Acta Scientiarum. Biological Sciences
ISSN: 1679-9283
eduem@uem.br
Universidade Estadual de Maringá
Brasil

Marques Freitas-Ribeiro, Gláucia; Fonseca, Cláudio César; Souza Rodrigues, Sirlene; Pinto da Matta, Sérgio Luis; Andrade Neves, Clovis
Quantification of argyrophillic, argentaffin and insulin immunoreactive cells in the small intestine in the opossum Didelphis aurita (Wied-Neuwied, 1826)
Acta Scientiarum. Biological Sciences, vol. 33, núm. 4, 2011, pp. 479-485
Universidade Estadual de Maringá
.png, Brasil

DOI: 10.4025/actascibiolsci.v33i4.864

Quantification of argyrophillic, argentaffin and insulin immunoreactive cells in the small intestine in the opossum *Didelphis aurita* (Wied-Neuwied, 1826)

Gláucia Marques Freitas-Ribeiro[1*], Cláudio César Fonseca[2], Sirlene Souza Rodrigues[1] Sérgio Luis Pinto da Matta[1] and Clovis Andrade Neves[1]

*[1]Departamento de Biologia Celular e Estrutural, Universidade Federal de Viçosa, Av. Peter Henry Rolfs, s/n, Campus Universitário, 36570-000, Viçosa, Minas Gerais, Brazil. [2]Departamento de Medicina Veterinária, Universidade Federal de Viçosa, Minas Gerais, Brazil. *Author for correspondence. E-mail: glauciamfr@yahoo.com.br*

ABSTRACT. The objective of this study was to quantify argyrophillic, argentaffin and insulin immunoreactive endocrine cells in the different segments of the small intestine of *Didelphis aurita* and measure probable differences in the number of these cells between adult and post-pubertal animals. Biological material consisted of ten male and female opossums specimen, divided in two groups according to weigh. The utilized staining techniques were Grimelius, modified Masson-Fontana and direct immunoperoxidase. Results indicated a predominance of argyrophillic cells in the small intestine of opossums from class 1 and 2, with an average of 52.58 and 56.15 cells mm^{-2}, respectively; of which, the average number of total endocrine cells, argyrophillic and argentaffin cells decreased distally in the intestinal segments of opossums from classes 1 and 2. No significant difference was observed for the insulin immunoreactive cells between the intestinal segments of animals from class 2. A greater number of insulin immunoreactive cells was encountered in the jejunum and ileum of animals from class 2 when compared to the same segment in animals from class 1.

Keywords: digestive tract, endocrine cells, immunoreactive cells, mammal, marsupial.

RESUMO. Quantificação das células argirófilas, argentafins e imunorreativas à insulina nos diferentes segmentos do intestino delgado do gambá *Didelphis aurita* (Wied-Neuwied, 1826). Os objetivos deste trabalho foram quantificar as células endócrinas argirófilas, argentafins e imunorreativas à insulina nos diferentes segmentos do intestino delgado de gambás *Didelphis aurita* e mensurar prováveis diferenças no número destas células entre animais adultos e pós-púberes. Dez exemplares de gambás *D. aurita* machos e fêmeas foram divididos em dois grupos de acordo com o peso. As técnicas de coloração utilizadas foram Grimelius, Masson-Fontana modificado e Imunoperoxidase direta. Os resultados indicaram um predomínio das células argirófilas no intestino delgado de gambás da classe 1 e 2, com uma média de 52,58 e 56,15 células mm^{-2}, respectivamente. O número médio de células endócrinas totais, células argirófilas e argentafins decresceu distalmente nos segmentos intestinais dos gambás das classes 1 e 2. Nenhuma diferença significativa foi observada para as células imunorreativas à insulina entre os segmentos intestinais dos animais da classe 2. Foi encontrado maior número de células imunorreativas à insulina no jejuno e íleo de animais da classe 2 quando comparado ao mesmo segmento em animais da classe 1.

Palavras-chave: trato digestivo, células endócrinas, células imunorreativas, mamíferos, marsupial.

