

Case Report

A case report of ventricular septal defect in an adult Sprague Dawley rat

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Abstract: A 20-week-old male Sprague Dawley rat noted with decreased body weight, dyspnea, and anorexia beginning 2 days before death was necropsied in the recovery period of a sub-chronic toxicity study. The heart was severely enlarged (30 × 20 × 20 mm), 3–4 times larger than normal, with an approximately 6 mm wide defect in the upper, membranous portion of the ventricular septum. Both ventricles measured 4 mm in thickness, and the right ventricle was 4 times thicker than normal. According to a microscopic examination, the myocardial fibers were severely hypertrophic in the right ventricle and mildly hypertrophic in the left ventricle and septum. Myocardial vacuolation, focal hemorrhages with hemosiderin-laden macrophages, myocardial necrosis, focal fibrosis, hyalinized myocardial fibers, and multifocal adhesive pericarditis were also present. This is the first report concerning severe ventricular septal defects in an adult Sprague Dawley rat with a detailed histopathological examination. (DOI: 10.1293/tox.2016-0073; J Toxicol Pathol 2017; 30: 327–332)

Key words: congenital heart anomaly, histopathology, spontaneous, Sprague Dawley rat, ventricular septal defect

A ventricular septal defect (VSD) indicates failure of complete development of the interventricular septum that allows shunting of blood between the ventricles¹. The defect more commonly occurs in the upper part of the interventricular septum resulting from fusion failure of the membranous and muscular components^{1–3}.

VSD is present not only in humans but also in many animals such as horses, ruminants, pigs, dogs, cats, and laboratory animals¹. Rats, mice, and rabbits have been used as treatment-induced VSD models for evaluation of human congenital heart disease^{4–7}, which can be induced experimentally by agents that delay fusion of the cardiac septae. These include various chemicals, such as trypan blue, salicylate², dimethadione⁸, and trimethadione⁵; hypoxia; deficiencies of nutrients, such as vitamin A and folic acid; and nutritional excesses, such as vitamin A and copper excess².

Spontaneous cardiac VSD has been reported in IS/Kyo⁹ and Sprague Dawley (SD) rats¹⁰, specifically in fetal rats with a small VSD. To our knowledge, there are no reports of VSD in adult SD rats with detailed histopathological findings. Our report describes pathological findings of

VSD in an adult SD rat with a severe defect in the ventricular septum.

A 20-week-old male Crl:CD(SD) rat was used as a control, in which the vehicle was administered by gavage, in a toxicity study for 13 weeks followed by a 4-week recovery period. This animal was supplied by Charles River Laboratories Japan, Inc. (Kanagawa, Japan). The animal was housed and cared for according to the principles outlined in the guidelines for the care and use of laboratory animals prepared by the Japanese Association for Laboratory Animal Science and our laboratory.

Decreased body weight, dyspnea, and anorexia were noted in the rat beginning 2 days before death. An autopsy was performed a few hours after death. All tissues were fixed in 10% phosphate-buffered formalin, trimmed, processed, and embedded in paraffin according to the standard of procedure (SOP) of LSI Medience Corporation. Deparaffinized sections were stained with hematoxylin and eosin (HE), and Masson trichrome stain was applied in the heart. Since we judged that the histological findings did not reflect the cause of the severely enlarged heart after slide preparation, the heart tissue was taken out from the paraffin block by reversing the course of embedding, and it was then reexamined macroscopically.

Macroscopic findings: The heart was severely enlarged (30 × 20 × 20 mm), with pericardial adhesion over a wide area. On the embedding plane of the retrieved heart tissue cut longitudinally from base to apex, the heart was 3–4 times larger than normal, and no anomalies were identified in the heart (Fig. 1). The wall thicknesses were 2.5, 2.0, and

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Fig. 1. On the embedding plane of retrieved heart tissue cut longitudinally from base to apex, the VSD heart is severely enlarged (left), 3–4 times larger than normal (right, 13-week-old).



