toxylin and 1 percent aqueous eosin. Eighteen lobsters in a second control group were quickly killed and the tissue was stained and mounted without storage. Tissue from both control groups was similar. Among the various tissues from lobsters exposed to lethal phosphorus suspensions in the laboratory only the antennal gland and hepatopancreas showed signs of degeneration.

Normal antennal gland tissue consists of blood sinus, coelomosac, and tubular labyrinth (*3*). Degenerative change in this organ was quite variable, with the labyrinth affected more frequently and extensively than the coelomosac (Fig. 1F). Some degree of degeneration was noted in all but one of the lobsters exposed to phosphorus, but did not occur in any of the 20 controls. In both type and degree the histological damage is similar to that observed by Hubschman (*4*) in antennal glands of crayfish exposed to copper.

Yellow phosphorus caused degenerative changes in all four cell types in the hepatopancreas. Disorientation and cell membrane destruction occurs in embryonic and fibrillar cells, and vacuole size and number increase in the secretory and absorptive cells until the lumen of the tubule is obliterated (Fig. 1, B to D). These changes are strikingly similar to those produced in crayfish by eyestalk ablation (*5*).

Lobsters suffering from lethal exposure to yellow phosphorus die in a characteristic way: they become lethargic, lie on their sides, wave pereiopods slowly, and gradually lose muscle tone and coordination. After some 8 to 15 hours all muscle response ceases and the animal is clinically dead. In the final few hours blood in the cardiogastric region becomes noticeably thick, and coagulation continues until the thorax is filled with gelled blood. Except for the coagulated blood, this syndrome is also characteristic of death by asphyxiation, and this fact prompted the earlier suggestion (*2*) that phosphorus in some way activates the hemolymph clotting mechanism of *Homarus*, causing asphyxiation.

Unlike that in vertebrates, the lobster clot-initiating factor is not a proteolytic enzyme but an intracellular calcium-dependent transglutaminase "which is released from ruptured hemocytes upon suitable provocation" (*6*). Suitable provocation can be provided by such diverse events as injury, injection of isobutyl alcohol, immersion in fresh water and, apparently, exposure to yellow phosphorus. We suspect that clotting is not triggered by phosphorus directly, but is an indirect result of damage to the cells of antennal gland and hepatopancreas. Although this damage probably makes death inevitable, the evidence still points to hemolymph coagulation and asphyxiation as the direct cause of death.

Assimilation of yellow phosphorus is roughly proportional to tissue lipid content (*7*). Lobster hepatopancreas is a lipid-rich organ that can concentrate yellow phosphorus to a level 1000 to 2000 times that in surrounding seawater (*8*). Since the hepatopancreas, or "tomalley," is an edible organ, and even small amounts of yellow phosphorus can cause serious hepatitis in humans, it is fortunate that this element is rapidly cleared from animals transferred to uncontaminated water (*8*). However, the toxic effects of this element to lobsters are cumulative and are not rapidly reversed by transfer to uncontaminated water. Some of this toxicity is expressed in the tissue damage we have described here, and when that damage becomes sufficiently extensive the death of the animal is assured.

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Mixed Function Oxidase and Ethanol Metabolism in Perfused Rat Liver

Abstract: *Oxidation-reduction changes of cytochrome P-450 and oxygen consumption were measured in isolated perfused livers from normal and phenobarbital-treated rats. Phenobarbital treatment markedly increased the aminopyrine-induced reduction of cytochrome P-450, but ethanol did not cause any redox changes of this cytochrome. It was concluded that the microsomal ethanol-oxidizing system has an insignificant role in the metabolism of ethanol in intact liver.*

Orme-Johnson and Ziegler (*1*) reported that microsomal preparations of rat liver oxidize ethanol to acetaldehyde, and others have described a microsomal system oxidizing ethanol (*2*, *3*). The in vitro experiments with isolated rat microsomes are still partly at variance with many in vivo experiments with rats (*4*). The Michaelis constant for ethanol in the microsomal system has been reported to be 8 m*M* (*3*), but in man ethanol elimination from the blood shows zero-order kinetics in the concentration range of 2 to 50 m*M* (*5*).

