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

Abdulrazzaq B kadhim*, and Iman M Khaleel

1Department of Anatomy and Histology, College of Veterinary Medicine, University of AL-Qadisiyah, Iraq, 2Department of Anatomy, College of Veterinary Medicine, University of Baghdad, Iraq

**ABSTRACT**

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 hystricocretaste 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**

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 (Figure 1). 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.
The means of diameter of the epinephrine cells and their nuclei in the right adrenal gland were \(18\pm0.97\) μm and \(23.7\pm0.44\) μm, in the left adrenal gland were \(10.4\pm0.73\) μm and \(12\pm0.52\) μm, there is a significant difference between the diameter means of the epinephrine cells and 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).

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.
The means of the diameter of the epinephrine cells and their nuclei in the right adrenal gland were 19.2±1.29 μm and 10.7±0.69 μm, in the left adrenal gland were 20.9±1.62 μm and 11.5±1.01 μm, 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, 11, 12) in rats and with (13) in squirrel Sciurusanomalus, 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±0.44 μm and 7.69±0.36 μm, while in the left adrenal gland of squirrel were significantly greater (P<0.05) than those of hamster (Table 1). The statistical analysis showed that the means of diameter of norepinephrine cells in the right adrenal gland of squirrel were significantly lesser (P<0.05) than those of hamster, but in the left adrenal gland of squirrel the diameters were greater (P<0.05) than those of hamster (Table 1).

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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مقارنة نسجية شكلية لخلايا كرومافين في ذكور السنجاب Sciurusanomalus و الهامستر Mesocricetusauratus

عبد الرزاق باقر كاظم1، وايمن موسى خليل2

الخلاصة
هدفت الدراسة الحالية إلى التعرف على السمات النسيجية للخلايا الكرومافين للسنجاب الحديدي والهامستر. عشرون حيواناً، عشرة منها من السنجاب وعشرة أخرى من الهمستر. تم قتلهم رحمة عن طريق استنشاق الكلوروفورم. أزيلت الغدد الكظرية من الحيوانات ووضعت في محلول أورث لمدة 24 ساعة ثم عولجت باللويني للهيماوتوكسين وفوقين وكذلك الميل الأزرق 1% ويوزين. تم العثور على نوعين من خلايا chromaffin في كل الحيوانات، في السنجاب كانت الخلايا العمودية في الشكل وعندها نواة بيضاوية. وعندها بطلة خليجية إفراز الإبينفرين. كانت الخلايا الثانية متعددة الأضلاع ونواة بيضاوية تقع في وسط الخلية وهي بطلة خليجية إفراز الإبينفرين. تشكل البلاست الكظرى للهامستر بالكامل إلى خلايا كرومافين عمودية أو محددة المعلومة. تحتوي هذه الخلايا على نواة بيضاوية تقع في وسط الخلية وتحتوي على بطلة خليجية إفراز الإبينفرين. تشكل الخلايا الكظرية للهامستر، بالكامل عن طريق الأورام تشل خلايا كرومافين عن طريق الأورام. ويعتبر تفاعلها مع صبغة ميثيلين - يوزين قوية للتفاعل مع صبغة اليهاموتوكسين - يوزين قوي للتفاعل مع صبغة ميثيلين - يوزين. في الختام، أظهرت النتائج الحالية تفاعل خلايا الكرومافين للهامستر مع صبغة ميثيلين - يوزين قوي للتفاعل مع صبغة اليهاموتوكسين - يوزين. بينما كان السنجاب عكس الهمستر في الخلايا المفتوحة.

الكلمات المفتاحية: