Relationship of mammalian erythrocyte enzymes and their intermediary compounds among selected animals

Lorena Késsia de Figueiredo Silva FONSECA[1]
Raimundo Antonio Gomes OLIVEIRA[2]
José Daniel Luza FEDULLO[3]
Sheila Serra VIEIRA-PINTO[1]
Orlando César de Oliveira BARRETTO[1]

1 - LIM-23, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo-SP
2 - Divisão de Hematologia da Universidade Federal do Maranhão, São Luis-MA
3 - Fundação Parque Zoológico de São Paulo, São Paulo-SP

Correspondence to:
Orlando C de O Barretto, Av. Pedroso de Morais 70 São Paulo SP- 05420-000, ocdobarr@usp.br

Recebido para publicação: 23/04/2007
Aprovado para publicação: 03/07/2008

Abstract

As there are few comparative studies on mammalian red cell metabolism, it was decided to study the glycolytic enzyme activities as well as related ones, and the metabolites adenosine-5´-triphosphate (ATP) and 2,3-bisphosphoglycerate (2,3-BPG). Mammalia representatives from Primates, Rodentia, Carnivora, Lagomorpha, Artyodactyla, Didelphimorphia and Xenarthra orders, obtained from Fundação Parque Zoológico de São Paulo and Centro de Bioterismo da Faculdade de Medicina da USP, were studied. The blood was collected in EDTA and ACD, the red cells were washed in saline at 4º C, lysed 1:20 in hemolysing solution by freeze-and-thaw, and the following enzymes were assayed according to standard procedures: hexokinase, glucose-6-phosphate isomerase, phosphofructokinase, aldolase, triose phosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, monophosphoglycerate mutase, enolase, pyruvate kinase, lactate dehydrogenase, as well as 2,3-bisphosphoglycerate mutase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase activities at 37ºC , and adenosine-5´-triphosphate and 2,3-bisphophoglycerate concentrations. A remarkable variation among the studied species was observed. However, it was detected a significant positive correlation between the adenosine-5´-triphosphate concentrations and triose phosphate isomerase and 2,3-bisphosphoglycerate mutase activities, as well as significant positive correlation between 2,3-bisphosphoglycerate concentration and 2,3-bisphosphoglycerate mutase activity in all studied species as a whole. Most of studied species exhibited a steady ATP concentration range between 4 and 6 μ moles.g Hb $^{-1}$ except the Artiodactyla (*Cervus elaphus*) and Carnivora (*Panthera leo, Leopardus pardalis, Canis Lupus and Chrysocyon brachyurus,*) which presented values between 2 and 3 μ moles g Hb $^{-1}$. However, the 2,3-BPG concentration showed remarkable variation among the studied species and among the orders.

Key words:
Mammalian erythrocyte enzymes.
2,3-bisphosphoglycerate.
Adenosine triphosphate.

Introduction

The mammalian circulating erythrocytes are non-nucleated and deprived of cytoplasm organelles such as ribosomes, mitochondria, Golgi apparatus, counting just on the glycolytic pathway, the pentose monophosphate shunt and attached enzymes to guarantee the supply of caloric energy under the form of adenosine-5'-triphosphate (ATP) and reducing energy under the form of reduced nicotinamide adenosine dinucleotide, reduced nicotinamide adenosine dinucleotide phosphate and reduced glutathione . The glycolytic pathway also exhibits the attached

Luebering-Rappaport shunt, in which the 2,3-bisphosphoglycerate (2,3-BPG) is formed, the metabolite which regulates the hemoglobin affinity to the molecular oxygen.[1,2,3,4] The Embden Meyerhoff pathway enzymes and the intermediary compounds (ATP and 2,3-DPG) in the erythrocytes have been studied in several species of animals [5,6,7,8,9,10,11,12] as well as some other enzymes which protect the red blood cells against oxidative damage.[5,10,11,12] However, no reference was found about the correlation of the enzyme activities and the concentration of ATP and 2,3-BPG among mammalian species. That being so, the correlation of the red cell enzymes and the intermediary compounds of selected species of mammals of seven orders was investigated in this work.

