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Organ distribution of glutathione transferase isoenzymes in the human fetus: differences between liver and extrahepatic tissues

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Boyland and Williams [1] provided the first information on the enzymatic conjugation of epoxides with glutathione. The role of glutathione transferase (EC 2.5.1.18) in the detoxication of xenobiotics has subsequently been investigated by various research groups [2–5].

More recent studies have demonstrated that the hepatic glutathione transferase activity resides in a family of isoenzymes. The isoenzymes in different human and animal tissues have been isolated and studied [5, 6]. We have previously reported on the composition of isoenzymes in the human fetal liver [7]. A basic and an acid form of the enzyme were found in this organ. Earlier studies had demonstrated that several basic isoenzymes as well as a near-neutral one were detectable in the human adult liver [8–11]. The glutathione transferase isoenzyme pattern in human liver is thus dependent on the developmental state of the individual.

In contrast to the multitude of glutathione transferases in human liver, only an acidic type of enzyme was found in the human placenta [12, 13], lungs [14–16], erythrocytes [17] and brain [18, 19].

So far, the information on the isoenzymes of glutathione transferase in the extrahepatic organs of human fetus is meagre. The present report demonstrates the presence of a major acidic transferase in lung, kidney, brain and intestine, and that the adrenal gland in addition to an acidic form also contains a basic isoenzyme.

Materials and methods

Human fetal tissue specimens were obtained at legal abortion performed on socio-medical indication. The specimens were collected and frozen at −80° within 30 min after the abortion. Fetal age, which was estimated from the crown–rump length, varied between 14 and 24 weeks.

Unlabelled styrene oxide and styrene glycol were obtained from Aldrich (Beerse, Belgium). (7-^{3}H)Styrene-7,8-oxide (99.6 mCi per mmol) was purchased from the Radiochemical Center (Amersham, U.K.). Radioactive and unlabelled styrene oxide were purified as previously reported [20]. The final purity of ^{3}H-styrene oxide was at least 99%.

Assays. Tissue specimens were homogenized in 3 vols of 0.25 M sucrose and the postmicrosomal 105,000 *g* supernatant fraction, obtained by standard centrifugation procedures, was investigated as the cytosol fraction. The glutathione transferases were isolated from the combined lung or kidney cytosols from fetuses no. 4, 5, 6, 7. The cytosol fractions from brain and intestine were obtained from fetus no. 8 and the adrenal gland was from fetus no. 6. Clinical data of the mothers are given in Table 1. The cytosols were dialyzed against 10 mM sodium phosphate (pH 7) containing 1 mM EDTA for 18 hr before isoelectric focusing. Preparative isoelectric focusing in a sucrose gradient was performed according to instructions by the manufacturer (LKB Produkter, Stockholm, Sweden) using Ampholine pH 3.5–10 as the ampholyte. The glutathione transferase activity was assayed in each fraction using 1 mM 1-chloro-2,4-dinitrobenzene and 1 mM glutathione in 0.1 M sodium phosphate buffer (pH 6.5) [21]. The transferase activity towards styrene oxide in cytosol fractions was measured as described previously [22]. The final styrene oxide and glutathione concentrations were 6 and 10 mM, respectively. The assay was performed at 23°, pH 8.5 (0.1 M sodium pyrophosphate buffer), for 4 min in 0.1 ml final volume. Each assay was provided with two blanks containing boiled cytosol fraction instead of fresh sample. The enzyme activity was obtained after correction for the blanks.

Results and discussion

The profiles of the glutathione transferase activity obtained by isoelectric focusing of cytosolic fractions of human fetal lung, kidney, brain, intestine and adrenal gland are depicted in Fig. 1. The results show that the human fetal lung, kidney, brain and intestine contain only one major form of glutathione transferase which focuses at approximately pH 4.8. The same isoenzyme pattern was earlier described for the human placenta [12] and for various adult human extrahepatic tissues, such as brain [18, 19], lung [14–16] and erythrocytes [17]. In contrast to these tissues, the fetal adrenal gland exhibits a major basic isoenzyme in addition to an acidic isoenzyme. This pattern is identical to that of fetal liver [7], whereas the human adult liver and adrenal gland possess basic isoenzyme, only small amounts of an acidic form, and in certain individuals a neutral isoenzyme [9, 10, 16, 23]. The glutathione trans-

Table 1. Clinical data of the mothers

Fetus no.	Fetal age (weeks)	Regular maternal drug intake (mg daily)	Maternal smoking habits (cig/day)
1	17	0	20–25
2	?	?	?
3	21	0	? (smokes)
4	?	?	?
5	17	?	?
6	24	0	10
7	21	chlorzoxazone 375 acetyl salicyclic acid 1500 dextropropoxyphene 135 throughout pregnancy	0
8	14	0	0