Introduction

Endocrine control of secretion, absorption and intestinal motility is performed by endocrine cells dispersed along the digestive tract and pancreas (FUJITA, 1973). These cells can be classified as open or closed according to apical communication with the lumen (SANTOS; ZUCOLOTO, 1996); in argyrophillic and argentaffin cells by the capacity to retain and reduce silver salts (GRIMELIUS; WILANDER, 1980); and in hormone producing cells (insulin, secretin, somatostatin and others) principally based on the characteristics and content o their secretory granules (POLAK et al., 1993). It is believed that these cells have an endodermal origin from stem cells of the intestinal mucosa, thus negating the possibility of their origin from the neural cres (RODRIGUES et al., 2005).

Insulin, principally produced by the pancreas, was also detected by immunohistochemistry techniques in the prostate (STAHLER et al., 1988), nephron (COUTINHO et al., 1985), central nervous system

(DEVASKAR et al., 2002), retina (MEIMARIDIS et al., 2003) and intestine (BENDAYAN; PARK, 1991; COUTINHO et al., 1984; ITO et al., 1988; KENDZIERSKI et al., 2000).

Kendzierski et al. (2000) encountered intracellular immunoradioactivity of insulin in glandular cells of the stomach and the colon of rats, but not in the small intestine. Contrarily, Coutinho et al. (1984) observed positive insulin staining in the brush border and in some cells isolated in segments near the small intestine of adult opossums (*Didelphis albiventris*), although no evidence was observed in the mid and distal segments. Bendayan and Park (1991) located extrapancreatic islets between the duodenum crypts and muscle layer of the mucosa in rats. These islets were restricted to the duodenum region, crossed by the final portion of the bile duct, before its opening in the intestinal lumen. The islets were surrounded by conjunctive tissue and exhibited no direct contact with the epithelial cells of the ducts and crypts. Ito et al. (1988) also encountered a small number of insulin immunoreactive cells in the pyloric antrum and duodenum of pigs between 32 and 41 days old.

In the study of the endocrine system, the marsupial opossum has become a model due to the simultaneous differentiation of the gastrointestinal tract and endocrine glands when the animal is found in the intra-marsupial stage (KRAUSE et al., 1989b; FONSECA et al., 2002b). Since its birth also occurs in a precocious development stage, is it possible to perform experiments without the need for pre-, trans- and post-operatory procedures as a result of prenatal surgeries (COUTINHO, 1985; PAIVA et al., 1992). However, there are still few immunocytochemical studies on the enteroendocrine cells of opossums (BARBOSA et al., 1987, 2006; FONSECA et al., 2002a; KRAUSE et al., 1985, 1989a; TAKAGI et al., 1990).

According to Krause et al. (1985), the type and distribution of enteroendocrine cells in opossums are similar to the majority of eutherian mammals. Based on this observation, the confirmation and detailed acknowledgment of the distribution of these cells along the intestine of opossums is necessary. There are still uncertainties in regards to location of the insulin immunoreactive (IR) cells in the intestine of animals already studied. Therefore, the objective of the present study was to quantify the argyrophillic, argentaffin and insulin immunoreactive endocrine cells in the small intestine and verify possible differences between the number of cells between intestinal segments of post-pubertal and adult opossums (*Didelphis aurita*).

Material and methods

Ten adult opossums, both male and female, from the species *Didelphis aurita* were used in this experiment. The animals were captured between January and June of 2007, in the municipality of Viçosa, Minas Gerais State, Brazil. Hook traps were used to capture the opossums using bate composed of pineapple and cotton impregnated with cod liver oil. The animals were euthanized with a general anesthetic (sodium pentabarbital), followed by administration of potassium chloride. Capture of the animals was authorized by IBAMA (license no. 10168-1) and the experiment was evaluated by the Ethics Commission of the Veterinary Department of the Federal University of Viçosa (process no. 56/2007).

Despite the estimate of age fluctuate depending on the development stage. This work was not possible to follow the development stages through periodic sampling, so to standardize the data, the animals were divided into two groups according to the weight for the quantification of endocrine cells between the intestinal segments. Animals where characterized in two groups, in which Class 1 consisted of those weighing between 400 and 800 g (post-pubertal) and Class 2 weighing more than 800 g (adults).

The abdominal cavity of the animals was opened and the following segments of the digestive tract were identified: duodenum, from the initial extremity of the small intestine to the duodenum-jejunum flexure; jejunum, from the flexure up to the ileocecal fold; ileum, from this fold to the ileocecal junction (DYCE et al., 1997).

Two 1 cm^2 fragments were collected from each intestinal section of the animals for the histological study. They were fixed for 24h in Bouin's liquid for staining by the Grimelius technique (GRIMELIUS, 1968) and the direct immunoperoxidase technique (STERNBERGER, 1979), and in 10% buffered formalin for the modified Masson-Fontana technique (BARBOSA et al., 1984). The fragments were then dehydrated, diaphanized, embedded in paraffin and sectioned at a width of 5 μm with the assistance of a manual rotating microtome (model Leica, RM2155). Each slide contained four sections of the same fragment, which was established a distance of 30 μm between the sections during microtomy. It produced a total of six slides for each region by intestinal segment. Therefore, a total of 24 sections were analyzed for each region. After

removal of the paraffin, the histological sections were hydrated and stained. The staining techniques utilized aimed to identify and quantify argyrophillic cells (Grimelius), argentaffin cells (modified Masson-Fontana) and insulin immunoreactive (IR) cells (direct immunoperoxidase).

In the argyrophillic reaction (Grimelius technique), the silver salts in ammoniacal, aqueous or alcoholic solution bond to the cytoplasmic granules, being then reduced to silver metal by the exposure to an exogenous reducing substance. In the argentaffin reaction (modified Masson-Fontana technique), a reduction in the ammoniacal silver nitrate is a result of the reducing capacity of its cellular components (RODRIGUES et al., 2005).

The antibodies used in the immunohistochemical technique were produced by the Bethyl laboratory, lot no. A90-117P-4, and the opossum pancreas was used as a positive control. Processing of the material was performed at the Laboratory of Structural Biology of the General Biology Department, Federal University of Viçosa, Minas Gerais State.

Quantification of the argentaffin, argyrophillic, and insulin immunoreactive cells was performed in six random fields of mucosa sections, defined by the extension of the ocular micrometric scale coupled to the 10x ocular, equivalent to 300 μm in extension and objective of 40x. The area of the mucosa was obtained from the average thickness multiplied by the extension of the micrometric scale. The average number of endocrine cells was registered for each mm^2 of the mucosa layer.

Photomicrographs were obtained using a binocular Olympus BX 60 photomicroscope coupled to a Qcolar.3 digital camera (Olympus) in the Laboratory of Insect Cytogenetics of the General Biology Department, Federal University of Viçosa, Minas Gerais State.

With the objective of performing comparisons between the segments of the small intestine (duodenum, jejunum and ileum) in relation to the number of argentaffin, argyrophillic, and insulin immunoreactive cells, as well as the total number of endocrine cells, the Kolmogorov-Smirnov test was employed to examine the normality of the data ($p < 0.05$). In function of the rejection of the normality criteria for the compared groups, non-parametric tests were employed for analysis of central tendency measurements. Therefore, the Kruskal-Wallis test (H) was used to verify possible differences between the medians of the three small intestine segments, considering the data for the total counts of argentaffin, argyrophillic, and insulin IR cells ($p < 0.05$). Comparisons of the median number of cells for each of the segments between the two animal classes evaluated was performed using the Wilcoxon test (t) ($p < 0,05$).

Results

The average number and median of argentaffin, argyrophillic, and insulin immunoreactive endocrine cells per mm^2 of the mucosa layer in the duodenum, jejunum and ileum of the opossums of class 1 (400 g ≥ animal < 800 g) and class 2 (animal > 800 g) are represented in Tables 1 and 2, respectively.

Table 1. Number of endocrine cells per mm^2 (mean ± standard deviation and median) in the mucosa layer of the duodenum, jejunum and ileum of opossum *D. aurita* from class 1 (400 g ≥ animal < 800 g; n = 5).

Endocrine cells	Duodenum		Jejunum		Ileum	
	mean	median	mean	median	mean	median
Argyrophillic	61.27 ± 11.76	59.84[a]	45.01 ± 14.17	46.95[b]	51.46 ± 15.35	49,81[b]
Argentaffin	18.29 ± 6.84	20.00[a]	9.71 ± 4.17	10.68[b]	8.33 ± 3.89	8,49[b]
Insulin IR cells	0.52 ± 0.36	0.60[a]	0.25 ± 0.17	0.23[a]	0.68 ± 0.56	0,47[a]
Total Endocrine cells	80.08 ± 12.36	82.04[a]	54.98 ± 15.77	55.92[b]	60.47 ± 17.41	60,63[b]

Medians followed by the same letter on the same line do not differ at the significance level of 5% by the Krusskal-Wallis test.

