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Free Amino Acids in Plasma and Tissue of Rats Born to Underfed Dams (38693)

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The importance of prenatal and postnatal nutrition on the development of the progeny has been well documented. Severe food restriction results in infertility in women (1). Maternal malnutrition in rats retards DNA replication and the synthesis of RNA and protein (2–4). Results from our laboratory have shown that underfeeding of the dams during gestation and lactation had an adverse effect on the growth rate of the offspring (5). These stunted animals required more feed for growth and excreted more urinary nitrogen, urea, total amino acid (6), and hydroxyproline (7) than the controls. These changes suggest that the growth stunting may be related to some aspects in protein metabolism. The present experiment was undertaken to determine the effect of maternal dietary restriction on free amino acids in plasma, liver, and muscle of the progeny.

Materials and Methods. Animal and diet. Male rats of the McCollum strain were used. They were housed individually in stainless steel cages at room temperatures of 25–28° during the whole experiment. The method of maternal dietary restriction has been described in detail elsewhere (7). The following comparisons reported are between groups of male rats born of mothers on *ad libitum* feeding of Purina Chow and groups born of the mothers restricted during gestation and lactation to approximately 0.5 the amount of feed consumed by the control group. After 28 days of age, all progeny were housed individually and received Purina rat chow. Fresh water and food were freely available.

The weights of rats, as well as their food intake, were recorded weekly. At the end of 9 mo, the animals were fasted for 18 hr and then killed by decapitation. The fasting blood was collected in a heparinized tube, and the plasma was separated and stored in a freezer at −25°. The liver and a portion of gastrocnemius muscle were removed quickly and frozen in −80° until analyzed.

Amino acid analysis. An aliquot of each tissue was homogenized in distilled water to yield a 5% homogenate. This was followed by centrifugation after which the tissue supernatants and the plasma were deproteinized with equal volumes of 9% sulfosalicylic acid. Amino acids were analyzed by ion-exchange chromatography on 140 × 0.62 cm single column with a Technicon amino acid analyzer. Gradient elution was carried out with sodium citrate buffers (a) pH 2.88 (0.2 *N* for Na+) and (b) pH 4.74 (0.8 *N* for Na+), prepared as described in Technicon Monograph No. 1, 1966. Norleucine (0.125 μM) was always included as an internal standard and a commercially prepared amino acid standard was analyzed periodically. The data were analyzed statistically by means of Student's *t* test.

Results. During the 9-mo experimental period the food intake, calculated as gram per unit body weight, was consistently about 20% higher among the offspring of underfed mothers than *ad libitum*-fed mothers. The mean body weight for the restricted progeny was 285 ± 31 g which is about 75% of normal value. This difference is statistically significant.

The concentrations of plasma free amino acid were generally lower in the offspring of underfed dams then in those of unrestricted mothers. The most striking differences were the marked decrease in the concentration of alanine, glutamic acid, glycine, and proline in the progeny of restricted mothers as shown in Table I. The concentrations of two essential amino acids, arginine and valine were also significantly reduced.

In the progeny of restricted mothers, the alterations in the amino acid content of plasma were, for the most part, reflected by

TABLE I. FREE AMINO ACIDS IN PLASMA, LIVER AND MUSCLE OF PROGENY BORN TO MOTHERS FED THE NONRESTRICTED OR THE RESTRICTED DIET

