0031-3998/87/2205-0573$02.00/0
PEDIATRIC RESEARCH

Vol. 22, No. 5, 1987
Printed in U.S.A.

In Situ Morphology of the Heart and Great Vessels in Fetal and Newborn Rats

KAZUO MOMMA, ATSUYOSHI TAKAO, RYUICHI ITO, AND TOSHIO NISHIKAWA

Department of Pediatric Cardiology, The Heart Institute of Japan, Tokyo Women's Medical College, Tokyo, Japan

ABSTRACT. Morphology of the cardiac chambers and great vessels of fetal and neonatal rats was studied using the whole body freezing technique and by sectioning through the short axis of the heart with a freezing microtome. Compared to the fetal heart, the neonatal heart showed rapid change 2 to 8 days after birth. The ventricular sinus septum was straight in the fetus and became concave to the left ventricle after birth. The right ventricular wall was as thick as the left in the fetus and became thinner rapidly after birth. At the same time, the right ventricular cavity dilated. The right and left pulmonary arteries and pulmonary veins were small in the fetus and enlarged soon after birth. At the same time, the foramen ovale was closed and the diameters of the inferior vena cava and descending aorta were diminished. One-half-mm thick sections were cut serially and then photographed. Ventricular volumes and masses were calculated from summation of the areas of each chambers. Left ventricular mass per body weight increased rapidly after birth, whereas right ventricular mass per body weight remained constant from 0 to 8 days after birth. (*Pediatr Res* 22: 573–580, 1987)

Abbreviations

AAo, ascending aorta
DAo, descending aorta
IVC, inferior vena cava
LA, left atrium
LPA, left pulmonary artery
LSVC, left superior vena cava
LV, left ventricle
RA, right atrium
RPA, right pulmonary artery
RV, right ventricle

Rapid anatomical and physiological changes of the heart accompany the transition from the fetal to the newborn circulation (1, 2). Major transitional changes include stoppage of the placental circulation through the umbilical vessels, and closure of the ductus arteriosus, ductus venosus and foramen ovale, dilatation of the pulmonary vessels, and thinning of the right ventricular wall (1, 2). The rapid whole body freezing technique has been used to study *in situ* morphology of the ductus arteriosus in fetal and neonatal animals (3–10) and has proved to be an excellent method for studying the ductus *in situ* with minimal artefacts (10). This method is, therefore, expected to be useful for *in situ* studies of other vessels and cardiac chambers of fetal and neonatal animals. In addition to the academic interest, *in situ* morphology of the fetal and neonatal heart and great vessels is interesting clinically because of recent advances in fetal and neonatal echocardiography (11, 12). We report our observation on the *in situ* morphology of the cardiac chambers and great vessels in fetal and neonatal rats.

MATERIALS AND METHODS

Wistar rats were raised in separate cages and fed with solid foods, commercially obtained. Animals were mated overnight from 1600 h, and vaginal smears were checked at 900 h the next morning. Pregnancy day 0 was defined by the presence of sperm in the vaginal smear.

Three to six pregnant rats were used in each experimental group. Fetal rats were studied on the 22nd day of pregnancy. Pregnant animals were sacrificed by cervical dislocation, and fetuses were delivered quickly by cesarean section, and fixed immediately by the whole-body freezing technique (5–10) using acetone cooled to −80° C by dry ice. Newborn rats, which were delivered spontaneously on the 22nd day and fed by their mothers, were fixed by the whole-body freezing technique 2, 4, or 8 days after birth. The thorax of the frozen rat was trimmed and sectioned on a freezing microtome (Freezing Microtome, Komatsu Solidate Co., Tokyo, Japan) in the short axis plane of the heart. This plane was 45° to the right to left axis and 45° to the long axis in these animals. Sections, 0.5-mm thick, were cut serially from the cardiac apex to the cranial end of two atria. Sections of the heart and great arteries were photographed using a binocular stereoscopic microscope (Wild M 400 Photo-makroskope, Wild Heerbrugg Ltd., Heerbrugg, Switzerland) and Fuji color film (Fujicolor Super HR 100, Fuji Film Co., Tokyo, Japan). The interval of 0.5 mm was used, based on the study of isolated neonatal ventricles described in this report. A magnification of 10× was used for fetuses and 2- or 4-day-old neonates, and a magnification of 7× was used for 8-day-old neonates. In the fetal heart, 15–16 sections were recorded, and 15–25 sections were recorded in the newborn heart. Numbered section paper (1 × 1 mm) was also photographed and used for the scale. The pictures were printed in color on paper, either 174 × 124 or 116 × 81 mm in size.

Morphology of the cardiac chambers and great vessels *in situ* on these prints were studied. The wall thickness of the RV and LV and ventricular septum was measured at the level of the two papillary muscles of the LV. Only compact zones at the sinus portions were measured as the wall thickness, excluding trabeculas at the inner surfaces of the ventricles and infundibulum. The transverse diameters, perpendicular to the septum, of the right and the left ventricular cavities were measured at one-third and two-thirds from the apex to the atrioventricular valves in serial sections in the short axis. The diameter of the right ven-

Received April 16, 1987; accepted June 30, 1987.
Correspondence and reprint requests Kazuo Momma, M.D., The Heart Institute of Japan, Tokyo Women's Medical College, Kawadacho, Shinjuku-ku, Tokyo, 162, Japan.
Supported by a grant in aid from the Ministry of Education, Science, and Culture of Japan and by the Japan Promotion Society for Cardiovascular Diseases.

tricular infundibulum was also measured. The diameters of the ascending aorta and the descending thoracic aorta were measured at the middle part. Short diameters were accepted when these vessels were sectioned obliquely and they appeared eliptic. The diameter of the LSVC, which was present constantly in this species and drained to the coronary sinus, was measured at the middle, between the confluence of the hemiazygos vein and the junction to the right atrium. The diameter of the IVC was measured at the supradiaphragmatic portion. The diameter of the MPA was measured at the midway point between the pulmonary valve and the bifurcation. The diameters of the RPA and LPA were measured at a point near the pulmonary hilum and proximal to the branching to the upper lobe. The diameter of the LLPV was measured at a point just distal to the junction with the left upper pulmonary vein.

