after 30 minutes, the PMN were present at the margins of synovial venules. At this time numerous PMN were fragmented and had released dense bodies (Fig 2) and many had lost dense bodies but were otherwise intact. By 3½ hours, specimens exhibited much less degranulation. No degranulation was seen in controls injected with saline. In four dogs injected with urate crystals each week for 4 weeks and again 3½ hours before being killed, a chronic synovitis was produced and intraluminal degranulation of PMN was still very prominent 3½ hours after the last injection.

Most investigators of experimental inflammation have not reported such intraluminal degranulation (*3*). However, in systemic anaphylaxis (produced by injection of immune aggregates) in the rabbit, Movat *et al.* (*4*) noted that intraluminal PMN phagocytized the immune aggregates and also that they gradually degranulate. This suggested that the rise in serum cathepsins and acid proteases in anaphylaxis might be a result, at least partly, of an intraluminal release from the PMN's (*5*). Fragmentation of PMN has been observed in pulmonary capillaries after injection of endotoxin which produces the generalized Schwartzman reaction (*6*); we saw some similar disruptions of PMN's in synovial vessels. The relation of this fragmentation to the more common form of degranulation of otherwise intact cells in not clear. Peripheral blood with bacterial infection (*7*) did not have PMN with decreased numbers of granules but the PMN did degranulate in vitro more quickly than did controls where no bacterial infection was present. Degranulated PMN in vessels located at sites of inflammation may not survive to be reflected in any large numbers in the peripheral circulation.

The variety of diseases which are accompanied by intraluminal degranulation suggests that this occurrence is not a specific one, but we propose that intraluminal release of PMN granules that contain histamine, kinins, and other mediators of inflammation (*8*) may be an important mechanism in vascular injury in many diseases and at many sites.

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References and Notes

1. H. R. Schumacher, *Arthritis Rheum.* **11**, 426 (1968).
2. D. J. McCarty and J. S. Faires, *Curr. Ther. Res.* **5**, 284 (1963).
3. G. Majno and G. E. Palade, *J. Biophys. Biochem. Cytol.* **11**, 571 (1961); C. C. Cochrane, E. R. Unanue, F. J. Dixon, *J. Exp. Med.* **122**, 99 (1965).
4. H. Z. Movat, T. Uriuhara, N. S. Taichman, H. C. Roswell, J. F. Mustard, *Immunology* **14**, 637 (1968)
5. S. Wasi, R. K. Murray, D. R. L. McMorine, H. Z. Movat, *Brit. J. Exp. Pathol.* **47**, 411 (1966).
6. R. G. Horn and R. D. Collins, *Lab. Invest.* **19**, 451 (1968); D. G. McKay, W. Margaretten, I. Csavossy, *ibid.* **15**, 1815 (1966).
7. C. E. McCall, I. Katayama, R. S. Cotran, M. Finland, *J. Exp. Med.* **129**, 267 (1969)
8. A. Janoff, *Ser. Haematol.* **3**, 96 (1970).
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Synaptic Transmission Depressed by Colchicine Blockade of Axoplasmic Flow

Abstract. *Colchicine, which inhibits axoplasmic transport and induces organelle alterations in nerve terminals, was injected intraocularly in pigeons. Electrical stimulation of the optic nerve yielded normal evoked potentials in retinotectal fibers, whereas postsynaptic responses recorded in the tectum were reduced. Postsynaptic depression suggests a deficit of synaptic transmission, presumably dependent on colchicine interference with migrating material.*

Material that is involved in rapid axonal flow and is synthesized in the neuronal perikarion migrates at speeds greater than 40 mm/day and reaches the presynaptic nerve terminals (*1*). Ganglion cells of the retina provide a good experimental preparation for axonal flow studies (*2*), particularly in birds whose optic fibers are generally considered to be completely crossed (*3*). A few hours after intraocular injection of radioactive amino acids, labeled proteins can be detected in the optic nerve terminals (*4*). The role of this migrating material in the presynaptic terminals is unknown, although investigations on adrenergic neurons suggest its involvement in catecholamine metabolism and in formation of dense core vesicles (*5*). Interference with the supply of this fast flow material to nerve endings may shed light on synaptic as well as axonal functions.

