1971). Under these conditions, however, it is feasible that the net synthesis of nitro-
amines may be decreased by the degradative action of bacterial enzymes, since many
strains of E. coli, the most common bacterial type associated with urinary-tract infections,
could degrade both diphenylnitrosamine and dimethylnitrosamine (Table 1).

The expert technical assistance of Mrs. Cherry Smith is gratefully acknowledged.

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The Effect of some Inducers of Hepatic Xenobiotic-Metabolizing
Enzymes on the Urinary Excretion of D-Glucuronic Acid
Metabolites in the Rat

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A wide variety of drugs, food additives, pesticides and environmental chemicals have
been shown to enhance the activities of hepatic microsomal xenobiotic-metabolizing
enzymes in experimental animals (Conney, 1967). In addition, some of these compounds
increase the metabolism of both D-glucose and D-galactose via the D-glucuronic acid
pathway (Burns et al., 1960), resulting in the increased urinary excretion of components
of this pathway, e.g. L-gulonic acid, L-ascorbic acid, D-glucaric acid and D-glucaric
acid (Conney et al., 1961).

The urinary excretion of D-glucaric acid has thus been proposed as an index of hepatic
microsomal enzyme activities in both experimental animals (Hunter et al., 1973), and in
man (Aarts, 1965; Sotaniemi et al., 1974). Recently, we have developed a sensitive g.l.c.
procedure for the determination of D-glucaric acid and a number of intermediates of the
D-glucaric acid pathway in both human and animal urine (Gangolli et al., 1974). In
the present communication we report the results of investigations on the effect of treatment
with some model compounds, which induce microsomal enzymes in the rat, on the
urinary excretion of components of the D-glucaric acid pathway.

Male Wistar albino rats (60–80 g) were used in these studies. They were caged in pairs
and allowed free access to laboratory diet and water. Ethylmorphine N-demethylase
(Holtzman et al., 1968), aniline 4-hydroxylase (Nakanishi et al., 1971) and 4-methyl-
umbelliferyl glucurononyltransferase (Neale & Parke, 1973) were determined on hepatic
10000 g supernatant fractions. Microsomal cytochrome P-450 was measured by the
method of Omura & Sato (1964). The 24h urine samples were examined by g.l.c. as
described by Gangolli et al. (1974).

In the first series of experiments sequential investigations were conducted on the activities
of some hepatic microsomal enzymes and the urinary excretion of D-glucaric
acid, L-gulonic acid and xylitol both during and after the cessation of sodium pheno-
barbitone treatment. The barbiturate was administered daily by the intraperitoneal
route to groups of rats at a dose of 100mg/kg per day for 7 days. The hepatic activities of aniline 4-hydroxylase, ethylmorphine N-demethylase and 4-methylumbelliferyl glucurononyltransferase progressively increased during the treatment (Fig. 1a). Similarly, increases were observed in the hepatic cytochrome P-450 content and the relative liver weights of the treated groups. On cessation of the treatment the activities of all these parameters declined to 125–170% of control values by the fourth day. The urinary excretion of L-gulonic acid closely paralleled the pattern of induction of the hepatic parameters, being 390% of control values after 7 days (Fig. 1b). Similarly, the urinary excretion of xylitol was also enhanced to 410% of the control value, maximal stimulation occurring after 4 days of treatment. In contrast, the increase in the urinary excretion of D-glucaric acid lagged behind the induction of the microsomal enzymes, the maximal enhancement of excretion being only 170% of control. Phenobarbitone treatment also resulted in increased urinary excretion of D-glucuronic acid.

Additionally, studies were undertaken on the comparable effects of four other commonly used microsomal enzyme-inducing agents, namely 20-methylcholanthrene (a polycyclic hydrocarbon carcinogen), dichlorodiphenyltrichloroethane (a chlorinated hydrocarbon pesticide), 3,5-di-t-butyl-4-hydroxytoluene (a food antioxidant) and pregnenolone 16α-carbonitrile (a synthetic non-hormonally active steroid). 20-Methylcholanthrene and dichlorodiphenyltrichloroethane were administered by daily intraperitoneal injections at doses of 20 and 100mg/kg per day respectively and 3,5-di-t-butyl-4-hydroxytoluene and pregnenolone 16α-carbonitrile by daily gastric intubations at doses of 500 and 40mg/kg per day respectively. All four compounds were administered for 7 days, control animals receiving appropriate quantities of corn oil. Urine samples (24h) were collected after the last dose and the animals were then killed for the determination of hepatic enzymes.

