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Functional Morphology of the Bronchial Mucociliary Transport System in Rats During Postnatal Ontogenesis

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ABSTRACT

BACKGROUND: Age-related remodeling of the mucociliary transport system (MCTS) of the airways plays a significant role in the pathogenesis of respiratory diseases. However, the available published data lacks studies of the structural and functional parameters of bronchial epithelial lining throughout postnatal development conducted under standardized conditions and using unified methodological approaches, which highlights the relevance of further research in this area.

AIM: The work aimed to investigate age-related patterns of postnatal morphogenesis of the bronchial mucociliary transport system in rats based on structural and functional analysis of the respiratory epithelium components.

METHODS: The work utilizes in vivo measurement of ciliary activity, light and electron microscopy, immunohistochemistry (using antibodies against the proliferation marker Ki-67), and morphometry. The research material consisted of respiratory epithelium from the main, lobar, and segmental bronchi of Wistar rats ($n = 76$) at ages of 1, 8, 14 days, and 1, 3, 6, 14, 20, and 26 months, 6–9 animals per time point.

RESULTS: In newborn rats, the bronchial tree is lined with a single-layer epithelium composed predominantly of undifferentiated cells (59%–62%) and a few ciliated cells (21%–26%). During the first postnatal month, MCTS elements undergo the most intensive and asynchronous differentiation: in the first two weeks, active ciliogenesis predominates, with the number of ciliated cells increasing by 2.2–2.7 times ($p < 0.001$); between days 14 and 30; goblet cell subpopulations are formed. Ciliary apparatus development proceeds faster in segmental bronchi, whereas glandular element formation occurs primarily in the main bronchi. Ciliary beat frequency is the highest in newborns (25.0–25.9 Hz), decreasing to 14.9–18.6 Hz by one month of age ($p < 0.001$), and subsequently stabilizing at 13.2–16.2 Hz. The formation of the typical structure of the respiratory epithelium is completed during puberty; starting from 3 months of age and throughout the entire reproductive period (6–14 months), its main structural and functional characteristics remain largely unchanged. In aging (20 months) and old (26 months) animals, the number of ciliated and goblet cells reaches its maximum, and ciliary apparatus activity is preserved. However, there is a decrease in proliferative cell count, an increase in hypertrophic ciliated cells, and the appearance of ultrastructural signs of epithelial cell damage.

CONCLUSION: Postnatal morphogenesis of the bronchial MCTS in rats continues throughout life, with the most pronounced histogenetic changes occurring during the first month after birth. Subsequent age-related changes in the mature epithelial structure are aimed at maintaining stable mucociliary clearance.

Keywords: respiratory epithelium; bronchi; mucociliary apparatus; postnatal ontogenesis.

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Функциональная морфология мукоцилиарной транспортной системы бронхов у крыс в постнатальном онтогенезе

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АННОТАЦИЯ

Обоснование. Возрастные перестройки мукоцилиарной транспортной системы (МЦТС) воздухоносных путей играют существенную роль в патогенезе респираторных заболеваний. Однако в доступной научной литературе отсутствуют исследования структурных и функциональных показателей эпителиальной выстилки бронхов в ходе постнатального онтогенеза, выполненные в стандартных условиях и с использованием единых методических подходов, что обусловливает актуальность дальнейшего изучения данного вопроса.

Цель исследования — изучить возрастные закономерности постнатального морфогенеза мукоцилиарной транспортной системы бронхов у крыс на основе структурно-функционального анализа элементов респираторного эпителия.

Методы. В работе использовали методы прижизненного измерения двигательной активности цилиарного аппарата, световой и электронной микроскопии, иммуногистохимии (с использованием антител к белку пролиферации Ki-67) и морфометрии. Объектом исследования служил респираторный эпителий главных, долевых и сегментарных бронхов лёгких крыс линии Вистар (n=76) в возрасте 1, 8, 14 суток, 1, 3, 6, 14, 20 и 26 месяцев, по 6–9 животных на каждую временную точку.

Результаты. Бронхиальное дерево новорождённых крыс выстлано однослойным эпителием, содержащим преимущественно недифференцированные клетки (59–62%), а также немногочисленные реснитчатые клетки (21–26%). В течение первого месяца после рождения дифференцировка элементов МЦТС происходит наиболее интенсивно и отличается асинхронным характером: в первые 2 недели преобладает активный цилиогенез и количество реснитчатых клеток возрастает в 2,2–2,7 раза (p <0,001); с 14 по 30 день — формируются субпопуляции бокаловидных клеток. Развитие цилиарного аппарата идёт опережающими темпами в сегментарных бронхах, а формирование железистых элементов — преимущественно в главных бронхах. Частота биения ресничек в бронхах всех калибров максимальна у новорождённых животных (25,0–25,9 Гц), снижается до 14,9–18,6 Гц к возрасту 1 месяц (p <0,001) и далее стабилизируется на уровне 13,2–16,2 Гц. Завершение формирования типичной структуры респираторного эпителия происходит в период полового созревания; начиная с 3-х месяцев и на протяжении всего репродуктивного периода (6–14 мес.) его основные структурные и функциональные характеристики существенно не изменяются. У стареющих (20 мес.) и у старых (26 мес.) животных количество реснитчатых и бокаловидных клеток достигает максимальных значений, двигательная активность цилиарного аппарата сохраняется. Одновременно происходит снижение количества пролиферирующих клеток, увеличение доли гипертрофированных мерцательных клеток и развитие ультраструктурных признаков повреждения эпителиоцитов.

Заключение. Постнатальный морфогенез МЦТС бронхов у крыс продолжается на протяжении всей жизни, при этом гистогенетические процессы наиболее выражены в течение первого месяца после рождения. Последующие возрастные преобразования сформировавшейся дефинитивной структуры эпителиального пласта направлены на поддержание стабильного уровня мукоцилиарного клиренса.

Ключевые слова: респираторный эпителий; бронхи; мукоцилиарный аппарат; постнатальный онтогенез.

