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HISTOCHEMICAL OBSERVATIONS OF CARBOHYDRATE IN THE KIDNEYS OF POSTNATAL SUCKLINGS OF INDIAN FALSE VAMPIRE *MEGADERMA LYRA LYRA* (GEOFFROY) AND INDIAN LEAF NOSED BAT *HIPPOSIDEROS SPEORIS* (SCHNIDER), CHIROPTERA; MAMMALIA.

KISHOR GOPAL PATIL

E-mail address: drkgpatil@gmail.com

Address: 54, Old Jagruti Colony,

Katol Road, Nagpur 4400013,

(M.S.) India.

KISHOR S. JANBANDHU

Department of Zoology,

Government Institute of Science,

R T Road Civil Lines Nagpur, (M.S.)

India 440001.

ABSTRACT

The glycogen activity is observed histochemically by Periodic Acid Schiff's (PAS) technique in the kidneys of postnatal sucklings of two species of animalivorous bats; *Megaderma lyra lyra* (carnivorous) and *Hipposederos speoris* (insectivorous). The proximal uriniferous tubules, Bowman's capsules and

macula densa in outer cortex of the kidneys examined shows high glycogen activity. The PAS reaction decreases in the thin loop of Henle, distal tubules, collecting tubules and collecting ducts of thick medulla which decreases further in the region of the large papilla. The luminal border, nuclei and the basement membrane of the epithelial cells lining the uriniferous tubules show more activity as compared to the cytoplasm. The carbohydrate activity in the kidneys of postnatal sucklings is related to the functional phyosiology as well the process of early post birth development in *Megaderma lyra lyra* and *Hipposederos speoris*.

Keywords: Bat; Carbohydrate; Histochemistry; Kidney; Postnatal; Uriniferous tubule

INTRODUCTION

PATIL & JANBANDHU (2011) examined the protein and glycogen activity histochemically in the prenatal, postnatal kidneys of frugivorous Indian bats, *Rousettus leschenaulti*. They observed high protein and glycogen activity is observed in the cortical region of the kidneys, which decreases in the medulla and further decreases in the region of the papilla. Increased protein and glycogen activity is observed in the Bowman's capsules, juxta-glomerular apparatus and proximal tubules as compare to the thin loop of Henle, distal tubules collecting tubules and collecting ducts. The activity is more in the nuclei and the basement membrane as compared to the cytoplasm of cells lining the uriniferous tubules. The kidneys in mammals play an important role in maintaining volume and the concentration of body fluids. In the carnivorous animals the morphological adaptations in the renal bodies is also influenced by the diversity in habitats and food they consume. The kidneys with relatively thick medullae which are subdivided into outer and inner zones representing the long uriniferous tubule are adapted for conservation of water in the animals with high protein rich diet (PATIL*et al*., 2010). The functional histology is the area of interest, especially when employed or combined with ultrastructural cell biology, cytochemistry or hstochemical visualization (ROUSENBAUM, 1970). In the hibernating bats, *Vespertilio* and *Pipistrellus* [=*vesperugo*] the proximal tubules showed increased accumulation of bacillus-like granules beneath the nucleus during October and November which disappeared by April at the time of arousal. During hibernation nuclei of proximal epithelia appeared paler than during arousal. The