According to Lieber and DeCarli (*3*), the microsomal ethanol-oxidizing system (MEOS) is sensitive to carbon monoxide and has enzymes in common with the mixed function oxidase that metabolizes drugs in the liver. Moreover, ethanol has been shown to produce a "modified type 2" spectral change in the spectrum of microsomal cytochrome P-450 (*6*, *7*). In contrast, others have interpreted their aminotriazole inhibition data and their findings of traces of catalase and alcohol dehydrogenase in the microsomal fraction to indicate that catalase plays an essential role in the microsomal oxidation of ethanol (*8*). The proportion of ethanol metabolism which can be accounted for by the microsomes varies between 3 and 36 percent in different reports (*1–3*, *8*). Discrepancy also exists between reports about the affinity of cytochrome P-450 for ethanol. Rubin *et al.* (*7*) showed that a half-maximal spectral change of cytochrome P-450 due to substrate binding was produced by 100 m*M* ethanol, whereas Imai and Sato (*9*) reported that 500 to 900 m*M* ethanol was required for the half-maximal change.

Neither in vitro experiments with microsomes nor in vivo measurements of the elimination kinetics of ethanol have given conclusive evidence about the functioning of the MEOS in vivo.

We have used isolated perfused rat livers, in which redox changes in cytochrome P-450 can be demonstrated (*10, 11*). Changes in flux in the electron transport system of the endoplasmic reticulum are reflected by changes in the steady-state redox level of cytochrome P-450. These changes are partly brought about by the effects of the substrates on the NADPH : cytochrome P-450 reductase (*12*) and partly by substrate binding to the cytochrome (*13*). The results we report show a differential effect of ethanol and of substrates of the mixed function oxidase on the redox state of cytochrome P-450.

Male Wistar rats were used, and no fasting preceded the experiments. One group of animals received daily doses of phenobarbital (100 mg/kg, intraperitoneally) for 7 days. The methods of liver perfusion and dual wavelength spectrophotometry of the liver lobe have been described (*14*). The slits of the monochromator for the measuring wavelength were set to give a spectral bandwidth of 6.6 nm. The oxygen concentration in the outlet cannula inserted into the inferior caval vein was monitored continuously with a Clark-type oxygen electrode. Formation of the carbon monoxide complex of cytochrome P-450 was measured by the difference between absorbance at 450 nm and that at 500 nm (*15*). Ethanol in the perfusion medium was measured by gas-liquid chromatography on a Porapak Q column (*16*). Gas coming from the oxygenator was bubbled through 100 ml of water at 0°C, and appropriate corrections for evaporation were made in calculations of oxidation rates of ethanol. The substrate concentration in the perfusion medium was kept constant by continuous infusion. Demethylation of aminopyrine (4-dimethylamino-1,5-dimethyl-2-phenyl-3-pyrazolone) was determined as described by Brodie and Axelrod (*17*).

When carbon monoxide at a final concentration of 3.5 percent was included in the gas phase of the oxygenator, no effect on oxygen consumption was observed but an absorption band at 450 nm appeared simultaneously. A marked increase in the absorbance at 450 nm occurred when 0.5 m*M* aminopyrine was added to the perfusion medium. This is in accordance with data on the in vitro stimulation of cytochrome P-450 reduction by "type 1" substrates of the mixed function oxidase (*13*). In contrast, no change in the carbon monoxide complex of cytochrome P-450 could be detected when 80 m*M* ethanol was added (Fig. 1).

Interaction between ethanol and the mixed function oxidase system can be ruled out on the basis of experiments like that in Fig. 1. First, if the oxidation of ethanol by MEOS were analogous to that of substrates of cytochrome P-450, as has been suggested (*3*), a change in the redox state of this cytochrome would be probable. The redox change, in turn, would be reflected in the amount of the carbon monoxide complex of reduced cytochrome P-450. Inhibition of the NADPH reduction of cytochrome P-450 by "type 2" substrates (*13*) occurs even in a perfused liver (*11*). If this is true for ethanol, there should be oxidation of cytochrome P-450 (that is, a decrease in the amount of the complex of reduced cytochrome) when ethanol is added. In conditions under which the carbon monoxide complex of reduced P-450 is detected, the demethylation rate of aminopyrine is still appreciable, as estimated from the oxygen trace of Fig. 1.