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大鼠出生后个体发育过程中支气管黏液纤毛清除系统的功能形态学研究

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摘要

论证。支气管黏液纤毛清除系统（mucociliary transport system, MCTS）的年龄相关重塑在呼吸系统疾病的发病机制中具有重要意义。然而，现有文献中尚缺乏在标准条件下、采用统一方法系统研究大鼠出生后支气管上皮结构和功能指标的研究，这凸显了进一步探讨该问题的现实意义与研究价值。

目的：基于对呼吸上皮结构与功能的分析，研究大鼠支气管黏液纤毛清除系统在出生后个体发育过程中的年龄相关形态发生规律。

方法。采用活体纤毛运动功能测定、光镜与电镜观察、免疫组织化学染色（使用Ki-67增殖标志蛋白抗体）以及形态计量学方法。研究对象为Wistar系大鼠肺部主支气管、叶支气管和段支气管的呼吸上皮（n=76），所取样本分别来自出生后第1、8、14天及第1、3、6、14、20和26个月龄的动物，每个时间点选取6-9只。

结果。新生大鼠支气管树被单层上皮覆盖，以未分化细胞为主（59-62%），仅少量为纤毛细胞（21-26%）。出生后第一个月为MCTS分化最活跃时期，过程呈非同步性：前两周纤毛生成活跃，纤毛细胞数量增加2.2-2.7倍（$p<0.001$）；第14至30天出现杯状细胞亚群。纤毛系统在段支气管中发育较快，而腺体成分主要形成于主支气管。所有级别支气管中，纤毛运动频率在新生期最高（25.0-25.9 Hz），1月龄降至14.9-18.6 Hz（$p<0.001$），随后稳定在13.2-16.2 Hz范围。呼吸上皮的典型结构在性成熟期完成，其后从3月龄开始，在整个生育期（6至14月龄）其主要结构和功能特征基本无显著变化。在老龄（20月龄）和高龄（26月龄）大鼠中，纤毛细胞和杯状细胞数量达到最大值，纤毛系统的运动功能仍然保持。同时观察到增殖细胞数量减少、肥大的纤毛细胞比例增加，以及上皮细胞出现超微结构损伤的表现。

结论。大鼠支气管MCTS的出生后形态发生过程持续终生，其中出生后第一个月的组织发生变化最为显著。其后不同时期的变化主要体现为对已建立的上皮结构和黏液纤毛清除功能的维持。

关键词：呼吸上皮；支气管；黏液纤毛系统；出生后个体发育。

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BACKGROUND

The pseudostratified columnar ciliated (respiratory) epithelium ensures the effective functioning of the mucociliary transport system (MCTS) of the airways. The formation and clearance of mucus along the luminal surface of the bronchi and trachea are achieved through the coordinated activity of the ciliary apparatus of ciliated cells and the secretory activity of glandular elements [1, 2]. Age-related remodeling of the epithelial layer, accompanied by alterations in its barrier and transport functions, may play an important role in the pathogenesis of respiratory diseases [3, 4]. The microscopic structure and motor activity of the airway epithelium in the upper airways at different stages of postnatal development have been studied in detail mainly [2, 5]; however, the studies in the bronchial tree are much less extensive [6]. Typically, studies of bronchi are limited by the use of a few control time points, and the methodological approaches utilized to assess ciliary activity vary significantly, complicating cross-study comparisons. Furthermore, there are no experimental studies describing the dynamics in quantitative structural and functional parameters of the bronchial epithelial lining throughout postnatal ontogeny under standardized conditions with uniform methodological approaches.

This study aimed to investigate the age-related patterns of postnatal morphogenesis of the mucociliary transport system in the bronchi of rats based on structural and functional analysis of the respiratory epithelium.

METHODS

Study Design

An observational, single-center, cross-sectional, uncontrolled study was performed on laboratory animals.

Eligibility Criteria

The study included intact male Wistar rats with known birth dates. Animals reaching the required age for analysis were randomly assigned to study groups. The rats were bred under vivarium conditions in compliance with GOST 33215-2014 (January 7, 2016) and GOST 33216-2014 standards for this species. The animals were housed with free access to water and a standardized dry feed (Stoylenskaya Niva Agro-Industrial Complex, Russia).

Study Setting

The experiments, histological, and immunohistochemical studies were conducted at the Department of Histology, Cytology, and Embryology, Yaroslavl State Medical University, Ministry of Health of the Russian Federation. Transmission electron microscopy (TEM) was performed at the Shared Research Facility for Electron Microscopy, Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences. Low-vacuum scanning electron microscopy (SEM) was conducted at the Yaroslavl Branch of the Institute of Physics and Technology, Russian Academy of Sciences.

Study Duration

The study was conducted between 2017 and 2024. During the first three years, the rats were bred, and biological material was collected at different stages of postnatal development (1, 8, and 14 days; 1, 3, 6, 14, 20, and 26 months after birth). Histological and morphometric processing of samples was also performed during this period. In 2023, TEM and SEM studies of bronchial samples were carried out, followed by final data analysis. In 2024, the manuscript was prepared.

Intervention

The lung tissue for analysis was obtained following intramuscular administration of a combination of tiletamine and zolazepam (Zoletil 100®, Virbac, France) at a dose of 10 mg/kg. The right lung was used for in vivo analysis, whereas fragments of the left lung were processed for histological examination. Euthanasia was performed before recovery from anesthesia by decapitation. Morphological and functional analyses were conducted separately for the main bronchi (MB), lobar bronchi (LB), and segmental bronchi (SB).

In vivo assessment. The motor activity of the ciliary apparatus was evaluated on thin (⩽1 mm) fragments of lung tissue using a modified method previously described in detail [7]. A hardware–software complex (Azimut-4 Research and Production Association, Russia) was used, consisting of a Biomed-2 var. 3 microscope (LOMO JSC, Russia) equipped with an electronic thermostatic control unit, a high-frequency digital video camera Grasshopper 3 2.3 MP Color USB3 Vision (FLIR Integrated Imaging Solutions Inc., Canada), and a personal computer with specialized MOSFRO software (v.4; Azimut-4 Research and Production Association, Russia).

Light microscopy. Lung fragments were fixed in 10% neutral buffered formalin (Element, Russia) and embedded in paraffin (Deltalab, Spain). Sections 4–5 μm thick were stained with hematoxylin and eosin (Emmonya Biotech Ltd, Bulgaria) and Schiff's reagent (Labiko, Russia) with counterstaining by hematoxylin.

