Decreased Plasma Proteins, Increased Total Plasma-Free Amino Acids, and Disturbed Amino Acid Metabolism in the Hereditary Severe Anemia of the Belgrade Laboratory (b/b) Rat (43613)

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Abstract. **The plasma amino acid pattern has been investigated in severely anemic Belgrade laboratory (b/b) rats. Nonanemic heterozygous (b/+) or normal homozygous (+/+) rats of the same age (six weeks) were used as controls. Decreased plasma proteins, increased total free amino acid, and urea concentrations in plasma associated with increased urea and 3-methylhistidine urinary excretion were found, indicating protein and amino acid metabolic alterations in anemic b/b rats. Plasma alanine, glutamine, tyrosine, and phenylalanine concentrations were increased. The significantly reduced molar ratio between valine + leucine + isoleucine and phenylalanine + tyrosine suggested severe disturbance in the hepatic energy-producing system and derangement of hepatic energy status. Partial or complete reversal of the anemia within 3 days by red blood cell transfusion or within 3 weeks by iron treatment resulted in normalization of tyrosine, alanine, glutamine, and total amino acid concentrations in plasma, as well as of molar ratio between valine + leucine + isoleucine and phenylalanine + tyrosine. This indicated a better oxygen supply to the liver and normalization of the hepatic energy status. These findings suggest that the metabolic disturbances in the b/b rat are the consequence of hypoxia due to the severe anemia.** [P.S.E.B.M. 1993, Vol 203]

The Belgrade laboratory b/b rat has severe hereditary hypochromic microcytic anemia (1, 2) due to defective transmembrane iron transport into erythroblasts (3) and a proliferative defect in the erythroid progenitor cells (4). Recently, it has been shown that the b/b rat also has abnormalities in megakaryocytopoiesis (5) and granulocytopoiesis (6) as a consequence of hypoxia due to the severe anemia. The affected animals are also retarded in growth (1). The whole body iron metabolism is disturbed and, despite the hyperferremia, reduction in the iron of body tissues exists (2).

Iron treatment improves hematopoiesis and body weight gain of the b/b rat (6), but the pathologic forms of erythrocytes remain in the peripheral blood (2). In addition, iron therapy showed some beneficial effects upon erythrocyte and plasma antioxidant systems (7).

The existence of severe anemia in combination with retarded growth prompted our interest in the plasma protein and amino acid status of the b/b rat. Plasma amino acid profiles can reflect alterations in protein and amino acid metabolism of the body in starvation (8), injury (9, 10), or sepsis (11). Hepatic and/or peripheral tissue disturbances in protein and amino acid metabolism are reflected by plasma-free amino acid pool characteristics (12, 13). The aims of the present work were to investigate the possible influence of severe anemia in the b/b rat on protein and amino acid metabolism and the effects of corrected

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anemia (either by red blood cell transfusion or iron treatment) on the plasma amino acid pattern.

Material and Methods

Animals. The anemic b/b rat colony raised at the Medical Military Academy from heterozygous b/+ male and female rats (kindly provided by Dr K. Keller, Centers for Disease Control, Atlanta, GA) was used for the examinations. The controls were nonanemic female heterozygous (b/+) or homozygous (+/+) rats of the same genetic background. We have also observed as noted previously (1) that b/+ rats, although not genotypically normal, do not express phenotypical differences from the +/+ rat. The experiments were done on 6-week-old female rats (39 b/b rats and 13 controls) fed a standard laboratory food mixture containing crude protein (20–23%), iron (50 mg/kg), copper (10 mg/kg), a mixture of other microelements, vitamins, and other nutrients (Agros, Subotica, Yugoslavia).

Experimental Design. In order to establish the amino acid status in anemic b/b rats and to examine the possible effects of hypoxia and feed intake on it, the following groups were studied: anemic b/b rats (Group A, $n = 18$); red blood cell-transfused b/b rats (Group B, $n = 7$); iron-treated b/b rats (Group C, $n = 7$ fed *ad libitum*; Group D, $n = 7$ pair-fed to anemic animals during the last 3 weeks); and controls (Group E, $n = 13$).