Amino acid	Plasma		Liver		Muscle	
	Nonrestricted	Restricted	Nonrestricted	Restricted	Nonrestricted	Restricted
	μmoles/100 ml		μmoles/100 g wet wt		μmoles/100 g wet wt	
Nonessential						
Alanine	64 ± 6.08[a]	43 ± 1.01[b]	653 ± 95	214 ± 37*	232 ± 88	205 ± 12
Aspartic acid	—	—	80 ± 16	83 ± 23	37 ± 1.9	36 ± 1.8
0.5 Cystine	13 ± 0.01	8 ± 2.82	—	—	—	—
Glutamic acid	52 ± 4.69	34 ± 4.01[b]	489 ± 58	232 ± 15[b]	79 ± 1.2	94 ± 5.4
Glycine	50 ± 4.58	39 ± 2.82[b]	578 ± 95	262 ± 15[b]	159 ± 61	139 ± 79
Ornithine	7 ± 0.01	7 ± 1.73	116 ± 31	56 ± 22	—	—
Proline	16 ± 0.01	12 ± 1.73[b]	92 ± 15	45 ± 37	—	—
Serine	26 ± 5.86	18 ± 2.23	139 ± 50	43 ± 12	36 ± 2.4	29 ± 2.4
Taurine	40 ± 8.60	23 ± 5.56	788 ± 255	306 ± 169[b]	1388 ± 456	1125 ± 367
Essential						
Arginine	11 ± 0.91	6 ± 0.11[b]	—	—	—	—
Histidine	7 ± 0.28	6 ± 0.03	76 ± 10	54 ± 37	—	—
Isoleucine	10 ± 0.02	9 ± 1.73	56 ± 10	35 ± 22	18 ± 9	19 ± 4
Leucine	22 ± 2.23	18 ± 0.03	128 ± 21	75 ± 41	32 ± 14	36 ± 23
Lysine	20 ± 0.41	16 ± 1.01	88 ± 13	39 ± 30[b]		
Methionine	—	—	49 ± 17	32 ± 14	—	—
Phenylalanine	9 ± 0.06	8 ± 1.02	49 ± 18	28 ± 9	18 ± 2	18 ± 12
Threonine	—	—	102 ± 58	41 ± 12	61 ± 3.8	60 ± 4.7
Tyrosine	10 ± 0.40	8 ± 0.88	56 ± 33	26 ± 9	19 ± 9	20 ± 14
Valine	22 ± 1.41	16 ± 0.01[b]	131 ± 39	52 ± 31	26 ± 11	27 ± 15

[a] Mean ± SD.
[b] $P < 0.05$ or $P < 0.01$.

alterations of free amino acid in liver tissue (Table I). However, the concentrations of amino acid in muscle of perinatally malnourished offspring appeared to be normal.

Discussion. The growth stunting of weanling rats from dams on restricted feed intake is associated with the disturbances of protein metabolism. This interpretation was based on the findings of increased amounts of urinary nitrogenous compounds (5), and hydroxyproline (7) in the progeny of underfed mothers. This view is now also supported by the present findings showing the reduction of certain free amino acids in the plasma of restricted progeny. In addition, the alterations of free amino acid concentrations induce a disruption of the normal balance of free amino acid pools and this in turn would affect the rate of protein synthesis.

Food intake as gram per unit body weight was higher in the offsprings of underfed mothers than *ad libitum*-fed mothers which would not account for these observations. Decreased absorption or increased tissue oxidation could have been contributing factors to the low concentrations of free amino acids in the plasma and liver. According to Lee and Chow (6), the increased amount of urinary amino acids in restricted progeny was associated with abnormal renal tubular reabsorption. If this is indeed the case a decrease of plasma amino acid in restricted progeny would be expected. Although only continued research will unravel further clues to the nature of the metabolic derangement, the importance of maternal nutrition in the maintenance of normal serum amino acids has been demonstrated.

Recently investigators (9–11) have been studying the change in amino acid patterns in the plasma of malnourished infants and children. They have sought in the plasma aminogram a clue to their nutritional status. The finding reported here that the maternal diet may influence the amino acid pattern of post weanling plasma, even though the current diet is an adequate one, is of utmost importance. This fact must be borne in mind whenever plasma aminograms are interpreted.

Summary. Amino acids were determined in plasma, muscle, and liver tissues of adult male rats born of mothers on restricted or nonrestricted diets during pregnancy and lactation. The concentrations of plasma alanine, glutamic acid, glycine, proline, arginine, and valine were significantly lower in the progeny of restricted mothers as compared to non-restricted mothers. Similar changes were observed in liver tissues. The concentrations of free amino acids in muscle of the perinatally malnourished progeny, however, did not differ significantly from those of the controls. The differences determined in this study are of special significance in view of the fact that the animals studied had been maintained on an adequate diet since weaning.

The demonstrated effect of the maternal diet on the plasma aminogram of the offspring some months later must be considered when interpreting plasma aminograms in studies of malnourished children.

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