0031-3998/92/3206-0669$03.00/0
PEDIATRIC RESEARCH

Vol. 32, No. 6, 1992
Printed in U.S.A.

In Situ Morphology of the Foramen Ovale in the Fetal and Neonatal Rat

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ABSTRACT. *In situ* cross-sectional morphology of the foramen ovale was studied after rapid whole-body freezing of the fetal and neonatal rat. In the fetus, the foramen ovale was open widely toward the left atrium with a thin, short primum septum. The opening area of the foramen ovale was 40% of the cross-section of the thoracic inferior vena cava, and the ratio of the long diameter to the short diameter was 2 to 1. After birth, the primum septum became longer, thicker, and straighter, with less leftward bowing. The opening of the foramen ovale diminished in the first 2 d and closed completely 3 d after birth. Postnatal thickening of the primum septum was very remarkable, increasing by 400% in the first 2 d, while only minimal change was noticed in the right and the left atrial walls. The length of the primum septum was short and was only 90% of the diameter of the fossa ovalis in the fetus. It increased and reached 97% and 111% of the diameter of the fossa ovalis 1 and 2 d after birth, respectively. The septum secundum also grew rapidly after birth, and its length and width increased by 40% and 29% after 1 and 2 d, respectively. These observations indicate a sudden, explosive growth of the atrial septum in the early neonatal period in the rat. (*Pediatr Res* 32: 669–672, 1992)

Neonatal adaptation of the cardiovascular system has been studied mainly in fetal and neonatal lambs (1, 2), and the physiologic aspects have been greatly clarified (1, 2). Neonatal closure of the foramen ovale is one of these major adaptive changes (1, 2). Recent advances in cross-sectional and Doppler echocardiography made it possible to observe the atrial septum and the foramen ovale in the fetus and newborn (3–6). However, the anatomical details related to the neonatal foramen ovale remain obscure because of difficulties encountered in human study. Recently, we developed a method to study the *in situ* morphology of the cardiovascular system of the fetal and newborn rat (7–10), and morphologic details of the foramen ovale in the fetal and neonatal rat are reported in this study.

MATERIALS AND METHODS

Animals. Twenty virgin Wistar rats (pregnancy period 21.5 d) were mated overnight from 1700 to 900 h, and the presence of sperm in vaginal smears fixed the zero day of pregnancy. These rats were fed commercial solid food and water. Treatment of the rats conformed to the guiding principles of the American Physiological Society.

Received April 29, 1992; accepted August 17, 1992.
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Supported by a grant-in-aid from the Ministry of Education, Science, and Culture of Japan and by the Japan Research Promotion Society for Cardiovascular Diseases.

Freezing, cutting, and photographing. Fetal and neonatal atrial morphology was studied using the rapid whole-body freezing technique as previously reported (7–10). In the study of the fetus, six pregnant rats were killed on the 21st d by cervical dislocation and frozen immediately in liquid nitrogen. Thereafter, frozen fetuses were taken out. In the study of newborn rats, eight mother rats nursed newborns for 1, 2, 3, or 4 d; these newborns were then frozen in dry ice-acetone.

Frozen thoraxes were trimmed and sectioned on a freezing microtome (Komatsu Solidate Co., Tokyo, Japan) in the frontal plane. Cross-sections were photographed with a binocular stereoscopic microscope (Wild M 400 Photomakroscope, Wild Heerbrugg Ltd., Heerbrugg, Switzerland) using color film (Reala, Fuji Film Co., Tokyo, Japan). Atrial cross-sections were photographed serially every 100 μm, beginning at the anterior end of the atrial septum and ending at its posterior end, encompassing the whole atrial septum, for morphologic study and for measurement of the opening area of the foramen ovale. In each rat, about 20 frontal cross-sections were photographed. Number section paper (1 × 1 mm) was photographed for a scale, and the pictures were printed on paper.

Measurement. We measured the following parameters of the foramen ovale on the printed paper (Fig. 1). The thickness of the valve was measured at the midpoint. The right-to-left opening distance of the foramen ovale was measured serially. The length and width of the septum secundum were measured as represented by *d* and *c* in Figure 1. The anteroposterior distance of the foramen ovale was calculated by the number of serial pictures with open foramen ovale multiplied by 100 μm. The area of the opening of the foramen ovale was calculated by adding the opening distances of the foramen ovale from the anterior end to the posterior end and multiplying the combined length by the thickness (100 μm). The arc length of the primum septum is measured from the cranial rim of the primum septum to its caudal end. The craniocaudal length of the foramen ovale is measured from the cranial ridge of the secundum septum to the caudal end of the primum septum. The arc length of the primum septum was compared to the craniocaudal length of the foramen ovale using the ratio of primum septum to foramen ovale and was expressed as a percentage. The inner diameter of the thoracic inferior vena cava was measured, and its inner cross-sectional area was calculated to be compared with the opening area of the foramen ovale.

