press the sensitivity of one mechanism so as to reveal others (*14*). The logic of the method lies in choosing a wavelength near the peak sensitivity of the mechanism one wishes to depress which is also far off the peak of the mechanism one wishes to express. Chromatic adaptation to light of a wavelength of 550 nm gave the next curve (closed circles) showing a peak near 450 nm. Adaptation to 600 nm light gave a spectral sensitivity with a peak near 520 nm (triangles), and adaptation to 400 nm light gave a spectral sensitivity peaking near 560 nm (squares). The smooth curves are modified Dartnall nomograms for peaks at wavelengths of 450, 520, and 560 nm (*15*). These curves provide evidence that when the rhodopsin rod mechanism is severely damaged by light other mechanisms mediated by pigments normally associated with cones survive in the albino rat (*16*).

While this work was in progress, LaVail (*17*) provided anatomical evidence that cones survive rods after long exposure to constant light in the Fischer albino rat. The percentage of cones detected by light and electron microscopy increased from 1.5 percent of all photoreceptors in the rat to about 60 percent with very long exposure. These findings are in conflict with earlier reports (*4*) that purport to find no photoreceptors surviving after exposure to lights of comparable intensity and shorter durations. The evidence presented here, showing greater initial damage to the rhodopsin rod system after much shorter exposure times, is consistent with LaVail's anatomical results and constitutes evidence linking anatomically described cones to a function distinct from that of rods.

In summary, two lines of physiological evidence show that the rods of the albino rat are initially more susceptible to the damaging effects of constant bright light than are the cones. In addition, the spectral sensitivities of the surviving mechanisms are consistent with single pigment Dartnall nomograms with peak sensitivities at wavelengths of 450, 520, and 560 nm.

CAROL M. CICERONE
Vision Research Laboratory,
Kresge Medical Research Building,
University of Michigan,
Ann Arbor 48109

References and Notes

1. W. K. Noell, V. S. Walker, B. S. Kang, S. Berman, *Invest. Ophthalmol.* **5**, 450 (1966).
2. R. A. Gorn and T. Kuwabara, *Arch. Ophthalmol.* **77**, 115 (1967); W. K. Noell and R. Albrecht, *Science* **172**, 76 (1971).
3. T. Kuwabara and R. A. Gorn, *Arch. Ophthalmol.* **79**, 69 (1968).
4. M. H. Bennett, R. F. Dyer, J. D. Dunn, *Exp. Neurol.* **34**, 434 (1972); K. V. Anderson and W. K. O'Steen, *ibid.*, p. 446; *Physiol Behav.* **12**, 633 (1974).
5. G. Walls, *J. Comp. Psychol.* **18**, 363 (1934).
6. R. Granit, *Acta Physiol. Scand.* **2**, 93 (1941); E. Dodt and K. Echte, *J. Neurophysiol.* **24**, 427 (1961); J. E. Dowling, *Science* **157**, 584 (1967).
7. D. G. Green, *Science* **174**, 598 (1971).
8. Low-intensity adapting lights will give a single, branched dark-adaptation curve. Infrequent data points may also spuriously give a single, smoothly descending curve. Dodt and Echte (*6*) have previously identified the change in spectral sensitivity during dark adaptation following intense light adaptation.
9. Repeated recordings did not by themselves cause damage. When control animals were recorded multiple times without intervening light exposure, there was no decrement of the response. After each experiment, the eye was ophthalmoscopically examined and flushed with mammalian Ringer's. Prophylactic doses of tetracycline were routinely administered in the drinking water to reduce infection.
10. Animals were dark-adapted 24 hours or more, then anesthetized with intraperitoneal injections of sodium pentobarbital (5 mg per 100 g of body weight) initially. To maintain anesthesia for periods longer than an hour, additional doses of 2.5 mg at a time were administered. The animal was mounted in an upright position by earbars. An electric blanket controlled by a temperature probe maintained the animal's temperature at 37°C. Under dim red illumination, the eyelids of the left eye were gently pulled back by sutures that were secured so the eyeball was exposed. The pupil was dilated fully with atropine. A cotton wick electrode with saline solution leading to a chlorided silver wire was placed at the edge of the cornea. An indifferent electrode was placed in a small cut in the cheek. The recorded signals were amplified by a Tektronix 122 preamplifier with a 0.2-second time constant and were displayed on a storage oscilloscope and photographed when desired by an oscilloscope camera.
11. R. A. Cone, *J. Gen. Physiol.* **46**, 1267 (1963); J. E. Dowling, *ibid.*, p. 1287.
12. This estimate of pigment bleached was made by using Cone's (*11*) calibration. The intensity of my bleaching source was converted to Cone's absolute intensity units by using his own procedure of sliding the b-wave amplitude curves until they matched his.
13. The cone plateau is that portion of the first branch where the threshold is stable.
14. W. S. Stiles, *Proc. Roy. Soc. London Ser. B* **127**, 64 (1939).
15. T. G. Ebrey and B. Honig, *Vision Res.*, in press.
16. The mechanism best fit by a nomogram peaking at 520 nm may possibly reflect cones containing rhodopsin or the residual rhodopsin rod mechanism.
17. M. M. LaVail, *Invest. Ophthalmol.* **15**, 64 (1976).
18. Supported by postdoctoral fellowship EY 02161-01 from the National Eye Institute and PHS grant EY 00379. I thank Daniel G. Green in whose laboratory this work was conducted.

