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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.

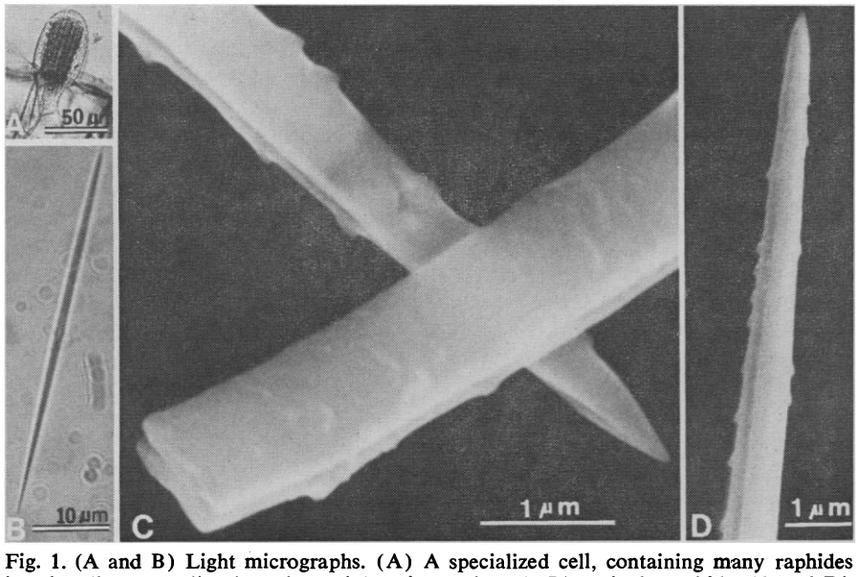

Fig. 1. (A and B) Light micrographs. (A) A specialized cell, containing many raphides in a bundle, protruding into the petiolar air canal, and (B) a single raphide. (C and D) Scanning electron micrographs. (C) Two raphides showing barbs and grooves, both at the broad, acute, abruptly pointed end. The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

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 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

broad end (Fig. 1C). These barbs protrude about 750 Å from the ridges of the raphide. This orientation of barbs on raphides of *X. sagittifolium* would make dislodging difficult once a raphide penetrated the mouth and throat tissues. Also, pressure against the abruptly pointed end of the crystal would force the raphide deeper into these tissues.

When examined in the scanning electron microscope, the raphides show a very complex morphology. They can no longer be viewed as simple needle-like crystals of calcium oxalate. It can also be seen that the monoclinic form of calcium oxalate monohydrate (*6*) is not the only factor in determining raphide structure. The occurrence of grooves and barbs is probably determined by biological processes under genetic control. In fact, in *Eichhornia crassipes* and *Yucca brevifolia* the raphides do not appear to have grooves (*5*). Haberlandt (*2*) suggested that raphides performed an ecological function by providing the plant with mechanical protection against noxious animals. Our findings are in agreement with this hypothesis. It appears that the raphides of *X. sagittifolium* would have a selective advantage in discouraging grazing animals.

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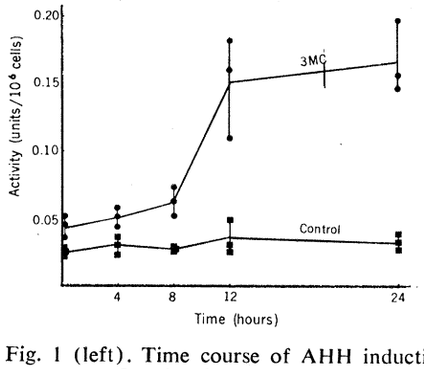
Aryl Hydrocarbon Hydroxylase Induction in Human Leukocytes

Abstract. *A method for determining aryl hydrocarbon hydroxylase induction in human leukocytes is described. Leukocytes from healthy volunteers were cultured in the presence of phytohemagglutinin, a mitogen. Addition of 3-methylcholanthrene to 72-hour cultures induced a fourfold increase in aryl hydroxylase activity. In the absence of a mitogenic agent, 3-methylcholanthrene stimulation of increased enzymatic activity did not occur.*