Statistical analysis. Morphometric data are presented as mean ± SEM. Comparisons between the two groups were subsequently submitted to *t* test at a confidence level of 95%.

RESULTS

The foramen ovale was an opening with a leftward-bending, thin, short primum septum in the fetus (Fig. 2). The anterior primum septum was long enough to reach the limbus fossa ovalis, although the primum septum was bent into the left atrium and the fossa ovalis was open (Fig. 2*a*). The middle and the

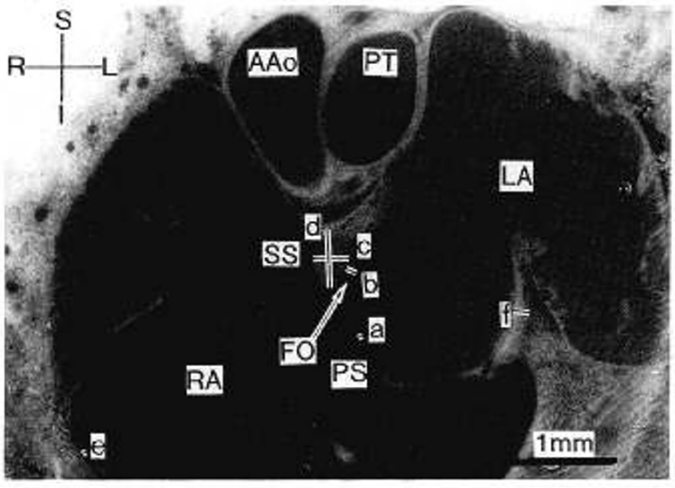
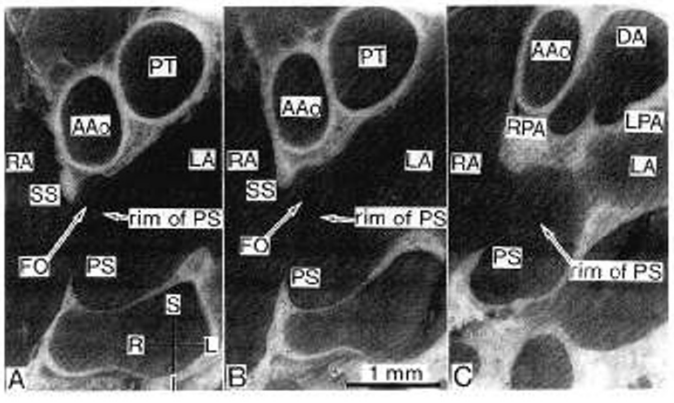


Fig. 1. Measuring points (*double lines*) on the frontal section of the neonatal rat atrium. In the 1-d-old neonate, the thickness of the primum septum was measured at the midpoint (*a*). The opening of the foramen ovale was measured as the shortest distance between the septum primum and the septum secundum (*b*). The length of the secundum septum was measured as a craniocaudal distance (*d*). The thickness of the secundum septum was measured at the middle (*c*). The wall thickness of the right and left atria was measured at the nonpectinated lateral wall (*e* and *f*). *AAo,* ascending aorta; *FO,* foramen ovale; *I,* inferior; *L,* left; *LA,* left atrium; *PS,* primum septum; *PT,* pulmonary trunk; *R,* right; *RA,* right atrium; *S,* superior; *SS,* septum secundum.