4 June 1976; revised 4 August 1976

Centrifugal Fibers to the Eye in a Nonavian Vertebrate: Source Revealed by Horseradish Peroxidase Studies

Abstract. *A source of efferent fibers to the eye of snakes of the genus* Thamnophis *has been identified by the use of the retrograde transport of horseradish peroxidase. Cell bodies of the contralateral nucleus of the ventral supraoptic decussation accumulate horseradish peroxidase after intraocular but not intraorbital injections. Intraocular injections also result in anterograde transport of horseradish peroxidase to retinofugal axon terminals. Intraorbital injections result in accumulation of horseradish peroxidase in the cell bodies of the cranial nerve nuclei of extraocular muscles.*

The existence of efferent fibers to the avian retina has been known since Cajal (*1*) first described these fibers in the retina. Efferent fibers in the retina have been described for several other vertebrate classes as well (*2*). However, it is only in the avian species that the cells of origin of the efferent fibers, the cells of the isthmo-optic nucleus, have been clearly demonstrated by means of anatomical techniques (*3*, *4*). That the source of the efferents to the retina has not been described in any other vertebrate class is surprising because the technology to identify the cells of origin of axon terminals has, over the past few years, become routine in many laboratories (*5*, *6*). In this report we describe a source of efferents to the eye of garter snakes as revealed by the use of the retrograde transport of horseradish peroxidase in neurons.

Fifteen garter snakes (ten *Thamnophis sirtalis sirtalis*, and five *Thamnophis radix*) weighing from 14 g to 92 g were anesthetized with a short-acting barbiturate (*7*). Type VI horseradish peroxidase (HRP) (0.1 to 8.0 μl of a 50 percent solution in saline) was injected unilaterally into the vitreous body or extraocular space through glass micropipettes with tip diameters between 30 and 40 μm. The micropipettes were inserted from the corneoscleral junction at the periphery of the eyeball into the vitreous body behind the lens for intraocular injections and into the orbital cavity for extraocular injections. Slow delivery of HRP through a microdrive system took from 5 to 20 minutes depending on the volume to be delivered. Animals were killed 24 hours to 5 days after injection; however, those killed after 2 to 3 days yielded the best results. The snakes were killed by intracardiac perfusion with cold solutions (4°C) of 0.18*M* sodium cacodylate buffer (*p*H 7.2) followed by fixative 1 (1 percent paraformaldehyde, 1 percent glutaraldehyde, in 0.1*M* sodium cacodylate buffer), and then fixative 2 (2 percent paraformaldehyde and 1 percent glutaraldehyde in 0.1*M* sodium cacodylate buffer). Brains were removed, stored, and processed according to the procedures described by Colman *et al.* (*8*) in which an unbuffered incubation medium containing *o*-dianisidine (3,3′-dimethoxy-

