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A simple new method to calculate small intestine absorptive surface in the rat

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Abstract The rat is an established model for studying intestinal adaptations following abdominal surgery. In the study of functional and morphological adaptations of the small intestine, it is helpful to estimate the mucosal surface area. In order to simplify measurements and calculation we developed a new mathematical model for calculation of the mucosal surface area on histological sections. In contrast to other methods, it requires only cross-sections of small intestine and includes the measurment of only three histological parameters: length and width of villus and width of crypt. The new approach was compared with the most commonly used procedures, the Harris and the Fisher-Parsons methods, under experimental conditions. An animal study including single-pass perfusion, fixation, staining and subsequent histomorphometry of jejunum and ileum using these different methods was performed. The new method showed the least work and presented no significant differences compared with the precise Harris method. In conclusion, the method described is an adeqate tool to estimate the mucosal surface area with less work and with comparable results to established methods. The less-complex method may be a valuable tool in experimental research of small intestine adaptations in rats.

Key words Rat • Small intestine • Morphometry • Absorptive surface area

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Introduction

In abdominal surgery research in rats [1–7] it is helpful to evaluate the surface area of intestinal mucosa [8] caused by villous alterations [9]. Thereby it is possible to quantify histological adaptations that enlarge or reduce small intestinal surface.

Many established methods to calculate intestinal absorption, i.e., single-pass perfusion, depend on knowledge of the mucosal surface area [7, 8, 10]. Therefore several complicated and less-complicated mathematical methods for the calculation of small intestinal surface in rats have been described [10, 11]. All require time-consuming procedures performed on longitudinal and transverse sections of the gut.

The new method described here is based on a simple mathematical model of mucosal architecture and therefore offers an easier mathematical approach (Fig. 1). For this method, only sections in one direction (cross-sections) are needed, and only three microscopic parameters of small intestine need be measured repeatedly: the villus length, villus width, and the width of crypts (Fig. 2).

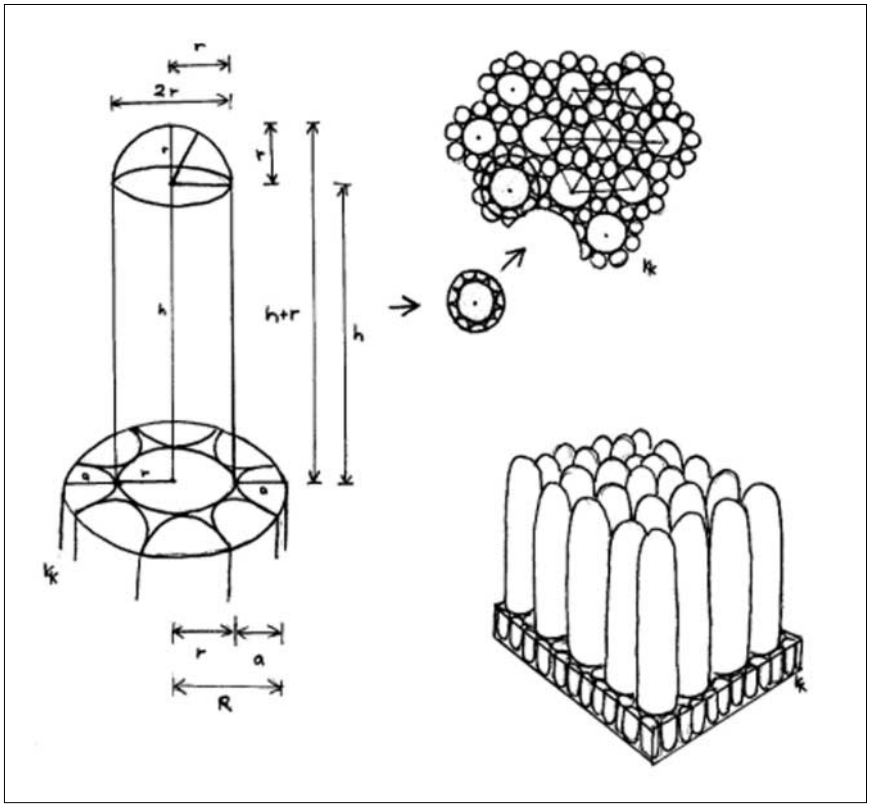

Fig. 1 Geometric model of totally symmetric intestinal mucosa with iteration of mucosal unit (*left side*) in order to constitute the whole small intestine mucosa (*right side*). Only the three parameters villus length (h+r), villus width (2r), and crypth width (2a) are needed to estimate the histological absorptive surface amplification, which is a result of both villus and crypt dimensions

