Morphometric Analysis of the Pulmonary Vessels in Premature Neonates

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In premature neonates with the respiratory distress syndrome the vessels of the pulmonary arterial system show stereotypical changes: dystonia with the predominance of vasoconstriction, a thickened muscular layer, and a narrowed lumen. A morphometric index is proposed: the coefficient of nonuniformity of the medial thickness of the vascular wall, which reflects a structural reorganization of the pulmonary vessels in different pathological processes.

Key Words: *pulmonary vessels; morphometry; neonates; prematurity; respiratory failure*

Disturbance of the pulmonary circulation during adaptation to postnatal life is a frequent cause of morbidity and death in neonates. Frequently, this manifests itself clinically in the respiratory distress syndrome resulting from perinatal hypoxia. It is well known that normally, as the lungs begin functioning, a rapid and radical transformation of the blood vessels of the pulmonary circulation occurs, promoting a drop of the hemodynamic resistance and an increase of the pulmonary blood flow [1,2]. At the same time, there are some specific features of the fetal and postnatal development of the pulmonary vessels which facilitate the prompt switch to independent respiration [5].

Morphometric analysis of the pulmonary vessels of the respiratory compartment has been performed in studies of adaptation to life outside the womb [7,8,14], as well as for different pathological states [6,9-13], but, for the most part, in experimental studies. Investigation of the pulmonary circulation during the neonatal period has demonstrated an increased muscularization of the small pulmonary arteries. [7]. Quantitative analysis of the parameters of the pulmonary arteries enables the level of disturbances of the pulmonary circulation in premature neonates with the respiratory distress syndrome to be objectively assessed.

The aim of the present study was to quantify changes in the structure of the vascular walls in the respiratory compartment of the lungs in premature neonates with the respiratory distress syndrome.

MATERIALS AND METHODS

We studied the pulmonary arteries in 31 dead neonates. In accordance with the main anatomicopathological diagnosis 4 groups of observations were distinguished: 1) pneumopathies; 2) pneumonias; 3) craniocerebral birth trauma; 4) congenital heart defects. The neonates were distributed among the groups depending on the degree of prematurity.

Morphometric analysis of the pulmonary arteries was performed using tissue sections stained after Van Gieson. Differentiation of the arteries was performed at the preacinar and intraacinar levels (taking into account the contact of the arteries with the aeriferous components of the lung) in accor-

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dance with the structure of the vascular wall (the vessels of the muscular and partially muscular type). The outer (D_o) and the inner (D_i) diameters of each artery, as well as the thickness of the medial layer (*TML*) of the walls of the muscular and partially muscular arteries, were routinely determined using an ocular micrometer mounted on an Epival microscope.

The resistance of the pulmonary vessels to the blood flow was assessed by calculating the Kernogan index (*KI*) by the formula: $KI=TML/D_i$, where *TML* was calculated by the formula $TML=(D_o-D_i)/2$, and the percentage thickening of the medial layer [11]: $TML,\%=2TML\times100/D_o$. *TML* was measured taking into account its nonuniform thickening in different portions of one vessel. To elucidate the importance of this phenomenon we proposed the coefficient of nonuniformity of the medial thickness (*CNMT*) calculated by the formula $CNMT=1-(2TML/D_o-D_i)$. This coefficient ranges from 0 to 1 and characterises the asymmetry of the vascular wall thickness.

The results were processed using the software developed on an SM-4 computer. This software is designed to handle the following operations: utilization of the data base obtained in primary observations, calculation of the above-mentioned morphometric parameters, sampling according to types of vessels, the above-mentioned nosological forms, and the degree of prematurity for statistical analysis, as well as statistical processing of the results [3].

RESULTS

Microscopic examination of the lungs of premature neonates [4] with pneumopathies and with the respiratory distress syndrome of different (respiratory, cerebral, and cardiac) genesis demonstrated stereotypical stromal-parenchymal changes (Fig. 1). Morphometric investigation of the arteries of the respiratory compartment showed changes in the vascular wall architectonics in all groups examined.

The intraacinar vessels of the muscular type were characterized by narrowed lumens along with an increased vascular resistance (maximum value of *KI* 2.59±0.20) and by a thickened medial layer (up to 82.12±0.75%) (Table 1). At this level *CNMT* was similar in all groups of diseases, except for pneumopathies (0.14±0.01, $p<0.05$).