benzidine dihydrochloride; Sigma), sodium nitroprusside, and hydrogen peroxide was used for the HRP reaction. Additional specimens were processed by the method described by Graham and Karnovsky (*5*, *6*) with an incubation medium containing phosphate buffer, 3,3′-diaminobenzidine tetrahydrochloride, and hydrogen peroxide. In several specimens two injections into the same eye were made, the first preceding the second by 24 hours. For each brain an unincubated series of sections was stained for Nissl substance with cresylviolet.

Horseradish peroxidase was transported to the brain by both anterograde and retrograde intraaxonal transport. The location of the HRP that reached the brain by retrograde transport was easily identified because it was totally confined to the soma and proximal portions of the dendrites of the nucleus of the ventral supraoptic decussation (*9*) and cranial nerve nuclei. On the other hand, the appearance of those regions to which the HRP was transported in an anterograde direction was distinctly different, with very small granules confined to the neuropil in which the retinofugal fibers terminate. Our observations on cellular labeling are described first.

In specimens treated with *o*-dianisidine (*8*) the reaction in the nucleus of the ventral supraoptic decussation contralateral to the injected eye was very clear and brilliant and could be observed through the light microscope under the lowest magnification, ×25 (Fig. 1, A and B). A very few cells of the ventral supraoptic decussation nucleus ipsilateral to the injected eye also accumulated HRP. Sections treated with diaminobenzidine (*5*, *6*) also revealed the nucleus of the ventral supraoptic decussation as a source of efferents to the eye. Using the latter technique we were able to see the accumulation of brownish granules in the cells of the ventral supraoptic decussation nucleus; however, these cells were not as readily visible as in sections treated with *o*-dianisidine, and dark-field microscopy greatly increased their visibility (Fig. 1C). Specimens in which the eye was injected twice reacted more brilliantly than specimens with single injections.

The nucleus of the ventral supraoptic decussation has been described previously in a study of the visual system of *Thamnophis* (*9*). This nucleus is located medial to the ventral portion of the optic tract in the ventrolateral posterior diencephalon and medial to the fibers of the ventral supraoptic decussation in the lateral, rostral mesencephalon. The nucleus extends for a considerable rostrocaudal distance. In its caudal extent it is interstitial to the medial fibers of the basal optic tract. The nucleus is composed of two parts: a rostral portion composed of large, elongated multipolar neurons (19 to 24 μm in their longitudinal axis, 10 to 16 μm in their short axis) which stain deeply with Nissl stain and a caudal portion which is somewhat smaller in rostrocaudal extent and contains neurons with somewhat rounder perikarya. The rostral neurons contain a dense accumulation of HRP after eye injection while the caudal neurons do not accumulate HRP as densely as the more rostral cells of the nucleus.

Cell bodies filled with HRP contain a dense accumulation of green granules (when treated with *o*-dianisidine) or brown granules (when treated with diaminobenzidine). These granules are located predominantly in the perinuclear region but are occasionally observed as well in the proximal portions of dendrites. In view of the HRP injection site, it is clear that the cells of the nucleus of the ventral supraoptic decussation must receive their HRP by retrograde transport. These neurons are very similar in appearance to the perikarya of the third, fourth, and sixth cranial nerve nuclei (*10*) which become labeled with HRP after extraocular injection (Fig. 2). The fact that the nucleus of the ventral supraoptic decussation is only labeled after intraocular injection and never labeled when HRP is injected intraorbitally indicates that this nucleus is not the source of an efferent system to some extraocular muscle or gland of the orbit.

