INCREASE IN LEVELS OF GLUCURONYLATING ENZYMES AND
ASSOCIATED RISE IN ACTIVITIES OF MITOCHONDRIAL OXIDATIVE
ENZYMES UPON PHENOBARBITAL ADMINISTRATION IN THE RAT

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Previous papers from this and other laboratories
have dealt with the increase of drug-metabolizing
activities of liver microsomes by administration of
certain drugs and with the metabolic events associated
with this increase (1). Heretofore, in this laboratory the greatest amount of attention has
been given to drug hydroxylation, although this is
only one of a number of detoxicating systems that
can be stimulated by the administration of drugs.
In the present paper we describe studies with an-
other detoxicating system which can be stimulated
by drug administration, namely the glucuronylating
system. We also describe studies on other metabolic events occurring in association with drug
administration, namely increases in the activities
of mitochondrial oxidative enzymes.

The glucuronylating pathway is one of the most
important routes for drug detoxication. A great
many drugs and foreign products, as well as natural products, are excreted in the urine as glucu-
ronides, by direct esterification or after prior hydroxylation. The glucuronylation pathway has
been well defined and is given in outline in Fig. 1.
The first enzyme involved, UDPG\(^1\) dehydrogenase,
is a supernatant enzyme. It has been purified by
Strominger et al. (2). The second enzyme, glucu-

1 Abbreviations employed in this paper: UDPG, Uridine diphosphoglucose; UDPGA, Uridine diphos-
phoglucuronic acid; NAD\(^+\), nicotinamide adenine dinu-
cleotide; NADP\(^+\), nicotinamide adenine dinucleo-
tide phosphate; ADP, Adenosine diphosphate; DCPIP, 2,6-dichlorophenolindophenol.

ronyl transferase, is a microsomal enzyme which
has been studied extensively by Isselbacher and
his associates (3).

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\begin{align*}
\text{UDPG} + 2 \text{NAD}^+ \xrightarrow{\text{dehydrogenase}} & \text{UDPGA} + 2 \text{NADH} + 2\text{H}^+ \\
\text{UDPGA} + \text{ROH} \xrightarrow{\text{transferase}} & \text{R-O-glucuronide} + \text{UDP}
\end{align*}
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**Figure 1** Metabolic pathway of glucuronolysis.

The results reported here with phenobarbital
administration, like those obtained by other work-
ers using a variety of drugs (see reference 4 for a
brief review), show that both UDPG dehydrogenase
and glucuronyl transferase activities are enhanced
by administration of the drug. This is in accord
with the work of Remmer (5) who showed that
administration of phenobarbital increases the met-
abolism of sulfadimethoxin via the glucuronic
acid pathway, as judged by urinary output of con-
jugated metabolites. The time course of the re-
sponse of the glucuronyl transferase activity is of
interest in that it seems to be characterized by a
lag period prior to the increase. The increment of
mitochondrial oxidative activities also follows this
pattern rather than the more rapid enhancement
exhibited by the oxidative demethylation system.

In all experiments male Sprague-Dawley rats
(200–300 g) were used. The animals were fasted overnight prior to the morning of the experiment. 
The experimental animals were given daily injections of sodium phenobarbital, 80 mg per kg of body weight, intraperitoneally.
Preparation of liver mitochondria was according to the procedure of Ernster and L6w (6).
Liver microsomes were prepared as follows. Tissue was homogenized in 5 volumes of 0.15 m KCl. The homogenate was centrifuged at 10,000 g for 10 min and the pellets were discarded. The supernatant was then centrifuged at 105,000 g for 1 hr, and the microsomal pellets thus obtained were washed two times in the same volume of 0.15 m KCl prior to suspension in the same medium at a concentration of 5–10 mg microsomal protein per ml. These suspensions were used to assay glucuronyl transferase and oxidative demethylating activities. The supernatant from the initial 105,000 g centrifugation was saved and used to assay UDPG dehydrogenase and DT diaphorase activities.
UDPG dehydrogenase was determined from the rate of NAD+ reduction, measured spectrophotometrically at 340 mμ. The method was essentially the same as that of Strominger et al. (2). Cuvettes contained, in addition to enzyme, 0.6 μmoles of UDPG and 3 μmoles of NAD+ in a total volume of 3 ml of 0.1 μ glycine buffer, pH 8.7. UDPG was omitted from control cuvettes. Activities were calculated by subtracting values of controls from experimental values.
Glucuronyl transferase was assayed according to the method of Hollmann and Touster (7) using p-nitrophenol as substrate, except that incubations were terminated at 5 min.
DT diaphorase was determined by the method of Ernster et al. (8) using both NAD+ and NADP+ as substrates.
Oxidative demethylation was measured as described previously (9), using aminopyrine as substrate.
The rates of oxygen consumption by rat liver mitochondria using succinate, pyruvate-malate, and alpha glycerophosphate as substrates in the presence of phosphate and phosphate acceptor were measured with a Clark oxygen electrode. To mitochondria (4 mg mitochondrial protein) in a total volume of 4 ml of Tris-KCl-phosphate buffer, pH 7.5, were added successively 20 μl of 1 μ M gSO4, 10 μl of 1 μ substrate, and 10 μl of 60 mm ADP. Buffer concentrations were: Tris-HCl, 50 mm; KCl, 100 mm; phosphate, 10 mm. All determinations were carried out at 30°C.
Protein was measured by the method of Lowry et al. (10).
Results obtained with the glucuronolyzing enzymes, DT diaphorase and oxidative demethylation are shown in Fig. 2. Daily injections of 80 mg phenobarbital per kg of body weight to rats resulted in an almost twofold increase in the activities of glucuronolyzing enzymes. The response of the UDPG dehydrogenase, however, was considerably faster than the response of the glucuronyl transferase which showed no obvious increase until the third day of drug administration. Oxidative demethylation, as observed previously (1), gave an immediate response, whereas there was no change in the level of DT diaphorase activity.

