study. This study adds the finding that purified myelin is capable of inducing both EAE and myelination inhibition factor, suggesting that the agent responsible for induction of the latter is a component of myelin. Our results also indicate that the ability of serums to block evoked electrical responses in tissue cultures is not specifically related to the presence of neurological deficit phenomena in EAE. The finding of neuroelectric blocking factor in serums from control animals as well as from animals with EAE argues against a specific role for this factor in the pathogenesis of EAE.

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References and Notes

1. M. B. Bornstein and S. H. Appel, *J. Neuropathol. Exp. Neurol.* **20**, 141 (1961); S H. Appel and M. B. Bornstein, *J. Exp. Med.* **119**, 303 (1964).
2. M. B. Bornstein and C. S. Raine, *Lab. Invest.* **23**, 536 (1970).
3. M. B. Bornstein, in *Progress in Neuropathology*, H. M. Zimmerman, Ed. (Grune & Stratton, New York, 1973), vol. 2, p. 69; M. B. Bornstein, *Mt. Sinai J. Med.* **41**, 46 (1974).
4. R. H. Laatsch, M. W. Kies, S. Gordon, E. C. Alvord, Jr., *J. Exp. Med.* **115**, 777 (1962).
5. F. J. Seil, G. A. Falk, M. W. Kies, E. C. Alvord, Jr., *Exp. Neurol.* **22**, 545 (1968).
6. M. W. Kies, B. F. Driscoll, F. J. Seil, E. C. Alvord, Jr., *Science* **179**, 689 (1973); F. J. Seil, H. C. Rauch, E. R. Einstein, A. E. Hamilton, *J. Immunol.* **111**, 96 (1973).
7. M. Dubois-Dalcq, B. Niedieck, M. Buyse, *Pathol. Eur.* **5**, 331 (1970); J. M. Fry, S. Weissbarth, G. M. Lehrer, M. B. Bornstein, *Science* **183**, 540 (1974).
8. P. Y. Patterson, *Annu. Rev. Med.* **20**, 75 (1969); F. J. Seil, *Calif. Med.* **116**, 25 (May 1972).
9. M. B. Bornstein and S. M. Crain, *Science* **148**, 1242 (1965).
10. ———, *J. Neuropathol. Exp. Neurol.* **30**, 129 (1971).
11. M. E. Smith, *Biochim. Biophys. Acta* **164**, 285 (1968).
12. M. B. Bornstein and M. R. Murray, *J. Biophys. Biochem. Cytol.* **4**, 499 (1958); S. M. Crain and M. B. Bornstein, *Exp. Neurol.* **10**, 425 (1964); F. J. Seil, *Brain Res.* **42**, 33 (1972); ———, J. M. Kelly, A. L. Leiman, *Exp. Neurol.* **45**, 435 (1974).
13. A. L. Leiman and F. J. Seil, *Exp. Neurol.* **40**, 748 (1973).
14. We thank R. A. Fisk for technical assistance. J.M.K. holds a Veterans Administration fellowship in neurobiology.

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Disc Shedding in Rodlike and Conelike Photoreceptors of Tree Squirrels

Abstract. *Electron microscopic observations suggest that the rodlike and conelike photoreceptors of diurnal tree squirrels shed outer segment discs. Twenty-four hours after injection of tritiated* L-*leucine, the rodlike photoreceptors show a band of radioactivity at the base of the outer segment. The conelike photoreceptor outer segments show only a pattern of diffuse labeling. These results strongly suggest that disc shedding can occur in photoreceptor outer segments in which proteins are diffusely renewed.*

Autoradiographic investigations of the outer segment renewal process in vertebrate photoreceptors show that a distinct band of radioactive material can be identified at the base of rod outer segments within hours after the administration of radioactive amino acids (*1*). It is thought that these labeled amino acids are incorporated into protein that is used in the assembly of new rod discs at the outer segment base (*2*). The labeled discs are continuously displaced from the base to the apex of the rod outer segment (*1, 3*). At this point the labeled discs detach from the outer segment tip and are withdrawn into the pigment epithelium as phagosomes (*4*). Electron microscopic evidence confirms that groups of rod discs detach intermittently from the outer segment tips, after which a process of degradation occurs within the pigment epithelium (*5, 6*).

