



## Comparison of Histomorphometric Study of Chromaffin Cells in Adult Males Squirrel (*Sciurus anomalus*) and Hamster (*Mesocricetus auratus*)

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Received: 2 December, 2020

Accepted: 11 January 2021

Published: 28 Jun 2021

**DOI:**

<https://doi.org/10.30539/ijvm.v45i1.1040>



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**Cite:**

Kadhim Ab, Khaleel IM. Comparison of Histomorphometric Study of Chromaffin Cells in Adult Males Squirrel (*Sciurus anomalus*) and Hamster (*Mesocricetus auratus*). Iraqi J. Vet. Med. 28 June. 2021; 45(1): 46-50.

### A B S T R A C T

The adrenal glands are endocrine glands that produce a diversity of hormones comprising of adrenaline, the aldosterone and cortisol. The present study aimed at investigation of the histomorphometric features of chromaffin cells. There were two types of chromaffin cells. In squirrel, the first type was columnar in shape and brownish in color contained spherical nucleus located at the base of cells, which represented the epinephrine secreting cells, and the second type was polygonal in shape and light brownish in color contained spherical nucleus located in the center of cells, which represented the norepinephrine secreting cells. The adrenal medulla of hamster consists almost entirely of columnar or polyhedral chromaffin cells forming clusters and anastomosing cords separated by sinusoids, giving a strong reaction with methylene eosin stain more than that seen in squirrel. The statistical analysis showed that the means diameter of epinephrine cells and norepinephrine cells in the right adrenal gland in squirrel were lesser than those of hamster significantly at  $P < 0.05$ , but in the left adrenal gland in squirrel the means were greater than those of hamster significantly at  $P < 0.05$ . In conclusion, the present findings showed the reaction of chromaffin cells of hamster with methylene-eosin stain to be stronger than with hematoxylin-eosin stain, while the opposite was true in case of the squirrel.

**Keywords:** chromaffin cells, squirrel, methylene blue, hamster

### INTRODUCTION

The African giant rat's adrenal medulla was found to consist of clusters of granular, mildly basophilic cytoplasm cells with multiple capillaries in its fine supporting stroma. The adrenal medulla contained around a quarter of the gland. The retained catecholamine granules of the adrenal medullary cells (chromaffin cells) were oxidized to a brown color when the gland had been fixed in potassium dichromate fixative (1). The medulla region was composed of ovoid group of cells (chromaffin cells) that arranged in irregular cords separated by blood sinusoid and surrounded by central vein, and there were two types

of cells, the first was columnar in shape and brownish in color representing the epinephrine secreting cell, and the second type was polygonal in shape and light brownish in color with spherical nucleus representing the norepinephrine secreting cells when fixed in chromate salts (2). The adrenal medulla parenchyma of porcupine was shown to consist of poorly arranged cells into clusters and strings. Chromaffin cells were columnar in form and the cells were darkly identified with a very basophilic nucleolus. The cytoplasm was treated basophilically and distinctly isolated from the cortex (3).

The adrenal medulla constituted approximately one quarter (25.7%) of the hystrixcristate gland area. The

chromaffin cells were found in irregular clusters and at the cortico-medullary cortical boundary cells. The cells that stored epinephrine (E-cells) were found to be more abundant and smaller than those that stored norepinephrine (NE-cells). The bulks were moderately dense electron E granules, but some were extremely dense NE granules. Rarely have the ganglion cells been detected. Instead of a large central venula, multiple central sinusoidal vessels were found. Both cells had one single, large spherical nucleus. Nucleoli have been well described, and up to three nuclei per cell have been observed on occasion. In terms of granulate size and form, E-cells are similar to NE-cells (4).

The adrenal medulla was observed to consist of chromaffin cells that formed clusters and cords forming anastomosis. In addition, adrenal medulla comprised of two types of chromaffin cells. The norepinephrine cells had a large spherical nucleus and granules extremely dense with electrons. The epinephrine cells were similar to norepinephrine cells, but their granules were less dense electrons, and there was a small empty space between granules and boundary membrane (5).