Material and Method

Fourteen adult mammalian species of seven different orders (Primates, Rodentia, Carnivora, Lagomorpha, Artiodactyla, Didelphimorphia and Xenarthra) obtained from Fundação Parque Zoológico de São Paulo and Centro de Bioterismo da Faculdade de Medicina da USP, were intestigated. Venous blood was collected in ACD (citric acid, citrate and dextrose) for enzyme assays within 5 days, and in EDTA (ethylenediamine tetraacetic acid, potassium salt) for 2,3-bisphosphate glycerate, adenosine-5'-triphosphate and hemoglobin concentration determinations soon after blood drawing.

Hexokinase (HX), glucose-6-phosphate isomerase (GPI), phosphofructokinase (PFK), aldolase (ALD), triose phosphate isomerase (TPI), glyceraldehyde-3-phosphate dehydrogenase (GAPD), phosphoglycerate kinase (PGK), monophosphoglycerate mutase (MPGM), enolase (ENO), pyruvate kinase (PK), lactate dehydrogenase (LDH), as well as 2,3-bisphosphoglycerate mutase (BPGM), glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (6-PGD) activities as well as adenosine-5'-triphosphate and 2,3-bisphosphoglycerate concentrations were assayed according to standard methods.[13]

The obtained data were submitted to variance analysis (ANOVA), and the correlation study (Pearson) between the enzyme activities and intermediary compounds was accomplished as well.[14,15]

Results

The results depicted in tables 1 to 3 show that there is a great variability of activities among the studied animals as well as adenosine-5'-triphosphate and 2,3-bisphosphoglycerate concentrations among the species. When the species are joined in their orders, the obtained data also vary a great deal among the orders.

Interestingly, the ATP concentrations present a moderate positive correlation with triosephosphate isomerase (r: 0.5) and 2,3-bisphosphoglycerate mutase (r: 0.5) activities, and 2,3-BPG concentration presents a positive correlation with 2,3-bisphosphoglycerate mutase (r: 0.6) activities.

Discussion

Comparative study of mammalian red cell metabolism has been performed by several researchers.[5,10,16,17] A remarkable variation among the studied mammalian species has been observed, consistent with other authors' findings.[6,9,10,11,12,18]

The present study demonstrated that most of the enzymes do not show any correlation with ATP and 2,3-BPG, but triose phosphate isomerase and 2,3-bisphosphoglycerate mutase showed significant coefficients of correlation of Pearson ($r : 0.5$, $p<0.001$) for ATP concentrations, as well as 2,3-bisphosphoglycerate mutase activity with the intracellular 2,3-BPG concentrations ($r : 0.6$, $p<0.001$). These findings suggest that most of glycolytic enzymes activities in normal individuals do not affect the rate and yield in terms of ATP and 2,3-BPG except triose phosphate isomerase and 2,3-

Table 1-A - Red cell enzymes activities (IUg $Hb^{-1}min^{-1}$ at 37ºC) and intermediary compounds concentrations (m M.g Hb^{-1}) among the studied species - Sao Paulo - 2008

