

# ELECTROLYTES, PROTEINS AND OSMOLALITY IN THE AMNIOTIC FLUID OF MICROCHIROPTERAN BAT *HIPPOSIDEROS SPEORIS* (SCHNEIDER)

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## ABSTRACT

The precise origin of the liquor amnii still remains unsolved. It is probably of mixed maternal and foetal origin. In the present paper osmolality or the measures of total concentration of all dissolved solutes was studied. The biochemical estimation of electrolytes, albumin, globulin and A/G ratio were estimated except variations in chloride values not much difference was observed in all estimated values. The purpose of this study is to present some observations on certain chemical constituents of amniotic fluid at term. The mean values for electrolytes *i.e.*  $\text{Na}^+$  -  $140.63 \pm 0.49$  meq/L,  $\text{K}^+$  -  $3.83 \pm 0.12$  meq/L,  $\text{Cl}^-$  -  $70.67 \pm 0.37$  meq/L. In proteins the concentration of albumin exceeds that of globulin. Albumin-  $4.10 \pm 0.06$  meq/L and globulin-  $1.87 \pm 0.20$  meq/L. The A/G ratio was  $1.67 \pm 0.09$  meq/L and osmolality was  $223.80 \pm 0.17$  mOsm. Despite all available information concerning the composition, origin and fate of liquor amnii our knowledge with regard to this fluid remains incomplete. The constitutions of the amniotic fluid are the principal source of information on foetal maturity. Due to differentiation of tissue and ensuing secretory functions no single value or combination of values provide absolute evidence of foetal maturation.

## INTRODUCTION

Chiropteran represents second largest order of mammals in number of species and the largest in overall abundance, excluding man and possibly rodents. They are also the most widely distributed land based mammals. The basic idea and purpose of this study is to present some observation on certain chemical constituents of amniotic fluid at term. The order chiropteran includes over 180 extant species incorporated in 17 families, (Simmons, 2005). Amniotic fluid has been referred to as a "private aquarium" for embryos of air breathing forms, in which recapitulation of the water existence of ancestral forms occurs. In humans, amniotic sac is formed by 11<sup>th</sup> and 12<sup>th</sup> day of conception and by the 11<sup>th</sup> week of gestation and contains about 50mL of colourless fluid. At mid pregnancy there are approximately 400mL of amniotic fluid and at near term the normal value is 1000mL (Albuquerque *et al.*, 2004). To this fluid has been attributed the protection of embryo, not only against mechanical injury but also from adhesion and consequent malformation. (Wirtschafter, 1957) the amniotic fluid could be regarded as ultrafiltrate of material plasma and contents corresponding with those of interstitial fluid elsewhere (Gillibrand, 1969a). The amniotic fluid may be formed, partially or entirely, by the amnion, amniotic skin, fetal kidney, fetal lung, buccal cavity and nasal cavity (Queenan, 1978; Hammer *et al.*, 1997). The mechanism of formation of amniotic fluid has not been clearly elucidated, in spite of extensive and elaborate research. It is generally accepted that the amniotic fluid is specifically circulated as are the other fluid compartments of the body. Amniotic fluid

steadily increases until 30-34 weeks gestation. Its composition also changes with a stable pattern throughout pregnancy (Wirtschafter, 1957). The total protein in the amniotic fluid of rats' increases rapidly at near term and a very large increase in albumin fraction accounted for most of the total protein increase. A large increase in albumin may correspond to increase viscosity. The precise relationship that volume bears to viscosity is not clear. The rapid increase in albumin at near term might agree with the concomitant increase in viscosity of rat amniotic fluid at near term (Marsh *et al.*, 1964). Osmolality is a measure of total concentration of all dissolved solutes. Early in gestation amniotic fluid osmolality is 280mOsm/kg, similar to blood osmolality and fetal blood osmolality. The measure solutes such as sodium and chlorides are present in amniotic fluid in essentially the same concentration as in maternal and fetal plasma. Beginning at the end of embryonic period, amniotic fluid osmolality begins a progressively decrease, which continues until term, when amniotic osmolality averages 265mOsm/kg. This fall in osmolality is paralleled by decrease in amniotic sodium and chloride concentration, whereas maternal and fetal plasma osmolality and electrolyte concentrations are unchanged (Gillibrand, 1969b). Such an investigation may help to elucidate the nature of the functional activity of the epithelium and the composition of the fluid in correlation of foetal health maturation of the foetus and of course its resemblance with other mammalian species. Therefore these pioneer studies on the amniotic fluid of bat would be important because of their unique volitional mode of life and with regard to the postnatal development of the foetus.