The mode of outer segment renewal described in mature vertebrate cones is thought to involve neither the synthesis of new discs nor the disposal of old discs, since radioactive label that is incorporated into cone outer segments does not accumulate in a distinct band as it does in rods (*7*). Rather, the labeled material is seen as diffusely incorporated throughout the cone outer segment (*8*). In this report we present electron microscopic evidence that implicates both the conelike and rodlike photoreceptors of diurnal tree squirrels in a process of disc shedding. We also show that proteins in the conelike outer segments are diffusely renewed like those in all other vertebrate cones examined (*7*).

Cohen (*9*) described the fine structure of two photoreceptor classes in the retina of the Eastern grey squirrel (*Sciurus carolinensis*), an arboreal species whose retina had been thought to contain only cones (*10*). He showed that the double-tiered photoreceptor layer was composed of an outer, more sclerad tier with short cylindrical outer segments whose discs were continuous with the outer cell membrane in the basal third of the outer segment. He also described an inner, more vitread tier with outer segments that were somewhat longer and of reduced diameter. In these receptors, only rare instances of disc-membrane continuity were observed. Cohen designated the inner tier photoreceptors as R or rodlike and the outer tier photoreceptors as C or conelike. This classification was confirmed by West and Dowling (*11*).

Laties and Liebman (*12*) showed that the outer segments of vertebrate cones selectively take up the dye Procion Yellow after it is injected in vivo into the vitreous humor. It is believed that the observed fluorescence in cone outer segments is due to the infiltration of the dye into the spaces between the infolded disc membranes which are open to extracellular space (*12, 13*). Only the conelike outer segments take up Procion Yellow in the retina of the Eastern grey squirrel (*14*). This observation lends support to the receptor designations originally proposed by Cohen (*9*).

We reexamined the fine structure of the photoreceptors in retinas of the Eastern grey squirrel and the closely related Western grey squirrel (*Sciurus griseus*). In all aspects examined thus far, the retinas of these two species are alike. In each case, fixation was accomplished by intracardiac perfusion or by immersion in a solution of 2.5 percent glutaraldehyde buffered with 0.067*M* sodium cacodylate (*p*H 7.4). After the initial fixation, the posterior segments of the eyes were briefly washed and then postfixed in 2 percent osmium tetroxide buffered with veronal acetate (*p*H 7.4). Sections for electron microscopy were stained with uranyl acetate and lead citrate and examined in a Siemens Elmiskop I or Siemens 101 electron microscope.

In both species a process of disc shedding, similar to that previously described only in vertebrate rods (*5, 6*),

is present in both the rodlike and conelike receptors. In the rodlike receptors, groups of detached discs can sometimes be identified within the villous processes (*9*) that typically "cap" the outer segment tip. In the conelike receptors, whose outer segments are flush with the apical zone of the pigment epithelium (Fig. 1A), disc packets in the process of detaching from the outer segment apex are surrounded by numerous pigment epithelial extensions (Fig. 1B). Whether such processes play an active role in the detachment phase is unclear (*6*). A characteristic, highly ordered rippling of the discs which appears in conelike outer segments fixed by immersion (*9*) can be occasionally identified in phagosomes, provided they have not undergone extensive degradation within the pigment epithelium. Observations of this kind strengthen the case for disc shedding from the conelike outer segments (*15*).

In order to determine the mode of outer segment renewal in these photoreceptors, 0.5-μm sections were prepared for light microscopic autoradiography. Two Eastern grey squirrels were each injected intraperitoneally with 40 mc of L-[4,5-^{3}H]leucine. After 24 and 72 hours, respectively, the animals were fixed by perfusion of the aldehyde fixative mentioned above (*16*). The results are shown in Fig. 2. At 24 hours conelike outer segments show a pattern of diffuse labeling like that of cone outer segments of other vertebrates studied (*7*), while the rodlike photoreceptors show a band of labeled protein that has accumulated at the outer segment base (Fig. 2A). At 72 hours after injection the conelike outer segments remain diffusely labeled. However, the band of labeled protein in the rodlike outer segments is displaced nearly halfway up the length of the outer segment (Fig. 2B). Our measurements indicate that the band of labeled protein is displaced 4.8 μm (mean) from the outer segment base after 72 hours, a rate of about 1.6 μm per day. At this rate the entire complement of rodlike discs should turn over in about 1 week ($\pm$1 day). Our calculations show the disc density in rodlike outer segments to be about 40 per micrometer. Thus, the estimated rate of renewal comes out to be approximately 65 discs per day. This figure is somewhat lower than those reported for other mammalian rods (75 to 90 discs per day) (*3, 17*).

