Number, size and distribution of ganglion neurons in urinary bladder of rodents

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Whole-mount preparations of urinary bladders stained with a modified Giemsa technique were obtained from three rodent species (Guinea-pig, ***Calomys callosus*** *and the C57/BLJ isogenic mouse) to identify and estimate the relative number and size of ganglionic neurons within the wall of the organ. The distribution of the ganglia was not uniform among the three species: ganglia were concentrated around the ureteral orifices in the Guinea-pig, they were scattered throughout the organ in* ***Calomys callosus****, and they were concentrated near the internal urethral orifice in the C57/BLJ mouse. In the Guinea-pig, the size of approximately 50% of the neurons lie in the range of 200 to 300 μm². In* ***Calomys callosus****, 40% of the neurons lie in the range of 200 to 250 μm², with 28% in the range of 50 to 100 μm². For the C57/BLJ mouse, approximately 60% of the neurons have an area of 250 to 400 μm².*

Key words: *autonomic nervous system, ganglionic neurons, rodents, urinary bladder.*

INTRODUCTION

The presence of ganglionic neurons within the wall of the urinary bladder has been found in several species, including man (Iwasaki, 1951; Gilpin *et al*, 1983), dog (Watanabe, 1954) and cat (Feher *et al*, 1979).

Although in mammals much is known about the general arrangement and histochemistry of the intramural plexus of the urinary bladder (El-Badawi and Schenk, 1966; Ek *et al*, 1977; Crowe *et al*, 1986), quantitative data on the neurons such as number and size, are available only for the Guinea-pig urinary bladder. In the urinary bladder of adult Guinea-pigs, counts on whole-mount preparations of entire bladders (Gabella, 1990) reveal the presence of 2000 to 2500 neurons per bladder, either as individual nerve cells or, more frequently, in the form of ganglia containing up to 40 neurons. In contrast, intramural ganglionic neurons have not been found in the urinary bladders of the rat and mouse, or in the ferret and rabbit, where they occur as a few dozen neurons at most, attesting that the extent of the intrinsic neuronal apparatus of the urinary bladder remains uncertain (Gabella, 1990).

Furthermore, estimates of the number and size of neurons in the urinary bladders of certain important laboratory animals, such as *Calomys callosus*, are not available in the literature. This animal is a cricetine rodent, similar to a mouse, commonly found in fields of South America, analyzed in some biological aspects as immunology and physiology (Petter *et al*, 1967; Justines and Johnson, 1970; Mello, 1978), and described in Brazil as harboring *Trypanosoma cruzi* (Ribeiro, 1973). Data on isogenic mouse C57/BLJ are also lacking.

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In the present study using whole-mount preparations, we intended to estimate the number of ganglia and neurons and their size and distribution in the urinary bladders of three rodent species: the Guinea-pig, *Calomys callosus* and the isogenic mouse (C57/BLJ). These data may be important for future physiological and pathological studies.

MATERIAL AND METHODS

Three adult Guinea-pigs, three wild mice (*Calomys callosus*) and three isogenic mice (C57/BLJ) were used. The animals were sacrificed with an overdose of ether and after opening the abdominal wall and cutting the pubic symphysis, the urinary bladder was removed with the distal segment of the ureter and the proximal portion of the urethra. The bladder was filled with Giemsa's fixative solution moderately distending the wall and immersed in the same fixative for 18 hours. It was then opened, the mucosa removed under a dissecting microscope and stained with a modified Giemsa's technique (Barbosa, 1978). Twelve hours later, the whole specimens were dehydrated in an alcohol series, diaphanized with xylene and mounted in resin as stretch or whole-mount preparations.

The numbers of ganglia and neurons were obtained by examining the whole-mount preparations under a binocular microscope at magnifications of 400 and 1000 X, respectively. All ganglia and neurons present in each bladder were counted. The profiles of 300 neural perikarya for each species were outlined on drawing paper using a *camera lucida* attached to a microscope. The areas of these nerve cell bodies were calculated using a digitizing pad and stereometric analysis on a personal computer.

