$$K_1 = \frac{[AuAlb]}{[Alb][Au]} \quad (4)$$

The K_1 value is equal to the sum of the calculated intrinsic association constants ($\Sigma_{i=1}^{n} k_i$) and was found to be $3.3 \times 10^4\ M^{-1}$ using $n = 4$. The range of K_1 values was $3.18 \times 10^4\ M^{-1}$ to $3.26 \times 10^4\ M^{-1}$ using $n = 3$ to $n = 8$. The value of k_1 corresponds to 95–96% binding of gold to human albumin under physiological conditions.

In the study by Mason [1] the binding parameters for binding of aurothiomalate to albumin were found to be $n_1 = 1.0$, $k_1 = 6.10 \times 10^3\ M^{-1}$ and $n_2 = 6.6$, $k_2 = 2.35 \times 10^2\ M^{-1}$. These results correspond to a thermodynamic constant $K_1 = 7.65 \times 10^3\ M^{-1}$. The higher degree of binding of aurothiosulphate to albumin found in this investigation might be an observation of practical importance as the two compounds on empirical base are administrated in equivalent doses with respect to gold content. Lederer [11] studied chromatographic properties of colloidal gold sulphide (Autosulfa Lab. G. Manzoni, Milan, Italy) and aurothiosulphate. He found that the two compounds had radically different properties in almost all systems, and thus presumably different transport properties in biological systems. As aurothiosulphate and aurothiomalate are respectively inorganic and organic gold compounds, the found differences in binding to albumin might conceivably be explained by different physical–chemical properties of the two compounds. However, the differences might also be due to the problems of drug–membrane binding in the ultrafiltration method and to the buffered solutions employed in the study by Mason [1]. Therefore, any conclusion obtained by comparison of the results of the two methods is uncertain.

In summary, the binding of aurothiosulphate to human serum albumin was studied by equilibrium dialysis at 37° in unbuffered solutions with pH 7.4 and ionic strength 0.15–0.16 M. At constant albumin concentration (in the *in vivo* range) and various gold concentrations aurothiosulphate was bound reversibly to human serum albumin at a single site with an intrinsic association constant of $3.0 \times 10^4\ M^{-1}$ and at 3 or more sites with intrinsic association constants of the order of $10^3\ M^{-1}$.

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Dependence of glucuronidation rate on UDP-glucuronic acid levels in isolated hepatocytes

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Glucuronidation is a major pathway in the biotransformation of foreign and endogenous compounds [1]. The reaction is catalysed by membrane-bound UDP-glucuronosyltransferases (GT) (EC 2.4.1.17) [2, 3] and requires uridine-diphosphoglucuronic acid (UDP-GA) as co-factor [1]. While there are some indications that the cellular UDP-GA level may be a determinant of glucuronidation rate *in vivo* [4, 5], studies performed with native microsomes show a relative independence of GT activity of UDP-GA at physiological and lower concentration [6]. Isolated hepatocytes offer the possibility to study the role of co-factor levels on GT activity under controlled *in vivo* conditions in the intact cell.

UDP-GA levels were modulated in this system by addition of various amounts of D-galactosamine [7], which has been shown to lower the concentration of the co-factor by trapping UTP [8] and inhibiting UDP-glucose dehydrogenase [9]. Glucuronidation activity of the intact cells was determined with 3-hydroxybenzo(*a*)pyrene (3-OH-BP) [10] which is a typical substrate for the late foetal and 3-methylcholanthrene-inducible GT form recently classified by the planar substrates 1-naphthol, 4-nitrophenol and *N*-hydroxy-2-naphthylamine [2, 11]. In order to minimise possible effects on the rate of glucuronide formation by sulphation of 3-OH-BP, an alternative pathway of metabolism for the phenol [12], experiments were performed in sulphate-free medium in which sulphate conjugation is decreased by about 80% [7, 13].