Anemic b/b rats (Group A) were not under any special treatment throughout the study. Red blood cell (RBC) transfusion (Group B) was given by intraperitoneal injections of 2.0 ml of a syngeneic RBC suspension (packed cell volume about 30%) as described in the previous paper (4). Transfusions were given during the 3 subsequent days and further examinations were made on the second day after the last transfusion, when the animals were 6 weeks old. Anemic and RBC-transfused b/b rats as well as controls were fed *ad libitum* throughout the lifetime of 6 weeks. The iron treatment (Group C) was started on the 21st day and the b/b rats were given an iron-dextran complex (Ferrum; Lek, Ljubljana), 3.5 mg ip, once per week as suggested by Sladić-Simić *et al.* (1). Groups B and C were used to examine the effect of corrected anemia on the amino acid and protein metabolic alterations.

In order to examine the possible effects of feed intake on amino acid metabolic alterations, during the last 3 weeks (from the third to the sixth week) some iron-treated animals were pair-fed by being offered the average daily amount of feed consumed by the anemic b/b rats (Group D).

Blood and Urine Sampling. On the day prior to the blood sampling, animals were placed in individual metabolic cages for urine collection (toluene was used as the preservative). All animals were allowed water *ad libitum*. Because preliminary investigations had shown that untreated anemic b/b rats could not survive food restriction for a period of 24 hr, the anemic b/b rats (Group A) were fed *ad libitum* during the period of urine collection. The other groups of animals (B, C, D, and E) were offered the same amount as that consumed daily on average by the group of anemic animals (8.6 g/day) and they consumed it. After urine collection for 24 hr, the animals were anesthetized (ether) and heparinized blood samples (a. abdominalis) were obtained.

Plasma and Urine Analysis. Plasma samples were obtained after centrifugation (4°C, 1500*g*, 10 min) and analyzed for total plasma proteins and albumin (biuret method), urea (diacetylmonoxime method), and creatinine (colorimetric method). After deproteinization of the plasma (sulfosalicylic acid) and adjustment of pH to 2.2, free amino acids were analyzed on an automatic amino acid analyzer (Beckman 121 M) employing five lithium citrate buffers (14). Urine samples were analyzed for urea, creatinine, and 3-methylhistidine. Urinary 3-methylhistidine was determined after deacetylation (6 moles/liter HCl, 110°C, 20 hr) using an ion-exchange chromatographic method (15).

Statistics. All data were expressed as mean ± SD. Analysis of variance (Duncan method) and correlation analysis were used for statistical evaluation. The statistical significance between means was taken at the 95% confidence level.

Results

In untreated (anemic) laboratory b/b rats fed *ad libitum* (Group A), hemoglobin and hematocrit values were significantly below control limits (Table I), indicating severe anemia. This was associated with reduced plasma concentrations of total proteins, particularly albumin, and a several times increased urea concentration, but plasma glucose concentration was within control limits (Table I).

Body masses of 6-week-old anemic b/b rats (Group A) were significantly lower compared with controls (Group E), but similar to RBC-transfused (Group B) and iron-treated pair-fed (Group D) animals (Table II). Body mass gain during the period of 3 weeks was the smallest in the anemic b/b rats (Table II). The amount of creatinine excreted by Group A was reduced, but the creatinine coefficient (CR/BM – excreted amount of creatinine/body mass) was within control limits (Table II). In the anemic b/b rats, the quantities of excreted urea, representing urea produced from both exogenous (feed) and endogenous (tissues) amino acids, were slightly reduced, while the quantities of urinary 3-methylhistidine were similar to control values. Considering the differences in body and muscle mass between anemic (Group A) and control (Group E) animals, urinary urea and 3-methylhistidine were expressed per amount of excreted creatinine (Table II). The results obtained indicated that in anemic rats, the urea to creatinine

Table I. Concentrations of Hemoglobin, Plasma Proteins, Albumin, Urea, Creatinine, Glucose, and Hematocrit Values in Anemic, Red Blood Cell-Transfused, Iron-Treated and *Ad Libitum*-Fed, and Iron-Treated Pair-Fed b/b Rats and Controls

Group	A (n = 18)	B (n = 7)	C (n = 7)	D (n = 7)	E (n = 13)
Hemoglobin (g/liter)	2.9 ± 0.4$^{*,b-d}$	14.5 ± 1.2^{a}	8.3 ± 1.4^{*,a}	8.6 ± 1.2^{*,a}	15.2 ± 0.8
Total protein (g/liter)	52.1 ± 5.9*	42.6 ± 4.4*	64.7 ± 5.6	57.9 ± 2.7	62.5 ± 4.1
Albumin (g/liter)	23.4 ± 4.8*	20.1 ± 3.9*	29.1 ± 2.8	30.3 ± 2.7	30.7 ± 2.3
Urea (mmol/liter)	20.2 ± 5.9$^{*,b-d}$	7.7 ± 1.4^{a}	9.0 ± 1.6^{*,a}	9.6 ± 1.9^{*,a}	6.3 ± 1.2
Creatinine (μmol/liter)	44.8 ± 14.8	32.5 ± 5.3	35.0 ± 5.1	33.3 ± 3.9	38.7 ± 6.9
Glucose (mmol/liter)	7.2 ± 1.3	7.6 ± 0.6	7.7 ± 1.8	6.7 ± 0.9	7.8 ± 1.5
Hematocrit (%)	17.5 ± 4.5$^{*,b-d}$	42.6 ± 2.6	40.4 ± 4.5	41.1 ± 4.5	41.2 ± 0.9

Note. Values are given as mean ± SD. Significant differences: * $P < 0.05$ vs controls (E group); a, b, c, d $P < 0.05$ vs A, B, C, D groups. A = anemic; B = red blood cell transfused; C = iron-treated and *ad libitum-fed*; D = iron-treated pair-fed b/b rats; E = control rats.

Table II. Body Mass, Urinary Creatinine, Urea, and 3-Methylhistidine Excretions in Anemic, Red Blood Cell-Transfused, Iron-Treated and *Ad-Libitum*-Fed, and Iron-Treated Pair-Fed b/b Rats and Controls

Group	A (n = 18)	B (n = 7)	C (n = 7)	D (n = 7)	E (n = 13)
Body mass (g)	60.4 ± 4.1^{*,c}	69.7 ± 5.4^{*,c}	103.0 ± 19.9^{a,b,d}	60.4 ± 9.2^{*,c}	124.2 ± 21
Body mass gain (g)	10.3 ± 2.5^{*,c,d}	—	26.0 ± 3.6^{*,a}	23.8 ± 6.7^{*,a}	35.0 ± 1.9
Creatinine (μmol/day)	27.5 ± 4.6*	47.4 ± 8.8	42.5 ± 10.2	30.9 ± 8.7	51.4 ± 8.8
CR/body mass ratio	0.456 ± 0.076	0.681 ± 0.127	0.420 ± 0.049	0.507 ± 0.108	0.42 ± 0.06
Urea (mmol/day)	3.28 ± 0.58	5.38 ± 2.08	4.11 ± 0.55	3.00 ± 0.87	4.64 ± 1.28
Urea/CR ratio	0.12 ± 0.01*	0.11 ± 0.03	0.10 ± 0.03	0.11 ± 0.05	0.09 ± 0.02
3-MeHis (μmol/day)	1.88 ± 0.65	2.58 ± 0.84	2.17 ± 0.87	2.15 ± 0.61	1.84 ± 0.45
3-MeHis/CR ratio	0.07 ± 0.02*	0.06 ± 0.02*	0.06 ± 0.04*	0.07 ± 0.03*	0.03 ± 0.01

Note. Values are given as mean ± SD. Significant differences: * $P < 0.05$ vs controls (E group); a, b, c, d $P < 0.05$ vs A, B, C, D groups. A = anemic; B = red blood cell transfused; C = iron-treated and *ad libitum*-fed; D = iron-treated pair-fed b/b rats; E = control rats; CR = urinary creatinine; 3-MeHis = 3-methylhistidine.

ratio was above control limits, suggesting increased protein and amino acid breakdown per unit of muscle mass. The increased urinary 3-methylhistidine to creatinine ratio found in the Belgrade rat indicated increased breakdown of myofibrillar proteins.

Concentrations of total free amino acids in the plasma of anemic b/b rats were increased compared with controls (Fig. 1). The increase was due mainly to an increase in total nonessential amino acids, as total essential amino acids were within control limits (Fig. 1). Thus, the molar ratio between essential amino acids and nonessential amino acids (Ea:nEa) in the anemic animals was significantly lower than that in controls (Fig. 1). The greatest differences between anemic and control animals were observed in the concentrations of alanine, glutamine, and tyrosine (Table III). However, branched-chain amino acids (BCA; valine + leucine + isoleucine) were not significantly altered, whereas aromatic amino acids (AA; phenylalanine + tyrosine) were more than three times greater than in controls (Table III). Thus, in the anemic animals, the molar ratio between valine + leucine + isoleucine and phenylalanine + tyrosine (BCA:AA) was reduced, whereas the ratio between phenylalanine and tyrosine Phe:Tyr was not altered (Fig. 2). While in anemic b/b rats the molar ratio between glycine and the branched-chain amino

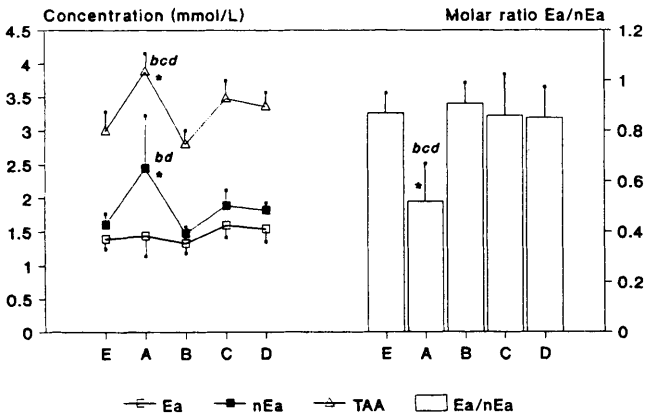

Figure 1. Plasma concentrations of total, essential, and nonessential amino acids (TAA, Ea, and nEa, respectively) and the molar ratio between essential and nonessential amino acids (Ea:nEa) in plasma of the Belgrade laboratory (b/b) rat. Values are given as mean ± SD. E = controls (n = 13); A = anemic (n = 18); B = red blood cell-transfused (n = 7); C = iron-treated and *ad libitum*-fed (n = 7); D = iron-treated pair-fed (n = 7) rats. Numbers of animals in the groups are given in parentheses. Significant differences: *$P < 0.05$ vs controls (Group E); a, b, c, d $P < 0.05$ vs A, B, C, D groups.

acids (Gly:BCA) in the plasma was within control limits, the molar ratio between alanine and these amino acids (Ala:BCA) was significantly above control values (Fig. 2). A correlation (r = 0.907, t = 4.414) between

Table III. Concentration of Free Amino Acids in Plasma (μmol/liter) in Anemic, Red Blood Cell-Transfused, Iron-Treated and *Ad Libitum*-Fed, and Iron-Treated Pair-Fed b/b Rats and Controls