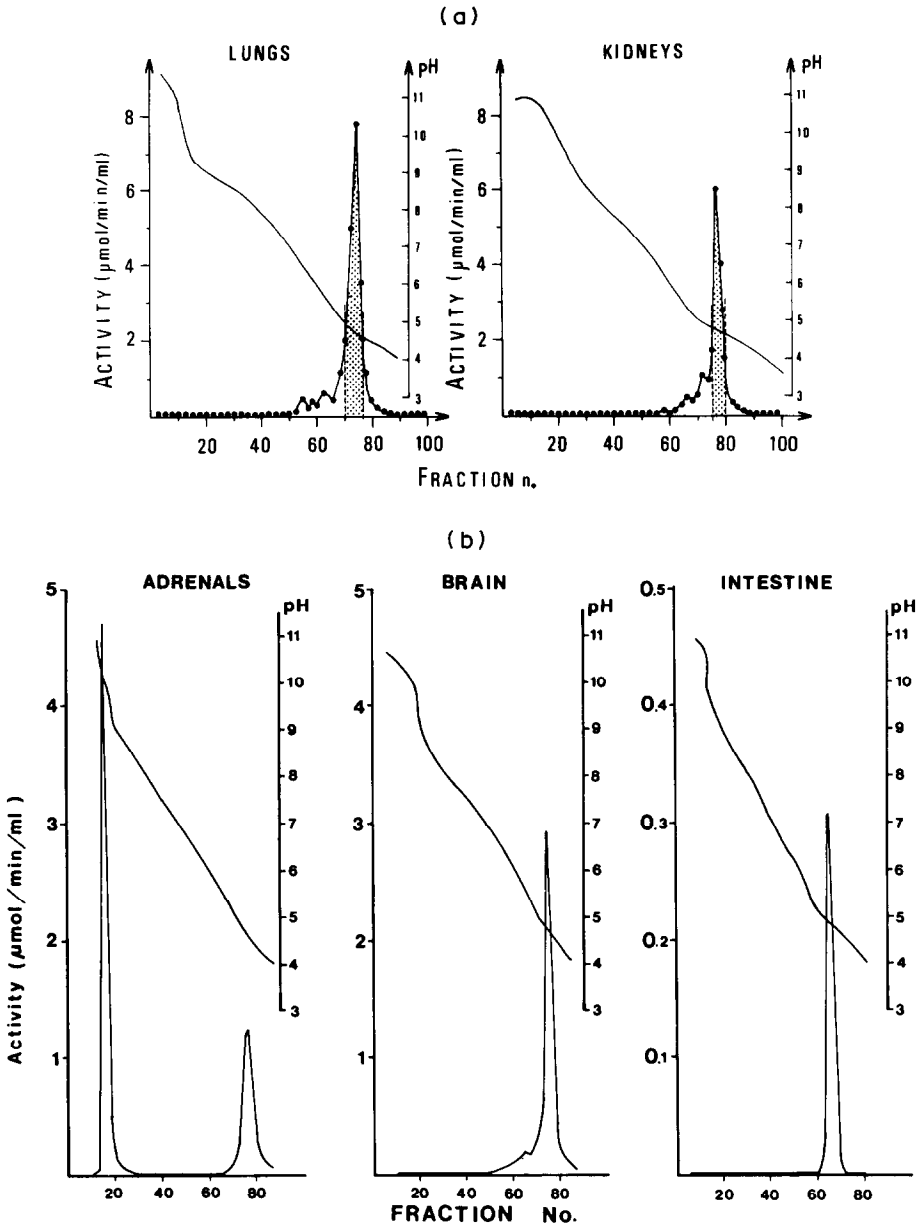

Fig. 1. (a) Isoelectric focusing of the cytosol fraction of human fetal lungs and kidneys. A mixture of cytosols from fetuses no. 4, 5, 6 and 7 were used (details in Table 1). The enzymatic activity was measured at 30° at pH 6.5 using 1 mM 1-chloro-2,4-dinitrobenzene and 1 mM glutathione as substrates. (b) Isoelectric focusing of the cytosol fraction of human fetal adrenals, brain and intestine. The intestine and brain cytosols were from fetus no. 8. The adrenal was from fetus no. 6 (details in Table 1). The enzymatic activity was measured at 30° at pH 6.5 using 1 mM 1-chloro-2,4-dinitrobenzene and 1 mM glutathione as substrates.

ferase isoenzyme composition is thus organ dependent and the most complex pattern is found in liver and adrenal gland. In addition to these inter-organ differences, which exist already at an early stage of gestation, there is evidently also a developmental change in isoenzyme pattern in certain organs, such as liver and adrenal gland.