Fig. 2. On the reverse plane, the VSD heart (left) has an approximately 6 mm wide defect (asterisk) in the upper, membranous portion of the ventricular septum. The right side of the heart is normal (13-week-old).

Table 1. Free Wall Thickness of the Each Chamber and Septum Thickness (mm)

	Embedding plane			Reverse plane		
	RV	Septum	LV	RV	Septum	LV
VSD	2.5	2	3.5	4	— [#] 6 ^s	4
Normal (13 w)	1	3	4	1	5	3.5

RV; right ventricle, LV; left ventricle. [#]patent portion of the interventricular septum. ^snon-patent portion of the interventricular septum.

3.5 mm for the right ventricle, septum, and left ventricle, respectively (Table 1). On the reverse plane, the heart had an approximately 6 mm wide defect in the upper, membranous portion and a 6 mm thick wall in the muscle portion of the ventricular septum (Fig. 2). The left and right ventricular walls measured 4 mm each in thickness, and the right ventricular wall was 4 times thicker than normal (Table 1). The left atrium was severely dilated (12 mm in diameter in the histological section). The lungs showed a dark reddish change due to chronic congestion with a large amount of serosanguineous fluid (approximately 20 mL) in the thoracic cavity. Perithymic lymph nodes showing a dark reddish change were 2 to 3 times larger than normal. The liver was severely enlarged and showed a dark reddish change. The abdominal cavity contained serosanguineous fluid (approximately 1.5 mL). There were no lesions in the vertebral bones at autopsy.

Microscopic findings: The myocardial fibers were severely hypertrophic, 4 to 5 times larger than normal, in the right ventricle (Fig. 3) and mildly hypertrophic, 1.5 to 2 times larger than normal, in the left ventricle and septum (Fig. 4). Myocardial vacuolation was seen in the all chambers; however, both the atrium (Fig. 5) and right ventricle

(Fig. 3) were more severe than the others. The dilated left atrium was mildly congested and had mild hemorrhages with small numbers of hemosiderin-laden macrophages (Fig. 5). The severity of hemorrhage and macrophage infiltration in the right atrium was less than that in the left atrium. In the septum, focal myocardial necrosis with inflammatory cells infiltration (Fig. 6) and hyalinized myocardial fibers (Fig. 7) were recognized. Although the myocardial vacuolation was mild in the left ventricle, small foci of myocardial necrosis (Fig. 8) and fibrosis were found sporadically. Multifocal adhesive epicarditis was also present with mild edema (Fig. 9). No coronary artery disease or myocardial infarction was found. In the lung, the alveolar septa were thickened due to a large number of erythrocytes in the dilated capillaries or due to edema with small amounts of inflammatory cell infiltration. The alveolar spaces contained large numbers of hemosiderin-laden macrophages (heart failure cells) (Fig. 10). The liver showed severe centrilobular congestion with hepatocellular degeneration/necrosis and fibrosis indicative of passive venous congestion (Fig. 11). There was also moderate to severe congestion of the parathymic lymph node, spleen, and adrenal inner cortex. Interstitial edema was seen in the pancreas, prostate, and dermis of the whole