epithelium of the thin loop of Henle is able to accumulate lipids during hibernation (BARONCINI & BERETTA, 1900). YOSHIMURA (1951) reported the accumulation of granules and secretory droplets in the collecting tubules in hibernating *Rhinolophus.* DAVIDHEISER (1966) reported that the enzyme involved in prophyrin metabolism and cytochrome oxydase activity in different organs including kidney of *Myotis lucifugus.* Rousenbaum (1968) described high enzymatic activity throughout hibernation in the kidney of *Myotis lucifugus*. Presence of several dehydrogenases (succinic, lactic, hydroxybutyric) was reported by him on unfixed tissues. PATANKAR & PATIL (1983) examined mucopolysaccharide histochemistry of glomeruli in nine species of bats with different feeding habits: *Cynopterus spinx* and *Rousettus leschenaulti* (frugivorous), *Megaderma lyra lyra* (carnivorous) and *Pipistrellus ceylonicus,Rhinolophus rouxi*, *Miniopterus schreibersii*, *Hipposideros fulvus*, *Scotophilus temmincki* and *Tadarida aegyptiaca* (insectivorous). The sialomucins were observed in the glomeruli of the frugivorous and carnivorous bats while the glomeruli of the insectivorous bats had both the sialomucins and sulphomucins. Semi hibernating insectivorous bats showed the presence of only neutral mucopolysaccharide in the basal border of glomeruli and Bowman's capsule, while in other species neutral mucopolysaccharides and sialomucins were observed. In the present report the activity of glycogen observed histochemically in the kidneys of postnatal sucklings of two species of Indian bats having animalivorous feeding habits viz.; *Megaderma lyra lyra* (carnivorous) and *Hipposederos speoris* (insectivorous).

MATERIALS AND METHODS

The postnatal sucklings of *Megaderma lyra lyra* attached to lactating females were collected from underground dilapidated mines around Nagpur. While the postnatals of *Hipposederos speoris* were collected from underground dilapidated dark rooms of an old fort at Ballarshah, Maharashtra, India. The colonies comprised of hundreds of bats. The bats were collected with the help of a butterfly net; the postnatal sucklings were separated from mothers and clinically anaesthetized. The sucklings attached to lactating females were selected. The body weight and sex of animals were recorded; the kidneys from the selected embryos were removed, measured, weighted and then fixed in different fixatives: Alcoholic Bouin's, Aqueous Bouin's, 10% Formalin for 24 hours for histochemical demonstration of carbohydrates (mucopolysaccharides). The kidneys were washed overnight in running tap water and dehydrated by passing through different grades

of ethyl alcohol, cleared in xylene and embedded in paraffin (58-60^{o}C). The mid sagittal sections of kidneys were cut at 5-7µm with the help of rotary microtome. Tissues fixed in Alcoholic Bouin's fluid and 10% formalin were used to demonstrate the presence of mucopolysaccharides by Periodic Acid Schiff's technique (McMANUS 1946 and HOTCHKISS 1948) and observed under light microscope.

RESULTS

The present study is based on the examination of kidneys of female postnatal suckling of *Megaderma lyra lyra* with body weight 21gm. (the right and left kidney weight was 0.08gm. and 0.073gm. respectively). The kidneys of female postnatal of *Hipposederos speoris* with a body mass 6.5gm. (and the weight of right and left kidneys was 0.011gm. and 0.010gm.).

The kidney of a postnatal suckling of *Hipposederos speoris* exhibits high glycogen concentration in the glomerular cells of the Bowman's capsules, cells of the juxta-glomerular apparatus and the membranes of cells lining the proximal convoluted tubules of the outer cortex. The cytoplasm of epithelial cells lining the proximal convoluted tubules show decreased glycogen concentration than their membranes. Accumulation of glycogen is not observed in the apical region of cells of the distal convoluted tubules towards the lumen but the nuclei of cells and the basement membrane exhibit weak PAS reaction. The uriniferous tubules in the medullary region show moderate glycogen activity in the nuclei of cells and their basement membranes, while very weak activity is observed in the apices of the cells. In the papilla the large collecting ducts and the collecting tubules show very weak glycogen reaction. The thick connective tissue and the interstitial cells inbetween the tubules exhibit an increased accumulation of glycogen.

The glycogen activity in the postnatal kidney of *M. lyra lyra* is similar to that of *H. speoris*; but in *M. lyra lyra* the brush border of the proximal convoluted tubules and the basement membrane of the cells of the uriniferous tubules in the medullary region exhibit increased glycogen concentration as compared to *H. speoris*.