RESULTS

The intramural neurons were readily identified in all whole-mount preparations of the bladders stained with Giemsa's method. The weakly stained muscle bundles and connective tissue did not obscure the neurons whose cell bodies stained dark blue, revealing very little variation in the staining intensity. The staining technique employed resulted in sharply delimited perikarya and clear visualization of the nuclei while the cell processes remained unstained.

The distribution of the ganglia was not uniform in the three species. Thus, in the Guinea-pig, although the ganglia were observed in all the extension of the urinary bladder, they were concentrated around the ureteral orifices. In *Calomys callosus*, the ganglia were scattered along the organ. In the C57/BLJ mouse, the ganglia were all concentrated near the urethral orifice. (See Fig 1).

Most of the neuronal cell bodies were circular in profile although some were elongated, with the long axis being twice the short axis (Fig 2A). While several isolated and paired neurons were found in all animals (Fig 2B), most intramural neurons were clustered in circular or elongated ganglia, containing a variable number of nerve cell bodies (Fig 2C-D).

The mean areas of the urinary bladders, the mean number of neurons and the mean number of ganglia obtained from the three species are presented in Table I, together with the mean area of the nerve cell bodies. While 2043 neurons per bladder were seen in the Guinea-pig, they were 1593 in the C57/BLJ mouse, and only 38 neurons per bladder were found in *Calomys callosus*.

The areas of the neurons (maximal cellular profiles) in the urinary bladder of the three rodent species are shown in Figure 3. In the Guinea-pig, neuron area ranges from 100 μm^2 to 400 μm^2, with approximately 50% of the neurons in the range of 200 μm^2 to 300 μm^2. In *Calomys callosus*, the neurons range in area from less than 50 μm^2 to cell bodies of 250 μm^2; approximately 40% of the neurons lie in the range of 200 μm^2 to 250 μm^2, with 28% in the range of 50 μm^2 to 100 μm^2. For the isogenic mice (C57/BLJ), the size range extends from 100 μm^2 to 600 μm^2, with approximately 60% of the neurons in the range of 250 μm^2 to 400 μm^2.

