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# A Simple Method to Record Parietal Cells in the Fundic Mucosa in Baboons

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**Abstract.** *Background: Gastric parietal cells in a baboon were recently found to be auto-fluorescent. Aim: To study gastric sections with a fluorescent microscope in a cohort of baboons. Material and Methods: Gastric sections from 38 baboons were stained with hematoxylin-eosin (H&E) and examined in a fluorescence microscope (FLM). The thickness of the parietal cell population was assessed at ×10 magnification. Results: H&E stained all mucosal cells: fovelar, parietal and chief cells. When the same sections were analyzed with an FLM, only parietal cells were auto-fluorescent, whereas fovelar and chief cells remained non-fluorescent. Parietal cells formed a distinct, continuous auto-fluorescent band. The ratio of the auto-fluorescent parietal cell band/total mucosa ranged between 0.20 and 0.30. Conclusion: Gastric parietal cells became auto-fluorescent when H&E-stained sections from baboon stomachs were observed with an FLM. Eosin was the stain responsible for this optical phenomenon.*

Approximately two-thirds of the stomach in baboons is lined by fundic mucosa (1). When gastric sections are stained with hematoxylin and eosin (H&E) several cell populations are identified in the fundic mucosa; the upper one fifth contains surface epithelial cells and neck cells, the underlying two to three fifths, parietal (oxyntic) cells, and the remaining deeper part, dark chief cells (1).

Various methods have been applied to study parietal cells, such as isolation and separation of enriched fractions of viable parietal cells in mucosal samples (2), stereology (3) and immunochemistry (4). These methods are, however, cumbersome, time-consuming and not in use in diagnostic veterinary pathology.

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To date, no studies have been undertaken to correlate the parietal cell population with increased or decreased gastric secretion of hydrochloric acid in non-human primates (5). One possible cause may be that that despite the easy recognition of parietal cells in H&E stain, they have to be histologically differentiated from other H&E-stained fundic cells such as surface, neck and chief cells. To address this important question, a simpler, more reliable method to visualize the actual thickness of the gastric parietal cell population is required.

While investigating H&E-stained gastric sections from a baboon with a fluorescence microscope, the Authors recently observed that parietal cells exhibited auto-fluorescence (unpublished). Surprisingly, the other mucosal components were dark (that is, non-fluorescent).

The purpose of the present work is to report findings obtained from the stomachs of a cohort of baboons using H&E-stained sections inspected under a fluorescence microscope.

## Materials and Methods

A cohort of 38 consecutive adult baboons were investigated. These baboons were members of colonies at the Southwest National Primate Research Center, Southwest Foundation for Biomedical Research. The housing conditions have been reported elsewhere (6). Briefly, the animals were housed in metal and concrete indoor-outdoor cages and were fed commercial monkey diets occasionally supplemented with a variety of fruit and vegetables. Water was available *ad libitum*. Baboons were euthanized with a commercial barbiturate euthanasia agent or died naturally. All procedures were carried out in accordance with the Institutional Animal Care and Use Committee.

On necropsy, tissue samples from the stomach were fixed in 10% neutral buffered formalin, processed conventionally, embedded in paraffin, cut at 5 μm, stained with H&E and evaluated both under transmitted light (TL) and under indirect light fluorescence (ILF) using a wavelength of 556 nm using a calibrated ocular scale and a ×10 objective.

## Results

*Assessing the parietal cell band with TLF in H&E stained sections*. The fundic mucosa clearly showed eosinophilic parietal cells (Figure 1). As foveolar and neck cells in the

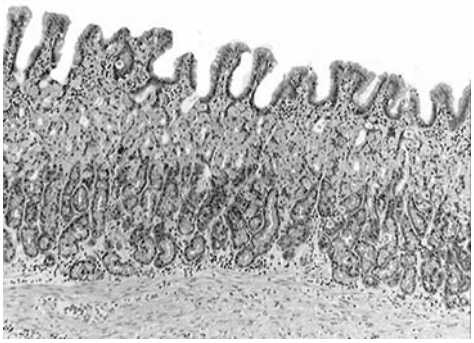

Figure 1. *Normal fundic mucosa of baboon. Superficial, foveolar, parietal and chief cells are readily seen (H&E stain, transmitted light, ×6).*