In a study in which the retinofugal pathways in *Thamnophis* were degenerated experimentally, the location of terminals of retinal ganglion cell axons was described. Treatment of our sections with *o*-dianisidine made these retinofugal fibers readily visible (Figs. 3 and 4). Sections treated with diaminobenzidine showed faintly stained retinofugal fibers. The retinofugal pathway revealed by these HRP techniques conforms precisely to that described previously in *Thamnophis* (*9*), with terminal fields located predominantly in the contralateral lateral geniculate complex, nucleus lentiformis mesencephali, nucleus posterodorsalis (area pretectalis), nucleus geniculatus pretectalis, basal optic nucleus, and superficial layers of the tectum. A sparse ipsilateral projection to the lateral geniculate complex and nucleus posterodorsalis has been observed in material stained for degenerating axons and ter-

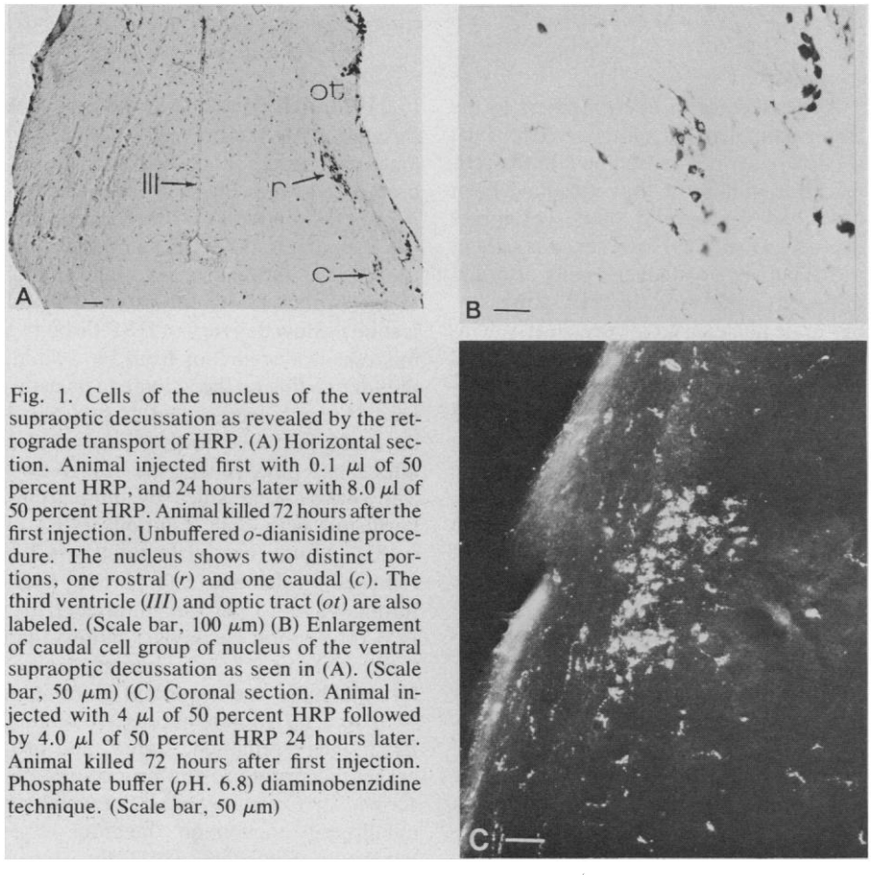

Fig. 1. Cells of the nucleus of the ventral supraoptic decussation as revealed by the retrograde transport of HRP. (A) Horizontal section. Animal injected first with 0.1 μl of 50 percent HRP, and 24 hours later with 8.0 μl of 50 percent HRP. Animal killed 72 hours after the first injection. Unbuffered *o*-dianisidine procedure. The nucleus shows two distinct portions, one rostral (*r*) and one caudal (*c*). The third ventricle (*III*) and optic tract (*ot*) are also labeled. (Scale bar, 100 μm) (B) Enlargement of caudal cell group of nucleus of the ventral supraoptic decussation as seen in (A). (Scale bar, 50 μm) (C) Coronal section. Animal injected with 4 μl of 50 percent HRP followed by 4.0 μl of 50 percent HRP 24 hours later. Animal killed 72 hours after first injection. Phosphate buffer (*p*H. 6.8) diaminobenzidine technique. (Scale bar, 50 μm)