Colchicine has been shown to interfere with axoplasmic transport, especially with the rapid flow processes (*6*). When injected intraocularly in the pigeon, colchicine inhibits the appearance in the optic tectum of labeled proteins after introducton of [^{3}H]leucine in the vitreous humor 24 hours earlier. This effect induced by colchicine is evident within 1 day, reaches a maximum at 4 days, and disappears after a few weeks (*7*). Moreover, intraocular injection of colchicine in the pigeon causes reversible alterations in the ultrastructure of optic nerve terminals (*8*); within a week synaptic vesicles enlarge, fibrils aggregate, mitochondria swell, and glycogen granules accumulate. The first ultrastructural alterations are evident 4 days after colchicine injection and disappear gradually by 4 to 6 weeks. These changes are strikingly similar to those observed after retinal ablation, except that colchicine-induced effects are reversible (*9*).

These observations suggested that the rapid phases of axoplasmic transport

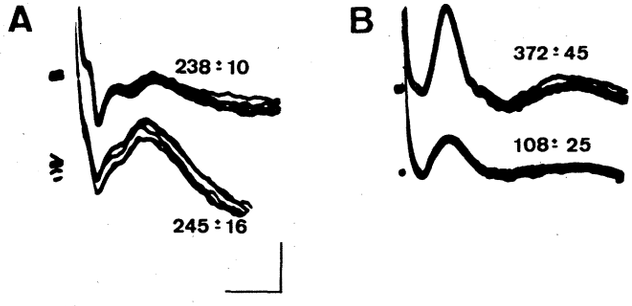

Fig. 1. (A) Optic tract responses and (B) tectal responses (depth, 500 μm) to optic nerve stimulation 3 days after injection of colchicine (100 μg) in the vitreous humor of one eye. Stimulus intensity, 1.0 ma; in both (A) and (B), upper trace from control side, lower trace from colchicine-treated side. Numbers indicate mean amplitude (μv) ± S.D. ($n = 10$); vertical calibration, 200 μv; horizontal calibration, 5 msec; positivity upward.

may contribute to the formation or maintenance of normal synaptic vesicles and perhaps other presynaptic contents implicated in synaptic transmission. We injected colchicine into one eye of a pigeon, stimulated the optic nerves electrically on both sides, and recorded evoked potentials in the retinotectal fibers and in the middle and deep layers of the optic tectum to determine integrity of the pre- and postsynaptic parts of the system (*10*). The contralateral visual pathway served as the control.

Colchicine (Fluka), in doses of 10 μg or 100 μg in 20 μl of Ringer solution, was injected into the vitreous humor of one eye of each of 13 pigeons (*Columba livia*) under methoxyflurane (Pentrane, Abbott) anesthesia. After 1, 3, 7, 14, 42, and 56 days, the animals were anesthetized with Equithesin (Jensen-Salsbery: chloral hydrate, $MgSO_4$, pentobarbital sodium), paralyzed by gallamine triethiodide (Flaxedil, Specia) and artificially ventilated. For the optic nerve stimulation, bipolar electrodes were inserted into the papilla of each eye after removal of the cornea and aspiration of the lens and crystalline body. Single square-wave pulses of 1 to 2 ma, 0.1-msec duration, and a frequency of 0.3 or 100 per second were delivered by a Grass S-8 stimulator. The posterior part of the telencephalon and anterior part of the optic lobes were exposed bilaterally and two tungsten electrodes (5 to 25 pfarad) were placed successively in the optic tecta and then in the optic tracts. Tektronix equipment was used for amplification and display of the evoked potentials. The electrode tracks were histologically verified for placement of electrode tips [technical details in (*4*, *8*, *11*)].

Responses evoked in the optic tracts did not differ in their shape, polarity, latency and amplitude by more than $\pm$ 10 percent between the colchicine-treated side and the control side (Fig. 1A). Similarly, with supramaximum stimulation, responses recorded at symmetrical points on the surface of the tectum were essentially identical (Figs. 2B and 3). These observations show that intraocular colchicine injection does not interfere with conduction along optic nerve fibers. In agreement with our observations, colchicine was found to have little effect on nerve fiber conduction in short-term experiments performed in vitro (*12*).