Group	A (n = 18)	B (n = 7)	C (n = 7)	D (n = 7)	E (n = 13)
Phenylalanine	139.3 ± 26.2$^{*,b-d}$	66.6 ± 8.6^{d}	86.8 ± 20.6^{*,a}	105.4 ± 14.3^{*,a,b}	59.8 ± 9.0
Valine	194.2 ± 70.7	207.3 ± 33.8	216.4 ± 47.7	209.3 ± 36.4	199.6 ± 31.8
Leucine	132.9 ± 53.6	142.9 ± 28.0	171.9 ± 26.4	168.7 ± 31.3	151.8 ± 26.0
Isoleucine	66.0 ± 19.7	71.6 ± 22.4	87.1 ± 21.9	98.0 ± 15.3	79.7 ± 15.7
Arginine	180.0 ± 39.6*	129.9 ± 27.9	178.4 ± 56.7	126.8 ± 14.1	126.5 ± 36.7
Lysine	298.0 ± 71.4	306.2 ± 37.4	338.5 ± 49.6	277.0 ± 27.3	317.6 ± 54.9
Histidine	97.4 ± 31.6$^{*,b-d}$	67.1 ± 5.1^{a}	73.1 ± 7.5^{a}	77.6 ± 11.0^{a}	59.7 ± 6.1
Threonine	184.9 ± 66.7	201.6 ± 53.9	258.5 ± 36.2	302.1 ± 84.6	240.9 ± 64.1
Tryptophane	116.4 ± 36.4^{*,b}	73.9 ± 15.0^{a}	116.2 ± 39.4*	113.3 ± 25.9*	81.1 ± 11.7
Methionine	79.8 ± 23.5^{*,b}	57.4 ± 9.7	66.2 ± 6.2	59.9 ± 6.1	52.7 ± 9.3
Tyrosine	251.4 ± 94.5$^{*,b-d}$	61.0 ± 5.9^{a}	83.7 ± 27.4^{a}	77.5 ± 4.9^{a}	67.2 ± 9.9
Alanine	998.6 ± 404.9$^{*,b-d}$	347.4 ± 58.7^{a}	658.1 ± 156.6^{*,a}	539.9 ± 70.4^{*,a}	443.6 ± 68.7
Glutamic acid	67.6 ± 31.1	104.4 ± 26.5*	66.0 ± 29.9	73.5 ± 24.1	44.0 ± 12.4
Glutamine	579.6 ± 82.0^{*,d}	485.5 ± 66.2	498.7 ± 130.9	459.7 ± 77.5	445.4 ± 84.6
Glycine	304.7 ± 60.2	211.4 ± 29^{*,a,c,d}	304.1 ± 28.9	330.0 ± 71.7	315.1 ± 56.1
Serine	256.4 ± 49.3	192.4 ± 38.2	234.8 ± 23.9	256.1 ± 48.2	226.2 ± 31.3
Ornithine	70.3 ± 25.2	54.9 ± 20.4	55.2 ± 10.0	51.0 ± 7.7	45.1 ± 15.9
Aspartic acid	16.7 ± 7.9	17.5 ± 3.2	14.5 ± 8.4	19.3 ± 3.7	16.6 ± 9.9
BCA	393.1 ± 141.4	421.8 ± 75.0	475.3 ± 93.5	476.1 ± 80.2	431.1 ± 61.8
AA	390.7 ± 51.8	127.6 ± 7.7	170.6 ± 47.7	183.0 ± 16.0	127.0 ± 16.7

Note. Values are given as mean ± SD. Significant differences: * $P < 0.05$ vs controls (E group); a, b, c, d $P < 0.05$ vs A, B, C, D groups. A = anemic; B = red blood cell-transfused; C = iron-treated and *ad libitum*-fed; D = iron-treated pair-fed b/b rats; E = control rats; BCA = branched-chain amino acids (valine + leucine + isoleucine); AA = aromatic amino acids (phenylalanine + tyrosine).

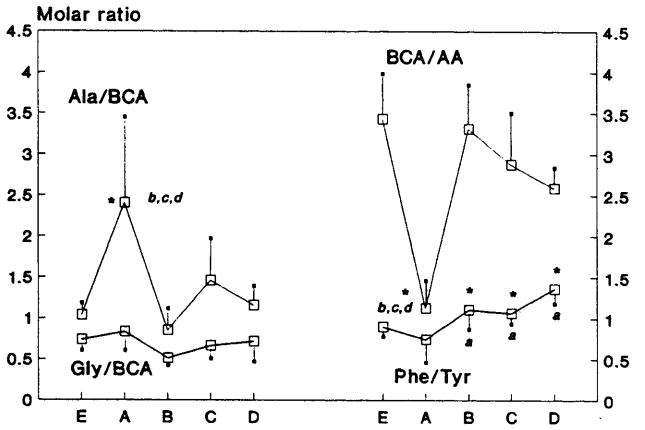

Figure 2. Alterations of molar ratio between glycine and branched-chain amino acid (Gly:BCA), branched-chain and aromatic amino acids (BCA:AA), and phenylalanine and tyrosine (Phe:Tyr) in the plasma of the Belgrade b/b laboratory rats. Values are given as mean ± SD. For details, see Legend to Figure 1.