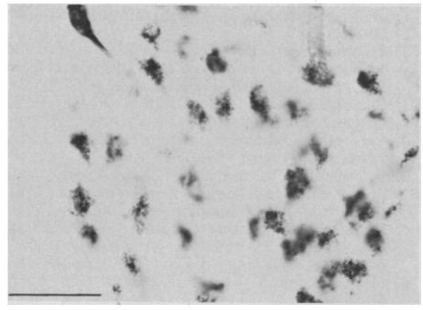

Fig. 2. Trochlear nerve nucleus on side contralateral to extraocular injection revealed by the retrograde transport of HRP. Horizontal section. Animal injected with 2 μl of 50 percent HRP and killed after 30 hours. Unbuffered *o*-dianisidine technique. (Scale bar, 50 μm)

minals but is just visible (Fig. 4) when HRP histochemical techniques are used.

One advantage of using the *o*-dianisidine incubation technique for viewing the retinofugal system is that terminal areas are distinctly visible and their presence need not be inferred from the appearance of the pattern of degenerating fibers. The appearance of the HRP reaction product in the retinofugal system corresponds precisely to the description of Scalia and Colman (*11*) and Colman *et al.* (*8*). Most optic tract fibers contain granules of reaction product while a few fibers, presumably injured during injection procedure, are homogeneously filled. The homogeneous coloration of axons probably results from intraaxonal diffusion of the enzyme, while the granules of reaction product are localized within membrane-bound organelles and are associated with rapid intraaxonal transport of the glycoprotein (*4, 8, 12*). The terminal fields of the retinal axons (Figs. 3 and 4) are characterized by an intricate plexus of finely "stained" fibers and very fine, but densely distributed granules.

Broadwell and Brightman (*13*) reported that large doses (30 to 50 mg) of HRP injected into the tail vein of mice result in selective acquisition of HRP in several nuclear groups in the hypothalamus. These hypothalamic neurons are presumed to acquire HRP from the circumventricular organs they innervate. We suggest that in the present study bloodborne HRP was not a confounding factor because (i) only the nucleus of the ventral supraoptic decussation selectively accumulated the HRP after intraocular injections; (ii) this nucleus accumulated HRP almost exclusively on the side contralateral to the intraocular injection with only a rare cell labeled on the side ipsilateral to the eye injection; and (iii) this nucleus does not concentrate HRP after extraocular injections. The concentration of HRP in the ventral supraoptic decussation nucleus is in all probability not the result of extracellular diffusion along the optic tract fibers, because if this were the case one would expect to see other neurons adjacent to the optic tract concentrating HRP. In fact the neurons of the supraoptic and suprachiasmatic nuclei which are both closer to the chiasma than the ventral supraoptic decussation nucleus do not concentrate HRP.

Repérant (*14*) reported an absence of orthograde movement of HRP in the optic nerve of the snake (*Vipera aspis*). It is unlikely that this contradictory result is a product of species differences (*15*); it is more likely to result from technique differences (*6, 8*). Orthograde transport is readily demonstrated by the use of the *o*-dianisidine incubation procedure and the resulting reaction product is considerably more visible under the light microscope than is the reaction product resulting from incubation with diaminobenzidine.

Miles (*16*) and Pearlman and Hughes (*17*) have recently suggested that the function of the efferent fibers to the avian retina is to alter the receptive field properties of retinal ganglion cells and that this effect is mediated through the termination of the efferent fibers on amacrine cells. However, it is too soon to speculate on the functioning of the efferent fibers of snakes. The major contribution of the present study is to extend the number of vertebrate classes in which a source of efferents to the retina has been identified.

