Journal of Cytology & Histology
ISSN: 2157-7099

Vidya and Naik, J Cytol Histol 2012, 3:3
DOI: 10.4172/2157-7099.1000143

Research Article Open Access

The Endocrine Pancreas of the Lizard *Calotes versicolor*: An Immunocytochemical and Physiological Study with Respect to its Reproductive Cycle

Vidya. R. Chandavar[1,2] and Prakash. R. Naik[1]*

[1]*Endocrinology laboratory, Department of Studies in Zoology, University of Mysore, Manasagangorti, Mysore 570006, India*
[2]*Molecular endocrinology laboratory,Yuvaraja's College, University of Mysore, Mysore- 570 005, India*

Abstract

The present investigation was undertaken to record the distribution and number of insulin immunoreactive (IR) and glucagon-IR cells in the pancreas and to find out their effect on plasma glucose level in *Calotes versicolor* during different stages of reproductive cycle. It is distinguished as preparatory, reproductive and recrudescent period. Plasma glucose was estimated by enzyme glucose oxidase method. There was variation in pancreatic endocrine cells and plasma glucose with respect to annual seasonal cycle of reproduction. Insulin-IR and glucagon-IR cells never appeared in the form of islets. Cell count for both IR cells of reproductive period was higher and differed significantly from preparatory and recrudescent periods. Plasma glucose recorded the highest value in preparatory period and differed significantly from other two periods. Insulin-IR cells always out numbered glucagon-IR cells. Morphological differences between two cell types were observed under electron microsopy and also pancreas exhibited the presence of nerves. Glycogen localization in liver was carried out by PAS method. Glucose is always utilized to accomplish energy demand rather than converting it as liver glycogen. Results are discussed with those of agamid and other lizards.

Keywords: Glucose; Glucagon-IR; Insulin-IR; Lizard; Season

Introduction

Reptiles were first to make transition from aquatic to terrestrial life and from them the birds and mammals evolved; inspite very little is known about their islets compared to fish and amphibians [1]. *Calotes versicolor* is an agamid lizard, a member of the family agamidae of order squamata, is widely distributed in Asia. They are commonly found in bushes and gardens feeding on insects. *C. versicolor* exhibits sexual dimorphism. Males have well separated spines in the mid dorsal line running from base of the head to root of the tail. Both males and females have light brown or grayish dorsal body color with transverse spots on back and sides [2]. They are seasonal breeders; males get a bright red throat in the breeding season. Histological and immunohistochemical investigation of endocrine pancreas of the grass lizard, *Mabuya quinquetaeniata*, and that of the desert lizard, *Uromastyx aegyptia* was reported [3]. Rhoten and Hall [4] examined the differentiation of islets of Langerhans in the lizard *Anolis carolinensis.* The endocrine pancreas of the lizard, *Podarcis hispanica,* consists of single scattered cells or small groups of two to five cells forming islet-like structures [5]. The endocrine pancreas of *Podarcis s. sicula* is concentrated more in the splenic than in duodenal region and never formed large clusters [6]. The islets in the pancreas consisted of central core of B (beta)-cells and A (alpha)-cells at the periphery with the predominance of earlier cells in 11 species of lacertids studied [7]. Morphology of the pancreas [8] and immunocytochemical study [9] of gekkonidae and lacertids were reported. The regional distribution and frequency of the pancreatic endocrine cells in the splenic lobe of the grass lizard, *Takydromus wolteri,* was studied by immunohistochemical method [10].

Regulation of blood glucose is a part of the energy management process and is a key homeostatic activity. Many of the above studies are confined to localize different cell types and their distribution in the pancreas. The present investigation was undertaken to record the distribution and number of insulin immunoreactive (IR) and glucagon-IR cells in the pancreas and to find out their effect on plasma glucose level in *C. versicolor* during different stages of reproductive cycle.