Ala:BCA and BCA:AA molar ratios in the plasma of anemic b/b rats was observed.

RBC transfusion quickly (within 3 days) and iron treatment slowly (within 3 weeks) reversed the anemia in b/b rats (Table I). RBC transfusion normalized both hemoglobin and hematocrit values as well as the plasma urea level in b/b rats, but decreased the concentrations of total proteins and albumin in the plasma (Table I, Group B). Iron treatment increased the hemoglobin concentration compared with the anemic animals, normalized hematocrit values, affected protein concentration, and lowered but did not normalize the urea level in the plasma (Table I, Group C). The concentrations of glucose were within control limits in both Groups B and C. The quantities of creatinine, urea, and 3-methylhistidine excreted in the urine in Groups B and C were within control limits (Table II). Both iron treatment and RBC transfusion normalized the plasma concentration of total amino acids mainly due to recovery of the level of nonessential amino acids in the b/b rats (Fig. 1). In addition, the treatments examined brought the molar ratio between valine + leucine + isoleucine and phenylalanine + tyrosine into control limits (Fig. 2). However, compared with the controls, both treatments produced an increase in the molar ratio between phenylalanine and tyrosine (Fig. 2), indicating increased net protein catabolism in peripheral tissues.

In the group of iron-treated b/b rats pair-fed to the anemic animals for 3 weeks (Group D), hemoglobin increased and urea concentration decreased compared with the anemic animals, while hematocrit values, protein, and albumin concentrations were normalized (Table I). Namely, the observed alterations were like those in the iron-treated b/b rats fed *ad libitum* throughout the same period (Table I), although the quantity of feed consumed during the 3-week period was different (average amount consumed 184 g and 158 g in Groups C and D, respectively). Body mass gain in Group D was similar to that in Group C and significantly above the values for anemic b/b rats (Table II). The lack of significant differences in the characteristics of plasma-

free amino acid pools between Groups C and D (Table III, Figs 1 and 2), regardless of the feed intake during the 3-week period, indicated that the abnormalities observed in plasma protein and amino acids in the anemic b/b rats were not the consequence and reflection of a smaller feed intake.

Discussion

The present investigations have shown severe disturbances in protein and amino acid metabolism in anemic b/b rats. Those abnormalities can be ascribed to: (i) protein and/or calorie malnutrition (16, 17) and (ii) hypoxia due to severe anemia.

The reduced body mass accompanied with decreased concentrations of albumin and total proteins in plasma, which we found in the b/b rats, could be the consequence of severe malnutrition (16). The disturbed molar ratio of nonessential to essential amino acids, observed in anemic b/b rats, could be ascribed to the decreased level of protein in the diet (17). However, our finding that iron-treatment of pair-fed b/b rats reversed the anemia excludes the possibility that disturbances in plasma amino acids are due to malnutrition, since neither plasma proteins nor the molar ratio between essential and nonessential amino acids was altered. The evidence that plasma branched-chain amino acids, glycine, and their molar ratio were not altered in anemic b/b rats supported this. Low protein or protein-free diets lead to a decline in the concentration of branched chain amino acids, increased glycine, and a concomitant increase in the molar ratio between glycine and branched-chain amino acids (Gly:BCA) in plasma (18). Namely, on the basis of amino acid changes in the plasma and urine of the anemic and iron-treated pair-fed b/b rats, the alterations in protein and amino acid metabolism in anemic b/b rats could not be explained by differences in exogenous protein and calorie supplies.

Since long-lasting fasting is associated with reduced urinary excretion of 3-methylhistidine, i.e., an adaptive decrease in the rate of catabolism of muscle protein as starvation progresses (19), it should be expected that the lower feed intake by anemic b/b rats would be followed by reduced 3-methylhistidine urinary excretion. On the contrary, in anemic b/b rats, urinary excretion of 3-methylhistidine, an amino acid with a specific metabolism (20) widely used for the assessment of skeletal muscle protein breakdown under different conditions (21–24), suggests increased skeletal muscle protein catabolism. The increased plasma concentration of phenylalanine, an essential amino acid which cannot be degraded in skeletal muscles but can in the liver (25), indicates increased protein degradation in the peripheral tissues of anemic animals, too. Alterations in alanine, glutamine, and urea levels in the plasma of anemic b/b rats could be associated with that, too.

