Histological examination of kidney sections stained with Eosin and Haematoxylin demonstrated that mercuric chloride at the dose used caused necrosis of the proximal convoluted tubule, while p-nitrophenylarsonic acid caused necrosis of both the proximal and distal convoluted tubules; no glomerular damage was detected in either case. Protein excretion in the rats rose from a normal range of 38 ± 13 mg/day to 115 ± 31 mg/day for p-nitrophenylarsonic acid and 71 ± 31 mg/day for mercuric chloride within 24 h of the injection but returned to normal within 10 days. Maximum polyuria occurred within 2 days after p-nitrophenylarsonic acid injection, rising from 9.0 ± 2 ml/day in the untreated animals to 23.0 ± 0.6 ml/day. Within 4 days of mercuric chloride administration a maximum of 13.5 ± 5.3 ml/day was excreted. No change in creatinine excretion was detected. Normal male adult rat urine proteins migrated in the albumin and prealbumin regions in vertical gel electrophoresis (Fig. 1d). After administration of either nephrotoxin this pattern was disturbed, and the principal urinary proteins migrated in the α- and β-globulin and albumin regions (Fig. 1b, a). Since one of the forms of the major protein found in the urine of adult male rats migrates similarly to albumin in certain electrophoretic systems (Lane & Neuhaus, 1972), two-dimensional gel electrophoresis was used to show that increased albumin excretion had occurred. According to analysis by this method normal urine contains little or no albumin. Albuminuria was also confirmed by gel filtration and subsequent electrophoresis of the pooled and concentrated fractions in polyacrylamide gel. Gel filtration (Fig. 2) showed that within 24 h after injection albumin excretion contributed 55% (p-nitrophenylarsonic acid) and 39% (mercuric chloride) of the total protein excretion. This decreased in 5 days to 33% (p-nitrophenylarsonic acid) and 29% (mercuric chloride). Results of serum albumin determination showed a decrease of albumin concentration from a value in untreated rats of 31.2 ± 1.6 mg/ml to 24.2 ± 1.6 mg/ml in the case of p-nitrophenylarsonic acid, and from 31.2 ± 1.6 mg/ml to 29.7 ± 1.6 mg/ml in the case of mercuric chloride, within 24 h of injection.

Mercuric chloride and p-nitrophenylarsonic acid at the doses used in these studies cause proteinuria, a partial analysis of which indicates that in addition to damaging the kidney tubules p-nitrophenylarsonic acid may also affect glomerular function, whereas mercuric chloride does not appear to do so.


The Effect of Ethanol Administration on Drug Oxidations and Possible Mechanism of Ethanol-Barbiturate Interactions

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Although it is well known that ethanol is metabolized by alcohol dehydrogenase of the liver cytosol (Morrison & Brock, 1967), another enzyme system capable of metabolizing...
ethanol and present in the hepatic microsomal fraction has been described (Orme-
Johnson & Ziegler, 1965; Lieber & DeCarli, 1968). Subsequent investigations of this
system revealed many similarities with the hepatic microsomal system known to
metabolize a diversity of drugs including the barbiturates (Rubin et al., 1971; Lieber &
DeCarli, 1970). Administration of ethanol has been shown to stimulate many hepatic
microsomal drug-metabolizing enzymes (Rubin & Lieber, 1968; Mezey, 1972), and has
led to the suggestion that the oxidation of ethanol by microsomal fractions was re-
sponsible for the decreased pharmacological response to barbiturates seen in non-
intoxicated chronic alcoholics and the increased response observed in intoxicated
persons (Wiberg et al., 1969; Coldwell et al., 1970). The present work was undertaken
to investigate the mechanism of metabolic interaction between ethanol and barbiturates.

Male albino Wistar rats, 21-days old, were allocated into four groups of six animals
treated as follows: A, controls; B, 10% (v/v) aq. ethanol as the only drinking liquid for
6 weeks; C, daily intraperitoneal administration of phenobarbitone (75 mg/kg) for 3 days
before killing; D, combination of B and C. Animals were killed by cervical fracture
24h after the last injection of phenobarbitone, and livers were immediately removed,
washed with ice-cold 1.15% (w/v) KCl, minced and homogenized with 4 vol. of KCl.

The liver homogenates were centrifuged at 9000g for 20 min to obtain the '9000g super-
natant fraction'. This was further centrifuged at 105000g for 60 min and the supernatant
obtained ('soluble fraction') was decanted and the microsomal pellet was resuspended
in KCl ('microsomal fraction'). The 9000g supernatant was used for the determination
of biphenyl hydroxylase [Creaven et al. (1965), modified by Neale (1970)], aniline
hydroxylation (Guarino et al., 1969), ethylmorphine demethylation (Holtzman et al.,
1968) and p-nitrobenzoate reductase (Fouts & Brodie, 1957). The microsomal fraction
was used for the determination of NADPH-cytochrome c reductase (Williams &
Kamin, 1962), cytochrome b5 (Schenkman et al., 1967), cytochrome P-450 (Sladek &
Mannering, 1966) and protein (Lowry et al., 1951). Sleeping times were measured by
intraperitoneal administration of hexobarbitone (100 mg/kg) and paralysis times by
intraperitoneal administration of zoxazolamine (100 mg/kg; 2-amino-5-chlorobenz-
oxazole). Plasma pentobarbitone concentrations were determined by gas-liquid
chromatography on 10% SE.30 on Diatomite C by using N2 saturated with formic acid
as carrier gas, in a Pye 104 Series Gas Chromatograph.

