**Free Amino Acid Changes in Serum throughout Rat
Gestation and Lactation.
Evolution of the
Plasma/Serum Relationships**

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Summary Serum amino acid levels in young, adult, pregnant (day 12 and 21) and lactating (day 20) rats have been described and compared with plasma concentrations. Serum values systematically overestimate the amino acid plasma content. Changes reported for plasma amino acid levels in midpregnant and lactating rats are well reflected in the serum values. This is not the case for adult and late pregnant rats.

Key Words: amino acids, serum, gestation, lactation, rat

The measurement of free amino acid levels in serum has been often used as an indicative parameter of the metabolic status, specially in clinical investigations [1, 2]. The validity of serum amino acid determinations seems controversial when results are compared with plasma levels [3–5]. In many cases, serum levels are higher than plasma ones [4, 5], but different from the whole blood content [3]. We have previously shown that blood amino acid compartmentation in the rat is affected by certain physiological situations such as aging [6], pregnancy [7, 8], and lactation [8]. These states are associated with important changes in the whole blood amino acid concentrations as well as in their compartmentation. The widely used clinical serum estimations are evaluated in the present work in order to test whether or not they reflect the known alterations in amino acid plasma composition reported in the rat under the same physiological conditions.

MATERIALS AND METHODS

Female Wistar rats, weighing initially 172 ± 3 g, were fed *ad libitum* with a standard diet (Sandermus S-10, Sanders, Spain), and were kept under controlled

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conditions of temperature ($23 \pm 1^\circ\text{C}$) and humidity ($65 \pm 5\%$) with a dark-light cycle of 12 h (on at 9:00 a.m. and off 9:00 p.m.). They were mated with adult males and after verification of impregnation by the presence of spermatozoa in daily vaginal smears (considered as the first day of pregnancy), they were kept alone until the day of the experiment (on days 12 and 21 of pregnancy) or on day 20 after delivery. Eight-(young controls) and eighteen-(adult controls)-week-old virgin females were also used in this experiment.

Animals were sacrificed by decapitation at the beginning of the light cycle. Blood was collected from the neck and one sample was used to obtain serum (after centrifugation at $5,000 \times g$) and another was collected into heparinized beakers and used for preparation of plasma. Both samples were deproteinized in capillary tubes with cold acetone [9], and the clear supernatant was used for amino acid determination by a radiochemical method [10], based on the specific reaction of amino acids with [^{14}C]dansyl-chloride (Amersham, U.K.) and subsequent thin-layer chromatographic separation of dansyl amino acid derivatives on polyamide sheets (Schleicher & Schüll). Individual amino acids were determined from the radioactivity of the spots, and calculations were performed using L-norvaline as internal standard and an amino acid solution (Sigma) as external standard. All the reagents for chromatography were of analytical grade (Carlo Erba).

Calculations were performed with an Apple microcomputer. Statistical differences were established by Student's *t* test.

RESULTS

Table 1 shows the free amino acid levels in serum through gestation and lactation. On day 12 after delivery there was a generalized decrease in serum amino acid levels, the differences in Ala, Glu+Gln, Gly, His, Met, ornithine, Phe and Ser being significant. This pattern varied as pregnancy progressed, thus Met and ornithine recovered to their normal values at the end of pregnancy. On the other hand, Ala, Glu+Gln, Phe, and Ser maintained their decreased values during the entire pregnancy. Glycine maintained a progressive decrease in its values during pregnancy, while Leu+Ile, taurine, Thr, Trp, and Val were decreased only at the end of the period. On day 20 after delivery there were increases in the levels of Cys, citrulline, ornithine, and Pro and a decrease in the Phe levels. Comparison of the two control groups revealed increases in Cys, citrulline and Tyr in the Adult group and a decrease in Lys levels.

