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Morphological Manifestations of Hereditary Hypertrophic Cardiomyopathy in W/SSM Rats

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A W/SSM strain of rats with hereditary hypertrophic cardiomyopathy has been created by inbreeding Wistar rats selected for an increased sensitivity to the cataractogenic effect of high doses of galactose. It is shown that myocardial hypertrophy attended by a diffuse stroma collagenization, focal sclerotic changes, and signs of chronic heart failure spontaneously develops in these animals.

Key Words: *W/SSM rats; hereditary hypertrophic cardiomyopathy; myocardium; cardiomyocyte count*

Hereditary factors play an important role in hereditary hypertrophic cardiomyopathy (HCM) etiology [5]. In this connection the creation of the animal strain with a hereditary pathology of the myocardium similar to HCM in humans is of special interest. A W/SSM rat strain has been created by the inbreeding of Wistar rats selected for an increased sensitivity to the cataractogenic effect of high doses of galactose [8]. In these animals cataracts, hepato- and splenomegaly, and kyphoscoliosis develop spontaneously, and an increased weight of the heart, delayed growth and development, and reduced fertility are observed [2,8,9]. In parallel, we have selected rats resistant to the damaging effect of galactose. In this strain (W/SSM-R) of rats obtained by selection and inbreeding no pathological manifestations have been observed in response to galactose loading [8].

Some biochemical characteristics and their heritability have been studied in W/SSM rats, and

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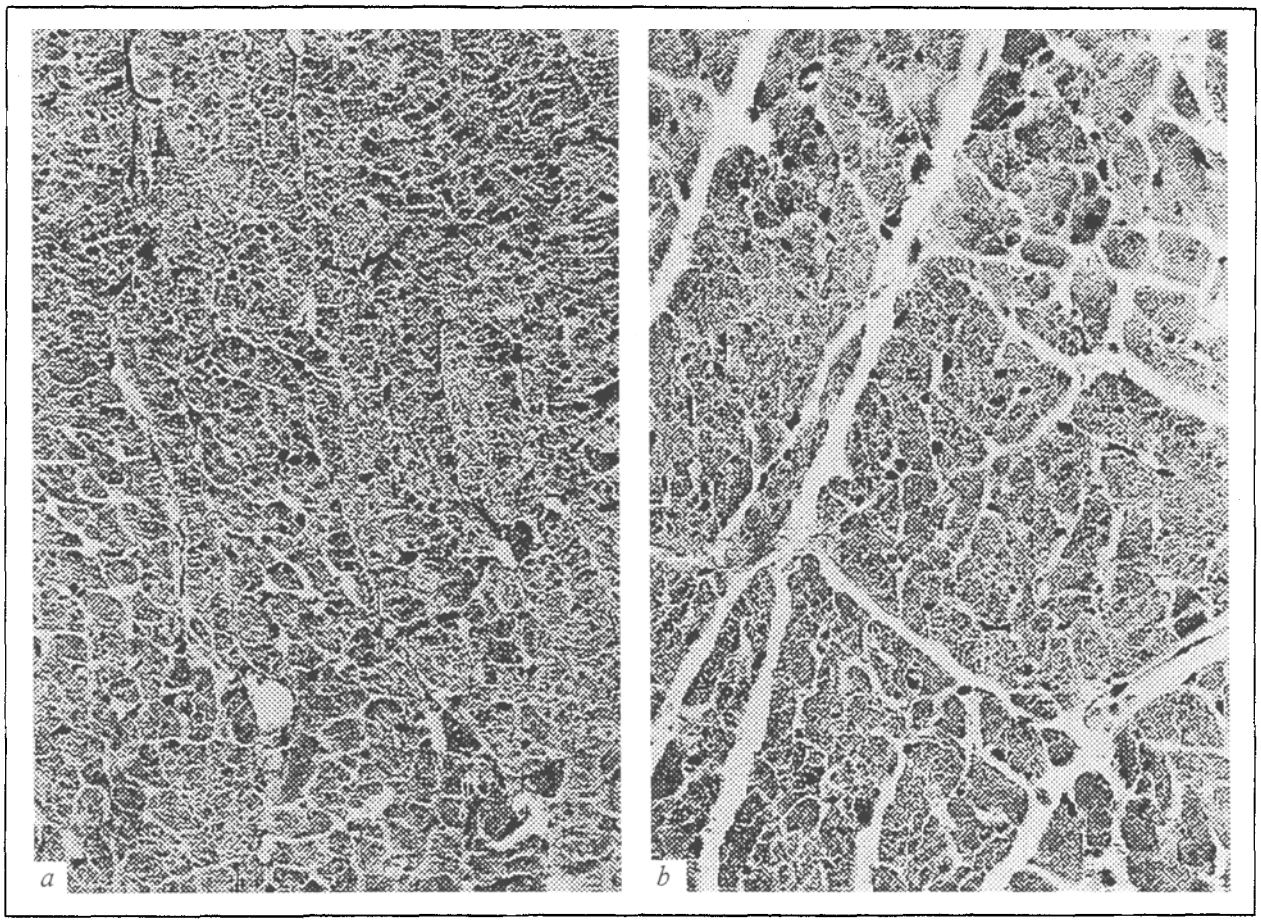

