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permanent cell lines (BHK-21, H-4-II-E) did not affect glucuronidation of 3-OH-BP in the intact cell (Singh and Wiebel, unpublished results).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

the method described by Harper [9] based on the phosphate determination of Fiske and Subbarow [10]. Citrate buffer was used in these experiments. Other marker enzymes were determined according to the standard methods adopted to placental conditions [5]: a mitochondrial marker enzyme, cytochrome oxidase [5]; a lysosomal marker enzyme, arylsulphatase A+B [11]; and a microsomal marker enzyme, arylsulphatase C [12].

In preliminary experiments the placental homogenate was centrifuged at 10,000 *g* for 15 min both in the absence, and presence, of heparin. Glucose-6-phosphatase activity was measured in resuspended pellets, because measurements in the supernatant were unreliable (high blanks), and 7-ethoxycoumarin *O*-deethylase activity was measured in the supernatant fraction. Figure 1 shows that glucose-6-phosphatase activity declines in the 10,000 *g* pellet with the increasing concentration of heparin and deethylase activity increases in the supernatant fraction. Studies on the effect of heparin on the subcellular distribution of deethylase activity are presented in Table 1. The most dramatic effect on heparin was on the amount of deethylase activity in the 700 *g* pellet ('nuclear' fraction). In the absence of heparin, over 70 per cent of the enzyme activity was recovered in this low-speed fraction, but in the presence

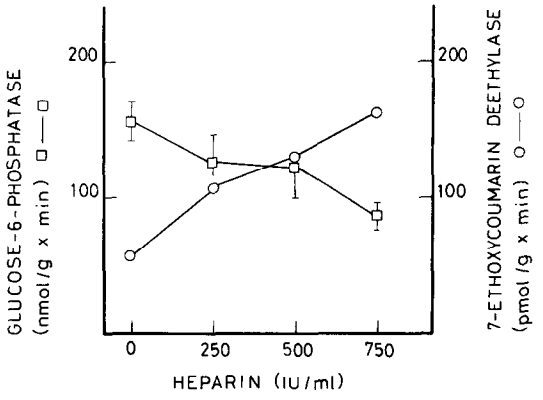

Fig. 1. Effect of heparin on the distribution of glucose-6-phosphatase and 7-ethoxycoumarin *O*-deethylase activities after the 10,000 *g* centrifugation of the placental homogenate in the absence and presence of heparin. Glucose-6-phosphatase activity was measured in the 10,000 *g* pellet (four different experiments) and 7-ethoxycoumarin *O*-deethylase activity in the 10,000 *g* supernatant (one representative experiment because of large variations in absolute activities between different placentas).

of 1000 IU/ml of heparin in the homogenate, only about 30 per cent of the enzyme activity remained in this fraction. The effect of heparin was concentration-dependent (data not illustrated). To elucidate whether heparin caused this effect via direct effects on the enzyme or via changing the sedimentability of the subcellular fraction containing deethylase activity, the effect of heparin on deethylase activity *in vitro* (Fig. 2) and on the distribution of some marker enzymes (Table 2) was studied. At best there was a slight inhibitory effect on deethylase activity *in vitro* (Fig. 2). Marker enzyme determinations (Table 2) demonstrated that heparin decreased the fraction of activity of all marker enzymes remaining in the 700 *g* pellet. There was an enrichment of the mitochondrial (cytochrome oxidase) and lysosomal (arylsulphatase A+B) marker enzyme activities in the 10,000 *g* pellet and an enrichment of lysosomal (arylsulphatase A+B) and microsomal (arylsulphatase C) marker enzyme activities in the 100,000 *g* pellet. It can be concluded that heparin causes its effect on the distribution of deethylase activity by affecting the sedimentability of subcellular structures and not by direct effects on enzyme activity.

The fractionation schedules have been developed mainly with the liver and often cannot be applied to other organs, as is well illustrated by the placenta. Heparin tends to 'normalize' the fractionation of subcellular organelles in the placenta, although the underlying mechanism is not clear. Actually, heparin has been used earlier in improving the recovery and purity of microsomes from rat intestinal mucosa [13], although at a very low concentration of 3 units per ml as contrasted with the efficient concentration of up to 1000 units per ml in the present study.