## MATERIALS AND METHODS

The procedures used in this study were reviewed and approved by the scientific committee at the University of Baghdad's College of Veterinary Medicine in compliance with animal welfare ethical standards.

In this study, 20 animals were used, 10 of which were squirrels and the other 10 were hamsters. They were euthanized by inhaling chloroform. The adrenal glands were removed from and placed in Orth's solution for 24 hours, and then treated with the routine staining of hematoxylin and eosin as well as methylene blue (1%) and eosin for studying the histological characteristics. Then, the slides were examined with the light microscope, and by using an ocular lens, the measurements of the diameter of cells and the nuclei of adrenaline and nor adrenaline cells were determined for both animal groups. Statistical analysis was applied using two ways ANOVA and T-test, the means were considered statistically significant at the level of  $P \leq 0.05$ .

## Reagents

Orth's stock solution consisted of potassium dichromate (25 g) and sodium sulfate (10 g) dissolved in 1000 mL of distilled water.

## Orth's Working Solution

Orth's working solution was made up of 50 mL of the stock solution. Just before use, 5 mL of 37% formaldehyde were added and fixed for 24 h, and after fixation the

samples incubated in water bath. The fixed samples were stored in 70% alcohol.

## RESULTS AND DISCUSSION

Medulla was composed of ovoid group of cells (chromaffin cells) that arranged in irregular cords separated by blood sinusoid and surrounded by central vein. There were two types of chromaffin cells, the first type was columnar in shape and brownish in color contained spherical nucleus located at the base of the cell, this represents the epinephrine secreting cell, the second type was polygonal in shape and light brownish in color contained spherical nucleus located in the center of the cell, this represents the norepinephrine secreting cell (Figures 1 and 2). These cells also appear as pale brown after fixation with potassium dichromate (Figure 3). Similar results were reported in the medulla of guinea pigs and vizcacha, in which the medulla composed of ovoid group of cells (chromaffin cells) that arranged in irregular cords separated by blood sinusoid (6, 16). These cells also appeared as light blue with Methylene-Eosin staining (Figure 4). The results obtained by the methods used by the above studies mainly depended upon the experience of the histologists. A successful preparation demonstrated well the nucleus, cytoplasm, and cell granules.

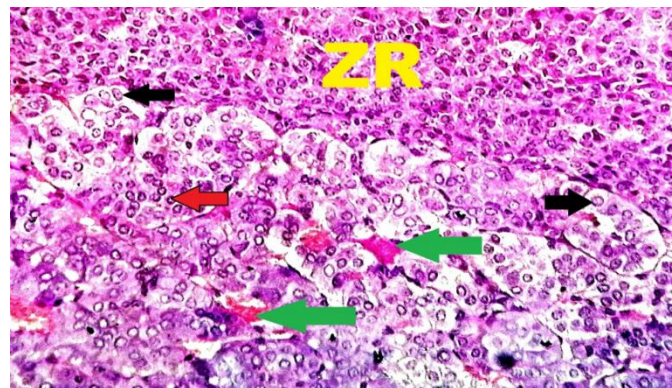


Figure 1. Squirrel histological section shows zonareticularis (ZR) and medulla. Chromaffin cells (black arrow) and sinusoid (green arrow). H&E, 40 $\times$