The preacinar vessels of the muscular type showed no marked changes in the parameters in question. The values of *KI* testified to a high vascular resistance at the preacinar level (1.12±0.06-1.57±0.13; $p<0.05$). Meanwhile, in pneumopathies

TABLE 1. State of the Pulmonary Vessels in Neonates with the Respiratory Distress Syndrome ($M\pm m$)

Type and level of vessel	Group	D_o, μ	D_i, μ	TML, μ	CNMT	KI	TML, %
Intraacinar vessels of muscular type							
	1	28.33±0.44	6.06±0.27	11.13±0.21	0.14±0.01	2.59±0.20	78.67±0.84
	2	28.55±0.39	6.60±0.27	10.98±0.16	0.09±0.01	2.12±0.11	77.68±0.80
	3	29.30±0.29	5.36±0.24	11.97±0.15	0.09±0.01	2.43±0.12	82.12±0.75
	4	29.05±0.37	6.60±0.36	11.23±0.18	0.09±0.01	2.00±0.12	77.72±1.06
Intraacinar vessels of partially muscular type							
	1	25.64±0.52	14.35±0.48	5.65±0.19	0.21±0.02	0.50±0.03	44.86±1.33
	2	26.19±0.40	15.46±0.40	5.36±0.11	0.18±0.01	0.41±0.02	42.23±0.92
	3	25.94±0.44	15.31±0.45	5.37±0.11	0.19±0.02	0.44±0.02	42.90±1.01
	4	26.23±0.58	15.31±0.58	5.51±0.14	0.18±0.03	0.42±0.03	43.03±1.31
Preacinar vessels of muscular type							
	1	45.99±0.90	14.88±0.81	15.55±0.36	0.16±0.06	1.57±0.13	68.66±1.34
	2	50.91±1.00	19.07±0.85	15.92±0.33	0.14±0.03	1.12±0.06	63.73±1.11
	3	46.17±0.83	13.80±0.76	16.18±0.31	0.10±0.01	1.56±0.11	70.93±1.18
	4	45.18±1.06	13.86±0.99	15.66±0.38	0.11±0.01	1.53±0.15	70.33±1.57
Preacinar vessels of partially muscular type							
	1	41.42±1.47	28.14±1.47	6.64±0.35	0.17±0.02	0.30±0.03	33.73±1.89
	2	44.35±1.39	31.41±1.58	6.47±0.27	0.21±0.02	0.24±0.02	30.36±1.64
	3	44.49±1.50	32.63±1.68	5.93±0.41	0.22±0.04	0.22±0.05	27.30±2.08
	4	53.34±4.44	36.65±5.10	8.35±2.18	0.30±0.07	0.32±0.15	31.17±6.79

Note. Here and in Table 2: $p<0.05$.

