Functional Morphology of Chorioallantoic Vascular Network in Chicken

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 142, No. 9, pp. 341-345, September, 2006
Original article submitted April 7, 2005

The formation, development, and reduction of the capillary network in the chicken chorioallantoic membrane on days 7-20 of egg incubation were studied by light and electron microscopy with morphometry. Specific features in the architectonics and structure of the mesodermal large vessels and their connection to the suprachorial capillaries for provision of adequate gas exchange are shown.

Key Words: *capillaries; blood vessels; chorioallantoic membrane; embryogenesis; birds*

The chorioallantoic membrane in birds is a provisional organ with important functions of embryo survival (respiration, water-salt homeostasis, thermal exchange) and therefore its development and functional potentialities largely determine the growth of the embryo. The chorioallantois is formed from the embryonic leaflets of the ectoderm (chorionic epithelium), entoderm (allantoic epithelium), while two mesodermal leaflets form the middle layer. This membrane grows tight to the shell membrane and is easily available for studies. Large vessels in its mesoderm grow rapidly and are well seen by naked eye. That is why the chorioallantois is widely used as a model for the study of angiogenesis. The capillary network providing gas exchange and forming by the middle embryogenesis is least of all studied in it. The structure of these capillaries remains not quite clear up to the present time; there are no even universal terms. They are described as fine-walled vascular sinuses located between two layers of the chorial epithelium, whose cells support and divide them [1,2], or they are called capillary plexus, network, or mesh on the surface of the epithelium [4]. Comparison of pictures on sections and total preparations gives the most complete notion of this capillary network.

The aim of this study was quantitative and qualitative evaluation of the topography and structure of the chorioallantoic vascular network and of the formation, development, and reduction of the capillary network at different stages of chicken embryogenesis.

MATERIALS AND METHODS

Eggs of Shaver hens (Novosibirskii State Poultry Factory) were studied on days 7, 10, 12, 15, 18, and 20 of incubation, 5-7 eggs per term. The chorioallantoic membrane together with the shell membrane was fixed in buffered neutral formalin. After hydropreparation, the total chorioallantois preparations were impregnated with silver after Masson and dried. Morphometry of the vessels was carried out using an ocular 1.54-μ scale. The area of capillaries on the surface of chorial epithelium was evaluated using a square test grid (8×8, 81 points) in 10 visual fields for each case at ×450. Histological preparations were stained with hematoxylin and eosin. For electron microscopy the material was fixed in 2.5% glutaraldehyde and 2% paraform, postfixed in 1.0% osmium tetroxide, and embedded in epon and araldite mixture.

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0007-4888/06/1423-0367

Variation series were statistically processed using Statistica 6.0 software and the results were presented as $M \pm m$. The differences were considered significant at $p<0.05$.

RESULTS

On day 7 of incubation the chorioallantois covers $^1/_3$ to $^1/_2$ of the yolk surface and is not connected to the shell membrane. Only major pathways from paired afferent and efferent vessels and a large-loop network of protovessels (17.60±0.59 μ in diameter) lying on the allantoic epithelium were seen in the most distal compartments of the membrane. The protovessels virtually immediately close the main vessels one by each other, and the bloodflow direction cannot be determined in multiangular honeycomb cells (182×312 μ) formed by them (Fig. 1, *a*). Large loops are separated by "blind" lateral processes into smaller cells.

The blood circulation tree starts to form in the mesoderm closer to the embryo. Afferent and ef-