Chronic administration of ethanol to rats stimulated the activities of biphenyl 4-
hydroxylase, aniline hydroxylation and p-nitrobenzoate reductase (see Table 1) but not to
as great an extent as was observed after phenobarbitone administration. No significant
changes occurred in the activities of NADPH-cytochrome c reductase and ethyl-
morphine demethylation, nor in the contents of cytochromes b5 and P-450. Simultaneous
administration of ethanol and phenobarbitone resulted in a greater increase in the
amounts of the cytochromes than was observed after administration of phenobarbitone
alone, but the enzyme activities in vitro were not similarly further enhanced. In con-
trast, the activities of the drug-metabolizing enzymes observed in the sleeping-time and
paralysis studies in vivo were more enhanced by phenobarbitone plus ethanol pre-
treatment than by phenobarbitone alone. The observations that ethanol per se has no
stimulatory effect on the drug-metabolizing enzymes in vitro and in vivo have been
further confirmed by the determination of the effects of ethanol on the rate of metabolism
of pentobarbitone. Pretreatment of rats with ethanol given as 10% (v/v) aq. ethanol for
2 weeks did not change the plasma half-life of pentobarbitone (t1/2 = 70min) but
simultaneous administration of ethanol (1200 mg/kg) intraperitoneally with pento-
obarbitone (75 mg/kg) increased the plasma half-life to an extent where no significant
metabolism was observed over a period of 90 min (see Fig. 1).

We have shown that chronic administration of ethanol has no effect on the plasma
half-life of pentobarbitone, which indicates that ethanol does not induce the microsomal
enzyme system and that the latter is therefore not identical with the 'microsomal
ethanol-oxidizing system'. The microsomal oxidation of ethanol observed in vitro is
probably the result of metabolism of ethanol by contaminating alcohol dehydrogenase
and by catalase peroxidation (Carter & Isselbacher, 1971; Thurman et al., 1972).
Table 1. Effect of chronic ethanol administration on rat hepatic microsomal drug metabolism

For details see the text. Results are presented as means±S.E.M. The values given in parentheses are the number of determinations. P values for groups compared to control are: *P<0.001; **P<0.01; ***P<0.05.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A Control</th>
<th>Group B Ethanol-treated</th>
<th>Group C Phenobarbitone-treated</th>
<th>Group D Ethanol+phenobarbitone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver wt./g body wt.</td>
<td>0.040±0.001</td>
<td>0.040±0.001</td>
<td>0.045±0.0002</td>
<td>0.045±0.001</td>
</tr>
<tr>
<td>Biphenyl 4-hydroxylase (µmol/h per g of liver)</td>
<td>0.72±0.01</td>
<td>1.5±0.13*</td>
<td>2.7±0.18*</td>
<td>2.8±0.06*</td>
</tr>
<tr>
<td>Aniline hydroxylase (µmol/h per g of liver)</td>
<td>1.4±0.08</td>
<td>1.9±0.13**</td>
<td>2.3±0.11*</td>
<td>3.0±0.13*</td>
</tr>
<tr>
<td>p-Nitrobenzoate reductase (µmol/h per g of liver)</td>
<td>6.3±0.19</td>
<td>7.0±0.25***</td>
<td>7.9±0.14*</td>
<td>7.9±0.06*</td>
</tr>
<tr>
<td>Ethylmorphine demethylation (µmol/h per g of liver)</td>
<td>20±2.0</td>
<td>18±3.2</td>
<td>28±2.2***</td>
<td>26±2.5</td>
</tr>
<tr>
<td>NADPH-cytochrome c reductase (E units/min per g of liver)</td>
<td>11±1</td>
<td>13±0.5</td>
<td>25±1.1*</td>
<td>23±1.6*</td>
</tr>
<tr>
<td>Cytochrome b₅ (E units/g of liver)</td>
<td>1.5±0.13</td>
<td>1.4±0.06</td>
<td>2.0±0.17***</td>
<td>2.4±0.11*</td>
</tr>
<tr>
<td>Cytochrome P-450 (E units/g of liver)</td>
<td>1.4±0.18</td>
<td>1.4±0.13</td>
<td>3.5±0.32*</td>
<td>4.2±0.34*</td>
</tr>
<tr>
<td>Microsomal protein (mg/g of liver)</td>
<td>35±3.2</td>
<td>40±2.6</td>
<td>39±2.8</td>
<td>45±3.3</td>
</tr>
<tr>
<td>Hexobarbitone sleeping time (min)</td>
<td>18±1 (9)</td>
<td>28±2 (7)</td>
<td>12±1 (7)*</td>
<td>6±1 (9)†</td>
</tr>
<tr>
<td>Zoxazolamine paralysis time (min)</td>
<td>153±4</td>
<td>151±20 (6)</td>
<td>48±9 (6)</td>
<td>0.00±(9)‡</td>
</tr>
</tbody>
</table>

