

lographic traces of compound action potentials obtained under experimental conditions identical to those of the upper portion of Fig. 2, except that the ganglion had been superfused for 24 minutes with 5 mM theophylline. The unconditioned test response (trace c) and the conditioning response (early part of trace d) were unaffected by theophylline. In contrast, the conditioned test response (later part of trace d) was considerably more reduced in amplitude (46 percent) and area (44 percent) than prior to theophylline. This increased inhibition of synaptic transmission after a conditioning stimulus, observed in the presence of theophylline, can be attributed to the potentiation of the slow-IPSP achieved by this phosphodiesterase inhibitor (Fig. 1A).

Our results indicate that cyclic AMP can mimic the electrophysiologic effects of dopamine, a putative ganglionic neurotransmitter. Cyclic AMP has been found to mimic the hyperpolarizing action of β -adrenergic agonists on the Purkinje cells of the rat cerebellum (12) and on the smooth muscle cells of the rabbit pulmonary artery (13). In the case of the Purkinje cells, theophylline potentiated, and PGE₁ blocked the inhibition of spontaneous discharge caused by application of exogenous norepinephrine (12).

Our electrophysiological data support the hypothesis that cyclic AMP plays a role in synaptic transmission in sympathetic ganglia. There appear to be both direct excitatory and interneuron-mediated inhibitory input from the preganglionic fibers to the postganglionic neurons of the superior cervical ganglion (10, 14). Our evidence supports the idea (2-4) that the slow-IPSP is generated by an increase in the amount of cyclic AMP in the postganglionic neurons in response to dopamine released from the interneurons. The hyperpolarization of the postganglionic neurons makes them less responsive to subsequent excitatory input. According to this scheme, cyclic AMP mediates dopaminergic transmission and, thereby, modulates cholinergic transmission in the ganglion, the modulation being of an inhibitory type that produces a negative feedback and limits the effectiveness of subsequent excitation.

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References and Notes

1. D. A. McAfee, M. Schorderet, P. Greengard, *Pharmacology* 12, 488 (1970).
2. ———, *Science* 171, 1156 (1971).
3. J. W. Keabian and P. Greengard, *ibid.* 174, 1346 (1971).
4. P. Greengard, D. A. McAfee, J. Keabian, in *Advances in Cyclic Nucleotide Research*, P. Greengard, R. Peoliti, O. A. Robison, Eds. (Raven, New York, in press), vol. 1.
5. B. Libet and T. Tosa, *Proc. Nat. Acad. Sci. U.S.A.* 67, 667 (1970); B. Libet, *Fed. Proc.* 29, 1945 (1970).
6. P. Greengard and D. A. McAfee, *Biochem. Soc. Symp.*, in press.
7. D. A. McAfee, P. Kall, M. Schorderet, P. Greengard, in preparation.
8. H. W. Kostelitz, G. M. Lee, D. I. Wallis, *J. Physiol. London* 195, 39 (1968). Ganglia were removed from New Zealand white rabbits under urethane anesthesia (1 g/kg), were decapsulated, and were positioned in the sucrose gap apparatus. The preganglionic (cervical sympathetic) nerve was stimulated through platinum bipolar electrodes. Locke solution containing various agents superfused the ganglion at 1 to 2 ml/min. A short segment of the proximal portion of the postganglionic (internal carotid) nerve was superfused with 215 mM sucrose, at 0.3 ml/min, and normal Locke solution superfused the distal end of the postganglionic nerve at 0.5 to 1 ml/min. Solution flow through the various chambers was monitored by precision flow meters. The 6-c potential difference between the ganglion and the end of the postganglionic nerve (that is, across the sucrose gap) was amplified and displayed on an oscilloscope and on a strip-chart servo recorder. The potential difference across the sucrose gap with the ganglion in normal Locke solution was 3 mV or less, and the resistance was usually 0.8 to 0.9 megohm. The design of the sucrose gap apparatus and the precise control of flow rates reduced flow artifacts and eliminated associated problems. The composition of the Locke solution (in millimoles per liter) was: NaCl, 136; KCl, 5.6; NaHCO₃, 30.0; NaH₂PO₄, 1.2; CaCl₂, 2.3; MgCl₂, 1.2; and glucose, 5.5. The Locke solution was equilibrated with a gas mixture of 95 percent O₂ and 5 percent CO₂ and had a pH of 7.2 to 7.3 at the temperature of the experiments (23° to 25°C).
9. W. J. George, J. B. Polson, A. G. O'Toole, N. D. Goldberger, *Proc. Nat. Acad. Sci. U.S.A.* 66, 398 (1970); J. A. Ferrendelli, A. L. Sierner, R. McDougal, D. M. Kipnis, *Biochem. Biophys. Res. Commun.* 41, 1061 (1970); J. F. Kuo, T.-P. Lee, P. L. Ryeck, R. G. Walton, T. E. Donnelly, P. Greengard, *J. Biol. Chem.* 247, 16 (1972).
10. R. M. Eccles and B. Libet, *J. Physiol. London* 157, 484 (1961).
11. A. P. McAfee, A. V. Somlyo, N. Friedman, *Ann. N.Y. Acad. Sci.* 188, 108 (1971).
12. C. G. W. Hoar, J. Hoffer, P. E. Bloom, *Science* 188, 1019 (1969).
13. A. V. Somlyo, G. Haesler, J. P. Somlyo, *ibid.* 189, 490 (1970).
14. T. H. Williams and S. L. Pelay, *Brain Res.* 15, 17 (1969); A. Björklund, L. Cegrell, B. Falck, M. Riisen, E. Rosenkrantz, *Acta Physiol. Scand.* 78, 334 (1970).
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Polychlorinated Biphenyls: Metabolic Behavior of Pure Isomers in Pigeons, Rats, and Brook Trout

