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Morphometric analysis of the small intestine in wild type mice C57BL/6J — a developmental study

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Recently the increasing prevalence of gastrointestinal diseases, including neoplasm, has resulted in the necessity of characterising not only the tumours, but also healthy mucosa. Research into the morphological changes of healthy mucosa under different experimental conditions, including drugs, special diets and the use of probiotic bacteria, is greatly facilitated by the availability of animal models. In spite of the widespread use of mice in gastrointestinal research, there is a lack of information on the qualitative and quantitative histological characteristics of the intestinal mucosa of the mouse.

The aim of this study was to assess the morphological characteristics and the postnatal development of the small intestine of wild type mice — C57BL/6J.

The mice were aged either 5 weeks or 12 weeks. The 12-week-old mice had been weaned at the age of 5 weeks. After dissection the small intestine was divided into 5 equal portions and randomly chosen microscopical sections from each were stained with haematoxylin and eosin. The parameters describing the morphology of the small intestine (villus height, depth of the crypt, villus width near the crypt, width of the villus connective tissue near the crypt, thickness of the muscular layer and the height of the enterocytes and their nuclei) were evaluated under a light microscope.

In both age groups the height and width of the villi decreased, while the thickness of the muscular layer increased in the distal direction. The height of the enterocytes decreased and the height of the enterocyte nucleus increased towards the colon in both age groups. The depth of the crypts was greater in the younger animals than in the older ones.

Our data provides the baseline morphological description of the small intestinal mucosa in wild type mice, strain C57BL/6J, which can be used as a reference for testing the influence of drugs, toxins, nutrients and inborn mutations on the mouse intestine.

Key words: mouse, morphometry, development

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INTRODUCTION

Morphometric analysis is widely used in gastrointestinal research, since it is a quantitative assessment and thus more reliable and reproducible than a subjective assessment, which is especially important in the diagnosis of different pathological conditions not readily apparent during routine histological assessment. For example, computerised image analysis has been used to discriminate between various types of inflammatory bowel disease [42] and has enabled various childhood enteropathies to be differentiated, showing that villous atrophy and crypt hyperplasia were most severe in patients with coeliac disease [20]. In addition, morphometry has been used to evaluate the condition of the intestinal mucosa after antibiotic treatment in patients with small intestinal bacterial overgrowth [12], the effectiveness of treatment and the prognosis in patients with coeliac disease [7], and the influence of environmental factors on mucosal morphology [19].

Morphometric analysis is also of great importance in cancer research where early diagnosis determines successful treatment. This method has been shown to be reliable in making a distinction between normal mucosa, early adenoma and adenocarcinoma and has assisted in distinguishing regenerative hyperplasia from dysplasia and separating high grade dysplasia from adenocarcinoma [8].

A combination of morphometric analysis and animal experiments is increasingly providing important information relevant to cancer and its genetic background. Most animal work has focused on the familial cancer genes because of their clear mechanistic relationship with cancer. In this respect the mouse is most commonly used to obtain transgenic animals which mimic hereditary human syndromes. In colon cancer research mice with mutation in the APC gene (APC mouse models) are most commonly used. The inactivating mutations of this gene in humans are responsible for familial adenomatous polyposis, an autosomal dominant predisposition to the development of hundreds to thousands of adenomatous polyps in the colon and rectum [3, 4]. There are several APC mouse models for intestinal cancer produced by introducing specific germline mutations into the mouse *Apc* gene (Apc^{Min}, Apc^{716}, Apc^{1638N}, Apc^{1638T}) on the same inbred genetic background — C57BL/6J [9]. The molecular and phenotypic analyses of these mice have provided very important clues to the understanding of the function of the APC gene in homeostasis and tumorigenesis. The close phenotypic resemblance to the human disease makes these mice unique pre-clinical models for intestinal cancer, providing highly informative examples of the efficacy of the combination of genetics and pharmacology in assessing the chemopreventive and/or therapeutic effects of experimental drugs and different diets on intestinal cancer [1, 16, 17, 21, 24, 29–32, 36, 37, 39]

Although transgenic mice are commonly used in intestinal cancer research, data regarding the morphology of healthy intestinal mucosa in mice are still scanty. The existing morphological studies of the intenstine of Apc^{Min} mice are limited only to assessment of the tumours that occur [10]. The characteristics of macroscopical healthy tissue would appear to be an important element in describing the pathological processes of the intestine, since the morphology of the mucosa reflects the influence of the certain pathogenic factors and is a valuable diagnostic element when taken as a biopsy. Moreover, the prevention of cancer is mainly directed to the tissue that is still healthy in order to protect it from the development of a lesion. In view of this, we decided to describe the morphological characteristics and the postnatal development of the small intestine of wild type mice — C57BL/6J.

