An Amphoteric Character in Mast Cell Granules of *Amphisbaenia fuliginosa*, a Snakelike Lizard

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Summary. By the use of haematoxylin-eosin and Masson's trichrome staining techniques, and of histochemical tests with Alcian blue and metachromasia with toluidine blue, the mast cell granules of the snakelike lizard *Amphisbaenia fuliginosa* are shown to present an amphoteric character: they are stained by the acid as well by the basic dyes.

This property is here discussed in function of the literature, with the main concern directed toward the taxonomic position of this animal. The Amphisbaenidae seem to represent an intermediate stage between lizards and ophidians.

The mast cell granules of lizards are basophilic and metachromatic; in the ophidian, typical mast cells have not been evidenced yet, but a granular acidophilic cell has been demonstrated in the connective tissue. The basophilic and acidophilic characters of these two cells seem to be intermingled in the mast cell of the Amphisbaenidae.

In a general way, the identification of mast cells is made by the use of histochemical techniques that can detect acid mucopolysaccharides, mainly the sulfated (heparin), in the cytoplasmic granules.

Mast cells have been demonstrated by several authors in higher and lower vertebrates. They have thus been evidenced in fishes (MICHELS, 1922b; BOLTON, 1933; STOLK, 1960a; ARVY, 1962); in amphibians (MAXIMOW, 1927; ARVY, 1962; SACERDOTE and PENNIZI, 1965); in lizards (STOLK, 1958; 1960b); in alligators (ARVY, 1962); in turtles (JORDAN and FLIPPIN, 1913; MICHELS, 1922a); in birds (DANTSCHAROFF, 1916; ARVY, 1955) and in mammals (NAGAYO, 1928; MICHELS, 1938; ARVY, 1955; 1962; MOTA et al., 1956; RILEY and WEST, 1956; GRUMBERG, 1965).

In our recent work using ophidians we noticed that mast cells could not be identified in this animal group (SOTTOVIA-FILHO and JUNQUEIRA, 1972a; SOTTOVIA-FILHO et al., 1972b).

In continuing our phylogenetic studies of the mast cells in reptiles, we have decided to investigate the presence as well as the histochemical behavior of these cells in the Amphisbaenidae. The main interest in the choice of this animal was its particular systematic position, between lizards and ophidians, which might give a clue to explain the absence of mast cells in ophidians, since they have been demonstrated in all the vertebrates which have been studied.

Material and Methods

Three samples of the species *Amphisbaenia fuliginosa* were used, two males and one female. The animals were sacrificed by decapitation and pieces of all of the main organs were obtained. The pieces were fixed in alcohol-formol and 4% basic lead acetate, both prepared according to SELYE (1965); Gendre's fluid and buffered neutral
formalin, according to Conn et al. (1965). The pieces were then embedded in paraffin and serial sections 7 μ thick were cut.

The staining techniques used for the morphological analysis were: haematoxylin-eosin and Masson's trichrome, according to Conn et al. (1965). For the histochemical study, the following reactions were examined: periodic acid-Schiff (PAS), according to McManus and as described by Lison (1960); metachromasia with 0.01% toluidine blue and acetic-Alcian blue staining, according to Conn et al. (1965); PAS plus Alcian blue, according to Vialli and as described by Lison (1960).

**Results**

Mast cells have been evidenced by metachromasia with toluidine blue as well as by Alcian blue staining in all the organs examined, mostly near blood vessels and nerve bundles (Fig. 1, 3). The cytoplasmic granules were PAS positive and, by

![Fig. 1. Mast cells of *Amphisbaenia* near a nerve bundle (NB). Toluidine blue. ×250](image1)

![Fig. 2. The same field of Fig. 1 (serial sections). Note the cytoplasmic granules of mast cells stained by eosin (arrows). Hematoxylin and eosin. ×250](image2)
Vialli's combined technique, PAS and Alcian blue positive granules were intermingled within the same cell.

Curiously, a certain acidophilia was recognized by haematoxylin-eosin and by Masson's trichrome techniques, as some granules were stained by eosin and by ponceau-acid-fuchsin (Fig. 1, 2).

Discussion

The basophilia of mast cell granules is well demonstrated, being responsible for the metachromasia. It is known that these staining properties are related to the presence of a sulfated acid mucopolysaccharide, heparin (SYLVÉN, 1954; HALE, 1957; LISON, 1960). The metachromatic reaction as well as the Alcian blue positiveness of the mast cell granules of the *Amphisbaenia fuliginosa* may then be related to the presence of heparin.
As to the results obtained by combined technique, PAS positive and Alcian blue positive granules were found to coexist within the same cell. These different reactions have been attributed by several authors (Hale, 1957; Arvy and Rancurel, 1958; Takeda, 1958; Combs et al., 1965; Grumberg, 1965) to a variation in the composition of the cell secretion. According to them the immature granules containing heparin precursors (monosulfated heparin acid) would be PAS positive and after the esterification of these precursors by sulfate radicals this positiveness would be lost. The mature granules (polysulfatic esters of heparin) would then be metachromatic and Alcian blue positive. Our results, obtained by Vialli’s combined technique, could be related to this same process.

In earlier works (Sottovia-Filho and Junqueira, 1972a; Sottovia-Filho et al., 1972b,) we could not evidence mast cells in the studied ophidians, by the use of histochemical and radioautographic methods nor by biological assays. On the other hand, granular acidophilic cells were found in the connective tissue of these animals (Sottovia-Filho et al., 1972c, d). The granular acidophilic cells present cytoplasmic granules which are acidophilic with haematoxylin-eosin and Masson's trichrome techniques; they do not incorporate sulfur radioactive isotope ($S^{35}$) nor are degranulated by compound 48/80.

The mast cells of Amphisbaenidae have an amphoteric staining character, i.e., they can be stained by acidic dyes (eosin and ponceau-acid-fuchsin), basic dyes (toluidine blue) as well as by Alcian blue which is considered an amphoteric dye (Gurr, 1962).

The acidophilia might be related to the PAS positive material or other components which do not present acid mucopolysaccharidic nature. This is obviously a hypothesis that requires deeper analysis for an eventual elucidation.

The position of the Amphisbaenidae in the order Squamata is subject of controversy. Some studious place them in the suborder Lacertilia while others locate them in a special suborder, the Amphisbenia (Grasse, 1970).

According to Grasse (1970), most of the taxonomists which classify the Amphisbaenidae in a special suborder consider them an evolutionary stage between lizards and ophidians. One of their basic arguments are the modifications in the scapular and pelvic arches, leading to the atrophy of limbs. From the evolutionary standpoint, this atrophy begins in some representatives of the suborder Lacertilia (the Anguimorpha), being emphasized in the suborder Amphisbaenidae and reaching its maximum in the suborder Ophidia.

Analysing the literature cited, it can be observed that typical mast cells have been evidenced in several animals of the class Reptilia, except for the suborder Ophidia, where granular acidophilic cells were described.

If the Amphisbaenidae really represent an intermediate stage between lizards and ophidians one might admit the hypothesis that in their mast cell granules the basophilic and metachromatic properties of typical mast cells are intermingled with the acidophilic character of the granular acidophilic cells. Comparing Figures 2 and 4, a great structural similarity can be noticed between the granular acidophilic cells of snakes and the mast cells of Amphisbaenia fuliginosa.
References


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