Reports of Morphology, Vol. 24, №2, Pages 66-72

ISSN 1818-1295 eISSN 2616-6194



Planimetric correlations between Peyer's patches and the area of small intestine of white rats

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ARTICLE INFO

Received: 9 March, 2018 Accepted: 30 April, 2018

UDC: 616.341: 612.1

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e-mail: vogrin034@gmail.com Hryn V.H. The largest concentration of the local immune system in the form of the nodular associations of the lymphoid tissue with the epithelium of the mucous membranes (tonsils, single lymphoid nodules and their aggregations, named Peyer's patches) is located in the gut. The paper is aimed at the study of the quantitative and planimetric correlations between the Peyer's patches and the area of the small intestine of white rats based on the visual assessment and comparative analysis of some metric values. The study was conducted on 60 white male rats, which were assigned into 2 groups of 30 animals: after the morning feeding (controls) and after a daily fasting. After the removal made in advance, the gut-associated complexes have been studied from the stomach to the cecum. The resulting experimental data have been processed on a personal computer using the EXCEL 2010 (Microsoft Excel Corp., USA) software. No significant difference in the diameter and length of the small intestine of white rats of the first and second groups was found. The total area of the small intestine wall in the control group ranged from 8666 mm² to 20724 mm², and from 8496 mm² to 20573 mm² in group II (after a daily fasting), ranking equally. Thickness parameters of the small intestine in two groups of animals were almost similar in its unchanged, within the limits of individual variability, longitudinal length. To conduct an accurate quantitative and planimetric analysis of the aggregated lymphoid nodules of the white rat's small intestine, it was advisable to distinguish 3 groups, namely: small-, medium- and large-sized. Thus, their total number varies from 12 to 28 units. Among them 8 to 17 units (on the average of 12.60±0.40) were small-sized, 2 to 11 (5.800±0.500) were medium-sized, and the large ones were not always found. For example, in the studied samples, they were absent in 6 animals, whereas 1 to 5 units were presented in the rest of animals. The area of single small Peyer's patches ranged from 1.570 to 9.800 mm², and their total area was 64.90±2.90 mm²; the area of medium samples individually ranges from 10.60 to 27.50 mm². Totally, they occupy an average area of 97.60±8.00 mm². The same value of individual large aggregated nodules is between 31.40 and 60.40 mm², which totally accounted on the average of 58.40±10.30 mm². Thus, the average statistical value of the total areas of Peyer's patches is 220.9±14.4 mm², accounting for only 2% of the total area of the small intestine wall of white rats, not including single lymphoid nodules, not exceeding 1 mm.

Keywords: Peyer's patches, small intestine, metric values.

Introduction

Currently, it has been found that the immune system of the mucous membranes of the gut is functionally consolidated with the immune mechanisms of the mucous membranes of other hollow organs (the phenomenon of the "immune solidarity of the mucous membranes") [6, 14, 16. 22, 32, 35, 36]. Apparently, the gut is characterized by the highest concentration of local immune system in the form of nodular associations of the lymphoid tissue with the epithelium of the mucous membranes [2, 8, 10, 21, 25], involving the tonsils, single lymphoid nodules, as well as their aggregations, named Peyer's patches. Moreover, the highest concentration of the latter is found in distal part of the human ileum and appendix, which is absent in rats, as well as the presence of tonsils is unusual for this species of rodents [9, 11, 12, 19, 33, 40].

Consequently, in white rats, according to the publications,

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aggregated lymphoid nodules (Peyer's patches) are located mainly in the wall of the small intestine, which is a transitive section between the stomach and cecum, accounting for about one meter in length, which is just 4-5 times shorter than in humans, being completely disproportionate relative to their body mass [3, 5, 13, 20, 24]. Taking into account that the lymphoid structures of the small intestine provide with immune control over its antigenic contents and are the initial links in the mechanisms of formation of the corresponding immune responses, it can be considered that the following planimetric parameters will serve as the most informative quantitative indices to estimate this correlation: (1) the total area of the small intestine walls; (2) the overall dimensional area of the Peyer's patches, and (3) index of the latter to the first ratio.

The paper was *aimed* at the study of the quantitative and planimetric correlation between the Peyer's patches and the area of the white rats small intestine based on the visual assessment and comparative analysis of some metric values.