Materials and methods

Hepatocytes were isolated from male Wistar rats (200 g body wt) by perfusion of the liver with collagenase [7]. Ca^{2+}-free perfusion was performed in the presence of 0.5 mM ethyleneglycol bis-(2-aminoethylether)-*N,N,N',N'*-tetra-acetic acid (EGTA). Viability of the preparations was routinely tested [14]. More than 92% of the cells excluded trypan blue and oxygen consumption was increased more than two fold in the presence of the uncoupler carbonyl cyanide-*m*-chlorophenylhydrazone (2 μM). Cells were incubated at a final concentration of 1.25×10^6/ml in sulphate-free Hank's solution containing vitamins and non-essential amino acids of Minimum Essential Medium (Seromed, Munich, West Germany), buffered with 15 mM Hepes. To determine glucuronidation activity of the intact cells, 3-OH-BP was added in DMSO (2.5 μl DMSO per ml incubation mixture) to a final concentration of 100 μM and benzo(*a*)pyrene-3-glucuronide formation was assayed fluorometrically in 200 μl aliquots of the incubate as previously described [10]. Under these conditions formation of benzo(*a*)pyrene-3-sulphate may not appreciably interfere with the glucuronidation assay in view of the fact that sulphation in the sulphate- and cysteine-free medium used is greatly depressed [7], and that the sulphate conjugate is considerably extracted into the organic phase during preparation for the fluorometric test (F. J. Wiebel, personal communication). Moreover, the two conjugates possess different fluorescence maxima [10, 12]. To stop the reaction and extract cellular UDP-GA, 200 μl of the cell suspensions were boiled with 300 μl water for 3 min. The amount of UDP-GA present in 100 μl of this extract was tested using an enzymatic assay as described previously [15]. UDP-GA levels determined in duplicate varied by not more than 6% from the mean.

Results and discussion

The hepatocytes contained about 4.6 nmole UDP-GA/10^6 cells at the start of the experiment, i.e. after the 10 min preincubation period; when calculated on a basis of 120×10^6 cells/g liver (wet wt) [18] this level (0.55 mM) is somewhat higher than those previously reported for rat liver (0.3 μmole/g^{-1}) [8, 16, 17]. Addition of D-galactosamine to the cell suspension decreased UDP-GA levels in isolated hepatocytes in a concentration- and time-dependent fashion (Fig. 1). The concentration of the co-factor decreased to 78, 61 and 31% of control after 20 min incubation in the presence of 1, 2 and 4 mM of D-galactosamine, respectively. A similar decrease has also been reported for the precursors of UDP-GA, UTP and UDP-glucose, in isolated hepatocytes after addition of D-galactosamine [19]. In untreated hepatocytes the level of the co-factor slightly increased during the 20 min incubation period (Fig. 1). A tendency to rise with incubation time has recently also been shown for UTP and UDP-glucose [19].

When glucuronidation activity of intact hepatocytes was determined after addition of D-galactosamine, i.e. at different intracellular concentrations of UDP-GA, we observed a linear relationship between co-factor levels and benzo(*a*)pyrene-3-glucuronide formation (Fig. 2). This effect cannot be attributed to a direct interference of the hexosamine with the enzyme molecule since D-galactosamine did not decrease glucuronidation activity mediated by microsomal preparations fortified with UDP-GA. The marked decrease in glucuronidation rates observed with decreased co-factor levels suggests that physiologic UDP-GA concentrations in hepatocytes are not far above the K_m value of the GT. Apparently the relative independence of GT activity of UDP-GA at lower co-factor concentrations found with hepatic microsomal preparations with 3-OH-BP (Singh and Wiebel, unpublished results) or *p*-nitrophenol [6] as substrate do not reflect the *in vivo* situation. Our data support the previous observations of Bock and co-workers [16], who showed that formation of 1-naphthol glucuronides in the isolated perfused liver was decreased when UDP-GA was lowered by pretreatment of the animals with D-galactosamine. However, not all substrates may depend so strictly in their rate of glucuronidation on the availability of UDP-GA since glucuronidation of bilirubin appeared to be independent of the co-factor concentration in the isolated perfused liver [16]. This may be attributable to the presence of multiple forms of the enzyme [2, 3]

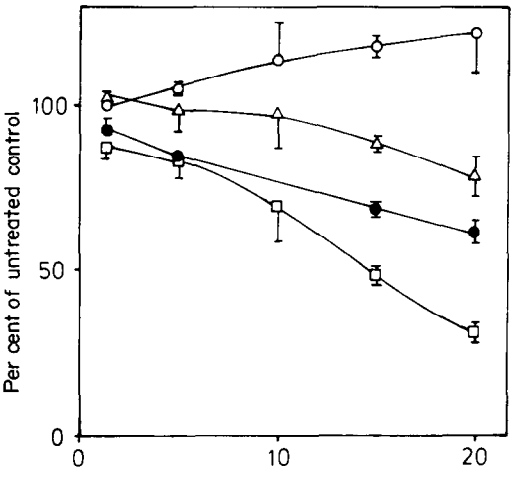

Fig. 1. Effect of D-galactosamine on UDP-GA levels in isolated hepatocytes. After preincubation of the cells (1.25×10^6/ml) for 10 min, various amounts of D-galactosamine were added to the cell suspension. Final concentrations of D-galactosamine: (○) 0.0 mM, (△) 1 mM (●) 2 mM and (□) 4 mM. For details of cell incubation and determination of UDP-GA levels see Materials and Methods. The data are expressed as per cent of the UDP-GA concentration found in the absence of D-galactosamine after a total incubation time of 12 min. 100% amounts to 4.6 ± 1.4 nmole UDP-GA/10^6 cells. The values give mean and range from two experiments.