Abstract. The metabolic behavior of pure mono-, di-, tetra-, and hexachlorobiphenyl isomers in pigeons, rats, and brook trout was investigated. Excreta from these animals were extracted and examined by chromatographic and mass spectrometric techniques. The results showed conversion of the 4-chloro-, 4,4'-dichloro-, and 2,2',5,5'-tetrachlorobiphenyl isomers into monohydroxylated derivatives by the rat and pigeon whereas no hydroxymetabolites were detected in the excreta of the brook trout. No hydroxylated products of 2,2',4,4',5,5'-hexachlorobiphenyl were detected in the excreta of pigeons, rats, or brook trout.

Polychlorinated biphenyls (PCB) are now recognized as almost universally distributed pollutants which are generally considered to be quite resistant to chemical and enzymatic degradation. Recent evidence suggests that chloro-

Table 1. Data on hydroxylated metabolites from rat urine and pigeon excreta. This-layer chromatography on silica; solvent A, hexane-acetone, 2.5 : 1; solvent B, benzene-ethyl acetate, 12 : 1.

Compound administered*	Hydroxylchlorobiphenyl R _f in solvent†		Mass spectrum	
	A	B	Molecular ion	Number of chlorine atoms in the metabolite
4-Chlorobiphenyl	0.5	0.5	204	1
4-Chlorobiphenyl	<0.3		220	1†
4,4'-Dichlorobiphenyl	0.58	0.6	238†	2
2,2',5,5'-Tetrachlorobiphenyl	0.558	0.55	306	4

* No hydroxymetabolites could be detected in the excreta of rats or pigeons treated with 2,2',4,4',5,5'-hexachlorobiphenyl. † The PCB isomers move with the solvent front in these systems. ‡ This compound was found in rat urine only (that is, not in pigeon excreta). § Several minor bands in this region were extracted together; peaks due to impurities were present in the mass spectrum at different temperatures. Compounds were made visible by ultraviolet light or by spraying a portion of the plate with a 1 percent solution of 2,4,7-trinitro-9-fluorenone in acetone. ¶ The accurate mass was determined for this compound. Mass calculated for C₁₂H₆Cl₄O: 237.9952; mass found: 237.9959.

approximately 30 seconds. The slow-IPSP, but not the initial EPSP, was potentiated in amplitude and duration in the presence of theophylline (Fig. 1A). Theophylline did not inhibit either the amplitude or the duration of the slow-EPSP (not shown). The hyperpolarization of the postganglionic neurons induced by exogenous dopamine was also potentiated by theophylline (Fig. 1C). The response to dopamine was tested by switching for 3 minutes from a superfusate of normal Locke solution to one containing $50 \mu\text{M}$ dopamine (prepared 2 to 5 minutes previously) and then by returning to the normal Locke solution. Theophylline (1 to 5 mM) in nine experiments caused an increase of 44 ± 7 percent (mean $\pm$ S.E.M.) in the amplitude of the slow-IPSP, and an increase of 54 ± 9 percent in the amplitude of the hyperpolarization induced by 50 to $200 \mu\text{M}$ dopamine. The effect of the theophylline could be reversed by superfusion of the ganglion with normal Locke solution for 90 minutes.