DISCUSSION

The carbohydrate (glycogen) activity was observed histochemically in the kidneys of the postnatal sucklings of two species of Indian bats *Megaderma lyra lyra* (carnivorous) and *Hipposederos speoris* (insectivorous). Carbohydrates act as source of nutrients and energy for the metabolic reactions.

According to ROUSENBAUM *et al.*, (1965) in the kidneys of *Myotis lucifugus* the Golgi elements were not involved in any of the relations. The areas of condensed enzymatic activity were associated with a vacuolar system, originating from beneath the microvilli of the brush border by means of extending channels, which terminate in vacuoles of varying size which are located in the supranuclear zone of the proximal tubular epithelium. The postnatal kidneys of *Megaderma lyra lyra* and *Hipposederos speoris* also shows an instanced reaction of PAS in the microvillar brush border of the proximal convoluted tubules; indicating the increased absorptive function in this part of uriniferous tubules. McFARLAND & WIMSATT (1965 and 1969) corelated the effect of ecological habitat and diet on the kidney functions of bats, *Desmodus rotundus* and *Pizonyx vivesi* in captivety. They observed a variable rate of urine flow is related to increase in extracellular space, an adaptation for water conservation to reduce evaporative water losses during flight. *P. vivesi* depended on saline water and maintained the body weight only when frozen shrimps soaked in saline was given, which was indicated by special adaptation in the structure of kidney. No such extratubular spaces were observed in the kidneys of postnatal sucklings of both*Megaderma lyra lyra* and *Hipposederos speoris*. But the high PAS reaction in the membranes of epithelial cells lining the tubules suggest the more re-absorption of water; thus helping in the conservation of water which is an adaptation of the kidneys of the animals studied here, which correlates the animalivorous feeding habits of these bats. PATANKAR & PATIL (1983) observed the carbohydrate histochemistry in the kidneys of nine species of bats. They noticed the presence of sialomucins in the glomeruli of the frugivorous and carnivorous bats while the glomeruli of the insectivorous bats had both the sialomucins and sulphomucins. In semi hibernating insectivorous bats only neutral mucopolysaccharide in the basal border of glomeruli and Bowman's capsule were present, while in other species neutral mucopolysaccharides and sialomucins were observed. In the postnatal kidneys of both *Megaderma lyra lyra* and *Hipposederos speoris*, the high activity of glycogen in the Bowmans capsules, macula densa, nuclei and the basement membranes of cells indicating that the mucopolysaccharides are involved in the development as well as in the of process of excretion and water re-absorption in these part of uriniferous tubules. The very low concentration of glycogen in the cytoplasm of

epithelial cells lining the developing uriniferous tubules indicates decreased metabolic activity as compare to nuclei and the basement membranes.

As observed in the cortex of the kidney of a posnatal suckling of Indian fruit bat *Rousettus leschenaulti* (PATIL & JANBANDHU, 2011) the postnatal kidneys if animalivorous bats studied here also show high glycogen concentration in the glomerular cells of the Bowman's capsules, cells of the juxta-glomerular apparatus and the membranes of cells lining the proximal convoluted tubules. The cytoplasm of cells of the proximal convoluted tubules show decreased glycogen concentration than their membranes. Accumulation of glycogen is not observed in the apical region of cells of the distal convoluted tubules towards the lumen but the nuclei of cells and the basement membrance exhibit weak PAS reaction. The uriniferous tubules in the medullary region show moderate glycogen activity in the nuclei of cells and their basement membranes, while very weak activity is observed in the apices of the cells. In the papilla the large collecting ducts and the collecting tubules show very weak glycogen reaction. The thick connective tissue and the interstitial cells lying in-between the tubules exhibit an increased accumulation of glycogen. But the activity of carbohydrate was intense in the postnatal kidneys of *Rousettus leschenaulti* than the postnatal sucklings of two species of bats; *Megaderma lyra lyra* (carnivorous) and*Hipposederos speoris* (insectivorous). Which may be due to the diverse feeding habits of lactating females; as the former feed on mainly fruits, rich in carbohydrate as compare to both the animalivorous bats examined in this report.