Enzymatic activities and intermediary compounds	*Homo sapiens* (Human) n: 8	*Mandrillus sphinx* (Mandrill) n: 2	*Chrysocyon brachyurus* (Maned Wolf) n: 6	*Panthera leo* (Lion) n: 4	*Leopardus pardalis* (Ocelot) n:1	*Canis lupus* (Grey Wolf) n: 2	*Canis lupus familiaris* (Dog) n: 6
HX	1.2 ± 0.3	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.2	0.8	0.7 ± 0.03	0.9 ± 0.1
GPI	61.1 ± 6.3	88.5 ± 9.2	79.7 ± 16.2	72 ± 10.4	72.5	36.5 ± 4.9	55.3 ± 4.7
PFK	11.6 ± 1.3	7.8 ± 1	12.5 ± 3.1	12.2 ± 1.7	8	5.1 ± 0.3	11 ± 0.4
ALD	3.3 ± 0.3	2.8 ± 0.4	3.1 ± 0.6	4.3 ± 0.3	2.3	1.2 ± 0.1	4 ± 0.2
TPI	1843 ± 343	1250 ± 212	1516 ± 239	887 ± 68	2044	1085 ± 162	2112 ± 20
GAPD	207± 18.6	161± 12	186 ± 33.2	231 ± 35.5	183	100 ± 2.8	172 ± 18.2
PGK	311 ± 21.8	310 ± 29	282 ± 17.6	320 ± 29.9	401	123 ± 9.2	193 ± 10.2
BPGM	4.8 ± 0.3	10.4 ± 3.4	5 ± 0.4	4.5 ± 0.5	4.6	1.6 ± 0.3	5.1 ± 0.94
MPGM	21.5 ± 5.4	8.8 ± 0.3	16.8 ± 1.6	17.7 ± 2.7	11.9	5.8 ± 0.2	12.7 ± 2.4
ENO	5 ± 0.5	4.5 ± 1.3	3.9 ± 0.9	5.3 ± 0.4	2.2	2.0 ± 0.4	4.0 ± 0.2
PK	14 ± 1.7	16.2 ± 3.9	12.9 ± 2.2	15.2 ± 1.3	26.3	4 ± 0.1	9.5 ± 0.5
LDH	196 ± 14	161 ± 26.8	191 ± 36.7	207 ± 19	164	56 ± 5.6	159 ± 13.7
G-6PD	12 ± 1	8.1 ± 1.6	15.9 ± 0.9	16.8 ± 2	16.6	13.9 ± 1.6	13.1 ± 1.9
6-PGD	8.2 ± 0.8	2.7 ± 0.6	6.8 ± 0.5	8.3 ± 0.9	3.8	3 ± 0.2	7.5 ± 0.8
ATP	4 ± 0.1	5.1 ± 0.2	1.7 ± 0.3	1.9 ± 0.2	2	2.8 ± 0.2	4.3 ± 0.1
2,3-BPG	12.1 ± 0.5	25.9 ± 1.4	19.8 ± 1	3.9± 0.6	4.2	12.2 ± 0.6	14.2 ± 0.7

The statistical analysis of variance (ANOVA) revealed that there is significant variability ($p < 0,05$) among the studied species. HX: Hexokinase, GPI:glucose-6-phosphate isomerase, PFK: phosphofructokinase, ALD: aldolase , TPI: triose phosphate isomerase , GAPD: glyceraldehyde-3-phosphate dehydrogenase, PGK: phosphoglycerate kinase, MPGM: monophosphoglycerate mutase, ENO: enolase, PK: pyruvate kinase , LDH: lactate dehydrogenase, BPGM: 2,3-bisphosphoglycerate mutase , G6PD: glucose-6-phosphate dehydrogenase, 6-PGD: 6-phosphogluconate dehydrogenase, ATP: adenosine-5'-triphosphate, 2,3-BPG: 2,3-bisphosphoglycerate

bisphosphoglycerate mutase.

Triose phosphate isomerase is by far the most active enzyme in the glycolysis, and its role is transforming dihydroxyacetone phosphate in its isomer glyceraldehyde-3-phosphate, which is promptly metabolized by glyceraldehyde-3-phosphate dehydrogenase, preserving the glycolytic pathway, and ATP production. The initial six carbon glucose which is splitted in two 3 carbon molecules by aldolase yields its maximum rate in terms of ATP production by TPI activity which shifts towards glyceraldehyde-3-phosphate and keeps the glycolysis pathway. That being so, it is not unexpected that TPI activity may be positively correlated with ATP production.

Although 2,3-bisphosphoglycerate mutase does not belong to glycolysis, its product 2,3-BPG is an important cofactor in monophosphoglyceric mutase activity the in the transformation of 2-phosphoglycerate to 3-phosphoglycerate, affording a fair glycolytic rate, explaining the positive correlation with ATP yield.