The next experiments were done in order to select the optimal interval for sectioning and photographing on the freezing microtome and also to confirm the accuracy of the measurements of ventricular mass and volume and atrial volume from these serial sections. Fetuses were delivered from three mother rats on the 21st day of pregnancy by cesarean section and sacrificed by decapitation within 30 min after birth. The ventricles were immediately isolated from the atria and great vessels with scissors under a stereoscopic microscope and placed in a physiologic saline solution. The heart was weighed, embedded with an embedding medium of frozen tissue specimens (Tissue-Tek II, OCT Compound, Lab-Tek Products, Napaville, IL) in a 10 × 10× 7 cm paper chamber, and frozen in acetone cooled to −80° C. A small amount of carbon powder was mixed in the embedding medium before it was used to make the contrast sharper. The frozen heart was sectioned in short axis planes of the heart on the freezing microtome and photographed at every 0.25 mm, in the same way as the fetal and neonatal hearts *in situ*. Numbered section paper (1 × 1 mm) was also photographed for a scale. Because isolated ventricles were contracted maximally and no cavity was noticed, as shown in Figure 1, only total ventricular mass was measured.

Photographs of the individual heart were numbered serially from the apex of the ventricle to the base of the heart. On each color print, the ventricular margin was clearly demarcated (Fig. 1). Each ventricular picture was cut off and weighed. A paper area of 20 mm^2 was cut off from the photographs of the section paper and weighed for the scale. Using this scale, the cross-sectional area of the ventricles was calculated from the weight of the ventricular picture. Three formulas were tested for calculation of the ventricular mass and the results were compared.

In formula 1, all the cross-sectional areas of the ventricles multiplied by 0.25 mm (thickness) were added. In formula 2, the cross-sectional areas of the ventricles with odd numbers (1, 3, 5,) multiplied by 0.5 mm (thickness) were added together. In formula 3, Simpson's rule was applied. S1 was the first and the last area. Another number with zero area was added in cases where the total number of ventricular pictures was odd. S2 was the summation of the cross-sectional areas with even numbers (2, 4, 6,), excluding the last number. S3 was the summation of the cross-sectional areas with odd numbers, excluding number 1 (3, 5, 7,). In this formula, the ventricular mass was calculated as follows:

$$\frac{(S1 + 2S2 + 4S3) \times 0.25}{3}$$

In order to test the accuracy of the calculations of ventricular masses obtained with these three formulas, the weight of the ventricles was divided by the ventricular mass calculated with the formulas to obtain specific gravity of the frozen ventricles. These indirectly measured specific gravities were compared with the following directly measured specific gravities.

The specific gravity of the ventricles was measured directly as follows. Ventricles of the newborn rat were isolated and were contracted in the same way as described above. The specific gravity of the wet ventricle was measured using the copper sulfate solution method (13) which has been used widely to measure the specific gravity of the blood. For measurement of the specific gravity of the frozen ventricles, 10 solutions of methyl alcohol in water with a specific gravity ranging from 0.90 to 0.99 at 0° C were prepared. The ventricles of newborn rats were frozen in acetone cooled to −80° C and the specific gravity was measured using the alcohol solutions at 0° C.

Ventricular masses and atrial and ventricular volumes of fetal and neonatal rats were measured *in situ* as follows. On the color photograph the sections of the frozen thorax, atrial and ventricular cavities, and ventricular muscle were clearly demarcated and easy to cut and separate. The ventricular cavity was cut as shown in Figure 2. Because small trabeculae were difficult to cut, a smooth cutting line across the trabeculae which separated accurately the ventricular cavity and the muscle was used. The ventricular septum was divided into the right and the left ventricles according to Emery and McDonald (14), in the same proportion as the right and left ventricular free wall thicknesses (Fig.

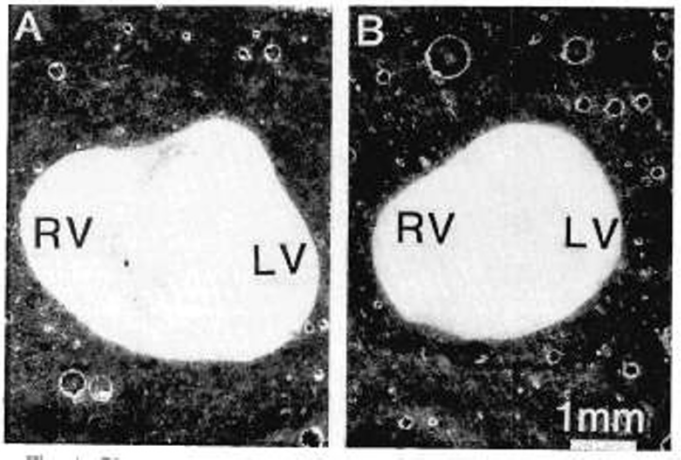

Fig. 1. Photograph of the cross-sections of the frozen isolated contracted ventricles of the newborn rats. *A* and *B* are from two different rats.

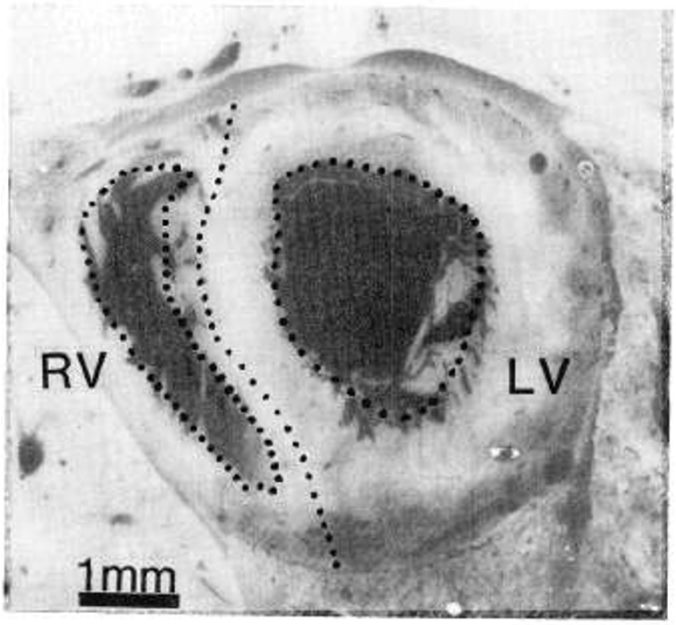

Fig. 2. Photograph of the cross-section of the heart through the short axis of a 4-day-old newborn rat. *Dotted lines* show the cutting lines through the ventricular septum and at the margins of ventricular cavities.