MATERIAL AND METHODS

The experiment was performed on the small intestines of 15 wild type mice — C57BL/6J of both sexes. The animals were divided into 2 groups. The first consisted of 7 suckling 5-week-old mice, while the second consisted of 8 12-week-old mice, weaned at the age of 5 weeks and fed on a high-fat fibre-free semi-synthetic diet and tap water. The animals were cared for and treated in accordance with the guidelines for laboratory animals established by the National Institute of Health as well as those laid down by the local ethical committee. The material was collected at the University of Helsinki. The mice were terminated by CO_2 asphyxiation. Immediately after termination the small intestine was dissected, opened along the longitudinal axis and rinsed with ice-cold saline. Afterwards the small intestine was divided into 5 equal portions (D1–D5). The D5 was the most proximal portion, corresponding to the duodenum, while the D1 was the most distal portion, corresponding to the ileum. All portions were fixed in 4% paraformaldehyde and then embedded in paraffin blocks and cut into 5 μm-thick sections.

Histochemical and morphometrical analysis

In each case a minimum of 5 transverse sections were stained from each portion of the small intestine using the haematoxylin and eosin technique. The sections for staining were selected in a systematic random manner.

The stained sections were examined using the light microscope DMLS (Leica, Germany) connected to a computer equipped with a QWIN morphometric system (Leica, Germany). All measurements were performed on images grabbed on a SONY 19" monitor with no prior knowledge of the age of the animal.

The following parameters were established: the height of the villus (Hv) under magnification × 10, the width of the villus near the crypt (Wv), the width of the villus connective tissue near the crypt (Wc), the depth of the crypt (Dc), and the thickness of the muscular layer (Tm) under magnification × 40. The parameters concerning the enterocytes were the height of the enterocyte (He) and the height of its nucleus (Hn) established under magnification × 100 in two characteristic places, at the beginning of the villus and at the mid-point of its length (Fig. 1).

In order to obtain valuable data we evaluated variation coefficients (SD/mean) and on the basis of these we sampled each portion of the intestine in each animal according to the following protocol: at least 25 villi for measurement of their height and at least 50 for measurement of their width. Additionally, in each portion of the intestine we measured the depth of 30 crypts and the height of at least 100 enterocytes.

The mean values of each measured parameter for each portion of the intestine in each animal were evaluated and entered as raw data on the database.

The analysis of variance using Statistica v.6.0 (Statsoft, USA) was performed to test whether the values of each measured parameter were dependent on the portion of the intestine and the age of animal. The interaction effect between these main factors was also tested. The relation between the portion of the intestine and a given parameter was tested by regression analysis. In all statistical analyses $p = 0.05$ was the level of significance.

RESULTS

The height of the villus decreased from the proximal to the distal end of the small intestine in the case of both the 5-week and the 12-week-old mice ($r = -0.76$ and $r = -0.93$, respectively; Fig. 2A). An interaction was present between the age of the animal and the portion of the small intestine. Age influenced the villus height predominantly in the proximal portions (D5, D4) of the intestine and older mice had longer villi than younger mice. The mean value of villus height in D5 was 436.7 μm in younger mice and 586.4 μm in older mice, while in D1 it was 229.4 μm and 202.6 μm in younger and older mice, respectively (Fig. 2B, C).

The width of the villus near the crypt diminished on approaching to the colon in both groups of animals studied (Fig. 2D). This effect was stronger in the 12-week-old group than in the 5-week-old group ($r = -0.79$ and $r = -0.5$, respectively). There was no interaction between the age of the animals and the portion of the small intestine.

The width of the villus connective tissue near the crypt was greater in the 12-week-old than in the 5-week-old mice (Fig. 2E–G). The mean value of this parameter in D5 was 34% and in D1 11% higher in the older than in the younger mice. Only in the 12-week-old animals did the mean value of this parameter decrease from the proximal towards the distal part of the intestine ($r = -0.37$). There was no interaction between the age of the animals and the portion of the small intestine.

The thickness of the muscular layer (Tm) in both age groups was greater in the distal part of the small intestine (5 weeks $r = +0.44$, 12 weeks $r = +0.36$, Fig. 3A). No significant differences between the age groups were observed. There was also no interaction between the age of the animals and the portion of the small intestine.