Materials and Methods

Animals

The animals were collected from Chamundi Hills (Latitude 12°27′N; Longitude, 74°00′ to 78°20 E; Altitude 1046 Meters above sea Level) 13 kms from the city of Mysore during 2007- 2009. They were maintained in the reptile house (20 × 20 × 10 feet, covered with mesh all around) in the open and fed with silk moth ad-libitum. "Guidelines for Care and Use of Animals in Scientific Research" were followed [11]. Experimental protocols were approved from Institutional Animal Ethics Committee (IAEC). They were studied in preparatory , reproductive and recrudescent periods of the reproductive cycle. Ten adult lizards of *C. versicolor* weighing 20-30 g were utilized in each period. As the animals were collected from the natural habitat their body length and weight were recorded. The length of the animal was based on the measurements from tip of the snout to the tip of the tail. The animals were sacrificed using 50 mg/kg body weight of sodium pentobarbital intra peritoneal injections. The pancreas was freed, its length measured,

***Corresponding author:** Prakash. R. Naik, Endocrinology laboratory, Department of Studies in Zoology, University of Mysore, Manasagangorti, Mysore 570006, India, Tel: 91-821-2419771; Fax: 91-821-2419301, 2419363; E-mail: prakashrnaik@yahoo.co.in

Received March 03, 2012; **Accepted** April 23, 2012; **Published** April 25, 2012

Citation: Chandavar VR, Naik PR (2012) The Endocrine Pancreas of the Lizard *Calotes versicolor*: An Immunocytochemical and Physiological Study with Respect to its Reproductive Cycle. J Cytol Histol 3:143. doi:10.4172/2157-7099.1000143

weighed and fixed in Bouin-Hollande sublimate solution for 18-20 h for light microscopy and immunocytochemistry, mean while pancreases of 0.5 mm^3 were also fixed for electron microscopy. The presence of abdominal fat was also observed and recorded.

Plasma glucose

Simultaneously, blood samples from carotid artery were collected and centrifuged at 4°C at 10000 rpm for 10 minutes. The separated serum was immediately used for estimation of glucose by enzyme glucose oxidase method of Trinder [12] as described earlier [13]. Plasma glucose (mg/dl) was determined for all the seasons using blank and by averaging the values of ten active animals (Ten samples from each animal).

Histology

Paraffin embedded pancreas from each of the animals was sectioned at 4-5 µm in series. Ten to 15 sections were mounted on a slide and every second slide was used for light microscopy. Chrome alum hematoxylin and phloxin (CHP) staining method [14] was employed for light microscopy. Sections were deparaffinised processed through grades of alcohol, washed in running water. Then the sections were treated with acidified $KMnO_4$ and subsequently decolourised with sodium bisulphite, stained with hematoxyline, differentiated in 1% acidified water, counter stained in phloxin for few minutes and mordant in phosphotungstic acid.

Immunocytochemistry

All the chemicals used in immunocytochemistry were purchased from Sigma-Aldrich, USA. Every third slide was used for glucagon cell localization and the fourth slide having sections of the same islet was used for insulin cell localization. They were immunolocalized by the extravidin-biotin peroxidase method after Yang et al. [15] and as per the instruction manual provided with the kit. The paraffin embedded sections were deparaffinised processed through grades of alcohol, washed in running water, pretreated with 3% H_2O_2 in methanol, rinsed with Phosphate Buffered Saline (PBS, pH 7.6) and non-specific reactive sites blocked with 5% normal goat serum. They were then incubated for 1 h at 37°C in a humidified chamber with the respective primary monoclonal mouse antibody (porcine glucagon was used as immunogen, product no G2654; diluted 1:2000 and human insulin was used as immunogen, product no I2018; diluted 1:1000). The sections were carefully washed 10 to 15 times with PBS and incubated for 30 min with biotinylated goat anti-mouse immunoglobulin secondary antibody and extravidin-peroxidase (Mouse extravidin peroxidase staining kit Stock No. EXTRA-2, Sigma), each diluted 1:20. PBS with 5% normal goat serum was used as diluent. The peroxidase activity was demonstrated using 0.7 mg/ml 3,3′-Diaminobenzidine Tetrahydrochloride (DAB) in 0.17 mg/ml urea hydrogen peroxide and 0.06 M Tris buffer for 1-3 min.To show that the labeling is specifically due to the primary antibody, the primary antibody was replaced with similarly diluted normal serum from the same species, keeping all the other steps the same in controls [16]. Another control for specificity which included omission of primary monoclonal antibody and parallel incubation with antibody reabsorbed with excess of respective antigen. No immunostaining was obtained in the controls. This further confirms that the immunolocalization has taken place in islets only.

