

## My Research Interests

### The Kinetics of Ethanol Metabolism in Human Tissue

Ingested ethanol is metabolized in the body predominantly by two enzymes. The enzymes believed most responsible for ethanol metabolism is a class of cytosolic alcohol dehydrogenase isoenzymes expressed in many tissues, including liver and stomach. A second enzyme is a part of the microsomal cytochrome P450 family of isoenzymes that are designed to eliminate foreign substances in the body. My laboratory seeks to characterize the kinetics of ethanol metabolism by these two enzyme systems, and to understand the relative importance of the two in metabolism of ingested ethanol. We use steady-state kinetics, stopped-flow kinetics, and computer simulation. We also use protein purification, and characterization.



#### Alcohol Dehydrogenase Isoenzymes (ADH)

Human tissue expresses a variety of ADH isoenzymes. These enzymes catalyze the breakdown of ethanol, as well as other alcohols. They are believed to function as general detoxifying enzymes. The human ADH isoenzymes are characterized by different specificities toward alcohols, including ethanol. The isoenzymes also exhibit different  $K_m$  values for ethanol and  $V_{max}$  for ethanol oxidation. The enzymes are active as dimers, with a subunit molecular weight of 40 KD. They catalyze alcohol oxidation as oxidoreductases, using NAD(H) as a coenzyme.

#### Cytochrome P450 2E1 Isoenzyme

Xenobiotics are environmental molecules that find their way into our bodies. Tissues in the body express a variety of membrane-bound enzymes called cytochrome P450 (CYP) isoenzymes. Like ADH isoenzymes, the CYP isoenzymes are believed to function in detoxification of tissue cells, oxidizing the xenobiotics. One such CYP enzyme, CYP2E1, catalyzes the oxidation of ethanol. Its presence in tissues can be induced by ethanol, so its role in ethanol metabolism increases among individuals who drink ethanol regularly.

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## **Research Interests and Directions**

### **The Kinetics of Human Ethanol Metabolism**

Ingested ethanol is metabolized in the body predominantly by two enzyme classes. The class of enzymes believed most responsible for ethanol metabolism is cytosolic alcohol dehydrogenase. A second enzyme is a part of the microsomal cytochrome P450 family of isoenzymes that are designed to eliminate foreign substances in the body. My laboratory seeks to characterize the kinetics of ethanol metabolism by these two enzyme systems, and to understand the relative importance of the two in metabolism of ingested ethanol. We use steady-state kinetics, stopped-flow kinetics, and computer simulation. We also use protein purification, and characterization. I am currently funded by NIAAA, through NIH, and submission of an R01 is currently pending.

#### ***Alcohol Dehydrogenase Isoenzymes (ADH)***

ADH catalyzes the reversible oxidation of alcohols using NAD(H) as a coenzyme. The enzyme in mammals is active as a dimer, with a subunit molecular weight of 40 KD. A variety of ADH isoenzymes are expressed in human tissues such as liver and stomach, and they are believed to function as general detoxifying enzymes. The human ADH isoenzymes are characterized by different specificities toward alcohols, including ethanol. The isoenzymes also exhibit different  $K_m$  values for ethanol and  $V_{max}$  values for ethanol oxidation. We are currently working to characterize the human ADH isoenzymes at physiological conditions. Using stopped-flow and steady-state kinetics, we are determining the sequence mechanisms for these isoenzymes with ethanol oxidation and acetaldehyde reduction. We are also evaluating individual rate constants that describe specific steps in the sequence mechanism. Using this data, we are using computer simulation software to 1) compare predicted ethanol elimination profiles with clinical data, 2) evaluate the relative importance of each ADH isoenzyme in ethanol metabolism, 3) simulate the effect of acetaldehyde levels on ethanol metabolism by ADH, and 4) simulate the effect of ethanol flux on cellular energy levels.

Simulating the pharmacokinetics of ethanol elimination in human liver requires an estimation of the amount of ADH present in a liver, and the relative amounts of each isoenzyme present in that liver. This data has been impossible because the isoenzymes are very similar in amino acid sequence, and even monoclonal antibodies cross-react. Inhibitors specific for a given isoenzyme are also unavailable. We are working in collaboration of Richard Smith at Pacific National Laboratories, who has separated the human ADH isoenzymes by capillary electrophoresis and mass spectrometry. A manuscript describing this work was recently accepted for publication in *Electrophoresis*. We are developing strategies to use this amazing technology to quantify the amount of each isoenzyme in a homogenate of human liver. Differences in the amount of ADH isoenzymes expressed in different tissues, and differences in gender, age, and ethnic background will be possible. This data will be used in the computer simulations of ethanol elimination.

We are also characterizing the sequence mechanism of ADH metabolism of retinoids, and the effect of ethanol on retinoid metabolism. Retinol (Vitamin A) is oxidized in the liver to retinal, which is used for night vision, and further processed into retinoic acid. Retinoic acid is a cell growth regulator. It has been postulated that ADH isoenzymes in the liver are primarily responsible for retinol oxidation, and that ingested ethanol interferes with retinoid metabolism. Clinically, chronic alcoholics suffer from night blindness, and some children born of alcoholic mothers have fetal alcohol syndrome, a set of abnormalities also associated some children born in geographical areas deficient of vitamin A. To test this hypothesis, we are evaluating the

sequence mechanism by which ADH oxidizes retinol and reduces retinal, and we will use computer simulation to evaluate the effect of ethanol on retinol oxidation.

### ***Cytochrome P450 2E1 Isoenzyme***

Xenobiotics are environmental molecules that find their way into our bodies. Tissues in the body express a variety of membrane-bound enzymes called cytochrome P450 (CYP) isoenzymes. Like ADH isoenzymes, the CYP isoenzymes are believed to function in detoxification of tissue cells, oxidizing the xenobiotics. One such CYP enzyme, CYP2E1, catalyzes the oxidation of ethanol. The presence of CYP2E1 in human tissues is induced by ethanol, so its role in ethanol metabolism increases among individuals who drink ethanol regularly. Activity of the enzyme is absolutely linked to the presence of another enzyme, NADPH-dependent oxidoreductase, and the presence of cytochrome  $b_5$  is reportedly also important for proper ethanol oxidation. The multi-enzyme complex is only active within a lipid matrix that contains phosphatidylcholine.

We are currently in collaboration with Jerry Lasker at Mt. Sinai to develop a rate equation for CYP2E1 activity toward ethanol that includes concentrations of its coupled electron carriers, NADPH-dependent oxidoreductase, and cytochrome  $b_5$ , as well as the concentration of phosphatidylcholine. Dr. Lasker has supplied us with each of the three purified protein components, and we are currently evaluating the effect of varying simultaneously each of the four complex components. The complete data set will be fit to varying kinetic models to sift out the effect of each substituent on the kinetics of ethanol oxidation. We will then have a general rate equation that describes the rate of ethanol oxidation at any concentration of CYP2E1, oxidoreductase, cytochrome  $b_5$ , and phospholipid.

Using the above rate equation, and the concentrations of each of the substituents determined by kinetic assay and western blotting, we will 1) determine the relative importance of CYP2E1 in ethanol metabolism by gender, age, and ethnic background in the normal liver as well as the ethanol-induced liver, 2) determine under what conditions the CYP2E1 can be significantly increased, 3) simulate the effect of ethanol on CYP2E1 activity toward xenobiotics such as acetaminophen (Tylenol) or chloroform.