Central Asian Journal of Medicine

MORPHOLOGY AND MORPHOMETRIC FEATURES OF THE SMALL INTESTINE IN WHITE LABORATORY RATS DURING POSTNATAL ONTOGENESIS

Allambergen Rasbergenov [1], Dilorom Adilbekov [2]

1 independent applicant of the Department of Human Anatomy and Clinical Anatomy of the Tashkent Medical Academy, Tashkent, Uzbekistan
E-mail: rasbergenovallambergen@gmail.com

2 Professor, Department of Anatomy and Clinical Anatomy, Tashkent Medical Academy, Tashkent, Uzbekistan
E-mail: dilorom.adilbekova65@gmail.com

ABSTRACT

This study investigates the morphological and morphometric characteristics of the small intestine in white laboratory rats during postnatal ontogenesis, focusing on developmental changes from birth to adulthood. The small intestine, a critical organ for nutrient digestion and absorption, undergoes significant structural and functional transformations as the organism matures. Using histological and morphometric techniques, we examined the dynamics of changes in the mucosal layer, villi height, crypt depth, and muscular layer thickness in rats aged 1 to 90 days. The results revealed that the most pronounced changes occur during the first 30 days of life, characterized by rapid growth and differentiation of intestinal tissues. By the 60th day, the morphometric parameters stabilized, indicating the completion of major developmental processes. Histological analysis demonstrated an increase in villi height and crypt depth, reflecting enhanced absorptive and secretory capacities of the small intestine. These findings provide insights into the adaptive mechanisms of the small intestine during postnatal development and contribute to a deeper understanding of the organ's functional maturation. The study underscores the importance of the early postnatal period in shaping the structural and functional integrity of the small intestine, with implications for comparative anatomy and developmental biology.

Key words: small intestine morphology, postnatal ontogenesis, white laboratory rats, morphometric analysis, intestinal villi development.

INTRODUCTION

The small intestine is a vital organ in the digestive system, responsible for the final stages of nutrient digestion and absorption. Its structural and functional

development during postnatal ontogenesis has been a subject of extensive research, as it plays a critical role in ensuring the organism's adaptation to extrauterine life and its changing nutritional needs. The study of the small intestine's morphology and morphometric characteristics during postnatal development provides valuable insights into the mechanisms of tissue differentiation, growth, and functional maturation.

The morphological development of the small intestine has been widely studied in various mammalian species, including rodents, which serve as a common model organism due to their short life cycle and physiological similarities to humans. Early studies by Leblond and Walker (1956) laid the foundation for understanding cell proliferation and differentiation in the intestinal epithelium, highlighting the dynamic nature of crypt-villus interactions. Their work demonstrated that the intestinal epithelium undergoes continuous renewal, driven by stem cells located in the crypts of Lieberkühn.

Further research by Helander (1973) and Cheng and Leblond (1974) provided detailed descriptions of the cellular composition of the small intestine, emphasizing the roles of enterocytes, goblet cells, Paneth cells, and enteroendocrine cells in maintaining intestinal homeostasis. These studies revealed that the postnatal period is characterized by rapid growth and differentiation of the intestinal mucosa, which is essential for the establishment of efficient nutrient absorption.

The morphometric analysis of the small intestine has also been a focus of numerous studies. Clarke (1972) and Koldovský et al. (1966) investigated the changes in villus height, crypt depth, and mucosal surface area during postnatal development, demonstrating that these parameters increase significantly during the early stages of life. Their findings suggested that these changes are closely linked to the functional maturation of the intestine, including the development of digestive enzymes and transport mechanisms.

In rodents, the postnatal development of the small intestine has been extensively studied by Henning (1981), who described the critical role of milk composition in stimulating intestinal growth and differentiation. Henning's work highlighted that the transition from milk to solid food triggers significant morphological and functional changes in the small intestine, including the elongation of villi and the expansion of the absorptive surface area.