In each of 15 ganglion preparations studied, 10^{-7} to 10^{-4} M PGE₁ virtually abolished the slow-IPSP within 10 to 20 minutes and substantially reduced the slow-EPSP, but had no effect on the initial EPSP. (A transient hyperpolarization of the postganglionic neurons of 5 to 10 minutes duration, was usually observed on starting PGE₁ superfusion.) The effect of $3 \times 10^{-7} \text{ M}$ PGE₁ on the slow-IPSP is illustrated in Fig. 1B. A concentration of PGE₁ of $1 \times 10^{-4} \text{ M}$ caused a 50 percent decrease in the amplitude of the slow-IPSP. In nine ganglion preparations, the effect of PGE₁ was tested on the hyperpolarization induced by 50 to $200 \mu\text{M}$ dopamine. It was found that PGE₁ abolished, or largely reduced, the dopamine-induced hyperpolarization at the same concentrations as were effective in abolishing the slow-IPSP (Fig. 1D). The effects of PGE₁ could be largely reversed on prolonged superfusion with normal Locke solution.

Monobutyl cyclic AMP, applied in a concentration of 1 to 2.5 mM , hyperpolarized 8 of 11 ganglia tested (Fig. 1E). Qualitatively similar results were obtained with cyclic AMP and dibutyl cyclic AMP, but not with adenosine 5'-monophosphate, adenosine, or butyric acid. Cyclic AMP and its derivatives were never observed to cause depolarization. The lack of any response, by some of the preparations, to the direct application of cyclic AMP or its derivatives may be due to a low

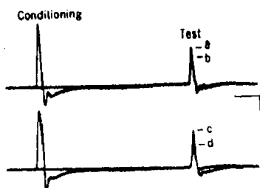


Fig. 2. Compound action potentials, elicited by submaximum stimulation of the preganglionic (cervical sympathetic) nerve, and recorded from platinum bipolar electrodes on the postganglionic (internal carotid) nerve 2 to 3 mm distant from the pole of the ganglion. Upper row, superimposed traces a and b recorded during superfusion of the ganglion with normal Locke solution. Lower row, superimposed traces c and d recorded 24 minutes after start of superfusion with Locke solution containing 5 mM theophylline. Traces a and c, response to a single test stimulus only. Traces b and d, response to a conditioning stimulus followed 600 msec later by response to a test stimulus. Conditioning stimuli were just maximum for the S₁ elevation (see (10)); voltage of the test stimuli was one-half that of the conditioning stimuli; pulse width for both kinds of stimuli was 0.5 msec . Calibration mark: $200 \mu\text{V}$, 100 msec . Bandwidth, 2 Hz to 10 kHz .

permeability of neuronal membranes to cyclic AMP.

Studies have shown that acetylcholine can cause an increase in the amount of guanosine 3',5'-monophosphate (cyclic GMP) in heart and brain tissue (9). Moreover, available evidence indicates the existence, on postganglionic neurons of the superior cervical ganglion, of muscarinic-type receptors that respond to acetylcholine by causing a prolonged depolarization of the neurons (10). These same receptors are probably involved in the generation of the slow-EPSP (10). In view of the possibility that cyclic GMP might mediate this muscarinic depolarizing action of acetylcholine, we have studied the effect of cyclic GMP and dibutyl cyclic GMP on the resting membrane potential of postganglionic neurons. Cyclic GMP itself did not cause a change in membrane potential. However, exposure of the ganglia to dibutyl cyclic GMP, in low concentrations (2.5 to $5.0 \times 10^{-6} \text{ M}$) for 4 minutes, caused a small, transient hyperpolarization followed by a depolarization of the postganglionic nerve cells in each of seven preparations tested (Fig. 1F). A depolarization of several millivolts could be achieved by maintaining the