Benzo[*a*]pyrene (BP), 3-methylcholanthrene (3MC), and related polycyclic hydrocarbons are carcinogens commonly present in tobacco smoke (*1*), polluted city air (*2*), and certain foods (*3*). The enzyme system aryl hydrocarbon hydroxylase (AHH) functions in the biotransformation of BP and related compounds to hydroxylated metabolites with resultant alteration of their carcinogenic activity. The exact relation between AHH activity and resistance to tumor initiation by environmental carcinogens is not well understood. This inducible enzyme occurs in many mammalian tissues including liver, lung, intestinal mucosa, thyroid, testis, adrenal cortex (*4*), striated muscle (*5*), placenta (*6*), and skin (*7, 8*). The inducibility of AHH shows genetic variation in the mouse, and is controlled by a single autosomal dominant locus (*9*). If this genetic control is as simple in man the implications would be profound.

Increased activity of AHH has been found in human placenta from cigarette smokers (*6*), and in cultured human foreskin from newborn infants (*8*). The lack of a method for assaying readily available human tissues has confined previous studies of AHH induction and activity to select populations. The demonstration of AHH induction in rat Kupffer cells (*10*) suggested agranular leukocytes (precursors of Kupffer cells) (*11*), may be inducible under appropriate conditions. We describe here a method for measuring the induction of AHH activity in normal human leukocytes.

To 10 ml of heparinized blood we added an equal volume of 3 percent dextran in normal saline and allowed the erythrocytes to sediment for 45 minutes at room temperature. The leukocyte-rich supernatant was centrifuged at 1000*g* for 3 minutes. The pellet was suspended in Gibco chromosome medium 1-A which contains phytohemagglutinin. Culture tubes were prepared containing 2×10^6 to 4×10^6 cells in 5 ml of medium. After incubation for specified intervals at 37°C appropriate tubes received 5 μl of 0.75 m*M* 3MC in methanol. Control tubes received only methanol. The cells were harvested 24 hours after addition of 3MC, and were suspended in 1.0 ml of 50 m*M* tris-HCl, *p*H 7.5, containing 3 m*M* $MgCl_2$ and 0.2*M* sucrose (TMS). Cell counts were made from the 1.0 ml cell suspension after a 1 : 1 dilution of a 25-μl portion with 1 percent acetic acid containing a trace of gentian violet. Leukocytes were homogenized in a glass tissue grinder, and



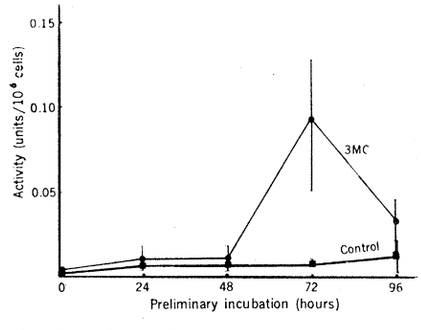


Fig. 1 (left). Time course of AHH induction by 3-methylcholanthrene (*3MC*) Leukocytes were first incubated for 72 hours in medium containing phytohemagglutinin. 3-Methylcholanthrene was added to a final concentration of 0.75 μM. Cells were harvested at the indicated intervals and were assayed for AHH activity. Each point represents a single determination; vertical bars represent the range of values. A unit of activity is the fluorescence produced equivalent to a picomole of 3-hydroxybenzopyrene per minute. Fig. 2 (right). Response of leukocytes to 3-methylcholanthrene during cultures. Leukocytes were maintained in phytohemagglutinin medium for the indicated times prior to addition of 3-methylcholanthrene and harvested after 24 hours. Each circle represents the mean of six determinations and each square represents the mean of three determinations. The vertical bars give the range of values.