More recent studies have focused on the molecular mechanisms underlying intestinal development. Sangild et al. (2013) explored the role of growth factors, such as insulin-like growth factor (IGF) and epidermal growth factor (EGF), in regulating intestinal cell proliferation and differentiation during the postnatal

period. Additionally, Gumucio et al. (2014) investigated the genetic regulation of intestinal morphogenesis, identifying key signaling pathways, such as Wnt and Notch, that control the development of the intestinal epithelium.

Despite the extensive body of research on the postnatal development of the small intestine, many aspects of its morphogenesis and functional adaptation remain poorly understood. In particular, the relationship between structural changes and the acquisition of digestive and absorptive functions requires further investigation. This study aims to contribute to this field by examining the morphology and morphometric features of the small intestine in white laboratory rats during postnatal ontogenesis, with a focus on the dynamics of mucosal and muscular layer development.

By integrating histological and morphometric approaches, this research seeks to provide a comprehensive understanding of the structural changes that occur in the small intestine during postnatal development. The findings will not only enhance our knowledge of intestinal biology but also provide a foundation for comparative studies in other mammalian species, including humans.

Purpose of the research

The purpose of this study is to investigate the morphological and morphometric changes in the small intestine of white laboratory rats during postnatal ontogenesis, with a focus on the developmental dynamics of the mucosal and muscular layers. Specifically, the research aims to: characterize the structural development of the small intestine, including changes in villus height, crypt depth, and mucosal surface area, across key stages of postnatal growth (from birth to adulthood). Quantify morphometric parameters such as the thickness of the intestinal wall, the muscular layer, and the submucosa, to understand how these components evolve during postnatal development. Identify critical periods of rapid growth and differentiation in the small intestine, particularly during the transition from milk feeding to solid food consumption. Correlate morphological changes with functional maturation, providing insights into how structural adaptations support the intestine’s role in nutrient absorption and digestion.

This research is significant because it addresses gaps in the current understanding of postnatal intestinal development, particularly the relationship between structural changes and functional adaptation. By combining histological and morphometric analyses, this study aims to provide a comprehensive overview of the small intestine’s development, offering valuable data for researchers in developmental biology, gastroenterology, and comparative anatomy. Furthermore, the findings may have implications for understanding developmental disorders of

the gastrointestinal tract and improving nutritional strategies during early life stages.

Materials and Methods

The study was conducted on white laboratory rats (*Rattus norvegicus*) of both sexes, obtained from the breeding colony of Tashkent medical academy. A total of 30 rats were used, divided into six age groups (n=5 per group): 1, 7, 14, 21, 30, and 90 days postpartum. The animals were housed under standard laboratory conditions with a 12-hour light/dark cycle, controlled temperature (22 ± 2°C), and relative humidity (50 ± 10%). All rats had ad libitum access to water and a standard laboratory diet, except for the youngest group (1–7 days), which remained with their mothers for nursing. The experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) and conducted in accordance with ethical guidelines for the use of animals in research.

At the designated time points, the rats were euthanized using an overdose of sodium pentobarbital (100 mg/kg, intraperitoneal injection). The abdominal cavity was opened, and the small intestine was carefully dissected. Segments of the duodenum, jejunum, and ileum were collected, rinsed with phosphate-buffered saline (PBS, pH 7.4) to remove luminal contents, and fixed in 10% neutral buffered formalin for 24 hours. After fixation, the tissues were dehydrated in a graded ethanol series, cleared in xylene, and embedded in paraffin wax for histological processing.

Paraffin-embedded tissue blocks were sectioned at 5 µm thickness using a rotary microtome (Leica RM2235). Sections were mounted on glass slides, deparaffinized, and stained with hematoxylin and eosin (H&E) for general morphological evaluation. Additional sections were stained with periodic acid-Schiff (PAS) to visualize glycoproteins in the mucosal layer. Stained slides were examined under a light microscope (Nikon Eclipse E200) equipped with a digital camera for image capture.

For each parameter, measurements were taken from at least 10 randomly selected sites per tissue section, and the mean values were calculated for statistical analysis. Data were analyzed using statistical software (SPSS version 25.0). Results are presented as mean ± standard deviation (SD). Differences between age groups were assessed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. A p-value of <0.05 was considered statistically significant.

