of the study were recovered separately, the respective fluids were combined and dried by lyophilization, and the residues extracted with methanol.

Three series of experiments were performed in Krebs-Ringer medium prepared as follows. (i) In the first series, the medium was buffered ($NaHCO_3$) at *p*H 7.4, and the substrate was $10^{-5}M$ 1-[1-^{14}C]naphthyl *N*-methylcarbamate. (ii) In the second series, the medium (*p*H 7.4; $NaHCO_3$ buffer) contained $10^{-5}M$ 1-[1-^{14}C]-naphthol substrate. (iii) In the third series, the medium was buffered (KH_2PO_4) at *p*H 6.5, and the substrate was $10^{-5}M$ 1-[1-^{14}C]naphthyl *N*-methylcarbamate.

The polar metabolites were recovered by partitioning the residue of the methanol extract between water and benzene, which removed the substrate from the aqueous phase. The aqueous-phase metabolites were purified by column chromatography and were isolated (*6*); they were then purified further on a Bio-Gel P-2 column. Only the major radioactive peak eluted from the respective columns was saved and processed further; the minor peaks did not contribute enough mass for spectral analysis.

The ^{14}C-labeled material eluted as a single peak from the last column in each cleanup procedure and was concentrated, and the infrared spectrum was examined (*6*).

The mass of ^{14}C-labeled material recovered from the serosal fluid of sacs incubated in *p*H 6.5 medium (there were fewer rats in this group) was not sufficient to obtain an infrared spectrum; consequently, 1 mg of 1-naphthyl β-D-glucuronide was added to the crude extract of this sample before column chromatography to determine whether the major ^{14}C-labeled metabolite behaved throughout the entire chromatographic procedure as naphthyl glucuronide.

It was established that 1-[1-^{14}C]-naphthyl *N*-methylcarbamate degraded spontaneously in *p*H 7.4 medium in blank incubations (14 percent in 2 hours) yielding free 1-[1-^{14}C]naphthol and that breakdown was negligible (< 1 percent) in *p*H 6.5 medium.

The major water-soluble metabolite isolated from the respective mucosal and serosal fluids from sacs incubated in media containing either 1-[1-^{14}C]-naphthyl *N*-methylcarbamate or 1-[1-^{14}C]naphthol was identified as naphthyl glucuronide by comparing its infrared spectra to that of an authentic sample of 1-naphthyl β-D-glucuronide (Pierce Chemical).

The result showed that 1-[1-^{14}C]-naphthyl glucuronide was present in both mucosal and serosal fluids of sacs incubated in *p*H 7.4 medium containing either free 1-[1-^{14}C]naphthol or 1-[1-^{14}C]naphthyl *N*-methylcarbamate, and in the serosal fluid of sacs incubated in *p*H 6.5 medium containing 1-[1-^{14}C]naphthyl *N*-methylcarbamate.

Recovery of ^{14}C from the columns was not always complete; nevertheless, labeled 1-naphthyl glucuronide represented a large proportion of the ^{14}C partitioned into the aqueous phase (Table 1). This was true whether the medium was *p*H 7.4 or 6.5, or whether the 1-naphthol was free or a constituent of 1-naphthyl *N*-methylcarbamate.

Hydrolytic liberation of naphthol from 1-naphthyl *N*-methylcarbamate apparently is an active intestinal function since an abundant quantity of naphthyl glucuronide was synthesized in the *p*H 6.5 medium, in which less than 1 percent of the substrate decomposed nonenzymatically.

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Reduced Nicotinamide-adenine Dinucleotide Phosphate Oxidase: Activity Enhanced by Ethanol Consumption

Abstract. *Prolonged consumption of ethanol enhances the activities of the hepatic microsomal ethanol oxidizing system and of reduced nicotinamide-adenine dinucleotide phosphate oxidase, but not of catalase. The oxidase-catalase system is not part of the microsomal ethanol oxidizing system since catalase inhibitors dissociate ethanol oxidation by the two pathways. Enhanced reduced nicotinamide-adenine dinucleotide phosphate oxidase activity may contribute to liver injury, possibly by favoring lipoperoxidation.*

Ethanol feeding increases the activity of a variety of hepatic microsomal drug-metabolizing enzymes, including that of the microsomal ethanol oxidizing system (MEOS) (*1*). Because ethanol oxidation by MEOS requires reduced nicotinamide-adenine dinucleotide phosphate (NADPH) and O_2 (*2*), it has been suggested (*3*) that the mechanism of ethanol oxidation by MEOS involves NADPH oxidation by the microsomal NADPH oxidase. This generates H_2O_2 (*4*) which in turn can oxidize ethanol to acetaldehyde in the presence of catalase (*5*). The present study was undertaken to test this hypothesis (Fig. 1) and to determine to what extent ethanol feeding affects the activities of hepatic NADPH oxidase and catalase.