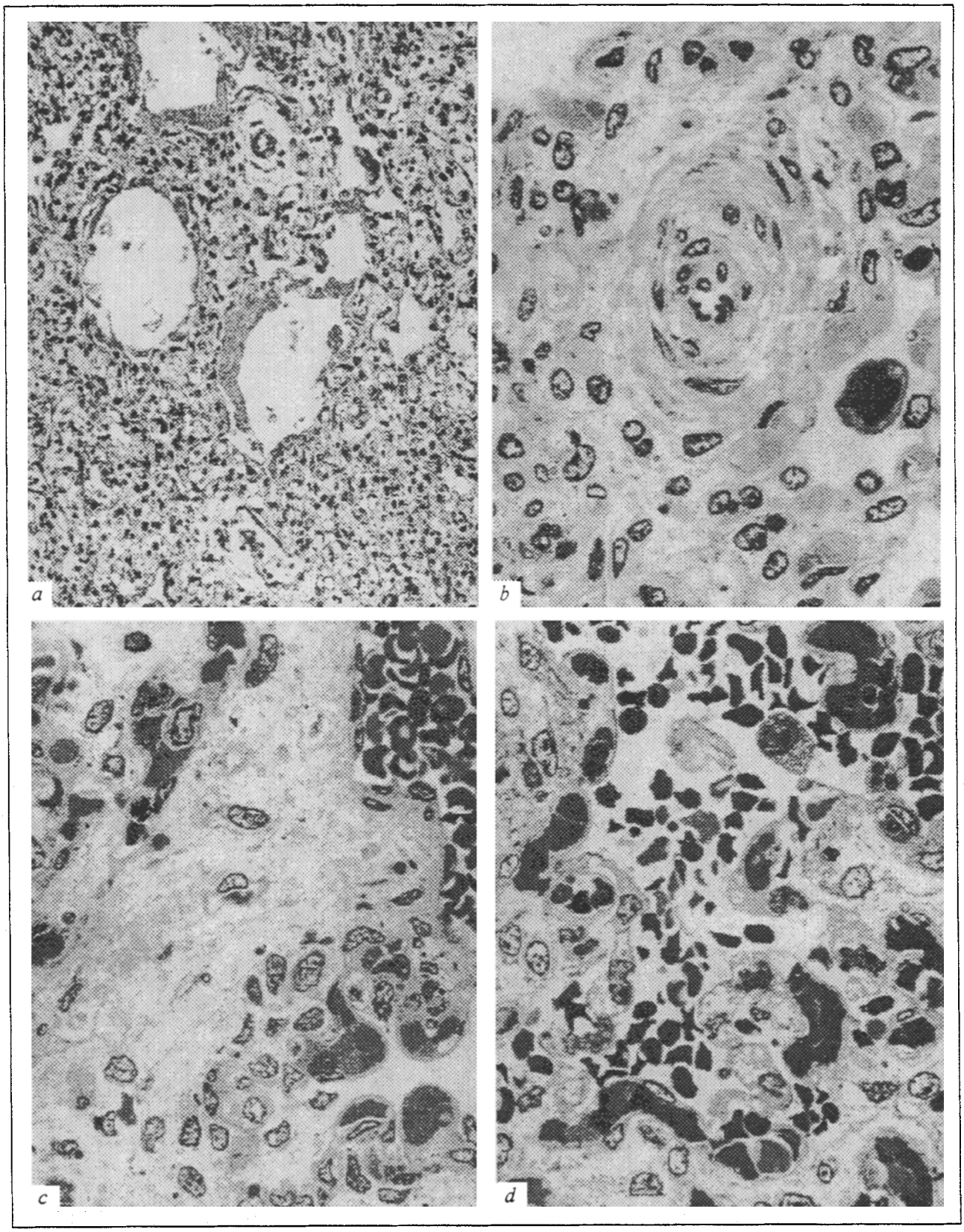

Fig. 1. Pathomorphological changes in respiratory tissue of lungs of neonates with the respiratory distress syndrome. *a*) atelectasis, hyaline membranes; *b*) vessel of pulmonary artery system with thickened muscular layer and fragmentation of elastic membrane; *c*) edema of interlobular septum, capillary ectasia into alveolar lumens; *d*) intraalveolar hemorrhage, plethoric capillaries, sludge phenomenon. Staining: *a*) hematoxylin–eosin, ×200; *b*, *c*, and *d*) Schiff–iodine acid reaction, ×800.

TABLE 2. Morphometric Indexes of Pulmonary Arteries as a Function of the Degree of Prematurity of Neonates ($M \pm m$)

Type and level of vessel	Degree of prematurity	D_o, μ	D_i, μ	TML, μ	CNMT	KI	TML, %
Intraacinar vessels of muscular type							
	I	28.45±0.38	5.84±0.26	11.31±0.18	0.11±0.01	2.43±0.12	79.87±0.78
	II	28.91±0.33	5.92±0.24	11.50±0.17	0.10±0.01	2.48±0.17	79.74±0.77
	III	29.00±0.28	6.37±0.22	11.32±0.13	0.11±0.01	2.10±0.10	78.60±0.67
Intraacinar vessels of partially muscular type							
	I	25.70±0.44	15.37±0.46	5.16±0.13	0.21±0.02	0.42±0.02	41.59±1.12
	II	26.08±0.40	15.07±0.38	5.55±0.13	0.21±0.02	0.46±0.02	43.49±0.95
	III	26.16±0.40	15.00±0.39	5.58±0.09	0.16±0.01	0.45±0.02	44.09±0.87
Preacinar vessels of muscular type							
	I	46.59±1.07	16.11±0.96	15.24±0.35	0.14±0.03	1.30±0.08	66.95±1.35
	II	48.29±0.81	15.16±0.74	16.57±0.30	0.11±0.01	1.66±0.12	69.76±1.13
	III	47.42±0.75	16.05±0.64	15.68±0.27	0.14±0.04	1.29±0.07	67.12±0.97
Preacinar vessels of partially muscular type							
	I	43.60±1.48	31.94±1.57	5.83±0.28	0.19±0.02	0.21±0.02	27.99±1.61
	II	41.41±1.50	28.85±1.56	6.28±0.31	0.20±0.03	0.28±0.03	32.24±1.95
	III	46.65±1.49	31.46±1.65	7.59±0.55	0.22±0.03	0.30±0.04	33.11±2.17