Detection of the proliferation marker Ki-67 protein was performed on paraffin sections using rabbit monoclonal antibodies (Ventana Medical Systems, USA; catalog no. 790-4286; dilution 1:100) and the ultraView Universal DAB Detection Kit (Ventana Medical Systems, USA).

Electron microscopy. For TEM, samples were fixed in 2.5% glutaraldehyde (Electron Microscopy Sciences, USA), postfixed in 1% osmium tetroxide (Aurat, Russia), dehydrated in acetone, and embedded in Epon (Sigma-Aldrich, USA). Ultrathin sections were prepared using an ultramicrotome Leica-EMU6C (Leica Microsystems, Germany), contrasted with uranyl acetate (Sigma-Aldrich, USA), and examined with a JEM-1011 transmission electron microscope (JEOL Ltd, Japan). The images were digitized. For SEM, lung fragments were fixed in 2.5% glutaraldehyde (Electron Microscopy

Sciences, USA) and analyzed using a Quanta 3D 200i scanning electron microscope (FEI Company, USA) under low-vacuum conditions at a water vapor pressure of 70–130 Pa.

Main Study Outcome

The primary endpoint of the study was the determination of morphometric and functional characteristics of the bronchial epithelial cells of rats throughout postnatal ontogeny.

Subgroup Analysis

Animals were allocated into age groups according to the postnatal development periodization of rats proposed by Zapadnyuk et al. [8]: day 1, newborns; days 8 and 14, suckling period; 1 month, infantile; 3 months, juvenile; 6 months, young; 14 months, adult; 20 months, aging; and 26 months, old rats.

Outcomes Registration

In vivo assessment. Quantitative processing of video recordings was performed using the MOSFRO software (v.4), which provided automatic calculation of ciliary beat frequency (CBF, Hz) as well as the duration of effective and recovery stroke phases of the ciliary beat (ms) [7].

Morphometry. The percentage of the main morphological types of epithelial cells was determined by counting 1000 cells per animal. A single animal served as the unit of observation for subsequent statistical analyses. The relative number of Ki-67–positive cells was determined by counting 500–1000 cells per animal. Frequency parameters were calculated using the Universal Histological Counter 2.0 application.[1] Ciliary length was measured on digital images using ImageJ software [9] at a total magnification of ×1000, with 100 ciliated cells assessed per animal.

Statistical Analysis

Sample size calculation principles. The sample size was not calculated in advance.

Statistical analysis methods. Statistical processing was performed using Microsoft Office Excel 2019 (Microsoft, USA) and Statistica 8.0 (StatSoft Inc., USA). The normality of distribution was verified using the Shapiro–Wilk test, and homogeneity of variances was confirmed with the F-test. Quantitative data are presented as mean (M) and standard error of the mean (*m*). Statistical significance between independent samples was assessed using Student *t*-test, with differences considered significant at $p < 0.05$.

RESULTS

Study Objects

The work was performed on 76 male Wistar rats without external signs of disease and with precisely known dates of birth. For in vivo and histological examinations, fragments of the main, lobar, and segmental bronchi were obtained from animals of different ages: 1 day (n = 9), 14 days (n = 6), 1 month (n=6), 3 months (n = 6), 6 months (n = 9), 14 months (n = 8), 20 months (n = 6), and 26 months (n = 5). SEM was performed on 3 samples of bronchi of different calibers from rats aged 1, 8, and 14 days and 1 month (12 animals in total). TEM was conducted on 3 samples of bronchi of different calibers from rats aged 1 day, 6 months, and 26 months (9 animals in total).

Primary Results

A characteristic feature of the histological structure of the rat bronchial tree is the absence of the submucosal glands, which makes it a convenient model for studying the subpopulations of motor (ciliated epithelial cells), secretory (goblet cells), and progenitor (basal cells) elements of the MCTS within a single epithelial layer.

In newborn rats, the bronchial tree in all regions is lined with a simple epithelium composed predominantly of undifferentiated (intermediate) cells, whereas ciliated cells are relatively scarce (Fig. 1, *a–c*). During the first two weeks after birth, the proportion of ciliated cells in the epithelial layer increases markedly because of active differentiation processes (ciliogenesis), accompanied by a proportional decrease in the content of poorly differentiated elements. Various stages of ciliary formation can be observed at the apical poles of differentiating ciliated cells (Fig. 1, *d, e*). The maturation of ciliated cells occurs in clusters, which is clearly visible under scanning electron microscopy (Fig. 1, *f*). By the end of the first month after birth, the typical structure of a pseudostratified ciliated epithelium is established.

In the definitive epithelial layer of sexually mature animals (6 months), ciliated epithelial cells predominate, whereas the subpopulations of goblet and basal cells are less numerous, and nonciliated (intermediate) cells are also relatively few (Fig. 2, *a, b*). Numerous fully differentiated cilia are clearly visible on the apical surface of ciliated epithelial cells (Fig. 2, *c*). In aging and old animals (20 and 26 months), the epithelium retains its differentiated structure with a well-developed ciliary apparatus (Fig. 2, *d, e*). However, in rats of these age groups, hypertrophied ciliated cells are consistently present, along with elements showing ultrastructural signs of damage: disruption of organelle architecture and cytoplasmic vacuolization, as well as areas of intercellular edema (Fig. 2, *f*).

Age-related dynamics of the quantitative characteristics of the bronchial epithelium across bronchi of different calibers are summarized in Tables 1 and 2. Based on light microscopy, the epithelial lining was classified into ciliated, goblet, basal, and intermediate (intercalated) cells.

[1] Kemoklidze KG. *Universal histological counter. Version 2.0*. Certificate of state registration of computer programs No. 2012617618 Russian Federation. Application No. 2012615714; application date 09.07.2012; registration date 23.08.2012.