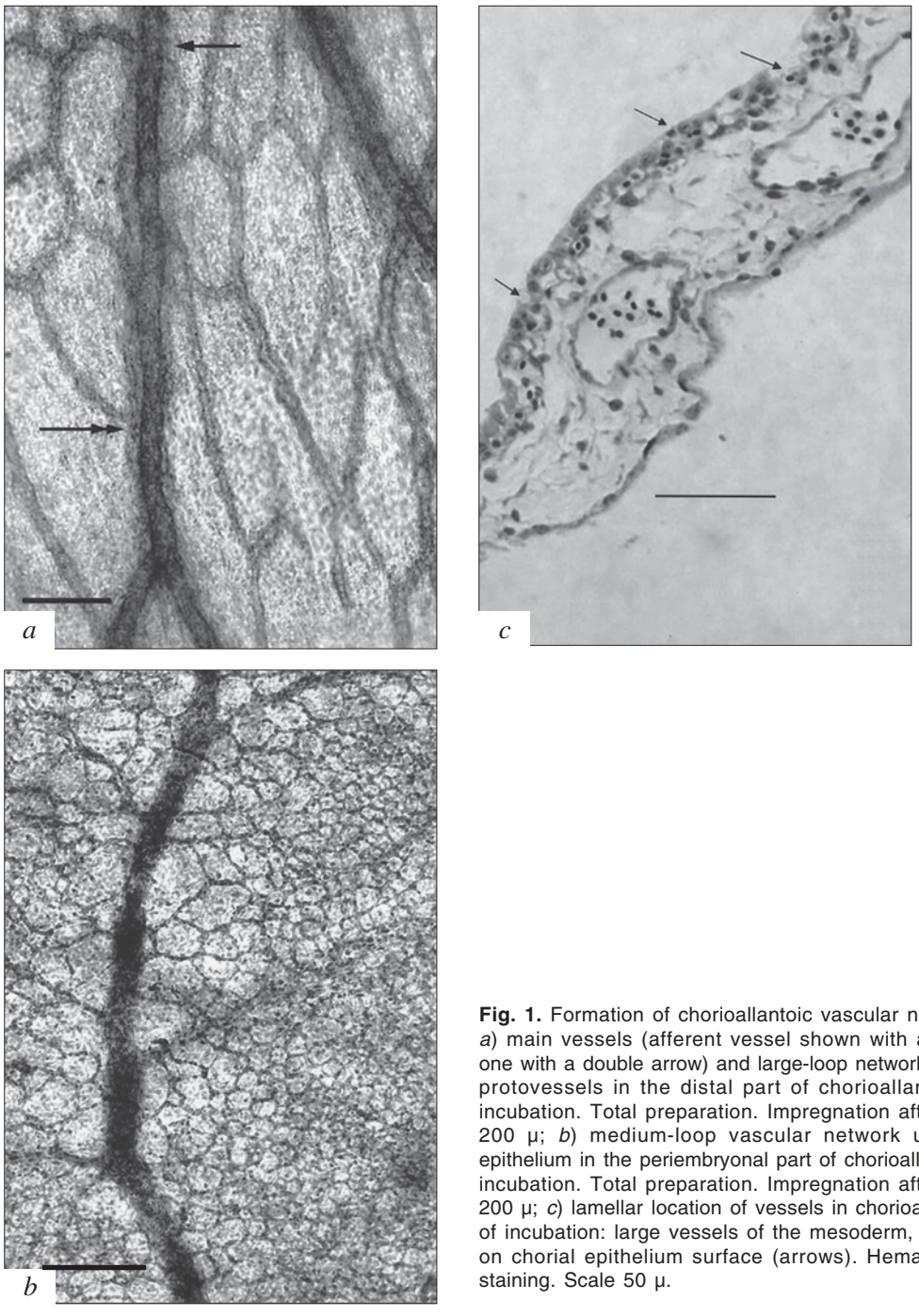

Fig. 1. Formation of chorioallantoic vascular network in chicken. *a*) main vessels (afferent vessel shown with an arrow, efferent one with a double arrow) and large-loop network of the mesoderm protovessels in the distal part of chorioallantois on day 7 of incubation. Total preparation. Impregnation after Masson. Scale 200 μ; *b*) medium-loop vascular network under the chorial epithelium in the periembryonal part of chorioallantois on day 7 of incubation. Total preparation. Impregnation after Masson. Scale 200 μ; *c*) lamellar location of vessels in chorioallantois on day 10 of incubation: large vessels of the mesoderm, vascular plexuses on chorial epithelium surface (arrows). Hematoxylin and eosin staining. Scale 50 μ.

ferent vessels give 3-4 and 5-7 generations, respectively, and form a closed medium-loop network presented by twisted vessels (9.50±1.52 μ in diameter and 145.0±6.2 μ long). The cells in the network are polyhedrons with the mean size of 65×100 μ. These networks penetrate through the entire mesenchyma and line the chorial epithelium (Fig. 1, *b*).