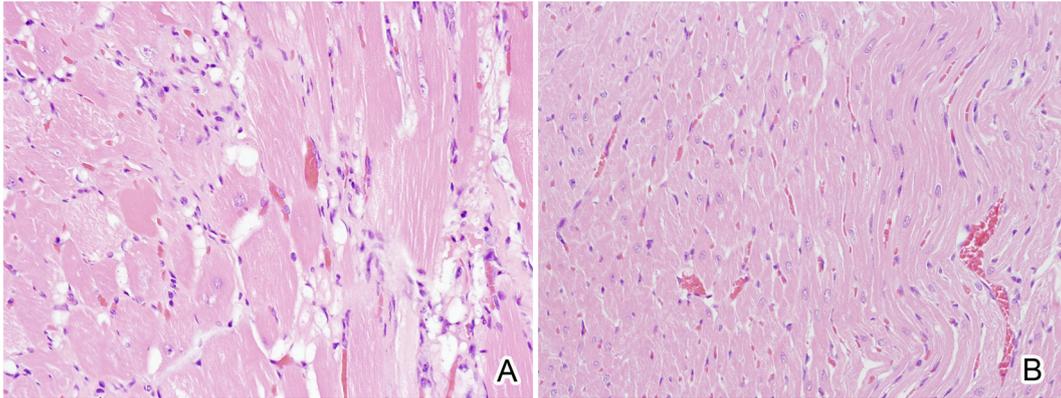


Fig. 3. Histopathology of the right ventricles in the VSD heart (A) and corresponding normal heart (B). The myocardial fibers of the VSD heart are severely hypertrophic with vacuolation. H&E stain. $\times 400$.

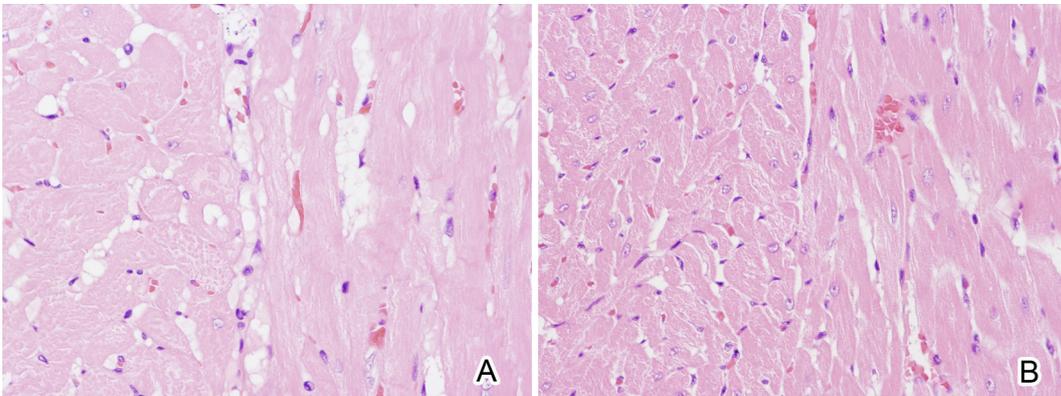


Fig. 4. Histopathology of the septum in the VSD heart (A) and corresponding normal heart (B). The myocardial fibers of the VSD heart are mildly hypertrophic with vacuolation. H&E stain. $\times 400$.

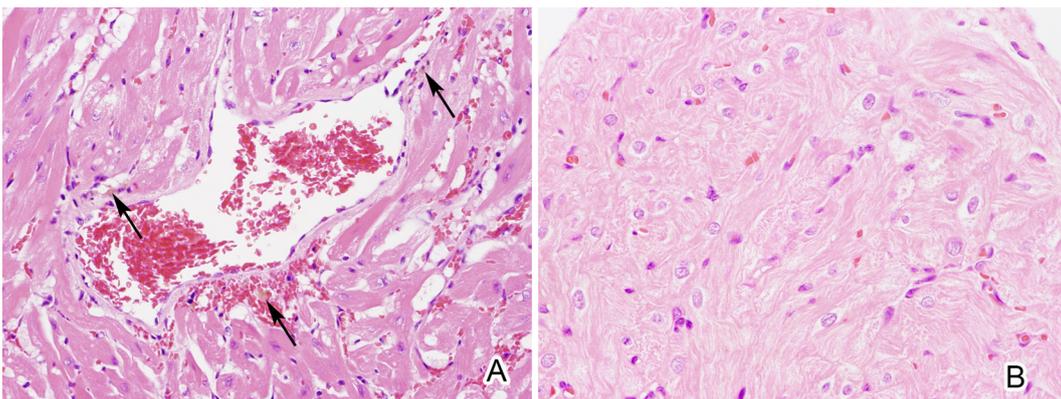


Fig. 5. Histopathology of the left atrium in the VSD heart (A) and corresponding normal heart (B). The dilated left atrium is mildly congested and has a small number of hemorrhages with hemosiderin-laden macrophages (arrows). H&E stain. $\times 400$.