† Only three animals slept.
‡ None of the animals was paralysed.
Chronic administration of ethanol to rats leads to stimulation of the activity of the hepatic drug-metabolizing enzymes in vitro which is not paralleled by any corresponding increase in the concentration of cytochrome P-450, the terminal enzyme in the microsomal mono-oxygenase system (Table 1). However, no changes were seen in hexobarbital sleeping time or zoxazolamine paralysis time, confirming that ethanol pretreatment does not lead to any significant stimulation of the hepatic microsomal drug-metabolizing enzymes in vivo. The observed inhibition of barbiturate metabolism after simultaneous administration of large amounts of ethanol may be attributed to non-specific interaction with the active-site of the microsomal drug-metabolizing enzymes.

Ethanol has also been shown to potentiate the enzyme induction effect of phenobarbitone in vitro in respect of cytochrome P-450 and cytochrome b5. The effect was even more pronounced in vivo when none of the animals pretreated with both phenobarbitone and ethanol exhibited zoxazolamine paralysis and only one-third of these animals exhibited narcosis after hexobarbitone administration.

The lipophilic character of ethanol allows it to penetrate the lipid environment of the endoplasmic reticulum and to interact with cytochrome P-450 giving a type II substrate interaction spectrum (Rubin et al., 1971), although it is not metabolized by the microsomal mono-oxygenase system. The molar concentration of ethanol required to give a type II spectrum is much higher than that required for aniline suggesting that the interaction of ethanol with cytochrome P-450 is a non-specific interaction with the lipophilic phase of the microsomal membrane adjacent to the haemoprotein (Imai & Sato, 1967). Ethanol may therefore act as an activator of the microsomal mono-oxygenase enzyme system either by modification of the membrane environment, by allosteric effects, or by displacement of other endogenous or exogenous substrates already bound to the enzyme sites. The potentiation of phenobarbitone induction of this enzyme system may be the result of stabilization of the substrate-enzyme complex, as it has been suggested that induction of the microsomal enzymes is a function of the stability of the enzyme-substrate complex (Ioannides & Parke, 1973).


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Adenosine 3':5'-Cyclic Monophosphate Response Elicited by Histamine and Adrenaline in Lungs of Normal and Allergic Guinea Pigs: Relationship of Histamine Release and Adenylate Cyclase Activity

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Sliced lungs of normal and ovalbumin-sensitized guinea pigs were preincubated in Krebs–Ringer-bicarbonate buffer, pH 7.4, for 30 min at 37\textdegree C and preincubated again for 20 min in the presence of aminophylline (20 \textmu M) and then incubated for 6 min with histamine, adrenaline (10 \textmu M) and/or neoantergane (1 mm), and/or propranolol (0.1 mm), and antigen (1 mg of ovalbumin/g wet wt.) respectively, at 37\textdegree C in the same buffer. The slices were homogenized in 0.25 M sucrose–1 mM ATP–1 mM EDTA–10 mM Tris–HCl buffer, pH 7.4, and centrifuged at 2000g for 30 min, at 4\textdegree C. Adenylate cyclase activity was assayed by the method of Krishna et al. (1968), modified by Ramachandran (1971). Histamine was assayed fluorimetrically by the method of Ruff et al. (1967).

In normal animals, the amount of cyclic AMP was increased about 10 times by histamine and its formation completely suppressed by neoantergane. Adrenaline increased the amount of cyclic AMP about 20 times. Propranolol caused an inhibition of adenylate cyclase activity by about 50\%. Histamine in the presence of adrenaline abolished adenylate cyclase activity completely (Table 1).

In the sensitized animals, addition of antigen produced histamine release: histamine release was increased by 75\%, the amount of cyclic AMP was doubled; in the presence of neoantergane the amount of cyclic AMP was unchanged. Neoantergane without addition of antigen had an analogous effect. Antigen in the presence of adrenaline revealed the same amount of histamine release as antigen without adrenaline; however, the amount of cyclic AMP was decreased. The histamine release by adrenaline without addition of antigen was smaller than that by antigen alone, but the amount of cyclic AMP was increased. There was no histamine release in the presence of propranolol, but cyclic AMP formation was augmented (Table 2).

From these results one can deduce that histamine release seems to implicate the \( \beta \)-receptor, because propranolol, an inhibitor of the \( \beta \)-receptor, inhibited histamine release; the formation of cyclic AMP was not inhibited by propranolol and it was even increased. It seems that in the absence of a \( \beta \)-adrenergic agent, such as adrenaline, the propranolol has no blocking effect on the adenylate cyclase activity.