Glutathione S-Transferases in Human Fetal Liver *

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Recent work on glutathione *S*-transferases (EC 2.5.1.18) in human tissues has revealed the existence of several forms with different physico-chemical properties. A group of basic enzyme forms, named α, β, γ, δ and ε, were found in adult human liver.[1] Another form (ρ), with an acidic isoelectric point, was isolated from human erythrocytes.[2] An acidic enzyme, very similar to, or identical with, the enzyme from erythrocytes was also purified from human placenta.[3] Thus, two categories of glutathione *S*-transferases have been identified in human tissues, one comprising basic proteins and the second one acidic proteins. Both of these categories appear to be present in all individuals, although not in all tissues or fluids. An additional form of glutathione *S*-transferase (form μ) has been discovered in livers of some individuals.[4] This new hepatic form of human transferase is of special interest with respect to the suggested role of the glutathione *S*-transferases in detoxication of carcinogens, because transferase μ is significantly more active with benzo[*a*]pyrene-4,5-oxide than any other of the human enzymes.[5] The present investigation was undertaken to make a comparison between the glutathione *S*-transferase activities in human adult and fetal liver. The results show that significant differences do exist.

Experimental. Liver tissue specimens were obtained from human fetuses at legal abortions *via* Cesarean section or *via* induction by prostaglandins or ethacridine (Rivanol®). The gestational ages of the fetuses varied between 17 and 25 weeks. Liver pieces were frozen at −80 °C within 45 min after death and the cytosol fraction was prepared on a later occasion. Homogenates were made in 0.25 M sucrose (1:3 w/v) and the postmicrosomal 105 000 g supernatant fraction obtained by standard centrifugation procedures. This fraction, representing the cytosol, was used in the experiments described here.

Isoelectric focusing was performed according to instructions of the manufacturer (LKB Produkter, Stockholm) using Ampholine pH 3.5 – 10 as the ampholytes. The samples of cytosol to be analyzed were dialyzed against 10 mM sodium phosphate (pH 7) containing 1 mM EDTA for 18 h before isoelectric focusing.

Glutathione *S*-transferase activity was measured spectrophotometrically at 340 nm using 1 mM 1-chloro-2,4-dinitrobenzene and 1 mM glutathione as substrates in 0.1 M sodium phosphate buffer (pH 6.5) containing 1 mM EDTA (*cf.* Ref. 6).

Results and discussion. Cytosol fractions from 8 fetal livers were analyzed by isoelectric focusing. A typical glutathione *S*-transferase activity profile after focusing is shown in Fig. 1. The activity was measured by use of 1-chloro-2,4-dinitrobenzene as the electrophilic substrate; activities with other substrates (*cf.* Ref. 4) could not be measured accurately owing to the low concentration of enzymes after the separation. Two major peaks of glutathione *S*-transferase activity were found in all samples analyzed. One peak focused at a high pH value (pH > 9) and the other peak focused at pH 4.7 (at 4 °C). The isoelectric point of the basic peak could not be determined accurately, because the pH gradient is not well controlled at pH > 9. In addition to the two major forms some livers appeared to contain trace amounts of additional enzyme forms (*e.g.* fractions 20 – 25 in Fig. 1), but in no case did any of these contribute significantly to the total activity of the sample.

The basic species of the glutathione *S*-transferases present in fetal liver cytosol should be assigned to the group of basic forms ($\alpha - \varepsilon$) purified from human adult liver.[1] In the fetal tissue only one major form has so far been found, whereas in adult liver one or several forms have been demonstrated depending on the liver analyzed.[1,4,7] The reason for the occurrence of multiple basic forms is unknown, but the present results show that the picture is simpler in fetal liver.

