tographic 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 m*M* 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 PGE_1 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, *Pharmacologist* **12**, 488 (1970).
2. ———, *Science* **171**, 1156 (1971).
3. J. W. Kebabian and P. Greengard, *ibid.* **174**, 1346 (1971).
4. P. Greengard, D. A. McAfee, J. Kebabian, in *Advances in Cyclic Nucleotide Research*, P. Greengard, R. Paoletti, G. A. Robison, Eds. (Raven, New York, in press), vol. 1.
5. B. Libet and T. Tosaka, *Proc. Nat. Acad. Sci. U.S.A.* **67**, 657 (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. Kalix, M. Schorderet, P. Greengard, in preparation.
8. H. W. Kosterlitz, G. M. Lees, D. J. 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 315 m*M* 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 d-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 5 mv or less, and the resistance was usually 0.4 to 0.8 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; $NaHCO_3$, 20.0; NaH_2PO_4, 1.2; $CaCl_2$, 2.2; $MgCl_2$, 1.2; and glucose, 5.5. The Locke solution was equilibrated with a gas mixture of 95 percent O_2 and 5 percent CO_2 and had a *p*H 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. Goldberg, *Proc. Nat. Acad. Sci. U.S.A.* **66**, 398 (1970); J. A. Ferrendelli, A. L. Steiner, D. R. McDougal, D. M. Kipnis, *Biochem. Biophys. Res. Commun.* **41**, 1061 (1970); J. F. Kuo, T.-P. Lee, P. L. Reyes, K. 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. Somlyo, A. V. Somlyo, N. Friedman, *Ann. N.Y. Acad. Sci.* **185**, 108 (1971).
12. G. R. Siggins, B. J. Hoffer, F. E. Bloom, *Science* **165**, 1018 (1969).
13. A. V. Somlyo, G. Haeusler, A. P. Somlyo, *ibid.* **169**, 490 (1970).
14. T. H. Williams and S. L. Palay, *Brain Res.* **15**, 17 (1969); A. Björklund, L. Cegrell, B. Falck, M. Ritzén, E. Rosengren, *Acta Physiol. Scand.* **78**, 334 (1970).
15. Supported by PHS grants NS 08440 and MH 17387, NSF grant GB 27510, and a postdoctoral fellowship from the Connecticut Heart Association to D.A.M.

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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. Thin-layer chromatography on silica; solvent A, hexane-acetone, 2.5 : 1; solvent B, benzene-ethyl acetate, 12 : 1.

Compound administered*	Hydroxychlorobiphenyl 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	0.5§	0.6	238‖	2
2,2',5,5'-Tetrachlorobiphenyl†	0.55§	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 viewing with 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 $C_{12}H_7Cl_2O$: 237.9952; mass found, 237.99[illegible]

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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 8*N* 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.

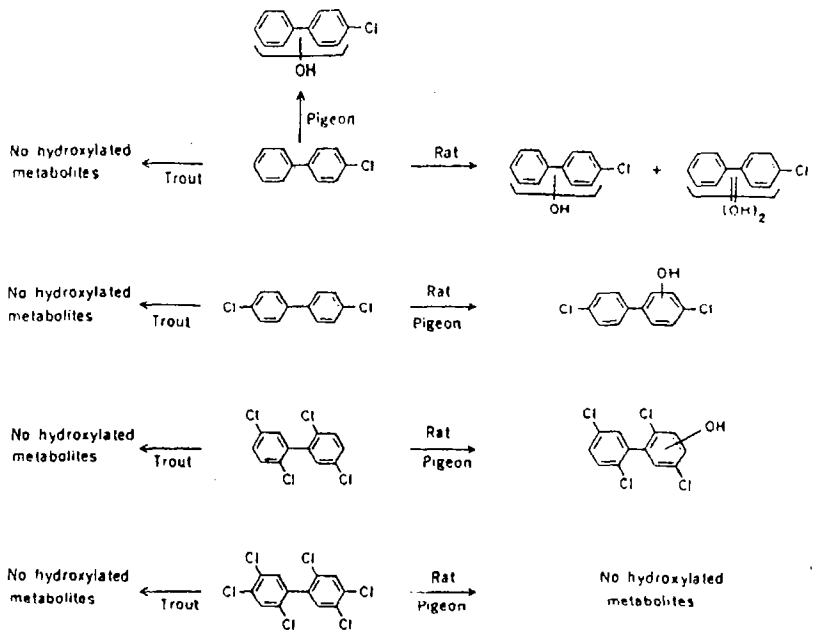


Fig. 1. Scheme for the metabolism of chlorobiphenyls in three animal species.