DISCUSSION

The stretch or whole-mount preparations have been used to estimate the number of

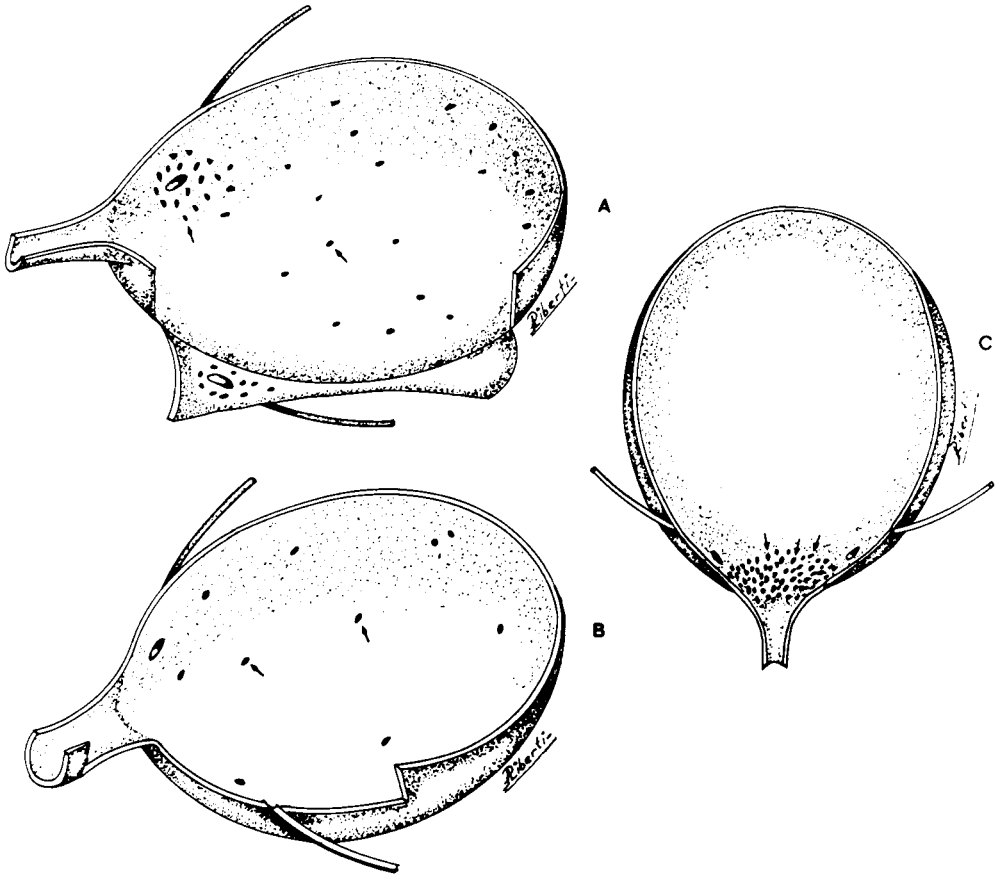

Fig 1. Schematic representation of the ganglia's distribution in the urinary bladder. A. Guinea-pig. B. *Calomys callosus*. C. C57/BLJ isogenic mouse. In this figure we do not attempt to express the total number of ganglia.

neurons in hollow viscera, as the esophagus (Wells *et al*, 1987; Kumar and Phillips, 1989), small and large intestines of humans (Murat, 1933; Sternini, 1988; De Souza *et al*, 1993), as well as the trachea, gut, gall bladder and urinary bladder of many kinds of animals (Irwin, 1931; Matsuo, 1934; Tafuri, 1957; Ali and McLelland, 1979; Chiang and Gabella, 1986; Gabella, 1987).

The staining method employed to identify ganglionic neurons in whole-mount preparations of the urinary bladder was developed long ago by Barbosa (1978) to study the enteric ganglia and has since been widely used by others investigators (*e.g.*, De Souza *et al*, 1982, 1988; Ferraz de Carvalho *et al*, 1983). We found this method suitable for studying the ganglionic plexus in the bladder, because the method selectively stains the nerve cells, leaving others cells unstained or only faintly stained.

Although there is some variation in the intensity of staining among the nerve cells, there is no evidence that any significant number of intramural neurons remained undetected. The cells which stained intensely were undoubtedly neurons owing to their typical morphology. Furthermore, the results of this study confirm those of Gabella (1990) who demonstrated, with the aid of an NADH stain, that the intramural ganglia present in the Guinea-pig urinary bladder contain from 2000 to 2500 neurons per bladder. The staining method we employed resulted in a mean of 2043 neurons for the Guinea-pig bladder.

As seen in the Guinea-pig (Crowe *et al*, 1986), intramural neurons are also found in the urinary bladder of man (Gilpin *et al*, 1983) and cat (Feher *et al*, 1979). However, these types of neurons have not been found in the bladder of the mouse or the rat. In the rabbit and ferret such neurons amount to a

