

# The cellular composition of the adrenal medulla of sexually mature guinea pigs

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*The adrenal glands from 15 guinea pigs aged 90 days were used in the study. Paraffin slices were stained with Mayer haematoxylin and eosin with Masson method and silvered. The histochemical reactions were carried out for catecholamines and the biochemical determinations of catecholamines. Ultrastructural observations were also performed.*

*In the adrenal medulla of sexually mature guinea pigs numerous epinephrocites and no norepinephrocites were observed when histochemical methods were used. The small and large ganglion cells accompanying the supporting and neurolemmal cells formed typical gangliar weaving (medullary ganglia). Apart from epinephrocites, small and large ganglion cells and supporting and neurolemmal cells, small intensively fluorescent (SIF) cells were also detected.*

*Biochemical investigation revealed that the mean content of adrenaline calculated for a pair of adrenal glands was 98.87% of the pool of catecholamines, whereas the mean content of noradrenaline was 1.00%, and of dopamine 0.13%.*

**Key words:** adrenal glands, chromaffin cells, ganglion cells, SIF cells

## INTRODUCTION

Medullary cells (i.e. chromaffin and ganglion cells) develop from sympathoadrenal cells originating from neural crests. The presence of two cell phenotypes in the adrenal medulla of mammals, namely epinephrocites and norepinephrocites has been reported by many investigators. In the anteater *Myrmecophaga jubata* and the sand hare, however, adrenal medullas were composed only of epinephrocites [18], and in many fish species (e.g. carp) only of norepinephrocites [12]. However, there is still some controversy as to how many cell types are present in the rabbit and guinea pig, since some researchers have reported the presence of both epinephrocites and norepinephrocites, whereas others have mentioned only one type of these cells [3, 12]. Because the guinea pig is a commonly used laboratory animal, the aim of the study was to determine the cellular com-

position of the adrenal medulla with particular attention to the phenotypes of the chromaffin cells.

## MATERIAL AND METHODS

The adrenal glands from 15 guinea pigs aged 90 days, i.e. sexually mature, and of both sexes were used in the study. After decapitation of the animals the adrenal glands were excised for examination and paraffin slices were stained with the Mayer haematoxylin and eosin, with the Masson method and then silvered using the Bodian and the Loots methods [16]. Histochemical reactions for catecholamines were carried out according to Kmiec [13], and Hopsu and Mäkinen [10]. The cellular content was visualised with the use of a EM-300 electron microscope [14, 15]. Biochemical determination of the catecholamines (adrenaline, noradrenaline and dopamine) in the homogenates from the gross adrenal glands was

performed using the HPLC method [17]. All the procedures were approved by the University of Medicine in Łódź Animal Care and Use Committee.

## RESULTS

18- $\mu\text{m}$ -in-diameter cells were predominantly observed in the adrenal medulla of sexually mature guinea pigs. The diameter of the nuclei in these cells was approximately 7  $\mu\text{m}$ , and the diameter of the nucleoli located near the nuclear membrane was approximately 1.5  $\mu\text{m}$ . The chromatic stroma was distinct and the cytoplasm of these cells was granular and slightly basophilous. Single or numerous cytoplasmic granules (0.2–1.0  $\mu\text{m}$  in diameter) were detected and the chromaffin cells showed granular vesicles (250–350 nm in diameter), corresponding to vesicles containing adrenaline.

Histochemical reactions for noradrenaline (Hop-su and Mäkinen method) in the medullary cells were negative (Fig. 1), although in some specimens ganglion nerve cells gave a trace reaction for this catecholamine. The histochemical reaction for both adrenaline and noradrenaline (according to Kmiec) revealed no cells with a positive reaction for noradrenaline, but only cells with a strong positive reaction for adrenaline (Fig. 2).

The ganglion cells of the adrenal medulla formed small groups or large aggregations, showing the typical ganglionic weaving (medullary ganglia). The diameters of these cells were variable, ranging from 19 to 50  $\mu\text{m}$ . In this group small (19 to 24  $\mu\text{m}$  in diameter) and large (35 to 50  $\mu\text{m}$  in diameter) cells were observed. In the vicinity of the ganglion cells numerous supporting and neurolemmal cells were detected. The cytoplasm of the ganglion cells was granular and very basophilous, particularly at their circumference. They contained large vesicular nuclei (approximately 12  $\mu\text{m}$  in diameter), which were located eccentrically. The latter showed 1–3 large nucleoli of around 3  $\mu\text{m}$  in diameter, predominantly located near the nuclear membrane. In the electron microscope these cells demonstrated granular vesicles of 40–200 nm in diameter.