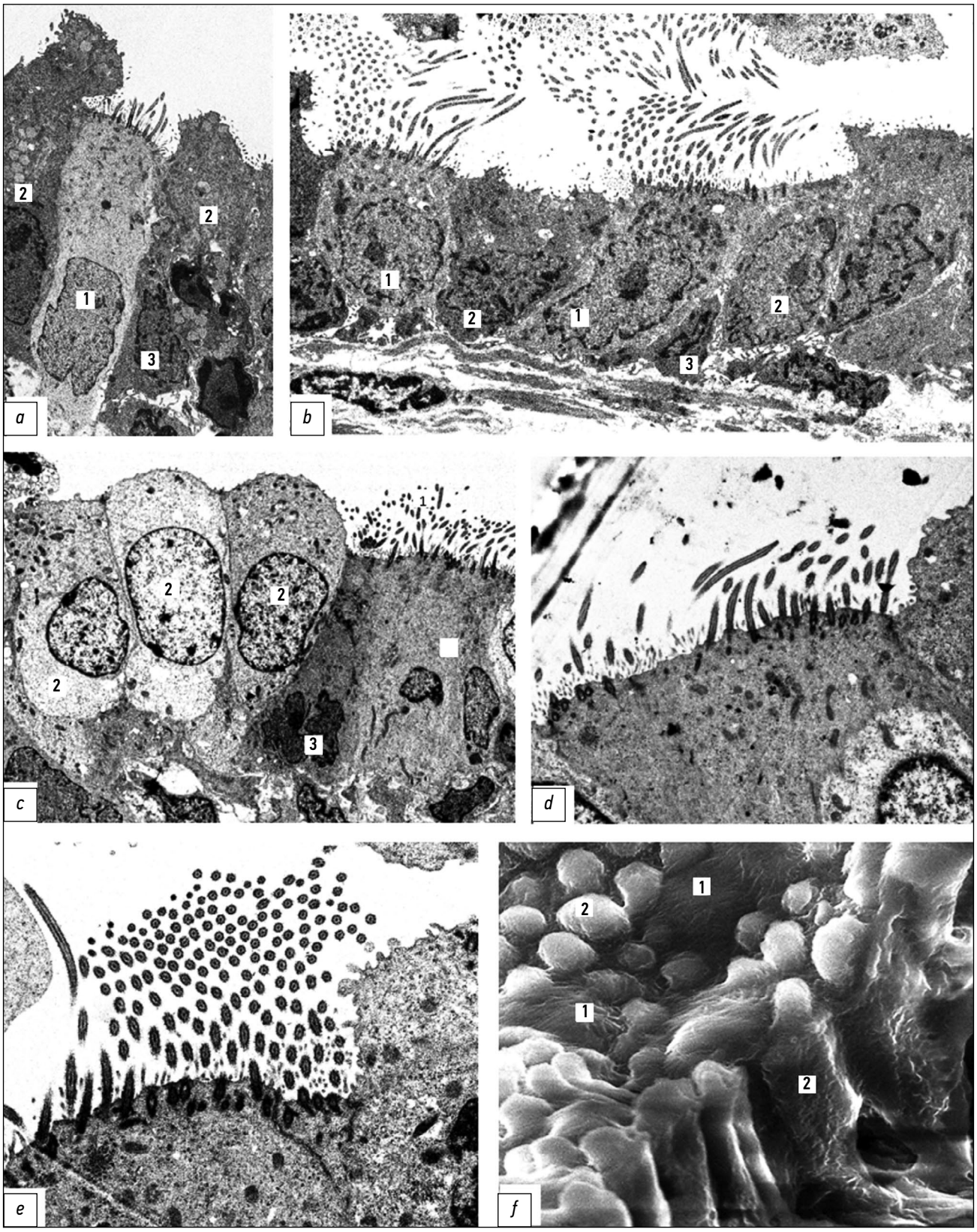

Fig. 1. Bronchial epithelial lining in rats: *a*, main bronchus; *b*, lobar bronchus; *c–e*, segmental bronchi in newborns; *f*, segmental bronchus in 8-day-old rats. *1*, ciliated cells; *2*, brush (non-ciliated) cells; *3*, basal cells. Transmission electron microscopy: *a–c*, ×5000; *d*, *e*, ×8000; *f*, scanning electron microscopy, ×4500.

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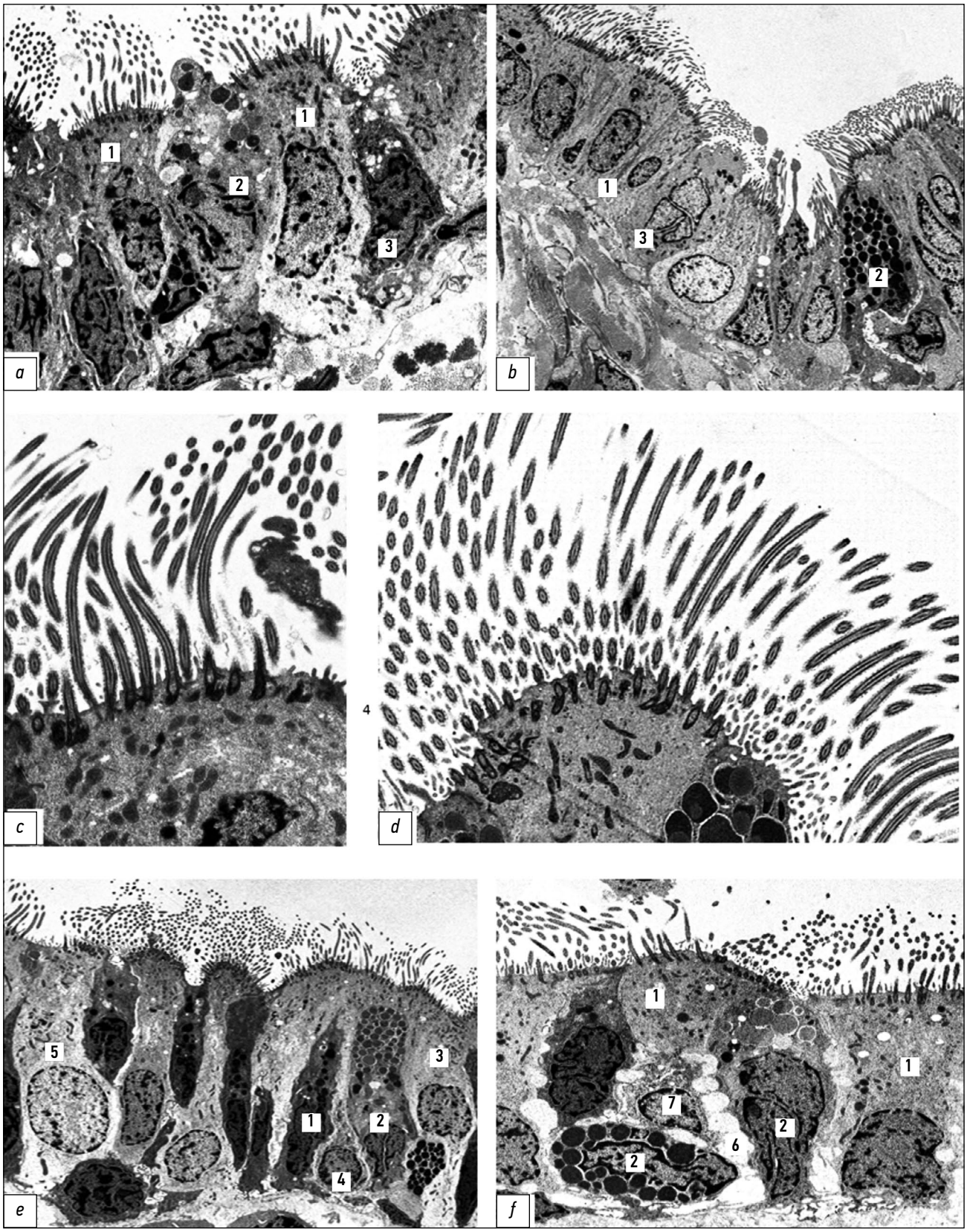