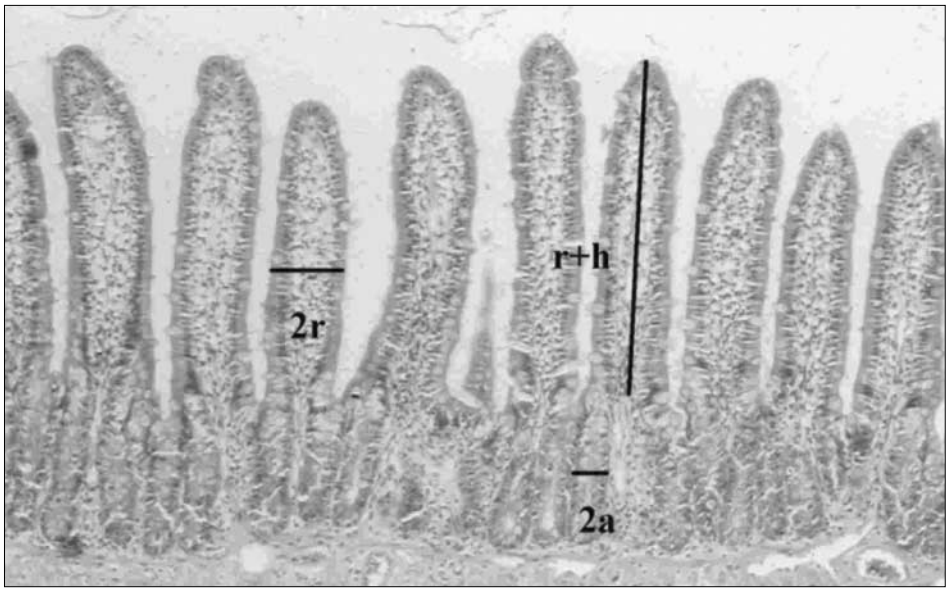

Fig. 2 Measurement of the three parameters villus length (h+r), villus width (2r), and crypt width (2a) on hematoxyalin and eosin-stained, formalin-fixed cross-section of rat small intestine according to the presented method

Materials and methods

In order to evaluate this new approach we performed an animal study. The approach was compared with the most commonly used procedures in rats, the Harris method [10] and the Fisher-Parsons method [11].

The work load and the results of the three methods were compared. Ten male Wistar rats (350–400 g) underwent a laparotomy after anaesthesia with a 10% ketamine and 2% rompune solution applied intramusculary (0.1 ml/100 g body weight). For the experiment, approximately 200 mm of proximal jejunum and 200 mm of distal ileum were isolated with intact blood supply. To obtain standardized conditions an isotonic and iso-osmolar solution, as used for absorption studies [12–15], was pumped at 300 µl/min through these intestinal segments. After 20 min of perfusion, cylindrical tissue samples from selected gut segments were obtained; 5 samples from each rat underwent standardized treatment (2 h fixation in 10% formalin solution, paraffinembedding, cutting of 4 µm longitudinal and transverse sections and staining). Each specimen was

Table 1 Histological surface magnification ratio measured on the same samples with three different methods (n=50) [a]

	Fisher-Parsons [11]	Harris [10]	New approach
Jejunum	8.5±1.2*,**	6.4±1.0	6.9±1.6
Ileum	6.3±1.1*	5.6±0.8	5.3±1.1

*P<0.05 versus new method; **P<0.005 versus Harris
[a] Mean±SD

examined histologically. On microphotographs of each representative specimen, an examiner blind to the experimental conditions performed morphometry of the parameters under study (Table 1).