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 hydroxychlorobiphenyls 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 monohydroxy 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 ≧ 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* 232, 641 (1971); K. Hustert and F. Korte, *Chemosphere* 1, 7 (1972); O. Hutzinger, S. Safe, V. Zitko, *Environ. Health Perspect.* 1, 15 (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. DeFreitas, 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. Zitko and O. Hutzinger, in preparation.
6. W. D. Block and H. H. Cornish, *J. Biol. Chem.* 234, 3301 (1959).
7. D. Sissons and D. [illegible], 15 (1971); R. G. Webb and A. C. McCall, *J. Ass. Off. Anal. Chem.* 55, 746 (1972).
8. O. Hutzinger, S. Safe, V. Zitko, *Bull. Environ. Contam. Toxicol.* 6, 209 (1971); S. Safe and O. Hutzinger, *J. Chem. Soc. Perkin Trans.* 1, 686 (1972).
9. H. H. Cornish and W. D. Block, *J. Biol. Chem.* 231, 583 (1958).
10. D. V. Parke and R. T. Williams, *Biochem. J.* 74, 5 (1960), and references cited therein.

11. We thank Dr. D. J. Ecobichon who supplied samples of rat urine and feces and Dr. W. D. Jamieson who carried out the high-resolution mass spectral measurements. National Research Council of Canada contribution No. 12733.

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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 many Pacific islands (*4*). The corm i baked or boiled and eaten as a sourc of starch. Leaves of other species o *Xanthosoma* are cooked and eaten i the same way as spinach. Specialize cells containing raphides occur in al organs of these plants.

In this study petiolar material wa collected from plants grown at th Lyon Arboretum at the University o Hawaii. Fresh material was observe 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 μm 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

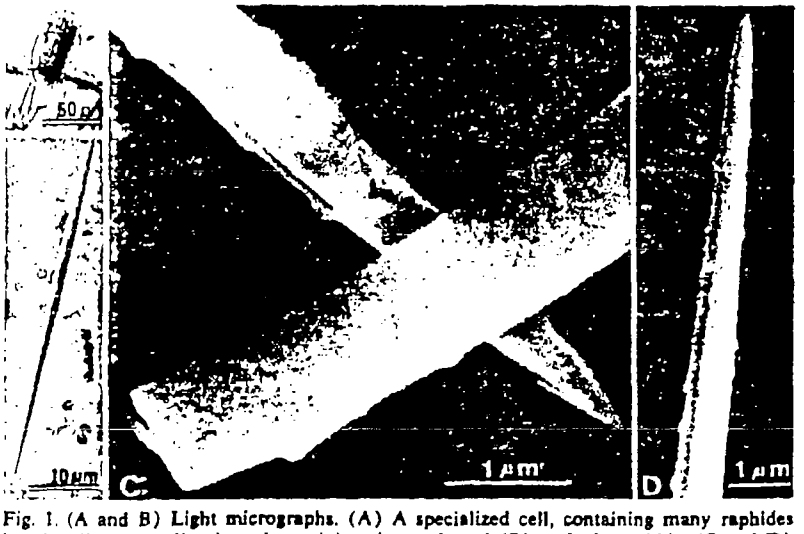


Fig. 1. (A and B) Light micrographs. (A) A specialized cell, containing many raphides in a bundle, protruding into the petiolar air canal, and (B) a single raphide. (C and D) Scanning electron micrographs. (C) Two raphides showing barbs and grooves, both at the broad, acute, abruptly pointed end. The 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.

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