Moreover, we found that the plasma Phe:Tyr ratio, which characterizes increased muscle protein breakdown (26, 27) in catabolic states of various origins (9, 10), was not elevated, but was even slightly reduced in anemic b/b rats. This was mainly due to a simultaneous large increase in the plasma tyrosine level. Such alterations may have been caused by overloading the system which transformed phenylalanine to tyrosine and by inhibiting amino acid entrance into the citric acid cycle in the liver, which occurs in hepatic dysfunction (13). Severe disturbances in the hepatic energy-producing system in the laboratory b/b rat were indicated by the reduced molar ratio between valine + leucine + isoleucine and phenylalanine + tyrosine in the plasma, a parameter that is positively correlated with the hepatic mitochondrial redox potential (13). The increased molar ratio between alanine and branched-chain amino acids could be a consequence of such alterations, too (18).

Thus, the derangement of hepatic energy status seems to be the most important factor in the plasma amino acid disturbance in the anemic b/b rat. It seems that the hepatic energy status in the anemic b/b rat is reduced due to prolonged hypoxia resulting from the severe anemia. An extremely low arterial oxygen content has indeed been found in b/b rats (6). Our results obtained after reversal of the anemia in b/b rats either by transfusion or by iron treatment showed normalization of almost all disturbances of plasma-free amino acid pool characteristics, strongly suggesting an association with better oxygen supplies to the liver and normalization of the hepatic energy status. These findings confirmed the role of anemia-derived hypoxia in the disturbed protein and amino acid metabolism found in anemic b/b rats.

1. Sladić-Simić D, Zivkovic N, Pavic D, Marinkovic D, Martinovic J, Martinovitch PN. Hereditary hypochromic microcytic anemia in the laboratory rat. Genetics **53**:1079–1089, 1966.
2. Sladic-Simic D, Martinovich PN, Zivković N, Pavić D, Martinovic J. A thalassemia-like disorder in Belgrade laboratory rats. Ann NY Acad Sci **165**:93–99, 1969.
3. Edwards J, Huebers H, Kunzer C, Finch C. Iron metabolism in the Belgrade rat. Blood **67**:623–628, 1986.
4. Pavlović-Kentera V, Basara N, Biljanović-Paunović L, Vasiljevska M, Rolović Z. Erythroid progenitors in anemic Belgrade laboratory (b/b) rats. Exp Hematol **17**:812–815, 1989.
5. Rolović Z, Jovanović T, Stanković Z, Marinković N. Abnormal megakaryocytopoiesis in the Belgrade laboratory rat. Blood **65**:60–64, 1985.
6. Rolović Z, Basara N, Stojanović N, Suvajdžić N, Pavlović-Kentera V. Abnormal megakaryocytopoiesis in the Belgrade laboratory rat. Blood **77**:456–460, 1991.
7. Simović M, Ristović B, Vasiljevski M, Buzadžić B, Saičić Z, Spasić M. The activity of some antioxidative enzymes in the