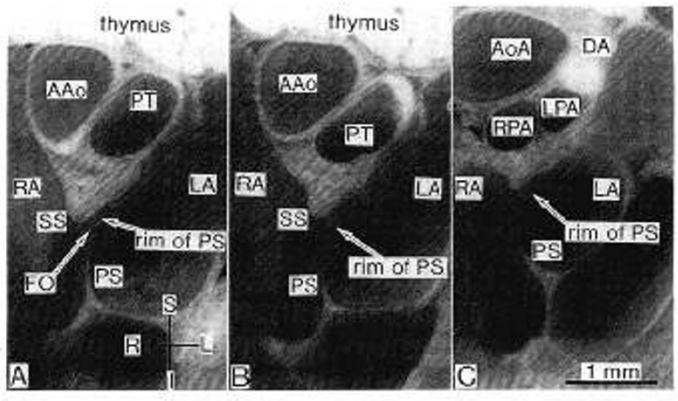
Fetus

Fig. 2. Frontal sections of the frozen fetus. The anterior primum septum at the level of middle pulmonary trunk is shown in *A*, the middle primum septum at the level of the posterior pulmonary trunk in *B*, and the posterior primum septum at the level of the anterior ductus arteriosus in *C* in Figures 2–4. The primum septum was bent into the left atrium in the fetus and the foramen ovale was widely open. The primum septum was very thin, and its cranial rim was slightly thickened. It was short and did not reach the secundum septum. *DA,* ductus arteriosus; *LPA,* left pulmonary artery; *RPA,* right pulmonary artery. Other abbreviations are the same as in Figure 1.

posterior primum septum were not long enough to reach the limbus fossa ovalis (Fig. 2*B* and *C*).

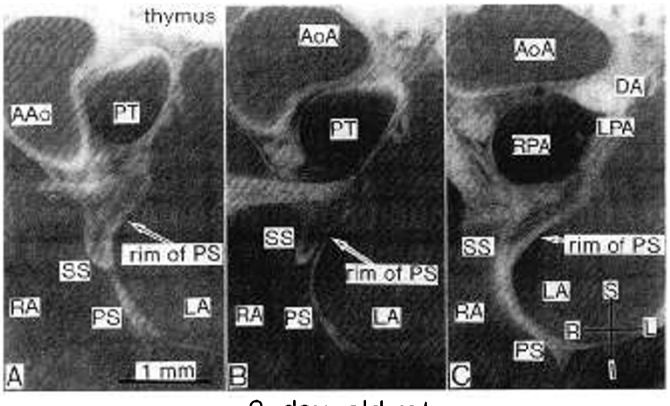
After birth, the primum septum became longer, thicker, and straighter, and the opening of the foramen ovale diminished rapidly in the first 2 d (Figs. 3 and 4). At 24 h after birth, the primum septum was just long enough to reach the caudal edge of the secundum septum (Fig. 3*A–C*). The foramen ovale was open and small in both the anterior and posterior septum.

At 48 h after birth, the anterior primum septum was thick and long, and it adhered to the secundum septum (Fig. 4*A*). The foramen ovale was small and open only at the middle part (Fig. 4*B*). The posterior primum septum was long and thick, and it



1-day-old rat

Fig. 3. Frontal sections of the 1-d-old newborn rat. The primum septum was longer, thicker, and closer to the secundum septum. The foramen ovale was smaller. *AoA,* aortic arch. Other abbreviations are the same as in Figures 1 and 2.



2-day-old rat

Fig. 4. Frontal sections of the 2-d-old rat. The primum septum was longer, thicker, and adhered to the secundum septum at the anterior and posterior parts (*A* and *C*). At the middle part, a slit-like opening of foramen ovale was present (*B*). Abbreviations are the same as in Figures 1–3.

adhered to the secundum septum (Fig. 4*C*). At 3 d after birth, the foramen ovale was always closed completely. These measurements are shown in Figure 5.

Postnatal thickening of the primum septum was very rapid and remarkable (Fig. 4*A–C*). The postnatal thickening was more prominent at the caudal two thirds of the primum septum (Fig. 4*A*). The postnatal thickness of the middle point of the primum septum increased progressively during the first 3 postnatal d (Fig. 6). The septum secundum also grew rapidly after birth. The length and width of the septum secundum increased 40% and 29%, respectively, 2 d after birth (Figs. 3–5). In contrast, the wall thickness of the right and left atrium showed no significant change in these postnatal d (Fig. 6).

In the fetus, the foramen ovale had a crescent opening, and its shorter and longer diameter ratio was about 1 to 2 (Table 1). The opening area of the fetal foramen ovale was 40% of the cross-sectional area of the thoracic inferior vena cava. One d after birth, the opening of the foramen ovale became narrower, and its shorter and longer diameter ratio was about 1 to 4. The opening area of the foramen ovale decreased rapidly in the first 2 d (Table 1).