Twelve pairs of Sprague-Dawley rat littermates (seven females, five males) (120 to 180 g) were pair-fed nutritionally adequate liquid diets with 36 percent of the total calories as ethanol or isocaloric carbohydrate (*6*). After 24 days, microsomes were obtained and measurements were made of the activities of MEOS and catalase as described (*2*) and of NADPH oxidase activity according to Gillette *et al.* (*4*), with final microsomal protein concentrations of approximately 0.2 mg/ml. Alcohol dehydrogenase (ADH) activity was measured in the hepatic cytosol (100,000*g* supernatant) as described (*2*). In the rats fed ethanol, catalase and ADH activities did not increase, but MEOS and NADPH oxidase activities were enhanced significantly. The MEOS activity (expressed as the number of nanomoles of acetaldehyde produced per minute per gram of liver) increased from 248 ± 21 to 387 ± 44 ($P < .001$) in male rats. The corresponding values for NADPH oxidase activity (expressed as the number of nanomoles of NADPH oxidized per minute per gram of liver) were 65 ± 3 (controls) and 118 ± 16

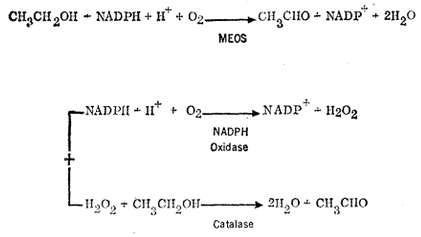

Fig. 1. Ethanol oxidation by way of the hepatic microsomal ethanol oxidizing system (MEOS) or by way of a combination of NADPH oxidase and catalase.

(after ethanol) ($P < .01$). The MEOS and NADPH activities also increased in female rats (Fig. 2). The observed enhancement of NADPH oxidase activity raised the question of its possible role in the increase in MEOS. Since the H_2O_2 generated by NADPH oxidation requires catalase to oxidize ethanol (Fig. 1), advantage was taken of the observation that administration of pyrazole in vivo results in a striking reduction of catalase activity, both in total liver homogenates and in microsomes (*2*, *7*). After a 2-hour fast, five rats received by gastric tube a single dose of pyrazole (300 mg/kg) dissolved in the liquid diet described previously (*6*) at a concentration of 10 mg/ml. The controls received the liquid diet alone. During the subsequent 23 hours, the animals had free access to water and laboratory chow. They were then killed by decapitation, and washed microsomes were obtained as described (*2*). Whereas activities of both MEOS and NADPH oxidase were unaffected, the activity of catalase was inhibited by 90 percent in the rats given pyrazole ($P < .001$) (Fig. 3). Since catalase is required for ethanol oxidation by way of the H_2O_2 generated from NADPH

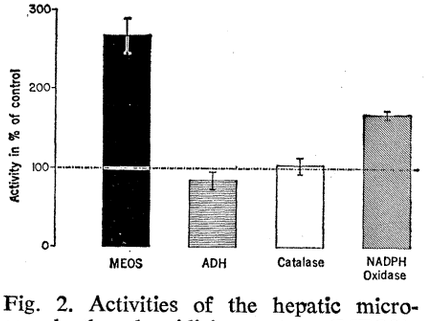

Fig. 2. Activities of the hepatic microsomal ethanol oxidizing system (MEOS), alcohol dehydrogenase (ADH), catalase, and NADPH oxidase in seven pairs of female rat littermates pair-fed with a liquid diet containing either ethanol (36 percent of calories) or isocaloric amounts of carbohydrate over a 24-day period.