For the Harris evaluation, villus diameters both on transverse (X) and longitudinal (Y) sections in three different positions (a tip, b midsection, c base) were measured (Xa, Xb, Xc, Ya, Yb, Yc). Additional villus height (h) and tip (t) were evaluated. Villus density was determined by multiplication of counted villi per length on transverse and longitudinal sections, respectively. Under low microscopic power, the inner (i) and outer (o) major (Mi, Mo) and minor (Ni, No) diameters of whole intestine in cross-section were measured. The mucosal-to-serosal amplification ratio M was estimated following the equation after Harris et al. [10]:

$$M=\frac{\left[2\pi\left(\frac{Xa^2+Ya^2}{8}\right)+2\pi\left(\frac{Xb^2+Yb^2}{8}\right)^{1/2}\right]\cdot\left(\frac{h-t}{4}\right)\cdot\text{villus density}}{2\pi\left(\frac{Mo^2+No^2}{8}\right)^{1/2}}+$$

$$\frac{\left[2\pi\left(\frac{Xb^2+Yb^2}{8}\right)^{1/2}+2\pi\left(\frac{Xc^2+Yc^2}{8}\right)^{1/2}\right]\cdot\left(\frac{h-t}{4}\right)\cdot\text{villus density}}{2\pi\left(\frac{Mo^2+No^2}{8}\right)^{1/2}}+$$

$$\frac{\left[\pi\left(\frac{Xa+Ya}{4}\right)^2+t^2\right]+\frac{Mi^2+Ni^2}{Mo^2+Ni^2}-\pi\left(\frac{XcYc}{4}\right)\cdot\text{villus density}}{2\pi\left(\frac{Mo^2+No^2}{8}\right)^{1/2}}$$

For Fisher-Parsons evaluation, the mucosal length not occupied by villi (f_c, f_l) was measured on transverse (c) and longitudinal (l) sections of small intestine. The mucosal (m_c, m_l) and serosal (s_c, s_l) outlines on transverse and longitudinal sections were determined. The following equation after Fisher-Parsons was used to calculate the mucosal-to-serosal amplification ratio M [11]:

$$M=\left(\frac{m_l}{s_l}-1\right)\cdot(1-f_c)+\left(\frac{m_c}{s_c}-1\right)\cdot(1-f_l)+1$$

The new approach for calculation of mucosal-to-serosal amplification ratio M was as follows. A geometric mucosal unit consisting of a cylindrical villus with rounded tip surrounded by cylindrical crypts was defined (Fig. 1). It was assumed that the whole mucosa is an iteration of this unit and the surface area can be calculated with mean values of structures that define the mucosal unit: villus length, villus width, and crypt width (Fig. 2), since the small intestine of rat does not posses circular plicae [10]. Crypt length is important to the overall function of the mucosa, but for the calculation of absorptive surface it is not necessary, because there is only secretion within crypts [12]. Therefore the histological surface magnification ratio M consists of mean values of villus surface (calculated using length and width of the villus), villus bottom (determined by villus width), and mucosal unit bottom (determined by villus and crypt width); r=radius of villus, 2r=villus width, a=radius of crypt, 2a=crypt width, h+r=villus length, R=a+r=radius of mucosal unit (Figs. 1 and 2):

$$M=\frac{(\text{villus surface}+\text{unit bottom}-\text{villus bottom})}{\text{unit bottom}}$$

with:

$$\text{villus bottom}=\pi\cdot r^2=\pi\cdot\left(\frac{\text{villus width}}{2}\right)^2$$

$$\text{villus surface}=2\cdot\pi\cdot r\cdot h+2\cdot\pi\cdot r^2=\pi\cdot(\text{villus length}\cdot\text{villus width})$$

$$\text{unit bottom}=\pi\cdot R^2=\pi\cdot(r+a)^2=\pi\cdot\left(\frac{\text{villus width}}{2}+\frac{\text{crypt width}}{2}\right)^2$$

Together this leads to an equation including the three variables villus width, crypt width and villus length:

$$M=\frac{(\text{villus width}\cdot\text{villus length})+\left(\frac{\text{villus width}}{2}+\frac{\text{crypt width}}{2}\right)^2-\left(\frac{\text{villus width}}{2}\right)^2}{\left(\frac{\text{villus width}}{2}+\frac{\text{crypt width}}{2}\right)^2}$$

Results

The results of the calculations using the three methods showed a normal curve of distribution according to the Kolmogorov-Smirnov test. Results were presented as mean±SD and were evaluated using the analysis of variance. If the probability level was <0.05, values were considered to be significantly different and individual post-hoc comparison was performed using Bonferroni's multiple comparison test.