When the tectal electrodes were lowered into the optic tectum, however, striking differences between responses on the two sides were evident by 3 days after monocular injection of colchicine. From a depth of 300 μm downward, the amplitude of evoked potentials was reduced by 65 to 85 percent on the side corresponding to the eye that was injected (Figs. 1B, 2A, and 3). No latency changes or shifts in the direction of evoked potentials were observed. This implies that impulses conducted along the optic nerve fibers were not being adequately conveyed to the tectal neurons. From this experiment, the possibility of a partial block in the fiber terminals cannot be excluded and may play a role, although a deficit in synaptic transmission seems the more likely explanation.

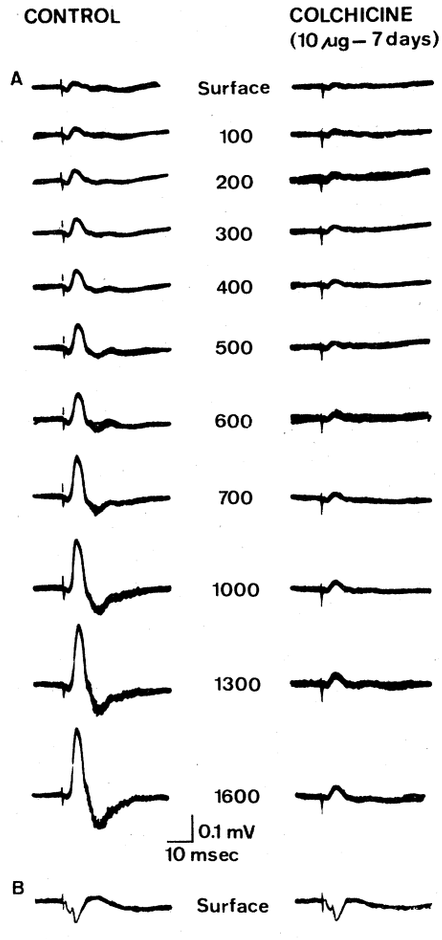

Fig. 2. Response of optic tectum to electrical stimulation of optic nerve 7 days after injection of colchicine (10 μg) in the vitreous humor of one eye. (A) Responses from the surface of the tectum to a depth of 1600 μm to a single shock of 1.0 ma. (B) Surface response to a single shock of 2.0 ma. The early negative waves, presumably corresponding to fiber activation, have the same amplitude on both sides. Positivity upward.

This colchicine-induced synaptic failure was studied with different doses and time intervals between injection and physiological assay. With both 10 μg and 100 μg of colchicine, the effect is evident 1 day and maximum 3 to 8 days after injection. After the 10-μg injection, normal potentials can be recorded from the depth of the tectum after 14 days, whereas with the 100-μg dose, recovery does take place within 6 to 8 weeks (Fig. 3). The effects of intraocular injection of colchicine, presumably by affecting synaptic transmission in the optic nerve terminals, reach a maximum within a few days and are reversible.

On the surface of the tectum, the response evoked by supramaximum optic nerve stimulation (1.5 ma) is characterized by a large, late negative wave that was practically not affected by colchicine (Fig. 2B). This response, although diminished by stimulation at a frequency of 100 per second, followed such a frequency better than the

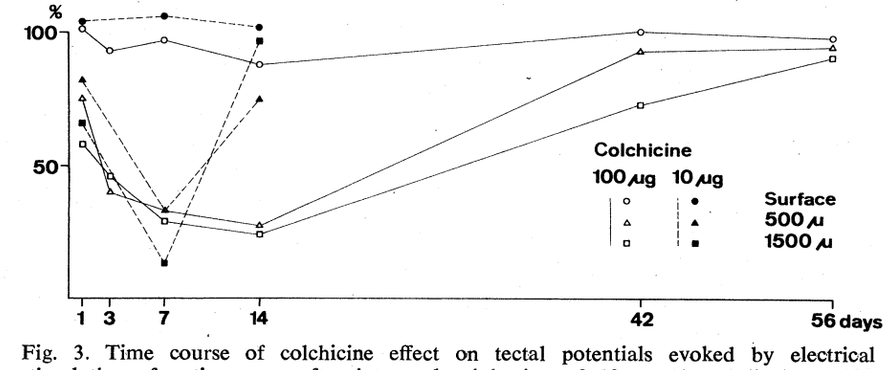

Fig. 3. Time course of colchicine effect on tectal potentials evoked by electrical stimulation of optic nerves after intraocular injection of 10 μg (dotted line) or 100 μg (solid line) of colchicine. Amplitude of potentials expressed as percent of control response in the same animal, recorded on the tectal surface (○) and at depths of 500 μm (△) and 1500 μm (□).

deep tectal, positive response. Contrary to the usual interpretation, this wave may be due to activation of slow conducting fibers. The deep response was depressed by both threshold and supramaximum stimulation.