Materials and methods

The study was conducted on 60 mature Wistar white male rats, weighing 200.0±20.00 g, one half of which was on a daily fasting regimen before vivisection, and euthanasia of the others was carried out immediately after the morning feeding. Before the experiment, all animals were kept in standard conditions of the experimental biological clinic (vivarium) of the Ukrainian Medical Stomatological Academy in compliance to the regulations on keeping experimental animals adopted by the European Parliament and Council Directive (2010/63/EU), the Order of the Ministry of Education and Science, Youth and Sports of Ukraine as of 01.03.2012. №249 "On approval of the procedure for conducting tests, experiments on animals by research institutions" and "General ethical principles of experiments on animals", adopted by the V National Congress on Bioethics (Kiev, 2013) [7, 31, 34].

After euthanasia made under the overdose of thiopental sodium anesthesia (75 mg/kg animal body weight intramuscularly in the upper third of the hip of the hind paw), the anterior abdominal wall was removed from all animals. The total organs of the gut were removed from the abdominal cavity, which, with the preservation of the natural position between them, were embedded into 10% neutral buffered formalin and photographed [1, 4, 39, 41]. Within few days, after washing in running water, in the area of the pyloric sphincter, loops of the small intestine were cut off from the stomach and cecum. It was measures flat, placed on a sheet of laminated graph paper with refinement using a metal ruler (GOST 427-75) and caliper (SHTS-1 DSTU 8.113: 2009; GOST 166-89 as of 14.05.17.Cert. No.1188/ 0315), which was calibrated by the territorial body of the State Consumer Standard of Ukraine.

Considering that the small intestine is a tubular formation, the calculations used in mathematics for

measuring the lateral surface area of cylindrical figures are quite applicable for calculating the area of its wall. Consequently, it is necessary to know only the size of the diameter of the figure and its height or length, which is easy to measure on the selected specimens of the small intestine. With these data, the calculation of the area of its wall is initially reduced to determining its perimeter according to the formula:

 $S=2\pi RL=\pi DL,$

where R is the outer radius of the intestine, D is the outer diameter, L is the longitudinal length of the intestine.

The resulting value was then multiplied by the actual length of the small intestine. It would be appropriate to point at this, the only possible, simple way of calculation of the area of the small intestine, since, for some reasons, it was not mentioned in the related descriptions of the authors, cited above.

The initial digital parameters of aggregated lymphoid nodules are represented by their number in the small intestine wall and two-dimensional size of isolated ones. Given that they all have a rounded shape (orbicular or oval), their area can be calculated by conventional formulas. The area of the oval lymphoid nodules was calculated using the formula for calculating the area of an ellipse:

 $S = \pi a b$,

where S is the area of the ellipse, π is the pi-number (3.1415), a is the length of the semimajor axis, b is the length of the minor semiaxis.

The calculation of the area of orbicular lymphoid nodules was carried out according to the formula for calculating the area of a circle:

 $S = \pi R^2$,

where S is the area of a circle, π is the pi-number (3.1415), r is the radius of a circle [18, 38].

The resulting experimental data were processed on a personal computer using the EXCEL 2010 (Microsoft Excel Corp., USA) software.

Results

Noteworthy, the analysis of the resulting digital data has shown no significant difference in the major metric parameters of the small intestine (its diameter and length) of white rats of the first and second groups, being within the statistical accuracy, which depends entirely on the variation of quantitative indicators (Table 1). Therefore, the calculation made on the basis of these initial data of the total area of its wall in animals of the control group is from 8666 mm² to 20724 mm²; in the group of animals undergone daily fasting, the value ranges from 8496 mm² to 20573 mm², ranking equally. The average value was 13127±644 mm².

For better representation of the morphophysiological features of the digestive system of white rats, 2 groups of animals were formed; animals of one of the group underwent evening fasting. By this, on the one hand, the representativeness of metric studies was twice higher, and on the other hand, it has been possible to identify some

No.	Groups								
	After mor	rning feeding (control group) ((n=30)	After daily fasting (n=30)					
	D (diameter), mm	L (longitudinal length), mm	S (area), mm ²	D (diameter), мм	L (longitudinal length), мм	S (area), mm ²			
M±m	4.200±0.200	998.6±9.0	13127±644	4.200±0.200	1005±9	13313±638			
Min	3	920	8666	3	902	8497			
Max	6	1100	20724	6	1092	20573			

Table 1. The mean values of the area of the outer surface of small intestine of white rats of both groups.

Notes: S - area, D - diameter, L - longitudinal length, M - mean value, m - error of mean, Min - minimal value, Max - maximum value.



