

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 another 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 glucuronides, 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, UDPG¹ dehydrogenase, is a supernatant enzyme. It has been purified by Strominger et al. (2). The second enzyme, glucu-

¹Abbreviations employed in this paper: UDPG, Uridine diphosphoglucose; UDPGA, Uridine diphosphoglucuronic acid; NAD⁺, nicotinamide adenine dinucleotide; NADP⁺, nicotinamide adenine dinucleotide 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).

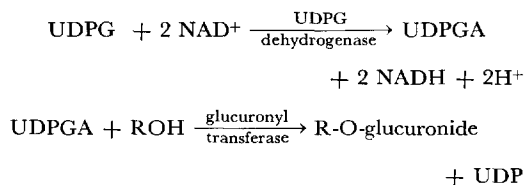


FIGURE 1 Metabolic pathway of glucuronylation.

The results reported here with phenobarbital administration, like those obtained by other workers 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 metabolism of sulfadimethoxin via the glucuronic acid pathway, as judged by urinary output of conjugated metabolites. The time course of the response 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 Löw (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 m μ . The method was essentially the same as that of Strominger et al. (2). Cuvettes contained, in addition to enzyme, 0.6 μ moles of UDPG and 3 μ moles of NAD⁺ in a total volume of 3 ml of 0.1 M 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 μ l of 1 M MgSO₄, 10 μ l of 1 M substrate, and 10 μ l 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 glucuronylating 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 glucuronylating 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.

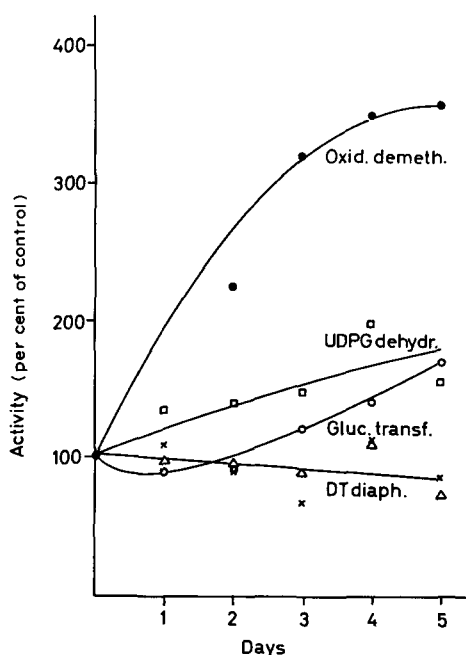


FIGURE 2 Activities of glucuronyl transferase (○), UDPG dehydrogenase (□), DT diaphorase, using NAD (×) and NADP (△) as hydrogen donors, and oxidative demethylation (●), all expressed as per cent of control values. Control values were as follows. Glucuronyl transferase: 0.678 μ moles *p*-nitrophenol conjugated/min/mg protein; UDPG dehydrogenase: 1.25 μ moles NAD⁺ reduced/min/mg protein; DT diaphorase: 1.96 (NAD) and 2.14 (NADP) μ moles DCPIP reduced/min/mg protein; Oxidative demethylation: 5.33 μ moles formaldehyde/min/mg protein. All points represent the average of four or more animals. Phenobarbital was injected once daily.

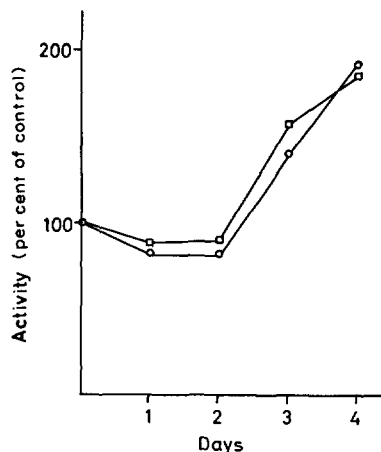


FIGURE 3 Rates of oxidation of pyruvate-malate (O) and succinate (succ.) in the presence of phosphate and phosphate acceptor catalyzed by mitochondria from control and phenobarbital-treated rats. Values are expressed as per cent of control values which were 0.045μ atoms O_2 /min/mg protein for pyruvate-malate and 0.115μ atoms O_2 /min/mg protein for succinate. Each point is the average of four separate 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 sub-

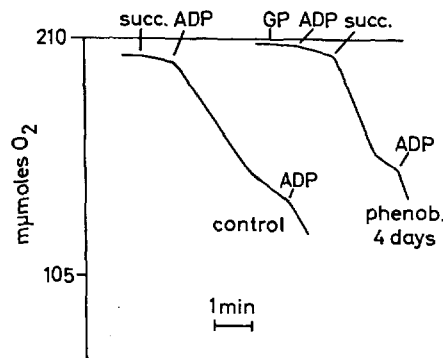


FIGURE 4 Oxygen electrode recording of oxidation of succinate and alpha glycerophosphate (GP) by rat liver mitochondria from animals receiving one daily injection of phenobarbital (phenob.) (80 mg/kg) for 4 days. Result with control mitochondria (succinate only) is also shown.

strate 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 P_i^{32} 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-

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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