Uridine Diphosphate Glucuronyltransferase Activity in Nuclei and Nuclear Envelopes of Rat Liver and its Apparent Induction by Phenobarbital

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The presence of UDP-glucuronyltransferase (EC 2.4.1.17) activity in chick-embryo liver nuclei and nuclear envelope and its inducibility by phenobarbital has been previously reported (Fry & Wishart, 1976). This enzyme has also been found in adult rat liver, with 64-76% in the microsomal and 14-19% in the unpurified nuclear fractions (Amar-Costesec et al., 1974). In view of the heterogeneity of such unpurified nuclear fractions and the high microsomal activity, much, if not all, of such apparent nuclear activity could be due to microsomal contamination. In the present study a reliable estimate of nuclear and nuclear-envelope activity was sought. Purified nuclear preparations have been prepared, with the degree of contamination of these quantified by electron-microscope morphometry (Weibel & Bolender, 1972), and the nuclear envelopes were isolated from these fractions as described previously (Fry & Wishart, 1976). The results on the nuclear and nuclear-envelope preparations were compared with those on standard microsomal preparations and on microsomal preparations subjected to the nuclear-envelope-isolation procedures ('treated microsomes'). As the microsomal enzyme is latent in broken-cell preparations, such comparison has little meaning without some standardization of the degree to which the latent enzyme has been activated (Leakey & Donald, 1976). All fractions were therefore assayed for glucuronyltransferase activity, by the procedure described by Winsnes (1969) with o-aminophenol as substrate, at a range of digitonin concentrations, so that estimates of maximal activity could be made. Female Wistar rats (3 months old) were used for all experiments and the effect of phenobarbital was determined by comparing results on phenobarbital-treated and control rats sampled on the same day, and with their liver fractions prepared in parallel. Maximal phenobarbital induction was achieved by introducing 1-2g/l into the drinking water for over 3 weeks before the experiment, or by injecting 100mg/kg intraperitoneally daily for 3 days and assaying on the fifth day.

In control rat livers the purified nuclear fractions contained less than 10% non-nuclear membrane and showed few non-membranous contaminants. The purified nuclear fraction contained 19.6% of the total homogenate DNA, but its maximal glucuronyltransferase activity was only 0.5% of the total. The true nuclear glucuronyltransferase activity therefore appears to be approx. 2.5% of the homogenate activity, a value substantially less than indicated by crude cell fractionation. The nuclear envelopes retained over 80% of the total nuclear activity, indicating that the envelope is the primary, if not the sole, location of the nuclear enzyme. Maximal nuclear, nuclear-envelope and microsomal glucuronyltransferase activities per mg of phospholipid were comparable (230nmol of o-aminophenyl glucuronide/h per mg of phospholipid). Similar results were obtained when 5-hydroxytryptamine or bilirubin was used as substrate instead of o-aminophenol. With o-aminophenol as substrate, the maximal activity obtained after digitonin treatment of 'treated microsome' preparations was nearly twice that obtained with the standard microsomal preparations, and it seems that the envelope-isolation procedure used (low ionic concentration, pH 8.5, deoxyribonuclease) has an activating
effect on the enzyme in addition to that produced by detergent. The apparent absence of this effect on the nuclear-membrane enzyme may be due to the greater ability of this membrane to retain its structural integrity during the procedure. Comparison of the maximal activities with the unactivated values showed that in the microsomal fractions digitonin increased the glucuronyltransferase activity 5.5±0.8-fold (mean±S.E.M., n = 9), in the ‘treated microsome’ fractions 2.8±0.5-fold, in the nuclear fractions 2.2±0.4-fold, and in the nuclear-envelope fractions 2.2±0.5-fold. This suggests that the last three fractions were already partly ‘activated’ before treatment with detergent.

In phenobarbital-treated rats the pattern of results was similar, but the maximal activities in all fractions were higher. Maximal specific activities were 1.8 times those in the controls indicating a significant degree of induction by the drug. This is in contrast with the lack of effect of phenobarbital treatment on benzopyrene hydroxylase (EC 1.14.14.2), N-demethylase and cytochrome P-450 (Kasper, 1971). However, it was only after detergent treatment that the difference between phenobarbital-treated and control livers was significant: in all fractions the specific activities without detergent treatment of control and phenobarbital-treated material were comparable. This suggests that any induction is of a latent enzyme. Phenobarbital treatment has no significant effect on nuclear volume density in liver, nor on nuclear shape (Staubli et al., 1969; Hooper & Dick, 1976), so that any inductive effect on nuclear membrane is unlikely to be due to change in surface area.

On the basis of the present work it seems that UDP-glucuronyltransferase activity towards a variety of substrates is present in rat liver nuclei and nuclear envelopes, that in these and the microsomal fractions it is partly present in a latent form activatable by digitonin and that phenobarbital treatment increases the amount of the latent form in a given area of membrane.

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3-Methylhistidine as a Measure of Skeletal-Muscle Protein Catabolism in the Adult New Zealand White Rabbit

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3-Methylhistidine has long been known as a constituent of both animal and human urine (Tallan et al., 1954), where it occurs together with 1-methylhistidine. The source of the 3-methylhistidine was subsequently identified as skeletal muscle and, within the muscle, to be exclusively confined to actin and myosin, where it occurred in peptide linkage (Asatoor & Armstrong, 1967; Johnson et al., 1967). Later studies established that the 3-methylhistidine was formed by methylation in situ of histidine in the polypeptide chain and that the 3-methylhistidine released during catabolism of muscle proteins was not reutilized (Young et al., 1972). Although 3-methylhistidine is now known to occur in other proteins, its content in actin and myosin makes muscle tissue the most important source (Haverberg et al., 1975), since actin and myosin together constitute about 50% of total muscle protein and muscle tissue comprises about 45% of adult body weight. Because 3-methylhistidine is not reutilized in protein synthesis,