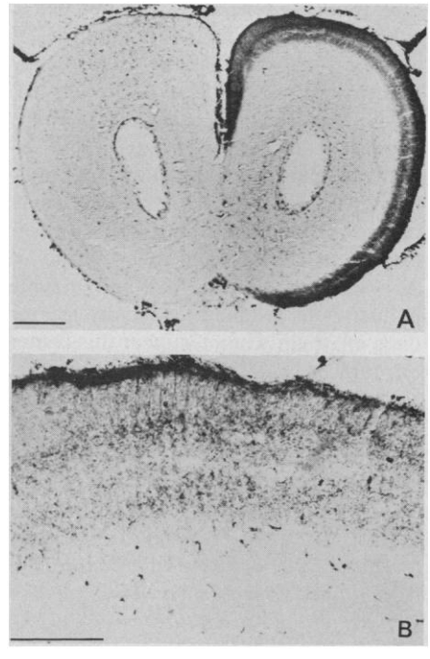

Fig. 3. Optic tectum contralateral to eye injection as revealed by anterograde labeling with HRP. Injection and time of death as in Fig. 1. Horizontal section; top-rostral. Unbuffered *o*-dianisidine procedure. The clear band in the tectum probably corresponds to a region occupied by axons, but not terminals of retinal ganglion cells. [Scale bar, 500 μm in (A), 50 μm in (B)]

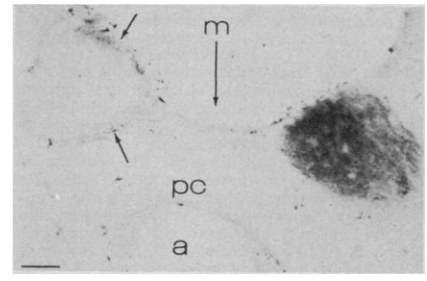

Fig. 4. Nucleus posterodorsalis (area pretectalis) as revealed by anterograde labeling with HRP. Coronal section; right side, contralateral to eye injection. Animal injected with 6 to 8 μl of 50 percent HRP and killed after 48 hours. Unbuffered *o*-dianisidine technique. The left nucleus posterodorsalis along the dorsal and medial edges contains HRP. The midline (*m*), posterior commissure (*pc*), and cerebral aqueduct (*a*) are indicated. (Scale bar, 50 μm)

MIMI HALPERN, RUU TONG WANG
DAVID R. COLMAN
Department of Anatomy, Downstate Medical Center, Brooklyn, New York

References and Notes

1. S. R. Cajal, *Anat. Anz.* **4**, 111 (1889).
2. R. N. L. Brooke, J. C. Downer, T.P.S. Powell, *Nature (London)* **207**, 1365 (1965); S. R. Cajal, *Die Retina der Wirbelthiere* (Wiesbaden, Bergmann, 1894); A. S. Dogiel, *Arch. Mikrosk. Anat. Entwicklungsmech.* **44**, 622 (1895); F. M. Honrubia and J. H. Elliott, *Arch. Ophthalmol.* **80**, 98 (1968); S. Polyak, *The Vertebrate Visual System* (Univ. of Chicago Press, Chicago, 1957); P. W. Witkovsky, *J. Comp. Neurol.* **142**, 205 (1971).
3. See W. M. Cowan [*Br. Med. Bull.* **26**, 112 (1970)] for a history of the problem and a review of the literature. See also W. M. Cowan, L. Adamson, T. P. S. Powell, *J. Anat.* **95**, 545 (1961); J. H. LaVail and M. M. LaVail, *Science* **176**, 1416 (1972).
4. J. H. LaVail and M. M. LaVail, *J. Comp. Neurol.* **157**, 303 (1974). K. Rubinson [*Brain Behav. Evol.* **1**, 529 (1968)] observed degenerating fibers in the optic nerves of *Rana pipiens* in which tectal lesions had been made, but these fibers were not traced to the retina so their terminus is unknown. Vanegas *et al.* [H. Vanegas, J. Amat, E. Essayag-Millan, *Brain Res.* **54**, 301 (1973)] present electrophysiological evidence of tectal efferents to the eye of the perciform teleost *Eugerres plumieri*.
5. R. C. Graham, Jr., and M. J. Karnovsky, *J. Histochem. Cytochem.* **14**, 291 (1966).
6. J. H. LaVail, in *The Use of Axonal Transport for Studies of Neuronal Connectivity*, W. M. Cowan and M. Cuenod, Eds. (Elsevier, Amsterdam, 1975).
7. We used Brevital Sodium (methohexital sodium, Eli Lilly).
8. D. R. Colman, F. Scalia, E. Cabrales, *Brain Res.* **102**, 156 (1976).
9. M. Halpern and N. Frumin, *J. Morphol.* **141**, 359 (1973). The nucleus of the ventral supraoptic decussation in snakes appears to be similar to the nucleus geniculatus medialis pars ventralis as described by J. W. Papez [*J. Comp. Neurol.* **61**, 433 (1935); *ibid.* **64**, 41 (1935)] in the turtle