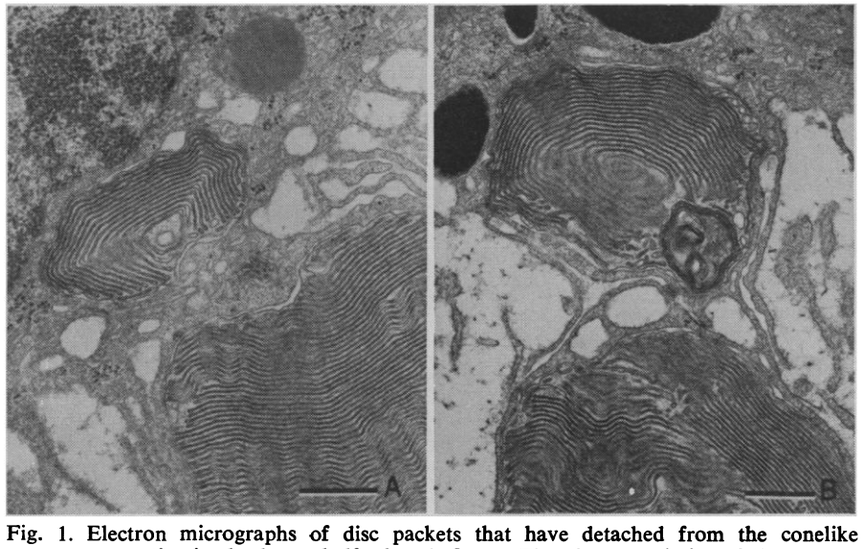

Fig. 1. Electron micrographs of disc packets that have detached from the conelike outer segment tips in the lower half of each figure. The close proximity of these outer segments to the pigment epithelium identifies them as conelike (scale, 0.5 μm).

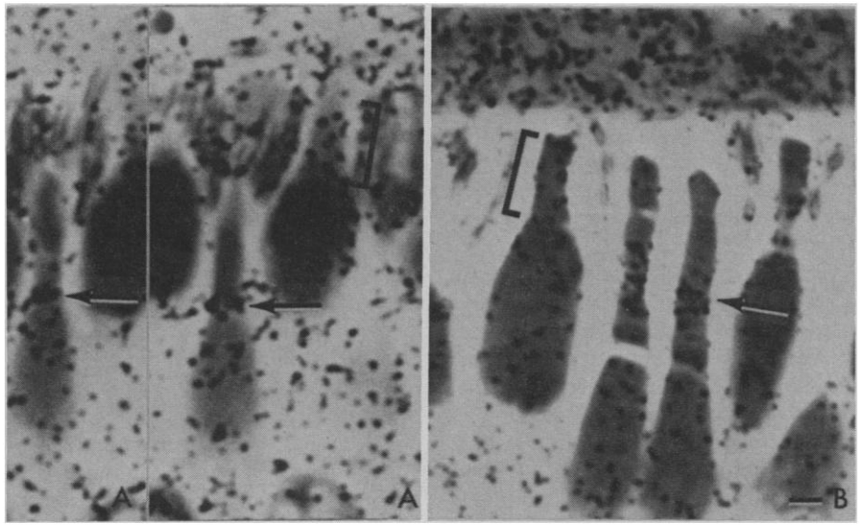

Fig. 2. Autoradiograms of photoreceptors of Eastern grey squirrel after intraperitoneal injection of L-[4,5-^{3}H]leucine; scale, 2 μm. (A) Twenty-four hours after injection, a band of labeled material appears at the base of the rodlike outer segments (arrows) while the conelike outer segment shows diffuse labeling over its entire length (bracket). (B) Seventy-two hours after injection, the band of labeled material has moved nearly midway up the rodlike outer segment (arrows) while the conelike outer segments remain diffusely labeled (bracket).