oxidation (Fig. 1), the dissociation between catalase inhibition and unaltered MEOS activity suggested that the NADPH oxidase-catalase system is not a component of MEOS. The possibility remained, however, that the activity of NADPH oxidase rather than that of catalase is rate limiting and that, even when inhibited, sufficient catalase activity persists to allow ethanol oxidation from H_2O_2 produced by NADPH oxidation. To test this, the NADPH generating system of our incubation media was replaced by a H_2O_2 generating system modified from Keilin and Hartree (*5*); hypoxanthine (0.45 mg/ml) and 1 unit of xanthine oxidase (Sigma Chemical Co., St. Louis, Mo.) per milliliter were added to the incubation media. In the presence of such an H_2O_2 generating system, even washed microsomes contain sufficient catalase for ethanol to be oxidized at a rate higher than, or at least equal to, that achieved with the NADPH generating system (MEOS). After pyrazole treatment, however, not only was there a 90 percent reduction in microsomal catalase activity, but ethanol oxidation by way of the H_2O_2 generating system was also reduced by 80 percent ($P < .001$) whereas ethanol oxidation by MEOS was unaffected (Fig. 3). A similar, but less striking, dissociation between MEOS activity on one hand, and ethanol oxidation by a H_2O_2 generating system and catalase activity on the other hand, was achieved by addition of $10^{-4}M$ azide (a catalase inhibitor) to microsomes (Fig. 3). When azide was then added in vitro to microsomes of pyrazole-treated animals, the activities of microsomal catalase and of ethanol oxidation with the H_2O_2 generating system were virtually abolished, whereas two-thirds of the activity of MEOS remained (Fig. 3). Hence when microsomal ethanol oxidation is dependent on H_2O_2 generation, it is also sensitive to catalase inhibition. Therefore, if MEOS activity were due to ethanol oxidation by H_2O_2 generated from NADPH oxidation, one would expect that inhibition of catalase activity should reduce MEOS activity to the same extent that it diminishes ethanol oxidation from a H_2O_2 generating system. Since this was not the case, it is unlikely that either catalase or NADPH oxidase are involved to any major extent in MEOS activity. This conclusion is supported by the observation that activity of MEOS can be inhibited by CO (*2*), whereas that of NADPH oxidase is not (*8*). Cholate,

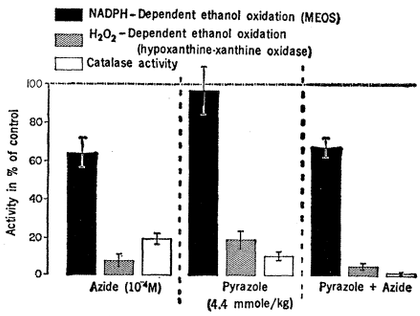

Fig. 3. Differentiation of the activity of the NADPH-dependent microsomal ethanol oxidizing system (MEOS) from that of catalase or of H_2O_2-dependent ethanol oxidation. Azide was added in vitro to washed microsomes of rats given pyrazole in vivo 23 hours before death.

which inhibits NADPH oxidase (*4*), also reduced the activity of MEOS (*9*). This does not mean, however, that NADPH oxidase is necessarily a component of MEOS since cholate is a nonspecific inhibitor which reduces the activity of many unrelated reactions, including that of a variety of microsomal drug detoxifying enzymes (*10*).

Although the increase in NADPH oxidase activity after ethanol consumption does not appear to be responsible for the enhanced MEOS activity, it may nevertheless play an important role in the development of liver damage. Enhanced lipid peroxidation has indeed been implicated in the pathogenesis of various toxic liver injuries (*11*). Enhanced NADPH oxidase activity may be associated with increased H_2O_2 production from NADPH and O_2, which in turn may favor lipid peroxidation in the endoplasmic reticulum, a key metabolic site. The role of such a mechanism in the development of ethanol-induced liver injury remains to be determined.

In conclusion, ethanol feeding was found to produce a striking enhancement of hepatic microsomal NADPH oxidase activity. This does not account for the previously described rise in MEOS activity, since ethanol oxidation by MEOS can be dissociated from that dependent on H_2O_2 generation. The rise in NADPH oxidase activity, however, could play a role in the pathogenesis of alcohol-induced hepatic liver injury.

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Symbiosis between Euglena and Damselfly Nymphs Is Seasonal

Abstract. *An endosymbiotic association has been demonstrated between* Euglena *and nymphs of three species of damselfly. The hindgut of the nymphs is inhabited by the euglenoid only during the winter. Symbiotic associations involving green euglenoids and insects are virtually unknown.*

Damselfly nymphs (Zygoptera: Odonata) collected during the last three winters from freshwater, interdunal ponds (*1*) have their rectums so densely packed with green unicells that the terminal four segments of the abdomen are dark green (Fig. 1). These algae (tentatively identified as *Euglena*) infect almost the entire population of each of three damselfly species, *Anomalagrion hastatum* (Say), *Lestes vigilax* Hagen, and *Ischnura verticalis* (Say). We have not seen this condition in other localities, nor do we find the algal masses on or in other members of the macrofauna, such as dragonfly nymphs (Anisoptera: Odonata), other aquatic insects, isopods, amphipods, and snails.