The occurrence of a major acidic form (isoelectric point at pH 4.7) of glutathione *S*-transferase in fetal liver (Fig. 1) is at variance with the activity profiles of adult liver.[4] Recent studies show the existence of acidic transferases in the adult liver tissue,[8,9] but their contribution to the total activity is small or negligible with 1-chloro-2,4-dinitrobenzene as electrophilic substrate.[4] In 2 of the 8 samples of fetal liver in the present study the acidic form showed two peaks of activity focusing at about pH 4.7 and 5.0, respectively, but the cause of the appearance of an additional acidic form can not be stated.

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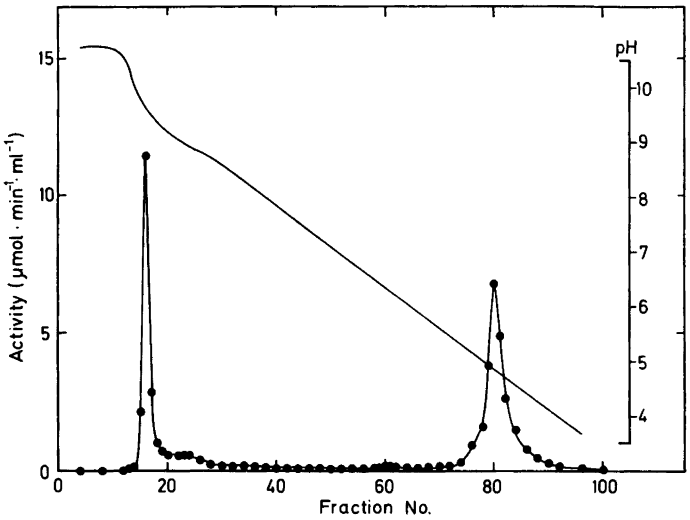

Fig. 1. Glutathione *S*-transferase activity profile obtained after isoelectric focusing of the cytosol fraction of human fetal liver. Fractions of about 1 ml were collected. The enzymatic activity (●) was determined by use of 1-chloro-2,4-dinitrobenzene as electrophilic substrate. The pH gradient (−) was determined at 4 °C. The liver sample was from a fetus of 25 weeks gestational age. The mother was not a smoker and did not use any drugs.

The acidic form in fetal liver appears to be identical with the transferase present in human placenta [3] and erythrocytes.[2] Antibodies raised against the purified placental transferase gave continuous precipitin lines with the acidic form from fetal liver in Ouchterlony double diffusion analysis (data not shown). The human erythrocyte transferase (form ρ) also reacts with the antibodies (indicating identity with the placental enzyme), but neither the basic transferases of adult or fetal liver nor transferase μ (*cf.* Ref. 5) do react.

The possibility that transferase ρ of residual blood in the fetal liver could account for the acidic transferase found in this tissue was considered. The maximal amount of contaminating blood was estimated by measuring the absorbance at 410 nm of the hepatic cytosol fraction, assuming that the absorbance was entirely due to heme and that hemoglobin accounted for all heme. Using the known concentration of glutathione *S*-transferase in erythrocytes [2] the maximal contribution of transferase ρ to the acidic form in fetal liver was estimated as $<1\%$. In this calculation it was assumed that the transferase activity of erythrocytes in fetal blood was the same as that in blood of adults.

The results of the present investigation show clear differences in the occurrence of various glutathione *S*-transferase forms in adult and fetal liver cytosol. The adult liver has most of the activity associated with the basic transferases ($\alpha-\varepsilon$). Some individuals, have a near neutral transferase (form μ) as an additional dominating form. In fetal liver transferase μ has never been found as a major component, although trace amounts appear to be present in some samples. The fetal liver has two dominating enzyme forms, one basic and one acidic. In no case has an acidic transferase been demonstrated in large quantities in adult liver. Accordingly, these differences in the spectrum of hepatic glutathione *S*-transferases reflect the ontogeny of the human organism. The functional significance of the occurrence of various forms of the enzyme remains to be established, but differences in their substrate specificities (*cf.* Ref. 5) would be expected to afford variation in the ability of the liver to detoxify xenobiotics.

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