It is interesting that none of the kynases, even pyruvate kynase, did not show correlation with ATP generation. Although pyruvate kynase deficiency in

Table 1-B - Red cell enzymes activities (IUg Hb^{-1} min^{-1} at 37ºC) and intermediary compounds concentrations (μ M.g Hb^{-1}) among the studied species - Sao Paulo – 2008

Enzymatic activities and intermediary compounds	*Cervus elaphus* (Red Deer) n: 2	*Didelphis Marsupialis* (Opossum) n: 2	*Myrmecophaga tridactyla* (Giant Anteater) n:2	*Oryctolagus cuniculus* (Europian Rabbit) n: 6	*Rattus norvegicus* (Wistar Rat) n: 6	*Cavia porcellus* (Guinea Pig) n: 2	*Mus musculus* (BALB/c Mouse) n: 6
HX	0.7 ± 0.2	1.1 ± 0.1	1 ± 0.1	1 ± 0.2	1.1 ± 0.1	1.1 ± 0.1	1.6 ± 0.1
GPI	36 ± 5.7	178 ± 11.3	65.5 ± 0.7	64.5 ± 5.7	94.5 ± 7.7	94.5 ± 20.5	125.7 ± 25.5
PFK	3.6 ± 0.6	12 ± 0.8	10.2 ± 0.3	13.3 ± 2.1	11 ± 0.3	11 ± 2	4.1 ± 0.2
ALD	1.4 ± 0.3	4.3 ± 0.1	3.1 ± 0.1	4.4 ± 0.4	4.3 ± 0.4	4.8 ± 0.1	3.9 ± 0.2
TPI	610 ± 70	2350 ± 71	2315 ± 21	1930 ± 119	1949 ± 39	2490 ± 14	2843 ± 129
GAPD	126 ± 19.8	212 ± 10.6	181 ± 15.6	141 ± 13.4	82 ± 12	44 ± 5.7	20.8 ± 2.7
PGK	123 ± 24	270 ± 14.1	346 ± 9.2	236 ± 21.3	288 ± 26	230 ± 14.1	130 ± 15.1
BPGM	3.2 ± 1.1	5.9 ± 0.2	8.9 ± 0.6	6 ± 0.6	5.4 ± 0.5	4.9 ± 0.9	5.3 ± 0,8
MPGM	7.5 ± 0.6	15 ± 1.4	22.5 ± 3.5	16.5 ± 3	16.6 ± 1.1	18.3 ± 2	25.7 ± 2.8
ENO	2.9 ± 0.2	4.8 ± 0.1	3.7 ± 0.4	5.3 ± 0.4	3.9 ± 0.1	5.1 ± 0.5	4.4 ± 0.4
PK	4.9 ± 1.3	24 ± 2.8	28 ± 1.4	10.9 ± 1.7	12.3 ± 0.7	14.1 ± 0.6	12 ± 0.6
LDH	79 ± 12.7	185 ± 7	137 ± 3.5	163 ± 32.2	194 ± 19.6	192 ± 31.8	203 ± 13.9
G-6PD	8.2 ± 2	200 ± 14.1	10.8 ± 0.3	13 ± 1.5	18.9 ± 3	14.4 ± 4.2	25.3 ± 3.2
6-PGD	2.7 ± 0.6	8.5 ± 0.1	7.9 ± 0.1	5.6 ± 1.3	10.1 ± 1.7	10.5 ± 1.5	10.8 ± 0.3
ATP	2.3 ± 0.1	5.2 ± 0.1	6 ± 0.1	6.7± 0.2	4.3 ± 0.1	4.5 ± 0.3	4.2 ± 0.2
2,3-BPG	8 ± 0.1	5.9 ± 0.1	28.2 ± 0.3	13.3 ± 0.5	14.9 ± 0.2	13.9± 0.2	15.8 ± 0.6

The statistical analysis of variance (ANOVA) revealed that there is significant variability ($p < 0,05$) among the studied species. HX: Hexokinase, GPI:glucose-6-phosphate isomerase, PFK: phosphofructokinase, ALD: aldolase , TPI: triose phosphate isomerase, GAPD: glyceraldehyde-3-phosphate dehydrogenase, PGK: phosphoglycerate kinase, MPGM: monophosphoglycerate mutase, ENO: enolase, PK: pyruvate kinase, LDH: lactate dehydrogenase, BPGM: 2,3-bisphosphoglycerate mutase, G6PD: glucose-6-phosphate dehydrogenase, 6-PGD: 6-phosphogluconate dehydrogenase, ATP: adenosine-5'-triphosphate, 2,3-BPG: 2,3-bisphosphoglycerate

humans leads to hemolytic anemia, with reduced ATP production, it is possible that in normal human states and in the studied species its activity is not rate limiting.