Table 2. Number of endocrine cells per mm^2 (mean ± standard deviation and median) in the mucosa layer of the duodenum, jejunum and ileum of opossum *D. aurita* from class 2 (animal > 800 g; n = 5).

Endo-crine cells	Duodenum		Jejunum		Ileum	
	mean	median	mean	median	mean	median
Argyro-phillic	68.70 ± 15.26	65.63[a]	50.53 ± 9.83	50.20[b]	49.23 ± 15.96	46.87[b]
Argentaffin	13.58 ± 5.80	11.83[a]	9.06 ± 4.55	10.48[ab]	5.34 ± 2.92	4.91[b]
Insulin IR cells	0.91 ± 0.63	0.88[a]	0.96 ± 0.60	1.04[a]	1.83 ± 1.47	1.18[a]
Total Endo-crine cells	83.19 ± 14.08	78.41[a]	60.57 ± 12.51	60.32[b]	56.42 ± 16.50	52.32[b]

Medians followed by the same letter on the same line do not differ at the significance level of 5% by the Krusskal-Wallis test.

The duodenum of the opossums in class 1 presented an average of 80.08 endocrine cells per mm^2, of which 76.52% were argyrophillic cells, 22.83% argentaffin cells and 0.65% insulin IR cells. An average of 60.47 cells mm^{-2} (Table 1) was encountered in the ileum, composed of 85.10% argyrophillic, 13.77% argentaffin and 1.13% insulin IR cells. The jejunum was the intestinal segment with the smallest number of total endocrine cells, 54.98 cells mm^{-2}, where 81.87% were argyrophillic, 17.67% argentaffin and 0.36% insulin IR cells (Tables 1 and 3).

Table 3. Values obtained from the Kruskal-Wallis test ($p < 0.05$) by comparison of the number of endocrine cells in the mucosa layer of the three segments in the small intestine of opossums in Class 1 (400 g ≥ animal < 800 g) and Class 2 (animal > 800 g).

	Class 1	Class 2
Argyrophillic	H = 10.12; df = 2; p = 0.0063	H = 12.5; df = 2; p = 0.0001
Argentaffin	H = 17.19; df = 2; p = 0.0002	H = 7.76; df = 2; p = 0.011
Insulin IR cells	H = 8.89; df = 2; p = 0.011	H = 3.02; df = 2; p = 0.23
Total Endocrine cells	H = 16.98; df = 2; p = 0.0002	H = 12.5; df = 2; p = 0.0001

The number of total endocrine cells was smaller in the distal segments of the intestine in opossums from class 2 ($p < 0.05$) (Tables 2 and 3), accounting for 83.19% in the duodenum, 60.57% in the jejunum and 56.42% in the ileum. In the duodenum, endocrine cells were composed of 82.58% argyrophillic cells, 16.33% argentaffin cells and 1.09% insulin IR cells. In the jejunum, 83.5% were argyrophillic, 14.97% argentaffin and 1.60% insulin IR cells. In the ileum, 87.26% were argyrophillic, 9.48% were argentaffin and 3.26% were insulin IR cells.

The number of argyrophillic and argentaffin cells per mm^2 was smaller in the distal segments ($p < 0.05$) of the small intestine of animals from class 1 and 2. No difference was observed for the insulin IR cells between the intestinal segments of class 1 and class 2 ($p > 0.05$) (Tables 1, 2 and 3).

A greater number of insulin IR cells was observed in the jejunum and ileum of animals from class 2 ($p < 0.05$) when compared to the same segment in animals of class 1 (Tables 1, 2 and 4), while the number of argyrophillic cells was smaller in the duodenum of animals from class 2 ($p < 0.05$). No difference was detected for the other cell types in relation to the compared segments of the two classes (Table 4).

Table 4. Values obtained from the Wilcoxon test ($p < 0.05$) when comparing the number of endocrine cells between opossums in Class 1 (400 g ≥ animal < 800 g) and Class 2 (animal > 800 g).