2). As the ratio of the wall thickness was unity in the fetal heart, the dividing line was at the center of the ventricular septum. At the age of 8 days, this ratio was about 1:3, and the dividing line was at a quarter of the total thickness of the septum from the right ventricular side (Fig. 2).

The cut pictures of each chamber and ventricular mass were weighed, and areas were calculated using the weight of the photograph of the section paper as a scale. The volume was calculated by area times thickness (0.5 mm).

Two-way analysis of variances and Duncan's multiple range *t* test were used for statistical analysis to determine the significance of difference of the mean value (15). Five percent was accepted as the level of significance.

RESULTS

The cross-sections of the fetal heart revealed very prominent right and left atria (Figs. 3–6). The left atrial appendage extended near to the cardiac apex and was always present at the cross-section through the apical end of the left ventricular papillary muscles (Fig. 3). The fetal right atrium was also prominent and was larger than the left atrium (Figs. 5 and 6). After birth, the right and left atria were less prominent and their volumes were smaller at 2 days of age than in the fetus (Table 1). Because of progressive dilatation of the RV and concentric hypertrophy and apical dilatation of the LV, both atria looked relatively smaller at 4 and 8 days of age (Figs. 4 and 5). The interatrial septum was open at the foramen ovale in the fetal heart and it was always closed at 2 days of age or later (Fig. 6). The IVC was larger than the RSVC or LSVC in the fetal heart, but it was smaller than the superior vena cava after birth (Fig. 6). Prominent eustachian valves were present at the junctions of the venae cavae to the right atrium (Fig. 6). The LSVC was the same size as the RSVC in the fetal and neonatal rats and was enlarged at 4 days of age or later (Figs. 7 and 8).

The morphology of the ventricles in the fetal rat was characteristic. The RV and the LV had the same wall thickness as the ventricular septum at the sinus portion, and the ventricular septum was straight at this portion (Figs. 3 and 4). The cardiac apex was formed with both the RV and the LV in the fetus, whereas it was formed with only the LV after birth. Two atrioventricular valves and two semilunar valves were in a half-opened position (Fig. 5). Both ventricles were dilated after birth, compared with the fetal ventricles. Postnatal dilatation of the RV was most apparent at the infundibulum and the inflow portion (Figs. 5 and 9), whereas postnatal left ventricular dilatation was most prominent at the sinus portion from the apex to the papillary muscle level (Figs. 3 and 10). The free wall of the RV became thinner and that of the LV became thicker after birth (Fig. 11). At 4 or 8 days after birth, the ratio of the right and left ventricular free walls was about 1:3 (Figs. 4 and 11).

The inner diameter of the ascending aorta was always smaller than that of the main pulmonary artery in fetal and neonatal rats by about 10% (Figs. 8 and 12). The diameters of the

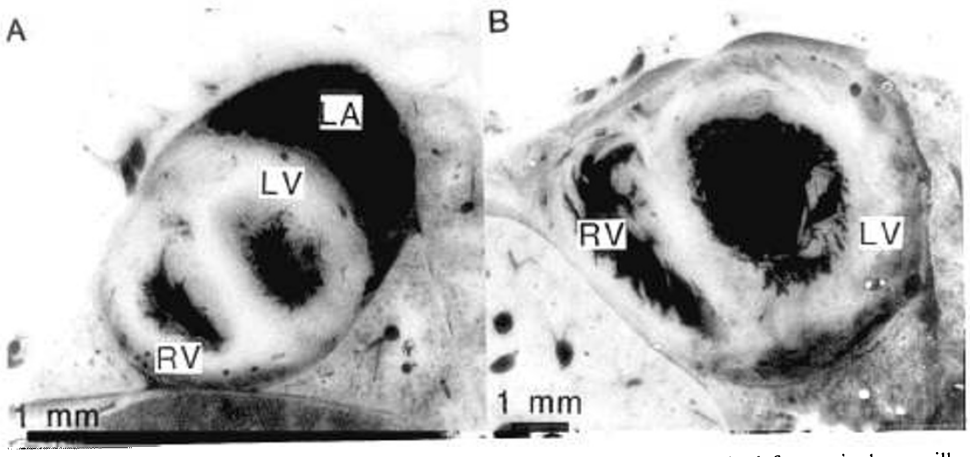

Fig. 3. Photographs of the cross-sections of the heart through a short axis at a level just below the left ventricular papillary muscles. Note large left atrial appendage and thick wall of the right ventricle in the fetal heart (*A*). At 4 days after birth, the right ventricular wall was thinner, and the left ventricle showed an enlarged cavity and thick wall (*B*).