ganglion in solutions of dibutyl cyclic GMP for longer periods of time (5 to 10 minutes). Higher doses (100 to $250 \mu\text{M}$) of dibutyl cyclic GMP greatly enhanced the rate of depolarization of the postganglionic neurons but either had no effect on, or decreased the size of, the transient hyperpolarization. These observations are in contrast to those made on liver slices where both cyclic AMP and cyclic GMP caused a hyperpolarization (11). Conceivably, cyclic GMP may mediate the slow-EPSP and, thereby, increase the responsiveness of the postganglionic neurons to subsequent excitatory input. If so, this would indicate that cyclic AMP and cyclic GMP function in opposite directions, that is, in a push-pull fashion to exert long-term control over neuronal excitability in the sympathetic ganglion.

Dopamine, theophylline, PGE₁, cyclic GMP, and cyclic AMP and its butyryl derivatives, when tested on axons of the cervical vagus nerve in the same concentrations that had been used on the ganglion, were found to cause little or no effect on the membrane potential. High concentrations of dibutyl cyclic GMP (1 to $4 \times 10^{-4} \text{ M}$) had an effect on the vagus nerve (Fig. 1G) similar to that which had been observed on the ganglion with lower concentrations.

We have been able to demonstrate consistently and reproducibly, with each of 13 preparations, that the excitability of postganglionic neurons in the superior cervical ganglion was diminished during the period of the slow-IPSP and that this inhibition was markedly potentiated by theophylline. These effects are illustrated in Fig. 2 where the unconditioned response to a submaximum test stimulus is compared with the response to the same strength of stimulus applied 600 msec after a stronger conditioning stimulus, that is, at the time of maximum development of the slow-IPSP. The upper portion of Fig. 2 contains two superimposed oscillographic traces of compound action potentials derived from the postganglionic nerve with platinum bipolar recording electrodes. The test response in trace a was elicited without a prior conditioning stimulus and therefore did not occur during a slow-IPSP. The test response elicited 600 msec after a conditioning response (trace b) and, therefore, during the slow-IPSP, was reduced 24 percent in amplitude and 20 percent in area compared to the unconditioned test response. The lower portion of Fig. 2 contains two superimposed oscil-

biphenyls do show chemical changes when exposed to ultraviolet light (1). Experiments with commercial PCB preparations also indicate that certain components, mainly those of lower chlorine content, are metabolized by rats (2), pigeons (3, 4), and Japanese quail (3). The evidence is based on the disappearance of certain peaks in the gas chromatograms of PCB extracted from tissue. A similar disappearance of peaks, on the other hand, was not observed in fish fed food containing Aroclor 1254 (5).

Because of their complex isomer composition, commercial PCB preparations are unsuitable for a study of the metabolic behavior in which individual compounds (metabolites) are to be identified. We know of only one study of the metabolism of a chemically defined chlorobiphenyl in the literature (6). In this case 4-chlorobiphenyl was shown to be converted to 4-chloro-4'-hydroxybiphenyl and its conjugates by the rabbit.

We have examined the metabolic behavior of 4-chlorobiphenyl, 4,4'-dichlorobiphenyl, 2,2',5,5'-tetrachlorobiphenyl, and 2,2',4,4',5,5'-hexachlorobiphenyl in laboratory rats, Carneau pigeons, and brook trout in an effort to observe (i) species differences and (ii) differences in the metabolic behavior of PCB components of different chlorine content. The compounds were chosen for their occurrence in commercial PCB preparations (7).

An intraperitoneal injection of the chlorobiphenyl (8) dissolved in oil (50 mg/kg) was administered to each of the young male rats housed in metabolic cages each day for 3 days. A capsule containing a solution of the chlorobiphenyl in corn oil (15 to 20 percent) (60 to 100 mg/kg) was fed to each of the pigeons each day for 3 days. Urine and feces were collected for 1 week from the start of the treatment from treated and untreated rats. Urine samples were hydrolyzed by refluxing with an equal volume of 8N sulfuric acid for 1 hour and extracted with ether. The feces were extracted with hexane at 50°C. In the case of the pigeons, the total excreta for 1 week from the start of the treatment were hydrolyzed and extracted. By comparing thin-layer chromatograms (Table 1) of samples from the treated and untreated animals, the extra bands due to the chlorobiphenyls administered could be located, extracted, and analyzed by gas chromatography and mass spectrometry.