Pancreatic sections containing islets were observed throughout the pancreas. Though *C. versicolor* exhibits sexual dimorphism there was no variation in the morphology and histology of the pancreas. Immunoreactive cell count was done by random selection of 100 sections in each period. Glucagon-IR and insulin-IR cell counting was done separately by using software Image pro express, version 5.1.Total number of glucagon-IR and insulin-IR cells of all ten animals in every period was considered as 100 percent and quantitative analysis in terms of percentage of glucagon-IR and insulin-IR cells was calculated. Digital photographs were taken using Olympus B x 60.

Electron microscopy

Pancreases of 0.5 mm^3 were fixed for 24 h at 4°C in 3% glutaraldehyde in 0.1 M phosphate (pH 7.2-7.4), then post fixed in 1% buffered osmium tetroxide, en-bloc stained with 2% uranyl acetate in 95% ethanol and embedded in araldite-cx resin after polymerizing it at 60°C for 48 h. Ultrathin sections were obtained with LKB ultracut microtome, stained with uranyl acetate fallowed by lead citrate, and examined by FM Jeol, EM, electron microscope [17].

Liver histochemistry

Specimens autopsied to collect blood and pancreases were also used for histochemical localization of liver glycogen. Liver was fixed in Rossman's fixative and then processed, sectioned at 9 -10 µm and stained following periodic acid-schiff (PAS) technique of Hotchkiss [18]. The PAS positive masses localized in the cytoplasm was taken into consideration for qualitative analysis of glycogen.

Statistical analysis

The values were expressed as mean ± SD for cell count and plasma glucose level in mg/dl during different periods was carried out using analysis of variance (ANOVA). Wherever the ANOVA values *(F)* were found to be significant, Duncan's multiple range test (DMRT) was applied. The *P* value < 0.05 was considered significant.

Results

The animals of *C. versicolor* were studied in three distinct periods according to their annual seasonal cycle of reproduction (Table 1). Different periods of reproductive cycles were assigned by careful observation of the status of the gonad during two successive cycles of reproduction. Initiation of gonad activity occurred during Feb-April in both the sexes of *C. versicolor* and this duration is designated as preparatory period, which corresponded with summer. Peak of gonad activity was observed during May-July, which is referred to as reproductive period. The gonads were regressed in the months of Aug-Jan, considered as recrudescent period.

During preparatory period, the mean weight and length of the animals were 29.2 ± 10.58 g and 29.4 ± 5.29 cm respectively, where

	Periods		
	Preparatory	Reproductive	Recrudescent
Months	Feb-April	May-July	Aug-Jan
Season	Summer	Monsoon	Winter
Gonad activity	Preparative	Peak	Regression
Temperature: Max:	37 ± 2°C	35 ± 1°C	29 ± 2.2°C
Min:	20 ± 2°C	18 ± 2°C	11 ± 2°C

Table 1: Annual cycle of reproduction in *C. versicolor*.

as pancreas of these animals weighed 0.056 ± 0.015 g, its mean length measured 3.12 ± 0.84 cm. In reproductive period, animals weighed 29.90 ± 14.67 g and length was 29.90 ± 5.84 cm, the pancreases on an average weighed 0.073 ± 0.026 g and measured a length of 3.70 ± 0.48 cm. During recrudesent period, *C. versicolor* weighed 33.8 ± 16.23 g and possessed a body length of 30.4 ± 4.88 cm. The weight and length of the pancreas of these animals were 0.053 ± 0.018 g and 3.90 ± 0.57 cm respectively. All these parameters indicate similarity in their pattern and no significant variation throughout the year irrespective of the period in *C. versicolor*.

