is a marker of DA receptors (*20*), our results can be taken to indicate the presence in the nigra of DA receptors localized on connections afferent to it.

Based on our findings and on recent results indicating DA release within the substantia nigra (*2*), a new mechanism of regulation of the activity of dopaminergic neurons may be postulated: nigral DA, by activating nigral DA-sensitive adenylate cyclase, would modulate transmitter release from nigral afferent connections, thus influencing the activity of the DA neurons. In particular, nigral DA might inhibit the release onto DA neurons of an excitatory transmitter (such as substance P) (*17*) or stimulate that of an inhibitory one (such as GABA) (*7, 17*), thus mediating a depression of dopaminergic activity. This model would explain the stimulation of DA firing produced by neuroleptics as due to blockade of nigral DA-sensitive adenylate cyclase and the inhibition produced by amphetamine as due to activation of the cyclase by DA released within the nigra.

This interpretation appears to reconcile the findings of Groves *et al.* (*4*) and of Bunney and Aghajanian (*5*) on the inhibition of dopaminergic firing by amphetamine. If one assumes with Groves *et al.* that amphetamine acts by releasing DA onto nigral DA receptors located on DA neurons, it is not possible to explain why interruption of afferent nigral connections blocks amphetamine effects, as shown by Bunney and Aghajanian. Conversely, if one postulates with Bunney and Aghajanian that amphetamine inhibits its DA firing by releasing DA onto striatal postsynaptic DA receptors, then it is difficult to justify its effectiveness when infused within the substantia nigra, as shown by Groves *et al.* Our model provides a unitary interpretation of these results. However, our findings do not exclude the existence of DA receptors localized on the membrane of DA neurons (autoreceptors) (*21*). Stimulation of these receptors would explain the finding of Bunney and Aghajanian that inhibition of dopaminergic firing by apomorphine is not abolished by interruption of afferent nigral connections. The significance of autoreceptors for the physiological regulation of dopaminergic activity, however, remains to be established (*22*).