The contralateral visual pathway used as control excludes a systemic effect of colchicine. One hour after direct colchicine application to the tectum the postsynaptic response to optic nerve stimulation was unaffected. It thus seems unlikely that the depression of synaptic transmission could result from a direct effect of colchicine on the nerve terminals, as might be anticipated from its prompt and drastic inhibitory effect on the release of catecholamine in the adrenal medulla (*13*). Intraocular injection of Ringer solution (20 μl) did not affect the tectal potentials recorded 7 days thereafter.

There is a striking convergence among the effects of intraocular injection of colchicine, that is, reduction of axonal transport (*6, 7*), alteration of organelles in the nerve terminals (*8*), and depression of synaptic transmission. The time course of these alterations is similar; the effect on protein transport appeared earlier than the other two, and all three were reversible within the same period. From the early appearance of these effects, we are tempted to conclude that proteins or other components migrating with the fast flow are more likely to be implicated in synaptic transmission than the material migrating slowly. Colchicine interference with slowly migrating material may contribute, however, to the relatively slow recovery of the phenomena. It should be noted that length of the nerve segment distal to the site of sectioning determines the time of onset of synaptic failure; the longer the axon, the later the alteration of the postsynaptic response (*14*). These observations point also to the dependence of the synaptic transmission on material supplied by the axon. In conclusion, although other effects of colchicine cannot be excluded, our results would be consistent with the notion that material, which is normally provided by the ganglion cell body to nerve terminals and which may be arrested by this drug, plays an essential role in the maintenance of synaptic functions.

