Reprod. Nutr. Dévelop., 1985, **25** (5), 861-866.

Activities of amino acid metabolizing enzymes in the stomach and small intestine of developing rats

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Summary. The activities of aspartate and alanine transaminase, serine dehydratase, arginase, glutamate dehydrogenase, adenylate deaminase and glutamine synthetase were determined in the stomach and small intestine of developing rats. Despite the common embryonic origin of the intestine and stomach, their enzymes showed quite different activity levels and patterns of development, depending on their roles. Most enzyme activities were low during late intrauterine life and after birth, attaining adult levels with the change of diet at weaning. No arginase activity was found in the stomach and no changes were detected in adenylate deaminase in the stomach or intestine throughout the period studied. Alanine transaminase, serine dehydratase and, to some extent, glutamine synthetase levels, significantly higher in late intrauterine life, decreased after birth, suggesting that the foetal stomach has a transient ability to handle amino acids.

Introduction.

The stomach and small intestine clearly have a common embryonic origin ; both, and especially the intestine, are smooth muscular organs and have a high weight relative to body weight ; their blood supply is high and they are both implicated in active transport processes. The size of the alimentary canal is considerable when compared with other well-studied organs, such as the kidneys, involded in amino acid metabolism. The stomach and intestine play an important role in the post-prandial catabolism of some amino acids and a significant part in the inter-organ handling of amino acids in the whole splanchnic bed system (Aikawa *et al.*, 1973). However, the enzymes of amino acid metabolism have been seldom studied (Burdett and Reek, 1979), despite their relevance to amino acid metabolism. During development, significant metabolic changes take place in the rat ; these are enhanced by profound alterations in the size and quality of the

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food ingested and that has to be processed by a fast-growing gut. These modifications can be expected to induce alterations in the enzyme activity of amino acid metabolism in response to changes in the modes of handling nitrogen, such as those response to changes in the modes of handling nitrogen, such as those found in the liver (Arola *et al.*, 1982).

To broaden our knowledge of the development of the activity of enzymes metabolizing amino acids and as continuation of our previous study of other organs (Arola *et al.*, 1982), we determined the activities of alanine transaminase (E.C. 2.6.1.2.), aspartate transaminase (E.C. 2.6.1.1.), serine dehydratase (E.C. 4.2.1.13), arginase (E.C. 2.4.2.3.), adenylate deaminase (E.C. 3.5.4.6.), glutamate dehydrogenase (E.C. 1.4.1.2.) and glutamine synthetase (E.C. 6.3.1.2.) in the stomach and small intestine of developing rats.

Material and methods.

Virgin female Wistar rats initially weighing 165 $\pm$ 5 g were mated with adult males until the presence of spermatozoa in daily vaginal smears was detected. The animals were then put into individual cages at 21 $\pm$ 1 °C and subjected to light between 8 and 20 h and to dark between 20 and 8 h. They were fed rat chow pellets (Sandermus S-10 from Sanders, Spain : total lipid 3.36 %, total protein 17.28 %, total free sugars 3.9 %, fibre 4.05 %, starch 45 %, minerals 4.6 %) and had free access to tap water. At days 19 or 21 of pregnancy, the rats were beheaded and their foetuses removed and sacrificed. Other groups of pregnant rats were allowed to give birth to their pups. No litter with less than 8 or more than 12 pups was used. Rat pups from different litters and 1, 5, 10, 20 and 30 days old were sacrificed at the beginning of a light cycle. A group of adult virgin female rats was used as a control.

After the animals were beheaded, they were dissected ; their stomach and small intestine were extracted, opened, cleaned of their contents, washed in saline, blotted, weighed and homogenized in chilled modified Krebs-Ringer bicarbonate buffer (Arola *et al.*, 1978). Coarse homogenates were filtered through nylon mesh and used directly as an enzyme source.

Glutamate dehydrogenase, alanine and aspartate transaminases, arginase, adenylate deaminase, serine dehydratase and glutamine synthetase activities were estimated using the standard methods described previously (Arola *et al.*, 1982). The statistical significance of the differences between groups was estimated by Student's t-test.

Results and discussion.

Table 1 presents amino acid activities in the small intestine and stomach of developing rats. Following a pattern similar to that in liver (Arola *et al.*, 1982), intestinal alanine transaminase activity slowly increased, reaching adult values on day 30 ; this supports the increasing role of hepatic gluconeogenesis from low

TABLE 1

Amino acid metabolism enzyme activities in the stomach and small intestine of developing rats.

