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.

WILLIAM S. SAKAI
Department of Agronomy and Soil Science, College of Tropical Agriculture, University of Hawaii, Honolulu 96822

MICHAEL HANSON
Department of Botany, University of Hawaii

ROLLIN C. JONES
Department of Agronomy and Soil Science, University of Hawaii

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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

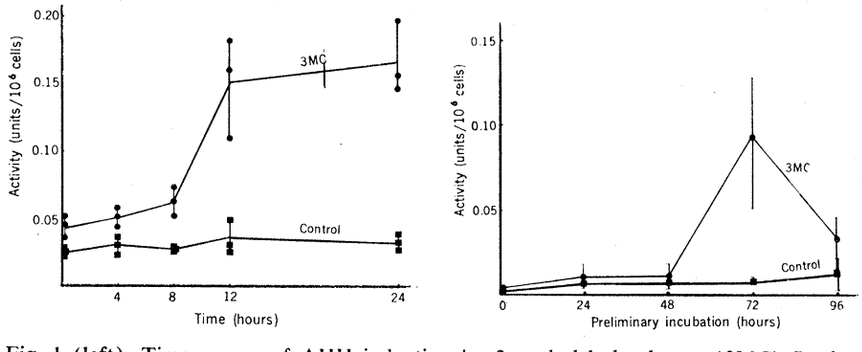

Fig. 1 (left). Time course of AHH induction by 3-methylcholanthrene (*3MC*) Leukocytes were first incubated for 72 hours in medium containing phytohemagglutinin. 3-Methylcholanthrene was added to a final concentration of 0.75 μM. Cells were harvested at the indicated intervals and were assayed for AHH 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.

AHH activity was determined by a modification of the method of Nebert and Gelboin (*12*). Homogenates were incubated for 30 minutes at 37°C in a final volume of 1.1 ml containing 1.0 mg of reduced nicotinamide adenine dinucleotide (NADPH) and 25 μg of BP. The reaction was stopped by addition of 4 ml of an acetone, hexane mixture (1 : 3) and vortex mixing for 1 minute. The phases were separated by centrifugation, and the organic phase was extracted with 0.5 ml of 1*N* NaOH for 1 minute. The aqueous and organic phases were separated by centrifugation and fluorescence of the hydroxylated BP in the aqueous phase was determined in an Aminco-Bowman spectrophotofluorometer with excitation at 396 nm and emission at 522 nm.

The AHH activity in leukocytes cultured for 72 hours in medium containing phytohemagglutinin was increased after treatment with 3MC, reaching a fourfold maximum by 12 hours (Fig. 1). In control leukocytes cultured under identical conditions the baseline levels of AHH activity was higher than that in fresh leukocytes. Fresh leukocytes were not induced by 24 hours of exposure to 3MC (Fig. 2). However, after 72 hours of preliminary incubation in phytohemagglutinin medium, the leukocytes responded to 3MC with a marked rise in AHH activity.

Activity of AHH in inducible experimental animals varies in response to environmental conditions, whereas the ability to be induced is expressed as a simple Mendelian characteristic (*9*). The AHH activity also varies in humans, notably, between cigarette smokers and nonsmokers (*6*). This cultured leukocyte system provides a simple method for studying the inducibility of AHH activity in the human population (*13*).

D. L. Busbee
C. R. Shaw
Section of Medical Genetics, University of Texas M. D. Anderson Hospital and Tumor Institute, Texas Medical Center, Houston 77025
E. T. Cantrell
Department of Pharmacology, Baylor College of Medicine, Texas Medical Center, Houston 77025

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Chronic Thyroiditis in the Rabbit Induced with Homologous Thyroid Microsomes

Abstract. *Rabbits immunized with freshly isolated homologous thyroid microsomes in complete Freund's adjuvant developed a severe chronic thyroiditis with the concomitant production of antimicrosomal antibodies. The antibody response was measured by indirect immunofluorescence and complement fixation, employing isolated microsomes as the antigen. Microsomes but not thyroglobulin absorbed out the immunologic activity of the rabbit serum.*

In view of the high incidence of antimicrosomal antibodies and the lack of correlation of disease with antithyroglobulin antibodies in Hashimoto's chronic thyroiditis, experiments designed to evaluate the role of microsomal antigen or antigens were indicated. Surprisingly, the number of investigators who have reported on this is small and the data conflicting. Jones and Roitt (*1*) attempted the experiment with homologous microsomes in the rat but reported failure, as did Roitt *et al.* (*2*) in the sheep. In contrast, Kite *et al.* (*3*) have been successful in producing transient mild thyroiditis in the monkey with human thyroid extract as well as obtaining an antimicrosomal antibody which was reactive with human target tissue as based upon cytotoxicity and complement fixation.

This report presents data on the production of a severe chronic thyroiditis in rabbits induced with homologous thyroid microsomes and the concomitant production of antimicrosomal antibody which appears to correlate with the severity of the disease.

Rabbit thyroid microsomes were isolated from an average pool size of no less than 200 thyroids (fresh iced, Pel-Freez, Rogers, Arkansas) by a modification of the method of Campbell and Kernot (*4*). After the thyroids were freed of superficial fat they were homogenized in a Sorvall Omni-Mixer at a ratio of 2.5 volumes of microsomal buffer (0.01*M* $MgCl_2$, 0.025*M* KCl, 0.035*M* tris buffer, *p*H 7.8, and 0.15*M* sucrose) to 1 gram weight of tissue. The homogenate was then filtered through gauze pads to remove much of the lipid material and centrifuged at 12,000*g* in a Sorvall RC-2 for 10 minutes to remove cell debris, nuclei, and mitochondria. The congealed upper lipid layer was removed with a spatula and the aqueous layer was decanted. This layer was then centrifuged in a Spinco model L at 105,000*g* for 50 minutes. The gelatinous button was homogenized in microsomal buffer with the aid of a hand-operated tissue grinder and recentrifuged at the same speed and time. This procedure was repeated twice and the pellet was finally suspended in microsomal buffer at a concentration of 10 mg of protein (as determined by micro-Kjeldahl) per milliliter. Microsomal preparations were declared as such and used only after evaluation by electron microscopy.

Rabbit liver microsomes were prepared in an identical manner from a pool of five normal rabbit livers.

Twelve female New Zealand white rabbits (2 to 2.5 kg) were each injected with 5 mg of microsomal protein incorporated in complete Freund's adjuvant (CFA) distributed among the four footpads. Fourteen days later they received 2.5 mg of freshly isolated microsomal protein antigen in CFA intramuscularly and another 2.5 mg of freshly isolated microsomal protein antigen in CFA intramuscularly 7 days