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References

1. N. E. Anden, A. Carlsson, A. Dahlström, K. Fuxe, N. A. Hillarp, K. Larsson, *Life Sci.* **3**, 523 (1964); P. Bedard, L. Larochelle, A. Parent, L. Poirier, *Exp. Neurol.* **25**, 365 (1969); R. Y. Moore, R. Bhatnagar, A. Heller, *Brain Res.* **30**, 119 (1971); U. Ungerstedt, *Acta Physiol. Scand. Suppl. 367* (1971); O. Lindvall and A. Björklund, *ibid. 412* (1974).
2. J. Parizek, R. Hassler, I. J. Bak, *Z. Zellforsch.* **115**, 137 (1971); A. Björklund and O. Lindvall, *Brain Res.* **83**, 531 (1975); V. M. Pickel, T. H. Joh, P. M. Field, C. G. Becker, D. J. Reis, *J. Histochem. Cytochem.* **23**, 1 (1975); L. B. Geffen, T. M. Jessell, A. C. Cuello, L. L. Iversen, *Nature (London)* **260**, 258 (1976); J. Korf, M. Zieleman, B. H. C. Westerink, *ibid.*, p. 257.
3. G. K. Aghajanian and B. S. Bunney, in *Frontiers in Catecholamine Research* (Pergamon, Oxford, 1973), p. 643.
4. P. M. Groves, C. J. Wilson, S. J. Young, G. V. Rebec, *Science* **190**, 522 (1975).
5. B. S. Bunney and G. K. Aghajanian, *ibid.* **192**, 391 (1976).
6. O. T. Phillipson and A. S. Horn, *Nature (London)* **261**, 418 (1976); J. W. Kebabian and J. M. Saavedra, *Science* **193**, 683 (1976); P. F. Spano, G. Di Chiara, G. C. Tonon, M. Trabucchi, *J. Neurochem.* **27**, 1565 (1976).
7. P. L. McGeer *et al.*, *Adv. Neurol.* **5**, 153 (1974).
8. J. F. R. König and R. A. Klippel, in *The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Part of the Brain Stem* (Krieger, Huntington, N.Y., 1963).
9. B. Costall and R. J. Naylor, *Eur. J. Pharmacol.* **24**, 8 (1973).
10. J. W. Kebabian, G. L. Petzold, P. Greengard, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2145 (1972).
11. J. Kuo and P. Greengard, *Adv. Cyclic Nucleotide Res.* **2**, 41 (1972).
12. M. L. Tappaz, M. J. Brownstein, M. Palkovits, *Brain Res.* **108**, 371 (1976).
13. F. Fonnum, *J. Neurochem.* **24**, 407 (1975).
14. J. M. Saavedra, M. Brownstein, M. Palkovits, S. Kizer, J. Axelrod, *ibid.* **23**, 869 (1974).
15. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
16. E. G. McGeer, H. C. Fibiger, P. L. McGeer, S. Brooke, *Brain Res.* **52**, 289 (1973).
17. J. Störm-Mathisen, *ibid.* **84**, 409 (1975).
18. T. Hattori, H. C. Fibiger, I. L. McGeer, *J. Comp. Neurol.* **162**, 487 (1975); K. Niimi, T. Ikeda, S. Kawamura, H. Inoshita, *Brain Res.* **21**, 327 (1970); J. Szabo, *Exp. Neurol.* **37**, 652 (1972); T. J. Voneida, *J. Comp. Neurol.* **115**, 75 (1960).
19. T. Hökfelt, J. O. Kellerth, G. Nilsson, B. Pernow, *Science* **190**, 889 (1975); M. J. Brownstein, E. A. Mroz, J. S. Kizer, M. P. Palkovits, S. E. Leeman, *Brain Res.* **116**, 299 (1976).
20. Y. C. Clement-Cormier, R. G. Parrish, G. L. Petzold, J. W. Kebabian, P. Greengard, *J. Neurochem.* **25**, 143 (1975); M. H. Makman, R. K. Mishra, J. H. Brown, *Adv. Neurol.* **9**, 213 (1975); A. S. Horn, A. C. Cuello, R. J. Miller, *J. Neurochem.* **22**, 265 (1974); M. Trabucchi, S. Govoni, G. C. Tonon, P. F. Spano, *J. Pharm. Pharmacol.* **28**, 244 (1976).
21. A. Carlsson, in *Pre- and Post-Synaptic Receptors*, E. Usdin and W. E. Bunney, Eds. (Dekker, New York, 1975).
22. G. Di Chiara, M. L. Porceddu, L. Vargiu, A. Argiolas, G. L. Gessa, *Nature (London)* **264**, 564 (1976).

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Lens Cataract Formation and Reversible Alteration in Crystallin Synthesis in Cultured Lenses

Abstract. *Embryonic chick lenses developed cortical cataracts and altered their pattern of δ-crystallin synthesis within 3 hours, if cultured without their vitreous body or traumatized with their vitreous body attached. δ-Crystallin reverted to the normal pattern by 24 hours in the cataractous lenses. Thus, biochemical differences that are only observable during the initial stages of cataractogenesis can exist between opaque and normal lenses.*

An ocular lens opacity, or cataract, often occurs naturally with age (senile cataract), develops as a congenital anomaly of chromosomal, genetic, or viral origin, or arises as a consequence of diseases occurring later in life, of metabolic stress, of trauma, or of various forms of physical or chemical insults to the lens (*1*). Cataracts can affect vision seriously and lead to blindness. Lens opacities cannot generally be reversed, and thus the usual treatment for an advanced cataract is surgery. Although many histological, cytological, and biochemical aberrations are associated with opaque lenses, the mechanisms underlying cataract formation are not known. Two very important advances concerning this problem include the recognition that polyol accumulation leads to osmotic imbalance in sugar-induced cataracts (*2*) and the evidence that protein aggregation leads to light scattering in senile cataracts (*3*). To the best of our knowledge, there has never been a direct demonstration of an alteration in protein synthesis associated with cataract formation. We now report that the synthesis of the principal crystallin of the embryonic chick lens, δ-crystallin (*4*), is reversibly affected during the initiation of cataract formation in vitro.

