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Electrophoretic study of haemoglobin components in Indian fruit bat *Rousettus leschenaulti* (Desmerest)

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Abstract-

Haemoglobin studies can be oriented as a tool in taxonomy, examining the immunological capacity of bats, or evaluating protein as an energy source. Electrophoretic examination of bat hemoglobin has at least a limited taxonomic value, however the greatest value of these examinations may be in phylogenetic analysis. Such studies are important contribution towards solving the problem of the origin of the bats and their relation to primates, and to find out the hemoglobinopathies ,if any, therefore are valuable tools in the study of mammals hence the present study has been undertaken. For haemoglobin electrophoretic study blood was analysed from 36 male Indian fruit bat *Rousettus leschenaulti* and human blood from a case sickle cell trait having A&S (adult and sickle Hb) component which was taken as control for electrophoresis in the run. The control AS blood from known sickle cell trait revealed A&S pattern –away from site of inoculation in alkaline PH. However, the blood of *Rousettus leschenaulti* showed only one electrophoretic band with very low mobility from the site of inoculation as is observed in agarose gel stripe. In conclusion the hemoglobin studies in *Rousettus leschenaulti* would be a significant contribution for finding out the abnormality in the haemoglobin.

Keywords: Hemoglobin Electrophoresis, Blood, Bat.

Introduction

Haemoglobin Electrophoresis is done for the identification and diagnosis of abnormal haemoglobins.It allows presumptive identification of haemoglobin phenotype i.e. one can differentiate HbS trait (AS pattern) from sickle cell disease (SS pattern).

Hemoglobin S and hemoglobin C are the most common types of abnormal hemoglobins that may be found by an electrophoresis test. Electrophoresis uses an electrical current to separate normal and abnormal types of hemoglobin in the blood. Hemoglobin types have different electrical charges and move at different speeds. The amount of each hemoglobin type in the current is measured. An abnormal amount of normal hemoglobin or an abnormal type of hemoglobin in the blood may mean that a disease is present. Abnormal hemoglobin types may be present without any other symptoms, may cause mild diseases that do not have

symptoms, or cause diseases that can be life-threatening. For example, hemoglobin S is found in sickle cell anemia, which is a serious abnormality of the blood and cause serious problems.

Likewise the electrophoretic **studies of Haemoglobin is** only restricted to human in various hemoglobinopathies (particularly sickle cell anaemia, percentage of reticulocytes; splenomegaly, osmotic fragility, red-cell morphology, hemolytic anemia, (Singer et al., 1995) cyanosis, polycythemia, hydrops fetalis (Schmidt and Holland, 1974) and a few notes are available on the Vespertilionid bats (Arevalo et al., 1987) and in other mammals (Hoff et al., 1976a; Asadi et al., 2007).

There is however, a relative paucity of information in the literature on the assessment of various parameters including the **haemoglobin studies,** since baseline data on physiological characteristics are essential in the management of wild animals and for finding out the abnormalities. It is also hoped that the information will serve a useful purpose as a guide for clinical diagnostic and research applications until comprehensive studies are available for other bats.

Materials & Methods:

Distribution-

Rousettus leschenaulti has a widespread distribution extending from Sri Lanka and Pakistan to Myanmar, Vietnam, Southern China, Java and Bali. In the Indian subcontinent, almost all states show localities of *Rousettus leschenaulti*. In Maharashtra *Rousettus leschenaulti* are distributed in Ghatmala; Chikalda; Elephanta; Jogeshwari; Kanheri; Khandala; Alibag; Mahabaleshwar; Aurangabad; Ratnagiri (Brosset, 1962); Marathwada; Satara ; Pune; Mansar; Kandri; Ellora (Gopalakrishna and Madhavan, 1970). This old world Indian fruit bat, *Rousettus leschenaulti* (Desmerest) is selected for the present study because of its easy availability in the vicinity of Nagpur city.

Collection of Animals-

Table – 1 Pertinent data regarding collection of *Rousettus leschenaulti*. The number in paranthesis is for number of animals used for the present study.

Specimen (n = 3)	Date of collection	Time of collection	Body weight(g)	Size of Testis (cm)	Length of body (cm)	Length of fore arm (cm)	Wing span (cm)	Reproductive status
Male	8/1/07	10.10am	111.33	2.47	14.0	12.1	22.5	Active male
Male	6/2/07	10.45am	113.33	2.60	14.5	12.9	22.6	Active male
Male	9/3/07	10.10am	128.00	2.90	15.5	13.5	24.7	Male active spermatogenesis + Leydig cells active, mating period (Peak-2)
Male	8/4/07	10.45am	100.00	2.17	14.0	12.0	23.9	Active male

Male	8/5/07	11.00am	98.00	2.03	14.0	12.1	23.6	Male inactive (Quiescence)
Male	9/6/07	10.10am	95.00	1.96	13.5	11.9	22.9	Quiescent male
Male	9/7/07	10.45am	93.00	1.83	13.0	12.0	22.5	Quiescent male
Male	6/8/07	10.35am	70.67	1.20	12.9	11.6	22.2	Male inactive (Quiescence)
Male	4/9/07	11.30am	71.00	1.03	13.3	12.1	21.3	Recrudescent male
Male	6/10/07	12.00noon	100.67	2.13	13.9	12.6	23.3	Active male
Male	9/11/07	11.30 am	121.67	3.07	14.9	13.2	24.2	Active male showing complete spermatogenesis, mating period (peak-1)
Male	11/12/07	11.30 am	99.00	2.33	13.5	11.3	21.9	Active male