On day 10 the structure of the blood vessel network acquires clearly seen "layers": large main vessels are seen in the mesoderm closer to the allantoic epithelium and a complete layer of small ves-

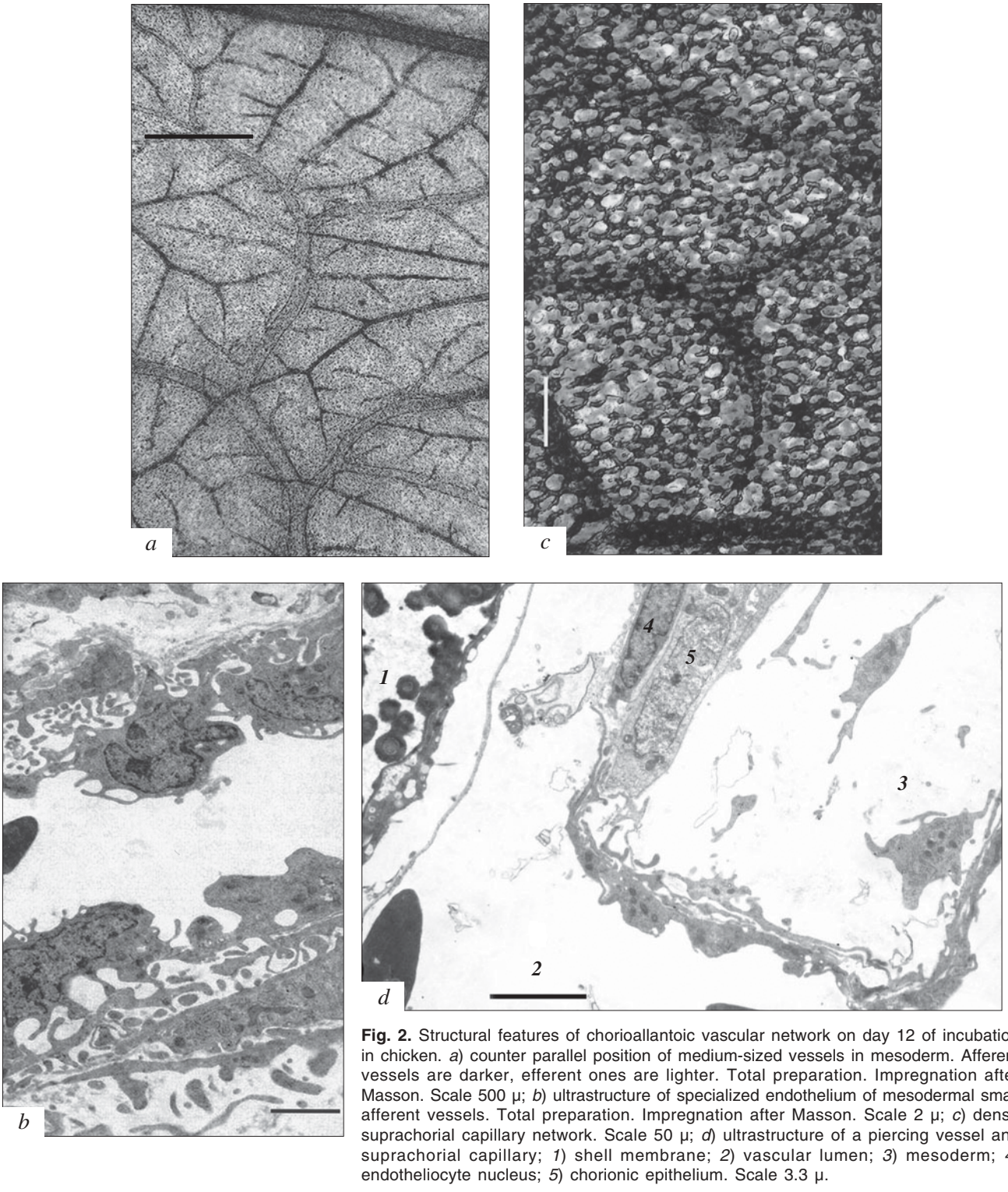

Fig. 2. Structural features of chorioallantoic vascular network on day 12 of incubation in chicken. *a*) counter parallel position of medium-sized vessels in mesoderm. Afferent vessels are darker, efferent ones are lighter. Total preparation. Impregnation after Masson. Scale 500 μ; *b*) ultrastructure of specialized endothelium of mesodermal small afferent vessels. Total preparation. Impregnation after Masson. Scale 2 μ; *c*) dense suprachorial capillary network. Scale 50 μ; *d*) ultrastructure of a piercing vessel and suprachorial capillary; *1*) shell membrane; *2*) vascular lumen; *3*) mesoderm; *4*) endotheliocyte nucleus; *5*) chorionic epithelium. Scale 3.3 μ.

TABLE 1. Morphometric Parameters of the Chorioallantoic Membrane during Different Periods of Chicken Egg Incubation (*M*±*m*)

Parameter	Day of incubation					
	7	10	12	15	18	20
Weight of embryo, g	0.7-0.9	3.0-3.4	4.7-5.6	7.6-10.2	16.8-22.8	32-41
Thickness (μ) of the						
chorioallantois	61.90±2.48	82.50±4.95	56.0±2.7	56.90±2.39	42.70±2.16	41.80±3.17
allantois	4.00±0.11	4.40±0.21	3.80±0.12	5.10±0.17	5.80±0.18	5.90±0.32
stroma	49.10±2.38	68.20±4.91	43.10±2.66	42.60±2.32	27.60±2.18	27.00±2.98
chorion	8.40±0.25	10.00±0.29	9.10±0.15	9.20±0.18	9.4±0.2	9.00±0.27
Capillary diameter, μ	9.10±0.35	7.70±0.26*	4.40±0.23*	5.30±0.12*	5.00±0.16	3.30±0.16*
Capillary length[1], μ	16.00±0.67	14.20±0.77	9.40±0.45*	9.10±0.26	12.00±0.42*	17.20±0.72*
S of capillaries on chorion, %		76.60±1.04	87.40±0.61*	89.30±0.43	76.60±1.01*	43.60±2.62*

