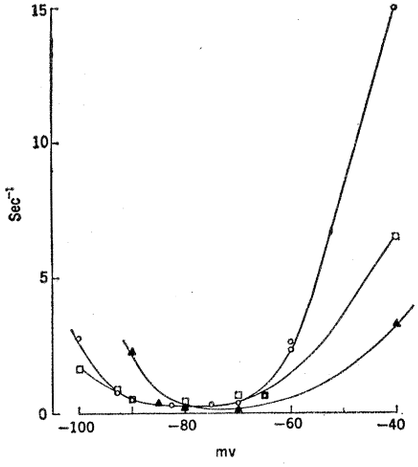

Fig. 3. Voltage dependence of rate of change of slow potassium current measured in terms of reciprocal of time constant (τ_s^{-1}) of current change. Symbols are the same as in Fig. 2.

than that of increasing the calcium ion concentration. Thus, increasing the external calcium concentration from 1.8 m*M* to 7.2 m*M* produces a shift of only 8 mv.

The physiological importance of these results lies in the fact that they account for the action of adrenaline in accelerating the pacemaker depolarization. At the beginning of the pacemaker potential the potassium current will decline more quickly, since the rate of change of i_{K_2} is faster in the presence of adrenaline (Fig. 3). Moreover, since the steady-state relation between *s* and membrane potential is shifted in the depolarizing direction (Fig. 2), i_{K_2} will fall toward a smaller value and so accelerate the depolarization throughout the pacemaker potential. Computer calculations have been done which show that these effects are quantitatively adequate to account for the chronotropic action of adrenaline.

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Ethanol Oxidation by Hepatic Microsomes: Adaptive Increase after Ethanol Feeding

Abstract. *Hepatic microsomes contain an ethanol-oxidizing system distinct from alcohol dehydrogenase. In vitro, it has characteristics comparable to those of microsomal drug-detoxifying enzymes and, in vivo, it is capable of adaptation to the administration of ethanol. The existence of this microsomal ethanol-oxidizing system may explain ultrastructural, pharmacological, and biochemical effects of ethanol.*

Ethanol administration, in man given a variety of diets (*1*) and in rats fed either an adequate (*2*) or a deficient diet (*3*), produced proliferation of the smooth endoplasmic reticulum (SER) of the liver. This proliferation was accompanied by an increase in the activity of various hepatic microsomal drug-detoxifying enzymes (*3*). These structural and functional changes are very similar to those produced by a variety of pharmacologic agents (*4*). The concept that ethanol affects the SER by acting as other drugs do produced the following difficulty. Most substances that induce microsomal drug-detoxifying enzymes are metabolized, at least in part, in the microsomes (which comprise the SER) (*4*). The hepatic enzyme responsible for ethanol oxidation is thought to be alcohol dehydrogenase (ADH) (*5*), which is located in another cell compartment, the cell sap or cytosol (*6*). This problem prompted our study of ethanol-oxidizing activity in various subcellular fractions of the rat liver.

Ten male Sprague-Dawley rats (150 to 250 g) were given Purina laboratory chow, and 28 rats (nine pairs of males and five pairs of females) were pair-fed nutritionally adequate liquid diets (containing 18 percent of the total calories as protein and 35 percent as fat) (*7*). One rat from each pair was given a diet containing 36 percent of the total calories as ethanol, whereas its littermate was fed the control diet in which ethanol had been isocalorically replaced by carbohydrate. After 24 days, the animals were killed; the liver and, in some animals, kidneys, stomach, heart, and brain were quickly excised.

The tissues were homogenized in 1.15 percent KCl, and the subcellular fractions were obtained by ultracentrifugation. The ADH activity was measured (*8*), and it was confirmed that ADH is limited to the cytosol. The following incubation technique was used to study ethanol-oxidizing activity in the microsomes. Microsomes (corresponding to 250 mg of tissue) were incubated at 37°C in a medium containing (per ml) 0.3 μmole of nicotinamide-adenine dinucleotide phosphate (NADP), 5 μmole of magnesium chloride, 20 μmole of nicotinamide, 8 μmole of sodium isocitrate, 2 mg of isocitrate dehydrogenase (crude-type I; Sigma Chemical Co.), 50 μmole of ethanol, and 80 μmole of phosphate buffer (*p*H 7.4). The incubations were carried out in the main chambers of stoppered 50 ml erlenmeyer flasks, with center wells containing 0.6 ml of 0.015*M* semicarbazide hydrochloride in 0.16*M* potassium phosphate buffer (*p*H 7.0). The reaction was stopped with 0.5 ml of 70 percent trichloroacetic acid, and, after an overnight diffusion period at room temperature, the concentration of acetaldehyde bound to the semicarbazide was determined (*9*). That the compound produced by the microsomes was identical with acetaldehyde was shown in two ways: (i) the retention times measured by gas-liquid chromatography (*10*) were equal; (ii) the absorption spectrum of an acetaldehyde-semicarbazone solution was the same as the spectrum of the compound obtained after incubation of microsomes with ethanol. For each determination, at least six incubation flasks were used, with duplicate incubations for 0, 5, and 10 minutes, to verify linearity of the reaction. Activity of the microsomal ethanol-oxidizing system (MEOS) was expressed in units corresponding to the number of nanomoles of acetaldehyde produced per minute per milligram of microsomal protein (*11*).

All hepatic microsomal preparations showed significant ethanol-oxidizing activity that was proportional to the quantity of microsomal suspension. In the ten male rats fed the Purina chow diet, MEOS activity averaged 8.6 ± 0.72 units. The cytosol, mitochondria, and nuclei of the liver, the microsomes of kidney, stomach, heart, and brain as well as purified liver alcohol dehydrogenase (purchased from Calbiochem, New York) had negligible ethanol-oxidizing activity under the conditions of our experiment.