The rectum of damselfly nymphs is functionally modified as an auxiliary respiratory organ. Although this is not the primary site of oxygen exchange, as it is in the dragonfly nymphs, water can be pumped in and out through the anus bringing oxygen to additional absorptive surfaces (*2*). The abdomen of damselfly nymphs is sufficiently transparent that the cells of its green partner can be observed through the body wall. The gentle water current created by respiratory movements of the insect causes the green cells to swing back and forth, but it does not flush them out of the chamber. The cells are embedded in the folds of the rectum wall (Fig. 2), or they are attached by their posterior end to the cuticular lining and to each other by an adhesive substance. Cells removed from the rectum are spherical or cuboidal, 13 to 14 μm in diameter, having 8 to 12 large chromatophores and small red or brown granules, which probably represent the dispersed eyespot. As the cells are warmed by the microscope lamp for 10 to 30 minutes, they exhibit an apparent euglenoid movement or metaboly. During this period, many cells elongate to dimensions of 7 to 11 by 30 to 35 μm. In each a single flagellum emerges, and the cell swims away with the characteristic euglenoid gyrating swimming motion (Fig. 3). Studies with light and electron microscopes reveal the presence of pyrenoids, paramylon, two flagella within a reservoir, an eyespot made up of eight or more granules, a large endosome in the nucleus, and a striated pellicle underlain by microtubules and muciferous bodies. These are characteristic of the genus *Euglena*. The shape and size of the cells and the arrangement of the chromatophores render the species unidentifiable at this time (*3*).

The damselflies complete one life cycle each year. Eggs are laid during the summer; they then hatch, and the young nymphs grow rapidly until the ice closes the ponds in winter. Then growth ceases, and the nymphs feed only occasionally. In the spring, they resume growth, and the adults emerge in June. The life history of the damselfly and *Euglena* are synchronized by the seasonal changes in the pond. Both forms live separately during the summer, and nothing is known of the ecology of this *Euglena* during this time. Late in the fall, the *Euglena* is somehow attracted to the insect.

We have collected damselfly nymphs, in November, with the external surface of the abdomen around the anus and the bases of the caudal lamellae coated with elongate, green cells. The rectum of each of these nymphs had many spherical cells within it. Both inside and outside the insect, the *Euglena* were nonflagellated, and we assume that they enter the rectum through the anus by a combination of metaboly and gliding motion. The damselfly nymphs present a protected, motile, translucent microhabitat and probably a source of dissolved organic nutrients for the *Euglena*. Since the ponds are shallow and eutrophic, the insects move, as the periphery freezes to the bottom, into deeper water or congregate in spring-fed areas where the ice remains thin. When the ice thaws in March, the damselflies move rapidly into the relatively warm shallows (8° to 10°C) carrying the *Euglena* into regions of relatively higher light intensity where the cells may transform back to the motile, flagellate form and leave their winter host or may be discarded with the cast skin, which includes the rectal lining, when the insect molts. If, during the winter, we bring an insect collected from under the ice into the warm laboratory, the cells will

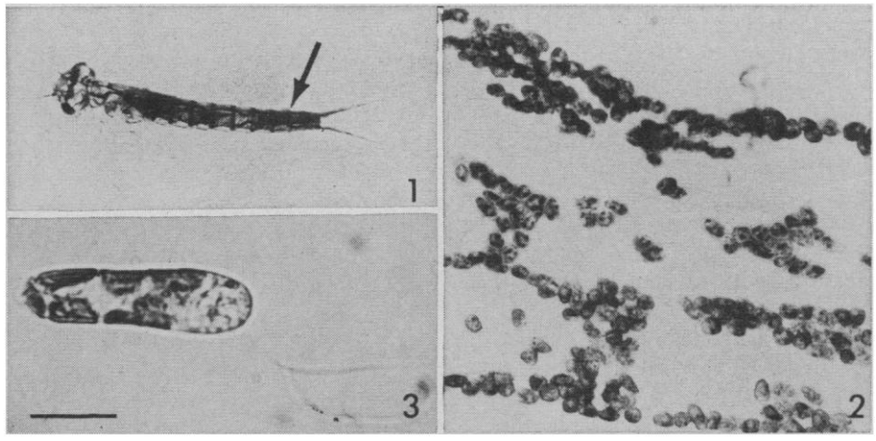

Fig. 1. Nymphal damselfly (*Anomalagrion hastatum*) carrying euglenoids in rectum (arrow). Fig. 2. Rectum removed from damselfly to show arrangements of euglenoids. Fig. 3. Flagellated *Euglena* (scale represents 10 μm).