percent (*15*); CB thus also interferes with glycolysis in nonmotile cells.

The various inhibitory effects of CB on leukocyte metabolism and function exhibit a similar dose-response relation, and they are all rapid and reversible, suggesting a single site of action for the drug with multiple effects. The findings reported here are compatible with the site of action of CB being at the plasma membrane, leading to blockade of certain transport systems, with resulting metabolic changes in the cell. The mechanisms responsible for inhibition by CB of cell functions such as locomotion and endocytosis are as yet undetermined; these effects, which have similar time and dose characteristics, may be related directly or indirectly to the action of CB on transport, or they may reflect multiple sites of drug action on membranes, microfilaments, or other structures.

Note added in proof: While the present work was in press, a report appeared showing inhibition by cytochalasins of deoxyglucose transport into certain cells in culture (*16*).

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Histological Changes in Lobsters (Homarus americanus) Exposed to Yellow Phosphorus

Abstract. *An industrial discharge of yellow phosphorus killed fish and crustaceans in Long Harbor, Placentia Bay, Newfoundland, in 1969. During subsequent toxicity studies various organs from lobsters killed by exposure to suspensions of yellow phosphorus were examined for histological damage. Antennal gland and hepatopancreas both showed degenerative changes, and cellular damage in the latter was extensive.*

In 1969 an industrial discharge of yellow phosphorus caused fish and crustacean mortality in Long Harbor, Newfoundland (*1*). In an ensuing study on lobsters (*Homarus americanus*) the toxicity of yellow phosphorus was determined (*2*). At that time no attempt was made to determine the exact cause of death, but it appeared that massive hemolymph clotting caused death by asphyxiation. An analysis of tissue samples from these lobsters killed by phosphorus has revealed a pronounced histological change in the hepatopancreas and variable destruction of parts of the antennal gland. This report details these findings.

Laboratory-conditioned juvenile lobsters of both sexes, in stage D_0-D_1 of the molt cycle, were held in tanks (1 by 1 by 0.3 m) containing 200 liters of continuously aerated seawater and various concentrations of phosphorus-contaminated mud obtained from the pollution site in Long Harbor. Tissue from the hepatopancreas, heart, carapace, intestine, gill, gastrolith plate, and supraesophageal ganglion was removed from 16 moribund lobsters contaminated by phosphorus and two controls that were killed by asphyxiation in oxygen-free seawater. Tissues were fixed in Bouin's fluid and stored in 70 percent ethyl alcohol. Carapace sections were stained with Mallory's trichrome; other tissue was stained with Delafield's hema-

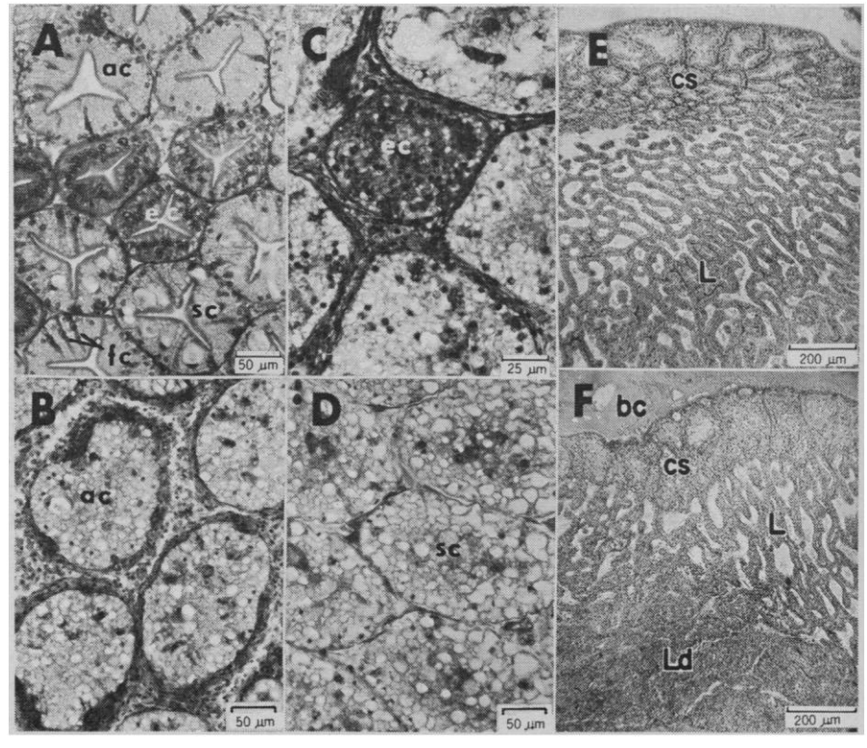

Fig. 1. Comparison of normal tissue from hepatopancreas and antennal gland with similar tissue from lobsters exposed to yellow phosphorus. (A) Sections of normal hepatopancreatic tubules showing four cell types: absorptive (*ac*), embryonic (*ec*), fibrillar (*fc*), and secretory (*sc*). (B to D) Effect of phosphorus on absorptive, embryonic, and secretory cells. Note loss of cellular integrity, extensive vacuolization, and obliteration of tubule lumen. (E) Normal antennal gland tissue showing labyrinth (*L*) and coelomosac (*cs*). (F) Antennae gland from lobster exposed to yellow phosphorus. Note degenerated region of the labyrinth (*Ld*), breakdown of the coelomosac basement membrane, and clotted blood (*bc*).

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.