It has been reported that the activity of MEOS is enhanced by in vivo administration of barbiturates and certain other drugs that induce the synthesis of cytochrome P-450 (*2, 3*). Aminopyrine is metabolized at a high rate in the livers of rats treated with phenobarbital, as judged from the marked stimulation of oxygen consumption (Fig. 2). However, the ethanol-dependent oxygen consumption was almost the same in the two groups of animals.

The wavelength dependence of the

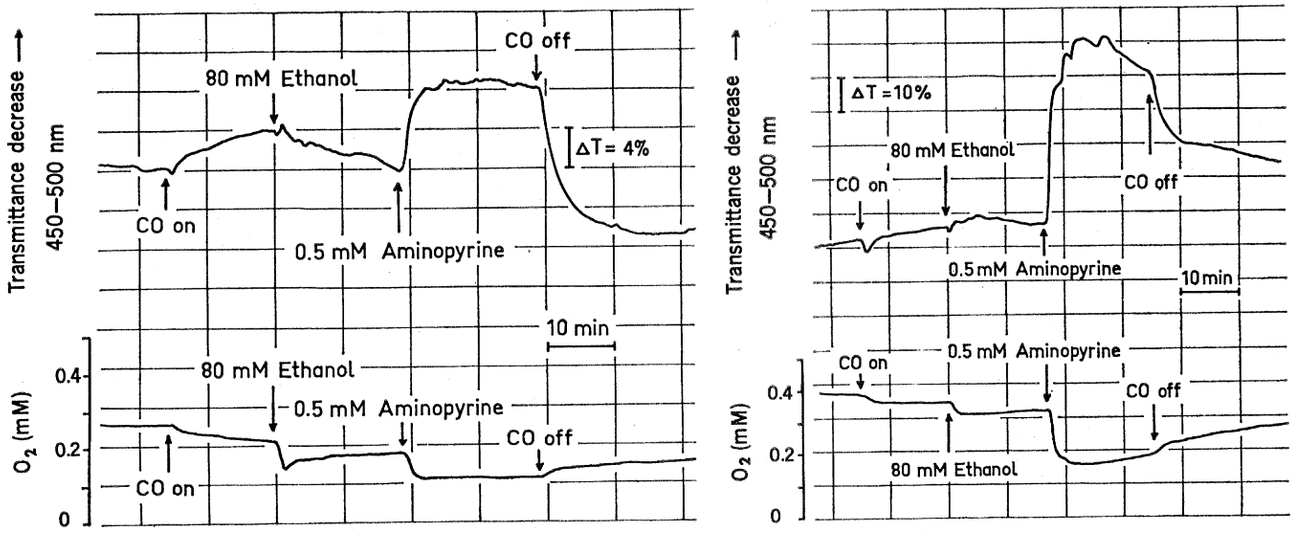

Fig. 1 (left). Effect of ethanol and aminopyrine on cytochrome P-450 reduction in the isolated perfused rat liver. The liver was perfused with medium consisting of 137 m*M* NaCl, 3 m*M* KCl, 0.5 m*M* $CaCl_2$, 0.5 m*M* $MgCl_2$, 0.7 m*M* NaH_2PO_4, and 24 m*M* $NaHCO_3$; the medium was equilibrated with 95 percent oxygen and 5 percent carbon dioxide at 35°C. Flow of the perfusion medium per gram of wet liver was 5 ml/min. During the time indicated, 3.5 percent of the gas phase was substituted by carbon monoxide. The difference between light transmittance at 450 and 500 nm was recorded in an area of a liver lobe about 2 mm thick. The upper curve shows absorption of cytochrome P-450, and the lower curve gives oxygen concentration in the venous perfusate. Fig. 2 (right). Effect of ethanol and aminopyrine on cytochrome P-450 reduction in the isolated perfused liver of a phenobarbital-treated rat. The animal had been given daily doses of phenobarbital (100 mg per kilogram of body weight) for 7 days. Assay conditions are the same as in Fig. 1.

spectral change induced by carbon monoxide in the presence of aminopyrine is presented in Fig. 3. The sharp absorption maximum at 450 nm is reminiscent of the spectrum of the carbon monoxide complex of reduced cytochrome P-450.