Another interesting finding, although expected, was the positive correlation between 2,3-bisphosphoglycerate mutase and 2,3-BPG ($r : 0.6$, $p < 0.001$), as this salt is the direct product of 2,3-DPGM catalytic action on its substrate 1,3-bisphosphoglycerate.

Reviewing the literature on enzymatic activity and red cell metabolites, we found an interesting paper of Goto, Agar and Maede[19], in which the glutathione metabolism in mammals was investigated, and described a positive correlation of glutathione-S-transferase activity and reduced glutathione concentrations ($r : 0.529$, $p<0.001$) in sheeps and mixed breed dogs.

In all investigated animals a slight ATP levels variation was found, what reinforces the crucial role of this phosphate salt rich in high energy bonds in erythrocyte metabolism, whatsoever the remarkable variation found in enzymatic activities among the studied species. The strong variation of enzymatic activities among the species might hint that ATP levels would exhibit great variation, but this did not occur indeed, as the red cells must count on the source of caloric energy kept in high energy bonds of ATP. The ATP is important in maintaining the

Table 2 - Red cell enzymes activities (IU . g Hb^{-1} . min^{-1} at 37ºC) and intermediary compounds concentrations (μ M.g Hb^{-1}) among the studied orders - Sao Paulo – 2008

Enzymatic activities and intermediary compounds	Primates n: 10	Carnívora n: 19	Artiodactyla n: 2	Didelphimorphia n: 2	Xenarthra n: 2	Lagomorpha n: 6	Rodentia n: 14
HX	1.1 ± 0.3	0.9 ± 0.1	0.7 ± 0.2	1.1 ± 0.1	1 ± 0.1	1 ± 0.2	1.3 ± 0.2
GPI	66.6 ± 13.2	65.4 ± 17.5	36 ± 5.7	178 ± 11.3	65.5 ± 0.7	64.5 ± 5.7	107 ± 23.7
PFK	10.9 ± 2	11± 2.9	3.6 ± 0.6	12 ± 0.8	10.2 ± 0.3	13.3 ± 2.1	8.1 ± 3.6
ALD	3.2 ± 0.3	3.4 ± 1	1.4 ± 0.3	4.3 ± 0.1	3.1 ± 0.1	4.4 ± 0.4	4.2 ± 0.4
TPI	1725 ± 399	1554 ± 509	610 ± 70	2350 ± 71	2315 ± 21	1930 ± 119	2409 ± 439
GAPD	198 ± 25.9	182 ± 44.1	126 ± 19.8	212 ± 10.6	181 ± 15.6	141 ± 13.4	50.7 ± 30.9
PGK	311 ± 21.5	252 ± 76.5	123 ± 24	270 ± 14.1	346 ± 9.2	237 ± 21.3	212 ± 78.8
BPGM	6 ± 2.6	4.5 ± 1.2	3.2 ± 1.1	5.9 ± 0.2	8.9 ± 0.6	6 ± 0.6	5.3 ± 0.7
MPGM	19 ± 7.2	14.3 ± 4.2	7.5 ± 0.6	15 ± 1.4	22.5 ± 3.5	16.5 ± 3	20.8 ± 4.9
ENO	4.9 ± 0.6	3.9 ± 1.1	2.9 ± 0.2	4.8 ± 0.1	3.7 ± 0.4	5.3 ± 0.4	4.3 ± 0.5
PK	14.5 ± 2.2	12.1 ± 5	4.9 ± 1.3	24 ± 2.8	28 ± 1.4	10.9 ± 1.7	12.5 ± 0.9
LDH	189 ± 21.5	169 ± 49.3	79 ± 12.7	185 ± 7.1	137 ± 3.5	163 ± 32.3	198 ± 17.9
G-6PD	11.2 ± 2	15 ± 2.1	8.2 ± 2	200 ± 14.1	10.8 ± 0.3	13 ± 1.5	21 ± 5.1
6-PGD	7.1 ± 2.4	6.8 ± 1.8	2.7 ± 0.6	8.5 ± 0.1	7.9 ± 0.1	5.6 ± 1.3	10.5 ± 1.2
ATP	4.2 ± 0.5	2.7 ± 1.2	2.3 ± 0.1	5.2 ± 0.1	6 ± 0.1	6.7 ± 0.2	4.3 ± 0.2
2,3-BPG	14.9 ± 5.8	13.1 ± 6.2	8 ± 0.1	5.9 ± 0.1	28.2 ± 0.3	13.3 ± 0.5	15.2 ± 0.8

