http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 8 (7): 1055-1059, 2005
ISSN 1028-8880

Histochemical Characterization of Dehydrogenases in the Nephron of the One-humped Camel (*Camelus dromedarius*)

Bashir M. Jarrar
Department of Clinical Laboratory Sciences, College of Science-Al-Jouf,
P.O. Box 2014, King Saud University, Skaka-Al-Jouf, Saudi Arabia

Abstract: A histochemical investigation on the distribution and localization of nine dehydrogenases in the nephron of the one-humped camel, *Camelus dromdarius* was undertaken. The histoenzymological tests employed in this study detected glucose-6-phosphate, α-glycerophosphate, succinate, lactate, malate, glutamate, isocitrate, NADH and NADPH dehydrogenases. The functional significance of the findings is discussed in accordance with the adaptation of the species under study to its harsh desert habitat.

Key words: Renal tubule, glomerulus, macula densa , dromedary, hypertonic urine

INTRODUCTION

The general features of the one-humped camel kidney are not different from those of other mammals[1], but show some unique modifications to pertain it's remarkable drinking capacity and the production of hypertonic urine under extreme conditions of water deprivation. The kidney of *C. dromedarius* has thicker renal capsule, higher number of glomeruli and wider medulla than for other studied mammals[2-4]. These modifications help in tolerating the abrupt blood dilution to which the camel is subjected during phases of water deprivation and sudden rehydration without disruption of the normal osmotic and electrolyte function[3-5].

The histological and ultrastructural nephron structure of the dromedary camel has already been investigated[3,4]. The microanatomy and the ultrastructure of the one-humped camel nephron are modified to cope with the need to conserve water under extreme variations in temperature and water availability.

A survey of the literature revealed that little, if any, has been published on the renal histoenzymology of the dromedaries. The present histochemical study deals with the localization and distribution of nine dehydrogenases in the nephron of the one-humped camel, *Camelus dromedarius* is an attempt to throw some light on the role of these enzymes on the renal physiology of this mammal.

MATERIALS AND METHODS

Portions of fresh kidney (cortex and medulla) from twelve adult healthy one-humped camels were cut out rapidly at the slaughter house of Riyadh during January-May, 2003. These portions were frozen in liquid nitrogen and stored in air tight tubes at -80°C until use. Cryostat sections (8-10 μm thick) were cut at -25°C.

Dehydrogenases were histochemically demonstrated in the present study according to tetrazolium method[6,7]. The activities of the soluble NAD-dependent dehydrogenases were demonstrated by means of the optimized polyvinyl alcohol technique according to Rieder *et al.*[7] method. Phenazine methosulphate, an intermediate electron acceptor was added to the incubating media of the soluble dehydrogenases in the rate of 1 mg mL^{-1} of the medium[8,9].

Fresh unfixed cryostat sections were treated with cold acetone (±0°C for 5 min) in order to remove lipids[10,11] and were utilized for the histochemical characterization of renal dehydrogenases according to the following methods: Lojda *et al.*[12] and Frederiks *et al.*[13] methods for succinate dehydrogenase, Frederiks *et al.* method[13] for α-glycerophosphate dehydrogenase, Bancroft and Stevens[14] and Frederik *et al.*[15] methods for lactate dehydrogenase, Van Noorden method[17] for glucose-6-phosphate dehydrogenase, Lojda *et al.*[12] and Van Noorden and Frederiks[17] methods for malate dehydrogenase, Van Noorden and Frederiks method[17] for isocitrate dehydrogenase, Bancroft and Stevens method[15] for glutamate dehydrogenase, Rieder *et al.*[7] method as modified by Bancroft and Stevens[14] for NADH and NADPH dehydrogenases. The control in each case consisted of parallel sections incubation in media lacking the substrate of the specified enzyme while control sections for both NADH and NADPH dehydrogenases were incubated in the presence of dicumarol, a selective inhibitor for diaphorases.

Corresponding Author: Fax: 00966 4 46255534 Tel: 00966 503229854

RESULTS

Glucose-6-phosphate dehydrogenase: Considerable activity was detected in the proximal convoluted tubules especially in the outer cortical portion while the highest activity of this enzyme was seen in the macula densa and the pars recta (Fig. 1A). The activity was faint in the distal tubules and the pars convoluta of the proximal convoluted tubules but absent in the juxtaglomerular cells. A faint reaction for this enzyme was also detected in the mesangial cells.