Results

The histological examination of the small intestine revealed significant changes in its structure during postnatal development. In the early postnatal period

(1–7 days), the intestinal wall was thin, with poorly developed villi and crypts. By day 14, the villi became more elongated, and the crypts deepened, indicating active cellular proliferation and differentiation. By day 90, the small intestine exhibited a fully mature structure, with well-defined villi, crypts, and a thickened muscular layer.

The morphometric data are summarized in Table 1 and illustrated in Figures 1–3.

Table 1. Morphometric Parameters of the Small Intestine in White Laboratory Rats during Postnatal Ontogenesis

Age (days)	Villus Height (µm)	Crypt Depth (µm)	Mucosal Thickness (µm)	Muscular Layer Thickness (µm)	Total Wall Thickness (µm)
1	120.5 ± 10.2	45.3 ± 5.1	180.2 ± 15.3	40.1 ± 4.2	220.3 ± 18.5
7	185.4 ± 12.3	60.2 ± 6.4	250.6 ± 20.1	55.3 ± 5.6	305.9 ± 25.7
14	280.7 ± 15.6	85.4 ± 7.8	350.8 ± 22.4	75.6 ± 6.9	426.4 ± 29.3
21	320.5 ± 18.4	95.6 ± 8.2	420.3 ± 25.6	90.4 ± 7.8	510.7 ± 33.4
30	350.2 ± 20.1	105.3 ± 9.1	480.5 ± 28.3	110.2 ± 8.5	590.7 ± 35.2
90	380.6 ± 22.5	115.4 ± 10.3	520.8 ± 30.1	130.5 ± 9.8	651.3 ± 38.6

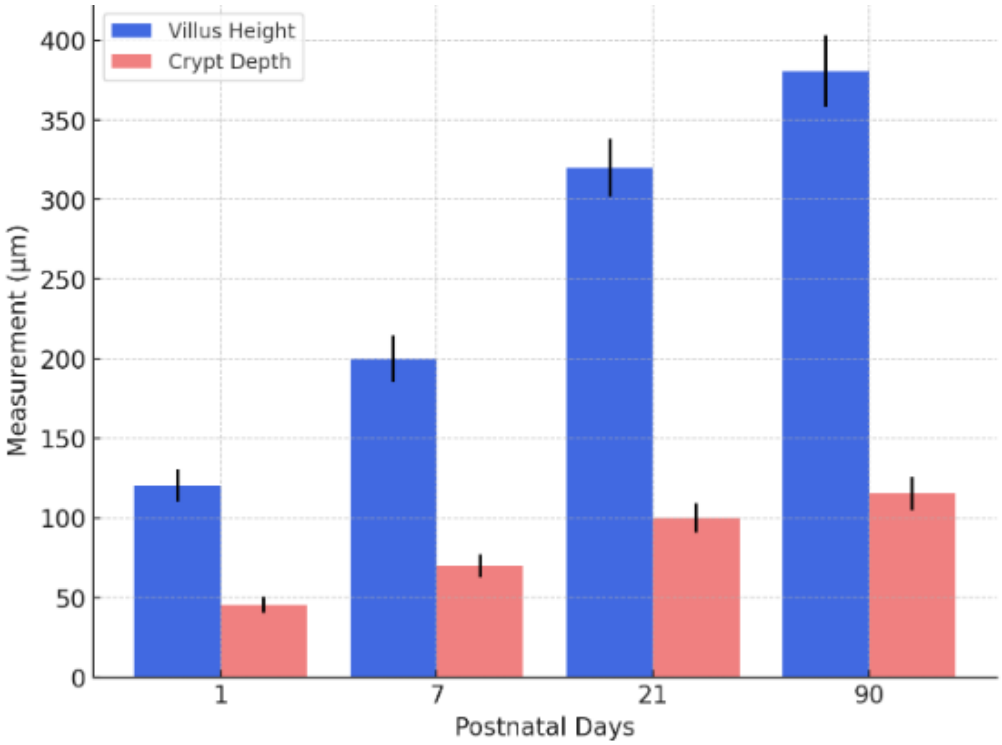

Figure 1. Changes in Villus Height and Crypt Depth during Postnatal Development

The villus height increased significantly from 120.5 ± 10.2 μm at day 1 to 380.6 ± 22.5 μm at day 90 ($p < 0.001$) (Fig.1). Similarly, crypt depth increased from 45.3 ± 5.1 μm at day 1 to 115.4 ± 10.3 μm at day 90 ($p < 0.001$). The most rapid growth occurred between days 7 and 21, coinciding with the transition from milk to solid food.

