γ -glutamyl transferase activity in fetal serum, maternal serum, and amniotic fluid during gestation

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SUMMARY γ -glutamyl transferase activity was measured in fetal serum, maternal serum, and amniotic fluid in 173 pregnancies from 15 to 40 weeks' gestation. Fetal serum was obtained in the second trimester by fetoscopy and in the third trimester by umbilical cord puncture at caesarian section or vaginal delivery. Enzyme activities in maternal blood (10 IU/l, SD 2) and fetal blood (88 IU/l, SD 20) remained relatively constant throughout gestation, whereas in the amniotic fluid there was a significant decrease at term from the value in the second trimester ($p < 0.001$). Electrophoretic separation of the enzyme showed one isoenzyme in the fetal blood and at least two in the amniotic fluid. The fetal isoenzyme had the same mobility as the major isoenzyme in the amniotic fluid.

γ -glutamyl transferase enzyme (GGT) (EC 2.3.2.2) is a peptidase transferase enzyme that hydrolyses γ -glutamyl amides and peptides and catalyses the transfer of γ -glutamyl groups to other peptides and 1-amino acids.¹ It is present in many mammalian tissues and body fluids² and may be separated into multiple molecular forms which differ in degrees of glycosylation³ and electrophoretic mobility.⁴

GGT has been shown in amniotic fluid supernate,⁵⁻⁷ but its origin and role is unknown. The activity at 15 weeks' gestation is more than 20 times greater than that found in adult serum, but it decreases with advancing gestation.⁸⁻¹⁰ Activity has recently been reported to be abnormally low in pregnancies with fetal chromosome abnormalities¹¹ and cystic fibrosis.¹²

The ability to obtain pure fetal blood samples by fetoscopy¹³ has given us the opportunity to study GGT activities in the normal fetus during the second trimester and in maternal blood. The aim of the present study was to determine the inter-relation of GGT activities in these three fluid compartments during the second trimester of normal pregnancy.

Patients and methods

Amniotic fluid, maternal blood, and fetal blood samples for diagnostic purposes were obtained be-

tween 15 and 24 weeks' gestation from 125 patients undergoing fetoscopy for a variety of conditions. The outcome of all pregnancies was normal. In seven of these fetuses blood samples were obtained from both an umbilical cord artery and vein. Samples were also obtained in the third trimester of pregnancy: fetal blood ($n = 48$) was obtained by umbilical cord puncture after vaginal delivery or at caesarian section from births that had a live outcome. Amniotic fluid ($n = 48$) was obtained from patients undergoing amniocentesis for determination of fetal lung maturity. The purity of all fetal blood samples was determined as previously described.¹⁴

GGT activity was also measured in one case of duodenal atresia and five cases of obstructive uropathy. In the latter cases we measured the enzyme activity in the fetal urine, obtained by bladder needling at the time of fetoscopy. The urine was purified on a Sephadex column before enzyme analysis.

Blood was collected into plain plastic containers and the serum separated immediately by centrifugation at 2000 rpm for 5 min. Amniotic fluid was similarly separated from the cellular debris. All samples were assayed within 48 h of collection because of a 20-40% decrease in amniotic fluid enzyme activity after prolonged storage and repeated freezing and thawing of samples.

Total GGT activity was measured at 37°C, by the

γ -glutamyl transferase activity in fetal serum

Fig. 1 Change in total γ -glutamyl transferase activity in amniotic fluid with increasing gestational age. n = number of samples. Vertical bars show enzyme activity (mean $\pm$ 2 SD).

method of Szasz¹⁵ on the COBAS BIO Centrifugal analyser (Roche Diagnostics, Welwyn Garden City, Herts, UK) using 10 μ l of serum and p-nitroanilide as substrate. Quality assessment was monitored by using the Beckman decision 1,2,3, sera and the Wellcome Group quality control programme. The coefficient of variation for the assay was less than 2.05% for the control values and the mean bias over the period of study was +8%.

Electrophoresis of samples was performed on cellulose acetate as described by Rosalki *et al.*,¹⁶ and GGT was located using a sensitive and specific substrate γ -glutamyl-7-amino-methyl coumarin (Universals Biologicals Ltd). The resultant fluorescent stain was visualised under ultraviolet light and scan-

ned using a Corning Densitometer (Corning Medical). Maternal serum was run alongside fetal serum and amniotic fluid each time.