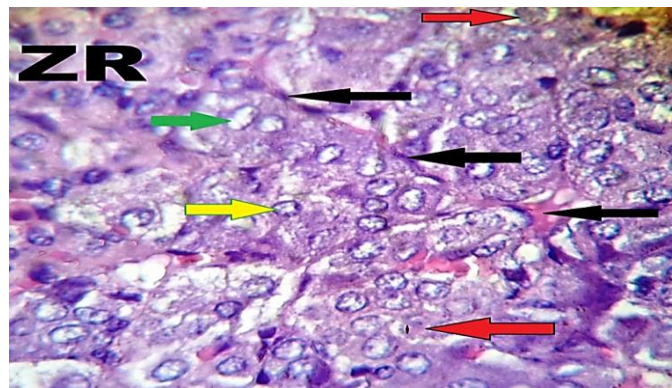
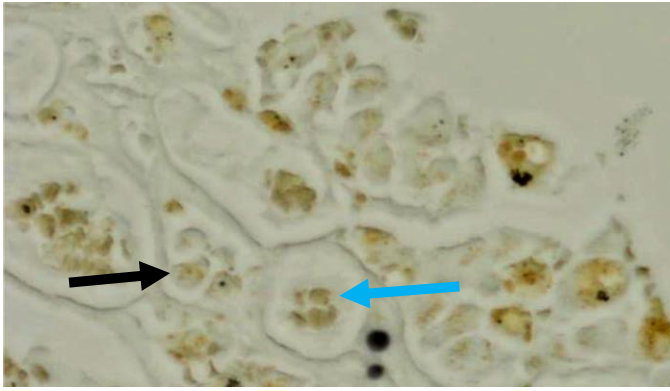


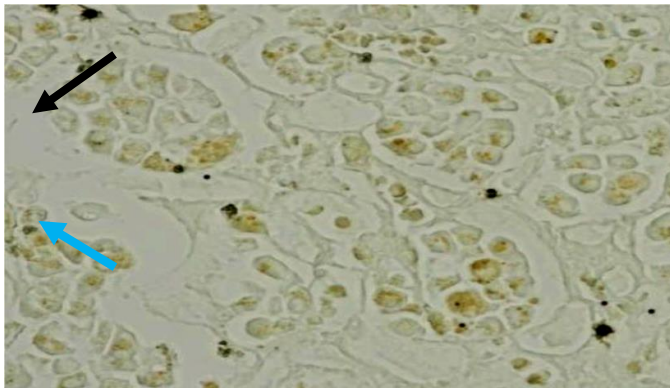
Figure 2. Hamster histological section shows medulla and zonareticularis (ZR) sinusoid (black arrow), chromaffin cell (yellow arrow) (green arrow) and postganglionic cells (red arrow). H&E, 100 $\times$



**Figure 3.** Histochemical section of medulla of squirrel adrenal gland showing a noradrenaline cells (blue arrow) and adrenaline cells (black arrow). Potassium dichromate salts. H&E,1000×



**Figure 4.** Histochemical section of medulla of squirrel adrenal gland showing medulla (red star), cortex (yellow star), chromaffin cells (green arrow) and central vein (black star). Potassium dichromate salts, methylene blue-eosin stain, 400×



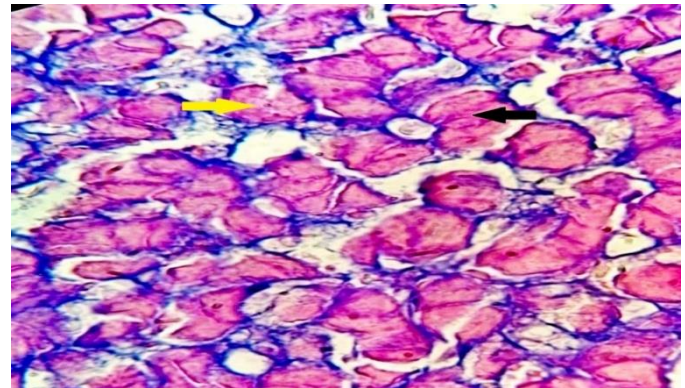
**Figure 5.** Histochemical section of medulla of squirrel adrenal gland showing adrenaline cells (black arrow) and noradrenaline cells (blue arrow). Potassium dichromate salts. H&E, 1000×

The means of diameter of the epinephrine cells and their nuclei in the right adrenal gland were  $18 \pm 0.97 \mu\text{m}$  and  $23.7 \pm 0.44 \mu\text{m}$ , in the left adrenal gland were  $10.4 \pm 0.73 \mu\text{m}$  and  $12 \pm 0.52 \mu\text{m}$ , there is a significant difference between the diameter means of the epinephrine cells and nuclei in the right and left adrenal gland of squirrel at  $p < 0.05$ , in which the left adrenal gland was greater than the right one (Table 1).