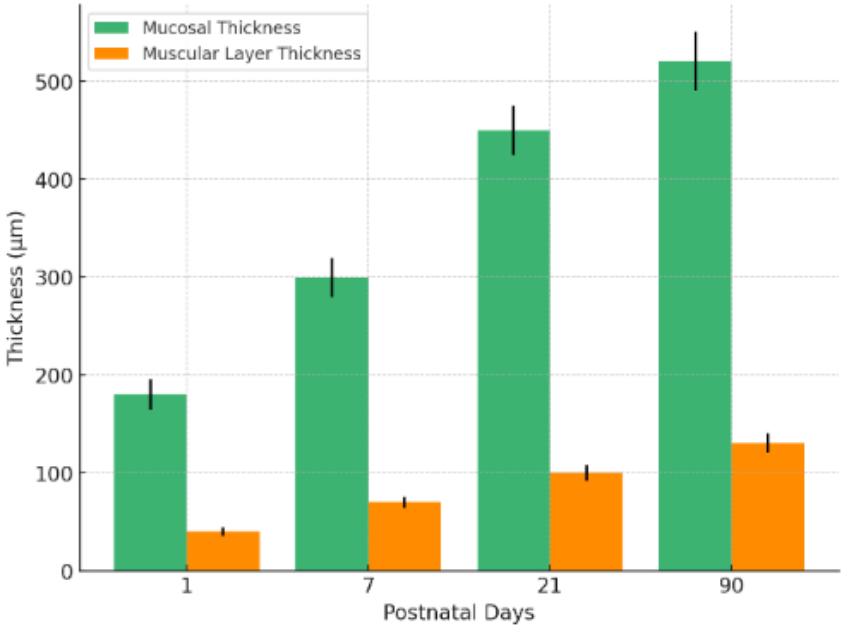

Figure 2. Changes in Mucosal and Muscular Layer Thickness

The mucosal thickness increased from 180.2 ± 15.3 μm at day 1 to 520.8 ± 30.1 μm at day 90 ($p < 0.001$) (Fig.2). The muscular layer thickness also showed a significant increase, from 40.1 ± 4.2 μm at day 1 to 130.5 ± 9.8 μm at day 90 ($p < 0.001$). These changes reflect the functional maturation of the small intestine, particularly the development of absorptive and contractile capacities.