body. Moderate hyperplasia of erythrocytic hematopoietic cells was seen in the bone marrow of the femur and sternum. Atrophy was noted in the thymus, mesenteric lymph node, and white pulp of the spleen.

This report described a VSD in an adult Crl:CD(SD) rat that was characterized macroscopically by a severe and wide defect of ventricular septa and microscopically by myocardial hypertrophy, vacuolation, necrosis with inflam-

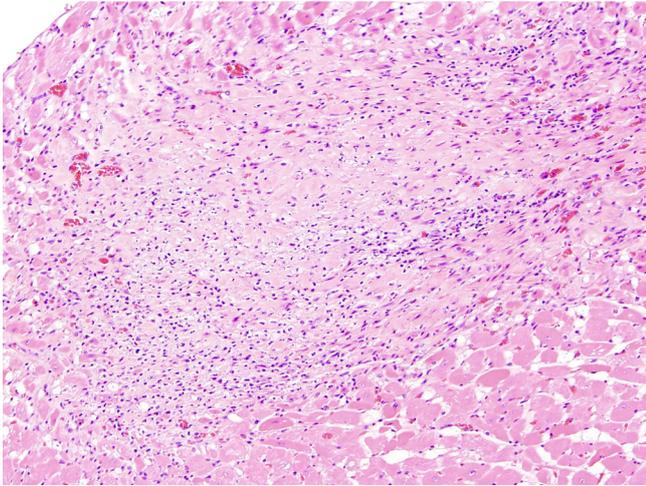


Fig. 6. Histopathology of the septum in the VSD heart. Focal myocardial necrosis with inflammatory cells infiltration is apparent. H&E stain. $\times 200$.

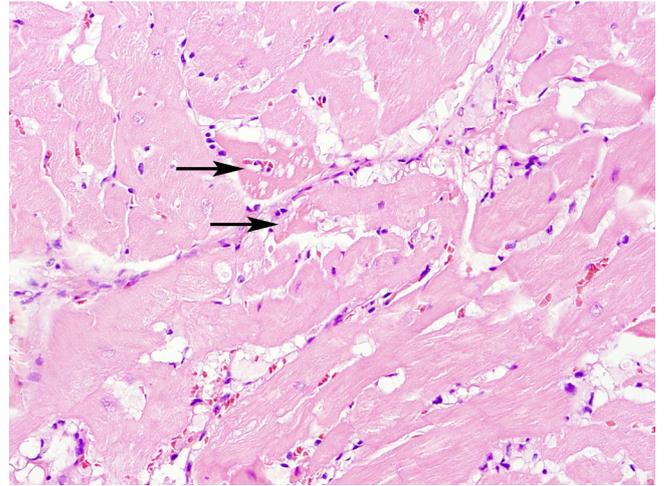


Fig. 7. Histopathology of the septum in the VSD heart. Myocardial vacuolation and hyalinized myocardial fibers (arrows) are slightly apparent. H&E stain. $\times 400$.