	Duodenum of opossum from class 1 and class 2	Jejunum of opossum from class 1 and class 2	Ileum of opossum from class 1 and class 2
Argyrophillic	t = 40, p = 0.25	t = 32, p = 0.11	t = 56, p = 0.22
Argentaffin	t = 22, p = 0.03	t = 48, p = 0.49	t = 31, p = 0.09
Insulin IR cells	t = 26, p = 0.05	t = 3, p = 0.001	t = 8, p = 0.003
Total Endocrine cells	t = 56, p = 0.82	t = 37, p = 0.19	t = 47, p = 0.46

Discussion

According to Polak et al. (1993) and Grimelius e Wilander (1980), endocrine cells are argyrophillic or argentaffin, with the exception of insulin immunoreactive, cholecystokinin and somatostatin cells, which are not labeled by silver (POLAK et al., 1993). Based on this information, here we encompassed an analysis of nearly all endocrine cells, lacking only a future study of the cholecystokinin and somatostatin producing cells.

Argyrophillic cells were predominant in the small intestine of opossums from Classes 1 and 2. These cells represent a heterogeneous population of endocrine cells, presenting diverse types of peptides and biogenic amines as a product of secretion (POLAK et al., 1993). Fonseca et al. (2002a), when identifying argyrophillic (argyrophil) cells in the ileum of the opossum *D. albiventris*, encountered approximately 130 cells mm^{-2} in animals in the intra-marsupial period, 133 cells mm^{-2} in weaned animals and 211 cells mm^{-2} in adults. Here, approximately 49 argyrophillic cells mm^{-2} were observed in the ileum of *D. aurita*. Differences in quantification of these cells by the Grimelius (1968) method can be attributed to the fact that the researcher may have considered their presence only when cytoplasm appeared to be impregnated with silver salts, associated or not to the negative image of the nucleus or only traces of silver. In this study, cells with the cytoplasm area totally impregnated with silver salts were considered positive.

Argentaffin cells were considered to be less in number than argyrophillic (argyrophil) cells, with an average of 12.11 cells mm^{-2} (class 1) and 9.33 cells mm^{-2} (class 2) in the small intestine of *D. aurita*. Bressan et al. (2004) identified 235.7 argyrophillic (argyrophil) cells mm^{-2} in the ileocecal region of the capybara *H. hydrochaeris*, and 228 argentaffin cells mm^{-2} in the same region. The elevated number of argentaffin cells encountered by Bressan et al. (2004) may have been a function of the thickness of the ileocecal region, where there is probably greater neuroendocrine control.

The population of enteroendocrine cells was less distal (smaller in the distal segments of the small intestine) in the two classes of animals studied. Inversely, Krause et al. (1985), when quantifying the various types of enteroendocrine cells in the gastrointestinal mucosa of the opossum species *D. virginiana*, registered a greater number in the final portion of the small intestine, highlighting the similarity of their results in relation to other mammals. The authors also observed a considerable variation in the distribution of enteroendocrine cells within a determined segment in different species. Takagi et al. (1990) encountered similarities in the distribution and relative frequency of endocrine cells in the duodenum of eight marsupial species as well when compared to eutherian mammals, showing no difference between marsupials, herbivores, omnivores or carnivores.

Visually, a greater abundance of total enteroendocrine cells was detected in the crypts, with a gradual reduction in the intestinal villi and no occurrence in the Brunner's glands, as observed by Takagi et al. (1990). Contrarily, Krause et al. (1985) identified an increase in the variety of enteroendocrine cell types in the villi and a smaller number in the crypts of the intestinal epithelium of *D. virginiana*. The results of Krause et al. (1985) were different from the aforementioned, probably in function of the employed methodology for detection of enteroendocrine cells. The technique used by these authors was peroxidase anti-peroxidase with polyclonal antibodies, while in this study, the immunohistochemical technique was used with monoclonal antibodies.

When the number of argyrophillic and argentaffin endocrine cells was compared between the two classes, no difference was observed in the small intestine of animals in Class 1 in relation to animals of Class 2 of *D. aurita*. However, in different stages of development, Fonseca et al. (2002a) encountered a greater number of argyrophillic cells in the ileum of *D. albiventris* adults in relation to the young marsupials. In the jejunum and ileum of animals from Class 2 of *D. aurita*, a greater number of insulin IR cells was observed when compared to the same segments of animals in Class 1. These results probably reflect the fact that the adult animals present the formation of a definite number of insulin IR cells, different from young animals in which the number of these cells is not yet constant.