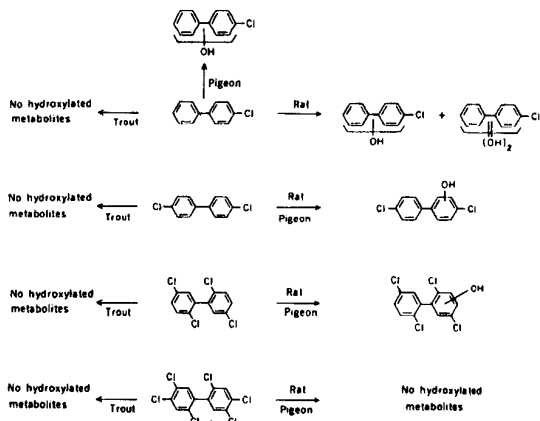


Fig. 1. Scheme for the metabolism of chlorobiphenyls in three animal species.

Each brook trout was fed a single dose of the chlorobiphenyl (460 mg to 1.2 g/kg) (the compound was concealed in chunks of beef liver). Immediately after being fed, the trout were transferred to a 5-liter tank and kept there for 4 days. The water in which the fish were kept during the 4 days after treatment was concentrated to 1 liter, acidified with concentrated hydrochloric acid, and refluxed for 3 hours. The acidified solution was extracted with ether, and the extracts were prepared and examined for hydroxy-chlorobiphenyls as described above.

Data for products obtained from animals treated with chlorobiphenyls are reported in Table 1 and Fig. 1. In agreement with earlier observations on the metabolism of chloronaphthalenes (9) and chlorobenzenes (10), mammalian metabolism (hydroxylation) of chlorobiphenyls seems to become increasingly more difficult as the number of chlorine atoms in the molecule increases. In the rat, 4-chlorobiphenyl is metabolized to a mono- and a dihydroxychlorobiphenyl, and only little starting material could be recovered from the feces (no starting material could be recovered from the urine). In the case of 4,4'-dichloro- and 2,2',5,5'-tetrachlorobiphenyl, large quantities of the unchanged material could be extracted from rat feces and only a mono-hydroxy derivative was identified in rat urine. With the method used in this experiment, no hydroxymetabolites

could be detected in the urine of rats treated with 2,2',4,4',5,5'-hexachlorobiphenyl but the unchanged compound was found in the feces. In all instances no evidence was found for reductive dechlorination in the animals tested.

The metabolism of the four chlorobiphenyls in pigeons appears to be similar to that in rats except that no dihydroxychlorobiphenyl was present in excreta from birds fed 4-chlorobiphenyl. The specificity of the hydroxylation reactions is not known. On the basis of qualitative observations, the ease of formation of hydroxychlorobiphenyls in rats and pigeons seems to be as follows: 4-chloro $\approx$ 4,4'-dichloro $>$ 2,2',5,5'-tetrachloro $>$ 2,2',4,4',5,5'-hexachloro.

For the conditions and methodology used here, there was no evidence for the excretion of hydroxylated chlorobiphenyls in brook trout.

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References and Notes

1. S. Safe and O. Hutzinger, *Nature* **233**, 641 (1971); K. Huster and F. Korte, *Chemosphere* **1**, 7 (1972); O. Hutzinger, S. Safe, V. Zizko, *Environ. Health Perspect.* **1**, 13 (1972).
2. D. L. Grant, W. E. T. Phillips, D. C. Villeneuve, *Bull. Environ. Contam. Toxicol.* **6**, 102 (1971).
3. S. Bailey and P. J. Bunyan, *Nature* **236**, 34 (1972).
4. A. S. W. DeFries, R. T. Norstrom, O. Hutzinger, paper presented at the 164th meeting of the American Chemical Society, New York, 1972 (paper No. 32, Division of Water, Air, and Waste Chemistry).
5. V. Zizko and O. Hutzinger, in preparation.
6. W. D. Block and H. H. Cornish, *J. Biol. Chem.* **234**, 3301 (1959).
7. D. Stinson and D. Wehl, *J. Chromatogr.* **60**, 15 (1971); R. G. Webb and A. C. McCall, *J. Ass. Off. Anal. Chem.* **53**, 746 (1972).
8. O. Hutzinger, S. Safe, V. Zizko, *Bull. Environ. Contam. Toxicol.* **6**, 209 (1971); S. Safe and O. Hutzinger, *J. Chem. Soc. Protein Trans.* **1**, 486 (1972).
9. H. H. Cornish and W. D. Block, *J. Biol. Chem.* **231**, 513 (1958).
10. D. V. Parke and R. T. Williams, *Biochem. J.* **74**, 5 (1960), and references cited therein.
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Raphides with Barbs and Grooves in *Xanthosoma sagittifolium* (Araceae)