The depth of the crypts was greater in the younger animals than in the older ones, especially in D2 (Fig. 3B). There was no interaction between the age of the animals and the portion of the small intestine.

The height of the enterocytes decreased towards the distal end of the small intestine in both groups (5 weeks $r = -0.51$, 12 weeks $r = -0.68$). The mean values for the height of the enterocytes were greater in the 5-week then in the 12-week-old mice (Fig. 3C). There was no interaction between the age of the animals and the portion of the small intestine.

The height of the enterocyte nucleus increased towards the colon in both age groups (5 weeks $r = +0.39$, 12 weeks $r = +0.64$; Fig. 3D). In the younger mice the nuclei of the enterocytes were larger than in the older ones. There was no interaction between the age of the animals and the portion of the small intestine.

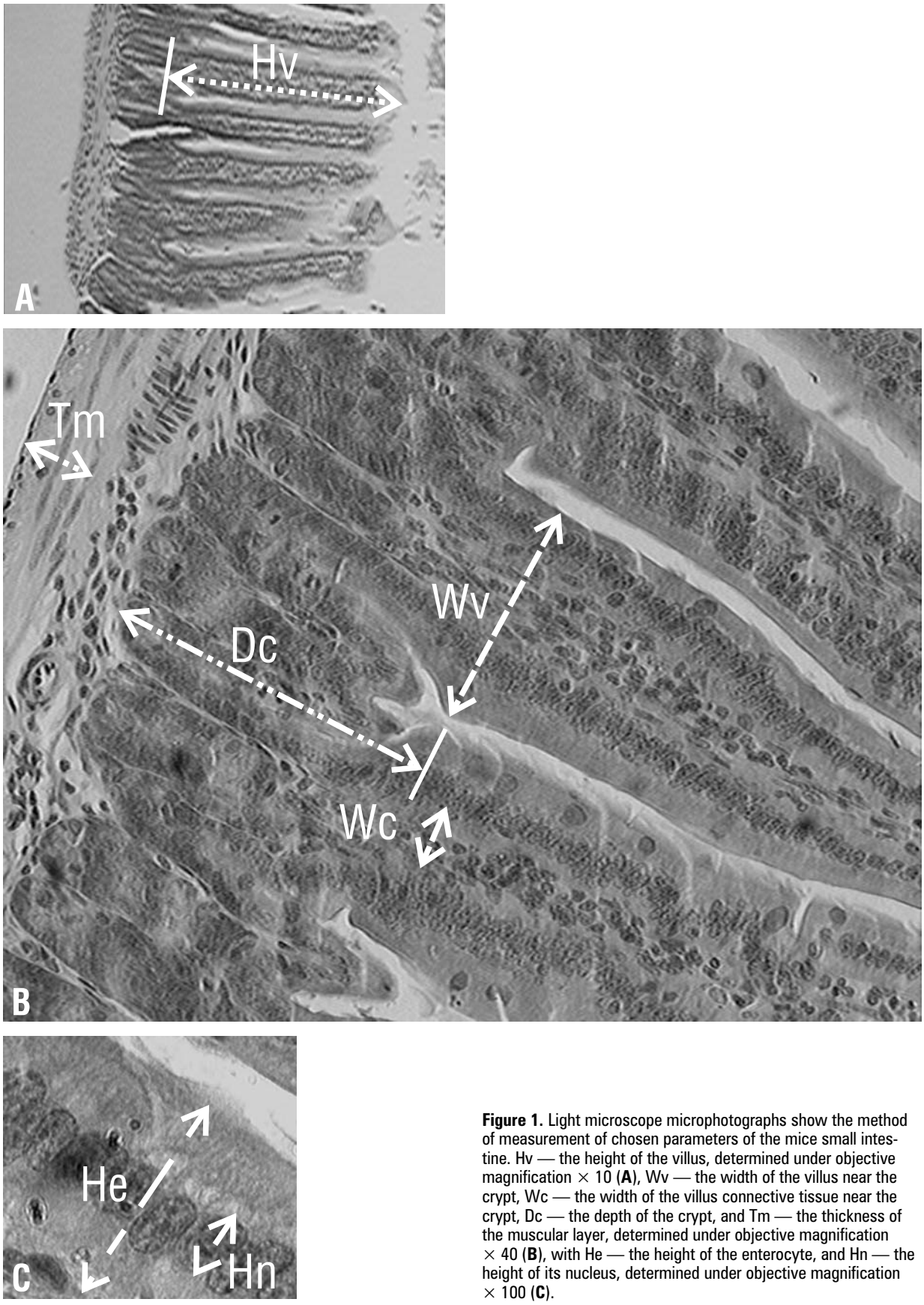

Figure 1. Light microscope microphotographs show the method of measurement of chosen parameters of the mice small intestine. Hv — the height of the villus, determined under objective magnification × 10 (**A**), Wv — the width of the villus near the crypt, Wc — the width of the villus connective tissue near the crypt, Dc — the depth of the crypt, and Tm — the thickness of the muscular layer, determined under objective magnification × 40 (**B**), with He — the height of the enterocyte, and Hn — the height of its nucleus, determined under objective magnification × 100 (**C**).