Failure to respond to ethanol would also be expected if the cytochrome P-450 were in the oxidized state. However, when the cytochrome was reduced by addition of 0.5 m*M* aminopyrine in the presence of carbon monoxide, no effect on the redox state of cytochrome P-450 could be observed when 80 m*M* ethanol was subsequently added.

Using these experimental conditions and livers from noninduced animals, we determined the accumulation rate of 4-amino-1,5-dimethyl-2-phenyl-3-pyrazolone, which in isolated microsomes accounts for only one-third of the metabolic products of aminopyrine (and probably much less in the intact liver). Expressed per gram of liver, this rate was 7.6 ± 0.84 nmole/min (mean ± standard error of the mean) and was reduced 18 percent in the presence of 8 percent carbon monoxide. The rate of ethanol oxidation per gram of liver was 1.36 ± 0.13 μmole/min at a substrate concentration of 80 m*M*, and was not affected by 8 percent carbon monoxide. Higher concentration of carbon monoxide cannot be used in studying intact tissue, because the mixed function oxidase system is sensitive to metabolic changes not directly related to it, for instance, changes in the nutritional or redox status of the liver (*18*). Thus, monitoring of the redox state of cytochrome P-450 in the intact liver seems to be a more sensitive method for detecting interactions with the mixed function oxidase system than is the inhibition of substrate conversions by carbon monoxide. Also, a partial inhibition of cytochrome oxidase and an accompanying change in the cellular redox state would completely mask the direct action of effective concentrations of carbon monoxide on microsomal electron transport.

Our results do not lend support to the view that a close similarity exists between MEOS and the oxygenase system that metabolizes drugs in intact tissue. The results are also in accordance with the data of Thurman *et al.* (*19*) on the role of catalase in the ethanol oxidation by microsomes in vitro. Our results were obtained under conditions when turnover of cytochrome P-450 was high, in contrast with the anaerobic in vitro experiments with added carbon monoxide. Because of this, our results also have a bearing on the physiological significance of the cooperative interaction of NADH and NADPH in microsomal electron transport reactions (*20*).

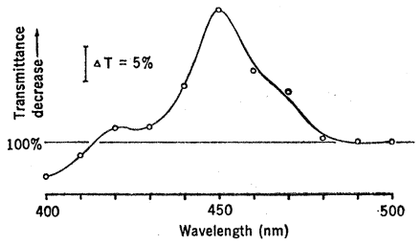

Fig. 3. The spectral change induced by carbon monoxide during aminopyrine demethylation in the isolated perfused liver of a rat treated with phenobarbital. A dual wavelength spectrophotometer was used to measure the transmittance changes in one liver at the wavelengths indicated; 500 nm was the reference wavelength. Five-minute pulses of carbon monoxide were given at intervals of 10 minutes, and recordings for one wavelength were made during each pulse. Other conditions are the same as in Fig. 1. The initial aminopyrine concentration was 0.5 m*M*. Oxygen consumption was kept constant by a continuous infusion of aminopyrine into the perfusion medium at 60 μmole/hour.

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Wiswesser Line Notation: Simplified Techniques for Converting Chemical Structures to WLN

Abstract. *Techniques have been developed for the generation of Wiswesser Line Notations (WLN), which require knowledge neither of rules for manual conversion of structures to line notations nor of computer programming. The desired WLN are obtained simply by drawing the structures of the compounds of interest on a tablet, which is linked to an appropriately programmed computer.*

As more and more scientific data are handled by computer techniques (*1*), there is an increasing tendency to use line notations to encode chemical structures (*2*), since such notations may be filed within a data bank and subjected to computer search. Of the various line notations that have been described, Wiswesser Line Notation (WLN) (*3*) seems to have received more general acceptance than any other. The manual conversion of WLN to structure is straightforward with a symbol key, and the reverse process is, for commonly encountered structures, not overly difficult.

Several schemes (*4*) have been proposed for converting structure diagrams to WLN descriptions, in addition to manual translation. In the scheme described here the input is the drawing of the diagram at a writing tablet. This scheme was tested in the course of preparation of a second edition of the handbook *Psychotropic Drugs and Related Compounds* (*5*). It was necessary that the chemical structures be filed in line notation form, both to allow the con-