Fig. 2. Bronchial epithelial lining in rats: at 6 months of age, *a*, main bronchus; *b*, segmental bronchus; at 26 months of age, *c*, segmental bronchus; *d–f*, main bronchi. *1*, ciliated cells; *2*, goblet cells; *3*, brush (non-ciliated) cells; *4*, basal cells; *5*, hypertrophic ciliated cell; *6*, areas of intercellular edema; *7*, epithelial cell with morphological signs of damage. Transmission electron microscopy: *a*, *b*, *e*, *f*, ×5000; *c*, *d*, ×10000.

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Table 1. Quantitative parameters of the structure and function of the bronchial mucociliary apparatus in rats during postnatal ontogenesis: ciliated cells

Bronchus Type	Age							
	1 day	14 days	1 month	3 months	6 months	14 months	20 months	26 months
Content in the epithelial layer, %								
Main Bronchi	20.6±1.2	46.7±1.5 [3]	49.5±2.5	59.3±2.3 [1]	56.9±3.0	58.0±2.1	64.3±2.1	64.0±2.3
Lobular Bronchi	26.5±1.7 *	60.8±1.6 [# 3]	70.5±0.7 [# 3]	68.0±2.4 *	63.4±2.4	69.0±1.9 [&]	70.6±2.7	71.0±1.6 *
Segmental Bronchi	21.0±0.5	58.4±0.7 [# 3]	71.7±0.9 [# 3]	71.1±1.8 [&]	69.1±1.9 [&]	75.8±1.7 [&]	70.2±1.5 *	71.8±0.9 *
Ciliary Length, μm								
Main Bronchi	2.5±0.2	3.0±0.1 [1]	3.2±0.1	3.8±0.2 [1]	3.8±0.3	3.9±0.2	3.7±0.2	3.2±0.2
Lobular Bronchi	2.8±0.1	3.3±0.1 [2]	3.6±0.1 *	4.5±0.3 [1]	3.1±0.1 * [2]	2.9±0.1 [&]	2.8±0.2 *	2.9±0.2
Segmental Bronchi	2.3±0.2	3.2±0.1 [2]	3.1±0.1	3.3±0.1 *	2.6±0.2 * [1]	2.5±0.1 [#]	2.6±0.2 [&]	1.9±0.2 [&]
Ciliary Beat Frequency, Hz								
Main Bronchi	25.3±0.8	16.6±0.9 [3]	18.6±0.8	15.1±1.2 [1]	14.1±0.6	16.2±0.6	15.9±0.6	15.3±0.7
Lobular Bronchi	25.9±0.6	15.2±1.0 [3]	13.6±0.7 [&]	13.6±0.6	13.2±0.4	15.1±0.4	16.1±0.7	14.2±0.9
Segmental Bronchi	25.0±0.8	14.1±1.2 [3]	14.9±0.8 [&]	13.9±0.9	14.1±0.3	14.6±1.5	15.7±0.8	15.0±0.7
Effective stroke phase, ms (absolute) and relative duration (fraction of cycle, 1.0)								
Main Bronchi	16.6±0.9 0.41	26.1±2.1 [2] 0.42	22.2±2.2 0.41	27.7±2.8 0.41	28.3±1.4 0.39	21.8±1.4 [1] 0.35	25.8±1.2 0.40	27.2±1.7 0.40
Lobular Bronchi	16.5±0.7 0.42	27.0±1.8 [3] 0.40	30.7±3.0 0.41	30.7±3.0 0.42	32.2±2.5 0.42	25.1±1.0 0.38	23.4±0.8 0.37	28.9±2.0 0.40
Segmental Bronchi	16.6±0.3 0.41	31.1±3.4 [2] 0.42	28.2±2.0 0.41	30.3±3.5 0.41	27.0±1.3 0.37	30.1±4.2 0.41	24.8±1.2 0.38	27.7±2.2 0.41

Note: Data are presented as M ± m, where M is the arithmetic mean and m is the standard error of the mean; [1] $p < 0.05$; [2] $p < 0.01$; [3] $p < 0.001$ vs the preceding age group for bronchi of the same type; * $p < 0.05$; [&] $p < 0.01$; [#] $p < 0.001$ vs the main bronchi.