Fig. 1. Location, configuration and general dimensions of the stomach and cecum of white rats after daily fasting. 1 - esophagus; 2 stomach; 3 - duodenum; 4 - loops of the small intestine; 5 - cecum.

morphological peculiarities of changes in the gut that occur during their daily feeding cycle. Here it is reasonable to give them a general assessment. Notably, in animals after a daily fasting, the stomach is collapsed, while the cecum looks more dilated (Fig. 1). At the same time, the small intestine, which is the transitive part between them, has heterogeneous lengthwise alternation of distensions, containing portions of chyme of different size, which are separated from each other by constrictions of different expressions. And the dimensional frequency of these distensions increases in the distal direction, i.e., towards the cecum (Fig. 2). A completely opposite picture occurs in animals, killed immediately after the morning feeding. The difference was mainly in their small intestine which was of homogenous thickness that significantly simplified



Fig. 2. Total specimen of the white rats' gut after daily fasting. 1 - esophagus; 2 - stomach; 3 - loops of the small intestine; 4 - cecum; 5 - colon.

measuring of its thickness, whereas in the first group of animals, calculation of its average thickness had to be made from the measurements in the region of several thickenings and narrowings. Finally, the thickness parameters of the small intestine in two groups of animals turned out to be similar, as mentioned above, with an unchanged, within the limits of individual variation, its longitudinal length. Consequently, in the process of transitive movement of the chyme from the stomach into the cecum the white rats' small intestine undergoes only homogenous, isometric deformation while preserving its basic dimensional parameters.

Peyer's patches are clearly visualized without optical instruments in the form of somewhat whitish orbicular or oval protrusions of various sizes that are faintly visible on



Fig. 3. Outer surface of the small intestine loops. 1 - small intestine, 2 - Peyer's patches.

Table 2. The resulting data of the quantitative and planimetric analysis of the aggregated lymphoid nodules (Peyer's patches) of the white rats small intestine.

No.	Total number of PPs	Number and area (S) of PPs size						
		Small		Medium		Large		Total area of PPs (mm ²)
		Number	S (mm²)	Number	S (mm²)	Number	S (mm²)	,
M±m	19.90±0.70	12.60±0.40	64.90±2.90	5.800±0.500	97.60±8.00	1.500±0.300	58.40±10.30	220.9±14.4
Min	12	8	1.570	2	10.60	0	31.40	87.30
Max	28	17	9.800	11	27.50	5	60.40	406.7

Notes: PPs - Peyer's patches, S - area, M - mean value, m - error of mean, Min - minimal value, Max - maximum value.

the outer surface of the white rats' small intestine along its entire length, starting from the duodenal part and reaching almost the cecum (Fig. 3). Moreover, their alternation in shape and size in such longitudinal direction is rather voluntary and changeable. However, in the total big combinational variability of their distribution along the length of the small intestine, certain regularity is observed in the form of smoothly increasing concentration of lymphoid tissue towards the cecum, which specifically results in enlargement of Peyer's patches and the last of them is the largest. If the shape of the aggregated lymphoid nodules is not an essential morphological criterion in their evaluation, then their dimensions should be taken into account, since they directly depend on the number of single lymphoid nodules associated in them. In this regard, for more clear quantitative and planimetric analysis of aggregated lymphoid nodules of the white rats' small intestine, it is reasonable to distinguish 3 groups among them, namely: small-, medium- and large-sized, which were separately subjected to mathematical analysis (Table 2). The findings show, first of all, the great variability of the total quantitative composition of lymphoid formations and their metric parameters. Thus, their total number varies from 12 to 28 units (the average value was 19.90 ± 0.70). Among them 8 to 17 units (on the average of 12.60 ± 0.40) were small-sized, 2 to 11 (5.800 ± 0.500) were medium-sized, and the large ones were not always found. For example, in the studied samples, they were absent in 6 animals, whereas 1 to 5 units were presented in the rest of the animals.

It has been subsequently established that the area of single small-sized Peyer's patches ranged from 1.570 to

9.800 mm², and their total area was 64.90 ± 2.90 mm²; the area of medium-sized samples individually ranges from 10.60 to 27.50 mm². In aggregate, they occupy an average area of 97.60\pm8.00 mm². The same value of individual large aggregated nodules is between 31.40 and 60.40 mm², which totally accounted on the average of 58.40 ± 10.30 mm².

Discussion

The anatomy of the gut of laboratory rats has not been fully elucidated in publications, and the available scarce data are not systematized, while it is known that the shape, structure and topography of the organs of the gastrointestinal tract in vertebrates are quite variably [23, 30, 37, 40].