In the silvered slices adrenal medulla ganglion cells with a variable number of argyrophilous granules were observed (Fig. 3). In the vicinity of the adrenal medulla ganglion cells numerous nerve fibres were observed, which extended to the chromaffin cells, amongst which they disappeared.

Apart from the medullary cells, small and large ganglion cells, supporting cells, neurolemmal cells and small intensively basophilous cells were ob-

served. These contained small chromaffin granules with a diameter of 60–120 nm (Fig. 4).

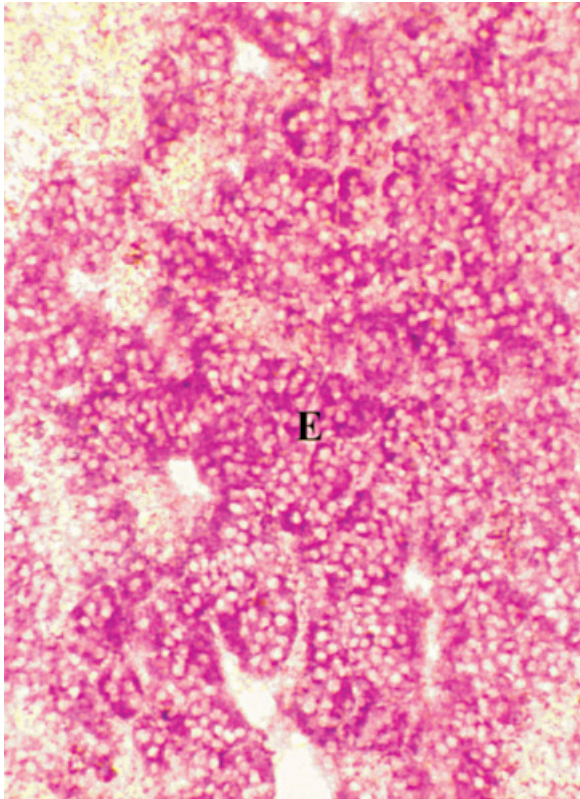
Biochemical investigation showed that the mean content of adrenaline in the adrenal medulla homogenates of sexually mature guinea pigs (calculated for a pair of adrenal glands) was 48388.3 ng (98.87% of the pool of catecholamines determined), whereas the mean content of noradrenaline was 491.8 ng (1.00%) and of dopamine 62.2 ng (0.13%).

## DISCUSSION

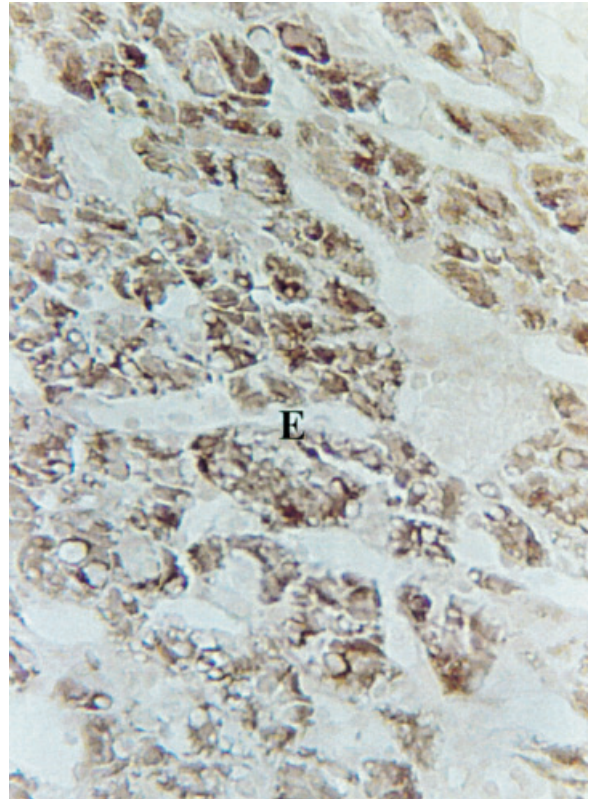
Our study has revealed that the adrenal medulla of sexually mature guinea pigs shows type IV weaving according to the Suzuki and Kach classification [19, 20]. The presence of cells producing and secreting adrenaline amongst the chromaffin cell phenotypes supports [1, 3, 4, 19, 20, 24] or is at variance [6, 7, 12] with the results of other investigators. Presumably this is the result of the disclosure of the small intensively fluorescent (SIF) cells, which were considered norepinephrocytes [4]. The extremely low content of noradrenaline in the adrenal gland homogenates of sexually mature guinea pigs in comparison with other animals only testifies to the presence of epinephrocytes. Noradrenaline and dopamine are presumably the pool of catecholamines present in the ganglion cells and the SIF (small granule containing SGC) cells.