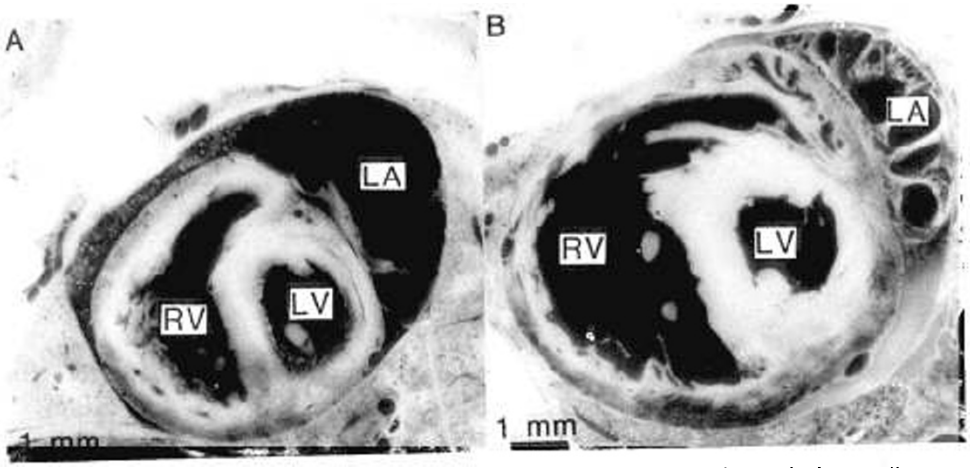

Fig. 4. Photographs of the cross-sections of the heart through a short axis at the level of the left ventricular papillary muscles. Compared with the fetal heart (*A*), the heart of 4-day-old rat (*B*) showed a right ventricle with a thinner wall and enlarged cavity.

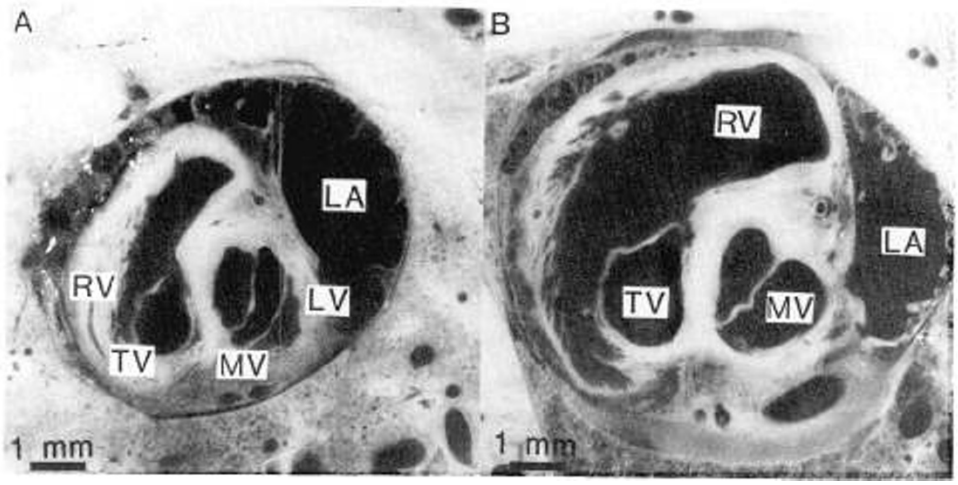

Fig. 5. Photographs of the cross-sections of the heart through a short axis at a level just below the tricuspid and mitral valves. Both atrioventricular valves were half-open. Note enlarged right ventricular outflow and smaller left atrium in 4-day-old newborn rat (*B*) compared with the fetal rat (*A*).

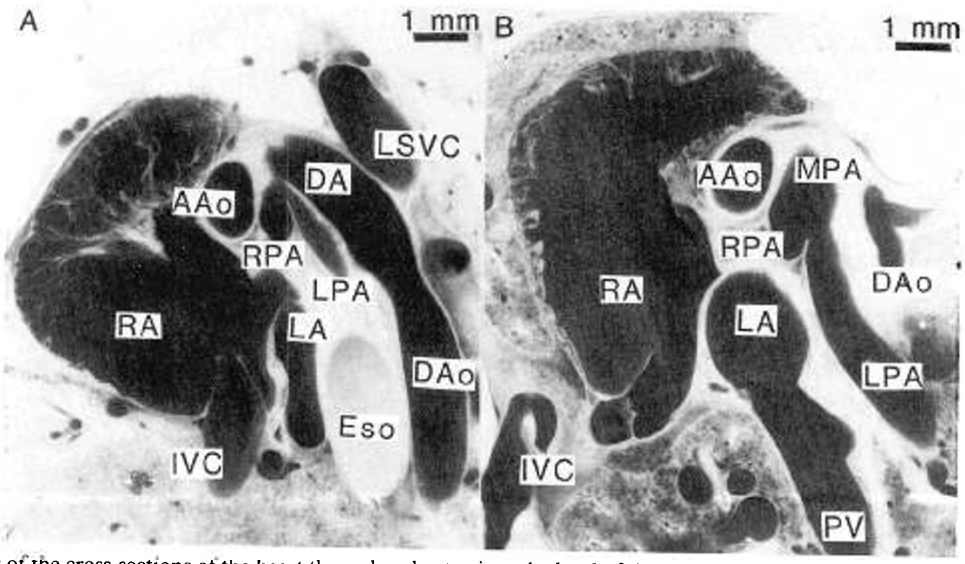

Fig. 6. Photographs of the cross-sections of the heart through a short axis at the level of the ascending aorta and the bifurcation of the pulmonary artery. Note a large ductus arteriosus, relatively large IVC, relatively small right and left pulmonary arteries, and expanded esophagus (*Eso*) in the fetus (*A*). The atrial septum was open as a flap toward the left atrium. At 4 days after birth (*B*), the ductus was closed, and the right and left pulmonary arteries and the left pulmonary vein were enlarged. The atrial septum was closed.

Table 1. *Body wt (g) and ventricular masses (cmm) of fetal and neonatal rats (mean ± SEM)*

Age (days)	Nos. studied	Body wt (g)	RV mass (cmm)	LV mass (cmm)	RV/LV ratio
0	15	5.6 ± 0.5	18.5 ± 1.0	14.9 ± 1.0	1.26 ± 0.05
2	15	6.1 ± 0.1	18.0 ± 0.8	20.6 ± 1.2*	0.90 ± 0.05*
4	15	8.0 ± 0.5*	26.2 ± 1.0*	30.2 ± 0.8*	0.87 ± 0.04*
8	12	15.1 ± 1.5*	44.5 ± 1.8*	55.6 ± 2.0*	0.81 ± 0.04*

* $p < 0.05$ compared to the fetal values.

ascending aorta and the main pulmonary artery increased after birth. In contrast, the inner diameter of the descending aorta decreased significantly after birth by about 20% compared with the fetus at 2 and 4 days after birth (Figs. 8 and 12). The ductus arteriosus was widely open in the fetus and was closed after birth as reported previously (5, 10).

