Acknowledgements. This study was partially supported by grants from the Plan de Movilidad de Investigadores of the University of Salamanca and by the Institut Menorqui d’Estudis, Consell Insular de Menorca, and by project PB98-0270 of the Spanish Ministry of Education and Culture. The work was conducted according to research protocol 00-037 of the Purdue Animal Care and Use Committee.

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Received: April 22, 2002. Accepted: August 25, 2002.

Mucosubstance histochemistry in the esophagus of the lizard *Agama stellio stellio*

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Although numerous histochemical studies have been made on mammalian gastrointestinal mucosubstances (Obuofuribo, 1975; Shehan and Jervis, 1976) and reptilian oral glands (see review by Kochva, 1978) less information is available on the gastrointestinal mucosubstances of reptiles (Luppa, 1977; Ferri and Liquori, 1992). This is a first attempt to
express quantitatively the cytological changes occurring during the secretory process of the goblet cells, using image analysis tools.

Seven adult lizards *Agama stellio stellio*, three males and four females were collected in the summer 2000 from the Kolhiko village, 27 km NE of Thessaloniki, Northern Greece. The animals were killed with an intraperitoneal injection of nembutal and the tissues were quickly removed and fixed in 4% neutral buffered formaldehyde at 4°C for 48 h and after that they were embedded in paraffin and sectioned at 6 μm. For routine studies, the sections were stained with Harris Hematoxylin and Eosin.

For the demonstration of mucosubstances, the following methods were used:

- The Periodic Acid-Schiff after α-amylase (α-am.-PAS) treatment for neutral polysacharides.
- The Alcian blue at pH 2.5 (AB 2.5) (Kiernan, 1990) both, carboxylated and sulfated, for acid mucosubstances.
- The AB pH 1 (AB 1) by blotting dry the sections after AB staining (Lev and Spicer, 1964) for sulfated acid mucosubstances.

The combined High Iron diamine-Alcian blue pH 2.5 (HID-AB 2.5) (Spicer, 1965) to distinguish between carboxylated and sulfated acid mucosubstances in the same section.

- The AB 2.5-PAS method (Mowry, 1963) for the differentiation between neutral and acid mucosubstances.
- The mild periodate oxidation-PAS (m-PAS), according to Roberts (1977) for sialomucins.

To avoid differences in staining intensity and thus great differences in threshold setting, all the sections which were stained with the same stain were processed together. Similarly, in all the pictures stained with the same stain, the light intensity of the microscope was always set at 10 volts.

Because of the small sample size, statistical analysis was impossible, the relative results being indicative.

The esophagus of *Agama stellio stellio* exhibits the typical structure of that of other lacertilia and ophidia (fig. 1). The esophageal epithelium contains in its entire length variably distributed columnar ciliated cells and, interspersed between them, goblet cells.

On the basis of their staining characteristics, five secretory states of goblet cells might be distinguished. It is necessary to make clear that neither were these states stable in all the animals examined, nor were all the cells in a section in the same secretory state.

The first one, is classified as containing exclusively neutral polysacharides.

The second state includes cells which are classified as carboxylated acid mucosubstances containing cells. With regard to the kind of the carboxylated mucosubstances present, application of more specialised methods disclosed that these are most probably sialomucins, since they are stained intensely magenta with m-PAS.

The third state includes cells in which, the intense stainability with AB 1 and HID, shows that these cells contain only sulfated acid mucosubstances.

The cells of the fourth state contain sulfated and carboxylated (presumably sialomucins) mucosubstances together, since these cells are stained variably, namely all of them blue with AB 2.5 method and a part of them brown with HID method or blue with AB 1 method.

The fifth state was the most numerous. It seems that these cells contain a mixture of neutral and acid mucopolysacharides. The area occupied by goblet cells stained with this technique is the most representative, since no goblet cells remain unstained.

On the basis of these secretory states, the animals were divided into three groups, each group including animals with nearly similar secretory conditions.

In the first group, all the goblet cells gave a strong PAS positive reaction, which has not been altered after amylase treatment. The results were nearly similar after AB 2.5 staining (fig. 2a), an indication that nearly all the goblet cells stained for neutral polysacharides