Chelone midas and the snakes *Thamnophis sirtalis* and *Natrix sipedon*. Papez considered this nucleus to be similar to nucleus Z in the alligator as described by C. C. Huber and E. C. Crosby [*J. Comp. Neurol.* **40**, 97 (1926)].

10. The third and fourth cranial nerve nuclei are located below the floor of the cerebral aqueduct ventral to the central gray in the caudal midbrain. The third nerve nucleus is split by the fibers of the medial longitudinal fasciculus into a dorsal and ventral division. The sixth cranial nerve nucleus is located just below the central gray at the level of attachment of the fifth cranial nerve to the brainstem. The neurons of the third and fourth cranial nerve nuclei accumulate a much greater amount of HRP than the neurons of the sixth cranial nerve nucleus, as judged from the intensity of the reaction in the third and fourth nerve nuclei and the relative paucity of reaction product in the sixth nerve neucleus.
11. F. Scalia and D. R. Colman, *Brain Res.* **79**, 496 (1974).
12. H. A. Hansson, *Exp. Eye Res.* **16**, 377 (1973).
13. R. D. Broadwell and M. W. Brightman, *J. Comp. Neurol.* **166**, 257 (1976).
14. J. Repérant, *Brain Res.* **85**, 307 (1975).
15. We have repeated these experiments with four reptiles (*Cordylus cordylus*, common cape girdled lizard; *Gerrhonotus coeruleus coeruleus*, San Francisco alligator lizard; *Gerrhosaurus validus*, Smith's plated rock lizard; and *Eumeces laticeps*, broad-head skink). Orthograde transport of HRP to the terminal regions of the retinofugal axons occurred in all specimens. In addition, a nucleus was identified which was labeled by retrograde transport of HRP following intraocular injection in these animals. The location of the nucleus varies in the four species, being located, for example, in the diencephalon adjacent to the optic tract in *Cordylus cordylus* and located in the caudal mesencephalic tegmentum in *Gerrhonotus coeruleus coeruleus*.
16. F. A. Miles, *Brain Res.* **48**, 115 and 131 (1972).
17. A. L. Pearlman and C. P. Hughes, *J. Comp. Neurol.* **166**, 123 (1976).
18. We thank D. H. Ford and K. Koizumi for their critical review of the manuscript. Supported by NIH grant NS12152.

23 July 1976

Aspergillus oryzae (NRRL Strain 1988): A Clarification

El-Hag and Morse (*1*) chose to ignore two opinions regarding the identity of their aflatoxin-producing "variant of *Aspergillus oryzae* NRRL 1988," and reported incorrectly the two opinions they acknowledged (*2*). The culture, as eventually sent to the Food and Drug Administration by Morse and to NRRC by El-Hag, proved to be a strain of *Aspergillus parasiticus* contaminated with a yeast. The subculture sent to me also was heavily infested with culture mites. This information was made available to El-Hag well in advance of publication.

We had supplied El-Hag with two freeze-dried preparations of *A. oryzae* NRRL 1988 (lyophilized on two different dates) prior to his first report of aflatoxin production on millet by this strain (*3*). Immediately following this report, I opened one preparation from each of the two dates and compared the subculture with the stock culture in our collection. All three were identical and were *A. oryzae* as it is known to me. Subcultures from each source were tested for production of aflatoxin on wheat, corn, millet, and rice. Later, when El-Hag stated that the aflatoxin production on millet was an "artifact" (*4*) and changed the substrate, the stock culture was also tested on cowpeas and soybeans. No aflatoxin was produced on any of these substrates by any of the three subcultures. El-Hag was informed of these results.