DISCUSSION

The perinatal morphologic changes in the cardiovascular system of the rat are different from what is seen in humans and

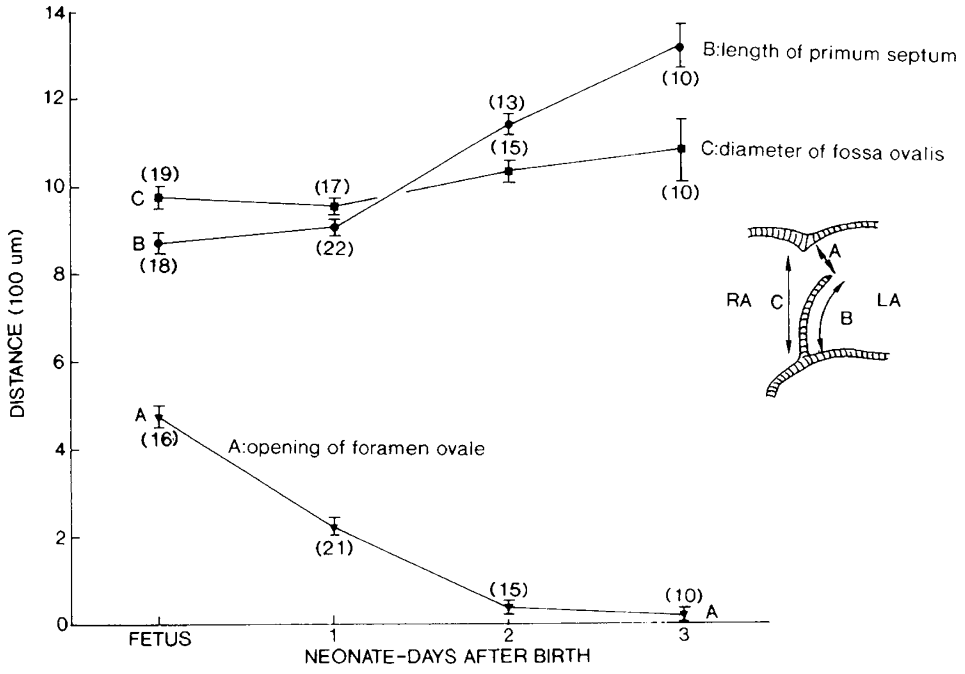


Fig. 5. The craniocaudal lengths of the foramen ovale (*C*), the valve (*B*), and the opening (*A*) in the fetal and neonatal rats. The sketch of the cross-section of the foramen ovale shows the measurements *A*, *B*, and *C*. These were measured at the maximum opening of each serial cross-section. Values are mean ± SEM; numbers of animals are shown in parentheses. RA, right atrium; LA, left atrium.