The statistical analysis of variance (ANOVA) revealed that there is significant variability ($p < 0,05$) among the studied orders. HX: Hexokinase GPI:glucose-6-phosphate isomerase, PFK: phosphofructokinase, ALD: aldolase, TPI: triose phosphate isomerase, GAPD: glyceraldehyde-3-phosphate dehydrogenase, PGK: phosphoglycerate kinase, MPGM: monophosphoglycerate mutase, ENO: enolase, PK: pyruvate kinase, LDH: lactate dehydrogenase, BPGM: 2,3-bisphosphoglycerate mutase, G6PD: glucose-6-phosphate dehydrogenase, 6-PGD: 6-phosphogluconate dehydrogenase, ATP adenosine-5'-triphosphate, 2,3-BPG: 2,3-bisphosphoglycerate.

membrane Na-K pump and other vital red blood cells metabolic functions.

The interaction of 2,3-bisphosphateglycerate with hemoglobin regulates its affinity to molecular oxygen, generally decreasing it when 2,3-BPG increases and vice-versa. Moreover, it is well known that at alkaline pH in lungs the oxygen is linked to hemoglobin, and at low pH in tissues the oxygen is released as well.[20,21] The 2,3-bisphosphateglycerate levels herein found exhibited a great variation among the studied species, confirming the Isaaks et al. [9] report, in which a great variation of red cell organic phosphates has been encountered in different classes of vertebrates. It is quite possible that this variation of 2,3-BPG among the species may be due to differences of hemoglobin structures and their affinities to oxygen.[3, 11]

The data herein reported show a striking variability of red cell metabolism among mammals which challenge any foresight, hinting that there is still a great deal of studies to be undertaken in this fascinating field.

Table 3 - Correlation (r of Pearson) between enzymatic activities and adenosine-5 '-triphosphate and 2,3-bisphosphoglycerate concentrations - Sao Paulo – 2008

Enzymatic activitie (IU. g Hb^{-1} . min^{-1} at 37ºC)	ATP (Coefficient of correlation r)	2,3-BPG (Coefficient of correlation r)
HX	r: 0.3	r : 0.06
GPI	r: 0.1	r : 0.01
PFK	r: 0.1	r : - 0.1
ALD	r: 0.4	r : - 0.1
TPI	r: 0.5*	r : 0.2
GAPD	r: - 0.2	r : - 0.2
PGK	r: - 0.1	r : 0.03
BPGM	r: 0.5*	r : 0.6*
MPGM	r: 0.1	r : 0.1
ENO	r: 0.3	r : - 0.2
PK	r: 0.1	r : 0.1
LDH	r: - 0.05	r : - 0.05
G-6PD	r: 0.1	r : - 0.3
6-PGD	r: 0.05	r : - 0.03

* P < 0,001; HX: Hexokinase, GPI:glucose-6-phosphate isomerase, PFK: phosphofructokinase, ALD: aldolase , TPI: triose phosphate isomerase , GAPD: glyceraldehyde-3-phosphate dehydrogenase, PGK: phosphoglycerate kinase, MPGM: monophosphoglycerate mutase, ENO: enolase, PK: pyruvate kinase , LDH: lactate dehydrogenase , BPGM: 2,3-bisphosphoglycerate mutase , G6PD: glucose-6-phosphate dehydrogenase, 6-PGD: 6-phosphogluconate dehydrogenase, ATP: adenosine-5'-triphosphate, 2,3-BPG: 2,3-bisphosphoglycerate

Correlação of enzimas eritrocitárias e seus compostos intermediários de mamíferos selecionados