Note. S is the area of chorial epithelium, covered by capillaries; [1]distance between capillary network nodes. *$p<0.05$ compared to previous term of observation.

sels forming a medium-loop network is located under the chorial epithelium. Many vessels pierce the chorial epithelium, their wide lumens (7.70±0.26 μ) seen between epitheliocytes (Fig. 1, *c*). They separate forming the upper layer: a capillary network from small (8×12 μ) polygonal cells (Table 1).

On day 12 the main vessels in the mesoderm are located always by pairs, the more narrow afferent above the wider efferent ones. This order is retained in the first 3-4 parallel ramifications. When the vascular diameter decreases to 80-20 μ, afferent and efferent vessels ramify in a strict order after equal distances. These branches, up to the smallest ones, are located counter parallel (Fig. 2, *a*). The wall ultrastructure these vessels varies. Endotheliocytes in the afferent vessels are elevated above the basal membrane due to numerous thick and thin processes, their large nuclei protruding into the lumen (Fig. 2, *b*). The efferent vessel wall consists of one layer of closely contacting endotheliocytes, flattened on the basal membrane.

The suprachorial capillary network is very dense (5.3×7.0 μ cells), the capillaries are short and narrow (Fig. 2, *c*). It covers 87.5% of the chorial epithelium surface (Table 1). Electron microscopy shows that capillary wall is located very close to the shell membrane; the endotheliocytes contain virtually no cytoplasm and therefore the wall consists of the basal membrane and two plasmalemma membranes. The main part of their cytoplasm with the nucleus and solitary pericytes are located on the side of the chorial epithelium (Fig. 2, *d*).