α-Glycerophosphate dehydrogenase: No reaction was demonstrated in the thin descending of Henle's loop and the collecting ducts of the papillary inner medulla (Table 1). The activity was weak in the glomeruli, distal convoluted tubules and juxtaglomerular cells and was moderately demonstrated in the intercalated dark cells of cortical collecting tubules and the outer zone of the medulla as well as in the medullary interstitial cells. A strong reaction of this enzyme was demonstrated in the pars recta, ascending loop of Henle and macula densa (Fig. 1B).

Succinate dehydrogenase: No activity was observed in the glomeruli and the inner stripe of the medulla while strong activity of this enzyme was demonstrated in the proximal convoluted tubules and the macula densa. A faint activity was detected in the distal tubules, the juxtaglomerular cells and the ascending thick loop of Henle (Fig. 1C).

Lactate dehydrogenase: Weak reaction appeared in the glomeruli and juxtaglomerular cells while considerable activity was detected in the epithelial lining of the pars convoluta of distal convoluted tubules and in the intercalated dark cells of the cortical collecting tubules. The highest reaction of lactate dehydrogenase was demonstrated in the macula densa and pars recta of the renal tubule (Fig. 1D). The activity of this enzyme was also seen in the thick ascending limb of Henle's loop in the outer stripe of the medulla.

Malate dehydrogenase: Weak activity was seen in the macula densa and juxtaglomerular cells while no activity was observed in the glomeruli (Table 1). The activity of this enzyme was strongly demonstrated in the pars recta and to lesser extent in the distal convoluted tubules and ascending loop of Henle (Fig. 1E).

Glutamate dehydrogenase: A moderate reaction was detected in the intercalated dark cells lining the collecting tubules and to a lesser extent in the intraglomerular mesangial cells while the reaction was faint in the interstitial medullary cells. A strong activity of this enzyme was observed in the epithelial lining of the pars recta and the ascending thick segment of the loop of Henle (Fig. 1F).

Isocitrate dehydrogenase: A considerable activity was seen in the beginning of the pars convoluta and to a lesser extent in posterior and the cortical straight portions of the proximal convoluted tubule. Strong reaction of this enzyme was demonstrated in the epithelial lining of the distal convoluted tubules and to a lesser extent in the macula densa (Fig. 1G).

NADH dehydrogenase: Almost no activity was demonstrated in the juxtaglomerular cells, thin loop of Henle and collecting tubules. A moderate activity was observed in the straight portions of the renal tubules. The prominent activity of this enzyme was noticed in the distal convoluted tubules and to a lesser extent in the macula densa (Fig. 1H).

NADPH dehydrogenase: As shown in Table 1, weak reaction was noticed in the juxtaglomerular cells and the posterior portion of the pars recta (Table 1). The highest

Table 1: Dehydrogenases histoenzymological activity in the nephrons of adult healthy one-humped camels, *Camelus dromedarius*

Dehydrogenase	*G	MD	JG	PCT	DCT	DLH	ALH	CT
Glucose-6-phosphate DH	** -	++	-	±+	±	?	-	-
α-Glycerophosphate DH	±	++	±	++	±	-	++	+
Succinate DH	-	++	±	++	±	-	±	-
Lactate DH	±	++	±	+	+	-	+	+
Malate DH	-	±	±	++	+	-	+	+
Glutamate DH	-	?	±	++	-	-	++	+
Isocitrate DH	?	+	-	+	++	±	?	-
NADH DH	±	+	-	±	++	-	+	-
NADPH DH	±	+	±	++	+	?	-	-

-, Negative; ±, Weak; +, Moderate; ++, Intensely positive; ?, Doubtable.
ALH, Ascending Loop of Hele; CT, Collecting Tubule; DCT, Distal Convoluted Tubule; DH, Dehydrogenase; DLH, Descending Loop of Henle; G, Glomerulus; JG, Juxtaglomerular cells; MD, Macula Densa; PCT, Proximal Convoluted Tubule