The specimens of *Rousettus leschenaulti* were collected with the help of mist net placed in the underground mines of Mansar / Kandri Near Nagpur, Maharashtra (20°92"N 78°95"E). Time of collection, body mass, wing span, length of forearm and other salient features of each specimen were maintained in the field diary (Table –1). Bat was transferred to an individual comfortable cage. These traps were transported to the RTM Nagpur University Laboratory. Minimum noise, human exposure and handling were employed to minimize capture stress and excitement. For each sampling, three bats were used.

Blood Sampling

The bats were held in hands and no anaesthesia was used at the time of sample collection. 2 ml of blood was collected into sterile tube, for haemoglobin electrophoresis EDTA was used. After blood sampling each bat was released.

Haemoglobin Electrophoresis at Alkaline p^H

Two ml. of venous blood was collected for haemoglobin electrophoretic studies using EDTA as anticoagulant. Haemolysate, agar plates were prepared and the sample was run in the alkaline medium (Upadhyay et al., 2007).

Observation & Results-

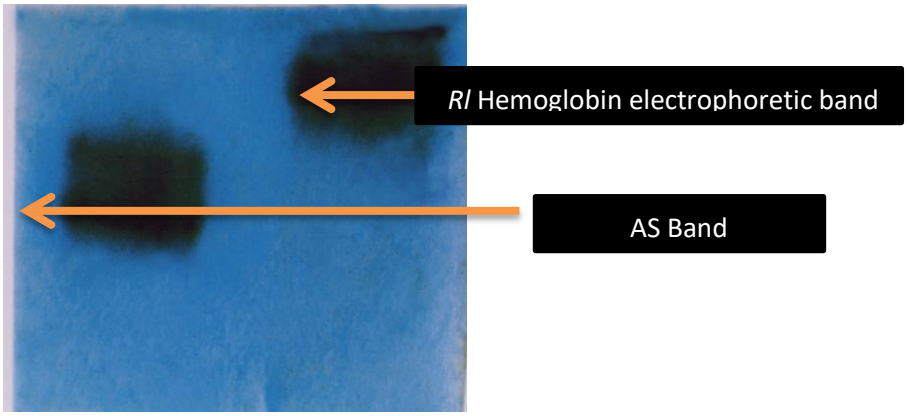

Fig.1- Electrophoretogram of haemoglobin from the bat *Rousettus leschenaulti* and human sickle cell trait.

For the present study as the control bat from other families of *Megachiroptera* were not utilized, the human blood from a case sickle cell trait having A and S (Adult and Sickle Hb) component was taken as control for electrophoresis in the run. The control AS blood from known sickle cell trait revealed A and S pattern – away from site of inoculation in Alkaline p^H. However, the blood of *R. l.* showed only one electrophoretic band with very low mobility from the site of inoculation as is observed in Agarose gel strip depicted by Agarose gel electrophoretogram of hemoglobin of *R. leschenaulti*. Thus, the upper two bands were of sickle cell trait showing AS band while lower band of *R.l.* deposited a single band with very low mobility hence very near to the site of inoculation as compared to AS band of sickle cell trait (fig.1).

Discussion-

Hemoglobin studies can be oriented as a tool in taxonomy, examining the immunological capacity of bats, or evaluating protein as an energy source. Electrophoretic examination of bat haemoglobins has at least a limited taxonomic value as molossid bats can be distinguished from other families (Valdivieso et al., 1969), however, the greatest value of these examinations may be in phylogenetic analysis (Foreman, 1964; Manwell and Kerst, 1966). Such studies are important contribution towards solving the problem of the origin of bats and their relation to primates, therefore are valuable tools in the study of mammals and of course for the order Chiroptera, similarly for phylogenetically correlating the sub order mega and microchiroptera hence the present study has been undertaken, however, with limited facilities we were not able to study a detailed account as described by previous workers.

A perusal of literature on the studies of bat haemoglobin (Kleinschmidt et al., 1986; Singer et al., 1995), rodents (Hoff et al., 1976), bovines (Scaloni et al., 1998) and human (Bulgir, 2005) have revealed that our studies on the bat haemoglobin are not comparable for the want of defining the percentage of haemoglobin I and II as well as various components for the want of facilities in Nagpur city, still our results are in agreement with them in defining the abnormalities in the haemoglobin and hence are important clue for localizing the

haemoglobinopathies in the wild population of bats- *Rousettus leschenaulti*, which would be of great significance in the conservation of wild population.