The means of diameter of the norepinephrine cells and the nuclei in the right adrenal gland were  $13.2 \pm 0.38 \mu\text{m}$  and  $14.25 \pm 0.75 \mu\text{m}$ , while in the left adrenal gland were

$7.25 \pm 0.16 \mu\text{m}$  and  $8.12 \pm 0.72 \mu\text{m}$ , there is a significant difference between the means of diameter of the epinephrine cells and their nuclei in the right and left adrenal glands of squirrel at  $P < 0.05$ , in which the left gland was greater than the right one (Table 1). There is a significant difference in the diameter mean of cells of the right and left adrenal glands between squirrel and hamster depending on the activity of the gland. Also, the statistical analysis showed that the means of diameter of nuclei in the left and right adrenal glands in squirrel were significantly lower ( $P < 0.05$ ) than those of hamster due to variations in species and nutrition.

The adrenal medulla of hamster consists almost entirely of columnar or polyhedral chromaffin cells forming clusters and anastomosing cords separated by sinusoids. The outer and inner zone of the medulla can sometimes be separated. While the outer zone composed of larger and darker stained cells, the inner zone comprised of smaller and lighter stained cells, since the reticularis projections that appear within the medulla at the junction of the cortex and medulla interdigitate. There are two types of chromaffin cells, the type of secreting epinephrine has bigger and less dense granules, and the type of secreting norepinephrine has somewhat smaller dense granules. The medulla consists of mainly modified postganglionic sympathetic neurons with heavy chromium salt stains and multiple brown granules in the cytoplasm (Figure 3).



**Figure 6.** Histochemical section of hamster medulla showing adrenaline cells (black arrow) and noradrenaline cells (yellow arrow). Methylene blue-eosin stain,1000×

The chromaffin cells had been observed in irregular clusters. Most of them had fewer electron-dense granules with an open boundary, but others had very dense electron granules.

These results are similar with results reported by (7) in African giant rats, (8) in domestic animals and (9) in Guinea pigs. These cells also look pale blue after fixation with potassium dichromate and staining with methylene blue-eosin (Figure 5), and pale brown after staining with H&E (Figure 6). Aqueous or alcoholic solutions of eosin and aqueous solutions of methylene-blue require to be independently and consecutively employed for the double staining of sections.

**Table 1.** The diameter of the cells and nuclei of different parts of the adrenal glands of squirrel and hamster

Type of animal	Layers of gland	Diameter of Cell ( $\mu\text{m}$ )			Diameter of Nucleus ( $\mu\text{m}$ )		
		Right adrenal	Left adrenal	t-test	Right adrenal	Left adrenal	t-test
Squirrel	epinephrine cell	18.0 $\pm$ 0.97	23.7 $\pm$ 0.44	0.002 NS	10.4 $\pm$ 0.73	12 $\pm$ 0.52	0.182*
	norepinephrine cell	13.2 $\pm$ 0.38	14.25 $\pm$ 0.7	0.125*	7.25 $\pm$ 0.16	8.12 $\pm$ 0.72	0.141*
Hamster	epinephrine cell	19.2 $\pm$ 1.29	20.9 $\pm$ 1.62	0.437*	10.7 $\pm$ 0.69	11.5 $\pm$ 1.01	0.551*
	Norepinephrine cell	13.7 $\pm$ 0.44	13.6 $\pm$ 0.90	0.922*	7.69 $\pm$ 0.36	8.67 $\pm$ 0.85	0.311*