There was no difference in the quantity of insulin immunoreactive cells between the duodenum, jejunum and ileum of the two classes studied, despite appearing to be more abundant in the ileum. Due to the fact that this last portion of the small intestine is involved in the absorption o water, it is speculated that insulin produced in thi segment participates in this process.

When the number of insulin immunoreactive cells was compared between the two classes, a increase in 0.75 cells mm^{-2} was observed in the smal intestine of animals weighing more than 800 g. Ito et al. (1988) encountered a small number of these cells in the duodenum and pyloric antrum of pig between 33 and 41 days old. Coutinho et al. (1984 also encountered these cells in the still immature region of the small intestine in nursing marsupials Coutinho et al. (1984) believe that the proxima portion of the intestine functions as an absorption point of the insulin secreted directly in the interio of this organ and, that the number of insulin IR cell in adult animals is not a function of the larg number of cells encountered in the embryo/young animal stage.

This study focused on the location of endocrin cells in the small intestine of *D. aurita*, while Kendzierski et al. (2000) verified the biosynthesis o preproinsulin in the epithelial cells of the colon, i the intestinal crypts and in some glandular cells i the rat stomach. Insulin liberated by endocrine cell present in the intestinal epithelium may locally stimulate neighboring cells (enterocyte), which block the absorption of glucose on the intestina wall. Similarly, peptide hormones liberated by th intestine stimulate liberation of pancreatic hormones, particularly insulin, which causes a increase in glucose uptake by tissues. The insulin producing endocrine intestinal cells, either autocrin or paracrine, stimulate other cells which cause a reduction in peristalsis, generating enough time fo effective absorption of nutrients by the intestin (KENDZIERSKI et al., 2000).

Pancreatic insulin reduces glucose in blood increases the deposit of glycogen in the muscles an the metabolic use of the glucose (GANONG, 1998) while enteral insulin controls intestinal motility (KENDZIERSKI et al., 2000). Eliasson et al. (1995 observed alterations in the gastrointestinal trac when parenterally inducing hyperinsulinemia (4 +/- 4 mU L^{-1}) in healthy humans, principally in th stomach and proximal duodenum. In this case insulin, when reaching the intestine via bloo channels, reduces intestinal mobility an carbohydrate absorption. The presence of insulin receptors in the intestinal epithelium also suggest that it may possess an autocrine or paracrine rol when produced in this organ (BERGERON et al. 1980; PILLION et al., 1985).

In our work, insulin immunoreactive cells were encountered insolated in the intestinal crypts an

villi. On the other hand, Bendayan and Park (1991) detected, by the use of histological and immunohistochemical techniques, insulin immunoreactive cells forming islets in the duodenum of rats. These cells were found in the center of the islets surrounded by glucagon, somatostatin and pancreatic polypeptide immunoreactive cells. The enteroendocrine cells were on the conjunctive tissue, between the duodenal crypts and muscular layer, near the final portion of the bile duct. All insulin immunoreactive cells exhibited developed rough endoplasmic reticulum and Golgi complexes, as well as numerous secretory granules.

Despite not observing the presence of endocrine cell islets in the small intestine of *D. aurita*, according to Bendayan and Park (1991), intestinal islets are very similar to pancreatic islets, with the glucagon, somatostatin and pancreatic polypeptide producing cells perfectly organized around the insulin producing cells.

Conclusion

Fewer argyrophillic, argentaffin and insulin IR cells were encountered in the small intestine of *D. aurita* when compared to animals from the groups metatheria (FONSECA et al., 2002a) and eutheria (BRESSAN et al., 2004; ITO et al., 1988). Differences in the employed methodology, in the region of the selected digestive tube and in the animal specimen utilized can reveal varied results. The population of enteroendocrine cells was smaller in the distal segments of the small intestine, and among the studied cells, the argyrophillic cells were more abundant. Insulin IR cells did not form islets, being encountered isolated in the intestinal villi and crypts.

References

BARBOSA, A. J. A.; CASTRO, L. P. F.; NOGUEIRA, A. M. F. A simple and economical modification of the Masson-Fontana method of staining melanin granules a dn enterochromaffin cells. **Stain Technology**, v. 59, n. 4, p. 193-196, 1984.

