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Maintenance of osmotic tonicity in *Rana tigrina* and *Bufo melanostictus* during their annual biological cycle

AMJAD FATMI*, D.S. RUBY and MD. AKHTAR

[1]Department of Zoology, Govt. P.G. College Dholpur (India).
[2]Department of Zoology, B.S. College Danapur, Patna (India).
[3]Department of Zoology, B.R.A. University, Muzaffarpur, Patna (India).

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ABSTRACT

Serum electrolyte blood glucose, blood urea and serum cholesterol are estimated in four phases of annual biological cycle of *Rana tigrina* and *Bufo melanostictus.* Electrolytes level was high during aestivation and hibernation but level of Serum cholesterol, blood glucose and blood urea was low, During breeding and post breeding season electrolytes level was low but that of serum cholesterol, blood urea and blood glucose was high.

Key words: Serum electrolyte blood urea, blood glucose, serum cholesterol, *Rana tigrina*; *Bufo melanostlctus,* annual biological cycle,

INTRODUCTION

Rana tigrina and *Bufo melanostictus* are two common Indian amphibians and like other anurans are markedly influenced by changes in abiotic factors like temperature and water. Indian anurans undergo four different physiological states - Hibernation or wintering (Dec-Feb) and aestivation or summer torpidity (Apr-June) are in active phases with low metabolic rates (Flanengan 1993). During these two periods they are completely terrestrial. Breeding season is the period of high sexual activities and is a completely aquatic phase which coincides with monsoon rain (Mukharjee and Deb 1960). Post breeding, season is the period of hyperphagia which is a preparation to under go wintering and then summer torpidity. Amphibians skin is highly permeable to water and it has been known that these animals absorb water (Jorgensen 1994). During terrestrial phase these anurans face high environmental temperature which leads to evaporative cutaneous water loss and during aquatic phase they encounter the passive inflow of water through skin and buccopharynx which may results in flooding of tissues. In bufo an area of skin on the lower abdomen is termed as seat patch or pelvic patch. It constitutes about 10% of the skin area but is responsible for 70-85 percent of the total water uptake by dehydrated toads (McClanahan and Baldwin 1969).

These two amphibians in order to adjust themselves to both aquatic and terrestrial life, undergo a series of physiological adjustments to decrease osmotic gradients during the breeding and post breeding periods, in order to reduce passive inflow of water through the skin. In aestivation and hibernation they increase the osmotic gradient to actively absorb water from the soil.

In the present work serum sodium, potassium, chloride, glucose, urea and cholesterol has been estimated during the four phases in view of exploring the role of these parameters in physiological adjustments in response to different environment condition.

MATERIAL AND METHODS

Normal healthy frog *Rana tigrina* and toad *Bufo melanostictus* were captured from Khagaul near Patna. Each toad weighed an average 150 gm. and a mean measurement of 90 mm. Each frog weighed an averahe 300 gm. and had a mean measurement of 150mm. Collection where made in the month of December and January for the study of hibernating condition. In April and May for aestivating period and in mid of June and whole of July for spawning season. Post spawning sample were obtained during August and September.

Ten each of toads and frogs were taken for investigation during each season. Blood were collected by inserting the syringe directly into ventricle after removal of the pericardium. Sodium and potassium were estimated by EEL flame photometer. Chloride was estimated by the method of Schales and Schales (1941) according to Wooten (1964). The quantitative analysis of serum urea was made by phenol hypochlorite method using Barthelot reaction (Fowcett and scott 1960 ; Channey and Marbach 1962). Blood glucose was analyzed by O-toluidine method of Cooper and McDanile (1970). Serum cholesterol was estimated quantitatively by the method of Zak (1957).

RESULTS AND DISCUSSION

Serum electrolyte (Na+, k+, Cl^-) showed a marked variation during the annual cycle of both Rana and Bufo. They showed high cumulative electrolyte level during the two predominantly terrestrial habitats aestivation and hibernation. However their cumulative electrolyte level was slightly higher in aestivation then hibernation probably due to effect of higher environmental temperature during summer (Ibraimova 1992). The cumulative electrolyte level was low during breeding and post breeding seasons (Table-1, and 2). Amphibians become aquatic during breeding seasons and in such condition they are surrounded by a hypotonic medium. *In vitro* studies with the isolated skin and urinary bladder wall has shown uptake of sodium and water (Harvey *et al.,* 1991, Malvin *et al.,* 1992, Van Der Goot *et al.,* 1992, ward J.M. *et al.,* 2004). Verma (1979) observed that aldosterone secretion is relatively higher during hibernation and aestivation than spawning and post spawning seasons in *Rana tigrina.*

The low aldosterone level during spawning and post spawning season permits a loss of sodium and a simultaneous escape of chloride under the influence of aldosterone significantly reduces

Table 1: *Rana tiqrina*

Season	Glucose Mg./100 ml.	Urea Mg/100 ml	Cholesterol Mg/100 ml	Na M eq/L	K M eq/L	Cl M eq/L
Aestivation	55.2±3.78	70.2+4.49	106.0±4.84	136.0±2.73	3.72±0.06	71.0±5.09
Breeding	51.0±3.53	107.8+ 3.70	133.8±3.03	108.2±3.22	4.9±0.27	50.6±2.40
PostBreeding	70.2±3.72	95.6±5.54	120.2±2.27	118.0±3.16	3.5±0.35	53.8±2.58
Hibernation	40.2±3.19	77.4±3.78	98.6±3.04	122.2±3.96	2.78±0.04	74.6±5.72