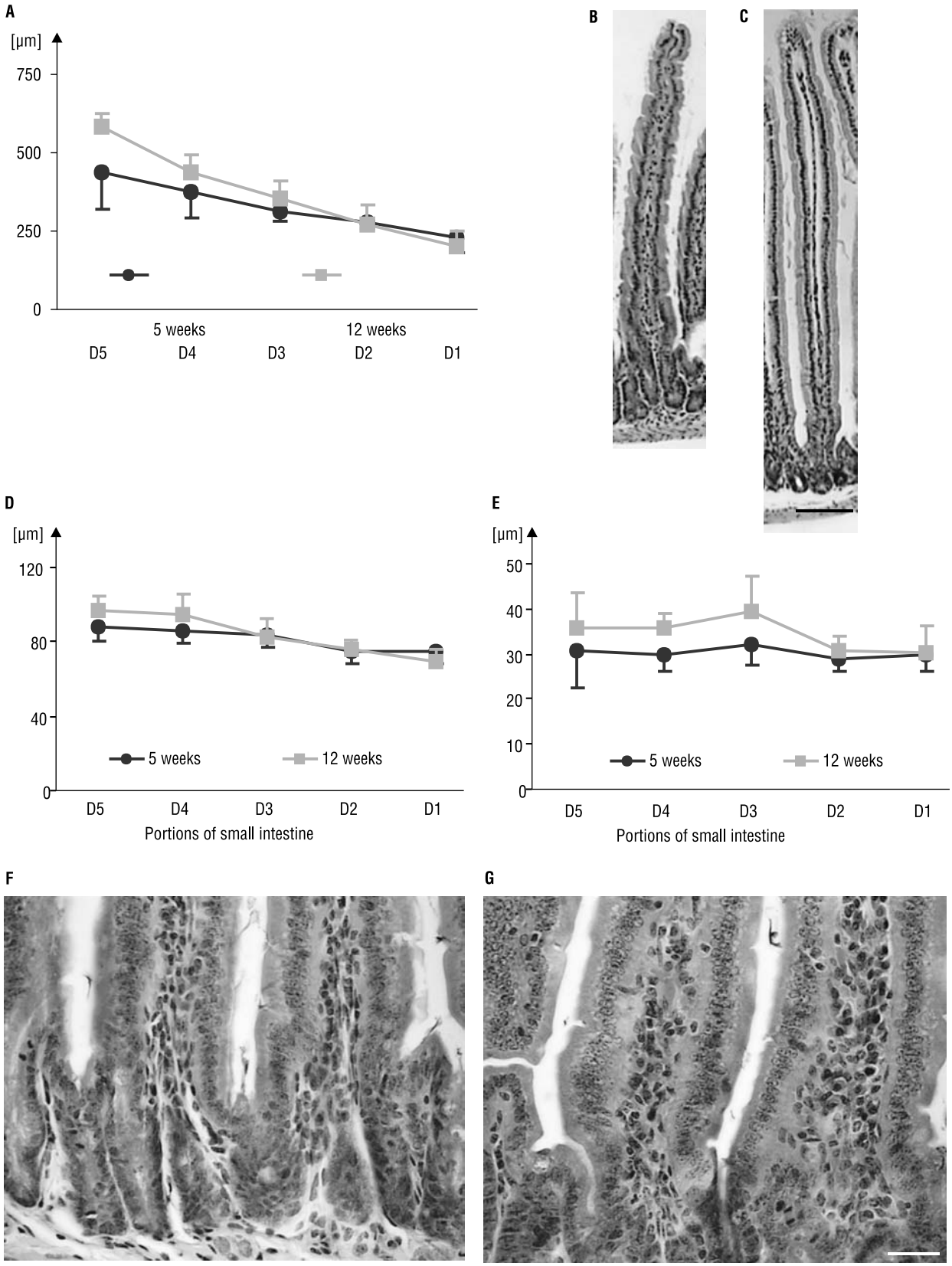

Figure 2. Morphological characteristics of the parameters evaluated in particular portions of the small intestine: the values of the height of the villi (**A**), the morphology of the villus from portion D5 in 5-week-old (**B**), and 12-week-old (**C**) mice, the values of the width of the villus near the crypt (**D**), the values of the width of the villus connective tissue near the crypt (**E**), the morphology of the villi near the crypt from