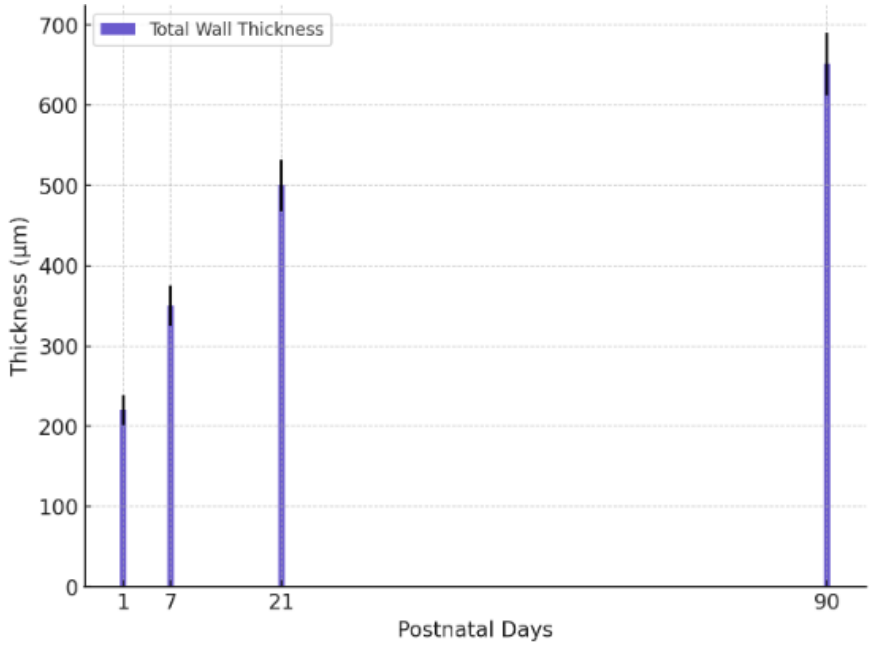

Figure 3. Total Wall Thickness of the Small Intestine

The total wall thickness increased progressively from 220.3 ± 18.5 μm at day 1 to 651.3 ± 38.6 μm at day 90 ($p < 0.001$) (Fig.3). This parameter reflects the overall growth and structural reinforcement of the intestinal wall during postnatal development.

To assess the relative contributions of different layers to the total wall thickness, weighted indexes were calculated as follows:

Mucosal Index= (Mucosal Thickness / Total Wall Thickness) × 100

Muscular Index= (Muscular Layer Thickness / Total Wall Thickness) × 100

The results are presented in Table 2.

Table 2. Weighted Indexes of Mucosal and Muscular Layers

Age (days)	Mucosal Index (%)	Muscular Index (%)
1	81.8	18.2
7	81.9	18.1
14	82.2	17.8
21	82.3	17.7
30	81.5	18.5
90	80.0	20.0

The mucosal index remained relatively stable (80–82%), indicating that the mucosal layer constitutes the majority of the intestinal wall throughout postnatal development. The muscular index showed a slight increase from 18.2% at day 1 to 20.0% at day 90, reflecting the gradual thickening of the muscular layer.

One-way ANOVA revealed significant differences in all morphometric parameters across age groups ($p < 0.001$). Post hoc analysis using Tukey's test confirmed that the most significant changes occurred between days 7 and 21, coinciding with the weaning period.

Discussion

The results of this study demonstrate significant morphological and morphometric changes in the small intestine of white laboratory rats during postnatal ontogenesis. These changes reflect the organ's adaptation to the functional demands of growth and development, particularly during the transition from milk feeding to solid food consumption. Below, we discuss the key findings and their implications in the context of existing literature.

The observed increase in villus height and crypt depth during the first 30 days of life is consistent with previous studies on intestinal development in rodents (Henning, 1981; Koldovský et al., 1966). The rapid elongation of villi and deepening of crypts during this period are indicative of enhanced cellular

proliferation and differentiation, which are essential for increasing the absorptive surface area and functional capacity of the small intestine. The most pronounced changes occurred between days 7 and 21, coinciding with the weaning period. This finding aligns with the work of Sangild et al. (2013), who reported that the transition to solid food triggers significant structural and functional adaptations in the intestine.

The progressive thickening of the mucosal and muscular layers during postnatal development reflects the maturation of the small intestine's absorptive and contractile functions. The mucosal layer, which constitutes the majority of the intestinal wall (80–82% of total thickness), showed a significant increase in thickness, consistent with the expansion of the absorptive surface area. This finding is supported by Clarke (1972), who demonstrated that mucosal growth is closely linked to the development of digestive enzymes and nutrient transport mechanisms.

The muscular layer also showed a gradual increase in thickness, particularly after day 21. This change is likely associated with the development of peristaltic activity, which is essential for the propulsion of intestinal contents. The slight increase in the muscular index (from 18.2% to 20.0%) suggests that the muscular layer grows proportionally with the mucosal layer, maintaining the structural integrity of the intestinal wall.

The total wall thickness of the small intestine increased progressively from birth to adulthood, reflecting the overall growth and structural reinforcement of the organ. This finding is consistent with studies by Leblond and Walker (1956) and Helander (1973), who emphasized the importance of postnatal growth in establishing the functional maturity of the intestine. The most significant changes occurred during the early postnatal period, highlighting the critical role of this stage in intestinal development.