Benedeczy et al. [2] found three types of chromaffin cell in the adrenal glands of the grass snake *Natrix natrix*. Cells with granular vesicles of diameter of around 250 nm corresponded to norepinephrocytes and those of diameter of 125 nm to epinephrocytes. The third type of cell with both small and large granular vesicles as well as well-developed rough endoplasmic reticulum and numerous ribosomes presumably contained dopamine [11, 12]. Similar cells (so-called SGC cells) were also reported in the adrenal medulla of the rat [5] and of man during development [9] and in the adrenal glands of sexually mature birds and reptiles [21–23]. Our study supports previous opinions [21, 22] that the majority of these cells, if not the total number, appear to be identical with the SIF cells, which were firstly reported by Eränkö and Härkönen [8] in the superior cervical sympathetic ganglion of the white rat.

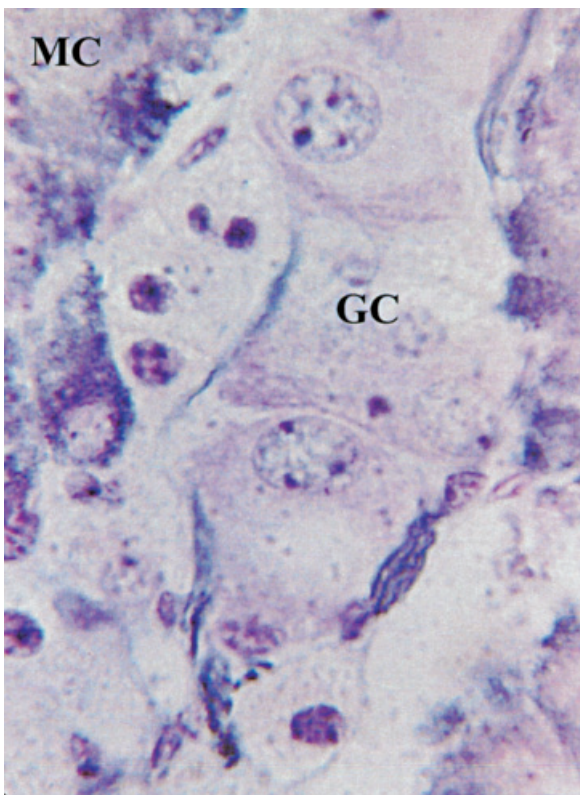
In conclusion, adrenal medulla cells, due to close "territorial" proximity, form a morphological and functional complex with the sympathetic nervous system and the adrenal cortex.



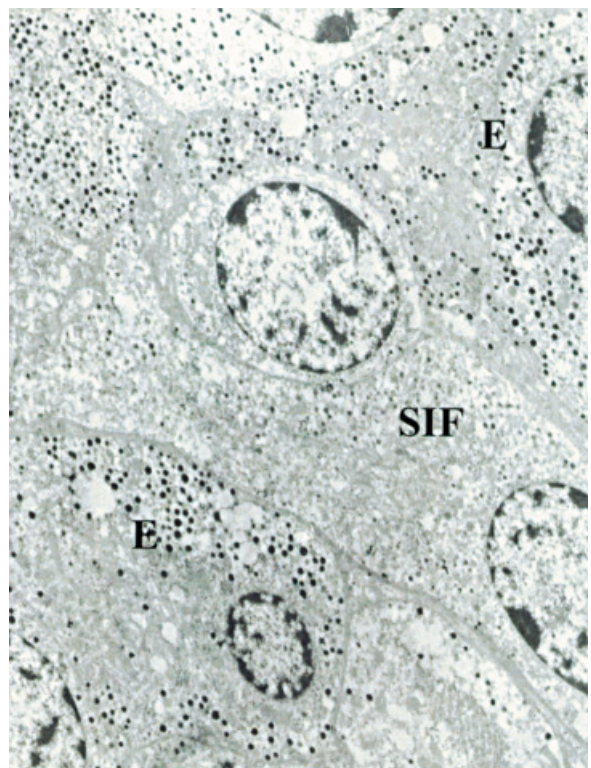
**Figure 1.** Epinephrocytes (E). Histochemical reaction for noradrenaline according the Hopsu and Mäkinen method. Enl. 220 ×.



**Figure 2.** Epinephrocytes (E). Histochemical reaction for noradrenaline according the Kmieć method. Enl. 220 ×.



**Figure 3.** Ganglion cells (GC), medullary cells (MC). Silvering according to Bodian. Enl. 560 ×.



**Figure 4.** Small intensively fluorescent cells (SIF) and epinephrocytes (E). Double immersion fixation of the slices in the Karnovsky and Dalton fluid with the Kmieć modification (chromaffin reaction). Enl. 4000 ×.

## ACKNOWLEDGEMENTS

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