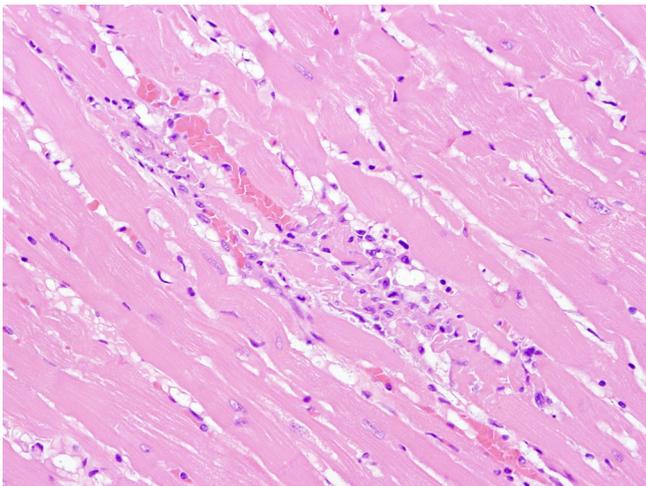


Fig. 8. Histopathology of the left ventricle in the VSD heart. Small foci of myocardial necrosis are sporadically apparent. H&E stain. $\times 400$.

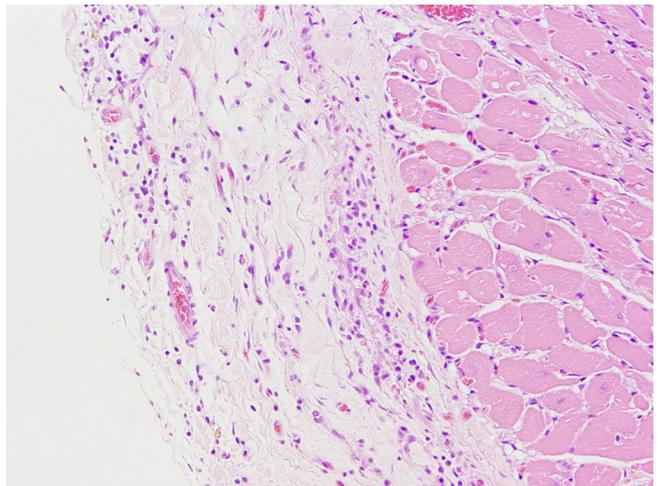


Fig. 9. Histopathology of the right ventricle in the VSD heart. Multifocal adhesive epicarditis is present with mild edema. H&E stain. $\times 200$.

mation and hyalinization, and congestion and hemorrhage with hemosiderin-laden macrophages. It has been reported that histological examination of spontaneously occurring VSD in rats revealed no morphologic changes in the ventricular walls or any evidence of inflammation, fibrosis, or cytologic changes⁹⁻¹¹. In contrast, myocardial changes are seen in various diseases. Abnormal metabolic, structural, and functional changes were found accompanied by hypertrophy that occurs in hypertension, obesity, and heart valve disease, after infarction, or with cardiomyopathy. Myocardial fiber damage takes the form of a cytoplasmic alteration such as vacuolation, and the irreversible consequence of myocyte damage is necrosis¹². Necrosis is accompanied by a variable degree of inflammation¹² and leads to cell loss

and fibrosis in all areas¹³. In degenerative myocardial disease, the lesion begins as coagulative necrosis of myocardial fibers, which become deeply eosinophilic and hyalinized with pyknotic nuclei¹³. Hemosiderin-laden macrophages are seen in the myocardium following hemorrhage or following repair of myocardial cell damage¹². Although the changes in the present case do not seem to be specific or uniform in VSD rats, this study would yield new information on the detailed histological findings of VSD in adult rats.

VSD usually has a relatively benign clinical course in humans, either closing spontaneously or causing congestive heart failure treatable by surgery¹⁴. In patients with large defects, however, symptoms of congestive heart failure become evident in the first few weeks of life, and infants show