Table 2 shows the plasma/serum relationship in rats during pregnancy and lactation (plasma/serum relationships have been established from individual comparisons in each animal). As a general trend, the relative maintenance of these ratios in most of the studied amino acids was noted, but there were notable exceptions. Thus, Ala, Glu+Gln, and Gly showed increased ratios in late pregnancy, and conversely, Asp+Asn, His, 4-OHPro, and taurine had diminished values in mid-pregnancy. On day 21 of pregnancy most of the plasma/serum

Table 1. Free amino acid levels in serum through gestation and lactation.

	C	P ₁₂	P ₂₁	M ₂₀	AC
Alanine	978 ± 19	693 ± 75**	840 ± 55*	1,268 ± 125	866 ± 52
Arginine	639 ± 298	274 ± 68	979 ± 74	630 ± 150	407 ± 66
Asp + Asn	247 ± 48	212 ± 41	201 ± 43	240 ± 49	167 ± 26
Cysteine	107 ± 20	131 ± 16	116 ± 22	282 ± 30**	337 ± 64**
Citrulline	88.9 ± 10	123 ± 11*	118 ± 26	188 ± 25*	289 ± 50*
Glu + Gln	1,085 ± 74	776 ± 75**	773 ± 60**	849 ± 77	881 ± 101
Glycine	508 ± 35	357 ± 45*	196 ± 18**	698 ± 120	388 ± 41
Histidine	200 ± 15	132 ± 20*	249 ± 52	186 ± 40	144 ± 21
4-OH Proline	29.4 ± 3.1	25.1 ± 1.4	35.4 ± 4.2	36.7 ± 5.2	32.8 ± 1.7
Leu + Ile	387 ± 52	325 ± 19	262 ± 14*	436 ± 24	390 ± 11
Lysine	418 ± 33	474 ± 72	379 ± 31	448 ± 29	325 ± 37*
Methionine	48.8 ± 15	10.1 ± 2.1**	47.6 ± 11	48.1 ± 9.1	60.3 ± 8.6
Ornithine	81.3 ± 11	41.1 ± 10*	73.1 ± 17	166 ± 27*	89.3 ± 11
Phenylalanine	122 ± 16	65.8 ± 9.6*	55.1 ± 7.2**	79.8 ± 9.7*	83.1 ± 9.1
Proline	445 ± 45	380 ± 71	418 ± 34	663 ± 71*	491 ± 29
Serine	419 ± 37	300 ± 15*	302 ± 16*	366 ± 27	365 ± 40
Taurine	908 ± 115	725 ± 116	290 ± 30*	720 ± 120	838 ± 90
Threonine	408 ± 43	333 ± 47	263 ± 21*	319 ± 27	304 ± 39
Tryptophan	143 ± 12	120 ± 13	81.8 ± 5.7*	116 ± 21	156 ± 27
Tyrosine	112 ± 11	138 ± 22	103 ± 8.6	157 ± 19	172 ± 14*
Valine	256 ± 39	182 ± 12	157 ± 19*	261 ± 43	196 ± 11

Results are the mean ± SEM of 5–7 different animals. Values are expressed as $\mu\text{mol/liter}$. C, Young controls; P₁₂, day 12 of pregnancy; P₂₁, day 21 of pregnancy; M₂₀, day 20 after delivery; AC, adult controls. Significance of the comparisons vs. young controls. * $p < 0.05$; ** $p < 0.01$.

ratios were close to 1. After delivery, Ala, Cys, Glu + Gln, Gly, taurine, and Trp showed decreased ratios. The comparison of both control groups showed notable differences; thus, adult controls showed increased values for Ala, Thr, and Trp and decreased ratios in Arg, Asp + Asn, His, 4-OHPro, and Met.

DISCUSSION

Changes in amino acid pools in serum of pregnant and lactating mammals are not well documented, although extensive information exists on amino acid levels in plasma under these physiological conditions [11, 12]. Pregnancy-associated plasma hypoaminoacidemia develops on day 12 [7, 11], probably due to an acceleration of hepatic metabolism [13]. Serum levels overestimate the amino acid plasma concentrations, but the drop at mid-pregnancy is of the same magnitude as that found in plasma. Furthermore it is accounted for by the same individual amino acids, especially Glu + Gln and Ala. The amino acid pattern in serum of lactating rats is also similar to that previously found in plasma [14]. This results in equal plasma/serum ratios in both physiological situations. Nevertheless some

Table 2. Plasma/serum relationship through pregnancy and lactation.

