Comparative ontogenesis of gamma-glutamyl transpeptidase in rat tissues[1]

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Summary. In rats, placental gamma glutamyl transpeptidase (GGTP) specific activity declined linearly during the last third of gestation. In contrast, the kidney enzyme activity progressively increased 15-fold. In the gut, peak activity was reached at mid-lactation (age 12 days). Hepatic GGTP levels were maximal during the foetal stage. Brain GGTP was negligible at birth and attained its highest specific activity at weaning.

Gamma-glutamyl transpeptidase [γ-glutamyl)-peptide: amino acid γ-glutamyl transferase, EC 2.3.2.2] (GGTP) is an enzyme widely distributed in mammalian tissues. Isozymes originating from the intestine, bone, brain, liver, pancreas, kidney and placenta have been identified[2-4].

GGTP is a key element of a proposed 'gamma-glutamyl cycle'[5,6] in which glutathione acts as a donor of a glutamyl fragment while effecting the membrane translocation of an amino acid or a small peptide. The contribution of this gamma-glutamyl cycle to the transport of protein breakdown products in organs where massive metabolic exchanges take place, such as the placenta, the kidney and the small intestine, has not yet been fully clarified. The ontogenesis of the enzyme in key tissues of the rat is only partially mapped[7].

Materials and methods. Female rats (Crl:(WI)BR, Charles River Breeding Labs., Wilmington, Ma., USA) were mated overnight and impregnation was checked by vaginal smears. Pregnant rats were anesthetized with 1.5 g/kg or urethane and the foetuses and placentas excised and chilled on ice. Tissues were mechanically homogenized in ice-cold deionized water with a teflon pestle. Suckling rats were decapitated and the tissues promptly removed and treated as described above. Male adult rats weighing 200–250 g were anesthetized, exsanguinated from the abdominal aorta and the tissues prepared similarly. The homogenates were spun down at 600 × g and 4 °C for 20 min. The supernatant was removed and used for the immediate assay of GGTP.

GGTP was assayed using 4 mM L-gamma-glutamyl-p-nitroanilide (Sigma Chem. Co., St. Louis, Mo., USA) as the substrate, 40 mM of glycylglycine as the acceptor in a tris-HCl 50 mM buffer pH 8.0 and 10 mM $MgCl_2$ in a final volume of 1.0 ml[8]. The reaction was carried out at 25 °C and the initial rate of reaction followed at 410 nm. The amount of p-nitroaniline cleaved was calculated from appropriate standards. Protein was determined by the method of Lowry et al.[9]. The results were expressed as nmoles (of p-nitroaniline) liberated per min, per mg of protein.

Results. The specific activity of placental GGTP (fig. 1) declined linearly with time in the period spanning the 14th to the 21st day of gestation. Linearity trend was assessed by testing the fit of typical equations[10].

The developmental patterns of GGTP in rat kidney, intestinal mucosa, liver and brain are presented in figure 2. Renal GGTP levels progressively increased from the late intrauterine period through lactation and adulthood. The adult enzymatic activity was about double than that of the weanling rat.

GGTP in the small intestinal mucosa was detectable 2 days before birth and reached a peak by the middle of the lactation period, returning to foetal levels at 21 days of age. The adult activity corresponded to foetal values of the enzyme, but the specific activity in the tissue during adult-

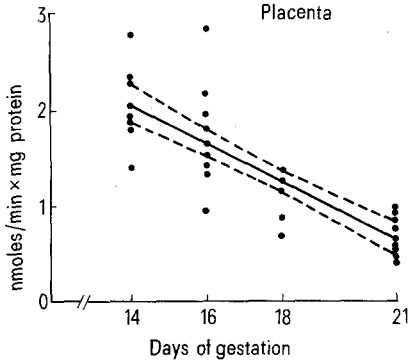

Figure 1. Changes in placental GGTP during the last third of gestation, in the rat. The units in the ordinate express the amount of p-nitroaniline liberated from the substrate. Points indicate values for individual litters. The solid tracing depicts the regression equation. The broken lines represent the 90% confidence interval.