Mean $\pm$ SE. \*P<0.05. NS= non-significant

The means of diameter of the epinephrine cells and their nuclei in the right adrenal gland were 19.2 $\pm$ 1.29  $\mu\text{m}$  and 10.7 $\pm$ 0.69  $\mu\text{m}$ , in the left adrenal gland were 20.9 $\pm$ 1.62  $\mu\text{m}$  and 11.5 $\pm$ 1.01  $\mu\text{m}$ , there is a significant difference between the diameters of the epinephrine cells and the nuclei in the right and left adrenal glands of hamster at P< 0.05, in which the left gland was greater than the right one (Table 1). These results coincide with (10-12) in rats and with (13) in squirrel *Sciurus anomalus*, and with domestic animals (14). However, these results disagree with (15) in *Galeaspixii*. The statistical analysis showed that the means of diameter of epinephrine cells in the right adrenal gland in squirrel were significantly lesser (P<0.05) than those of hamster (Table 1), but in the left adrenal gland of squirrel were significantly greater (P<0.05) than those of hamster (Table 1).

The means of diameter of the norepinephrine cells and their nuclei in the right adrenal gland were 13.6 $\pm$ 0.44  $\mu\text{m}$  and 7.69 $\pm$ 0.36  $\mu\text{m}$ , while in the left adrenal gland the means were 13.7 $\pm$ 0.90  $\mu\text{m}$  and 8.67 $\pm$ 0.85  $\mu\text{m}$ . There is a significant difference (P<0.05) between the mean of diameter of the epinephrine cells and their nuclei in the right and left adrenal glands of hamster, in which the left gland was greater than the right one (Table 1). The statistical analysis showed that the means of diameter of norepinephrine cells in the right adrenal gland of squirrel were significantly (P<0.05) lesser than those of hamster (Table 1), but in the left adrenal gland of squirrel the diameters were greater (P<0.05) than those of hamster (Table 1).

The current data revealed that the reaction of hamster chromaffin cells to methylene-eosin stain is stronger than that of hematoxylin-eosin stain, whereas the opposite was true in the case of the squirrel.

## ACKNOWLEDGEMENTS

The authors thank the College of Veterinary Medicine, University of Baghdad for providing supplies and devices for tissue processing, as well as the Department of Anatomy and Histology, College of Veterinary Medicine, University of AL-Qadisiyah for providing materials and stain.

## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

## REFERENCES

1. Al-Rubaie N. Anatomical and histological study of adrenal gland in Iraqi buffalo (*Bubalus bubalis*) with referring to the seasonal changes [Thesis]. Baghdad: Iraq. University of Baghdad; 2007.
2. Abbas L, Shakir M. Histomorphological and histochemical study of adrenal gland in adult male of guinea pigs (*Cavia porcellus*). The Iraqi. J. Vet. Med.2019; 43(1):59-66p.
3. Yilmaz S, Girgin A. Light and electron microscopic observations on the structure of the porcupine (*Hystrix cristata*) adrenal gland. Vet. Arhiv. 2005;75 (3): 265-272.
4. Stevens A, Lowe JS. Histology picture tests paperback. London: Gower medical pub; 1992 .260-263.
5. Dellman HD, Brown EM. Endocrinology textbook of veterinary histology. 3rd ed. Philadelphia: Lea and Febiger;1987. 361-381p.
6. 6-Sheikhian A, Saadatfar Z, Mohammad AA. A histological study of adrenal gland in guinea pig and hamster, Comp Clin Pathol., 2014;111:782-789.
7. Enemali FU. Anatomical studies on thyroid, parathyroid and adrenal gland of the African giant rat (*Cricetomys gambianus* Water House-1849) [Thesis] Zaria: Nigeria, Bello University, 2014.
8. 8- Bacha WJ, Bacha LM. Color atlas of veterinary histology. 2nd ed. USA, New York: John-Wiley-Sons-Inc; 2012; 191-200 p.
9. Al-sharoot HA. Histological study of the adrenal gland in guinea pig (*Cavia porcellus*). Int J Adv Res. 2014; 2(9): 755-760.
10. Mughal IA, Qureshi AS, Tahir MS. Some histological observations on postnatal growth of rat adrenal gland with advancing age. Int. J. Agri. Biol. 2005; 6(2):413-417.
11. Baiomy WK, Anas A, Ghaly M, Moustafa AM, Mohamed A W. Quantitative and histochemical study on the adrenal medulla in post-natal period of albino rat. Egypt J. Hosp Med. 2009; (36) 468-482.
12. Ezumi W, Amrah SS, Farid CG, Mohsin SJ. Morphological characteristics of the adrenals of *Rattus norvegicus*: a revisit by scanning electron microscopy. Annals of microscopy J. 200; 62-69.
13. Goodarzi N, Akbari G. Histomorphometry of adrenal gland of squirrel *Sciurus anomalus*. J Vet Res.2016; 20(11):691-697.
14. Sisson and Grossman. The anatomy of domestic animals revised by Grossman, J.D. 5th ed. WB. Saunders company: Philadelphia;1979: 573-580p.
15. Santos C, Viana DC, Bertassoli BM, Vasconcelos BG, Liveira DM, Rici EG. Adrenal glands of Spix's yellow-toothed cavy (*Galeaspixii*, Wagler, 1831): morphological and morphometric aspects. Braz. J. Biol. 2016; 76(3):.645-655.
16. Ribes AC, Mohamed F, Dominguez S, Delgado M, Scardapane L, Guzman J. Probable effect of photoperiod on seasonal variation in the nuclear volume of the adrenal cortex of viscacha (*Lagostomus maximus maximus*). Braz J Med Biol Res.1999;32(9):1115-1120.