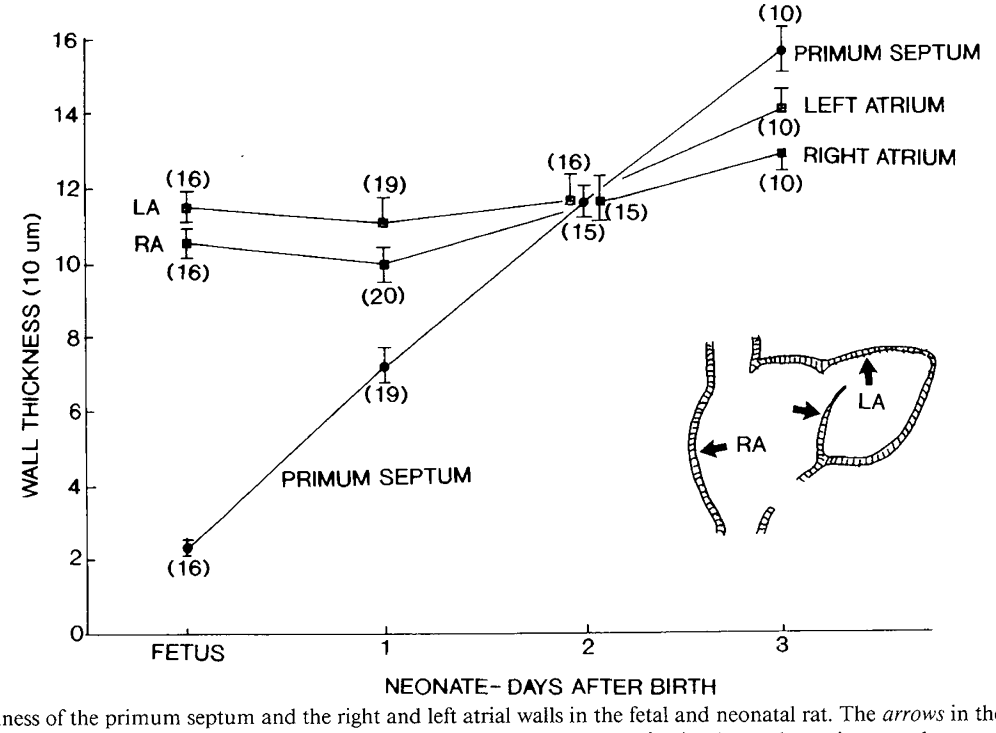


Fig. 6. The thickness of the primum septum and the right and left atrial walls in the fetal and neonatal rat. The *arrows* in the sketch indicate the measuring points. *LA*, left atrium; *RA*, right atrium. Values are mean ± SEM; numbers of animals are shown in parentheses.

occur much faster. The ductus arteriosus in the rat closes within 1.5 h (10) in contrast to about 15 h in the human (2). Probably this is related to the rapid growth of the fetal and newborn rat. In the rat, the gestational period is only 21.5 d. The body weight in the near-term fetal rat increases by about 40% in a day. Postnatally, the body weight of the neonatal rat doubles in 4 or 5 d. Apparently the rapid changes in the cardiovascular system in the neonatal rat are related to the rapid growth.

Our study revealed morphologic details of the closure process of the neonatal foramen ovale. The rapid thickening and growth of the atrial septum are very remarkable, although these have not been given much attention in earlier studies. The postnatal rapid growth of the atrial septum is presumably important to secure neonatal closure of the foramen ovale. The mechanism of this sudden spurt of neonatal growth of the atrial septum is not known and remains to be clarified. As our recent study has shown (7), the right and left atrial volumes decrease about 20% 2 d after birth in neonatal rats, and the present study revealed no increase of the thickness of the atrial lateral wall. Therefore, the observed rapid growth of the atrial septum in the neonatal rat was a localized phenomenon. A recent study reported rapid accumulation of elastin and collagen in the aortas of sheep in

Table 1. *Morphologic perinatal adaptation of foramen ovale system in rats**

Measurement	Fetus	1-d-old	2-d-old
Body weight (g)	5.1 ± 0.1 (16)	5.2 ± 0.1 (21)	7.5 ± 0.2 (16)†
Vascular diameter (×10 μm)			
Ductus arteriosus	75 ± 2 (20)	0 ± 0 (20)†	0 ± 0 (11)†
Thoracic inferior vena cava	100 ± 3 (10)	80 ± 3 (12)	81 ± 2 (13)†
Interatrial septum and right atrial lateral wall (×10 μm)			
R-L opening of foramen ovale	47 ± 2 (16)	22 ± 2 (19)†	9 ± 4 (18)†
A-P opening of foramen ovale	98 ± 3 (13)	90 ± 3 (12)†	9 ± 4 (18)†
Ratio of primum septum to foramen ovale (%)	90 ± 2 (18)	97 ± 3 (17)†	111 ± 2 (13)†
Septum primum thickness	2.3 ± 0.1 (16)	7.2 ± 0.4 (19)†	11.6 ± 0.4 (15)†
RA lateral wall thickness	10.5 ± 0.3 (16)	9.8 ± 0.4 (19)	11.6 ± 0.4 (15)†
Septum secundum thickness	28 ± 1 (12)	36 ± 1 (14)	36 ± 1 (16)†
Septum secundum length	55 ± 2 (12)	62 ± 2 (14)	77 ± 3 (16)†
Opening area (×1000 μm^2)			
Foramen ovale	313 ± 14 (17)	93 ± 7 (10)†	4 ± 2 (18)†
Inferior vena cava	785 ± 52 (14)	503 ± 38 (12)†	511 ± 26 (13)†

* Values are mean ± SEM; numbers in parentheses represent number of rats. *A-P*, antero-posterior; *RA*, right atrium; *R-L*, right-left.
† $p < 0.05$ *vs.* fetus.

the immediate postnatal period and indicated that cell turnover plays an important role in postnatal arterial growth and development (11). A similar mechanism might be responsible for the neonatal growth of the atrial septum.

Acknowledgment. The authors thank Barbara Levene for her editorial help.

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