On day 15 of incubation the histological picture, morphometric parameters, and general architectonics of the vascular bed virtually do not differ from the previous stage (Table 1).

On day 18 of incubation, when the embryo pecks through the air chamber of the egg, the thickness of the chorioallantois decreases primarily at the expense of the mesodermal layer (Table 1). The content of the main amorphous substance shrinks in it, fibroblasts and numerous collagen fibers emerge. Morphological signs of hemodynamic disorders appear: hemorrhages into the space under the shell and detachment of the chorioallantois, partial or complete thrombosis of small vessels, parietal thrombi in large afferent vessels, devastation of the efferent vessels. Some impregnated preparations present the predominating picture of capillary network reduction: empty vascular lumens, low density of capillaries, and significantly larger cells in the network (10.1×17.9 μ) in comparison with the previous term of the study. In other cases a dense network of plethoric capillaries predominated.

On day 20 all pathomorphological changes in the chorioallantois and its vascular bed, described on day 18, augmented (Table 1).

It can be hypothesized that the protovessels of the mesodermal large-loop network, along with the yolk sac vessels, perform gas exchange function until days 6-7 of incubation. Oxygen demands increases with embryo growth. This is paralleled by the formation of a more mature blood circulation tree in the mesoderm, in which the filtering (afferent) and reabsorbing (efferent) compartments can be distinguished by the structure of their walls. The smallest branches (about 9 μ in diameter) strive to be maximally close to oxygen source, and that is why they support the chorial epithelium and form a medium-loop network under it. From this network capillaries penetrate to the surface along the gaps between epitheliocytes and contact the shell mem-

brane. The respiratory network of capillaries develops up to day 12 of incubation: they become thinner, shorter, the cells formed by them decrease, and the area of the chorion coverage approaches the maximum. Thus formed vascular system of the chorioallantois provides the increment in embryonic weight from day 12 to day 18 by an average of 14.5 g (almost 4-fold). After day 18, when the embryo gradually transfers to lung respiration, the significance of the respiratory function in the chorioallantois decreases. Morphologically these processes manifest by reduction and devastation of the capillary network, signs of circulation disorders, and degenerative changes in the mesodermal vessels. This is confirmed by experiments [5] demonstrating a more than 2-fold increase in the blood volume in chorioallantoic capillaries from day 10 to day 18 and its subsequent drop. Experiments with microspheres showed that about 50% cardiac output is directed into the chorioallantois from day 10 until day 17 of incubation, while on days 18-19 this value decreases to 30% [3,6].

Thus, the formation of the chorioallantois as the organ with all structural components is completed by day 12 of incubation. For the period of the maximum development (until day 18) the following scheme of functioning of chorioallantois vascular system can be proposed. Venous blood flows into mesodermal vessels via allantoic arteries. Liquid part of the blood is partially filtered in small afferent vessels due to specialized endothelium of these vessels. Blood concentration after filtration decreases its volume directed into capillaries of the flat monolayer network on the chorion surface. The erythrocytes have to be arranged into a one-by-one order because of narrow capillary lumen, this promoting effective gas exchange. Diffusion pathway of oxygen to hemoglobin is maximally shortened, because even the nucleus and cytoplasm of endotheliocytes are displaced to the contralateral side. Oxygen-rich erythrocytes return to mesodermal efferent vessels, where blood is reabsorbed and diluted with purified filtrate; blood rheology and hematocrit are restored. Ballast substances from the interstitial filtrate are absorbed by the allantoic epithelium and released into the urinary sac. Purified and oxygenated blood returns to the embryo via the allantoic vein. Presumably, these processes are provided or facilitated by the orderly counter parallel location of the afferent and efferent vessels and specialized endothelial lining in them. Hence, the problem of provision of the increasing metabolic requirements of the embryo is solved in the course of evolution due to development of the unique vascular system in a limited volume of the membrane in birds.

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