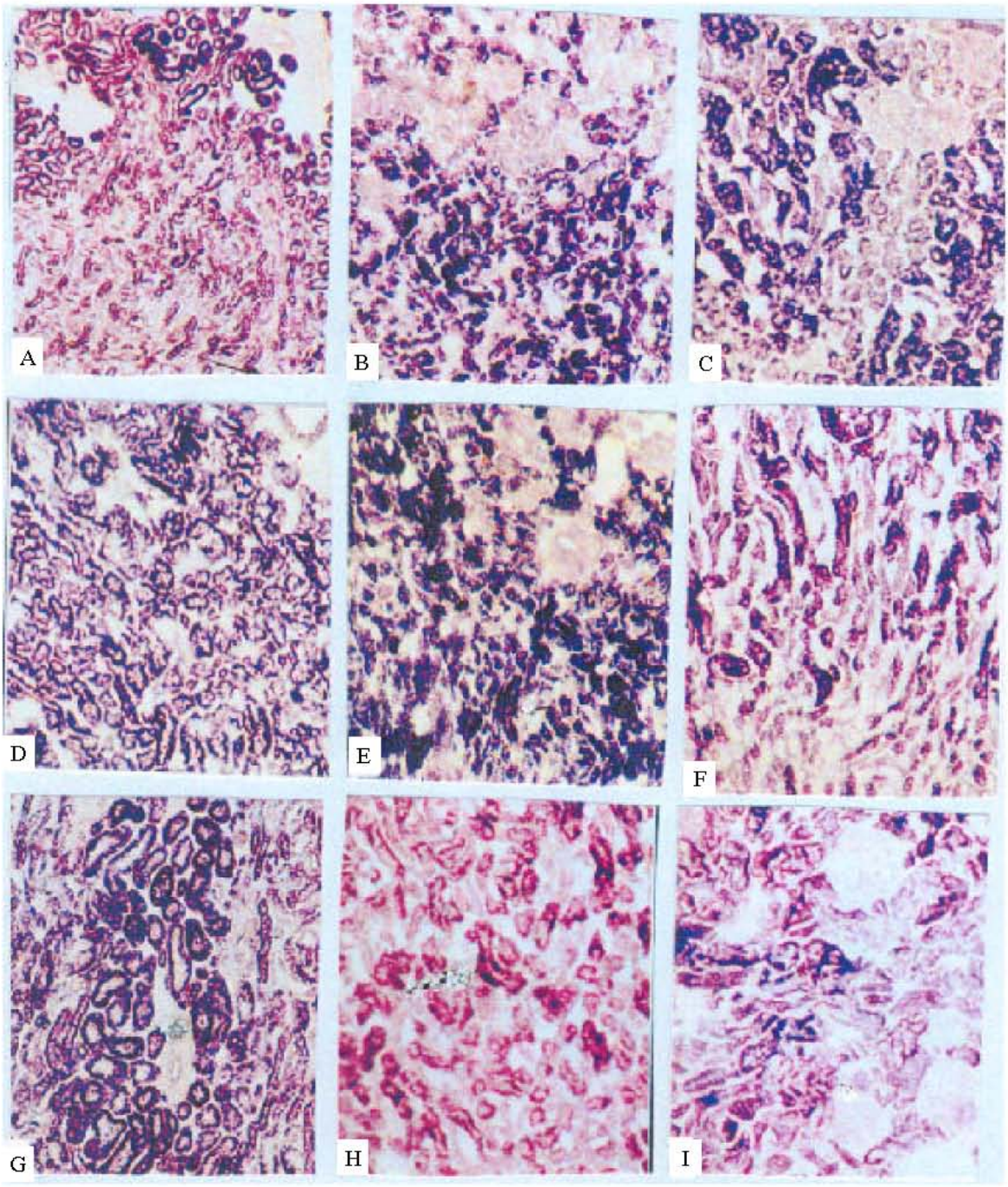

Fig. 1: Light micrographs in the nephrons of adult healthy one-humped camels (*Camelus dromedarius*) showing the activity of: A: Glucose-6-phosphate dehydrogenase; B: α-Glycerophosphate dehydrogenase; C: Succinate dehydrogenase; D: Lactate dehydrogenase; E: Malate dehydrogenase; F: Glutamate dehydrogenase; G: Isocitrate dehydrogenase; H: NADH dehydrogenase; I: NADPH dehydrogenase

activity of NADPH dehydrogenase was seen in the beginning of pars convoluta and to a lesser extent in the macula densa, distal convoluted tubules and the cortical portion of pars recta (Fig. 1I).

DISCUSSION

The results of the present investigation show high activity of dehydrogenases in the macula densa, the proximal convoluted tubule and the ascending loop of Henle. This finding copes with the fact that the epithelial lining of these portions of the dromedary nephron contain numerous mitochondria[4]. This might also explain the higher activity of dehydrogenases in the intercalated dark cells than the principal light ones of the collecting tubules where the former cells contain more mitochondria than the later ones.