The sizes of the right and LPA and pulmonary veins changed remarkably after birth. In the fetus, these pulmonary vessels were small. After birth, these vessels increased rapidly in diameter as shown in Figures 6 and 7. The diameters of the right and the LPA of the fetal and neonatal rats were about 20% larger near the pulmonary hilum than at the bifurcation (Figs. 6 and 7).

The volumes of frozen isolated contracted ventricle of the newborn rat were calculated using the three formulas. The results were presented in the form of specific gravity and the mean and SD in Table 2. The specific gravity of the wet ventricles that was measured directly with copper sulphate solution was 1.06 ± 0.01 (n = 10, mean ± SD). The specific gravity of frozen ventricles that was measured directly with methyl alcohol solution was 0.96 ± 0.01 (n = 9). As seen in Table 2, formulas 1 and 2 resulted in values which were close to the directly measured specific gravity, and formula 3 gave higher values. SD of the result using formula 2 was only slightly larger than that using formula 1. On the basis of these results, ventricular masses and volumes in fetal and neonatal rats were measured with formula 2, using a constant thickness of 0.5 mm.

The right and left ventricular masses in fetal and neonatal rats are shown in Table 3 and masses per body weight are shown in Figure 13. The right ventricular mass was larger than the left ventricular mass in the fetus, but this relation was inverted

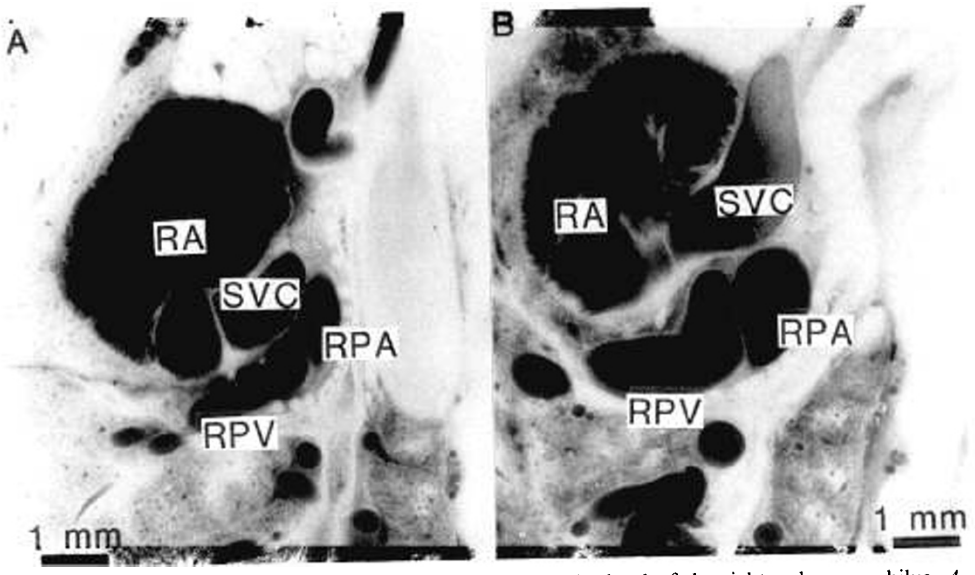

Fig. 7. Photographs of the cross-sections of the heart through a short axis at the level of the right pulmonary hilus. *A*, fetus. Note enlarged pulmonary vessels at 4 days after birth (B).

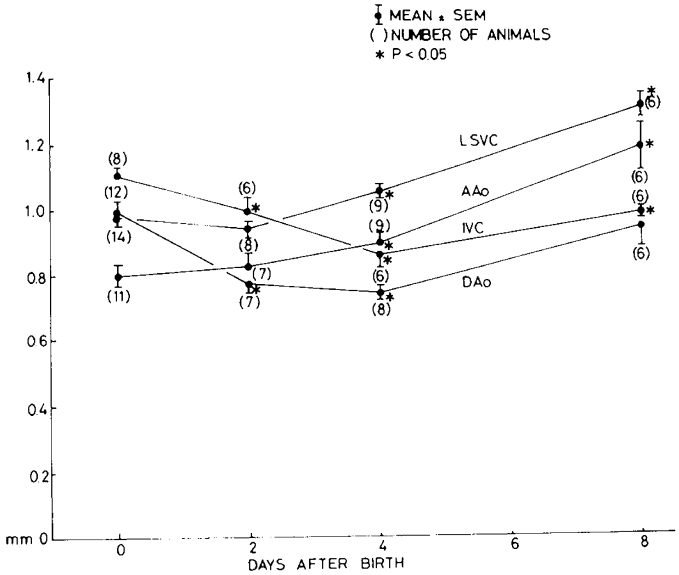

Fig. 8. Inner diameters of the venae cavae and the aorta in fetal and neonatal rats.

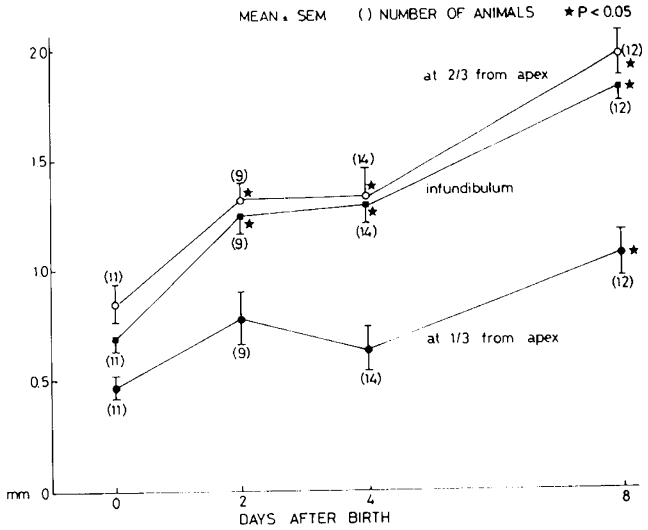

Fig. 9. Diameters of the right ventricular cavity one-third and two-thirds from the apex and at midinfundibulum in fetal and newborn rats (mean ± SEM). Numbers of animals in parentheses. Note more prominent enlargement of the right ventricular cavity at the infundibulum and at the inflow portion of the two-thirds than at one-third from the apex.

postnatally. The right ventricular mass per body weight remained fairly constant from the fetus through 8 days after birth while the left ventricular mass per body weight increased progressively after birth.

