to 43 ± 2.2 percent of control values. The uptake of [^{3}H]serotonin was not significantly altered (98 ± 6.3 percent of controls). Both spontaneous and L-dopa-induced release of [^{3}H]DA was significantly diminished by prior treatment with 6-hydroxydopamine, while the release of [^{3}H]serotonin remained unchanged (Fig. 1).

The foregoing observations provide direct evidence that L-dopa releases brain DA and serotonin and that this releasing action may be dependent on the conversion of dopa to DA. The effect of L-dopa appears to be specific, since it did not influence the release of [^{14}C]urea from striatal slices (*9*). Furthermore, selective destruction of catecholamine-containing nerve terminals by 6-hydroxydopamine substantially decreased the uptake and release of [^{3}H]DA but not that of [^{3}H]serotonin. Although it is likely that some decarboxylation of L-dopa to DA continues in catecholaminergic terminals remaining after prior treatment with 6-hydroxydopamine, it is improbable that the amine thus formed could account entirely for the unaltered release of [^{3}H]-serotonin. Our results thus suggest that the presence of catecholaminergic terminals are not necessary for the L-dopa-induced release of serotonin and that the uptake and decarboxylation of L-dopa to DA occur within serotoninergic as well as dopaminergic neurons.

These findings may have important clinical implications. Conceivably L-dopa when forming abnormally large concentrations of dopamine may interfere with the metabolism of some other essential amine or displace it from its normal storage sites. In patients treated with high doses of L-dopa a decrease of serotonin resulting from displacement by DA seems therefore highly probable. The role of DA as a central "false" serotoninergic transmitter may be relevant to the therapeutic effects or side effects, or both, observed in patients receiving high doses of L-dopa.

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Intestinal Hydrolysis and Conjugation of a Pesticidal Carbamate in vitro

Abstract. *The metabolite 1-[1-^{14}C]naphthyl glucuronide was isolated from mucosal and serosal fluids of everted sacs of rat small intestine incubated in media containing either the insecticide 1-[1-^{14}C]naphthyl N-methylcarbamate (carbaryl) or 1-[1-^{14}C]naphthol. The hydrolysis of carbaryl and conjugation of the liberated naphthol indicated some degree of metabolism by the intestine before absorption.*

Conjugation of one chemical with another is a common metabolic function in mammals. Conjugates of insoluble toxic chemicals are generally more water-soluble and less toxic than the parent chemical (*1*).

Although the liver has a high capacity to perform the metabolic process of conjugation, it is important to recognize that other tissues also have this capability. Metabolic conjugation occurs in the kidney, gastric mucosa (*2*), and intestine (*3*).

Pesticide chemicals, ingested as residues on foodstuffs, encounter the gastrointestinal tract before any other tissue. If these pesticides were conjugated by the intestine, their solubility, and consequently their absorption and toxicity, would be altered. Reduced absorption or toxicity or both would provide substantial protection against the chemical.

Sulfate and glucuronide conjugates of various carbamate pesticidal chemicals or their hydrolysis products or both were reported to have been found in urine (*4–6*). The 1-[1-^{14}C]naphthyl glucuronide conjugate was among the major polar metabolites of 1-[1-^{14}C]-naphthyl *N*-methylcarbamate in rat and guinea pig urine (*4*), and to a lesser extent in chicken urine (*6*). Since naphthyl glucuronide represents one of the major polar urinary metabolites of the rat and methods for its isolation from biological samples have been described (*6*), it was selected as a model compound to investigate the ability of rat intestine to synthesize this specific glucuronide from carbaryl.

The small intestines of male Sprague-Dawley rats (275 to 350 g) were divided into three approximately equal parts and everted by use of the general technique of Wilson and Wiseman (*7*). The serosal space of the sacs was filled with 4 ml of the appropriate Krebs-Ringer medium (*8*), containing 1 mg of glucose per milliliter, and the sacs were individually incubated for 2 hours at 37°C in 100 ml of the same medium (mucosal fluid) under an atmosphere of 95 percent O_2 and 5 percent CO_2. After incubation, the serosal and mucosal fluids from all sacs

Table 1. The percentage of the total ^{14}C in that portion of mucosal and serosal fluids which partitioned into the aqueous phase, and the *minimum* percentage of the carbon-14 in the aqueous phase that was accounted for as 1-[1-^{14}C]-naphthyl glucuronide (NG). The minimum percentage is the ratio of the disintegrations per minute of final isolated metabolite to the disintegrations per minute in the original aqueous phase after partition times 100.

Substrate	Fluid	Aqueous phase ^{14}C	
		Total (%)	As NG (minimum %)
Krebs-Ringer bicarbonate medium (pH 7.4)			
1-[1-^{14}C]naphthyl *N*-methylcarbamate	Mucosal	21	*
	Serosal	68	67
Krebs-Ringer phosphate medium (pH 6.5)			
1-[1-^{14}C]naphthyl *N*-methylcarbamate	Serosal	40	46
Krebs-Ringer bicarbonate medium (pH 7.4)			
1-[1-^{14}C]naphthol	Mucosal	91	35
	Serosal	99	58

* Insufficient quantity of compound for analysis.

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