![Figure 2](https://example.com/figure2.png)

**Figure 2** Activities of glucuronyl transferase (○), UDPG dehydrogenase (□), DT diaphorase, using NAD (×) and NADP (△) as hydrogen donors, and oxidative demethylation (●), all expressed as per cent of control values. Control values were as follows. Glucuronyl transferase: 0.678 μmoles p-nitrophenol conjugated/min/mg protein; UDPG dehydrogenase: 1.25 μmoles NAD+ reduced/min/mg protein; DT diaphorase: 1.96 (NAD) and 2.14 (NADP) μmoles DCPIP reduced/min/mg protein; Oxidative demethylation: 5.85 μmoles formaldehyde/min/mg protein. All points represent the average of four or more animals. Phenobarbital was injected once daily.
Results obtained with mitochondrial oxidative enzymes are shown in Fig. 3. An increment in mitochondrial oxidative utilization of succinate and pyruvate-malate occurred. As will be noted, both the magnitude and time course of the increment are comparable to those in the curve obtained with glucuronyl transferase. Respiratory control was as good in mitochondria from drug-treated rats as from controls, and P:O ratios were also unchanged (Fig. 4).

In connection with this phenomenon, the results of Lee, Takemori, and Lardy (11), on the increments of alpha glycerophosphate oxidation by rat liver mitochondria after treatment of the animals with thyroid hormone, were of interest. Experiments were carried out to see whether treatment of animals with phenobarbital would lead to an increase in utilization of alpha glycerophosphate as substrate, as was the case with succinate and pyruvate-malate. Oxidation of alpha glycerophosphate by rat liver mitochondria in the presence of phosphate and ADP was measured with the oxygen electrode. As will be noted in Fig. 4, alpha glycerophosphate was found to be a substrate of negligible significance for drug-treated mitochondria, whereas in mitochondria from thyroid-treated animals it has been shown to become almost as good a substrate as succinate (the response to which is also seen in Fig. 4). The same result was obtained twice with animals after 1, 2, 3, 4, and 5 days of phenobarbital administration, and in controls.

Previous work has been done in this laboratory to examine the possibility that mitochondria participate in the cellular response to administration of drugs, with essentially negative results. Orrenius, Ericsson, and Ernster (12) have shown that, whereas treatment of rats with phenobarbital causes a greatly enhanced incorporation in vivo of P32 into the microsomal phospholipid, incorporation into the phospholipid of mitochondria was only slightly increased. However, the present findings of increased oxidative utilization of succinate and pyruvate-malate suggests that the extensive metabolic response of the liver cell to phenobarbital also includes alterations in mitochondrial function.

From the experiments presented here using alpha glycerophosphate as substrate, it is quite clear that the drug response of the mitochondria is quite distinct from the response to thyroid hormone studied by Lardy and his associates, Lee et al., (11). This indicates that mitochondria, like microsomes, are capable of making specific responses to specific enhancing substances.

Since ethionine blocked the response of alpha glycerophosphate utilization to thyroid hormone, it was concluded (11) that the hormone was probably not functioning by activating latent enzyme but rather via de novo synthesis of enzyme protein. Whether the drug-induced enhancement of mito-

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**Figure 3** Rates of oxidation of pyruvate-malate (○) and succinate (•) in the presence of phosphate and phosphate acceptor catalyzed by mitochondria from control and phenobarbital-treated rats. Values are expressed as per cent of control values which were 0.045 μ atoms O2/min/mg protein for pyruvate-malate and 0.115 μ atoms O2/min/mg protein for succinate. Each point is the average of four separate animals. Phenobarbital was injected once daily.

**Figure 4** Oxygen electrode recording of oxidation of succinate and alpha glycerophosphate (GP) by rat liver mitochondria from animals receiving one daily injection of phenobarbital (phenob.) (80 mg/kg) for 4 days. Result with control mitochondria (succinate only) is also shown.
chondrial oxidation is due to de novo synthesis is a matter of considerable interest now under study.

It is of particular interest that brain mitochondria do not increase their respiration in hyperthyroidism and do not oxidize alpha glycerophosphate more rapidly after administration of thyroid substance. In connection with this, it should also be noted that drug-metabolizing enzymes in the brain do not respond to administration of drugs.

The increase in the activities of drug-hydroxylating enzymes after treatment with drugs is pronounced and rapid. A similar type of response is observed with UDPG dehydrogenase although the magnitude of the response is smaller. On the other hand, the responses of both glucuronyl transferase and mitochondrial oxidative enzymes are characterized by a 2 day lag period. This raises the possibility that there are two different types of response by the cell, an immediate one and a slower, possibly secondary response. The nature and significance of these differential responses requires further study.

SUMMARY
The administration of phenobarbital to rats results in an elevation of the hepatic levels of UDPG dehydrogenase and glucuronyl transferase. The activities of mitochondrial oxidative enzymes utilizing succinate and pyruvate-malate are also increased. The time courses for the increase of the mitochondrial enzyme levels and for that of glucuronyl transferase level are characterized by a lag period, whereas UDPG dehydrogenase gives a more rapid response resembling that of oxidative demethylation.

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