The right and left atrial volumes per body weight decreased after birth, and the decrease was more remarkable in the RA (Fig. 14). The volumes of the ventricles per body weight increased postnatally, and the increase was more prominent in the RV. In the fetal heart, the right ventricular volume was slightly larger than the left but the difference was not statistically significant. Postnatal differences between the volumes of the two ventricles were highly significant, and the ratio was more than 2:1 at 8 days after birth.

DISCUSSION

The technique of fixation using rapid whole-body freezing was developed for the study of the fetal and neonatal ductus arteriosus of experimental animals and has been proved to be good for studying the morphology of the ductus *in situ* with minimal artefacts (2–10). This technique was used in this study to study the morphology of the cardiac chambers and great vessels other than the ductus arteriosus and enabled us to examine the morphology clearly. One unsolved problem was the mode of cardiac arrest of the rats in this study. We tried electrocardiographical recording of the animal at the instant of rapid whole-body freezing, but failed because of the unstable baseline of the electrocardiography. Therefore, we have no definite information about the cardiac status of contraction before arrest at the instant of the whole-body freezing. Because cardiac arrest develops following ventricular fibrillation or sinus bradycardia in deep hypothermia (16, 17), either ventricular fibrillation or sinus bradycardia was assumed to be the preceding cardiac condition in our experiments. Both of these conditions seem to be concordant with the following observations. First, the ventricular figures in these frozen hearts were at some point between end-systole and end-diastole. Second, all four valves of the heart were half-open.

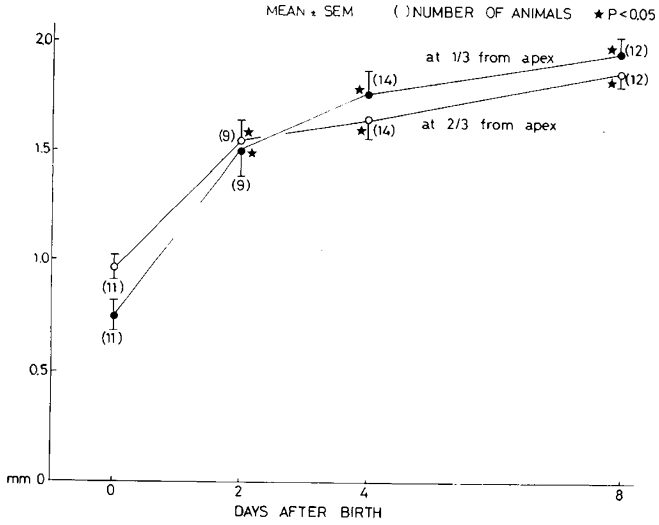

Fig. 10. Diameters of the left ventricular cavity at one-third and two-thirds from the apex in fetal and newborn rats (mean ± SEM). Numbers of animals in parentheses. Note more prominent enlargement of the left ventricle at one-third than at two-thirds from the apex.

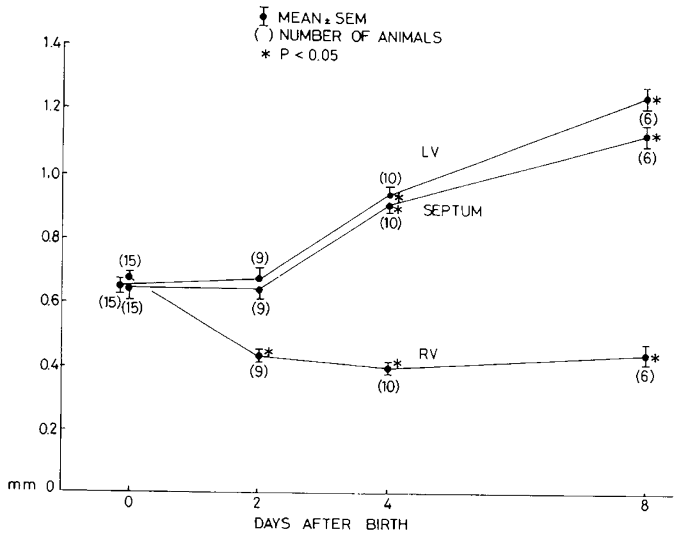

Fig. 11. Ventricular wall thickness of fetal and neonatal rats.

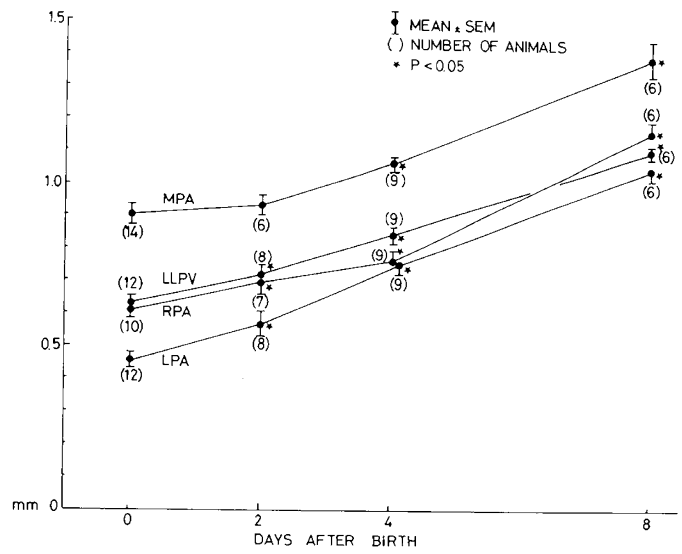

Fig. 12. Inner diameters of the pulmonary vessels of fetal and neonatal rats, *LLPV*, left lower pulmonary vein.