portion D5 in 5-week-old (**F**), and 12-week-old (**G**) mice. The graphs present mean value [mm] and standard deviation. Scale bare: B, C: 50 μm; F, G: 20 μm.

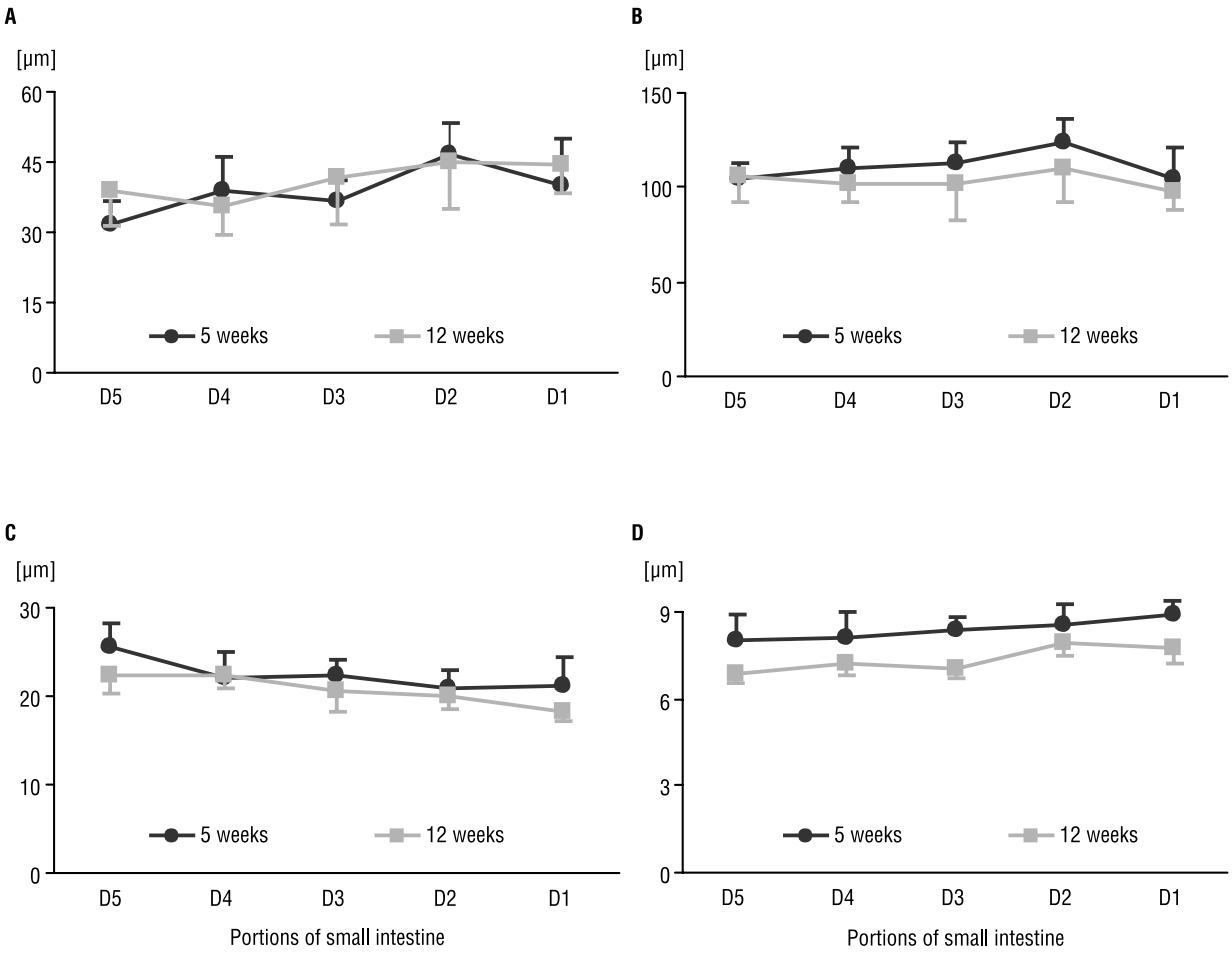

Figure 3. The graphs present the mean values [mm] and standard deviation of the parameters evaluated in particular portions of the small intestine: the muscular layer (**A**), the depth of the crypts (**B**), the height of the enterocyte (**C**), and the height of enterocyte nucleus (**D**).