Marker enzyme determinations indicated that the distribution of 7-ethoxycoumarin *O*-deethylase activity, and the effect of heparin thereon, resembled most closely the distribution of arylsulphatase C, a microsomal marker enzyme. Heparin also markedly affected the distribution of arylsulphatase A+B activity, which is a lysosomal marker enzyme. Although the recovery of 'microsomal' enzyme activities was improved by heparin from about 15 to 25–40 per cent, marker enzyme determinations when taken together still showed a marked heterogeneity of subcellular fractions, whether separated in the presence, or absence, of heparin.

A recent study of Tadolini [14] demonstrated that heparin can affect the subcellular fractionation of rat liver homogenate in the presence of polyamines. Because the placenta is a rapidly growing organ, and thus contains a large amount of polyamines [15, 16], a hypothesis can be presented that perhaps the anomalous sedimentation properties of placental subcellular organelles are due to the high polyamine content. Whatever the final mechanism for the

Table 1. The distribution of 7-ethoxycoumarin *O*-deethylase activity in different ultracentrifugal fractions in the absence and presence of heparin

Fraction	7-Ethoxycoumarin O-deethylase activity per cent of the total activity	
	no heparin	heparin (1000 IU/ml)
Homogenate	100*	100†
700 x g pellet	71.4	30.1
10 000 x g pellet	13.9	41.7
100 000 x g pellet	14.6	26.1
100 000 x g supernatant	<0.5	2.1

* Absolute activities varied from 2360 to 7180 pmoles/g × min in four placentas used for the experiment.

† Heparin slightly inhibits the enzyme activity in the homogenate and consequently 100% activity is calculated by adding separate activities in different fractions.

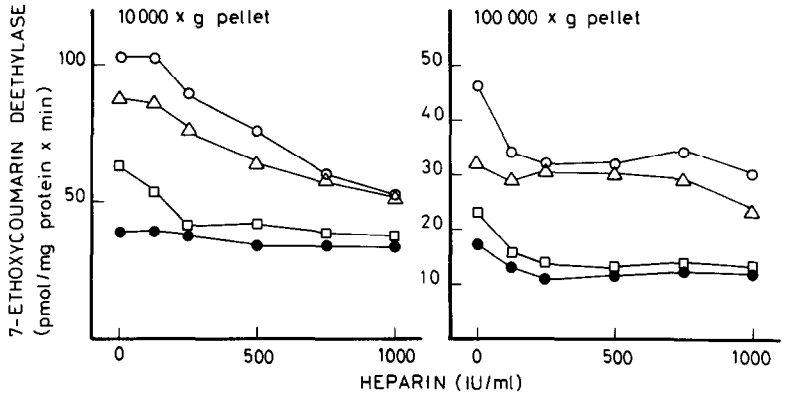

Fig. 2. Effect of heparin added to the incubation mixture on the activity of 7-ethoxycoumarin *O*-deethylase in the 10,000 *g* pellet ('mitochondrial') and 10,000 *g* pellet ('microsomal') fractions of the human placenta. Each symbol represents experiments done with a single placenta.

Table 2. Distribution of marker enzymes in human placental homogenate subfractions separated by differential ultracentrifugation in the presence or absence of heparin during separation (1000 IU/ml)

Marker enzyme	Heparin	700 x g pellet SA*	700 x g pellet F†	10 000 x g pellet SA	10 000 x g pellet F	100 000 x g pellet SA	100 000 x g pellet F	100 000 x g supernatant SA	100 000 x g supernatant F
Cytochrome oxidase‡	-	6.59	0.72	4.29	0.11	2.13	0.04	nd§	nd
	+	2.21	0.38	5.40	0.19	1.08	0.02	nd	nd
Arylsulphatase A+B	-	295	0.63	462	0.18	527	0.13	61	0.07
	+	191	0.42	1613	0.31	799	0.23	29	0.05
Arylsulphatase C	-	183	0.63	308	0.16	464	0.16	45	0.06
	+	99	0.40	287	0.15	649	0.39	55	0.07

Values are averages of duplicate determinations on two separate placentas with similar homogenate marker enzyme activities.

* SA indicates sp. act. expressed as nmoles (except μmoles with cytochrome oxidase) of the substrate metabolized per mg protein per min.

† F indicates the fraction of the homogenate activity in the subfraction indicated.

‡ Cytochrome oxidase measurements were very variable in the homogenate and consequently F values must be regarded only as approximations.

§ nd means that activity could not be determined reliably.

heparin effect, the fractionation of the placental homogenate in the presence of heparin improves the recovery of 'microsomes' and perhaps other subcellular organelles and can be used for obtaining enough material and purer fractions for enzymic and purification studies.

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