Annals of Anatomy 196 (2014) 41–47

Contents lists available at ScienceDirect

Annals of Anatomy

journal homepage: www.elsevier.de/aanat

Practical stereology of the stomach and intestine

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ARTICLE INFO

Article history:
Received 3 April 2013
Received in revised form 21 October 2013
Accepted 22 October 2013

Keywords:
Stomach
Intestine
Stereology
Number
Surface area
Volume

SUMMARY

We provide a practical review of the opportunities made available by design-unbiased stereology to estimate cell number, total volume, mean volume and mean height in the rat stomach using enterochromaffin-like cells as an example. The second example comprises estimation of the surface area of well-defined segments of rat colon and the volumes of different layers following surgery and/or treatment which may result in the atrophy or growth of the colon. The pros and cons of the stereologic designs are discussed and the pitfalls and some solutions to these are elucidated.

1. Introduction

This review presents some practical solutions using stereology to estimate number and size of different structures in the rat stomach and rat colon. The first example involves enterochromaffin-like (ECL) cells, which are found in gastric glands of the mucosa below the epithelium in the oxyntic part of the stomach. They synthesize and secrete histamine stimulated by pituitary adenylyl cyclase-activating peptide and gastrin hormones. Acetylcholine may also activate release of histamine from ECL cells via M1 receptors through vagal innervation. Histamine and gastrin are both potent important mediators of gastric acid secretion from parietal cells in the stomach. Stimulation of ECL cells may be followed by ECL cell hypertrophy, hyperplasia, dysplasia and formation of ECL cell carcinoids as seen in Zollinger-Ellison's syndrome (Masaoka et al., 2008; Zhao and Chen, 2012). Estimation of the number and size of ECL cells is therefore important and is illustrated using the rat stomach in the first practical example.

The small intestine follows the stomach in the course of the gastrointestinal tract, and is responsible for the digestion and absorption of food nutrients and minerals through processes of simple/passive diffusion, facilitated diffusion, primary/secondary active transport processes and osmosis. The large intestine and colon make up the distal part of the digestive system. The colon absorbs water from the remaining indigestible food and the remainder is eliminated from the body. The surface area of the intestine is an important parameter for understanding intestinal function and the second practical example shows how to estimate the surface area of defined segments of the rat colon as well as the size of the different layers of the rat colon wall.

2. Animal handling and sampling of the rat stomach

Female Wistar rats aged six months and weighing approximately 230 g were used. The rats were perfused with isotonic saline 0.9% solution at a low flow rate (about 10 ml/min) for 30 s through the left cardiac ventricle using pentobarbital anaesthesia (5 mg per 100 g body weight). Afterwards, a 4% carbodiimide solution (Sigma, E7750, 1-ethyl-3-(3-dimethyl-amino-propyl)carbodiimide) dissolved in 0.1 M sodium-phosphate-buffer (pH 7.4) was perfused at the same low flow rate lasting 0.1 min per gram body weight. The stomach was removed, opened along the greater curvature (Fig. 1A), and rinsed with ice-cold isotonic saline. The stomach was flattened and pinned to a wax-plate, the mucosa facing upwards and immersed in the 4% carbodiimide fixative overnight. After 24 h, the stomach was washed in 0.1 M sodium phosphate buffer (pH 7.4).

The stereologic design of the study is modified and simplified from the one described in detail in Bendtsen et al. (2002). The

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http://dx.doi.org/10.1016/j.aanat.2013.10.007