Some other electrophoretic studies on rodents, showed that, Squirrel Hgb migrated slightly ahead of human Hgb A, toward the anodal end when electrophoretic pattern of squirrel Hgb performed on cellulose acetate. Such a pattern has been shown by Hoff et al., (1976); Asadi et al., (2007) in the gray squirrel of America and Persian squirrels. Hoff et al., (1976) speculated that the faster mobility could be because of higher charges carried by squirrel Hgb at PH 8.4 or because squimel Hgb (Asadi et al., 2007) is a smaller molecule than human Hgb. Other differences observed in squirrel Hgb are the absence of the carbonic anhydrase band and Hgb A_2[4]. Six different hemoglobins have been demonstrated in a species of vole Pitymye duodecimcostatus (Perez - Suarez et al., 1985) and it was found to be of no significant difference with the rat (*rattus norvegicus*).

For haemoglobin electrophoretic study as the control bat from other families of Megachiroptera were not utilized, the human blood from a case sickle cell trait having A and S (Adult and Sickle Hb) component was taken as control for electrophoresis in the run. The control AS blood from known sickle cell trait revealed A and S pattern – away from site of inoculation in Alkaline PH. However, the blood of *R.l.* showed only one electrophoretic band with very low mobility from the site of inoculation as is observed in Agarose gel strip depicted by Agarose gel electrophoretogram of haemoglobin of *R. leschenaulti*. Thus, the upper two bands were of sickle cell trait showing AS band while lower band of *R.l.* depicted a single band with very low mobility hence very near to the site of inoculation as compared to AS band of sickle cell trait. The earlier studies on bats (Arevalo et al., 1987; Singer et al., 1995) and in other mammals (Hoff et al., 1976; Asadi et al., 2007) are comparable to our results.

Conclusion-

There is however, a relative paucity of information in the literature on the assessment of various parameters including the **haemoglobin studies,** since baseline data on physiological characteristics are essential in the management of wild animals and for finding out the abnormalities. It is also hoped that the information will serve a useful purpose as a guide for clinical diagnostic and research applications until comprehensive studies are available for other bats. **The haemoglobin studies in *R. leschenaulti* would a significant contribution well as for finding out the abnormality in the haemoglobin.**

References-

Arevalo F, Parez - Suarez G, Lopez - Luna P (1987) Haematological data and haemoglobin components in bats (Vespertilionidae). Comp. Biochem. Physiol. (A) **88**(3) : 447 – 450.

Asadi F, Rostami A, Asadian P, Pourkabiz M (2007) Serum biochemistry and hematology values and hemoglobin electrophoresis in persian squirrels (*Sciurus anomaius*). Vet. Clin. Path. **36**(2): 188-191.

Balgir RS (2005) The spectrum of hemoglobin variants in two scheduled tribes of Sundergarh district in north-western Orissa, India. Ann. Hum. Biol. **32** : 560-573.

Foreman CW (1964) Tryptic peptide patterns of some mammalian hemo-globins. J. Cell. Comp. Physiol. **63** : 1-6.

Gopalakrishna A and Madhavan A (1970) Sex ratio in some Indian Bats. J. Bom. Nat. Hist. **67** : 171 – 175.

Hoff GL, Lassing EB, Chan MS, Bigler WMJ, Doyle TJ (1976a) Hematologic values for free ranging urban gray squirrels (*Sciurus C. carolinensis*). Am. J. Vet. Res. **37** : 99-101.

Hoff GL, McEldowny LE, Bigler WMJ, Kuhns LJ, Toms JA (1976) Blood and urinary values in the grey squirrel. J Wildl. Dis. **12** : 349-352.

Kleinschmidt T, Koop BF, Braunitzer G (1986) The primary structure of a mouse eared bat (*Myotis velifer, chiroptera*) hemoglobin. Biol. Chem. Hoppe. Seyler. **367** : 1243-1249.

Manwell C, Kerst KV (1966) Possibilities of biochemical taxonomy of bats using heamoglobin, lactate dehydrogenase, esterases and other proteins. Comp. Biochem. Physiol. **17** : 741 – 754.

Perez-Suarez G, Arevalo F, Lopez-Luna P (1985) Hemoglobin components & plasma proteins in *Pitymys duodecimcostatus*. Comp. Biochem. Physiol. B. **80** : 145 - 147.

Scaloni A, Pieragostini E, Malorni A, Ferrara L, Di Luccia A (1998) Bovine hemoglobin alpha-globin chain polymorphism : primary structure determination of two new genetic variants by mass spectrometry and amino acid sequencing. Biochem. **80** : 333 - 338.

Schmidt RM, Holland S (1974) Standardization in abnormal haemoglobin detection. An evaluation of hemoglobin electrophorosis kits. Clin. Chem. **20**: 501-504.

Singer GA, Kleinschmidt J, Braunitzer G (1995) The primary structure of the hemoglobin from the lobe-lipped bat (*Chalinolobus morio* , Microchiroptera) : Biol. Chem. Hoppe Seyler. **376**(10) : 603 - 609.

Upadhyay A, Upadhyay K, Nath N (2007) **Principles and Techniques Biophysical Chemistry.** Himalay Publishing house Mumbai, India. **4**: 422-497.

Valdivieso D, Tamsitt JR, Conde-Del Pino E (1969) Electrophoretic properties of neotropical bat hemoglobin. Comp. Biochem. Physiol. **30** : 117-122.