A few individual cells stained dark blue between the acini (exocrine pancreas) under CHP method, which were difficult to identify under light microscope. Hence, immunocytochemistry was carried out to localize insulin and glucagon cells separately. Use of monoclonal antibodies is the most reliable method for localizing glucagon and insulin cells in pancreas. The immunoreactive (IR) cells were located in the exocrine pancreas as mainly solitary or two to three cell clusters throughout the pancreas in all the periods in both male and females. They were oriented towards the capillary pouring their content into the blood vessel indicating that they are endocrine cells.

During preparatory period, very few immunoreactive (IR) cells were found scattered throughout the pancreas (Figures 1 and 2) with moderate count of both insulin-IR (1946 ± 24) and glucagon-IR (85 ± 9) cells. The cell percent was 95% and 5% for insulin-IR and glucagon-IR cells respectively. The plasma glucose recorded the highest value of 194.31 ± 33.99 mg%. The preparatory animals exhibited no PAS positive masses in their liver samples. Moderate fat deposition in the abdominal cavity was observed.

During reproductive period, smaller aggregation of insulin-IR (4583 ± 32) and glucagon-IR (691 ± 15) cells were seen (Figures 3 and 4). The cell percent was 87% and 13%, respectively, with significantly higher number of both cell types than in earlier period. The plasma glucose recorded the moderate value of 167.02 ± 16.54 mg%. There was no localization of glycogen mass in liver sections. Excessive fat deposition was observed in the abdomen.

The number of insulin-IR (726 ± 12) and glucagon-IR (26 ± 4) cells were least in recrudescent period (Figures 5-7). Insulin-IR and glucagon-IR cell percent stood at 97% and 3%, respectively. The plasma glucose was low with a value of 157.85 ± 34.63 mg%. Liver sections of recrudescent period revealed very few PAS positive masses of glycogen. No fat deposition in the abdomen.

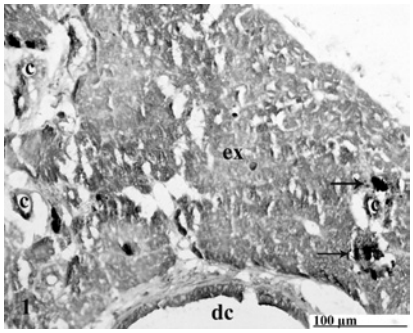


Figure 1: Pancreas of *C. versicolor* during preparatory period. Insulin-IR cells (arrows) are seen near the blood capillary (c), acinar (ex) cells and pancreatic duct (dc) are also seen. X200.

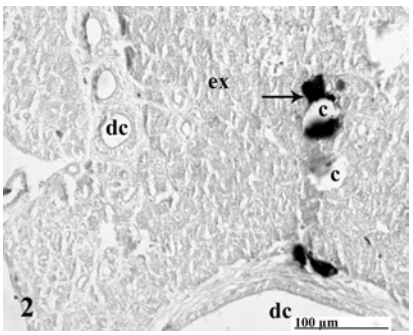


Figure 2: Succeeding section of preparatory period immunolocalized for glucagon-IR cells (arrow) pouring its secretion into blood capillary (c). A faint pool of granules moving towards the capillary, smaller and larger ducts (dc) are also seen. X200.

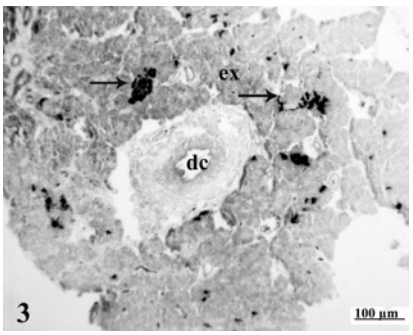


Figure 3: Pancreas of *C. versicolor* during reproductive period. Insulin-IR cells appearing in smaller groups (arrows) and also as individual cells between acinar (ex) cells around the pancreatic duct (dc). X100.