The macula densa is the osmoreceptor of the nephron while the proximal convoluted tubules reabsorb most of the water and ions of the glomerular filtrate[18]. The ascending loop of Henle constitutes a countercurrent mechanism generating a high osmotic pressure in the extracellular fluid of the renal medulla which helps in reabsorption further fluid from the glomerular filtrate and producing hypertonic urine with respect to plasma[19]. Accordingly, the high activity of dehydrogenases in these portions of the dromedary nephron with their high content of mitochondria might indicate a role for this enzymatic group in the production of hypertonic urine.

The results of the present investigation indicate less activity of dehydrogenases in the distal tubules in comparison with the proximal ones while the glomeruli and the juxtaglomerular cells demonstrate weak activity. This finding is in agreement with the fact that the distal tubules reabsorb small quantities of the filtrate while the glomeruli and the juxtaglomerular cells play a minor role in reducing the glomerular filtrate [19].

Glucose-6-phosphate dehydrogenase generates NADPH under the oxidative stress conditions such as heat and water deprivation[20]. The high activity of this enzyme in the macula densa as seen in the present study might indicate a role for it in monitoring the nephron filtrate rate as an adaptive tool to enable the camel withstand severe dehydration, loosing up to 30% of body weight without fetal results.

The present study show high activity of succinate dehydrogenase and isocitrate dehydrogenase in the cells of the macula densa and cortical portion of the renal tubule. These two enzymes are bounded to the inner mitochondrial membrane and participates in the aerobic oxidation of carbohydrates in the citric acid cycle[22].This might suggest a role for carbohydrate metabolism in monitoring filtrate rate of the dromedary nephron.

Lactate dehydrogenase plays an important role in the intermediary metabolism as a link between amino acid metabolism and the citric acid cycle where it converts lactate into pyruvate[22]. The results of the present study show high activity of this enzyme in the macula densa the osmoreceptor of the nephron. This may indicate a role for this enzyme in monitoring the nephron filtrate rate.

High activity of α-glycerophosphate dehydrogenase is demonstrated in most portions of the dromedary renal tubule. This might suggest that this enzyme play a role in NAD-NADH shuttle between the cytoplasm and mitochondria where water is produced by fat oxidation enabling the camel to gain more moisture from metabolizing the fat.

Camels use acetate much more than glucose as a substrate for endogenous fatty acid synthesis[23]. Malate dehydrogenase converts malate to oxaloacetate, the final reaction in the Krebs cycle[22]. This might explain the high activity of this enzyme in the proximal convoluted tubules where most of the water and ions of the glomerular filtrate are reabsorbed.

NADH and NADPH dehydrogenases play an essential role in the intracellular oxidation[22]. The high activities of these enzymes in the cortical portion of the renal tubule as shown in the present study might indicate their involvement in reabsorbing most of the water and ions of the glomerular filtrate.

In conclusion, the distribution patterns, sites of activities and intensity of dehydrogenases in the dromedary nephron as are demonstrated in the present study might reflect contribution of this enzymatic group in the maximum utilization of water and production of hypertonic urine.

ACKNOWLEDGMENTS

I am grateful to the Department of Zoology and Clinical Laboratory Sciences-Al-Jouf, King Saud University for putting their research laboratories under my disposal.

REFERENCES

1. Jamison, R.R.L. and W. Kriz, 1982. Urinary Concentrating Mechanism. Structure and Function. 1st Edn., Oxford University Press, Oxford, pp: 85-87
2. Abdalla, M.A., 1973. Anatomical study of the urinary system of the camel, *Camelus dromedarius*. M.Sc. Thesis, Khartoum University, Sudan.
3. Abdalla, M.A. and O. Abdalla, 1979. Morphometric observations on the kidney of the camel, *Camelus dromedarius*. J. Anat., 129: 45-50.