Resumo

Como há poucos estudos comparativos sobre o metabolismo eritrócitário dos mamíferos propôs-se estudar as atividades das enzimas glicolíticas, anexas e os metabólitos adenosina-5'-trifosfato e 2,3-difosfoglicerato. Foram estudados mamíferos das ordens Primata, Rodentia, Carnivora, Lagomorpha, Artiodactyla, Didelphimorphia e Xenarthra oriundos da Fundação Parque Zoológico de São Paulo e Centro de Bioterismo da Faculdade de Medicina da USP. O sangue foi colhido em EDTA e ACD, os eritrócitos foram lavados em solução fisiológica a 4º C e hemolisados em solução hemolisante 1:20 por congelamento e descongelamento e as atividades das seguintes enzimas foram determinadas de acordo com procedimentos padronizados: hexoquinase, glicose-6-fosfato isomerase , fosfofrutoquinase, aldolase, triose fosfato isomerase, gliceraldeído-3-fosfato desidrogenase, fosfoglicerato quinase, 2,3-difosfoglicerato mutase, monofosfogliceromutase, enolase, piruvato quinase, lactato desidrogenase, bem como a glicose-6-fosfato desidrogenase, 6-

Palavras-chave:
Enzimas eritrocitárias de mamíferos.
2,3-bisfosfoglicerato.
Adenosina trifosfato.

fosfogluconato desidrogenase a 37ºC e os metabólitos intermediários 2,3-difosfoglicerato e adenosina-5'-trifosfato. As enzimas e os compostos intermediários estudados apresentaram grande variabilidade entre as espécies de mamíferos estudadas. Foi observada correlação positiva entre a atividade da triose fosfato isomerase e a 2,3-difosfoglicerato mutase e os teores de adenosina-5'-trifosfato das espécies, bem como correlação positiva entre a 2,3-difosfoglicerato mutase em relação ao 2,3-difosfoglicerato. Os teores de adenosina-5'-trifosfato mantiveram-se dentro de uma faixa estável, ao redor de 4 a 6 μ moles / gHb, com as exceções das espécies das ordens Carnivora (Panthera leo, Leopardus pardalis, Canis lupus and Chrysocyon brachyurus) e Artiodactyla (Cervus elaphus), que exibiram 2 a 3 μ moles / g Hb. Já os valores da concentração de 2,3-difosfoglicerato apresentaram, por sua vez, variação considerável entre as espécies e as ordens estudadas.

References

1 BEUTLER, E. Composition of the erythrocyte. In: BEUTLER, E.; LICHTMAN, M. A.; COLLER, B. S.; KIPPIS, T. J.; SELIGSOHN, U. **Williams hematology**. 6. ed. New York: McGraw-Hill, 2001. cap. 23, p. 289-293.

2 BEUTLER, E. Energy metabolism and maintenance of erythrocytes. In: BEUTLER, E.; LICHTMAN, M. A.; COLLER, B. S.; KIPPIS, T. J.; SELIGSOHN, U. **Williams hematology**. 6. ed. New York: McGraw-Hill, 2001. cap. 26, p. 319-332.

3 JAIN, N. C. **Essentials of veterinary hematology**. 6. ed. Philadelphia: Lea & Febiger, 1993. 417 p.

4 TELEN, M. J.; KAUFMAN, R. E. The mature erythrocyte. In: LEE, G. R.; FOERSTER, J.; LUKENS, J.; PARASKEVAS, F.; GREER, J. P.; RODGERS, G. M. (Ed.). **Wintrobe's clinical hematology**. 10. ed. Philadelphia: Lippincott Williams & Wilkins, 1999. v. 1, cap. 10, p. 193-227.

5 AGAR, N. S.; SUZUKI, T. Red cell metabolism in buffaloes and camels. **The Journal of Experimental Zoology,** v. 223, n. 1, p. 25-28, 1982.

6 AGAR, N. S.; GODWIN, I. R. Studies on the blood of fallow deer (*Dama dama*) e red deer (*Cervus elaphus*): haematology, red cell enzymes, metabolic intermediates and glycolytic rates. **Comparative Biochemistry and Physiology, Part B: Biochemistry and Molecular Biology,** v. 103B, n. 4, p. 909-911, 1992.