The evidence for disc shedding from the conelike photoreceptor outer segments in tree squirrels strongly suggests that discs may be shed from outer segments that show a strictly diffuse pattern of protein renewal. However, it does not necessarily imply that all vertebrate cones are engaged in shedding discs. Young (*18*) found no evidence of phagosomes in the pigment epithelium of certain all-cone lizards. On the other hand, Hogan *et al.* (*19*) presented evidence of disc shedding from human cone outer segments. The critical factors that determine the presence or absence of disc shedding in vertebrate cones remain to be identified.

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References and Notes

1. R. W. Young, *J. Cell Biol.* **33**, 61 (1967).
2. ——— and B. Broz, *ibid.* **39**, 169 (1968).
3. R. W. Young, *ibid.* **49**, 303 (1971).
4. ——— and D. Bok, *ibid.* **42**, 392 (1969).
5. A. Bairati and N. Orzalesi, *J. Ultrastruct. Res.* **9**, 484 (1963).
6. M. Spitznas and M. J. Hogan, *Arch. Ophthalmol.* **84**, 810 (197
7. R. W. Young, *J. Ultrastruct. Res.* **34**, 190 (1971).
8. R. W. Young, *Vision Res.* **11**, 1 (1971).
8. ———, *Invest. Ophthalmol.* **8**, 222 (1969).

9. A. I. Cohen, *ibid.* **3**, 198 (1964).
10. G. B. Arden and K. Tansley, *J. Physiol. (Lond.)* **127**, 592 (1955).
11. R. West and J. E. Dowling, *J. Comp. Neurol.*, in press.
12. A. M. Laties and P. A. Liebman, *Science* **168**, 1475 (1970).
13. A. I. Cohen, in *Handbook of Sensory Physiology*, M. Fuortes, Ed. (Springer-Verlag, Berlin, 1970), vol. 7, part 1B, pp. 63–109.
14. D. H. Anderson and S. K. Fisher, in preparation.
15. The possibility that these observations may be the result of some fixation artifact or unidentified pathological condition is slight. Evidence of disc shedding can be seen in both osmium- and glutaraldehyde-fixed material, it can be found after fixation by immersion or by perfusion, and it can be found in both species of tree squirrels and in several species of ground squirrels.
16. We are indebted to R. Young (Department of Anatomy, School of Medicine, University of California, Los Angeles) for demonstrating his method of fixation.
17. M. M. LaVail, *J. Cell Biol.* **58**, 650 (1973).
18. R. W. Young, in preparation.
19. M. Hogan, I. Wood, R. H. Steinberg, *Nature (Lond.)*, in press.
20. Supported by NIH research grant EY 000888 and a University of California faculty research grant to S.K.F., and by NIH research grant EY 00105 to G. H. Jacobs. We thank A. Laties for his help in obtaining the Procion Yellow data and K. Esau for use of her electron microscope facilities.

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Blockade of Acetylcholine Receptors: A Model of Myasthenia Gravis

Abstract. *In order to block acetylcholine receptors of muscle, the alpha toxin of the Formosan cobra (*Naja naja atra*) was given intravenously to rats. Electrophysiological and pharmacological changes typical of myasthenia gravis were recorded, including decremental responses to repetitive stimuli, curare sensitivity, neostigmine reversal, and posttetanic phenomena. This model supports the concept that a reduction of available acetylcholine receptors may play an important role in myasthenia gravis.*

Myasthenia gravis is a neuromuscular disorder manifested by weakness and fatigability of muscle. It is generally accepted that the abnormality involves the neuromuscular junction, but the exact site and nature of the defect are not yet settled (*1*). There has been considerable debate as to whether the nerve terminal (*2; 3*, p. 305) or the postsynaptic region (*4*) of the muscle, or both (*5*), are affected by the disease process. We have found an abnormality in the acetylcholine (ACh) receptors of neuromuscular junctions from myasthenic patients (*6*). By means of techniques which utilize α-[^{125}I]bungarotoxin binding to receptors, we determined that the number of available ACh receptors was reduced in myasthenic junctions, averaging 80 percent below normal. This finding raised the question of whether the receptor reduction per se could explain the physiopathological defects of myasthenia gravis. In our study we have compared the physiological effects produced by experimental receptor blockade in animals with those occurring naturally in myasthenia gravis.