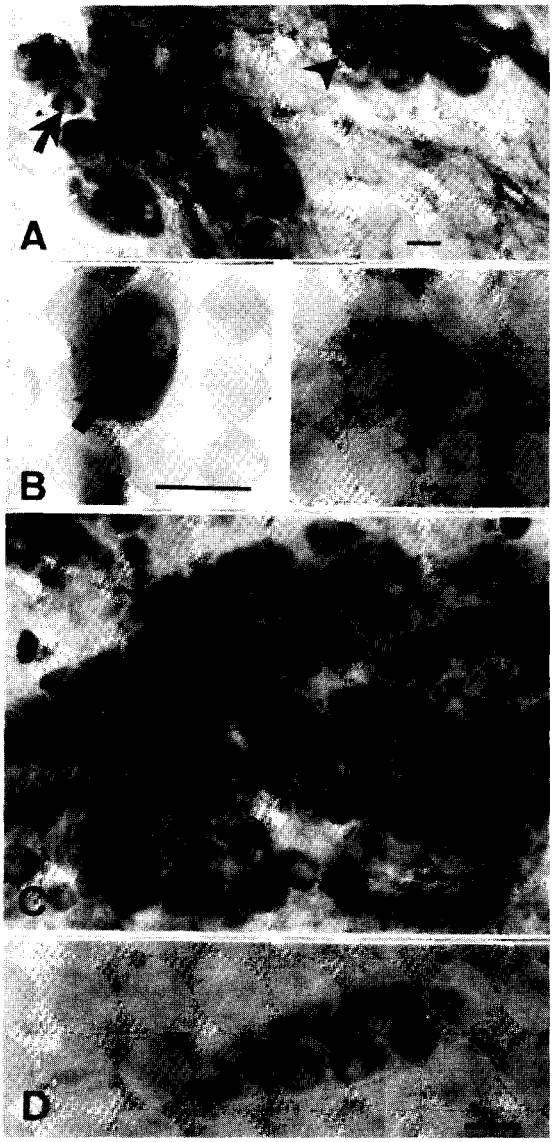

Fig 2. Whole-mount preparations of urinary bladders stained with Giemsa method. A. Ganglion from Guinea-pig with round neuronal cell bodies (arrow) and elongated neuronal cell bodies (arrowhead). Calibration bar 20 μm. B. Isolated (arrow) and "paired" neurons (arrowhead) from Guinea-pig. Calibration bar, 15 μm. C. Large ganglion from isogenic mouse C57/BLJ with a large number of neurons. Calibration bar, 20 μm. D. Small ganglion of *Calomys callosus* with a small number of neurons. Calibration bar, 20 μm.

few tens of cells at most (Gabella, 1990). Our results are in partial agreement with these data since the number of neurons was fewer in *Calomys callosus* than in the Guinea-pig although fairly high in the isogenic mouse (C57/BLJ). These findings corroborate Gabella's (1987) assertion that the packing density of intramural neurons is higher in species of smaller body size.

Ganglia and neurons were present in all the specimens we studied, although their densities were not uniform. Thus, although ganglia and neurons were found in all parts of the Guinea-pig bladder, most were located in the region near the entrance of the ureter. According to Gabella (1990), this area of the bladder is also the point of entry of the two major urinary arteries. The neuronal precursors that colonize the bladder during embryonic life may penetrate this organ by migrating along the vessels. Should this be so, then the distribution of the intramural neurons may reflect aspects of the migratory process of ganglion cells. In *Calomys callosus*, the few ganglia present were scattered throughout the entire bladder, while in the isogenic mouse (C57/BLJ) despite the abundant ganglia observed, their density was greatest near the internal urethral orifice. The presence of such a concentration of neurons in these regions may be related to control of the local sphincteric mechanism. Relationships of this type are known to occur in several sphincteric regions of the digestive tract (Palumbi, 1933; Indar-Jit, 1951; Damiani and Batistelli, 1956; Lorenz, 1962; Ferraz de Carvalho *et al*, 1983). Why these differences occur among these three rodents was not evident in the literature until now.

The number of neurons in the urinary bladders of the three rodent species is very low when compared with those seen in the enteric plexus along the digestive tracts of many other animals (Gabella, 1987), including the Guinea-pig and the mouse. Conversely, with the exception of *Calomys callosus*, the number of neurons is higher than that seen in the tracheal plexus of mice (Chiang and Gabella, 1986).