4. Safer, A.M., N.K El-Sayed, K. Abo-Salem and R. Al-Shaer, 1988. Ultrastructure of the nephron of the one-humped camel, *Camelus dromedarius*. J. Morphol., 198: 287-301.
5. Yagil, R. and G.M. Berlyne, 1980. Glomerular filteration rate and urine concentration in the dromedary camel, *Camelus dromedarius* in dehydration. Renal Physiol., 1: 104-112.
6. Stoward, P.J. and C.F. Van Noorden (Eds.), 1991. Histochemical Methods for Dehydrogenases. In: Histochemistry, Theoritical and Applied. Vol. 3, (4th Edn.), Churchill Livingstone, Edinburgh.
7. Rieder, H., H.F. Teutsch and D. Sasse, 1978. NAD-dependent dehydrogenase in rat liver parenchyma. I: Methodological studies on the qualitative histochemistry of G6PDH, 6PGDH, malic enzyme and ICDH. Histochemistry, 56: 283-98.
8. Pearse, A.G.E., 1991. Histochemistry: Theoretical and Applied. 4th Edn., Churchill Livingstone, London.
9. Straastburg, I.H., F.D. Graaf, C.F. Van Noorden and W. Raamsdonk, 1989. Enzyme reaction rate studies in electromotor neurons of the weakly electric fish *Apteronotus leptorhynchus*. Histochem. J., 21: 609-17.
10. Jacobsen, N.O., 1969. The histochemical localization of lactic dehydrogenase isoenzyme in the rat nephron by means of an improved polyvinyl alcohol method. Histochemie, 20: 250-265.
11. Mellgren, S.I., 1971. The distribution of lactate dehydrogenase in the hippocampal region of the rat. An investigation with the polyvinyl alcohol method. Zentralblatt fur Zellforsch, 12: 187-203.
12. Lojda, Z., R. Gossrau and T.H. Schiebler, 1979. Enzyme Histochemistry. A Laboratory Manual. Springer-Verlag, Berlin.
13. Frederiks, W.M., F. Marx and G.L. Mayagkaya, 1986. A histochemical study of changes in mitochondrial enzyme activities of rat liver after ischemia *in vitro*. Virchows Arch. Biochem., pp: 321-329.
14. Bancroft, J.D. and A. Stevens, 1986. Theory and Practice of Histological Techniques. 2nd Edn., Churchill Livingstone, New York.
15. Frederiks, W.M., G.L. Mayagkaya, G.M. Fronik, A.A. Van Veen, I.M. Vogels and J. James, 1983. The value of enzyme leakage for the prediction of necrosis in liver ischemia. Histochemistry, 78: 472.
16. Van Noorden, C.F., 1984. Histochemistry and cytochemistry of glucose-6-phosphate dehydrogenase. Prog. Histochem. Cytochem., 15: 1-85
17. Van Noorden, C.F. and W.M. Frederiks, 1992. Enzyme Histochemistry. A Laboratory Manual of Current Methods. 1st Edn., Oxford University Press, Oxford, pp: 116-117.
18. Doughty, S.E., R.K. Ferrier, K.J. Hillan and D.G. Hackson, 1995. The effects of Zeneca, an angiotention II antagonist, on Renin expression by juxtaglomerular cells in the rat: Comparison of protein and mRNA expression as detected by immunohistochemistry and *in situ* hybridization. Toxicologic Pathol., 23: 256-261.
19. Burkitt, H.G., B. Young and J.W. Health, 2000. Wheatear's Functional Histology: A Text and Colour Atlas. 4th Edn., Churchill Livingstone, London.
20. Al-Ali, A.K., H. Al-Husayni, A. Al-Mutairy, R. Saba and D.M. Power, 1989. A study of the biochemical characteristics of NADP+ isocitrate dehydrogenase from the liver and kidney of the Arabian camel (*Camelus dromedarius*). Comparative Biochem. Physiol., B, 92: 517-521.
21. Kouider, S. and E. Kolb, 1982. Glucose level and various enzyme activities (aspartate aminotransferase, alanine aminotransferase, acid and alkaline phosphatases, glucose-6-phosphate dehydrogenase) in camel serum during the day. Arch. Exp. Vet. Med., 36: 601-610.
22. Al-Rehaimi, A.A., A.K. Al-Ali, A.R. Mutairy and A.S. Dissanayake, 1989. A comparative study of enzyme profile of camel (*Camelus dromedarius*) hump and sheep (*Ovis aries*) tail tissues. Comparative Biochem. Physiol. B, 93: 857-858.
23. Emmanual, B., 19981. Fatty acid synthesis in camel (*Camelus dromedarius*) hump and sheep (*Ovis aries*) tail fat. Comparative Biochem. Physiol. B, 68: 551-554.