Our ability to study protein synthesis during cataract formation became possible when we discovered that surgically excised embryonic chick lenses develop cortical cataracts within 3 hours of culture unless the vitreous body is not detached from the posterior lens capsule (Fig. 1). Vitreous-associated lenses remained clear when cultured for 48 hours. A similar protective action by the vitreous body toward cataract formation has been observed earlier in cultured rabbit lenses, where Chylack and Kinoshita pointed out that a disordered relation between the vitreous body and the lens may contribute to the development of a posterior subcapsular human senile cataract or affect the ability of the human lens to withstand metabolic stress (*5*). Those authors provided evidence that

the cataracts resulted from trauma to the lens when the adhering vitreous was detached. We also believe that the cataracts observed in our investigation are due to lens trauma occurring when the vitreous is removed, since opacities developed when vitreous-associated lenses were injured by gently squeezing with jeweler's forceps (without disrupting the lens capsule), or when vitreous-free lenses were cultured on a vitreous bed. Moreover, occasionally a vitreous-free lens developed a less severe cataract which disappeared by 24 hours of culture; those lenses remained clear when cultured an additional 24 hours. The reversal of an experimentally induced cortical cataract is not unique and has been shown to occur in vivo in rats fed with galactose and then returned to a normal diet (*6*).

Our experiments comparing protein synthesis in the clear and cataractous lenses demonstrated a pronounced difference in the pattern of δ-crystallin synthesis, although the total amounts of [^{35}S]methionine incorporated into the lens proteins were not significantly different in the two cases. δ-Crystallin from the embryonic chick lens can be resolved into two bands by electrophoresis in polyacrylamide gels containing 0.1 percent sodium dodecyl sulfate and 8*M* urea (Fig. 2A) (*7*). A scan of the stained gel indicated that there is approximately three times as much protein in the lower molecular weight band (about 48,000 daltons) as in the higher molecular weight band (about 50,000 daltons). This mass ratio of the smaller to larger δ-crystallin band was the same in clear and cataractous lenses. The relative rates of [^{35}S]methionine incorporation into the two δ-crystallin bands, however, differed markedly in the clear and cloudy lenses. In the clear lens cultured with the vitreous body, the specific activity of each band of δ-crystallin was similar (Fig. 2B). By contrast, in the cataractous lens cultured without the vitreous body, the specific activity of the larger molecular weight band of δ-crystallin was much higher than that of the smaller molecular weight band (Fig. 2C). This was due to both a greater incorporation of [^{35}S]methionine into the larger band and less incorporation into the smaller band in the cataractous lens. Purification of the δ-crystallin before electrophoresis by differential centrifugation and solubilization at *p*H 5 (*8*) did not affect this result. Analysis of the tryptic peptides of the newly synthesized δ-crystallin showed that the [^{35}S]methionine incorporation was not limited to the NH_2-terminal positions. The abnormal pattern of δ-crystallin labeling is not due simply to an adaptation of the lens to the culture conditions, but is associated with the development of a cataract, since both cataract formation and alteration of δ-crystallin labeling occurred within 3 hours when the vitreous body was removed from lenses precultured for 24 hours before labeling. Short-term labeling experiments, performed in the presence or absence of protein synthesis (inhibited by cycloheximide at 10 μg/ml), showed that the newly synthesized bands of δ-crystallin were not degraded in the cultured lenses over the period of the experiment, and thus the noted change in [^{35}S]methionine incorporation represents a change in the relative rates of synthesis of the two bands.

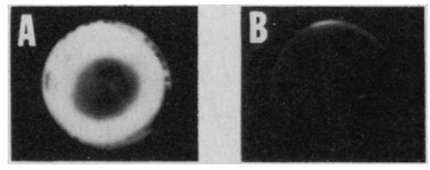

Fig. 1. (A) A 15-day-old embryonic, White Leghorn chick lens (fertile eggs obtained from Truslow Farms, Inc., Chestertown, Maryland) cultured without its vitreous body for 3 hours at 37°C, in an atmosphere of 5 percent CO_2 and 95 percent air, in 4 ml of Ham's F-10 medium (*10*) enriched with D-fructose (848 mg/100 ml; 329 milliosmols). Typically, two to four lenses were cultured per plastic tissue culture dish (Falcon Plastics; 15 by 60 mm). (B) An embryonic chick lens–vitreous preparation after 3 hours in vitro. The immersed lenses were photographed through a Wild stereoscopic microscope, with a light source at a 90° angle from the objective and a black hole under the lens. The black specks on the perimeter of the lens in (A) are remnants of the ciliary body; the white patch and gray perimeter of the lens in (B) represent reflected light, not an opacity (magnification, ×8.5).