I believe it most unlikely that the culture distributed by El-Hag arose as a variant of NRRL 1988, a strain of *A. oryzae* that has remained unchanged in our collection through 30 years of maintenance by periodic transfer. A more likely explanation, in my opinion, would be that *A. oryzae* NRRL 1988 has been replaced by their *A. parasiticus* through mite infestation. This opinion of its origin was transmitted to El-Hag. [A complete summary of our contacts with their investigations will be sent to any interested person (or persons) on request.]

The variability in aflatoxin production reported by El-Hag and Morse, and attributed by them to instability of the culture, probably reflects varying degrees of contamination of their fermentations. I am not surprised that the culture they returned to me produces aflatoxins B_1, B_2, G_1, and G_2. *Aspergillus parasiticus* is known for this ability.

In my opinion, manufacturers of industrial enzymes from *A. oryzae* and users of their products need not be disturbed. Strain NRRL 1988 and other strains of *A. oryzae* used in the food and enzyme industries have failed repeatedly to produce aflatoxin when tested at this center on corn, wheat, rice, millet, cowpeas, soybeans, and a liquid medium. Our results corroborate the extensive Japanese work (*5–8*) demonstrating that none of hundreds of industrial strains of *A. oryzae* produce this mycotoxin.

DOROTHY I. FENNELL
Northern Regional Research Center, Agricultural Research Service, Department of Agriculture, Peoria, Illinois 61604

References

1. N. El-Hag and R. E. Morse, *Science* **192**, 1345 (1976).
2. Personal communication from J. C. Ayres and T. Hamsa to D. I. Fennell (July 1976), and from J. L. Peterson to D. I. Fennell (July 1976).
3. Personal communication from N. El-Hag to T. G. Pridham (August 1974).
4. Personal communication from N. El-Hag to D. I. Fennell (August 1975).
5. S. Matsuura, M. Manabe, T. Sato, *Proceedings of the First U.S.–Japan Conference on Toxic Microorganisms* (unnumbered publication, U.S. Department of the Interior, and U.S.–Japan Natural Resources Panels on Toxic Microorganisms, Washington, D.C., 1970), pp. 48–55.
6. H. Murakami, S. Takase, T. Ishii, *J. Gen. Appl. Microbiol.* **13**, 323 (1967).
7. H. Murakami, S. Takase, K. Kuwabara, *ibid.* **14**, 97 (1968).
8. H. Murakami, H. Sagawa, S. Takase, *ibid.*, p. 251.

28 June 1976; revised 13 September 1976

We have read the comments by Fennell and think it is interesting to compare them with a text by the same author (*1*).

> The Aspergilli are characterized by great diversity and variability as they are isolated from nature, and these differences may be interpreted as having emerged by processes of variation and mutation similar to those that occur in the laboratory. . . .
>
> The term variant is applied to strains arising through gradual change from normal members of identifiable species. The characters of a variant are generally not stable but subject to continued change and further variation.

This statement appears to be at variance with the position taken by Fennell.

When we first encountered rat mortality, followed by isolation of aflatoxins, we were anxious to identify the cause. We sent samples of the culture to two collaborators and to the Northern Regional Research Center and the Food and Drug Administration. The two collaborators gave the identification described in our report. Prepublication copies of the report were sent to collaborators and to the Northern Regional Research Center. Collaborators acquiesced to the report as written; but phone calls from Fennell produced the reversal described in Fennell's comment.

All in all it has been an episode that we would like to have closed. In our view the question remains open.

ROY E. MORSE
Department of Food Science, Cook College, Rutgers–The State University of New Jersey, New Brunswick 08903

References

1. K. B. Raper and D. I. Fennell, *The Genus* Aspergillus (Krieger, Huntington, N.Y., 1965), chap. 6, pp. 68 and 69.

4 October 1976