Compared with the Harris procedure, the new method showed no significant difference for the mucosal-to-serosal amplification ratio in the jejunum as well as in the ileum (Table 1). The results obtained with the Fisher-Parsons formula differed significantly from both approaches above in jejunum and in the ileum. The greatest work was found for the Harris method and the least for the new method (Table 2).

Table 2 Volume of work of the three methods for calculating histological surface area magnification ratio (n=50 gut samples)

	Fisher-Parsons [11]	Harris[10]	New approach
Number of measurements	300	700	150
Number of cross-sections	50	50	50
Number of longitudinal sections	50	50	0
Number of stainings	100	100	50

Discussion

Rats are frequently used in experimental research of absorption and morphology as intestinal adaptations following surgery [1–7, 16]. Single-pass perfusion studies are generally accepted for estimating intestinal absorption. Knowledge of the exact mucosal surface area is an absolute prerequisite for performing such studies. The present evaluation included two established methods [10, 11] and a new method, which determine mucosal surface area in rats. Models based on other animals or humans have not been considered in this study.

Harris calculations [10] and those after Fisher-Parsons [11] are based on both cross- and longitudinal sections. However, such extensive measurement is not necessary, with an alternative approach, as shown by the present results. The new method for calculating the absorptive surface of rat small intestines is restricted to cross-sections. The Harris method is an exact calculation with much work, including 14 measurements of diverse parameters on longitudinal and transverse sections. It takes into account the complex histological mucosal architecture that enlarges the surface of the tube-shaped rat small intestine. Villus diameter as well as villus height and villus density are considered.

The Fisher-Parsons approach is less labor intensive, with measurement of six parameters on transverse and longitudinal sections, but the mathematical transformation into the third dimension with help of simple multiplication of length values (the mucosal outline on transverse and longitudinal sections) leads to results that are too high. This method does not take into account the complex three-dimensional villous structure of the small intestine and provides the investigator with inappropriate values of plane surfaces. Only the mucosal and serosal outline on transverse and longitudinal sections and the mucosal length not occupied by villi are considered. Another factor for possible abberations might be longitudinal and transverse sections not being exact, while in the rotation model this factor can be excluded. The difference in the results from the Fisher-Parsons method compared with the other methods may be due to this problem.

In the new approach presented, a mathematical rotation of two-dimensional structures allows creation of a three-dimensional gut structure. In contrast to the Harris procedure, this needs only cross-sections, without the need for longitudinal sections, and with only 3 parameters instead of 14 (Fig. 2). However, one must consider the high standard deviation values of the new procedure, which may correspond to this simplification.

The model does not account for gaps between adjacent unit bottoms, the portion of mucosal surface that is neither villus or crypt. However, when used in comparative investigations with an experimental group and controls, this imprecision is compensated for. The advantages of the new mathematical method could be shown for jejunum as well as for ileum, which have different mucosal architectures.

It is not clear what the "gold standard" is, but the new method is simple and has already been proven as practical in experimental research. In a rat model of adaptations after colectomy and ileal pouch-anal anastomosis, the new mathematical approach was successfully used to reveal a significant small intestine surface increase in test animals compared with controls [17]. This recent publication validates the method for pathological and experimental conditions and shows its sensitivity. This supplies evidence that the formula is applicable to study of adaptive changes of mucosal surface area and absorption in experimental research.

The new method gives results comparable to the precise method of Harris, but is superior due to its mathematical simplicity and lower work load. We think that it is a valuable tool for experimental research of small intestine adaptations in rats.

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