Table 2. *Specific gravities of frozen contracted ventricles of neonatal rats calculated from the weight and volumes using three different formulas (indirectly measured specific gravity) (mean ± SD)*

Formula	Specific gravity
1	0.974 ± 0.040
2	0.972 ± 0.050
3	1.000 ± 0.053

Table 3. *Atrial and ventricular volumes of fetal and neonatal rats in cmm (mean ± SEM)*

Age (days)	RV volume (cmm)	LV volume (cmm)	RA volume (cmm)	LA volume (cmm)
0	4.8 ± 0.6 (15)*	3.2 ± 0.5 (15)	28.4 ± 1.0 (11)	15.4 ± 0.8 (11)
2	11.5 ± 0.6† (15)	8.7 ± 1.0† (15)	20.8 ± 1.0† (6)	12.7 ± 0.8† (6)
4	20.5 ± 1.4† (15)	11.1 ± 0.5† (15)	25.2 ± 1.3† (9)	17.1 ± 0.9 (9)
8	43.7 ± 2.0† (12)	19.2 ± 1.3† (12)	41.9 ± 2.4† (8)	34.9 ± 1.4† (8)

* No. of studied animals in parentheses.
† $p < 0.05$ compared to the fetal values.

In this study the cardiac volumes were measured from the serial cross-sections of the fetal and neonatal rat. The following points are important to minimize potential errors of this relatively indirect method of measurements. Serial photographic recording should be made strictly at 500 μ on a freezing microtome. Tracing and cutting on a printed heart should be accurate. Cut papers of atria and ventricles of each heart were measured in total to avoid difficulty in weighing small pieces of paper. Scale-paper of each heart should be weighed. In fact, heart to heart or day to day differences of the weight of the scale paper were very small and ranged from 53 to 55 mg/mm^2. We used a microbalance which can weigh 0.1 mg accurately. The lightest paper that we measured was 80 mg. From these data, the potential error in measuring the weight of the paper is thought to be relatively small.

As shown in Table 2, calculation of the specific gravity using these volumes obtained from the serial cross-sections was very close to the value obtained by direct measurement using alcohol solutions. Comparison of three formulas showed that sectioning at either 0.5 or 0.25 mm made no significant difference. Calculation using Simpson's rule (formula 3) did not give values closer to the directly measured specific gravity. Based on these basic experiments, the volume study using the serial cross-sections were thought to be reasonably accurate in the study of the heart of these small animals.

Early studies of autopsied human fetuses and infants showed slight right ventricular preponderance over the left at the end of pregnancy and its reversal after birth (2, 18, 19). The present study showed the same trends in the rat, and postnatal reversal of ventricular preponderance was very rapid in the rat. The present study confirmed in rats and *in vivo* the following observations in human materials. First, the inner diameter of the main pulmonary artery was larger than that of the AAo in the fetus and infants (2, 11). Second, pulmonary arteries and veins were relatively small in the fetus (2) and became larger rapidly after

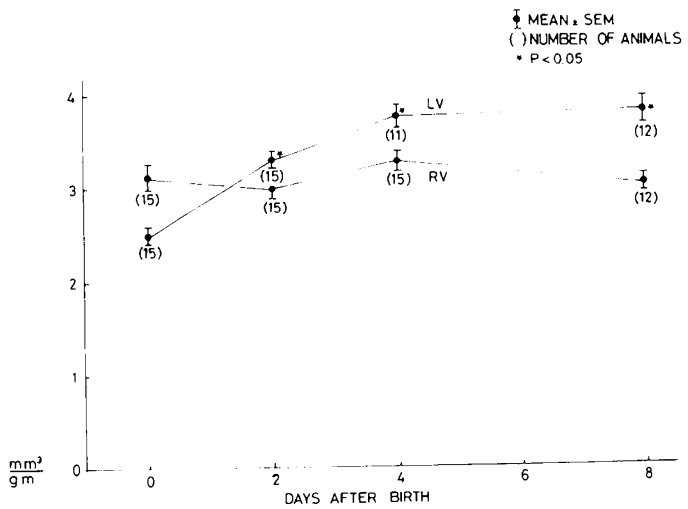

Fig. 13. Ventricular masses of fetal and neonatal rats.

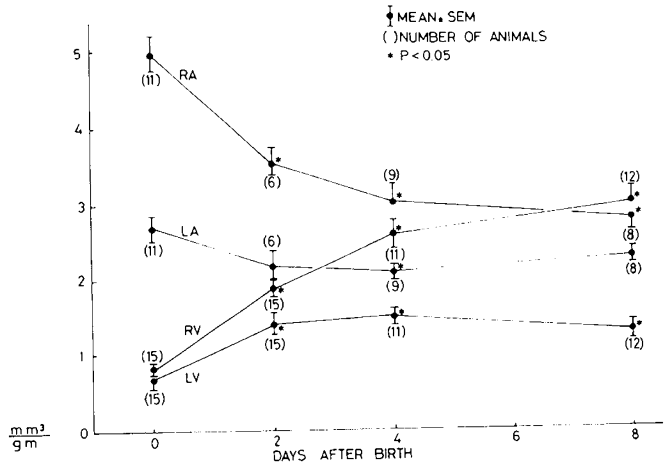

Fig. 14. Atrial and ventricular volumes of fetal and neonatal rats.

birth. Third, the DAo had a relatively large diameter in the fetus, and the present study showed further that its diameter became significantly smaller postnatally, a finding concordant with the postnatal cessation of umbilical circulation through the DAo and the iliac arteries. At the same time, the diameter of the IVC was decreased postnatally in this study. These postnatal changes of the great vessels in human infants remain to be studied with two-dimensional echocardiography.