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References and Notes

1. B. Grafstein, in *Advances in Biochemical Psychopharmacology*, E. Costa and P. Greengard, Eds. (Raven Press, New York, 1969), p. 11; S. H. Barondes, in *Handbook of Neurochemistry*, A. Lajtha, Ed. (Plenum Press, New York, 1969), vol. 2, p. 435; R. J. Lasek, *Int. Rev. Neurobiol.* **13**, 289 (1970); S. Ochs, in *Protein Metabolism of the Nervous System*, A. Lajtha, Ed. (Plenum Press, New York, 1970), p. 291; H. Koenig and B. Droz, *C. R. Acad. Sci. Ser. D* **272**, 2812 (1971).
2. A. C. Taylor and P. Weiss, *Proc. Nat. Acad. Sci. U.S.* **54**, 1521 (1965); B. Grafstein, *Science* **157**, 196 (1967); B. S. McEwen and B. Grafstein, *J. Cell Biol.* **38**, 494 (1968); J.-O. Karlsson and J. Sjöstrand, *Brain Res.* **11**, 431 (1968); *J. Neurochem.* **18**, 749 (1971); S. M. Chou, *Neurology* **20**, 607 (1970); J. S. Elam and B. W. Agranoff, *J. Neurochem.* **18**, 375 (1971).
3. M. Cuénod, in *The Structure and Function of Nervous Tissue*, G. H. Bourne, Ed. (Academic Press, New York, in press), vol. 5, p. 455.
4. ——— and J. Schonbach, *J. Neurochem.* **18**, 809 (1971); J. Schonbach and M. Cuénod, *Exp. Brain Res.* **12**, 275 (1971); J. Schonbach, C. Schonbach, M. Cuénod, *J. Comp. Neurol.* **141**, 485 (1971); A. E. Hendrickson and W. M. Cowan, *Exp. Neurol.* **30**, 403 (1971).
5. A. Dahlström, *Phil. Trans. Roy. Soc. London Ser. B* **261**, 325 (1971); L. B. Geffen and B. G. Livett, *Physiol. Rev.* **51**, 98 (1971).
6. F. O. Schmitt, *Proc. Nat. Acad. Sci. U.S.* **60**, 1092 (1968); G. W. Kreutzberg, *Int. Congr. Histo. Cytochem. Proc. 3rd* (1968), p. 133; *Proc. Nat. Acad. Sci. U.S.* **62**, 722 (1969); A. Dahlström, *Eur. J. Pharmacol.* **5**, 111 (1968); J.-O. Karlsson and J. Sjöstrand, *Brain Res.* **13**, 617 (1969); H. L. Fernandez, F. C. Huneeus, P. F. Davison, *J. Neurobiol.* **1**, 395 (1970); K. A. C. James, J. J. Bray, I. G. Morgan, L. Austin, *Biochem. J.* **117**, 767 (1970); J. Sjöstrand, M. Frizell, P.-O. Hasselgren, *J. Neurochem.* **17**, 1563 (1970); J.-O. Karlsson, H.-A. Hansson, J. Sjöstrand, *Z. Zellforsch. Mikrosk. Anat.* **115**, 265 (1971).
7. M. Cuénod, J. Boesch, C. Sandri, P. Marko, J. P. Susz, *Abstr. Int. Meet. Int. Soc. Neurochem. 3rd Budapest, 1971*, p. 155; J. Boesch, P. Marko, M. Cuénod, *Experientia* **27**, 719 (1971).
8. M. Cuénod, C. Sandri, K. Akert, *Brain Res.*, in press; M. Cuénod, J. Boesch, P. Marko, M. Perišić, C. Sandri, J. Schonbach, in *Information Transfer from a Neurophysiological and Neurochemical Point of View*, Gyllenberg Symposium, Helsinki, Finland; *Int. J. Neurosci.*, in press.
9. M. Cuénod, C. Sandri, K. Akert, *J. Cell Sci.* **6**, 605 (1970); K. Akert, M. Cuénod, H. Moor, *Brain Res.* **25**, 255 (1971).
10. J. L. O'Leary and G. H. Bishop, *J. Cell. Comp. Physiol.* **22**, 73 (1943); Y. Galifret, *Le Système Visuel du Pigeon* (Thèse, Paris, 1966); A. L. Holden, *J. Physiol. London* **194**, 75 (1968); F. Robert and M. Cuénod, *Exp. Brain Res.* **9**, 116 (1969).
11. M. Perišić, J. Mihailovic, M. Cuénod, *Int. J. Neurosci.* **2**, 7 (1971).
12. R. E. Hinkley and L. S. Green, *J. Neurobiol.* **2**, 97 (1971).
13. A. M. Poisner and J. Bernstein, *J. Pharmacol. Exp. Ther.* **177**, 102 (1971).
14. R. Miledi and C. R. Slater, *J. Physiol. London* **207**, 507 (1970); O. L. Vaccarezza, T. A. Reader, E. Pasqualini, J. Pecci-Saavedra, *Exp. Neurol.* **28**, 277 (1970).
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Release of Mercury from Contaminated Freshwater Sediments by the Runoff of Road Deicing Salt

Mercury is strongly held by the bottom sediments of natural water courses, and, as binding mechanisms, Krauskopf (*1*) has suggested (i) correlation or sorption on hydrated ferric oxide, (ii) surface sorption or ion exchange, or both, with naturally occurring mineral ion exchangers, such as montmorillonite, and (iii) sorption or chemical combination, or both, with organic material, such as peat and especially sulfur-containing matter. In view of these differ-

Table 1. Mercury partitioning at 25°C; S, sandy sediment; O, highly organic sediment. Both sandy and highly organic sediments are from Nagog Pond, Acton, Massachusetts. The moisture contents of the sandy and highly organic sediments were, respectively, 27 and 79 percent; the losses on ignition of the sandy and highly organic sediments were, respectively, 0.804 and 44.3 percent. Both types of sediments released some H_2S upon acidification; the highly organic sediments released more H_2S than the sandy sediments.

Sediment type	Salt added		Hg^{2+} content (ppm)		$(Hg^{2+})_{H_2O}/(Hg^{2+})_{sed}$	pH
	Formula	Amount (g/liter)	Dry sediment	Water		
S	None		41.2	0.024	5.8×10^{-4}	6.7
O	None		1430	< 0.00002	$< 1.4 \times 10^{-8}$	5.2
O	None		2670	0.0044	1.65×10^{-6}	5.1
S	NaCl	35	45.4	1.70	3.75×10^{-2}	6.6
O	NaCl	35	800	0.004	5.0×10^{-6}	4.8
O	NaCl	35	2670	25.0	9.4×10^{-3}	4.6
O	$CaCl_2$	165	800	1.58	1.9×10^{-3}	3.7
O	$CaCl_2$	165	1535	215.0	0.14	3.6