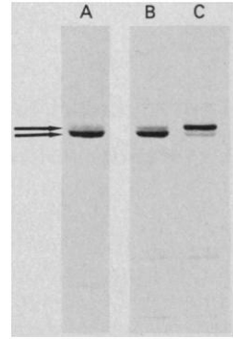

Fig. 2. Electropheregrams of proteins from clear and cataractous lenses. (A) Staining pattern of total proteins from a cataractous lens cultured for 3 hours without its vitreous body, as in Fig. 1A. The arrows denote the two bands of δ-crystallin. There is an extremely high proportion of δ-crystallin in these lenses (*4*). (B) Autoradiogram of the proteins from a clear lens cultured with its vitreous body and labeled for 3 hours with [^{35}S]methionine (125 μc/ml; 300 c/mmole; New England Nuclear) in 4 ml of Ham's F-10 medium lacking nonradioactive methionine. The vitreous body was removed before the proteins were prepared for electrophoresis. The specific activity of each δ-crystallin band appears approximately the same. (C) Autoradiogram of the proteins from a cataractous lens cultured for 3 hours without its vitreous body. There is a large increase in the labeling of the higher molecular weight band (upper band) and a decrease in labeling of the lower molecular weight band of δ-crystallin. The relative staining intensity of the two δ-crystallin bands was as in (A) in the clear and cataractous lenses. For analysis, individual lenses were homogenized in 0.2 ml of 0.01*M* sodium phosphate, *p*H 6.4, and approximately 2 μg of protein (50,000 count/min) of each sample was examined by discontinuous, 10 percent polyacrylamide gel electrophoresis in the presence of 0.1 percent sodium dodecyl sulfate and 8*M* urea in a 1.5-mm thick gel slab (*11*). Electrophoresis was for 5 hours at 100 volts in room temperature. The gel was stained with Coomassie brilliant blue R, destained electrophoretically, dried under vacuum, and autoradiographed on x-ray film (Kodak SB-5). Prints were made from negatives of the autoradiograms.

In one test, δ-crystallin was labeled for 5 hours in ovo by placing 200 μc of [^{35}S]methionine (405 c/mmole) on the chorioallantoic membrane of a 15-day-old chick embryo. Electrophoresis showed that the ratio of synthesis of the two δ-crystallin bands was the same as that in the clear lens cultured with its vitreous body attached.

The same alteration in [^{35}S]methionine incorporation also took place when vitreous-associated lenses developed cataracts after squeezing with forceps, suggesting that, like cataract formation, it is not the absence of the vitreous body per se which causes this specific change in incorporation, but rather trauma to the lens. Alternatively, culture of the lens without its vitreous body, and trauma induced by squeezing the excised lens associated with its vitreous body, may represent two ways of producing similar results through different mechanisms.

Unexpectedly, δ-crystallin synthesis reverted to the normal pattern in the lenses cultured for 24 hours without the vitreous body, despite persistence of the cataract, and was indistinguishable from the pattern of δ-crystallin synthesis in the clear lenses. Thus, the alteration in δ-crystallin synthesis in the cultured lenses during the development of the cataract is completely reversible. Traumatizing a cataractous lens by squeezing it with forceps after 24 hours of culture without its vitreous body did not cause the lens to assume the abnormal pattern of δ-crystallin synthesis.

We do not yet know whether this alteration in protein synthesis in the cultured lenses is due to a translational control of δ-crystallin synthesis or to a change in the rate of cleavage of the larger δ-crystallin band during or immediately after translation (*7*). The former would repre-

sent a particularly interesting example of translational control in view of the apparent homogeneity of δ-crystallin messenger RNA (*9*). Whatever the precise mechanism, our data show that embryonic chick lenses cultured under the described conditions without their vitreous body develop cortical cataracts and undergo a specific, reversible change in δ-crystallin synthesis. Although we do not know whether there is a causal relation between this particular transient change in δ-crystallin synthesis and cortical cataracts in the cultured lens, our study demonstrates that a reversible biochemical event can occur during the development of a cataract and, therefore, it may be insufficient to limit etiological studies of cataracts to lenses that already have pronounced opacities.