The volumes of the cardiac chambers in these fetal and neonatal rats showed very definite chronological trends from the fetus through the immediate postnatal period up to 8 days after birth. First, atrial volumes tended to decrease immediately after birth. This is concordant with the echocardiographic observation in the human fetal heart that the fetal LA was relatively larger and the fetal LA/AO was 1.4 (12). Second, ventricular volumes tended to increase. Third, the right ventricular mass per body weight remained constant, and left ventricular mass per body weight increased. These are concordant with the well-documented postnatal circulatory changes such as a decrease in right atrial pressure (2), relatively large cardiac output (20–22), and fall of the pulmonary arterial pressure (1, 2). One puzzling result of this study was the postnatal increase of the right ventricular volume in excess of the increase of the left ventricular volume. Three possible mechanisms include volume overload, pressure overload, and decreased contractility of the RV. Volume overload to the RV can be caused by neonatal tricuspid regurgitation (23) or stretched foramen ovale in these neonatal rats. An angiographic study in newborn lambs showed equal right and left end-diastolic volumes (24). A sudden increase of the right ventricular afterload or decrease of the right ventricular contractility at the instance of freezing can be speculated but must still be proved.

CONCLUSION

A new method to measure volumes of cardiac chambers and ventricular masses of fetal and neonatal rats is described. Combined with the rapid whole-body freezing technique, this new method made it possible to study quantitatively volumes of cardiac chambers and ventricular masses of the fetal and neonatal rat. The right ventricular mass was larger than the left in the fetus, but postnatal left ventricular dominancy was already present 2 days after birth. Pulmonary vessels showed a rapid increase in diameter, whereas the diameter of the DAo and the IVC decreased postnatally.

Acknowledgment. The editorial help of Barbara Levene is greatly appreciated.

REFERENCES

1. Rudolph AM 1974 Congenital Diseases of the Heart. Year Book Medical Publishers, Chicago, pp 1–48
2. Walsh SZ, Meyer WW, Lind J 1974 The Human Fetal and Neonatal Circulation. Function and Structure. Charles C Thomas, Springfield, IL, pp 3–329
3. Hornblad PY, Larson KS 1967 Studies on closure of the ductus arteriosus. I. Whole-body freezing as improvement of fixation procedures. Cardiologia 51:231–241
4. Sharpe GE, Thalme B, Larsson KS 1974 Studies on closure of the ductus arteriosus. XI. Ductus closure in utero by a prostaglandin synthetase inhibitor. Prostaglandins 8:363–368
5. Momma K, Uemura S, Nishihara S, Ota Y 1980 Dilatation of the ductus arteriosus by prostaglandins and prostaglandin's precursors. Pediatr Res 14:1074–1077
6. Momma K, Nishihara S, Ota Y 1981 Constriction of the fetal ductus arteriosus by glucocorticoid hormones. Pediatr Res 15:19–21
7. Momma K, Takeuchi H 1983 Constriction of fetal ductus arteriosus by nonsteroidal anti-inflammatory drugs. Prostaglandins 26:631–643
8. Momma K, Hagiwara H, Konishi T 1984 Constriction of fetal ductus arteriosus by nonsteroidal anti-inflammatory drugs: study of additional 34 drugs. Prostaglandins 28:527–536
9. Momma K, Takeuchi H, Hagiwara H 1984 Pharmacological constriction of the ductus arteriosus and the ductus venosus in fetal rats. In: Nora JJ, Takao A (eds) Congenital Heart Disease: Causes and Processes. Futura Co., New York, pp. 313–327
10. Momma K, Konishi K, Hagiwara H 1985 Characteristic morphology of the constricted fetal ductus arteriosus following maternal administration of indomethacin. Pediatr Res 19:493–500
11. Sahn DJ, Lange LW, Allen HD, Goldberg SJ, Anderson C, Giles H, Haber K 1980 Quantitative real-time cross-sectional echocardiography in the developmental normal human fetus and newborn. Circulation 62:588–597
12. St. John Sutton MG, Gewitz MH, Shah B, Cohen A, Reichek N, Gabbe S, Huff DS 1984 Quantitative assessment of growth and function of the cardiac chambers in the normal human fetus: a prospective longitudinal echocardiographic study. Circulation 69:645–654
13. Phillips RA, Van Slyke DD, Hamilton PB, Dole VP, Emerson K Jr, Archibald RM 1958 Measurement of specific gravities of whole blood and plasma by standard copper sulfate solutions. J Biol Chem 183:305–330
14. Emery JL, MacDonald MS 1960 The weight of the ventricles in the later weeks of intra-uterine life. Br Heart J 22:563–570
15. Armitage P 1971 Statistical Methods in Medical Research. Blackwell Scientific Publications, Oxford, pp 217–225
16. Barratt-Boyes BG, Simpson MM, Neutze JM 1971 Intracardiac surgery in neonates and infants using deep hypothermia with surface cooling and limited cardiopulmonary bypass. Circulation 48, 49(Suppl 1):25–30
17. Stephenson HE Jr 1974 Cardiac Arrest resuscitation, 4th ed. CV Mosby Co., St. Louis, MO, pp 185–188
18. Recavarren S, Aias-Stella J 1964 Growth and development of the ventricular myocardium from birth to adult life. Br Heart J 26:187–192
19. St. John Sutton MG, Raichlen JS, Reichek N, Huff DS 1984 Quantitative assessment of right and left ventricular growth in the human fetal heart: a pathoanatomic study. Circulation 70:935–941
20. Klopfenstein HS, Rudolph AM 1978 Postnatal changes in the circulation and responses to volume loading in sheep. Circ Res 42:839–845
21. Romero T, Friedman WF 1979 Limited left ventricular response to volume overload in the neonatal period: a comparative study with the adult animal. Pediatr Res 13:910–915

22. Berman W Jr, Musselman J 1979 Myocardial performance in the newborn lamb. Am J Physiol 237:H66–H70
23. Mahoney LT, Coryell KG, Lauer RM 1985 The newborn transitional circulation: a two-dimensional doppler echocardiographic study. J Am Coll Cardiol 6:623–629
24. Erath HG Jr, Graham TP Jr, Smith CW, Thompson SL, Hammon JW 1981 Comparative right and left ventricular volumes and pump function in the newborn lamb. Am J Cardiol 47:855–860