Enzyme		Intrauterine life (days)		Age after birth (days)					
		19	21	1	5	10	20	30	adults
Alanine transaminase	S :	100 ± 15	47 ± 12*	38 ± 7*	58 ± 5*	55 ± 5*	91 ± 26	121 ± 18	131 ± 15
	I :	40 ± 3*	58 ± 7*	143 ± 14*	101 ± 13*	120 ± 9*	196 ± 34	220 ± 42	201 ± 16
Aspartate transaminase	S :	206 ± 12*	199 ± 17*	200 ± 25*	263 ± 34*	368 ± 72	362 ± 13*	382 ± 32	520 ± 46
	I :	162 ± 25*	172 ± 16*	120 ± 8*	148 ± 12*	144 ± 13*	228 ± 24*	262 ± 57	328 ± 24
Serine dehydratase	S :	165 ± 56*	124 ± 108	40 ± 6	62 ± 18	35 ± 3	29 ± 6	45 ± 15	27 ± 4
	I :	2.5 ± 0.7	1.9 ± 0.6	6.9 ± 2.0	4.9 ± 2.0	2.6 ± 1.3	2.7 ± 0.6	4.7 ± 0.8	5.9 ± 1.3
Adenylate deaminase	S :	56 ± 14*	87 ± 12	45 ± 7*	65 ± 11*	97 ± 9	60 ± 22	73 ± 21	99 ± 10
	I :	48 ± 9	44 ± 9	59 ± 13	61 ± 12	78 ± 12	53 ± 10	83 ± 15	66 ± 8
Glutamate dehydrogenase	S :	109 ± 17	79 ± 11*	78 ± 9*	96 ± 19	91 ± 18	132 ± 21	127 ± 11	130 ± 9
	I :	161 ± 22	210 ± 28*	118 ± 20	81 ± 8*	123 ± 14	154 ± 20	182 ± 27	138 ± 16
Glutamine synthetase	S :	161 ± 19	96 ± 20	70 ± 10*	139 ± 18	77 ± 11*	83 ± 15*	129 ± 19	142 ± 11
	I :	4.0 ± 0.6*	5.8 ± 0.6	6.5 ± 0.9	15.2 ± 2.3*	14 ± 2.0*	4.9 ± 0.3	5.5 ± 1.4	7.6 ± 1.1
Arginase	I :	60 ± 21*	69 ± 19*	79 ± 5*	71 ± 19*	67 ± 20*	202 ± 30*	605 ± 70	675 ± 76

S : Stomach ; I : Small intestine. All values are the mean ± s.e.m. of 5-7 different animals and are expressed in nanokatals per gram of tissue weight. Significance of the differences versus adult controls : * = $p < 0.05$.

dietary protein amino acids (Phillippides and Ballard, 1969) such as those found in a solid diet. The same was true of aspartate transaminase which reached adult values on day 30 ; this activity was related to a general activation of amino acid catabolism. Serine dehydratase values in the intestine decreased near birth, increasing thereafter during weaning, finally recovering on day 30. This complex pattern resembled that of kidney enzyme development (Arola *et al.*, 1982) but individual variability was considerable because of the very low enzyme activity as compared with the liver, the main site of serine degradation (Schepartz, 1973). The actual meaning of this activity must be carefully considered.

Arginase, a typical liver enzyme also found in small proportions in the intestine (Konarska and Tomaszewki, 1975) and other tissues (Reddi *et al.*, 1975), is probably more related to polyamine synthesis and arginine removal. As in liver and kidney (Arola *et al.*, 1982), the actual levels of its activity in the intestine were low during the lactation period studied, rising to adult levels by the time of weaning and suggesting a common regulatory system. The low activity found in early suckling suggests the existence of a mode of high nitrogen storage in the whole developing rat (Hahn and Koldovsky, 1961) in a situation where growing is the main objective ; the whole supply of protein-rich milk is practically devoted to the buildup of body proteins (Miller, 1970), and the urea cycle enzymes are depressed in the liver (Miller and Chu, 1970).

The enzyme patterns found in the stomach were quite different from those in the intestine, indicating the strong biochemical differentiation of the two organs. Alanine transaminase activity in the stomach followed the pattern found in the intestine with a high 19-day level that dropped to half that 2 days later. This could be related to the high level of serine dehydratase in the foetal stomach which decreased progressively (about five-sixths) to low adult levels. It is significant that high levels of two unequivocal gluconeogenic enzymes were found in the stomach, showing that it has a transient role in amino acid conversion. Aspartate transaminase activity followed the same pattern in the stomach as in the intestine but was more intense.

