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DIFFERENTIAL EXPRESSION OF G_6PD IN HAEMOGLOBIN AND RBC DURING HIBERNATION IN ANURAN, *DUTTAPHRYNUS MELANOSTICTUS*

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ABSTRACT
In this study it was tried to investigate that the seasonal variation of Glucose-6-phosphate dedydrogenase activity was associated with hibernation in Indian common toad (*Duttaphrynus melanostictus*). The decline in Glucose-6-phosphate dedydrogenase activity had its behavioral and physiological response that leads to increase the efficacy of hibernator survival.

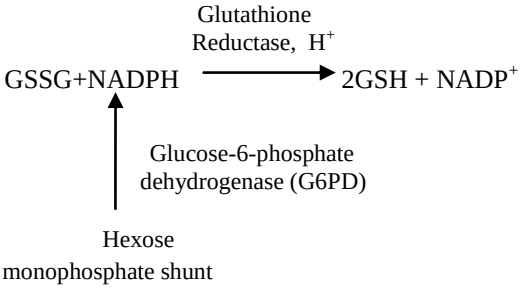
KEYWORDS: Hibernation, *Duttaphrynus melanostictus* , Glucose-6-phosphate dedydrogenase

INTRODUCTION

Hibernation allows animals to conserve energy during the winter when there is scarcity of food. During hibernation, animals drastically lower their metabolism so as to tap energy reserves stored as body fat at a slower rate. At the time of the hibernation animals such as anurans enter to a state of hypometabolism when the metabolic rate is suddenly reduced. As they there is opportunity to take little or no food during hibernation they depend on their reserve food material which is primarily in the form of fat stored in various tissues. Many endergonic reactions notably the reductive biosynthesis of fatty acids and cholesterol require NADPH in addition to ATP. Cells also have a second currency, reducing power. Despite of their close chemical resemblance, NADPH and NADH are not metabolically interchangeable. NADPH uses the free energy of metabolic oxidation to synthesize ATP (oxidative phosphorylation). NADPH uses the free energy of metabolic oxidation for reductive biosynthesis. Cells normally maintain their $(NAD^+)/(NADH)$ ratio near 1000, which favors metabolic oxidation while keeping $(NADP^+)/(NADPH)$ ratio near .01 which favors reductive biosynthesis. NADPH is generated by oxidation of glucose-6-phosphate via an alternative pathway to glycolysis, the pentose phosphate path way (also called hexose monophosphate shunt). G6PD is considered the starting point of the pentose phosphate pathway. This metabolite may arise through the action of hexokinase and glucose or from glycogen break down. The reaction is the source of NADPH production. There are inter-relationship between these two very important ways of the metabolism. The pentose phosphate path way, which begins with G6PD production in first step of glycolysis, generates NADPH for use in reductive reaction. NADPH is required for several reductive processes in addition to biosynthesis. Erythrocytes require a plentiful supply of reduced glutathione (GSH), a cys-containing tripeptide. A major function of GSH in the erythrocyte is to reductively eliminate H_2O_2 and organic hydro peroxides which are reactive oxygen metabolites that can irreversibly damage hemoglobin and cleave the C-C bond in the phospholipid tails of cell membranes. These unchecked buildings up of peroxides result in the premature cell lysis. Glucose 6 phosphate dehydrogenase (G6PD) deficiency is particularly sensitive to oxidative damage. This detoxification method is mediated by the glutathione peroxidase. G6PD stimulates the NADPH regeneration which helps to produce reduced glutathione.

$$2GSH + ROOH \xrightarrow{\text{Glutathione Peroxidase}} GSSG + ROH + H_2O$$

In order to study the hexose monophosphate shunt in hibernating condition and throughout the year, G6PD was selected. The expression of G6PD in erythrocytes was also studied throughout the year to elucidate the oxidative stress defense at the period of hibernation.



Glutathione (GSH) is the major thiol – disulfide redox buffer in the cells and is a critical component of antioxidant defense. The ratio of reduced GSH to its oxidized form (GSSG), which is an index of oxidative stress, was fivefold lower in hibernation as compared to the summer active squirrels, an effect due primarily to the elevated GSSG concentration in hibernation (Pratihar et al 2010). During hibernation, the total pool of GSH equivalents was lowest in squirrels undergoing arousal and highest in squirrels during interbout arousals (Carrey and Rhodes, 2003). Hibernation decreased intestinal GSSG

reductase activity by 50% but had no effect on glutathione peroxidase or glucose 6 phosphate dehydrogenase(G6PD). Ratios of reduced: oxidized GSH were lower in intestine of hibernators compared with summer active squirrels, an effect most pronounced for torpid animals. In the present study we determined whether hibernation is associated with changes in the intestinal glutathione redox system. However, very few studies have been performed to focus on the exact role and relationship of G6PD expression during hibernation in anurans.

Duttaphrynus melanostictus is a species of toad that is common in South Asia. It is inhabits southwestern and southern China (including Taiwan and Hainan) and throughout southern Asia from northern Pakistan and Nepal through India to Sri Lanka, Andaman Islands, Sumatra, Java, Borneo, and Bali. The species grows to almost 20 cm long. Commonly disturbed in open areas, villages, towns and only seen occasionally in primary forest. The species breeds during the monsoons.