	C	P ₁₂	P ₂₁	M ₂₀	AC
Alanine	0.77±0.02	0.68±0.06	0.95±0.05*	0.73±0.04	1.04±0.08*
Arginine	0.84±0.01	0.69±0.16	0.68±0.15	0.54±0.12*	0.58±0.08*
Asp+Asn	1.00±0.13	0.57±0.12*	1.09±0.22	0.67±0.09*	0.66±0.07*
Cysteine	1.22±0.44	0.55±0.12	1.18±0.10	0.55±0.16	0.82±0.13
Citrulline	0.92±0.19	0.67±0.10	0.98±0.03	0.69±0.12	0.67±0.13
Glu+Gln	0.62±0.03	0.76±0.05	1.15±0.06**	0.79±0.08	0.67±0.07
Glycine	0.62±0.03	0.72±0.11	0.90±0.05**	0.64±0.08	0.68±0.08
Histidine	0.88±0.11	0.46±0.04**	0.56±0.13	0.53±0.17	0.56±0.05*
4-OH Proline	1.05±0.05	0.76±0.09*	0.89±0.16	0.65±0.08**	0.77±0.07**
Leu+Ile	0.86±0.04	1.00±0.08	1.01±0.13	0.83±0.05	0.75±0.05
Lysine	0.79±0.10	1.00±0.06	1.00±0.07	0.94±0.11	0.98±0.20
Methionine	1.52±0.02	1.03±0.17	1.06±0.03**	0.93±0.17	0.92±0.01*
Ornithine	0.44±0.06	0.60±0.08	1.08±0.24	0.56±0.09	0.64±0.10
Phenylalanine	0.76±0.16	0.85±0.16	1.16±0.16	0.72±0.12	1.07±0.09
Proline	0.80±0.09	1.02±0.13	0.88±0.10	0.77±0.19	0.82±0.09
Serine	0.80±0.04	0.96±0.09	1.01±0.10	1.03±0.14	0.86±0.09
Taurine	0.56±0.06	0.37±0.04*	0.65±0.08	0.25±0.05**	0.41±0.05
Threonine	0.76±0.06	0.73±0.07	1.01±0.10	0.89±0.18	1.09±0.08*
Tryptophan	0.90±0.07	0.96±0.10	1.08±0.15	0.55±0.13	0.99±0.07
Tyrosine	1.02±0.07	0.79±0.11	0.91±0.10	0.82±0.10	1.29±0.08*
Valine	0.88±0.04	0.96±0.60	0.96±0.17	0.88±0.05	1.12±0.13

Results are the mean±SEM of 5–7 different animals. C, Young controls; P₁₂, day 12 of pregnancy; P₂₁, day 21 of pregnancy; M₂₀, day 20 after delivery; AC, adult controls. Statistical comparisons vs. young controls. * $p<0.05$; ** $p<0.01$.

slight variations were found, but only for those amino acids that have a minor contribution to the overall circulating levels. This is not the case in late pregnancy (21-day), because serum levels are lower than those found for virgin rats. This does not occur when plasma or whole blood contents are considered [3]. Thus, the plasma/serum ratio for Glu+Gln and Ala is significantly increased.

It is rather obvious that clinical serum amino acid determinations do not only give a clear overestimation of the plasma values, but may even mask the actual changes in plasma. The reasons for this controversial behaviour in both fluids are not evident. It is not known how blood clotting may induce such different values between serum and plasma amino acid levels. Care must be taken as to the procedure used to estimate free amino acids when changes in their free circulating concentrations are considered. Comparisons between plasma, blood and serum levels are not allowed, and probably the latter are mainly artificial.

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