## MATERIALS AND METHODS

### Breeding habits

*Hipposideros speoris* bat breeds once in a year. Ovulation and copulation in females occur during mid-December. The gestation period lasts for 135 days. Parturition takes place from last week of April to first week of May. Lactation extends upto June (Gopalkrishna, 1991).

### Collection of animals

The specimens of *Hipposideros speoris* were collected from abandoned mines in Khapa, Nagpur with the help of mist net.

### Collection of amniotic fluid

After anaesthetizing the animals with ether, abdomen walls were cut opened by a mid-incision. The gravid uteri of full term pregnant bats were slit opened without damaging the amnion. Amniotic fluid was drawn with 2mL sterile syringe and was collected into Eppendorf's tube for biochemical estimations. The tubes were kept in deep freezer at -20°C until estimations to be performed.

### Method of estimation of sodium, potassium, chloride, total proteins and osmolality

The sodium and the proteins are precipitated simultaneously by means of a reagent magnesium Uranyl acetate containing alcohol. The precipitate is separated by centrifugation, then measured absorbance of BST against distilled water on photocolormetry at 540nm within 10 minutes. Potassium was measured by the use of ion selective electrode. The method is based on the measurement of the turbidity of the reaction mixture containing Tetraphenyl Boron, alkaline EDTA, formaldehyde and sample containing potassium or standard potassium salt. The method accurate within the concentration of 2.0 to 7.0mm/L. This is also in good agreement with flame photometry. Chloride was estimated by colorimeter method with the principle that chloride ions of the specimen react with a solution of mercuric thiocyanate and ferric nitrate to form undissociated mercuric chloride and allow the formation of red brown ferric thiocyanate complex which is quantitatively proportional to concentration of chloride in specimen. Total protein (Albumin, Globulin and A/G ratio) was estimated by Biuret and BCG Dye binding method and the osmolality or the measures of total concentration of all dissolved solutes on an advanced osmometer (Henry, 1986).

## RESULTS

The various values recorded for different amniotic fluid constituents for *Hipposiderous speoris* at term are depicted in the tabulated form after calculating the mean values and analyzing it statistically (Table 1).

### Electrolytes

The mean values for the electrolytes in *H. speoris* ( $\text{Na}^+$  -  $140.63 \pm 0.49$ ,  $141.77 \pm 0.20$ meq/L,  $\text{K}^+$  -  $3.83 \pm 0.12$  to  $4.50 \pm 0.12$ meq /L,  $\text{Cl}^-$  -  $70.67 \pm 0.37$  to  $74.80 \pm 0.17$ )meq /L was recorded.

The above results indicate that the values for  $\text{Na}^+$  and  $\text{K}^+$  are in the same range but there are remarkably wide variations in the

concentration of chloride.

### Proteins

The total protein of the amniotic fluid is composed of albumin and globulin fractions. The concentrations of albumin exceed that of the globulin. In *H. Speoris* it was in the range of  $6.10 \pm 0.06$  to  $6.67 \pm 0.09$  g/100mL; albumin -  $4.10 \pm 0.06$  to  $4.60 \pm 0.15$  g/100mL; globulin -  $1.87 \pm 0.20$  to  $2.40 \pm 0.10$  g/100mL and their ratio  $1.67 \pm 0.09$  to  $2.50 \pm 0.35$  g/100mL.

### Osmolality

osmolality or the measure of the total concentration of all dissolved solutes were studied and the mean value range at term in *H. Speoris* it was  $223.80 \pm 0.17$  to  $229.57 \pm 0.35$ mOsm.