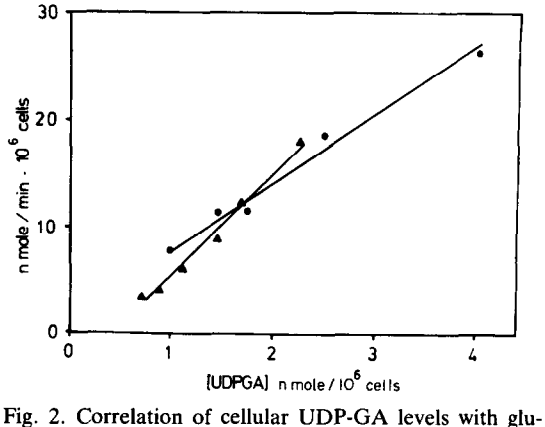

Fig. 2. Correlation of cellular UDP-GA levels with glucuronidation activity. Cells were incubated as described in Fig. 1. After incubation of hepatocytes for 19 min with D-galactosamine concentrations ranging from 1 to 4 mM, 200 μl aliquots were taken for determination of UDP-GA levels. One minute later, 3-OH-BP (100 μM) was added and glucuronide formation was assayed after 30, 60 and 90 seconds. Initial rates of glucuronidation are plotted versus the corresponding UDP-GA levels. Data are from two separate experiments represented by circles and triangles.

exhibiting different properties. Interestingly, modulation of UDP-GA levels in permanent cell lines (BHK-21, H-4-II-E) did not affect glucuronidation of 3-OH-BP in the intact cell (Singh and Wiebel, unpublished results).

Conclusions

The linear correlation of UDP-GA levels and glucuronidation activity observed in isolated hepatocytes support previous indications that pathological conditions such as diabetes [4] may decrease the capacity of the liver to glucuronidate via shortage of co-factor supply. Furthermore, these data stress the need for intact cells in studying co-factor dependence of GT.

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Effect of heparin on the subcellular distribution of human placental 7-ethoxycoumarin *O*-deethylase activity

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The human placenta has been shown to be able to metabolize a limited number of exogenous substances, but this activity is dependent on maternal cigarette smoking [1–3]. The characteristics of the placental enzyme system clearly classify it as a typical xenobiotic-metabolizing polysubstrate monooxygenase [4]. Early studies demonstrated that most of the activity could be recovered with very low speeds in the centrifugal fractionation and suggested that the sedimentability of placental microsomes differed drastically from that of liver microsomes [4–5]. However, because placental mitochondria contain a cytochrome P-450 system, it cannot be excluded that mitochondria may also be active in xenobiotic metabolism [6].

In search of ways to improve the recovery of microsomes from placental homogenates many different detergents, chelating agents and other compounds were tried. Amongst them, only heparin showed consistently promising results. This report shows that heparin does affect the recovery of 'microsomal' monooxygenase activities during the subcellular fractionation of placental homogenates.

Term human placentas were obtained following normal vaginal delivery, cut into small pieces and stored at −40°. There was no loss of activity during at least four months in storage. In the beginning of an experiment, a piece of placenta was thawed, washed, excised free from connective tissue and homogenized in four volumes of 0.25 M sucrose, with a Potter–Elvehjem-type all-glass homogenizer. In the experiments with heparin, a solution containing 5000 IU heparin/ml was added to the homogenate and mixed with several strokes of the homogenizer. The homogenate was centrifuged at 700 *g* for 10 min, the resulting supernatant at 10,000 *g* for 15 min and the microsomal pellet was obtained by centrifuging the postmitochondrial supernatant at 100,000 *g* for 60 min. Pellets were suspended in 0.25 M phosphate buffer, pH 7.4 containing 30% glycerol. Protein content was determined by the method of Lowry *et al.* [7] with crystalline bovine serum albumin as a standard.

7-Ethoxycoumarin *O*-deethylase activity was assayed according to the method of Greenlee and Poland [8]. The protein concentration used was about 200 to 600 μg per ml and the incubation time was 15 min. In the experiments with heparin, 25 to 200 μl of a solution containing 5000 IU of heparin per ml was added to the incubate.

Glucose-6-phosphatase activity was assayed according to