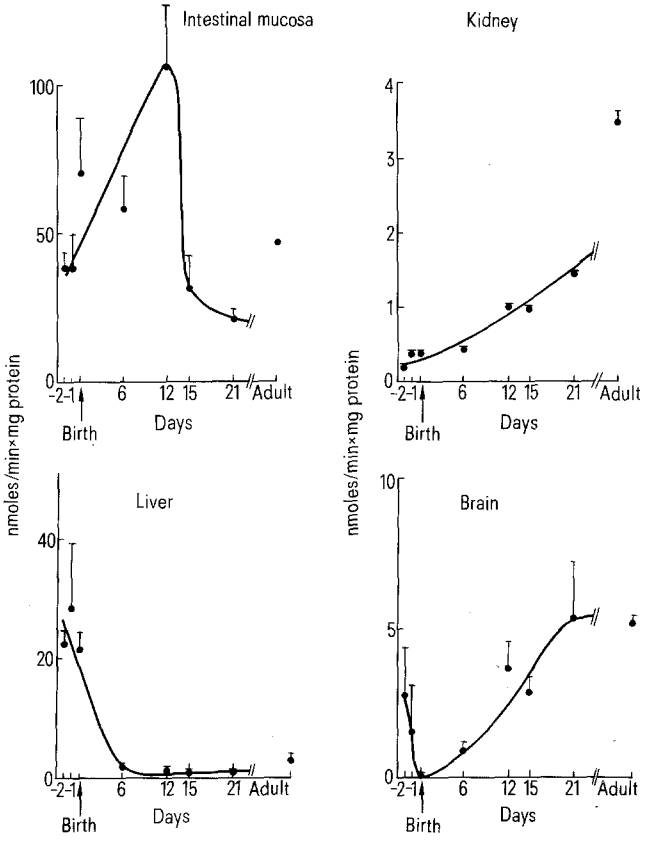

Figure 2. Ontogenesis of GGTP in rat small intestinal mucosa, kidney, liver and brain. The points indicate the means ± SEM for 6–8 determinations each. Pooled litters up to day-of-birth. Tissues from 2 or 3 animals thereafter for each determination. For the preparation of tissues and assay, see 'Materials and methods'. The units of the ordinate as explained under fig. 1.

hood represented less than 2% of the kidney enzymatic capability.

The ontogenesis of GGTP in rat liver was sharply different from that in other tissues. During the 2 days before term, GGTP specific activity was comparable to that of the small intestinal mucosa. However, after birth there was a sharp decline and during the lactation period GGTP levels were almost negligible. Ultimately, adulthood values were about 1000 times lower than in the kidney.

Rat brain GGTP showed still a different pattern to that observed in other tissues. In absolute terms, the activity demonstrated was minimal before birth. At delivery, GGTP became undetectable and a moderate surge followed during the subsequent three weeks, stabilizing, at that point, at levels that persisted through adulthood.

Discussion. The patterns of GGTP ontogenesis in various tissues can provide clues to the importance of GGTP activity for the physiologic function of different organs during development in regard to amino acid exchanges and/or cellular turnover. The steady decrease of placental specific activity during the last third of gestation may suggest a progressive loss of physiologic role as the delivery date approaches. A similar pattern occurs in the brain (figs 1 and 2). The declining trend is shared by the liver. In this tissue, however, GGTP enzymatic activity drops rapidly after the 2nd day of life. By day 6 it is already negligible.

The kidney is the organ with the highest GGTP specific activity. The ascending progression observed early in life continues through adulthood. The levels of renal GGTP by far exceed those of all other organs and has thus received the greatest attention. This phenomenon prevails in all mammals. Physiologically, it provides an effective mechanism for the salvage of amino acids and the production of ammonium ions[2,11].

To an extent 3 orders of magnitude smaller than in the kidney, a steady increase of GGTP is also found in the brain, from birth through the adult stage. The significance of this finding is not yet clear, but may relate to the active translocation of amino acids through the blood-brain barrier[2,12].

The rat small intestinal mucosa presents a peak of GGTP levels at mid-lactation. This is a period of rapid growth, almost continuous feedings and proliferation of the mucosal surface. Afterwards, GGTP specific activity declines sharply and never surpasses one fourth to one half the maximum levels recorded at the 12th day of life.

Different enzymes usually show recognizable developmental characteristics. Some typical patterns have been described in hepatic enzymes of energy metabolism[13,14]. GGTP presents the singularity of possessing tissue-specific isozymes which have varied ontogenic patterns. In general, most enzymes have relatively low activity before birth and show a rapid increase in the postnatal period, as is the case in rat kidney GGTP. Teleologically, tissues may require at various stages of development one or several active translocation mechanisms for amino acids. The orders of magnitude differences existing for GGTP in the organs studied here also suggest that the transport of protein breakdown products may be simultaneously carried out by more than one, unrelated, energy-requiring mechanisms.

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A comment on the estimation of times required for the attainment of equilibrium by noncooperative, single site ligand-receptor systems

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Summary. A table presents the number of hours required for binding to reach 80% and 95% of the equilibrium value for a noncooperative, single site ligand binding system. A 2nd table provides the fraction of binding sites occupied and the fraction of the total ligand bound at equilibrium under the same conditions.

The rate equation for noncooperative, single site ligand binding systems can be represented as follows: $\frac{dB_{sp}}{dt} = k_a (S_o - B_{sp}) (B_{max} - B_{sp}) - k_d B_{sp}$, where B_{sp} is the concentration of specifically-bound ligand, S_o is the total ligand concentration, B_{max} is the total concentration of binding sites, k_a and k_d are the 2nd-order and 1st-order association and dissociation rate constants, and t is the time of incubation. The exact solution to this equation gives the value of B_{sp} as a function of time for the given incubation conditions if the rate constants (or the equilibrium constant, K_d, and one of the rate constants) can be estimated and if the concentrations of ligand and binding sites are known[1,2]. Thus, if nonspecific binding and loss due to inactivation of binding sites may be neglected as an approximation, the solution to the rate equation provides an estimate of the time required for any arbitrary degree of approach to the equilibrium value of specific binding. For example, in recent studies[3,4] the solution to the rate equation has been used to examine the effect of inadequate incubation time (during which equilibrium is not attained under conditions of low ligand