DISCUSSION

The present study is the first to present a morphometric analysis of the chosen parameters of the small intestine in the C57BL/6J mouse strain. Two age groups were selected as a result of their key role during the maturation of the gastrointestinal tract. The 5th week of C57BL/6J mouse life is usually the weaning period [13, 34]. This is later than in the case of other mouse strains [25]. Weaning is a crucial factor and generally has a crucial influence on the morphology of the mucosal layer in the small intestine [5, 11, 40, 41]. The effect of weaning is at its most significant during the first week following. Later, approximately 2 weeks after weaning, the morphological parameters have stabilised and resemble the morphology characteristic for an adult animal [5, 14, 22]. After 12 weeks of life the gastrointestinal tract is indisputably mature.

The research conducted showed that in the mouse small intestine both the height and the width of the villi decreased on approaching the colon. This accords with the characteristics commonly considered valid for the histological structure of the small intestine mucosa. Similar results have been described in rat, human, mouse, guinea pig, rabbit and piglet intestines [6, 18, 23, 26, 27, 33].

The older group of C57BL/6J strain mice had higher villi than the younger one. Previous reports have indicated that jejunal villi were shorter in weaned pigs than in suckling pigs at the same approximate chronological age but that subsequently, two weeks after weaning, villus height increased [5, 14, 22]. The older group in our study was a post-weaned group, but our observations were performed 7 weeks after weaning and so our result was expected. Similar results were obtained in the rat by Clarke [6] and Wang et al. [38]. The former found that the height of the

proximal villi increased in successively older rats, while the height of the villi at the two distal sites decreased with age. Wang et al. [38] reported that the height of jejunal villi increased after birth and peaked in the 3rd postnatal month.

The widening of the villi near the crypt during ageing has previously been described in piglets [26]. In the present study no differences in this parameter were observed between the two age groups, although the higher values for the width of the villus connective tissue and the slightly lower values of the height of the enterocytes observed in the 12-week-old mice indicate the transformations which take place with ageing. In contrast, ageing had no effect on the size of rabbit enterocytes [18].

The lack of distinction in the thickness of the muscular layer between the 5-week and 12-week-old animals is in accord with the changes described by Wang et al. [38]. In rats the thickness of the jejunal muscular layer increased twofold until the 3rd week and between the 3rd and the 12th months. In our material the thickness of the muscular layer increased in the distal direction of the small intestine, which is in accord with the result of Ogiolda et al. [28].

The fact that the mice crypts become shallower with age is in agreement with the results obtained by Penna et al. [33] on the human intestine. However, Nunez et al. [26] observed that the crypts in 35-day-old piglets were deeper than in 5-day-old ones. The assumption that increased crypt depth is an indication of increased cell production is widely accepted. More intensive proliferation cannot be observed in younger transgenic animals of 5 weeks old, which can be explained by the lower metabolic activity of the cells.

The depth of the crypt in piglets decreases as it approaches the colon between the 3rd and 9th day after weaning, the pigs being weaned at 4 weeks of age [15]. The piglet's crypts described by Nunez et al. [26] were deeper in the duodenum than in ileum, but in the jejunum they achieved their highest value. The same differences were seen in the group of rabbits fed diets supplemented with 12% lignin. However, supplementation by cellulose, pectin or alfalfa respectively caused the crypts in the jejunum to reach their lowest depth.

In adult rats the crypts in the jejunum are deeper than in the ileum [2].

The depth of the crypt in rats fed a 2% or 20% casein diet as described by Qu et al. [35] also decreases on approach to the colon.

The enterocyte nuclei diminish with age. This decrease can be explained by the lower metabolic activity of the cells.

When taken together, our data provide the baseline morphological description of the small intestinal mucosa in wild type mice, strain C57BL/6J, which can be used as a reference for testing the influence of drugs, toxins, nutrients and inborn mutations on the mouse intestine.

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