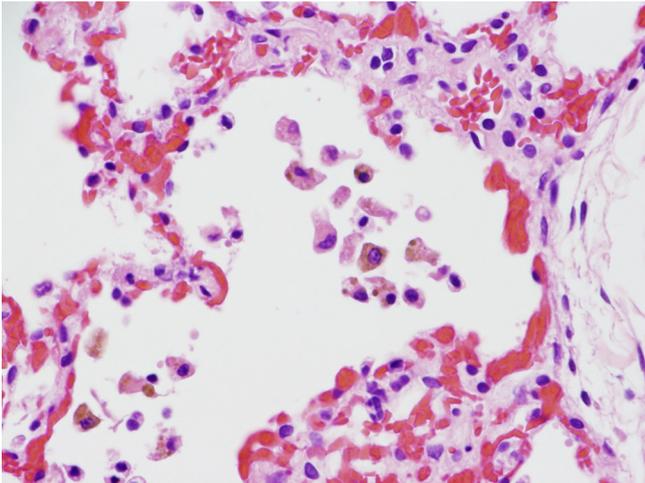


Fig. 10. Histopathology of the lung. The alveolar septa are thickened with a large number of erythrocytes in the dilated capillaries. The alveolar spaces contained large numbers of hemosiderin-laden macrophages (heart failure cells). H&E stain. $\times 800$.

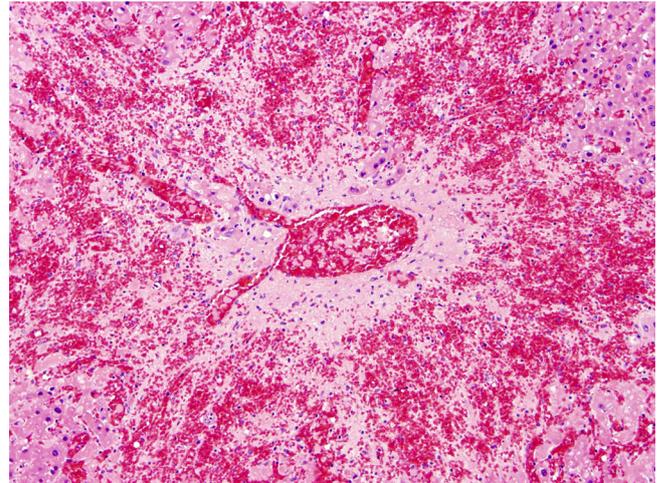


Fig. 11. Histopathology of the liver. The liver shows severe centrilobular congestion with hepatocellular degeneration/necrosis and fibrosis indicative of passive venous congestion. H&E stain. $\times 200$.

high pulmonary blood flow and high pulmonary artery pressures, poor growth, rapid/labored breathing, tachycardia, and diaphoresis¹⁵. This case was apparently healthy and had no problems in terms of body weight gain until 3 days before death. It is uncertain why dyspnea and anorexia were noted and weight loss was recorded only 2 days before death.

The causes of congenital cardiovascular anomalies are varied. A great variety of environmental and genetic factors are probably responsible for this outcome in rats^{1, 16, 17}. The pathogenic mechanism of the lesions detected in this case is speculated about below. As the right ventricular pressure equals the left ventricular pressure postnatally by left-to-right shunt in VSD, the right ventricle is confronted with a large systolic and diastolic load, which is followed by pulmonary hypertension with hypertrophy of the pulmonary arterioles upon histopathology¹⁶. The present case contained numerous hemosiderin-laden macrophages in the alveolar spaces, which indicated chronic passive congestion¹⁸, though hypertrophy of the pulmonary arterioles could not be distinguished. The liver showed passive venous congestion, which denotes elevation of pressure in the hepatic veins and venules relative to the pressure in the portal venules caused by congestive heart failure as a result of congenital cardiac disease¹⁹. These findings in this case are consistent with congenital cardiac disease (ventricular septal defect).

There is a surprising lack of information concerning the incidence of spontaneous VSD in these widely used Crl:CD(SD) rats. VSD in adult rats may be seen more frequently than has been recognized in the past. We would like to request that pathologists and lab technicians using small ruminants including Crl:CD(SD) rats pay more attention to not only necropsy but also to the process of making sections because VSD may be present even with no significant clinical histories.

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