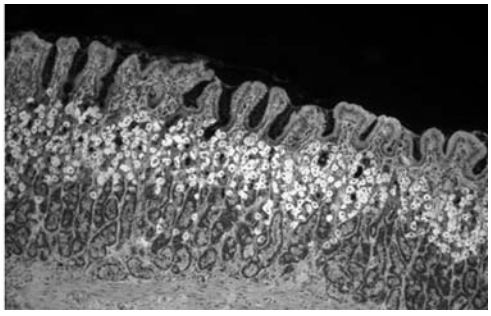

Figure 2. *Normal fundic mucosa of baboon. Note the band of auto-fluorescent parietal cells. Other cells in the fundic mucosa (superficial, foveolar and chief cells) remain non-fluorescent (H&E stain, indirect light fluorescent, ×4).*

upper part of the mucosa and the darker chief cells in the lower part were also stained with H&E, estimation of the limit between parietal cell and the rest of the stained fundic mucosa was time-consuming and difficult.

The mean ratio of H&E-stained parietal cell band to total mucosa could not be assessed with certainty.

*Assessing the parietal cell band with ILF in H&E-stained sections.* The fundic mucosa displayed a distinct band of parietal cells (Figure 2). In contrast, the mucus-producing foveolar and neck cells in the upper part of the mucosa and the darker chief cells in the lower part were non-fluorescent.

The mean ratio of the auto-fluorescent parietal cell band to total mucosa was 0.26 (range 0.20-0.30).

## Discussion

This study demonstrated that in H&E-stained sections from baboon stomachs, gastric parietal cells become auto-fluorescent. Eosin was the stain responsible for this optical phenomenon.

Several methods have been applied to study gastric parietal cells such as WGA and HPA-L lectins in guinea pigs (3), flow cytometry in mouse and guinea pigs (2, 4), huntingtin-interacting protein 1 in mice (7), parietal-specific $H^+K^+$-ATPase antibody in transgenic mice (8), as well as prostaglandin $E_2$ (9) and transmission electron microscopy in laboratory animals (10). The disparate methods used highlight the difficulties in finding an adequate technique to visualize gastric parietal cells in animals. None of them have been applied to retrospectively or prospectively investigate the bulk of the parietal cell population in health and disease.

In 1987, Gherardi *et al.* (11) tested four different stains to study parietal cells in the rat stomach: (i) a modification of the H&E method proposed by Drysdale and Marks (12), (ii) hematoxylin-eosin-saffron fluorochrome stain, (iii) hematoxylin-azophloxin-saffron fluorochrome stain and (iv) May-Grunwald-Giemsa stain on thin sections from plastic-embedded specimens. According to Gherardi *et al.* (11), plastic-embedded specimens stained with the May-Grunwald-Giemsa stain provided the best results for the visualization of parietal cells in rat stomachs. It should be stressed that by using this complex, costly and time-consuming method, only very small areas of gastric mucosa (1 to 2 mm) can be analyzed.

When H&E-stained sections from the gastric mucosa are observed with TL, a zone containing parietal cells is easily identified in baboons, thus contrasting with the more difficult recognition of parietal cells in humans (13) and in rodents (14). However, although parietal cells are readily identified in H&E-stained sections in baboons, a time-consuming differential histological identification with other stained fundic cells present in the rest of the mucosa is required and the exact limit between H&E-stained parietal cells and the rest of the fundic cells is difficult to assess.

Furthermore, one of the criteria for the recognition of parietal cells is their affinity for eosin stain. This attribute may be a drawback when male biologists/pathologists try to detect cells by their eosinophilic cytoplasm, since 10% of male biologists/pathologists, a sample from the general population, suffer from Daltonism (15).

One of the advantages of the method presented here is that by observing H&E-stained sections with ILF, only parietal cells exhibit auto-fluorescence while the other mucosal cells remain non-fluorescent.

Despite the fact that many common chronic gastric diseases affect the parietal cell population, no studies have been carried out to correlate the bulk of parietal cells to gastric acid secretion in primates (1, 16). Accordingly, the method described here will be applied to correlate the thickness of the auto-fluorescent parietal cell band to pH alterations of the gastric acid secretion in baboons.

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