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References and Notes

1. *Cataract and Abnormalities of the Lens*, J. G. Bellows, Ed. (Grune & Stratton, New York, 1975); R. van Heyningen, in *The Eye*, H. Davson, Ed. (Academic Press, New York, ed. 2, 1969), vol. 1, p. 381; J. F. R. Kuck, Jr., in *Biochemistry of the Eye*, C. N. Graymore, Ed. (Academic Press, New York, 1970), p. 319; J. Nordman, *Ophthalmol. Res.* **3**, 323 (1972); G. W. Barber, *Exp. Eye Res.* **16**, 85 (1973).
2. J. H. Kinoshita, *Invest. Ophthalmol.* **13**, 713 (1974).
3. A. Spector, L-K. Li, R. C. Augusteyn, A. Schneider, T. Freund, *Biochem. J.* **124**, 337 (1971); A. Spector, T. Freund, L-K. Li, R. C. Augusteyn, *Invest. Ophthalmol.* **10**, 677 (1971); A. Spector, *Isr. J. Med. Sci.* **8**, 1577 (1972); G. B. Benedek, *Appl. Opt.* **10**, 459 (1971); J. A. Jedziniak, J. H. Kinoshita, E. M. Yates, L. O. Hocker, G. B. Benedek, *Invest. Ophthalmol.* **11**, 905 (1972); A. Spector, *Exp. Eye Res.* **16**, 115 (1973); D. Roy and A. Spector, *Invest. Ophthalmol.* **15**, 394 (1976); *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3484 (1976).
4. M. Rabaey, *Exp. Eye Res.* **1**, 310 (1962); J. M. Genis-Galvez, H. Maisel, J. Castro, *ibid.* **7**, 593 (1968); J. Zwaan and A. Ikeda, *ibid.*, p. 301; K. Yoshida and A. Katoh, *ibid.* **11**, 184 (1971); J. Piatigorsky, H. deF. Webster, S. P. Craig, *Dev. Biol.* **27**, 176 (1972).
5. L. T. Chylack, Jr., and J. H. Kinoshita, *Exp. Eye Res.* **14**, 58 (1972); **15**, 61 (1973).
6. H. S. Mitchell and G. M. Cook, *Arch. Ophthalmol.* **19**, 22 (1937); V. N. Reddy, D. Schwass, B. Chakrapani, C. P. Lim, *Exp. Eye Res.* **23**, 483 (1976).
7. R. Reszelbach, T. Shinohara, J. Piatigorsky, *Exp. Eye Res.*, in press. In this study we have shown that both bands are immunologically related to δ-crystallin, and that neither band is contaminated by the other in the polyacrylamide gel. Both bands have the same two-dimensional peptide pattern of leucine-containing tryptic peptides, and thus the smaller band may be a cleavage product of the larger. It is equally possible, however, that the two bands have a separate genetic origin, since two bands which comigrate during electrophoresis with those of authentic δ-crystallin are synthesized when purified δ-crystallin messenger RNA is translated in cell-free extracts from rabbit reticulocytes or wheat germ. Moreover, neither band of δ-crystallin is preferentially labeled in short-term labeling experiments or processed into the other during subsequent incubations.
8. J. Piatigorsky, P. Zelenka, R. T. Simpson, *Exp. Eye Res.* **18**, 435 (1974).
9. P. Zelenka and J. Piatigorsky, *ibid.* **22**, 115 (1976).
10. R. G. Ham, *Exp. Cell Res.* **29**, 515 (1963).
11. J. V. Maizel, Jr., in *Methods of Virology*, K. Maramorosch and H. Koprowski, Eds. (Academic Press, New York, 1971), vol. 5, p. 179.
12. We thank Dr. A. J. Coulombre for help in photographing the lenses and labeling δ-crystallin in ovo, and Drs. J. H. Kinoshita, D. C. Beebe, A. J. Coulombre, L. T. Chylack, Jr., and P. Zelenka for critically reading the manuscript.