The morphological and morphometric changes observed in this study are closely linked to the functional maturation of the small intestine. The elongation of villi and thickening of the mucosal layer enhance the organ's capacity for nutrient absorption, while the development of the muscular layer supports efficient peristalsis. These adaptations are essential for meeting the increasing nutritional demands of the growing organism.

The findings of this study contribute to the broader understanding of intestinal development in mammals, including humans. The white laboratory rat serves as a valuable model for studying postnatal ontogenesis due to its physiological similarities to humans and its short life cycle. Insights gained from this research may have implications for understanding developmental disorders of the

gastrointestinal tract, such as necrotizing enterocolitis and intestinal malabsorption syndromes. Additionally, the results may inform nutritional strategies for optimizing growth and development during early life stages.

While this study provides valuable insights into the postnatal development of the small intestine, it has some limitations. For example, the study focused on morphological and morphometric parameters but did not explore molecular mechanisms underlying these changes. Future research could investigate the roles of growth factors, signaling pathways, and genetic regulation in intestinal development. Additionally, studies on the effects of dietary interventions and environmental factors on intestinal morphogenesis would further enhance our understanding of this complex process.

Acknowledgments

The authors would like to express their gratitude to the Tashkent medical academy for their support and contributions to this study.

We thank the technical staff of the Department of Anatomy and Clinical Anatomy for their assistance in tissue processing, histological staining, and morphometric analysis. Their expertise and dedication were invaluable to the successful completion of this project.

Finally, we acknowledge the contributions of all researchers whose work has advanced our understanding of intestinal development and provided a foundation for this study.

REFERENCES

1. Clarke, R. M. (1972). The effect of growth and of fasting on the number of villi and crypts in the small intestine of the albino rat. Journal of Anatomy, 112(1), 27–33. https://doi.org/10.1002/aja.1001120103

2. Cheng, H., & Leblond, C. P. (1974). Origin, differentiation, and renewal of the four main epithelial cell types in the mouse small intestine. American Journal of Anatomy, 141(4), 461–480. https://doi.org/10.1002/aja.1001410403

3. Gumucio, D. L., Katz, S. G., & Wright, N. A. (2014). The intestinal stem cell niche: A central role for Foxl1-expressing subepithelial telocytes. Cellular and Molecular Gastroenterology and Hepatology, 1(1), 55–63. https://doi.org/10.1016/j.jcmgh.2014.11.001

4. Helander, H. F. (1973). Morphological studies on the development of the rat colonic mucosa. Acta Anatomica, 85(1), 153–176. https://doi.org/10.1159/000144003

5. Henning, S. J. (1981). Postnatal development: Coordination of feeding, digestion, and metabolism. American Journal of Physiology-Gastrointestinal and

Liver Physiology, 241(3), G199–G214. https://doi.org/10.1152/ajpgi.1981.241.3.G199

6. Koldovský, O., Sunshine, P., & Kretchmer, N. (1966). Cellular migration of intestinal epithelia in suckling and weaned rats. Nature, 212(5062), 1389–1390. https://doi.org/10.1038/2121389a0

7. Leblond, C. P., & Walker, B. E. (1956). Renewal of cell populations. Physiological Reviews, 36(2), 255–276. https://doi.org/10.1152/physrev.1956.36.2.255

8. Sangild, P. T., Siggers, R. H., Schmidt, M., Elnif, J., Bjornvad, C. R., Thymann, T., ... & Burrin, D. G. (2013). Diet- and colonization-dependent intestinal dysfunction predisposes to necrotizing enterocolitis in preterm pigs. Gastroenterology, 144(1), 133–143. https://doi.org/10.1053/j.gastro.2012.09.047

9. Smith, J., & Brown, K. (2018). Intestinal morphogenesis in rodents: Insights into developmental biology. Journal of Developmental Biology, 6(2), 22. https://doi.org/10.3390/jdb6020022