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Influence of Hypoxia on the Behavior and Serum Proteins in Grey Quail (*Coturnix coturnix*)

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ABSTRACT

The behavioral changes observed due to induced conditions of hypoxia in experimental Grey Quails (*Coturnix coturnix*) included swelling, slower breathing, and later signs of unconsciousness. The changes observed due to induced conditions of hypoxia in serum protein after electrophoresis showed the absence of certain proteins, whose synthesis were checked when conditions were not favorable. There were indications of proteins whose synthesis were initiated in unfavorable conditions and proteins whose synthesis were enhanced under conditions of stress. Such kind of study has not been documented in aves very strongly and so this work has its importance in this aspect.

Key words: *Coturnix coturnix, electrophoresis, hypoxia, behavior, serum proteins*

Animals within the natural environment are confronted with adverse and challenging conditions that pose as a stress to them and can present a significant threat to their well-being or at worst, survival. The tolerance of stress by a species can influence its distribution and abundance (Sousa, 1984), the rate of population growth and the outcome of interactions with other species (Sagasti, *et. al*., 2001).

Hypoxia, even for brief periods, can be detrimental to humans and most mammals and birds. After hypoxia, four different parameters are reduced in the body: body temperature, heart rate, respiratory rate and blood pH. Hypoxia is a resultant of increasing pollution and decreasing oxygen content as a consequence in the environment.

Many studies on the behavioral, physiological, biochemical and molecular mechanisms of adaptation to life without oxygen have been conducted (Storey and Storey, 2001; Hermes-Lima and Zenteno-Sav´, 2002). Snyder, *et. al.*, 1984 found that bird embryos respond to hypoxia by increasing capillarity in hatchling of Canada geese, *Branta canadensis*. Physiologic consequences of hypoxia have been well documented in birds (Richard and Sykes, 1967) and mammals.

MATERIALS AND METHODS

Experimental Design

The birds were purchased from the local dealers and were checked for their health and activity before experimentation and were kept in an open aviary to acclimatize them before the experiment. Environmental factors like temperature, humidity etc. was dependent on the season. The study divided the birds into two categories, one the control group and other the experimental group (stressed).

The experiment was conducted in triplicate and every experiment had three birds each kept simultaneously in three glass chambers. They were given feed and water *ad. libitum*. The glass boxes were sealed from all sides so as to prevent the entry of any air inside. When the birds started showing signs of unconsciousness (which was a few hours depending upon the condition of the birds) the experiment was terminated. Along with these a set of three birds was kept as a control group in steel cage through out the experimental period. They were given feed and water *ad. libitum.*

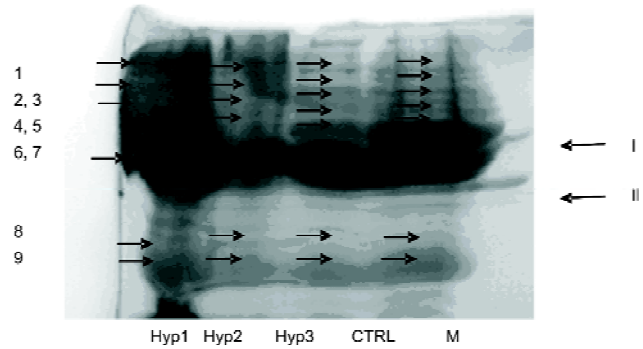
The bird behavior was monitored and after the completion of the experiment blood sample was collected and serum was separated. A lysis buffer was added to the serum to protect it from getting denaturated (25µl sample + 5 µl lysis buffer). The samples of the serum protein were marked and later electrophoresis (Sodium Dodecyl Sulphate-Polyacryamide Gel Electrophoresis, SDS-PAGE) was performed on the samples. The gel surfaces were run with one sample from control bird and three samples from each one of the experiment. After the electrophoresis, gel surfaces were photographed and analyzed for the bandwidth of the serum proteins.