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Stink of Stinkpot Turtle Identified: ω-Phenylalkanoic Acids

Abstract. *The exocrine secretion of the "stinkpot turtle,"* Sternotherus odoratus, *discharged by the animals in response to disturbance, contains four ω-phenylalkanoic acids (phenylacetic, 3-phenylpropionic, 5-phenylpentanoic, and 7-phenylheptanoic). The last two of these are new natural products. The first two are powerfully malodorous and responsible for the stench of the fluid. Lesser components, including several aliphatic acids, are also present. Only a few milligrams of secretion are discharged by a turtle at any one time. Although bioassays with fish suggest that the secretion has the potential to serve as a feeding deterrent to predators, it is argued that* Sternotherus *does not ordinarily discharge enough fluid to effect this action and may employ its secretion only as an aposematic signal that warns predators of its more generalized undesirability.*

The turtles of the family Kinosternidae include a number of species known as musk turtles, which eject an odorous secretion when disturbed (*1*). No chemical work had hitherto been done on these fluids, which in some species are strongly scented. We here report the isolation and characterization of the odorous constituents of the secretion of *Sternotherus odoratus*, a species from the eastern United States, inelegantly but appropriately called the "stinkpot." *Sternotherus* has four glands, morphologically identical, opening lateroventrally near the edge of the carapace, in front and behind the plastral bridges (Fig. 1A). They are small structures, comprising only about 0.1 percent of body weight (*2*), and capable of discharging no more than a small droplet at any one time (Fig. 1, B and C). The amount suffices, however, to impart a potent stench to a manipulated turtle. The glands are present in both sexes and are functional in juveniles as well as adults.

Two medium-sized *Sternotherus*, from Tampa, Florida, were "milked" of secretion by tapping their shells and prodding their bodies, and taking up the exuded fluid in glass capillary tubing. Analyses of the pooled milkings (in ether or amyl acetate) by gas chromatography–mass spectrometry (GCMS) revealed no highly volatile components (*3*), and exposure of the secretion to dampened lead acetate paper indicated that hydrogen sulfide was absent. Treatment of the secretion with ethereal diazomethane yielded a series of methyl esters; these were identified on the basis of GCMS data (*4*) as methyl esters of phenylacetic, 3-phenylpropionic, and 5-phenylpentanoic acids. The methyl ester of a fourth acid had a mass spectrum, *m/e* 220 (3), 188 (11), 129 (5), 117 (5), 105 (11), 104 (11), 97 (12), 92 (30), 91 (100), 87 (28), 84 (12), 74 (33), 69 (11), 65 (14), 59 (8), 55 (9), suggestive of methyl 7-phenylheptanoate. An authentic sample of this ester was prepared by treatment of 7-phenylheptanoic acid (*5*) with diazomethane, and was indistinguishable (by GCMS comparison) from the natural product. In addition, small amounts of 3-methylbutanoic, hexanoic, hexadecanoic, and heptadecanoic acids were also identified by the GCMS data obtained from their methyl esters (*4*). Upward of ten additional minor components were present, in amounts too small to permit identification.

Five additional turtles were milked, including a third unsexed medium-sized specimen from Tampa, and four sexed adults from Philadelphia, Pennsylvania, (three males and one female). The three males were milked twice, with 3 weeks between milkings. As is seen from Fig. 2, which incorporates the data from all seven turtles, the secretion consistently contained the four ω-phenylalkanoic acids, in relative percentages that varied somewhat between individuals and milkings (*6*). The aliphatic acids (not tabulated) were present at concentrations not exceeding 1 percent relative to the ω-phenylalkanoic acids. As is evident from the four cases where both secretory output was weighed and the total ω-phenylalkanoic acid content of the secretion was calculated (*7*), the turtles discharge the acids in microgram quantities, with only milligrams of fluid. Our own olfactory sensitivity to the acids is evidently high. Subjective sniff testing of the secretion and its components showed that the stench of the secretion is attributable