Abstract. *Raphides* in petioles of *Xanthosoma sagittifolium* are needlelike crystals about 50 micrometers long. The rectangular cross sections have maximum dimensions of approximately 850 by 250 nanometers. The raphides have two distinct end structures. One end is narrow, acute, and tapered to a point; the other is broad, acute, and abruptly pointed. Barbs, about 750 angstroms long with tips oriented away from the narrow end, occur along the length of the raphide on ridges on either side of two longitudinal grooves. These grooves, located opposite each other, give the raphide cross section an H-shape.

Raphides are needle-shaped crystals of calcium oxalate, occurring in bundles within specialized cells of certain flowering plants (1). Ingestion of fresh plant tissue containing raphides usually results in immediate and often severe irritation of the mouth and throat. Two reasons for this irritation have been suggested: (i) mechanical irritation by the crystal itself (2) or (ii) chemical irritation by a curare-

like drug associated with the crystal (3).

We describe barbs and grooves on raphides, which probably act as mechanical irritants and possibly act by carrying a chemical irritant into the wound produced by the crystal. To our knowledge, the barbs have not been reported before.

Xanthosoma sagittifolium (L.) Schott, commonly known as xannia or yautia, is an edible aroid grown as

a subsistence or commercial crop in many Pacific islands (4). The corm is baked or boiled and eaten as a source of starch. Leaves of other species of *Xanthosoma* are cooked and eaten in the same way as spinach. Specialized cells containing raphides occur in all organs of these plants.

In this study petiolar material was collected from plants grown at the Lyon Arboretum at the University of Hawaii. Fresh material was observed with a Zeiss RA light microscope. Sections of tissue for scanning electron microscopy were crushed on the sample holder to force mechanical release of the raphides from the specialized cells in which they developed (Fig. 1A). The samples were dried in air for 5 minutes, coated with an Au-Pd alloy in a vacuum evaporator, and viewed and photographed with a JEOL JSM-U3 electron microscope operated at 15 kv.

The use of crushed tissue in the scanning electron microscope is an excellent method for observing raphides. Raphides are held in the tissue, and the irregularity of the tissue surface makes it possible to observe the crystal morphology in various orientations. The needlelike raphides of *X. sagittifolium* are about 50 μ m in length (Fig. 1B). They have two distinct end structures. One end is broad, acute, and abruptly pointed (Fig. 1C), and the other is narrow, acute, and tapering (Fig. 1D). Two grooves located opposite each other run the length of the crystal. The cross sections have maximum dimensions of approximately 850 by 250 nm.

The grooves in the sides give the cross section an H-shape (Fig. 1C). Similar cross sections can be seen in transmission electron micrographs of *Lemna* and *Spirodella* (5). No previous description of grooves has been made. In *X. sagittifolium* the grooves are shallow near the narrow point, but they appear to extend almost to the tip (Fig. 1D). These grooves may allow material (possibly a chemical irritant) to be carried into tissues with the raphide. The grooves may also prevent throat or mouth tissue from sealing around the raphide. Their small size may allow tissue fluids to leak along the groove, as occurs in the blood channels of some weapons.

Prominent barbs occur on ridges on either side of the grooves along the length of the crystals. The tips of the barbs are oriented away from the narrow end (Fig. 1D) and toward the



Fig. 1. (A and B) Light micrographs. (A) A specialized cell, containing many raphides in a bundle, protruding into the petiolar air canal, and (B) a single raphide. (C and D) Scanning electron micrographs. (C) Two raphides showing barbs and grooves, both at the broad, acute, abruptly pointed end. The broken one shows the two grooves on the narrow sides of the raphide. (D) The narrow, acute, tapering point of the raphide. Note the orientation of the tips of the barbs away from this end.