In this experiment it was tried to investigate the changes in G6PD activity in haemoglobin and RBC during hibernation.

MATERIALS AND METHODS

Animals

Ten adults of common Indian toad, each weighing 80-100 g were collected from a selected site in Medinipore ($22^0 15^/$ N $87^0 39^/$ E) in May, (air temperature 34.4^0C-38.6^0C) as non hibernating toads. Ten adults were collected from the same site from the mud hole in hibernating state in mid Feb (air temperature ranging between 7.4^0C - 10.2^0C).

From hibernating and non-hibernating individuals blood samples were drawn via cardiac puncture immediately after euthanasia. Animals handling was performed following the ethical guidelines laid down by the committee for the purpose of control & supervision of experimental animals (CPCSEA) constituted by the Animal Welfare Division of Government of India on the use of animals in scientific research.

Glucose 6 phosphate analysis

This dehydrogenase is rather unique in that it possesses dual coenzyme specificity. When assayed under conditions that are optimal for the particular coenzyme, the ratio of observed catalytic activity is NAD/NADP = 1.8. The reaction velocity is determined by measuring the increase in absorbance at 340 nm resulting from the reduction of NAD or NADP. One unit reduces one micromole of pyridine nucleotide per minute at 30°C and pH 7.8 under the specified conditions. Statistical analyses were done using Microcal origin 6.0, statistical analysis software (Microcal Inc.USA). Each biochemical experiments were performed at least three times with 5 toads in each experimental group.

RESULTS

G6PD is considered the starting point of the pentose phosphate pathway. This metabolite may arise through the action of hexokinase and glucose or from glycogen break down. The reaction is the source of NADPH production. Variations of G6PD was studied in haemoglobin throughout the year (Fig.1). G6PD significantly lower ($P<0.05$) concentration during deep hibernation phase compared to other phases. G6PD was also studied in RBC throughout the year (Fig.2)

DISCUSSION

The pentose phosphate path way, which begins with G6P production in first step of glycolysis, generates NADPH for use in the reductive reaction. NADPH is required for several reductive processes in addition to biosynthesis. In this study Glucose 6 phosphate dehydrogenase (G6PD) decreased significantly both in hemoglobin and RBC (fig:1 and 2). NADPH production was decreased during the period of hibernation. NADPH helped to produce reduced glutathione (GSH). The tripeptide glutathione (GSH) is among several important naturally occurring antioxidants that detoxify ROS metabolites (Pratihar et al 2010). Decreased GSH caused an increase in cell's oxidative stress. As a result, cells remain under oxidative stress during the period of hibernation.

CONCLUSION

The tripeptide glutathione (GSH) is among the several important naturally occurring antioxidants that detoxify ROS metabolites through its contributation to cellular thiol status. During ROS detoxification GSH is oxidized to glutathione disulfide (GSSG) by glutathione peroxidase and regeneration of reduced GSH occurs at expense of NADPH in the presence of glutathione reductase. G6PD is the starting point of the hexose monophosphate shunt pathway. This metabolite may arise through the action of hexokinase and glucose or from glycogen break down. The reaction is the source of NADPH production. There are inter-relationship between these two very important ways of the metabolism. The pentose phosphate path way, which begins with G6PD production in first step of glycolysis, generates NADPH for use in reductive reaction. NADPH is required for several reductive processes in addition to biosynthesis. From this experiment it was cleared that reduced G6PD activity during hibernation has its physiological and evolutionary role to survive the individuals at that time.

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Figures

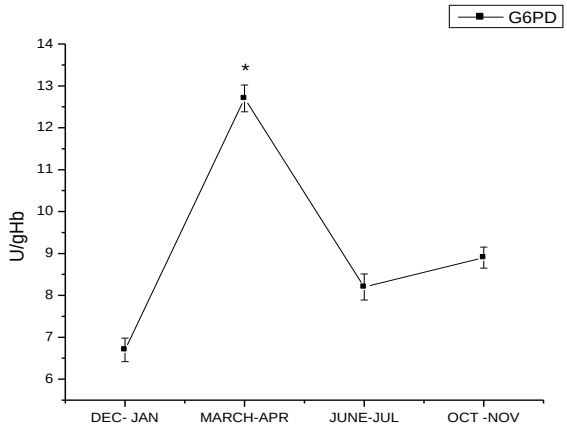

Fig: 1 Variation of G6PD in haemoglobin (* $P < 0.05$).

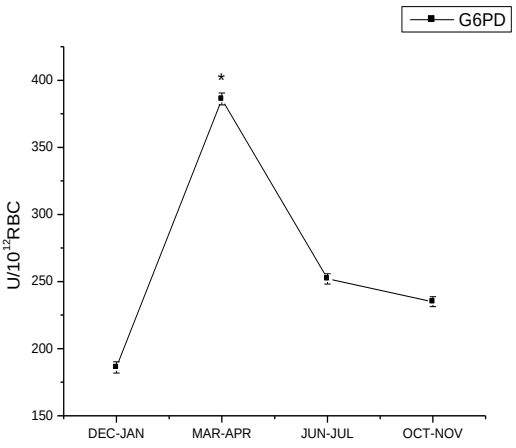

Fig: 2 Variation of G6PD in RBC (* $P < 0.05$).