Table 2. Quantitative parameters of the structure and function of the bronchial mucociliary apparatus in rats during postnatal ontogenesis: composition of goblet, basal, and intermediate cells as the main epithelial cell types in the epithelial layer

Bronchus Type	Age							
	1 day	14 days	1 month	3 months	6 months	14 months	20 months	26 months
Goblet Cells, %								
Main Bronchi	-	1.3±0.2	9.7±2.6 [2]	8.7±1.3	13.3±1.2 [1]	16.8±2.2	16.6±1.5	16.6±1.5
Lobular Bronchi	-	1.3±0.2	4.5±0.6 [3]	8.2±1.0 [2]	13.1±2.0 [1]	17.2±1.7	14.3±1.1	13.9±1.8
Segmental Bronchi	-	0.7±0.1 *	0.8±0.1 [#]	2.1±0.4 [#1]	5.0±0.6 [#2]	6.1±1.3 [&]	9.4±0.6 [&1]	11.2±1.0 *
Basal Cells, %								
Main Bronchi	20.7±1.8	24.6±1.7	18.4±2.0 [1]	21.3±1.3	16.5±2.4	19.6±0.4	20.7±0.7	15.5±0.9 [2]
Lobular Bronchi	12.1±1.4 [&]	15.5±0.7 [#]	8.5±0.5 [#3]	9.3±1.1 [#]	10.2±2.0	11.7±0.9 [#]	12.6±0.9 [#]	12.1±0.5 [&]
Segmental Bronchi	17.3±0.9	15.7±0.7 [#]	12.3±0.7 *[2]	19.1±2.0 [2]	14.3±3.1	13.2±0.7 [#]	10.7±1.1 [#]	14.4±1.3
Intermediate cells, %								
Main Bronchi	58.7±0.7	27.4±1.6 [3]	22.4±1.6	17.1±1.0 [1]	10.9±1.6 [1]	6.7±0.9 [1]	4.6±0.6	3.6±0.8
Lobular Bronchi	61.5±2.0	22.3±1.9 [3]	16.5±0.2 [&1]	11.5±0.9 [&3]	8.6±1.4	7.7±1.1	4.2±0.3 [1]	3.4±0.8
Segmental Bronchi	61.7±0.5	25.2±0.8 [3]	15.2±0.5 [&3]	11.1±1.1 [&2]	9.5±1.6	6.8±1.4	4.1±0.5	4.1±0.8

Note: Data are presented as M ± m, where M is the arithmetic mean and m is the standard error of the mean; [1] $p < 0.05$, [2] $p < 0.01$, [3] $p < 0.001$ vs the preceding age group for bronchi of the same type; * $p < 0.05$,[&] $p < 0.01$, [#] $p < 0.001$ vs the main bronchi.

Table 3. Ki-67-positive epithelial cells in the bronchi of rats during postnatal ontogenesis

Bronchus Type	Age					
	1 day	14 days	1 month	6 months	20 months	26 months
Main Bronchi	6.7±0.5	8.7±0.4 [1]	6.3±0.4 [2]	3.9±0.4 [2]	3.2±0.3	2.5±0.2
Lobular Bronchi	5.6±0.4	8.2±0.4 2	5.5±0.3 [2]	2.7±0.3 *[3]	2.1±0.2 *	1.8±0.2 *
Segmental Bronchi	8.2±0.5	8.0±0.4	4.5±0.3 [3]	1.9±0.2 [&3]	1.7±0.2 [&]	1.5±0.2 [&]

Note: Data are presented as M ± m, where M is the arithmetic mean and m is the standard error of the mean; [1] $p < 0.05$, [2] $p < 0.01$, [3] $p < 0.001$ vs the preceding age group for bronchi of the same type; * $p < 0.05$,[&] $p < 0.01$ vs the main bronchi.

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Ciliated epithelial cells. In newborn rats, the proportion of ciliated elements was minimal: 21% in the main and segmental (small) bronchi, and 26% in the lobar bronchi. During the subsequent two weeks, the number of ciliated epithelial cells in the layer increased most intensively: 2.2-fold in MB, 2.3-fold in LB, and 2.7-fold in SB. By day 30 after birth, the density of ciliated elements increased 2.5–2.7 times in the main and lobar bronchi and 3.3 times in the segmental bronchi compared with newborns ($p < 0.001$). These values remained stable throughout the subsequent observation period. At the same time, the proportion of ciliated cells in the lobar and segmental bronchi was, on average, 25%–30% higher than in the main bronchi.

The analysis of the ciliary apparatus of ciliated cells demonstrated that in newborn animals, the mean ciliary length was comparable across all bronchial segments (range: 2.3–2.6 μm, $p > 0.05$). After 2 weeks, this parameter increased 1.2-fold in MB and LB and 1.4-fold in SB. Between 6 and 20 months, the mean ciliary length stabilized at 3.8–3.9 μm in MB, 2.8–3.1 μm in LB, and 2.5–2.6 μm in SB. In old rats, the mean ciliary length in MB and SB was reduced by 18% and 27%, respectively, compared with 20-month-old animals.

The CBF in all bronchi was highest in newborns (25–26 Hz) but decreased by 37%–40% by day 14. Beginning at 3 months, and throughout the remaining observation period, CBF stabilized within 14.1–16.2 Hz. In 1-month-old rats, CBF in the lobar and segmental bronchi was 21%–25% lower than in the main bronchi; however, at later time points no significant inter-bronchial differences in ciliary activity were found.

The frame-by-frame analysis of video recordings revealed that in newborn rats, the rapid beating of short cilia had a poorly defined wave-like pattern and was insufficiently synchronized (Fig. 3, *b–d*). The appearance of typical ciliary beating cycles with distinct effective and recovery phases occurred by day 14 of postnatal development. Between 14 and 26 months, the wave-like character of long ciliary movement in the recovery phase was most pronounced (Fig. 3, *f–h*). These observations were confirmed by the phase analysis of ciliary beating. In newborn rats, the effective stroke phase was minimal (16.5–16.6 ms) across all bronchial regions. By day 14 and at all subsequent ages, this parameter stabilized at 26–32 ms. Regardless of CBF, the fundamental cycle structure was preserved: the ratio of the effective stroke phase to the total cycle duration (normalized to 1.0) remained stable across all time points (0.38–0.41).