No measurable arginase activity was found in the stomach. This could be related to the different way the stomach handles ammonia as compared with the intestine (Arola *et al.*, 1982). However, adenylate deaminase, an important ammonia producer, had comparable activity in both tissues. Glutamate dehydrogenase showed a pattern similar to that in the intestine that closely followed the pattern of aspartate transaminase, *i.e.* a constant increase from low foetal values troughout development. The pattern of this enzyme was comparable to that found in liver (Arola *et al.*, 1982) and could be related with the increasing role of amino acid catabolism in the tissue.

Intestinal glutamine synthetase activity reached a maximum between days 5 and 10 and decreased afterwards to adult levels. This increase during mid-lactation was related with the relative lack or urea cycle activity (Miller and Chu, 1970) during the initial stages of lactation and the need to detoxify excess ammonia produced in the gut (Arola *et al.*, 1981a). However, this role was probably of limited effectiveness because of strong intestinal glutaminasic activity (Pinkus and Windmueller, 1977). In addition, glutamine synthetase provides the

substrate for carbamyl phosphate synthesis when the circulating levels of glutamine are low because of the lower ability of the muscle to release and synthesize glutamine, as in the perinatal period (Alemany, 1979).

The activity of glutamine synthetase in he stomach was much higher than in the intestine as it has an important role in maintaining acid-base equilibrium (Arola *et al.,* 1981a). The pattern of this enzyme followed that of serine dehydratase (ammonia producer *vs* ammonia consumer) during development and until weaning, when it again increased to adult levels. This implies a dual role of the enzyme as a detoxifying agent compared to that in the liver and muscles where its activity increases as the rat develops (Arola *et al.,* 1982) ; this enzyme is probably more related to other pathways using glutamine as substrate (Lund, 1980) since its intestinal activity is low compared with other tissues (Arola *et al.,* 1981a). High levels of adenylate deaminase occur in muscular organs such as striated muscle (Arola *et al.,* 1981b). No significant changes were observed in the enzyme contents of the small intestine or the stomach during development, despite changes in function and gross size increases. This might be because enzyme action is related more directly to the muscular part of the organ and its operation than to the changing function of amino acid catabolism.

The *main conclusion* that can be drawn from the present data is that the developmental pattern and adult activity of the enzymes studied in the stomach and intestine are different. The fact that the stomach is small and secretes acid might account for some of the differences, the intestine still being the main splanchnic bed organ resembling the liver in size and probably in activity. The enzymes more directly involved in the catabolic role of the intestine in amino acid metabolism act as follows : both transaminases and arginase show high activities in adults while their activities are low during foetal life and then increase along with weaning. It must be noted that gut size actually increases faster in proportion to the rest of the body in order to cope with the increasing amounts of food ingested and to be processed in the alimentary canal (Palou *et al.,* 1983). Thus, the total ability of the organ for alanine synthesis actually increases more than one order of magnitude when the whole animal is considered. The patterns found suggest that there is no particular direct relationship between the amino acid catabolizing enzymes of the gut and the changing diet other than that found in adult system of the splanchnic bed as a whole in postprandial states (Aikawa *et al.,* 1973). The developmental patterns agree with the high nitrogen storage found in late foetal and early postnatal life (Hahn and Koldovsky, 1961 ; Miller, 1970).

Reçu en juin 1984.
Accepté en juin 1985.

Résumé. *Activités des enzymes du métabolisme des acides aminés dans l'estomac et l'intestin grêle du rat en croissance.*

Les activités de l'alanine transaminase, de l'aspartate transaminase, de l'arginase, de la sérine deshydratase, de la glutamate déhydrogenase, de l'adénylate deaminase et de la glutamine synthétase sont déterminées dans les tissus stomacal et intestinal du rat à la fin de

sa vie fœtale et pendant la croissance. Malgré l'origine commune des deux tissus, les activités enzymatiques étudiées sont très différentes. La plupart des enzymes étudiées ont une activité faible pendant la vie intra-utérine et l'allaitement. Leur activité augmente pour atteindre le niveau des adultes pendant le sevrage. Il n'existe pas dans l'estomac d'activité arginasique et celle-ci est très faible dans l'intestin. Les activités élevées de l'alanine transaminase, de la sérine déshydratase et aussi, dans une certaine mesure, de la glutamine synthétase pendant la vie fœtale suggèrent l'existence d'une importante aptitude de l'estomac et de l'intestin à métaboliser les acides aminés. Cette aptitude diminuerait après la naissance.

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