7 ARAI, T.; WASHIZU, T.; SAGARA, M.; SAKO, T.; NIGI, H.; MATSUMOTO, H.; SASAKI, M.; TOMODA, I. D-glucose transport and glycolitic enzyme activities in erythrocytes of dogs, pigs, cats, horses, cattle and sheep. **Research in Veterinary Science,** v. 58, n. 2, p. 195-196, 1995.

8 BALLAS, S. K. Comparative distribuition of glyceraldehydes-3-phosphate dehydrogenase activity in human, guinea-pig, rabbit and mouse erythrocytes. **Comparative Biochemistry and Physiology, Part B: Biochemistry and Molecular Biology**, v. 87B, n. 4, p. 837-842, 1987.

9 ISAACKS, R.; NICOL, S.; SALLIS, J.; ZEIDLER, R.; KIM, H. D. Erythrocyte phosphates and hemoglobin function in monotremes and some marsupials. **The American Journal of Physiology,** v. 246, n. 2, p. R236-R241, 1984.

10 PARKINSON, A. L.; WHITTINGTON, A. T.; SPENCER, P. B. S.; GRIGG, G.; HINDS, L.; GALLAGHER, C.; KUCHEL, P.; AGAR, N. S. Comparative erythrocyte metabolism in marsupials and monotremes. **Comparative Biochemistry and Physiology, Part C: Pharmacology, Toxicology and Endocrinology**, v. 110C, n. 3, p. 261-265, 1995.

11 SUZUKI, T.; AGAR, N. S.; SUZUKI, M. Red cell metabolism: a comparative study of some mammalian species. **Comparative Biochemistry and Physiology, Part B: Biochemistry and Molecular Biology**, v. 79B, n. 4, p. 515-520, 1984.

12 WHITTINGTON, A. T.; PARKINSON, A. L.; SPENCER, P. B. S.; GRIGG, G.; HINDS, L.; GALLAGHER, C. H.; KUCHEL, P. W.; AGAR, N. S. Comparative study of the antioxidant defense systems in the erythrocytes of Australian marsupials and monotremes. **Comparative Biochemistry and Physiology, Part C: Pharmacology, Toxicology and Endocrinology**, v. 110C, n. 3, p. 267-272, 1995.

13 BEUTLER, E. **Red cell metabolism**: A manual of biochemical methods. 3rd ed. Orlando: Grune & Stratton, 1984. 188 p.

14 MORETTIN, P. A.; BUSSAB, W. O. **Estatística básica**. 5. ed. São Paulo: Saraiva, 2004. 526 p.

15 NETER, J.; KUTNER, M. H.; NACHTSHEIN, C. J.; WASSERMAN, W. **Applied linear statistical models**. 4th ed. Chicago: Irwin, 1996. 1408 p. (The Irwin series in statistics).

16 KANEKO, J. J. Comparative erythrocyte metabolism. **Advance veterinary science and comparative medicine**, v. 18, p. 117-153, 1974.

17 TURNER, T. R.; WADE, P. T.; MOHRENWEISER,

H. W. Variation in erythrocyte enzyme activity among primate species. **Comparative Biochemistry and Physiology, Part B: Biochemistry and Molecular Biology**, v. 77B, n. 3, p. 541-545, 1984.

18 SHIMIZU, Y.; KAWARADA S. Y.; SUZUKI, M.; TANAKA, T. Interstrain differences in red cell enzyme activities in mice and rats. **Comparative Biochemistry and Physiology, Part B: Biochemistry and Molecular Biology**, v. 100B, n. 4, p. 687-690, 1991.

19 GOTO, I.; AGAR, N. S.; MAEDE, Y. The relationship between reduced glutathione level and glutathione-s-transferase activity in sheep erythrocytes**. Japanese Journal of Veterinary Research**, v. 40, n. 2/3, p. 99-104, 1992.

20 HARVEY, J. W. The erythrocyte: physiology, metabolism, and biochemical disorders. In: KANEKO, J. J.; HARVEY, J. W.; BRUSS, M. L. (Ed.). **Clinical biochemistry of domestic animals**. 5. ed. San Diego: Academic Press, 1997. cap. 7, p. 157-203.

21 NIKINMAA, M. **Vertebrate red blood cells:** adaptations of function to respiratory requirements. Berlin, New York: Springer-Verlag, 1990. 262 p. (Zoophysiology, v. 28).