Behavioral Changes Observed during the Experiment in *Coturnix coturnix*

During the initialization of the experiment the birds ran all along the chamber vigorously and fought with one another. After nearly one hour the birds slowly settled down decreasing activity, and the chamber was filled with moisture. Two hours later the birds were observed to be gaping with their beaks open towards the top of the chamber trying to gulp in air. In the third hour the birds were observed to be swelled up to the maximum and lying on their sides or simply flopped down on the base. Their breathing was deliberate and they showed no interest in either feeding or drinking. After another half hour the birds started showing signs of unconsciousness, no movement or activity. The experiment was then terminated for sampling. The birds under analysis showed little fecal matter during exposure to stress. They behaved normally showing active movements in the cage, feeding and drinking water and moved about effortlessly in control. They also showed play behavior. Both the groups were under normal conditions of temperature, humidity, photo cycle as existent.

Gel Surfaces Changes Observed during the Experiment in *Coturnix coturnix*

The gel surface photographs of the birds in the experimental group exposed to hypoxic conditions are presented. The right side of the surface shows roman numbers which denote the known marker (BSA-SHMT) with a limited range used along with the serum samples. The left side of the surface has been numbered according to the major visible bands formed in the control and experimental samples.

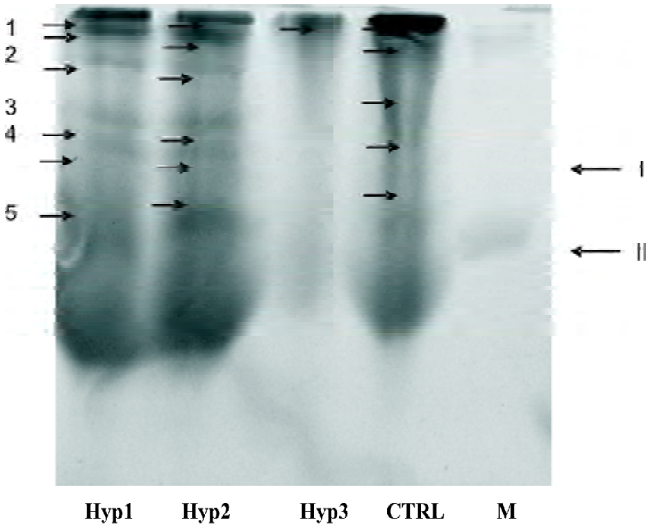


Hypoxia as a Stress Factor in Serum Protein (Exp. I)
(Fig. 1.)

The first band as seen in the control group has corresponding appearance in Hyp1, Hyp2 and Hyp3 (the three experimental samples). The second and the third bands in the control sample have equal correspondence in all the experimental samples. The fourth band in the control sample is thin; in Hyp3 and Hyp2 it is a bit thicker but is not distinct in Hyp1. The fifth band observed in the control sample has similar presence in Hyp3 but is absent in Hyp1 and Hyp2. The sixth band observed in the control sample does not have any correspondence in the experimental samples. The seventh band observed in the control sample is thick and it shows similar appearance in Hyp1 and Hyp3 although in Hyp2 it is a bit thinner than the rest. The eighth band formed in the control sample is a thin band and is found to be corresponding in position in all the experimental samples, except in Hyp1, where it is a little lower placed than the others. The ninth band observed in the control sample also corresponds in all the experimental samples.

Thus in the first experiment conducted on *Coturnix coturnix* the control sample showed two bands whose absence was marked in the experimental samples. Two protein bands were also found to be thicker in the experimental samples as when compared to the control sample.

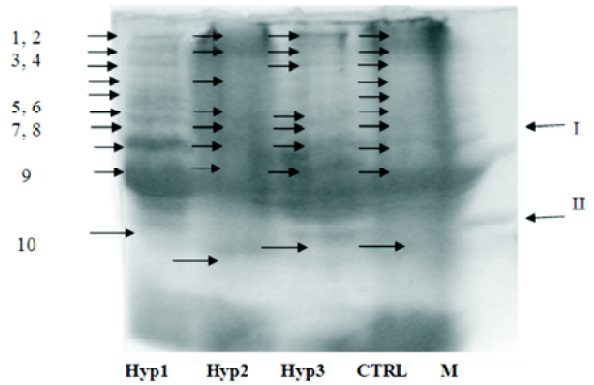
The Hyp3 sample of the experimental group is very feeble in appearance and could not register any band in the sample run so will not be utilized for comparison. The first and second band as seen in the control group has corresponding appearance in Hyp1, Hyp2 and Hyp3. Between the second and third band of the control sample a broad band is observed



Hypoxia as a Stress Factor in Serum Protein (Exp. II)
(Fig. 2.)

in the experimental samples Hyp1 and Hyp2. The third band observed in the control sample cannot be observed in the other experimental samples. The fourth band observed in the control sample shows an equal correspondence in Hyp1 and in Hyp2. Between the fourth and the fifth band in the control sample a thick band is observed in Hyp1 and Hyp2. The fifth band observed in the control sample is a thick band and it also shows similar correspondence in all the experimental samples.