BARBOSA, A. J. A.; NOGUEIRA, J. C.; PENNA, F. J.; POLAK, J. M. Distribution of enteroglucagon- and polypeptide YY-immunoreactive cells in the gastrointestinal tract of the white-belly opossum (*Didelphis albiventris*). **Journal of Histochemistry**, v. 88, n. 1, p. 37-40, 1987.

BARBOSA, A. J. A.; NOGUEIRA, J. C.; FONSECA, C. C. Células endócrinas (APUD) do sistema digestivo do gambá *Didelphis albiventris*. In: CÁCERES, N. C.; MONTEIRO-FILHO, E. L. A. (Ed.). **Os Marsupiais do Brasil**. Campo Grande: UFMS, 2006. p. 89-98.

BENDAYAN, M.; PARK, I. S. Presence of extrapancreatic islets of Langerhans in the duodenal wall of the rat. **Diabetologia**, v. 34, n. 8, p. 604-606, 1991.

BERGERON, J. J. M.; RACHUBINSKY, R.; SEARLEM, N.; BORTS, D.; SIKSTROM, R.; POSNER, B. I. Polypeptide hormone receptors in vivo: demonstration of insulin binding to adrenal gland and gastrointestinal epithelium by quantitative radioautography. **Journal of Histochemistry and Cytochochemistry**, v. 28, n. 8, p. 824-835, 1980.

BRESSAN, M. S.; FONSECA, C. C.; MENIN, E.; PAULA, T. A. R. Identificação e quantificação de gânglios nervosos, células argentafins, argirófilas e imunorreativas à serotonina no ceco de capivara (*Hydrochoerus hydrochaeris*). **Revista Ceres**, v. 298, n. 51, p. 729-739, 2004.

COUTINHO, H. B. Insulina de gambá. **Ciência Hoje**, v. 3, n. 18, p. 57-60, 1985.

COUTINHO, H. B.; SEWEL, H. F.; COUTINHO, V. B. Immunocytochemical demonstration of insulin in the mesonephros and metanephros of the brazilian opossum *Didelphis albiventris*. **Anatomisher Anzeiger Jena**, v. 159, n. 1-5, p. 97-103, 1985.

COUTINHO, H. B.; SEWEL, H. F.; SMITH, D. I.; COUTINHO, V. B.; PINHEIRO, P. B. N. Demonstration of insulin in the pancreas of the *Didelphis albiventris* (Opossum) by immunocytochemical techniques. **Anatomisher Anzeiger Jena**, v. 157, n. 2, p. 167-175, 1984.

DEVASKAR, S. U.; SINGH, B. S.; CARNAGHI, L. R.; RAJAKUMAR, P. A.; GIDDINGS, S. J. Insulin II gene expression in rat central nervous system. **Regulatory Peptides**, v. 48, n. 1-2, p. 55-63, 2002.

DYCE, K. M.; SACK, W. O.; WENSING, C. J. **Tratado de anatomia veterinária**. Rio de Janeiro: Guanabara Koogan, 1997.

ELIASSON, B.; BJÖRNSSON, E.; URBANAVICIUS, V.; ANDERSSON, H.; FOWELIN, J.; ATTVALL, S.; ABRAHAMSSON, H.; SMITH, U. *Hyperinsulinaemia impairs* gastrointestinal motility and slows carbohydrate absortion. **Diabetologia**, v. 38, n. 1, p. 79-85, 1995.

FONSECA, C. C.; NOGUEIRA, J. C.; BARBOSA, A. J. A. Argyrophilic and Glucagon-Immunoreactive cells in the ileum and colon of the developing opossum *Didelphis albiventris* (Marsupialia). **Cells Tissues Organs**, v. 170, n. 1, p. 20-33, 2002a.

FONSECA, C. C.; NOGUEIRA, J. C.; BARBOSA, A. J. A. Diâmetro das ilhotas pancreáticas do gambá *Didelphis albiventris* em desenvolvimento intramarsupial. **Archives of Veterinary Science**, v. 7, n. 2, p. 129-134, 2002b.

FUJITA, T. Insulo-acinar portal system in the horse pancreas. **Archivum Histologicum Japonicum**, v. 35, n. 2, p. 161-171, 1973.