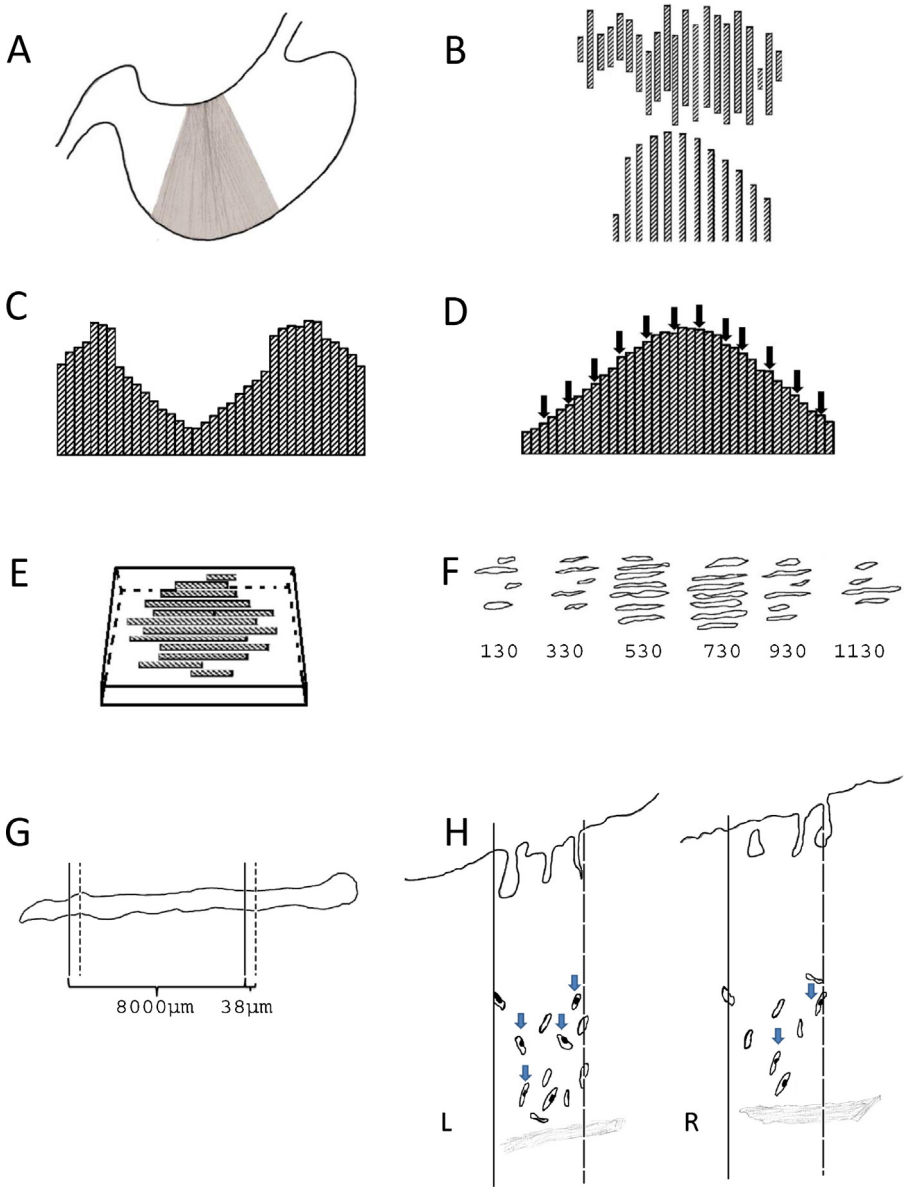

Fig. 1. (A) The oxyntic part of the rat stomach is demarcated. The rumen is to the right, the antrum is to the left. (B) The stomach was opened along the greater curvature, and the rumen and antrum were excised leaving the oxyntic resect, which contained all the ECL cells. The resect was divided along the minor curvature in order not to get strips too large for paraffin embedding. The resect was cut into parallel 2 mm wide strips, which produced strips from the anterior and posterior gastric wall. (C) Shows the bimodal distribution of the natural sequence of strips. (D) The strips were rearranged into a unimodal smooth spindle pattern. A random strip was selected from the first three strips (here 3) and then every third strip was sampled. (E) The sampled strips were embedded in a paraffin block and cut perpendicularly to the mucosal surface into an exhaustive series of sections with a thickness of 2 µm. (F) A random number between 1 and 200 was selected, here 120, and every 200th paraffin section was sampled from this section. For every sampling section, the look-up section was sampled as well. Six paraffin section pairs were sampled (section numbers indicated on the figure), and they contained 33 tissue sections. (G) A counting frame with width 38 µm was moved along a section. The starting position was before the tissue. A random number between 1 and 8 was selected (e.g. 4), and the stage of the microscope was moved 4 mm from the starting position. Hereafter the step length was 8 mm. (H) Six ECL cell profiles were observed in the sampling field (L), but one of the nuclei was intersected by the "exclusion" left border of the counting frame, which means that five ECL cell profiles are sampled, $Q(\mathrm{ECL}) = 5$. However, only four of these ECL cells are sampled by the disector because their nuclei were not seen in the look-up section as marked by arrows on section L, $Q^{-}(\mathrm{ECL}) = 4$. Analogously, the number of sampled ECL profiles in *R* is $Q(\mathrm{ECL}) = 3$, and the two arrows in R indicate the sampled cells, $Q^{-}(\mathrm{ECL}) = 2$. In this disector a total of 6 ECL cells are counted.

resected stomach was cut into parallel strips at 2 mm intervals with a set of razor blades in a fixed position. The order of the sequence of strips was rearranged before sampling, so the length of strips increased smoothly from each end towards the middle of the sequence according to the smooth fractionator principle (Gundersen, 2002). The first strip was sampled by choosing a random number between 1 and 3. From this strip onwards, every third strip was sampled. In probability terms, the systematic uniform strip sampling fraction contained on average $StripSF = 1/3$ of the total number of ECL cells. The sampled strips were embedded in two paraffin blocks, which were sectioned exhaustively with a microtome advance (Dorph-Petersen et al., 2001) of 2 µm on a calibrated microtome (Microm HM 360, Brock & Michelsen, Denmark) with stainless steel blades (S 35, Feather Safety Razor Co., Ltd., Medical Division, PFM medical ag, Cologne, Germany) under constant exposure to dry ice vapor. The cut surface was roughly perpendicular to the mucosal surface. This section sampling fraction contained every 200th section as well as the previous section (the look-up