Table 2: *Bufo melanostictus*

Season	Glucose Mg./100 ml.	Urea Mg/100 ml	Cholesterol Mg/100 ml	Na M eq/L	K M eq/L	Cl M eq/L
Aestivation	61.4±5.22	119.4±5.19	98.4±3.20	159.42+3.87	3.91+0.19	80.4+4.75
Breeding	64.45+3.32	139.6+5.98	112.8±1.91	122.8+5.58	5.10+0.14	64.8+4.14
PostBreeding	80.2±3.81	131.0+5.59	103.8±3.03	126.6+4.49	3.10+0.7	67.2+3.36
Hibernation	41.0±4.63	120.8+3.83	93.4±3.50	130.2+3.96	2.82+0.15	70.81+2.86

cumulative electrolyte level. A part of it is brought about by increase access to water during aquatic and semi-aquatic condition with high to massive inflow of water and a compensatory poly urea which may leads to ion lossy. When these amphibians are partly or wholly aquatic they surrounded by a hypotonic medium. It will more convenient for them to have a low tonicity of blood to reduce the osmotic gradient between the animal and its surrounding Takeuchi *et al.,* (1994) reported that an increase in chloride increases the volume of marginal cells and a decrease in chloride simultaneously decreases the cell volume. Thus a decrease in cumulative electrolyte load decreases their osmotic tonicity and simultaneously the osmotic gradient to minimize the passive inflow of water during the aquatic phases. Jorgensen *et al.,* (1993) reported that an increase in Na+ and Cl- increases cutaneous water uptake and reduces bladder urine accumulation in Bufo. The increase in cumulative electrolyte level is thus a preparation for a purely sub terrestrial existence during hibernation and aestivation. The high level of aldosterone after post breeding season results in increase retention of sodium and chloride leading to mark increase in electrolyte load. A part of increase in electrolyte load is brought about by non availability of water during terrestrial phases. Several investigations suggest forcible cutaneous water uptake under dehydrated condition in amphibians. Parson (1993) reported that cutaneous drinking is associated with empty urinary bladder. Baker *et al.,* (1993) reported that ADH. Arginine vasotocine stimulate osmotic water inflow. Calamita *et al.,* 1994 reported osmotic gradient water absorption from urinary bladder of xenopus laeves. Word *et al.,* (2005) reported that osmotically absorbed water preferentially enters the cutaneous capillaries of pelvic patch in B. Marinus. Veborg *et al.,* (2004) with their experiment on Bufo bufo reported that when the dehydrated animal is exposed to water, blood cell flux (BCF) in the ventral pelvic patch capillaries increases significantly resulting in rapid absorption of water through that area.

It suggest that the animal can sense the level of tissue hydration through the water potential receptors in skin and in dehydrated anuran water contact triggers increased BCF followed by water uptake.

In both Rana and Bufo cumulative electrolyte level is slightly higher in post breeding season than breeding season but significantly lower than hibernation and aestivation Rana is amphibious during post breeding so a low electrolyte load is an automatic choice but Bufo is predominately terrestrial during post breeding season. There are reports that feeding increases water retention and cutaneous drinking in toads (Jorgensen 1994, Sinsch 1992). So low level of electrolyte in Bufo during post breeding season is probably the effect of feeding.

In both the amphibians cumulative electrolyte level is much higher in aestivation than hibernation. It is probably due to increase in environmental temperature causing evaporative water loss through the skin. Ibraimova (1992) reported that when R temporaria acclimated to 4°C when transported to an aquatic medium at temperature 20°C caused hypernatremia.

Glucose cholesterol and urea are important components of blood. Level of these parameters in blood is determined by physiological activities of the animal. In the present investigation these parameters exhibit a marked variation during the annual biological cycle of both Rana and Bufo.

The pattern of changes of these parameters during annual cycle is almost opposite to that of serum electrolytes.

During breeding and post breeding season when both the animals are active the level of blood glucose, serum cholesterol and blood urea are high. cholesterol is required for reproductive activities particularly for synthesis of sex hormones resulting in its mobilization. So its level in blood rises. Glucose is an immediate source of energy. These animals are relatively active during breeding season so the energy requirement increases. To meet the energy requirement gluconeogenesis and glycogenolysis probably increases resulting in rise of blood glucose level. Increase in gluconeogenesis results in rise of urea level.

Feeding increases urea level (Sinsch 1992). Thus a higher level of blood glucose serum cholesterol and urea is probably the effect of

hyperphagia during the post breeding season. As both Rana and Bufo tends to maintain low osmotic tonicity during breeding and post breeding period, a high level of these parameters is probably compensated by lowering the level of serum electrolytes.

During hibernation and aestivation plasma level of glucose, cholesterol and urea decreases. Both aestivation and hibernation are relatively inactive phases and the energy requirement is low so the surplus blood glucose is removed from the blood to be stored in the form of glycogen. Low urea level is also related to less gluconeogenesis during these phases. The animals do no show any sexual activities so requirement of cholesterol is low resulting in its removal from the blood probably to be stored in the fat bodies.

The low level of glucose cholesterol and urea in serum is compensated by increasing the plasma level of serum electrolytes to maintain high osmotic tonicity during these two sub terrestrial phases when there is scarcity of water.

From the present findings its is apparent that in both Rana tigrina and Bufo melanostictus organic parameters like glucose, cholesterol and urea do not play any significant role in maintaining osmotic tonicity and is largely brought about by the serum electrolytes.

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