GANONG, W. F. **Fisiologia médica**. Rio de Janeiro: Guanabara Koogan, 1998.

GRIMELIUS, L. A silver nitrate for α2 cells in human pancreatic islets. **Acta Societatis Medicorum Upsaliensis**, v. 73, p. 243-270, 1968.

GRIMELIUS, L.; WILANDER, E. Silver stains in the study of endocrine cells of the gut and pancreas. **Investigative and Cell Pathology**, v. 3, p. 3-12, 1980.

ITO, H.; HASHIMOTO, Y.; KITAGAWA, H.; KON, Y.; KUDO, N. Ontogeny of gastroenteropancreatic (GEP) endocrine cells in mouse and porcine embryos. **Japanese Journal of Veterinary Science**, v. 50, n. 1, p. 99-110, 1988.

KENDZIERSKI, K. S.; PANSKY, B.; BUDD, G. C.; SAFFRAN, M. Evidence for biosynthesis of preproinsulin in gut of rat. **Endocrine**, v. 13, n. 3, p. 353-359, 2000.

KRAUSE, W. J.; YAMADA, J.; CUTTS, H. Quantitative distribution of enteroendocrine cells in the gastrointestinal tract of adult opossum, *Didelphis virginiana*. **Journal of Anatomy**, v. 140, n. 4, p. 591-605, 1985.

KRAUSE, W. J.; YAMADA, J.; CUTTS, H. Enteroendocrine cells in the developing opossum small intestine and colon. **Journal of Anatomy**, v. 162, p. 83-96, 1989a.

KRAUSE, W. J.; CUTTS, J. H.; YAMADA, J. Immunohistochemical study of the developing endocrine pancreas of the opossum (*Didelphis virginiana*). **Acta Anatomica**, v. 135, n. 1, p. 84-96, 1989b.

MEIMARIDIS, D. G.; MORSE, D. E.; PANSKY, B.; BUD, G. C. Insulin immunoreactivity in the fetal and neonatal rat retina. **Neuroscience Letters**, v. 118, n. 1, p. 116-119, 2003.

PAIVA, M. G. S.; CHAPLIN, E. L.; STOBBE, N. S.; ARAÚJO, F. A. P.; SILVA, N. R. S. Utilização de *Didelphis marsupialis* como animal de laboratório. **Pesquisa Agropecuária Brasileira**, v. 27, n. 1, p. 213-216, 1992.

PILLION, D. J.; GANAPATHY, V.; LEILABACH, F. H. Identification of insulin receptors on the mucosal surface of colon epithelial cells. **Journal of Biological Chemistry**, v. 260, n. 9, p. 5244-5247, 1985.

POLAK, J. M.; BISHOP, A. E.; BARBOSA, A. J. A. BLOOM, S. R. Hormônios gastrointestinais. In DANI, R.; CASTRO, L. P. (Ed). **Gastroenterologia clínica**. Rio de Janeiro: Guanabara-Koogan, 1993 p. 1446-1465.

RODRIGUES, S. S.; FONSECA, C. S.; NEVES, M. T D. Células endócrinas do sistema gastropancreático conceitos, distribuição, secreções, ação e controle **Arquivos de Ciências Veterinárias e Zoologia**, v. 8 n. 2, p. 171-180, 2005.

SANTOS, G. C.; ZUCOLOTO, S. Células endócrina gastrointestinais: breve histórico e principais métodos d identificação à microscopia óptica. **Arquivos d Gastroenterologia**, v. 33, n. 1, p. 36-44, 1996.

STAHLER, M. S.; PANSKY, B.; BUDD, G. C Immunocytochemical demonstration of insulin-lik immunoreactivity in the rat prostate gland. **Prostate** v. 13, n. 2, p. 189-198, 1988.

STERNBERGER, L. A. **Immunocytochemistry**. New York: John Wiley and Sons, 1979.

TAKAGI, C.; YAMADA, J.; KRAUSE, W. J. KITAMURA, N.; YAMASHITA, T. An immunohistochemical study of endocrine cells in the proximal duodenum of eight marsupial species **Journal of Anatomy**, v. 168, p. 49-56, 1990.

Received on November 1, 2009.
Accepted on April 16, 2010.