Goblet cells. The first isolated glandular elements appeared in the epithelial layer on day 14. By 6 months of age, their proportion in the main and lobar bronchi increased 10-fold ($p < 0.001$), after which it stabilized at 14%–17%. Throughout the observation period, the proportion of goblet cells in the segmental bronchi was consistently lower than in the main bronchi. In segmental bronchi, the number of goblet cells increased less intensively and reached the maximum (11.2%) only at 26 months.

Basal and intermediate cells. The proportion of basal cells in MB peaked at 25% in 14-day-old rats. Between 1 and 20 months, the proportion of progenitor elements remained stable at 20%–21%, with a decrease to 15.5% observed only in old rats. In smaller bronchi (lobar and segmental), the age-related dynamics of basal epithelial cell proportions followed a similar pattern; however, their numbers were statistically significantly lower than in MB in most observations.

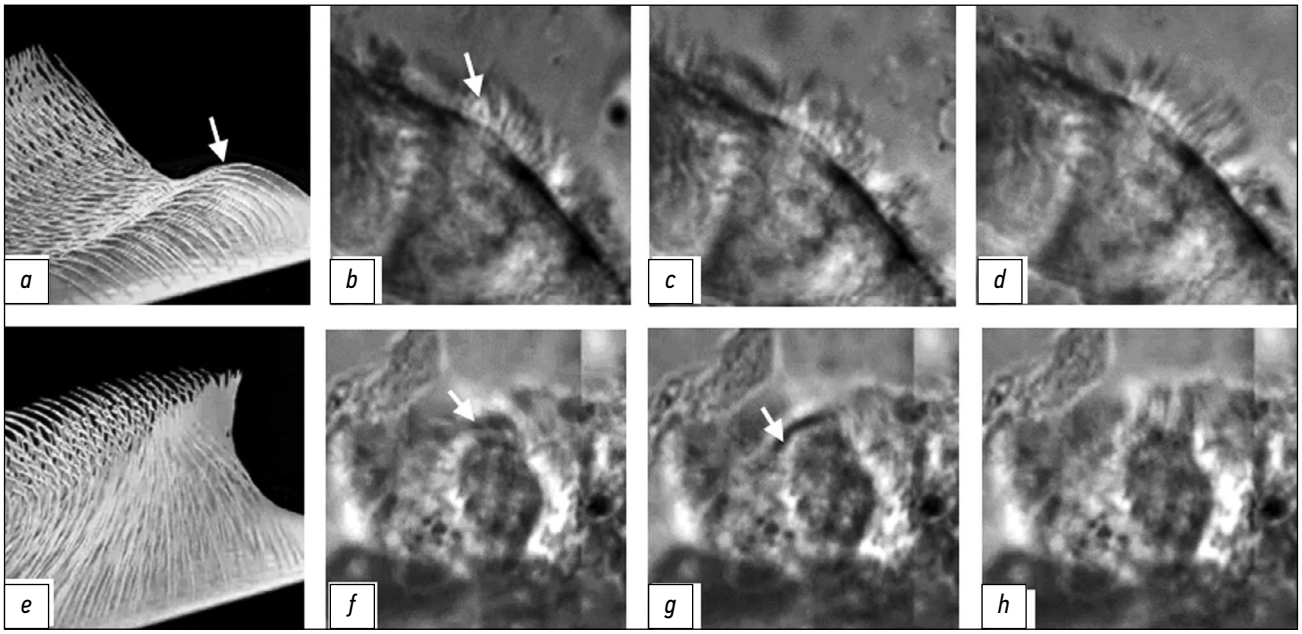

Fig. 3. Phases of ciliary beating in epithelial cells of the main bronchi in rats: *a*, *e*, diagram; *b–d*, *f–h* , frames from intravital microvideo recording; *b–d*, newborn rats; *f–h*, 26-month-old rats; *a*, *b*, *f*, *g*, recovery stroke phase, bending direction indicated by arrows; *c–e*, *h*, effective stroke phase. Objective lens, ×40.

Intermediate (nonciliated) cells constituted 59%–61% of epithelial cells in all types of bronchi in newborn rats. By day 14, their numbers had decreased 2.1-fold in MB and 2.5–2.7-fold in LB and SB. This trend persisted at 1 and 3 months. By 6 months, the proportion of intermediate cells stabilized at 9%–11% across all bronchial regions. In pre-senescent and senescent rats, against the background of a high proportion of differentiated elements, the content of intermediate cells reached its lowest values (3.4%–4.6%).

Cell proliferation. The results of quantifying Ki-67–expressing cells in the bronchial epithelium across bronchi of different calibers are presented in Table 3. The highest proportion of proliferating cells was observed during the first two weeks after birth, peaking on day 14 (8.2%–8.7%). Beginning at 6 months, the Ki-67 index in the main bronchi stabilized at 2.5%–3.9%. In LB and SB, the proportion of proliferating epithelial cells was substantially lower, averaging 66% and 51% of the values in MB, respectively. At all studied time points, Ki-67 expression was detected exclusively in the nuclei of basal and intermediate (intercalated) epithelial cells.

DISCUSSION

Summary of Primary Results

Morphogenetic processes in the respiratory epithelium of the bronchi are most active during the first postnatal month in rats and proceed asynchronously. During the first 2 weeks after birth, accelerated differentiation of ciliated cells predominates, and the formation of the goblet cell subpopulation becomes more prominent starting from day 14. Development of the ciliary apparatus progresses more rapidly in SB, whereas glandular differentiation is more pronounced in MB.

The establishment of the typical structure of the respiratory epithelium is completed during puberty (3 months). During the reproductive period (6–14 months), the quantitative and structural parameters stabilize at levels typical of each bronchial caliber. At the same time, the principal parameters of ciliary activity of ciliated epithelial cells remain consistent across all bronchial regions. In aging, preservation of high functional activity of MCTS is accompanied by morphological signs of cellular injury and reduced proliferative turnover of the epithelial layer.