blood of the Belgrade laboratory rats. Iugosl Physiol Pharmacol Acta **24**(suppl 6):73–74, 1988.
8. Adibi SA. Interrelationships between level of amino acids in plasma and tissues during starvation. Am J Physiol **221**:829–839, 1971.
9. Chang Xiao-Jun, Yang Chin-Chun, Hsu Wei-Shia, Hsu Wei-Tsung, Shih Tse-Grane. Changes of serum amino acids in severely burned patients. Burns **10**:109–115, 1983.
10. Žunić G, Savić J, Simović M, Todoric M, Erdeljan D, Albreht M. Early plasma free amino acid concentration changes in pigs wounded by high velocity missiles. J Trauma (China) Suppl **6**:204–209, 1990.
11. Metzler B, Rettenmeier AW, Bauer B, Schmahl FW. Serum amino acids in experimentally induced endotoxic shock: The prognostic significance of hyperalaninemia. Circ Shock **29**:1–12, 1989.
12. Hasselgren PO, Jagenburg R, Karlstrom L, Pedersen P, Seeman T. Changes of protein metabolism in liver and skeletal muscle following trauma complicated by sepsis. J Trauma **24**:224–228, 1984.
13. Ikai I, Ozaki N, Shimahara Y, Wakashiro S, Tokunaga Y, Tanaka A, Ozawa K. Significance of hepatic mitochondrial redox potential on the concentrations of plasma amino acids following hemorrhagic shock in rats. Circ Shock **27**:63–72, 1989.
14. Lee P. Single column system for accelerated amino acid analysis of physiological fluids using five lithium buffers. Biochem Med **10**:107–121, 1974.
15. Žunić G, Stanimirović S, Savić J. Rapid ion-exchange method for the determination of 3-methylhistidine in rat urine and skeletal muscle. J Chromatogr **311**:69–77, 1984.
16. Olusi SO, McFarlane H, Osunkoya BO, Adesina H. Specific protein assays in protein-calorie malnutrition. Clin Chem Acta **62**:107–116, 1975.
17. Suzić S, Janković V, Radunović LJ, Marinković J, Segović R. Influence of protein intake on the systemic and portal vein plasma amino acid ratios in fed rats. Iugosl Physiol Pharmacol Acta **28**:123–131, 1992.
18. Oberholzer VG, Briddon A. A novel use of amino acid ratios as an indicator of nutritional status. In: Lubec GA, Rosenthal GA, Eds. Amino Acids, Chemistry, Biology and Medicine. The Netherlands, Escom-Leiden, pp1079–1083, 1990.
19. Young VR, Haverberg LN, Bilmazes C, Munro HN. Potential use of 3-methylhistidine excretion as an index of progressive reduction in muscle protein catabolism during starvation. Metabolism **22**:1429–1435, 1973.
20. Young VR, Alexis SD, Baliga S, Munro HN. Metabolism of administered 3-methylhistidine—Lack of muscle transfer ribonucleic acid charging and quantitative excretion as 3-methylhistidine and its N-acetyl derivative. J Biol Chem **247**:3592–3600, 1972.
21. Long CL, Schiller WR, Blakemore WS, Geiger JW, ODell MO, Henderson K. Muscle protein catabolism in the septic patient as measured by 3-methylhistidine excretion. Am J Clin Nutr **30**:1349–1352, 1977.
22. Burman KD, Wartofsky L, Dinterman R, Kessler P, Wannemacher RW. The effect of T3 and reverse T3 administration on muscle protein catabolism during fasting as measured by 3-methylhistidine excretion. Metabolism **28**:805–813, 1979.
23. Calles-Escandon J, Cunningham JJ, Snyder P, Jacob R, Huszar G, Loke J, Felig P. Influence of exercise on urea, creatinine and 3-methylhistidine excretion in normal human subjects. Am J Physiol **246**:E334–E338, 1984.
24. Žunić G, Savić J, Pantelić D. Urinary excretion of 3-methylhistidine in injured rats. In: ZS Biro, AGB Kovach, JJ Spitzer, HB Stoner, Eds. Advances In Physiological Science, Vol 26. Homeostasis in Injury and Shock. England/Hungary, Pergamon Press/Akademia Kiado, pp285–286, 1981.
25. McGee MM, Greengard O, Know WE. The quantitative determination of phenylalanine hydroxylase in rat tissues—Its developmental formation in liver. Biochem J **127**:669–674, 1972.
26. Herndon DN, Wilmore DW, Mason AD, Pruitt VR. Abnormalities of phenylalanine and tyrosine kinetics—Significance in septic and nonseptic patients. Arch Surg **113**:133–135, 1978.
27. Wannemacher RW, Klainer AS, Dinterman RE, Beisel W. The significance and mechanism on increased serum phenylalanine-tyrosine ratio during infection. Am J Clin Nutr **29**:997–1006, 1976.