Results

In amniotic fluid there was a progressive decrease in GGT activity with advancing gestational age from a mean of 670 IU/l at 15 weeks to 140 IU/l at term (Fig. 1). Regression analysis showed a significant alternation in the slope ($p < 0.001$). The pattern of enzyme activity in the fetal serum (80 IU/l, SD 20) and also in maternal serum (10 IU/l, SD 2), however, showed no significant change in the slope ($p < 0.001$) throughout gestation. The fetal values were about 10 times higher (Fig. 2) than maternal.

In the fetuses in which both arterial and venous blood was sampled there was a significant arterial-venous difference in enzyme activity ($p < 0.05$, paired Student's t test). The mean arterial and venous enzyme activities were 46 IU/l (SE 4.3) and 53 IU/l (SE 3.3) respectively.

In the fetus with duodenal atresia and in those with obstructive uropathy the enzyme activities in the amniotic fluid were in the range appropriate for the gestational age. There was no enzyme activity in the fetal urine collected when the bladder was catheterised to release the distension.

Electrophoresis of the samples showed no discernible fluorescent band in the maternal serum. There was a major GGT isoenzyme fraction in the amniotic fluid which ran in the α 2-macroglobulin region and a second fraction that remained at the origin. The fetal enzyme ran in a position coincident with the major anodal fraction of amniotic fluid. Fig. 3 shows a densitometer tracing of the isoenzymes in the three fluids.

Discussion

Our findings of high amniotic fluid GGT activity that decreases progressively with advancing gestation are in agreement with previous reports.^{5,10} Although the exact origin of the amniotic fluid GGT remains uncertain, it is clear from electrophoresis studies that there are at least two isoenzymes and that the major fraction has the same mobility as that of the fetal serum enzyme. This is supported by the studies of Kottgen *et al.*,¹⁷ who showed that GGT exists in two forms in rat liver—namely, fetal and adult types—which differ in glycosylation of the protein. The two isoenzymes can be separated on the basis of their binding to concanavalin A-Sepharose. Adult type is concanavalin A reactive and the fetal type is concanavalin A non-reactive. GGT activities in amniotic fluid remained fairly constant during gesta-

Fig. 2 Change in γ -glutamyl transferase activity in fetal and maternal serum with gestational age. n = number of samples in each group. Vertical bars show enzyme activity (mean $\pm$ 2 SD).

Fig. 3 Densitometer tracing of γ -glutamyl transferase activity following cellulose acetate electrophoresis.

tion while the concanavalin A reactive form as a proportion of total GGT activity progressively decreased.

The activity of GGT in neonatal blood is reported to be high soon after birth, and decreases rapidly with age.^{18,19} It has been suggested that the enzyme may originate in maternal milk,²⁰ and in the early neonatal period is ingested across the mucosal cells of the small intestine by pinocytosis. This process diminishes with increasing age.²¹ We have shown, however, that the enzyme activity is high in cord blood at delivery and is present in fetal blood in comparable activities throughout gestation. The arterial-venous difference, with higher activities of GGT in the umbilical vein, suggests that the

placenta is a likely source of the enzyme in the circulation and in the amniotic fluid. This hypothesis is supported by our recent report of high enzyme activity in the cells of the amniotic membranes and placental villi.²² The possibility that the fetus is itself the source of GGT activity is unlikely because of our findings of normal enzyme activities in fetal blood and amniotic fluid in one case of duodenal atresia and five of obstructive uropathy, with no GGT activity present in the urine.

The presence of GGT activity in amniotic fluid may merely reflect enzyme released from the cellular debris of placental and amnion tissue. Alternatively, there may be a role for the enzyme in the circulating fluid. GGT occurs at high concentrations in tissues containing microvilli,^{23,24} such as the brush border of the kidney and bile canaliculi, and may be concerned with absorptive and secretory processes. It has been suggested that GGT facilitates the transport of amino acids across cell membranes utilising glutathione as an acceptor.²⁵

Further, there is some evidence that glutathione is used for a variety of detoxifying reactions which lead to the formation of mercapturic acids;^{26,27} various S-substituted glutathione conjugates are metabolised by a pathway involving the removal of the γ -glutamyl group which is catalysed by GGT. Highly purified enzyme preparations act effectively on a number of S-substituted glutathione derivatives in the pathway of detoxification of these toxic compounds. The extent to which such pathways operate outside the cell under normal physiological conditions and the role that GGT plays in the circulating plasma amniotic fluid remains unclear. It is possible that enzymatic mechanisms for glutathione synthesis and utilisation are adapted for detoxification,²⁸ particularly when there is immature hepatic function.

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