the tendency toward an increase in the *CNMT* was preserved (0.16±0.06; $p<0.05$).

In pneumopathies the intraacinar vessels of the partially muscular type were also characterized by high values of *CNMT* (0.21±0.02; $p<0.05$).

In congenital heart defects the preacinar vessels of the partially muscular type had the maximum values of medial thickness (8.35±2.18 m), of *KI* (0.32±0.15), and of *CNMT* (0.30±0.07).

Comparison of these types of vessels at these levels showed the presence of relationships attesting to the absence of marked differences in the above-mentioned morphometric parameters depending on the anatomicopathological diagnosis, except for changes in *CNMT*. In these groups, notably in pneumopathy, structural changes in the arterial wall were conducive to the preservation and to the increase of pulmonary hypertension during the postnatal period and impeded the normal switchover to independent respiration. The results of morphometric analysis, characterizing persistent pulmonary hypertension in premature neonates with the respiratory distress syndrome, were identical for different diseases. Comparison of the results depending on the degree of prematurity demonstrated a total identity of the parameters and indexes in the pulmonary arteries (Table 2).

Thus, morphometric analysis of the pulmonary arteries showed identical parameters for different nosological forms and no dependence on the degree of prematurity, as well as a characteristic relationship between these morphometric indexes and the level and the type of vessels. The value of *TML* was closely associated with the type of vessels and served as a criterion for their differentiation. The maximum value of *KI* was noted for the intraacinar vessels of the muscular type. The percentage *TML* was lower in the partially muscular and higher in the muscular vessels, exhibiting a pronounced tendency toward an increase from the preacinar to the intraacinar level.

The proposed morphometric index - *CNMT* of the vascular wall - defines the vascular wall asymmetry and probably reflects the dynamic process of thickening of the smooth muscle layer, due to smooth muscle cell hypertrophy and hyperplasia, and the structural reorganization of the pulmonary artery system. We found that in the lungs of premature neonates the walls of the small intraacinar arteries were thickened and muscle cells appeared in the walls of the peripheral arteries, which normally have no smooth muscles. Evidently, persistent pulmonary hypertension in neonates results from prenatal muscularization of the peripheral pulmonary arteries.

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Responses of the Arterial and Venous Vessels of the Skeletal Muscle to Norepinephrine Following Damage to Their Endothelium

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The functional activity of vascular endothelium in a muscle preparation from the feline gastrocnemius is impaired with ethanol, which results in an increase of an adrenergic responsiveness of the arterial compartment of the vascular bed. The exchange function of the microvessels changes little. Veins exhibit nonuniform changes in their responsiveness after exposure of their endotheliocytes to ethanol.

Key Words: *norepinephrine; blood vessels; skeletal muscle; endothelium; ethanol*

In studies carried out on strips and segments of major blood vessels, mainly arteries, endothelium has been shown to contribute to the pattern of their responses to humoral factors acting on vascular myocytes [7,10,11,14]. The role of endothelium in the vasomotor reactions of an organ as a whole has to be studied by a dosed, selective damage to the endothelium in consecutive portions of the vascular bed, while fully preserving the exchange function of the microvessels [1]. A dosed impairment of the function of endothelium without damage to its structural integrity in microvessels of the organ may be achieved using chemical agents [7].

The objective of this study was to examine the norepinephrine (NE)-induced responses of the arterial and venous vessels of the gastrocnemius muscle for different degrees of endotheliocyte dysfunction caused by ethanol.

MATERIALS AND METHODS

The experiments were carried out on 23 urethane-anesthetized (1 g/kg) cats of both sexes. The vas-

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