The glutathione transferase activity towards styrene oxide was also measured. The activities in lung and kidney cytosols were 3.7 and 5.4 nmol/min per mg protein, respectively.

Focusing our attention on the acidic form, the question was raised whether this isoenzyme had the same properties in different tissues. Antibodies raised against the purified placental transferase (transferase π) gave continuous precipitin lines, when the placental transferase was immunoprecipitated in parallel with cytosol fractions from fetal liver, lung, kidney, brain, intestine and adrenal gland. Part of the results are exemplified in Fig. 2.

The precipitin lines indicate that the acidic form of glutathione transferase in fetal liver, lung, kidney, brain, intestine and adrenal gland are very similar or immunologically identical to the placental transferase. Antibodies raised against the placental transferase also react with the erythrocyte enzyme [12], but not with the near-neutral or basic

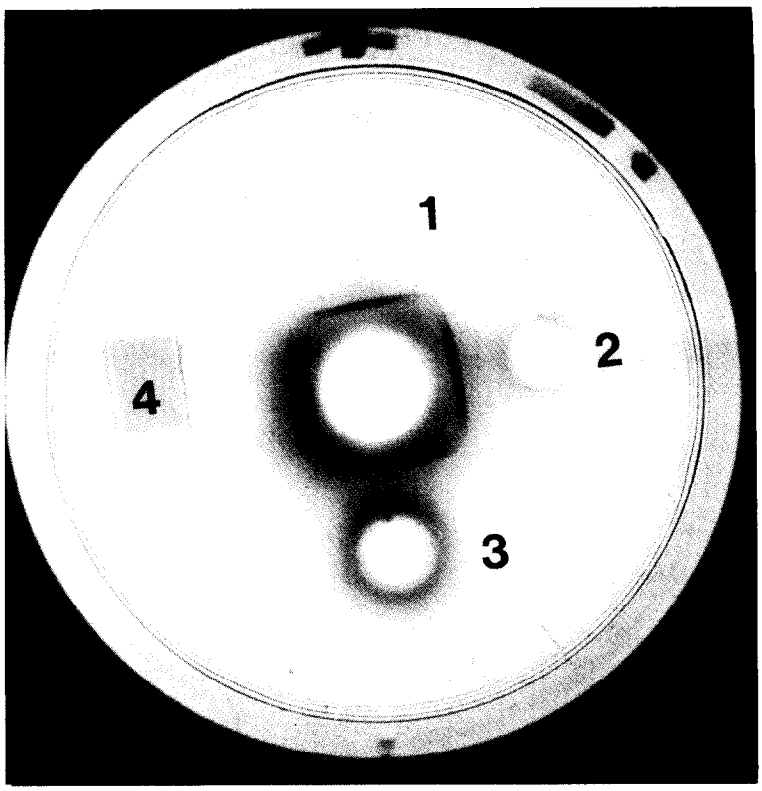

Fig. 2. Ouchterlony double immunodiffusion of glutathione transferase. Antiserum raised against glutathione transferase π from human placenta was placed in the center well and cytosol fractions from various organs in the peripheral wells: (1) fetal lung, (2) fetal brain, (3) fetal intestine, and (4) purified glutathione transferase π.

isoenzymes. The data obtained with human fetal lung, kidney, brain and intestine suggest that these tissues contain only the acidic form of the transferase. An acidic form of transferase has been reported also for adult human tissues such as lung [14–16], brain [18, 19], and placenta [12, 13], whereas the transferase composition in human adult kidney is more complex [15, 23]. Perhaps, the acidic transferase is a more primitive isoenzyme present in higher amounts in human fetal tissues compared to adult tissue. If so, this isoenzyme may have a multifunctional role with a wide substrate specificity.

The results presented here for human fetal lung and brain and the earlier data for the corresponding adult tissues [14–16, 18, 19] indicate that no developmental changes are detectable in these tissues, at least as regards the number of isoenzymes. In contrast, adult liver, kidney and adrenal gland have an isoenzyme pattern which is different from that of the corresponding fetal tissue.

In summary, the isoenzyme composition of glutathione transferase in the cytosol fraction of several human fetal organs was studied. Only one major isoenzyme, having an acidic isoelectric point, was detected in lung, kidney, brain and intestine using 1-chloro-2,4-dinitrobenzene as the electrophilic substrate. The cytosolic fraction from human fetal adrenal glands, like that from fetal liver, showed a more complex isoenzyme pattern in that a major basic isoenzyme was found in addition to the acidic form. In Ouchterlony double diffusion experiments the acidic form of glutathione transferase, occurring in all fetal organs tested, showed an identity reaction with transferase π, isolated from human placenta.

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