The similarity of the structural organization of the organs and tissues of humans and some animals determines the use of the latter for the experimental modeling of various diseases occurred in clinical practice [2, 17, 23]. However, for a deeper understanding and adequate interpretation of the findings of the experiment and the development of methods for their correction, certain species characteristics and differences that are typical for specific animals should be taken into account as well as the quantitative parameters of their tissues, organs and systems for comparison.

Accurate digital data on metric values of the albino rats' small intestine are presented in the "Results" section. However, such parameter as the mean statistical value of the area of the small intestine (13127±644 mm²) is noteworthy since it does not coincide with similar values obtained by S.A. Kashchenko et al. [15, 26, 27, 28, 29], stating that the surface area of the small intestine is at the average of 9121±35 mm² in albino male rats weighing 250-280 g, that is approximately 300.0 mm² less than the calculated value. Apparently, it can be explained differently. For example, when measuring dimensions of small intestine, which is, morphologically, rather variable gutassociated organ, the errors in the results are always inevitable; this entirely depends on its functional state on the eve of the vivisection of animals, as well as their individual status and weight. The latter factor can be minimized by selecting animals of approximately equal

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weight (200.0±20.0 g).

Notably, the similar algorithm have been used by other researchers in their investigations [2, 10, 13, 14, 22], and it would be appropriate to refer to their findings during the study. Specifically, the dependence of the above metric values on the functional state of the digestive system of the experimental animals is crucial and should be taken into account during the study.

Regarding the quantitative and topographical indices of Peyer's patches, they are mainly concentrated in the small intestine on the wall opposite to the site of the mesentery attachment [5, 9, 27, 30, 35]. Many morphologists have been conducting such studies, though no detailed analysis of Peyer's patches has been carried out, with the distribution of the latter into groups (small-, medium- and large-sized). The resulting quantitative data on the group lymphoid formations (Peyer's patches) of the small intestine, as well as the values of their size and area, contribute to enhancement of expertise, which is beneficial both for theoretical and practical medicine.

Prospects for further investigations are the study of the lymphoepithelial structures of the small intestine of white rats on the immunohistochemical level, as well as in the simulation of various pathological states.

Conclusions

The simple addition of the mean values of the total areas of small-, medium- and large-sized Peyer's patches gave a value of $220.9\pm14.40 \text{ mm}^2$, which is only 2% of the total area of the small intestine of albino rats, the metric value of which, according to above data, is equal to the average of $13.127\pm644 \text{ mm}^2$. However, the superficial contact of the lymphoid tissue with the contents of the small intestine is limited by this value; single lymphoid nodes are not taken into account, since only some of them can be visible on its outer surface in the form of single whitish spots of not more than 1 mm. In fact, their amount is much bigger; they are distributed in large quantities in the mucous membrane of the small intestine, occupying an intermediate position between the Peyer's patches.

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ПЛАНІМЕТРИЧНІ СПІВВІДНОШЕННЯ МІЖ ПЕЙЄРОВИМИ БЛЯШКАМИ І ПЛОЩЕЮ ТОНКОЇ КИШКИ БІЛИХ ЩУРІВ Гринь В.Г.