## DISCUSSION

In the present work, the osmolality in *H. speoris* were found to be in the range of  $223.80 \pm 0.17$  to  $229.57 \pm 0.35$ mOsm/kg. Several researchers have suggested that osmotic equilibrium during early pregnancy falls with advancing age. In human the range is between 223 to 280mOsm/Kg (Albuquerque et al., 2004, Gillibrand, 1969b, Lind et al., 1972), whereas (Daniel et al., 2004) recorded 274-298mOsm/Kg in sheep, whereas (Daniel and Morishma, 1968) found 304-320mOsm/Kg in guinea pig. Even though our results are in accord with the results of previous workers at term, we have not correlated our osmolality range with maternal serum. The significant and interrelationship of the electrolytes and osmolality changes confirms the view that the amniotic fluid could be regarded as an ultrafiltrate of maternal plasma (Gillibrand, 1969b). The relationship between the falls in these two factors was not constant or direct. This reflects the findings in a number of paired results of a considerable osmolality deficit with little or no sodium deficit indicating the involvement of other unmeasured osmotically active constituents (Wallenberg, 1977; Lind, 1972). Since our studies are mainly at term pregnancy the fall in  $\text{Na}^+$  and  $\text{K}^+$  concentrations cannot be interpreted but our values for  $\text{Na}^+$  and  $\text{K}^+$  at term pregnancy resemble largely the range recorded by earlier authors worked on human and other species (Gillibrand, 1969). For the justification of fall in amniotic fluid sodium concentration and osmolality with the advancement in pregnancy has long been attributed to the foetus passing hypotonic urine of low sodium concentration into the amniotic sac (Heron, 1966). Significantly higher level of total proteins as well as albumin and globulin in the maternal plasma than in amniotic fluid is confirmed by earlier findings by other authors (Sutcliffe and Brock, 1973; Sutcliffe, 1975; Ozegbe, 2005) who concludes that foetal circulation is the main source of amniotic fluid proteins. Some authors state that most of the proteins in the amniotic fluid are of maternal origin (Palliez et al., 1954; Ozegbe, 2005). The antenatal diagnosis of at least some genetic disorders associated with changes in the urinary compositions, by the biochemical analysis of amniotic fluid, therefore seems a distinct possibility (Ozegbe, 2005). The correlation between the falling sodium and chloride concentration in the amniotic fluid as pregnancy advanced did not suggest that the reduction in the concentration of these two ions as entirely independent. Although the osmolality of the amniotic fluid was largely dependent on the sodium concentration, the relationship between fall in these

**Table1: Amniotic fluid chemistry values of *H. speoris***

Date of collection	Sodium (meq/L)	Potassium (meq/L)	Chloride (meq/L)	Proteins (g/100mL)	Albumin (g/100mL)	Globulin (g/100mL)	A/G Ratio (g/100mL)	Osmolality (mOsm)
11.3.2004	141.00±0.12	4.13±0.15	70.67±0.37	6.50±0.06	4.10±0.06	2.40±0.10	1.67±0.09	228.43±0.66
14.3.2004	141.07±0.44	4.13±0.15	71.27±0.35	6.10±0.06	4.13±0.09	1.97±0.15	2.10±0.21	223.80±0.17
22.3.2004	140.63±0.49	4.00±0.12	72.13±0.18	6.43±0.20	4.50±0.12	1.93±0.09	2.27±0.03	225.80±0.26
24.3.2005	141.30±0.29	4.10±0.15	72.80±0.17	6.33±0.09	4.60±0.15	1.87±0.20	2.50±0.35	225.57±0.33
8.4.2005	141.40±0.44	4.33±0.09	71.07±0.35	6.40±0.17	4.10±0.06	2.23±0.24	1.83±0.23	226.73±0.20
10.4.2005	141.30±0.27	4.47±0.09	72.77±0.38	6.53±0.19	4.50±0.15	2.03±0.12	2.20±0.15	227.77±0.38
15.4.2005	141.60±0.23	3.83±0.12	74.20±0.17	6.37±0.20	4.40±0.06	1.97±0.18	2.23±0.18	228.13±0.33
12.3.2006	140.70±0.26	4.50±0.12	71.57±0.39	6.47±0.20	4.43±0.15	2.07±0.09	2.13±0.07	226.03±0.23
15.3.2006	141.77±0.20	4.13±0.09	73.77±1.04	6.67±0.09	4.43±0.18	2.23±0.09	1.97±0.15	229.57±0.35
p value	p < 0.1	p < 0.1	p < 0.1	p < 0.1	p < 0.4	p < 0.3	p < 0.1	p < 0.1

two factors was not constant or direct. Since our study is mainly at the term pregnancy the fall in sodium and potassium concentration cannot be interpreted but the values of sodium and potassium at term pregnancy resembles largely by the range recorded by authors earlier in human species (Gillibrand, 1969). The constitutions of the amniotic fluid are the principle sources of information on fetal maturity due to differentiation of tissue and ensuing secretory functions. It is inferred that presence of proteins in the amniotic fluid reflects the ageing of the foetus coming either from fetus or fetal serum (Wirtschafter, 1957). Moreover such studies would enrich the phylogenetic relationship between other suborders of chiroptera.

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