Discussion of Primary Results

At all levels of bronchial tree branching in rats, structural and functional differentiation of the respiratory epithelium proceeds at the highest rate during the first postnatal month. Our observations are generally consistent with published data describing the early stages of airway development in laboratory animals and humans [1, 5, 10]. The principal manifestations of morphogenesis aimed at establishing the MCTS include active proliferation of undifferentiated cells and the differentiation of ciliated and goblet cell subpopulations. These differentiation processes in rats occur asynchronously: ciliogenesis is most pronounced in the first two weeks after birth, whereas the system of secretory elements develops by the end of the first postnatal month. Moreover, the development of the ciliary apparatus progresses more rapidly in the distal regions of the bronchial tree (SB), whereas glandular elements differentiate predominantly in the main bronchi. The intensive differentiation of the epithelial lining during the first month (a period of maximal growth rates in rats [8]) is supported by high proliferative activity across all bronchial regions.

The CBF in newborn rats is higher than in any subsequent age period. This phenomenon, characteristic of the airways of both laboratory animals and humans [11, 12], when combined with the underdeveloped ciliary apparatus and the absence of differentiated secretory elements at this stage, indicates the immaturity of the MCTS at birth.

Completion of the structurally and functionally differentiated epithelial layer occurs during puberty. In 3-month-old rats, the quantitative parameters of ciliated cell subpopulations approach those of sexually mature animals. The analysis of ciliary motility demonstrated that starting at 3 months, CBF stabilizes and remains unchanged at all subsequent ages. Moreover, no inter-bronchial differences in ciliary activity were detected. Temporal parameters of ciliary beating cycles also remain stable throughout life. These observations are consistent with measurements of mucus transport velocity in the rat trachea, showing that the establishment of a stable level of mucociliary clearance (0.10–0.13 mm/s) is completed by 3 months of age [5]. Comparable results for CBF across specific age periods have been reported for both the bronchial tree of rodents and in human studies [13, 14].

In animals of reproductive age (6 and 14 months), MCTS of the bronchial tree achieves full structural and functional maturity. Across bronchi of different calibers, a distal gradient can be traced in the proportion of differentiated cell types (ciliated and goblet cells) and proliferating cells within the epithelial lining.

In aging (20 months) and old (26 months) rats, the previously established level of structural and functional differentiation of the epithelial layer is preserved: the proportion of ciliated and goblet cells reaches its peak values, and the ciliary motility remains maintained. Similar findings were previously obtained in our laboratory when examining age-related remodeling of the tracheal epithelium [5]. These results differ from reports of several authors who observed a decline in mucociliary transport with aging in the trachea of mice, guinea pigs, and in the human nasal cavity [15, 16]. In addition to interspecies differences, such discrepancies may be explained by variations in in vivo methods used to assess ciliary activity. Furthermore, in human studies, the influence of comorbid airway conditions cannot be excluded.

Importantly, the processes described in aging and old rats occur against the background of a marked decline in the proportion of epithelial cells entering the mitotic cycle.

With reduced proliferative turnover, previously differentiated ciliated cells are forced to maintain viability and function under increased workload. Morphologically, this is manifested by a higher proportion of hypertrophied ciliated cells and the appearance of ultrastructural features of epithelial injury, including disrupted organelle architecture and cytoplasmic vacuolization. The age-related changes identified in this study complement published evidence on involutional remodeling of the respiratory epithelium with aging: accumulation of ultrastructural defects in ciliary microtubules [15]; altered synthesis of proteins regulating epithelial barrier permeability in the areas of intercellular junctions [3]; weakening of adhesion to the basal lamina and enhanced desquamation of epithelial cells [17].

Study Limitations

No factors were identified that could substantially influence the study outcomes. However, it should be considered that the histological structure of the bronchial tree in rats differs significantly from that of humans and other mammals.

CONCLUSION

Postnatal morphogenesis of the bronchial respiratory epithelium in rats continues throughout life, with histogenetic processes being most pronounced during the first month after birth. The structural and temporal dynamics in differentiation of the motor (ciliated cells) and secretory (goblet cells) components of the MCTS demonstrate distinct regional features across the tracheobronchial system.

One hallmark of structural and functional maturity of the respiratory epithelium during reproductive age is the establishment of a gradient in the proportions of differentiated and proliferating epithelial cells across bronchi of different calibers, with their relative abundance decreasing in the distal direction. The preservation of high functional activity of the MCTS during aging is achieved at the expense of increased workload on ciliated and secretory elements, leading to cumulative cellular injury and reduced compensatory capacity of the epithelial lining.

Overall, the respiratory epithelium in all regions of the rat airways demonstrates uniform transport properties and functions as an integrated tissue system, ensuring a stable level of mucociliary clearance throughout the animal's lifespan.

ADDITIONAL INFORMATION

Author contributions: A.V. Pavlov: conceptualization, methodology, formal analysis, writing—original draft, writing—review & editing, visualization, search for sources; N.A. Tyumina: investigation, data curation, formal analysis, visualization, collection and analysis of sources, writing—original draft. All the authors approved the version of the manuscript to be published and agreed to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Data availability: All data obtained in the present study are presented in the article.

Generative artificial intelligence: No generative artificial intelligence technologies were used in the creation of this article.

Review and peer review: This work was submitted to the journal on an unsolicited basis and reviewed through the standard procedure. The review process involved two external peer reviewers, a member of the editorial board, and the journal's scientific editor.

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. А.В. Павлов — концепция и дизайн исследования, анализ, интерпретация данных, написание и редактирование текста, подготовка иллюстраций, поиск литературных источников; Н.А. Тюмина — проведение исследования, сбор первичных данных и их статистическая обработка, подготовка иллюстраций, сбор и анализ литературных источников, написание текста. Все авторы одобрили рукопись (версию для публикации), а также согласились нести ответственность за все аспекты работы, гарантируя надлежащее рассмотрение и решение вопросов, связанных с точностью и добросовестностью любой её части.

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Источники финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

Раскрытие интересов. Авторы заявляют об отсутствии отношений, деятельности и интересов за последние три года, связанных с третьими лицами (коммерческими и некоммерческими), интересы которых могут быть затронуты содержанием статьи.

Доступ к данным. Все данные, полученные в настоящем исследовании, представлены в статье.

Генеративный искусственный интеллект. При создании настоящей статьи технологии генеративного искусственного интеллекта не использовали.

Рассмотрение и рецензирование. Настоящая работа подана в журнал в инициативном порядке и рассмотрена по обычной процедуре. В рецензировании участвовали два внешних рецензента, член редакционной коллегии и научный редактор издания.

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