Variation of the *p*H of the incubation medium showed optimum activity from *p*H 6.8 to 7.5. The system was inactive in the absence of reduced NADP (or a NADPH-generating system). Lack of oxygen abolished MEOS activity; reduction of the O_2 from 21 to 4 percent diminished MEOS activity by 45 percent ($P < .01$). Carbon monoxide reduced MEOS activity even further, approximately 67 percent ($P < .001$). Sodium cyanide (0.1 m*M*) inhibited MEOS activity by 17 percent.

Feeding of ethanol resulted in a significant increase in the activity of hepatic MEOS; it averaged 7.70 ± 1.02 units in the nine male rats fed the control diets for 24 days, whereas in the littermates pair-fed daily with isocaloric amounts of the alcohol-containing diet, the activity was 9.95 ± 0.87 units ($P < .05$). A greater increase was observed in the female rats: 5.0 ± 0.59 units in the controls versus 11.1 ± 1.45 units after ethanol ($P < .01$). Contrasting with MEOS, ADH activity was unaffected by the alcohol feeding.

The results of the present investigation show that microsomes of liver tissue contain an active ethanol-oxidizing system. Preliminary results also indicate that a similar system exists in human liver (*12*). Hitherto, ADH was generally thought to be the only enzyme responsible for the oxidation of ethanol in vivo (*13*), though in vitro, catalase is also active (*5*). That MEOS activity is different from ADH is indicated by (i) the cofactor requirements (NAD for ADH and NADPH for MEOS); (ii) the effect of *p*H, the optimum *p*H for the oxidation of ethanol to acetaldehyde by ADH being 10.8 (*5*), whereas the optimum for MEOS is physiological *p*H. Consequently, under our standard conditions for measuring MEOS activity, purified ADH was inactive. That catalase is not likely to be responsible for MEOS activity is indicated by its distribution. Most of the hepatic catalase is localized in the cytosol and "mitochondria" (*14*). These fractions had negligible ethanol-oxidizing activity under our assay conditions for MEOS measurement. Moreover, cyanide only partially inhibited MEOS activity at a concentration (0.1 m*M*) which almost completely abolishes catalase activity (*15*).

Characteristics of MEOS (requirement for O_2, NADPH, and partial inhibition by CO) resemble those commonly found among microsomal drug-detoxifying enzymes (*4*), but differ from the microsomal system reported to oxidize primarily methanol and to be insensitive to CO (*16*). Like that for other drug-detoxifying enzymes, the MEOS activity increased significantly after induction by substrate administration. Though MEOS activity in control rats was lower in females than in males, the capacity for adaptation was greater in the females, a sex difference common for microsomal drug-detoxifying enzymes (*4*).

Though reports by others concerning the possibility of ADH adaptation have been conflicting (*17*), our observation of a lack of ADH adaptation is in accord with several previous publications (*18*). Furthermore, whereas ADH is found in the cytosol (*6*), a number of ethanol effects involve the microsomes (*17*). Though the quantitative role of the microsomal ethanol-oxidizing system (MEOS) in vivo, remains unknown, demonstration of the existence of MEOS and its adaptive increase helps to explain a number of poorly understood effects of ethanol (*17*).

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Phagocytosis of Inhaled Plutonium Oxide–^{239}Pu Particles by Pulmonary Macrophages

Abstract. *Pulmonary macrophages and plutonium particles were removed by washing the lungs of rats that had inhaled plutonium oxide–^{239}Pu. A significant amount of plutonium was found in multiple washings of the same lung. The removal of toxic particles by washing is of potential therapeutic value. Particles were phagocytized by macrophages during the first 3 hours and retained within these cells for up to 25 days. Nearly all particles in washings were found in macrophages after the second day. The percent of macrophages with engulfed particles increased with increasing amounts of plutonium deposited in the lungs. The ability of pulmonary macrophages to rapidly phagocytize and retain plutonium particles deposited in the lungs has been shown.*

The role of the pulmonary macrophage in the clearance of plutonium particles deposited in alveoli has remained somewhat speculative. The deposition, retention, translocation, and excretion of inhaled $^{239}PuO_2$ particles has been previously described (*1–4*). Studies of the rat peritoneal cavity demonstrated the ability of mononuclear phagocytes to rapidly phagocytize and accumulate plutonium particles (*5*, *6*). Studies were initiated in order to define the role of the pulmonary macrophage in determin-

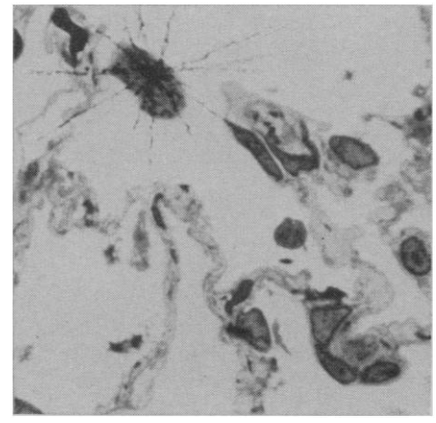

Fig. 1. Phagocytosis of plutonium particles by pulmonary macrophages. Section of unworked lung removed at 7 days after inhalation, fixed in glutaraldehyde, and embedded in epon. Autoradiogram, 14-day exposure, Richardson's stain (*10*). Note alveolar macrophage with alpha tracks from phagocytized plutonium particle(s) in upper left-hand corner within an alveolus. A similar particle distribution is seen on autoradiograms of cytosmears from lung washings. (×975)