Thus in the second experiment conducted on *Coturnix coturnix* the control sample showed one band whose absence was marked in the experimental samples. The experimental samples also showed two bands whose absence was marked in the control sample.



Hypoxia a Stress Factor in Serum Protein (Exp. III)
(Fig. 3.)

The first band as seen in the control group has corresponding appearance in Hyp1, Hyp2 and Hyp3. The second band observed in the control sample is quite thick and is observed to be equally thick in Hyp2, whereas in Hyp1 and Hyp3 it is thinner as compared to the control and Hyp2.

The third band observed in the control sample is present in all the experimental samples except in Hyp2 where it is absent. The fourth band observed in the control sample is present in all the experimental samples except in Hyp3 where it is not found. The fifth band observed in the control sample is a thin band and is found in Hyp1 but is absent in Hyp2 and Hyp3. The sixth and seventh band observed in the control sample is found corresponding in all the experimental samples. The eighth band observed in the control sample is thinner as compared to Hyp1, Hyp2 and Hyp3. The ninth band observed in the control sample is similar and corresponding in all the experimental samples. The tenth band that can be observed in the control sample has variations in position, and band for Hyp2 is a bit thicker also as compared to them.

Thus in the third experiment conducted on *Coturnix coturnix* the control sample showed a band whose absence was marked in the experimental samples. The experimental samples showed a band whose position was a little lower than the corresponding control sample's band. Two protein bands were also found to be thicker in the experimental samples as when compared to the control sample whereas a protein band was found to be thinner in the experimental samples as when compared to the control sample.

RESULTS AND DISCUSSION

The choice of hypoxia as a factor for the study was based on the observations which the prevailing environmental conditions create and also due to the consideration that it is a result of increasing pollution and decreasing oxygen content in the environment of cities due to urbanization (Jesko, *et. al.*, 2006). Hypoxia allows animals to adapt themselves in such conditions to assure their endurance and longevity (Hopkins and Powell, 2001).

Tolerance to hypoxia varies among taxonomic groups and habitats. Hypoxia depresses feeding in some species (Sobral and Widdows, 1997) while increasing it in others (Breitburg, *et. al.*, 1994; Sagasti, *et. al.*, 2001).

Much has been elucidated on the biochemical and physiological adaptation mechanisms that make these animals endure oxygen deprivation (Fritsche and Nilsson, 1993; Jackson, 2000; Hochachka and Lutz, 2001). Cáced *et. al.*, 2001 described energetic metabolism in mouse cerebral cortex during chronic hypoxic conditions. Chiral, *et. al.*, 2004 studied production of stress proteins in the Piglet brain at birth under conditions of hypoxia. Adaptation to hypoxia in birds helps them adjust to life in high altitudes and is in a way selective and helps in survival (Faraci, 1991).

The behavioral changes in birds led them to slowly settle down decreasing any activity, for lack of fresh air. They became inactive with lack of interest in feeding and drinking. Four bands on the whole in *Coturnix coturnix* were seen to be present in the control samples but could not to be traced in the experimental samples indicating towards proteins whose synthesis are checked when conditions are not favorable. Similarly two bands traced in the experimental samples were not observed in the control sample indicating towards protein whose synthesis were initiated when conditions were not favorable. Four bands are found to be thicker in the experimental samples as when compared to the control sample indicating towards proteins whose synthesis were enhanced under conditions of stress. One band was found to be thinner in the experimental samples as when compared to the control sample which indicated towards a protein whose synthesis was checked under conditions of stress. One band in the experimental sample showed a change in position with the corresponding control sample indicating towards a change in molecular weight of the protein concerned due to stress.

Thus the experimental samples show more pronounced effect of hypoxic conditions on these birds as compared to control group indicating the serious implications of stressful conditions on these animals.

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