Table I

Mean values of urinary bladder area (**UBA**), total number of neurons (**TNN**), number of ganglia (**NG**) and neurons area (**NA**) in three rodent species.

Species	UBA cm^2	TNN	NG	NA μm^2
Guinea pig	2.7	2043	202	331
C57/BLJ mouse	0.78	1593	58	321
Calomys callosus	0.65	38	17	148

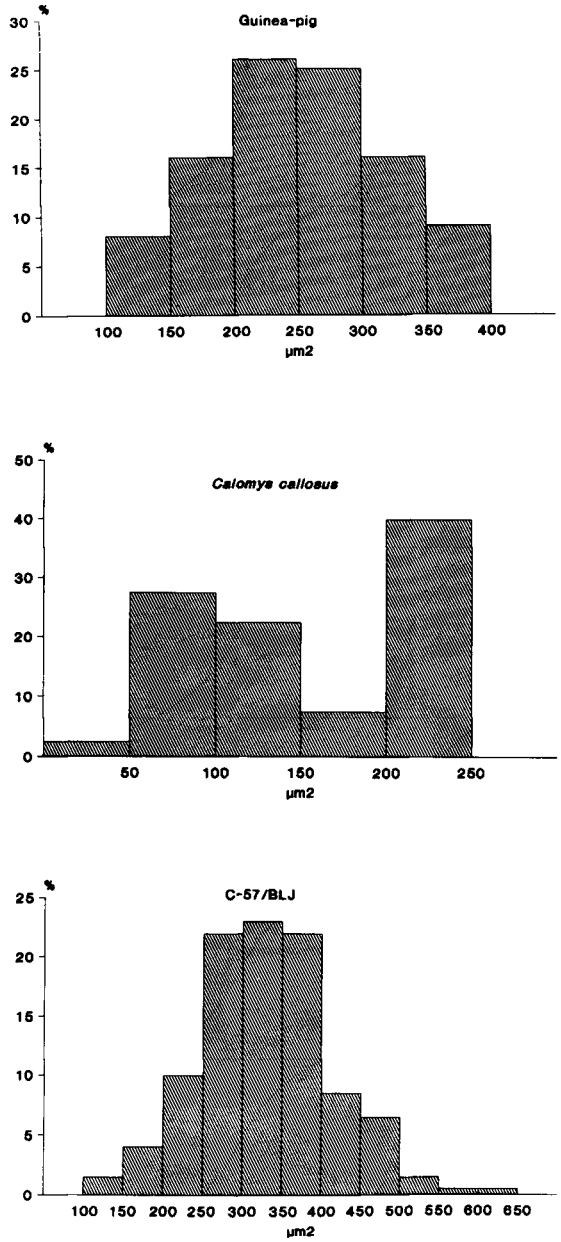

Fig 3. Histograms of percentages of neurons with different sizes in the plexuses of the urinary bladders of Guinea-pig (top), *Calomys callosus* (middle) and C57/BLJ isogenic mouse (bottom).

In terms of neurons size, the nerve cells of the urinary bladder do not form a uniform population. However, in the Guinea-pig and isogenic mouse (C57/BLJ), the variability in the area shows that the neuron size varies less in the urinary bladder than in the enteric ganglia (Gabella, 1971) and thus resembles the ganglia of the rat tracheal plexus (Gabella and Trigg, 1984). According to Gabella *et al* (1988), one of the characteristics of nerve cells, and particularly of those whose axons or cell bodies lie outside the CNS, is a large variation in perikaryon size; this variation in size within a population of neurons may be related to different functional specializations or to differences in the extent of their territories of innervation (Chiang and Gabella, 1986).

In the dog (Hamberger *et al*, 1965) and in the rat (Schulman *et al*, 1972), the majority of neurons in the urinary bladder plexus shows a positive reaction for acetylcholinesterase, while a minority shows fluorescence for catecholamines. However, little is known of the significance of this variability or of the underlying mechanisms.

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