The high glycogen activity in the nuclei and the basement membrane of cells lining the uriniferious tubules suggest that the carbohydrates are utilized for the development and maturation of the nephron as functional unit and also the surrounding tissue leading to self establishment of independent excretion. In the postnatal kidneys of both *Megaderma lyra lyra* and *Hipposederos speoris* the high glycogen concentration in the microvillar brush border of the proximal tubules indicate its absorptive nature for water glucose, minerals etc. The proximal convoluted tubules are one of the most important parts of uriniferious tubules which facilitated the active absorption of minerals. Thus presence of glycogen in membranes of these cells can be attributed for its role as energy substrate because active absorption takes place at the expense of energy. The brush border of the proximal convoluted tubules and the basement membrane of the cells of the uriniferous tubules in the medullary region exhibit increased glycogen concentration in the postnatal kidneys of *M. lyra lyra* as compared to *H. speoris*. Which indicating the kidneys of carnivorous *M. lyra lyra* are more adapted to conserve water than the insectivorous *H. speoris*. The proximal tubule is an

important part of uriniferious tubules because where the filtrate volume gets reduced to almost eighty percent in mammals; the condition is also established in the kidneys of postnatal sucklings of both*Megaderma lyra lyra* and *Hipposederos speoris* studied in this report as occurrence of high PAS reaction.

EXPLANATIONS OF FIGURES

Figure 1. In the cortex of postnatal kidney of *M. lyra lyra* the Bowman's capsules and proximal convoluted tubules show intense to high activity for glycogen. The distal convoluted tubules exhibit weak PAS reaction.

Figure 2. The medulla region of postnatal kidney of *M. lyra lyra* showing intense to high PAS positive reaction in the basement membrane of cells of different types of uriniferous tubules. The cell cytoplasm and luminal surface of the tubules show weak glycogen activity. Intense to high glycogen activity in the basement membrane of cells of the collecting ducts and collecting tubules. The cells linning the collecting ducts and the collecting tubules exhibit moderate to weak PAS reaction.

Figure 3. Cortex of the postnatal kidney of *H. speoris* showing high glycogen accumulation in the glomeruli, basement membranes and luminal surfaces of cells the proximal convoluted tubules and the basement membrane of the distal convoluted tubules. Cytoplasm of cells linning the uriniferious tubules and the luminal surfaces of the distal convoluted tubules exhibit moderate to weak PAS reaction.

Figure 4. Medulla of the postnatal kidney of *H. speoris* showing high glycogen accumulation in the basement membrane of cells linning the uriniferous tubules. The cytoplasm of cell of the uriniferous tubules, and the luminal surfaces exhibit weak PAS staining. The basement membrane of cells linning the collecting tubules and the collecting ducts show moderate to high activity of glycogen. The cytoplasm of cells of the tubules show weak PAS reaction and the luminal surface show no reaction for glycogen activity.

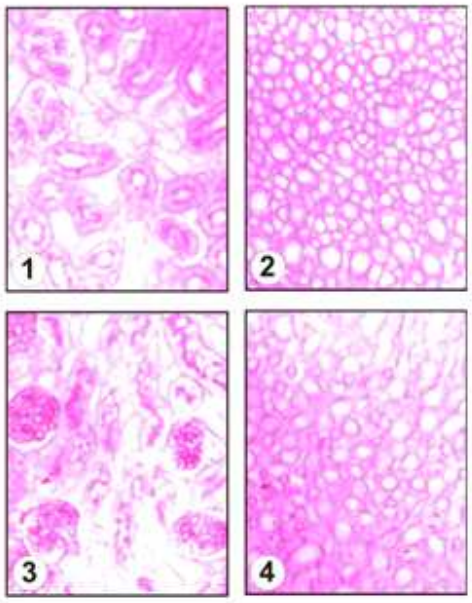

Figure.1

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