N U C L E A T E D R E D C E L L S

COMPARATIVE GLYCOLYTIC METABOLISM AND FUNCTION

A thesis submitted to the University of New England for the degree of Master of Science.

by

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"Perhaps the most valuable result of all education is the ability to make yourself do the things you have to do, when it ought to be done, whether you like it or not."

Thomas Henry Huxley.

1877.

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PREFACE

The studies presented in this thesis were completed by the author at The Royal Alexandra Hospital for Children and the Children's Medical Research Foundation, Sydney, Australia. The assistance received from other persons is indicated in the list of acknowledgements. All references cited are included in the bibliography. The work is otherwise original.

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I certify that this thesis has not already been submitted in substance for any degree and is not currently submitted for any other degree.

I certify that any help received in preparing this thesis and all sources used have been acknowledged.

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SUMMARY

Nucleated Red Cells - Comparative glycolytic metabolism and function.

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The aims of these experiments reported in this thesis were to characterize the red blood cells of healthy wild caught reptiles, in particular crocodilian, determine the presence of enzymes of glycolysis and test the efficiency of crocodilian erythrocytes in reducing methaemoglobin.

The composition of crocodilian blood is typical of that of vertebrates in general, with an MCHC close to that of humans, and a packed cell volume of 25% which is typical of reptiles. A haemoglobin electrophoretic pattern of one major band was found in two species of crocodiles, C. porosus and C. johnstoni. Blood glucose levels in the crocodile were similar to human levels, but the levels of lactate were high and variable, consistent with the metabolism of an animal dependent upon anaerobic glycolysis for major activity. The level of red cell ATP is high, similar to those of rabbits; but red cell 2,3-DPG is present in low concentration. Glutathione, an alternative method of modifying the oxygen affinity of haemoglobin, was considerably higher in reptilian red cells compared to human cells, but lower than that found in chickens.

Activities of several enzymes of the glycolytic pathway, and several enzymes involved in maintenance of red cell integrity were measured and partially characterized. HK and

6-PGD activities were the lowest among the glycolytic enzymes and could be rate-limiting steps. HK and LDH were both substrate inhibited at high concentrations, while HK and PK activities were strongly affected by pH.

Low levels of methaemoglobin and high levels of NADH-MR were found in several reptilian species. In vitro reduction of methaemoglobin with glucose and especially lactate was shown in crocodilian, snake and chicken red cells.

These studies have characterized crocodilian red cells and shown that these erythrocytes possess a functional glycolytic pathway, and are capable of maintaining haemoglobin in a reduced state.

ABBREVIATIONS

ACD	Acid-citrate-dextrose
ADP	Adenosine - 5' - diphosphate
ALD	Aldolase
ATP	Adenosine - 5' - triphosphate
DHAP	Dihydroxyacetone phosphate
1,3-DPG	1,3 - diphosphoglycerate
2,3-DPG	2,3 - diphosphoglycerate
DTNB	5,5 - Dithiobis (2-nitrobenzioc acid)
GAP	Glyceraldehyde - 3 - phosphate
GAPD	Glyceraldehyde phosphate dehydrogenase
α-GPDH	α- Glycerophosphate dehydrogenase
α-GP	α- Glycerophosphate
G-6-PD	Glucose - 6 - phosphate dehydrogenase
GR	Glutathione reductase
GSH	Glutathione reduced
GSSG	Glutathione oxidized
Hb	Haemoglobin
HbO_2	Oxyhaemoglobin
HK	Hexokinase
IHP	Inositol hexophosphate
IPP	Inositol 1,3,4,5,6 - pentophosphate
LDH	Lactate dehydrogenase
MCHC	Mean Corpuscular Haemoglobin Concentration
MPA	Meta-phosphoric ac
MR	Methaemoglobin reductase
NADH	Nicotinamide adenine dinucleotide (reduced)
NADPH	Nicotinamide adenine dinucleotide phosphate
Na,K-ATPase	Sodium, potassium - adenosine triphosphatase

PCV	Packed cell volume
PFK	Phosphofructokinase
2-PGA	2 - phosphoglycerate
3-PGA	3 - phosphoglycerate
6-PG	6 - phosphogluconate
6-PGD	6 - phosphogluconate dehydrogenase
PGK	Phosphoglycerate Kinase
PI	Inorganic phosphate
PK	Pyruvate kinase
ppt	Precipitate
RBC	Red blood cells
RCOP	Red cell organic phosphate
TCA	Trichloracetic acid
TCA cycle	Tricarboxylic acid cycle
TPI	Triose phosphate isomerase