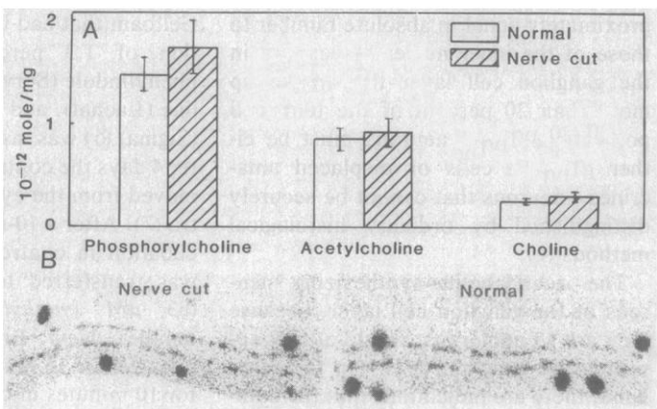

Fig. 2. (A) Radioactive phosphorylcholine, acetylcholine, and choline in retinas incubated in the presence of 0.8 μM [^{3}H]-choline. More than 95 percent of the total tissue radioactivity was in these compounds. Their amounts were identical in the normal retinas and in retinas in which 60 to 70 percent of the ganglion cells had degenerated consequent to optic nerve section. The slight increase in the synthesis of the choline metabolites in retinas with sectioned optic nerves may have been due to a slight decrease in their base of reference, caused by loss of the optic nerve fibers from the retinal surface; vertical sections showed, however, that the overall loss of volume in these retinas was very small. Data shown are the mean ± standard error of the mean of triplicate determinations for each of four normal retinas and four retinas whose optic nerves had been sectioned. (B) Autoradiographs of sections from the freeze-dried portions of a normal retina and one with a cut nerve. The distribution of radioactivity was identical in the normal and degenerated retinas (×370).

conventionally placed amacrine cells. Out of a total of 21 cells in the cat's ganglion cell layer injected with Procion dye, Nelson *et al.* (*12*) found three cells that had a combination of features suggesting that they were amacrines. Our studies confirm findings (*13*) that some of the cells of the ganglion cell layer fail to degenerate following optic nerve section. Vaney and Hughes (*14*) pointed out that fewer axons seem to be present in the rabbit's optic nerve than neurons in the ganglion cell layer. Studies in which the optic nerve is exposed to horseradish peroxidase, like the present experiment in which small transported molecules were used, show a population of neurons in the ganglion cell layer that do not become labeled by retrograde transport (*15*). Our experiments, in which acetylcholine serves as a cellular marker, allow an added conclusion: that at least some of the degeneration-resistant neurons are the same cells that cannot be backfilled. When all of this evidence is considered together, it seems quite certain that a substantial number of the cells of the ganglion cell layer are displaced amacrines and that many of these cells synthesize acetylcholine.

All of the cholinergic neurons of the rabbit retina would thus appear to be amacrine cells. We are impressed by the geometry with which the cholinergic cells are positioned. Essentially equal numbers are located on either side of the inner plexiform layer, and their processes arborize at roughly equal distances from the midplane of the plexiform layer. A prime feature of this symmetry is that the cell bodies of half of the amacrine cells involved have been "displaced" to the proximal side of the plexiform layer. It would be interesting to know the functional or developmental reasons for this arrangement.

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17. We thank C. Liu and M. Whalen for technical assistance. Supported by NIH grants EY 01075 and HLO 6664, by a Research Career Development Award to R.H.M., and by a King Trust award to J.W.M.

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Pollen Tube Growth Rates in *Zea mays*: Implications for Genetic Improvement of Crops

Abstract. *Speed of pollen tube growth is positively correlated with the quality of the resultant sporophytic generation. Therefore, gametophytic competition may be an important adaptive mechanism. Furthermore, pollen tube growth rates may be used to predict the quality of F_1 crosses in crop species.*

Plant breeders evaluate inbred lines according to how they perform in different hybrid combinations. The mean performance of each line (the mean performance of the F_1's in crosses with other lines) is indicative of its general combining ability (GCA). The performance of a particular cross may deviate from the average of the two parental lines; this deviation is the specific combining ability (SCA) of that cross (*1*). We consider that pollen tubes within a style, like parental genomes in an F_1 hybrid, represent an interacting combination that can be analyzed in terms of SCA's and GCA's. Pollen GCA, for example, is the mean growth rate of one pollen tube type in several style types, and stylar GCA is the mean growth rate of pollen tubes in that style.

We tested for correlations between pollen GCA and classical GCA and between pollen-style SCA and classical SCA. Positive correlations would imply useful applications, such as a rapid test for combining ability, and would support the hypothesis that pollen tube competition may be of importance in adaptive processes (*2*).

Eleven lines of corn (*Zea mays* L. Poaceae) were used in this study. All are standard inbred lines employed in the production of commercial hybrid corn. Pollen tube growth rates of pollen from five of these lines (WF9, C123, M14, B37, and H3025) were determined in the styles of five of the other lines (W23, 33-16, Sil-91, B73, and B14). This was accomplished by comparing the pollen tube growth rate from each pollen source with that of a standard inbred pollen source, W22. Fertilizations by the standard resulted in red aleurone, while those by the other five pollen sources produced colorless aleurone. Because aleurone color is expressed in the kernel, it allows immediate determination of which kernels resulted from fertilization by tester (W22) gametes and which did not.

Determination of the relative growth rates of pollen tubes is based on the observation (*3*) that, if a mixture of two pollen types is applied to stigmas, and if these types, tagged by genetic markers,

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