In order to reduce the number of free receptors, we have used the α-toxin derived from the venom of the cobra *Naja naja atra* as a pharmacological blocking agent (*7*). Its specificity has been confirmed by its use in an assay for purified ACh receptor (*8*). Lyophilized venom was obtained from the Miami Serpentarium, and the α fraction was isolated and purified by the ion exchange chromatography method of Lee *et al.* (*9*).

Female Lewis rats, anesthetized with chloral hydrate (40 mg/kg), were prepared as follows for recording of evoked muscle action potentials. Tracheostomy was carried out, and the tube was connected to a Harvard respirator. The skin temperature of the rat was maintained at 35°C by means of an infrared lamp regulated by a telethermometer (Yellow Springs Instruments). In order to obtain reliable and consistent records of muscle action potentials, mechanically secure mounting of the animal and of the electrodes proved essential. The animal was strapped to a Lucite platform, and the right leg was taped to a metal rod. One surface recording electrode was secured over the mid-belly of the triceps surae (calf) muscle with collodion and tape, and the other was fastened over the Achilles tendon with a 9-mm Clay-Adams wound clip. After the right sciatic nerve was surgically exposed, the stimulator probe was gently hooked around it and clamped in position. The overlying skin was closed around the probe with pins and kept moist with mineral oil.

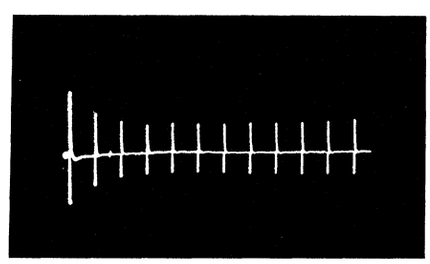

Fig. 1. Decremental response recorded in deltoid muscle of myasthenic patient (stimulus rate, 5 sec^{-1}).

Trains of stimuli were supplied by a Grass S-48 stimulator through a stimulus isolation unit. (The stimulus duration was 0.2 msec; the amplitude was 1.5 times maximal, usually 4 to 7 volts.) The muscle potentials were amplified by a differential amplifier (WPI Co. DAM-6) displayed on an oscilloscope (Tektronix), and recorded on Polaroid film for measurement.

Control records of the animal's responses to repetitive nerve stimulation were made at the start of each experiment. An injection of α-cobratoxin (12 to 20 μg) was then given intravenously, and recordings were made at intervals of approximately 30 to 60 minutes until the termination of the experiment, up to 14 hours later.

This method of giving a relatively large single dose of α-cobratoxin, followed by a long period of observation, was used to allow for adequate equilibration of the toxin throughout the neuromuscular junctional receptor pool. The toxin binds rapidly to receptors, but the toxin-receptor complex is slowly reversible (*10*). Although single evoked potentials decreased in amplitude for several hours after injection, they subsequently returned to normal or nearly normal levels (mean, 83 percent of original amplitude). All of the myasthenic features described below were present during the recovery phase, when the amplitude of single evoked potentials was approaching normal.

Affected muscles of patients with myasthenia gravis typically show a decrement in the amplitude of action potentials evoked by repetitive nerve stimulation (Fig. 1). Conventionally, a stimulus rate of 2 to 5 sec^{-1} is used, and the amplitude of the fourth potential is compared with that of the first (*11*). The first potential is usually normal or only slightly diminished and a decrement of more than 7 to 15 percent is considered to be consistent with a diagnosis of myasthenia.

In our experiment, we reproduced the clinical procedure by stimulating the sciatic nerve at 3 sec^{-1} and recording evoked muscle action potentials with surface electrodes placed over the calf muscles as described above. In control observations on 21 untreated