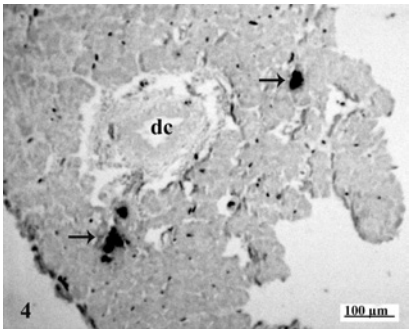


Figure 4: Succeeding section of reproductive period, immunolocalized for glucagon-IR cells. Glucagon-IR cells appear in smaller groups (arrows) and as individual cells between acinar (ex) cells around the duct (dc). X100.

Since there was no significant characteristic difference in the morphology of pancreas, distribution of immunoreactive cells and plasma glucose with respect to sex, the data were pooled together.

A difference in cell count and cell percent for insulin-IR and glucagon-IR was observed between the periods. Cell count for both IR cells of reproductive period was higher and differed significantly (P <0.05) from preparatory and recrudescent periods. Difference in mean plasma glucose level was significantly higher in preparatory period, and non significant difference existed between reproductive and recrudescent period.

None of the liver samples showed PAS positive masses in preparatory and reproductive periods, however, recrudescent animals had very few PAS positive masses in their liver sections.

Insulin-IR cells and glucagon-IR cells never formed islets in the pancreas of *C. versicolor*. This was further confirmed by electron microscopy (Figure 8 and 9). Morphological differences between two cell types were observed. Under electron microscopy the insulin cells were elongated or oval in shape with eccentric indented nucleus. Secretory granules were round or rectangular with polymorphic matrix (Figure 8). The glucagon cells were oval in shape. Their nuclei were placed in the centre. Cytoplasm showed the presence of round or oval electron dense granules (Figure 9). Under electron microscopy pancrases showed the presence of neve innervations in them. The neves are identified as medulated neurons because of the presence of medullary sheath (Figure 10). Among the endocrine cells insulin-IR cells were predominant and occasionally showed glucagon-IR cells.

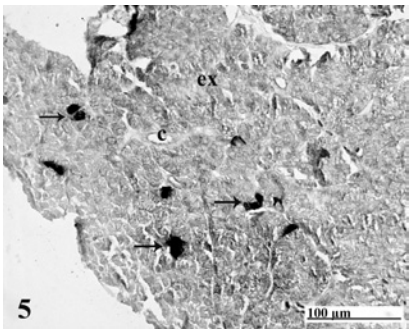


Figure 5: Pancreas of *C. versicolor* during recrudesent period. Insulin-IR cells and their secretions appear as dark spots (arrow), blood capillary (c) are seen between acinar cells (ex). X200.

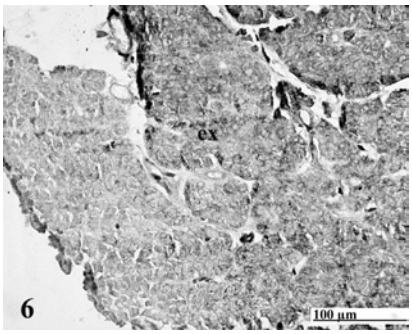
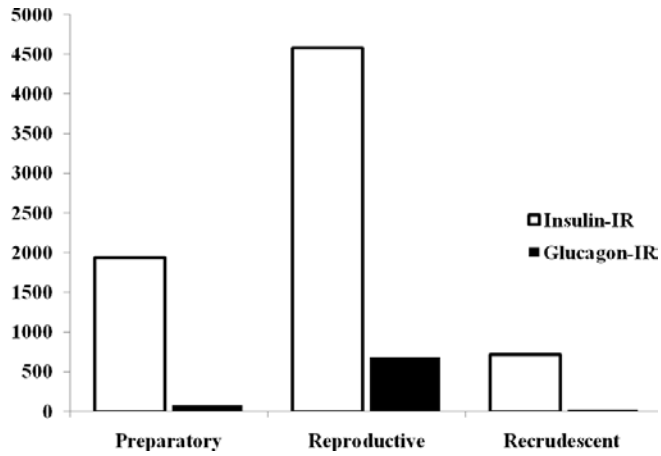


Figure 6: Succeeding section of recrudescent period, immunolocalized for glucagon-IR cells. No glucagon-IR cells are seen between acinar cells (ex). X200.