Травний тракт відрізняється найбільшою концентрацією місцевого представництва імунної системи у вигляді вузликових асоціацій лімфоїдної тканини з епітелієм слизових оболонок (мигдалики, поодинокі лімфоїдні вузлики і їх групові скупчення пейсрові бляшки). Мета - вивчити кількісне і планіметричне співвідношення між пейсровими бляшками і площею тонкої кишки білих щурів на основі візуальної оцінки і порівняльного аналізу деяких метричних показників. Дослідження проведено на 60 білих щурах-самцях, яких розділили на 2 групи по 30 тварин: після ранкового годування (контрольна група) і після добового голодування. Після попереднього видалення, досліджували комплекси шлунково-кишкового тракту від шлунку до сліпої кишки. Отримані експериментальні дані оброблені на персональному комп'ютері пакетом прикладної та статистичної програми EXCEL 2010 (Microsoft Excel Corp., США). Діаметр і довжина тонкої кишки білих щурів першої і другої групи між собою достовірно не відрізнялися. Загальна площа стінки тонкий кишки в контрольній групі склала від 8666 мм² до 20724 мм², а у 2 групі (після добового голодування) від 8496 мм² до 20573 мм², що виявилося практично рівнозначним. Параметри товщини тонкої кишки у двох груп тварин виявилися близькими при незмінній, в межах індивідуальної варіативності, її поздовжньої довжини. З метою проведення коректного кількісного і планіметричного аналізу групових лімфоїдних вузликів тонкої кишки білих щурів доцільно було виділити 3 групи, а саме: малого, середнього і великого розміру. Так, загальна їх кількість варіює в межах від 12 до 28 одиниць. Серед них малих форм налічується від 8 до 17 одиниць (в середньому - 12,60±0,40), середніх - від 2 до 11 (5,800±0,500), а великі зустрічаються не завжди. Наприклад, у вивчених вибірках вони були відсутні у 6 тварин, тоді як у всіх інших їх налічувалося від 1 до 5 одиниць. Площа окремих малих пейєрових бляшок перебувала в діапазоні від 1,570 до 9,800 мм², а загальна їх площа дорівнює 64,90±2,90 мм²; площа середніх зразків окремо коливається від 10,60 до 27,50 мм². В сукупності вони займають площу в середньому 97,60±8,00 мм². Те ж значення окремих великих групових вузликів знаходиться між 31,40 і 60.40 мм², що в їх сукупному значенні дорівнює в середньому 58,40±10,30 мм². Таким чином, встановлено середньостатистичне значення загальних площ пейєрових бляшок, яке дорівнює 220,9±14,4 мм², що становить лише 2% від загальної площі стінки тонкої кишки білих щурів, не враховуючи поодинокі лімфоїдні вузлики, величиною не більше 1 мм. Ключові слова: пейєрові бляшки, тонка кишка, метричні показники.

ПЛАНИМЕТРИЧЕСКИЕ ОТНОШЕНИЯ МЕЖДУ ПЕЙЕРОВЫМИ БЛЯШКАМИ И ПЛОЩАДЬЮ ТОНКОЙ КИШКИ БЕЛЫХ КРЫС *Гринь В.Г.*

Пищеварительный тракт отличается самой большой концентрацией местного представительства иммунной системы в виде узелковых ассоциаций лимфоидной ткани с эпителием слизистых оболочек (миндалины, одиночные лимфоидные узелки и их групповые скопления - пейеровы бляшки). Цель - изучить количественное и планиметрическое отношение между пейеровыми бляшками и площадью тонкой кишки белых крыс на основе визуальной оценки и сравнительного анализа некоторых метрических показателей. Исследование проведено на 60 белых крысах-самцах, которых разделили на 2 группы по 30 животных: после утреннего кормления (контрольная группа) и после суточного голодания. После предварительного удаления, исследовали комплексы желудочно-кишечного тракта от желудка до слепой кишки. Полученные экспериментальные данные обработаны на персональном компьютере пакетом прикладной и статистической программы EXCEL 2010 (Microsoft Ехсеl Согр., США). Диаметр и длина тонкой кишки белых крыс первой и второй группы между собой достоверно не отличались. Общая площадь стенки тонокй кишки в контрольной группе составила от 8666 мм2 до 20724 мм², а во 2 группе (после суточного голодания) от 8496 мм² до 20573 мм², что оказалось практически равнозначным. Толстотные параметры тонкой кишки у двух групп животных оказались близкими при неизменной, в пределах индивидуальной вариативности, ее продольной длины. С целью проведения корректного количественного и планиметрического анализа групповых лимфоидных узелков тонкой кишки белых крыс целесообразно было выделить 3 группы, а именно: малого, среднего и большого размера. Так, общее их количество варьирует в пределах от 12 до 28 единиц. Среди них малых форм насчитывается от 8 до 17 единиц (в среднем - 12,60±0,40), средних - от 2 до 11 (5,800±0,500), а большие встречаются не всегда. Например, в изученных выборках они отсутствовали у 6 животных, тогда как у всех остальных их насчитывалось от 1 до 5 единиц. Площадь отдельных малых пейеровых бляшек находилась в диапазоне от 1,570 до 9,800 мм², а общая их площадь равна 64,90±2,90 мм²; площадь средних образцов в отдельности колеблется от 10,60 до 27,50 мм². В совокупности они занимают площадь в среднем 97,60±8,00 мм². То же значение отдельных больших групповых узелков находится между 31,40 и 60,40 мм², что в их совокупном значении равно в среднем 58,40±10,30 мм². Таким образом, установлено среднестатистическое значение общих площадей пейеровых бляшек равно 220,9±14,4 мм², что составляет всего лишь 2% от общей площади стенки тонкой кишки белых крыс, не учитывая одиночные лимфоидные узелки, величиной не превышающей 1 мм.

Ключевые слова: пейеровы бляшки, тонкая кишка, метрические показатели.