مقارنة نسجية شكلية لخلايا كرومافين في ذكور السنجاب *Sciurus anomalous* و الهامستر *Mesocricetus auratus*عبد الرزاق باقر كاظم<sup>1</sup>، وإيمان موسى خليل<sup>2</sup><sup>1</sup> فرع التشريح و الانسجة، كلية الطب البيطري، جامعة القادسية، العراق، <sup>2</sup> فرع التشريح، كلية الطب البيطري، جامعة بغداد، العراق

## الخلاصة

هدفت الدراسة الحالية إلى التعرف على السمات النسيجية للخلايا الكرومافين للسنجاب والهامستر. عشرون حيوانًا، عشرة منها من السنجاب وعشرة أخرى من الهامستر. تم قتلهم رحيماً عن طريق استنشاق الكلوروفورم. أزيلت الغدة الكظرية منها ووضعت في محلول أورث لمدة 24 ساعة ثم عولجت بالتلوين الروتيني للهِماتوكسيلين و يوزين وكذلك الميتيل الأزرق 1% و يوزين. تم العثور على نوعين من خلايا chromaffin في كلا الحيوانين ، في السنجاب كانت الخلية الأولى عمودية في الشكل ولونها بني اللون تحتوي على نواة كروية تقع في قاعدة الخلية وهذا يمثل خلية إفراز الأدرينالين ، وكانت الخلية الثانية متعددة الأضلاع وذات لون بني فاتح يحتوي اللون على نواة كروية تقع في وسط الخلية وهذا يمثل خلية إفراز النوربينفرين. يتكون اللب الكظري للهامستر ، بالكامل تقريباً من خلايا كرومافين عمودية أو متعددة السطوح تشكل مجموعات وحبال مفصولة مفصولة عن طريق الجيوب الأنفية ، وتعطي تفاعلاً قوياً من صبغة الميتيلين إيوزين أكثر من السنجاب. أظهر التحليل الإحصائي أن متوسط القطر لخلايا الإبينفرين ولا خلايا الإبينفرين في الغدة الكظرية اليمنى في السنجاب كانت أقل بشكل ملحوظ من الهامستر عند  $P < 0.05$  ، ولكن في الغدة الكظرية اليسرى في السنجاب كانت أكبر من تلك الموجودة في الهامستر بشكل ملحوظ. عند  $p < 0.05$  في الختام أوضحت النتائج الحالية تفاعل خلايا الكرومافين للهامستر مع صبغة ميتيلين - يوزين قوية للتفاعل مع صبغة الهيماتوكسيلين يوزين ، بينما كان السنجاب عكس الهامستر

الكلمات المفتاحية: سنجاب، هامستر، كرومافين