Note: X-axis Periods; Y-axis Number of cells

Figure 7: Seasonal variation in number of insulin-IR and glucagon-IR cells. Data are the mean±SD of ten animals in each period.

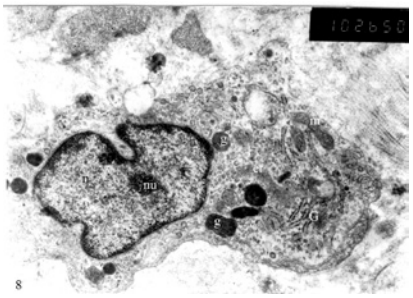


Figure 8: Electron micrograph of pancreas of *C. versicolor* showing insulin cell and no neighbouring cells of similar type. The cell shows the nucleus (n), nucleolus (nu), endoplasmic reticulum (er), mitochondria (m) and secretory granules (g). X20000.

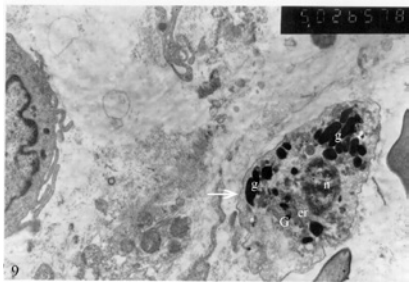


Figure 9: Electron micrograph of pancreas of *C. versicolor* showing individual glucagon cell (arrow) with no neighbouring cells of similar type. The cell shows nucleus (n), endoplasmic reticulum (er) and secretory granules (g) and Gogi (G). X10000.

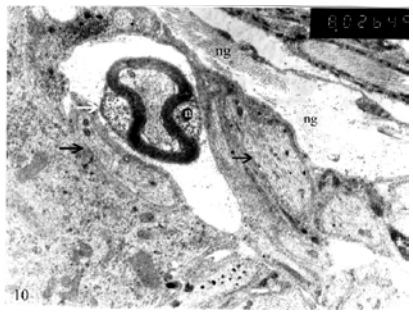


Figure 10: Electron micrograph of pancreas of *C. versicolor* showing medullated neuron (white arrow) with nucleus (n) in the medularty sheath, many nerve endings (black arrows) with pool of neurosecretory granules (ng) in them are seen. X16000.

Discussion

Calotes versicolor is a common tropical lizard, feeding on insects. It basks during winter and shifts to shady area in summer. Pancreas is a thread like organ as in other lizard studied, *Eutropis carinata* [19], running from spleen to gall bladder all along the duodenum.

Calotes versicolor exhibits moderate fat deposition in the abdominal cavity during preparatory period which reached to highest level in reproductive period but it was utilized by the end of recrudescent period. Abdominal fat and body mass of *C. versicolor* showed annual changes. Our findings are in accordance with Shanbhag and Prasad [20], fat bodies were absent in late breeding phase.

The food consumed by the animals during preparatory and reproductive period was converted and stored as reserve food in the form of fat and the animals weighed similar in both the periods.The significant rise in insulin-IR cells during reproductive period may be due to stimulus of higher plasma glucose of regenerative period. Glucose acts as stimulus for an increase in the beta cell number [21]. Earlier studies on agamid lizards were reported by Kumar and Khanna [22]; El-Salhy and Grimelius [3]. Kumar and Khanna [22] demonstrated preponderance of glucagon secreting alpha-cells in *Uromastix hardwicki,* after injection of insulin, caused hypoglycemia. In another agamid lizard *Uromastyx aegyptia* [3] histology and immunocytochemical studies showed the distribution of different cell types in the pancreas; however the quantification of B and A cells was not done. Present investigation deals with insulin-IR and glucagon-IR cells with respect to reproductive cycle.