All four compounds significantly increased the relative liver weight, cytochrome P-450 content and the activities of aniline 4-hydroxylase and 4-methylumbelliferyl glucurononyltransferase (Table 1). The activity of ethylmorphine N-demethylase was not enhanced by 20-methylcholanthrene administration, in accordance with the previous findings of Sladek & Mannering (1969). Moreover, the microsomal CO-binding haemoprotein present in the livers of the 20-methylcholanthrene-treated rats was cytochrome P-448 rather than cytochrome P-450 as previously observed by Alvares et al. (1967).

The urinary excretion of D-glucaric acid, L-gulonic acid and xylitol was also examined (Table 1). None of the four inducers studied significantly enhanced the excretion of all
Table 1. Comparison of the effects of some xenobiotics on hepatic microsomal enzyme activities and on the urinary excretion of D-glucaric acid, L-gulonic acid and xylitol

For details see the text. Results are presented as percentages of control values.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Hepatic parameters</th>
<th>Urinary parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Relative liver weight</td>
<td>Ethylmorphine N-demethylase</td>
</tr>
<tr>
<td>20-Methylcholanthrene</td>
<td>130‡</td>
<td>95</td>
</tr>
<tr>
<td>Dichlorodiphenyltrichloroethane</td>
<td>130‡</td>
<td>365‡</td>
</tr>
<tr>
<td>3,5-Di-t-butyl-4-hydroxytoluene</td>
<td>135‡</td>
<td>245‡</td>
</tr>
<tr>
<td>Pregnenolone 16α-carbonitrile</td>
<td>135‡</td>
<td>385‡</td>
</tr>
</tbody>
</table>

* Value significantly different from control, $P < 0.05$.
† Value significantly different from control, $P < 0.01$.
‡ Value significantly different from control, $P < 0.001$. 
three intermediates of the D-glucuronic acid pathway examined. Both dichlorodiphenyl-trichloroethane and pregnenolone 16α-carbonitrile enhanced the urinary excretion of both L-gulonic acid and xylitol; whereas 20-methylcholanthrene treatment enhanced the urinary excretion of both L-gulonic acid and D-glucaric acid. The administration of 3,5-di-t-butyl-4-hydroxytoluene resulted in a large increase in xylitol excretion. The results of our studies indicate that the measurement of a spectrum of metabolites of the D-glucuronic acid pathway provides a more reliable index of the induction of hepatic microsomal xenobiotic-metabolizing enzymes than the determination of a single urinary component such as D-glucaric acid.

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Hexachlorobenzene: Member of a New Class of Hepatic Microsomal Enzyme Inducers

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Glucose Utilization by the Avian Shell Gland

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The shell gland is the region of the avian oviduct where the calcareous shell is secreted on to the inner shell membranes of the egg. During the laying cycle of the domestic fowl each ovum reaches the shell gland approx. 5 h after the preceding oviposition, and remains there for some 20 h (Melek et al., 1973) while CaCO₃ is deposited at a rate exceeding 2.5 mmol/h (Talbot & Tyler, 1974). The secretory activity is accompanied by an increase in the blood flow through the gland (Moynihan & Edwards, 1974) and, according to studies in vitro (Beuving, 1971), increased O₂ uptake by the organ takes place. Such observations indicate a high metabolic activity within the organ, but the nature and source of the substrates utilized has not been investigated previously.

Seven groups of 1-year-old laying fowls were lightly anaesthetized at successive 4h intervals after oviposition. A sample of arterial blood and of shell-gland venous blood were taken from each hen via a mid-line incision. The animals were killed by cervical dislocation, the glands were removed immediately and the glycogen content was measured by the method of Walaas & Walaas (1950). D-Glucose and L-lactate in the blood plasma were measured by the methods of Huggett & Nixon (1957) and Hohorst (1963).