In the subsequent discussion, wherever the results of *E. carinata* are compared with those of *C. versicolor*, it pertains to the reports of Chandavar and Naik [19]. Immunocytochemical studies of recrudescent period, which corresponded with winter, was indicated by considerably low number of insulin-IR and glucagon-IR cells but was heigher in *E. carinata*. During winter months almost all the endocrine glands are inactive in *C. versicolor* [23]. The mean plasma glucose level was also low of all periods. Probably, the animals utilized plasma glucose and abdominal fat in order to over come cold winter months as in *E. carinata* though the reproductive periods deffered from one another between these two species.The relative number and percent of insulin-IR cells appeared significantly ($P < 0.05$) higher than glucagon-IR cells in *C. versicolor* where as in *E. carinata* glucagon cells (55%) were more numerous than insulin cells (45%). In most lacertids studied, the central core consisted of B and A cells at the periphery, with predominance of B cells [7,24]. It was observed that the insulin-IR and glucagon-IR cells never formed islets in *C. versicolor* instead they were individually distributed. This was further confirmed by electron microscopy (Figure 8 and 9). In another lizard *Takydromus wolteri,* endocrine cells were distributed as solitary cells among the exocrine parenchyma [10]. Under electron microscopy pancreases of *C. versicolor* showed the presence of neves in them (Figure 10). This indicates that regulation of insulin and glucagon secretion as well as exocrine function of pancreas may be regulated by the neves. López et al. [25] identified adrenomedullin immunoreactive cells in the pancreas of seven nonmammalian species including reptiles. The study of Trandaburu and Trandaburu [26] in turtles, lizards and snakes revealed serotonin-producing cells in the pancreas. In *E. carinata*, insulin-IR and glucagon-IR cells formed islets with intermingled glucagon and insulin cells with paracrine property where glucose and glycogen regulation is probably dependent on the insulin and glucagon cells.The regulation of hepatic glucose metabolism has a key role in whole-body energy metabolism, as the liver is able to store and to produce glucose [27]. In liver, where glycogen is stored as a reserve of glucose for extra-hepatic tissues, the glycogen-metabolizing enzymes have properties that enable the liver to act as a sensor of blood glucose and to store or mobilize glycogen according to the peripheral needs. In *C. versicolor,* they have no PAS positive masses in their liver samples. The available glucose might have been utilized by peripheral tissues with the aid of high number of insulin cells to accomplish energy demand rather than converting it into liver glycogen. Probably the reserve energy source is mainly abdominal fat in *C. versicolor*. Internal factors seem to play a major role in the determination of annual tissue sensitivity rhythms in *C. versicolor*.

Acknowledgements

The first author acknowledges the University of Mysore for the extending facilities, Neuropathology Department of National Institute of Mental Health and Neuro Science, Bangalore for Electron Microscopy work and the UGC for the grants to carry out immunocytochemistry.

References

1. Heller RS (2010) Comparative anatomy of islets. The islets of Langerhans: Advances in experimental medicine and biology. Springer Dordrecht Heidelberg, London, New York.
2. Pandav BN, Shanbhag BA, Saidapur SK (2010) Growth patterns and reproductive strategies in the lizard, *Calotes versicolor* raised in captivity. Acta Herpetologica 5: 131-142.
3. El-Salhy M, Grimelius L (1981) Histological and immunohistochemical studies of the endocrine pancreas of lizards. Histochemistry 72: 237-247.
4. Rhoten WB, Hall CE (1982) An immunocytochemical study of the cytogenesis of pancreatic endocrine cells in the lizard, *Anolis carolinensis*. Am J Anat 163: 181-193.
5. López J, Echevarría M, Vázquez JJ (1988) Histological and immunocytochemical study of the endocrine pancreas of the lizard *Podarcis hispanica* Steindachner, 1870 (Lacertidae). Gen Comp Endocrinol 71: 212-228.
6. Putti R, Varano L, Cavagnuolo A, Laforgia V (1991) The endocrine pancreas of Podarcis s. sicula Raf.: an immunocytochemical study at light and electron microscopic levels. Eur J Basic Appl Histochem 35: 145-159.
7. Putti R, Della Rossa A, Varano L, Laforgia V, Cavagnuolo A (1992) An immunocytochemical study of the endocrine pancreas in three genera of lacertids. Gen Comp Endocrinol 87: 249-259.
8. Buono S, Odierna G 2nd, Putti R (2006) Morphology of the pancreas of some species belonging to the genera Phelsuma and Gecko (family Gekkonidae): evidence of apoptotic process during the seasonal cycle. Anat Embryol (Berl) 211: 413-421.
9. Della Rossa A, Putti R (1995) The endocrine pancreas of lacertids: an immunocytochemical study of the genera Pedioplanis and Meroles. Eur J Histochem 39: 47-58.
10. Ku SK, Lee HS (2004) The distribution and frequency of endocrine cells in the splenic lobe of grass lizard (Takydromus wolteri). An immunohistochemical study. Eur J Histochem 48: 429-436.
11. Anonymous (2000) Guidelines for care and use of animals in scientific research. Indian National Science Academy, New Delhi.
12. Trinder P (1969) Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem 6: 24-27.
13. Chandavar VR, Naik PR (2004) Variation in plasma glucose and pancreatic β cells in the turtle, *Lissemys punctata* (Order: Chelonia; Family: Trionychidae). Acta Zool 85: 113-118.
14. Gomori G (1941) Observations with differential stains on human islets of Langerhans. Am J Pathol 17: 395-406.

15. Yang H, Morrison CM, Conlon JM, Laybolt K, Wright JR Jr (1999) Immunocytochemical characterization of the pancreatic islet cells of the Nile Tilapia (*Oreochromis niloticus*). Gen Comp Endocrinol 114: 47-56.

16. Burry RW (2000) Specificity controls for immunocytochemical methods. J Histochem Cytochem 48: 163-166.

17. Johannessen JV (1978) Electron microscopy in Human Medicine. Instrumentation Techniques. Mc Graw Hill International Book Company, New York.

18. Hotchkiss RD (1968) The Periodic Acid Schiff (PAS) Technique. Histochemistry theoretical and Applied. Edinburgh, Churchhill Living stone.

19. Chandavar VR, Naik PR (2011) Localization of glucagon and insulin cells and its variation with respect to physiological events in *Eutropis carinata*. Acta Herpetologica 6: 47-57.

20. Shanbhag BA, Prasad BS (1992) Fat body ovarian relationship in the garden lizard, *Calotes versicolor* (Daud.). J Exp Zool 264: 454-60.

21. Bonner-Weir S (2000) Perspective: Postnatal Pancreatic β Cell Growth. Endocrinology 141: 1926-1929.

22. Kumar S, Khanna SS (1977) Response of the blood glucose and the pancreatic islets of the lizard, Uromastix hardwicki (Gray) to exogenous insulin. Z Mikrosk Anat Forsch 91: 131-143.

23. Gupta BB, Thapliyal JP (1983) Adrenal hormones and oxidative metabolism of the garden lizard (*Calotes versicolor*). J Endocrinol 99: 211-216.

24. El-Salhy M, Abu-Sinna G, Wilander E (1983) The endocrine pancreas of a squamate reptile, the desert lizard (*Chalcides ocellatus*). A histological and immunocytochemical investigation. Histochemistry 78: 391-397.

25. Lopez J, Cuesta N, Cuttitta F, Martinez A (1999) Adrenomedullin in nonmammalian vertebrate pancreas: an immunocytochemical study. Gen Comp Endocrinol 115: 309-322.

26. Trandaburu T, Trandaburu I (2007) Serotonin (5-hydroxytryptamine, 5-HT) immunoreactive endocrine and neural elements in the chromaffin enteropancreatic system of amphibians and reptiles. Acta Histochem 109: 237-247.

27. Foufell F, Ferre P (2002) New perspectives in the regulation of hepatic glycolytic and lipogenic genes by insulin and glucose: a role for the transcription factor regulatory element binding protein-1c. Biochem J 366: 377-391.