Fig. 1. RV myocardium in 3-month-old W/SSM rats. Hematoxylin-eosin staining, ×320. *a*) W/SSM-R rat (control); normal packing of myofilaments in CMC; absence of focal and diffuse changes in the muscle cells and stromal elements. *b*) W/SSM rat; myofilament loosening in CMC.

speculations on the genetic and biochemical mechanisms of pathological processes have been made. These studies have demonstrated that in W/SSM rats a mutant gene is expressed which alters the properties of the glucose transporter, which results in the accumulation of sugars and aldolases in the cells [1,8]. In addition, the specific activity of lysosomal enzymes in the plasma and leukocytes has been found to be increased in the animals of this strain [1].

In the present study we morphologically investigated the heart of W/SSM rats with spontaneous hereditary HCM.

MATERIALS AND METHODS

The experiments were carried out on 40 rats of the pathological W/SSM strain; healthy W/SSM-R rats served as the control. Morphological and morphometric investigations were performed on young (1-3 months) and adult (10-12 months) animals. Rats were killed by decapitation. The myocardium was fixed in a 10% neutral Formalin, and 1 h later its weight was determined, after which the weights of the right ventricle (RV) and left ventricle (LV) myocardium with the ventricular septum were determined. After alkaline dissociation of the fixed myocardium [6] the ventricular cardiomyocyte (CMC) population was quantified. Histological investigation was performed on sections stained with hematoxylin-eosin in combination with Perls reaction and with colloid iron - Schiff-iodine acid (SIA) - hematoxylin [7]. The numerical results were processed by parametric statistics using Student's t test.

RESULTS

In 3-month-old W/SSM rats the weight of the heart was 17% higher, on average, than in the control rats of the same age with the same body weight; the LV weight was 15% higher on average, and the RV weight was 42% higher (Table 1).

In 10-month-old W/SSM rats the relative weight of the RV was 25% higher, on average, than in the control animals. The total weight of

TABLE 1. Weight of the Myocardium and Ventricles in Rats of the Pathological W/SSM Strain and in Control W/SSM−R Rats

Strain	3-month-old rats			10-month-old rats		
	myocardium	LV	RV	myocardium	LV	RV
W/SSM	328±7.65*	269±5.72*	64±3.42*	304±13.21	233±7.94	64±1.25*
W/SSM−R	281±5.25	234±6.03	45±0.87	274±9.5	224±8.21	50±1.88

Note. The total weight of the myocardium or ventricle (mg) is calculated per g body weight ×100 (the mean of 7−8 experiments). An asterisk denotes the reliability of differences from the control.

the myocardium and the LV weight in these animals did not differ from those in the age-matched controls (Table 1). The weight of the myocardium in healthy W/SSM-R rats did not differ from that in Wistar rats [8].

Histological study of the myocardium in 3-month-old W/SSM rats (Fig. 1) demonstrated that the epicardium and endocardium in all animals had a normal structure. In the RV and LV myocardium a predominantly compact distribution of muscle fibers was observed. In the median and subendocardial zones of some hearts a moderate edema of connective tissue between the fibers was noted, which was more pronounced near the apex of the heart.

The majority of CMC stained uniformly with acid stains, but at the same time we noted a mosaic staining, resulting from eosinophilia of the muscle segments, as well as from the presence of a small number of cells with a thinned, cleared sarcoplasm. Small foci of necrobiotically changed CMC were found in virtually all animals, and around these clusters of mononuclears formed. It is worth noting that diffuse mononuclear infiltration of the myocardial stroma was enhanced, notably in the zones where the muscle segments were predominantly eosinophilic.

Spasm or secondary paresis was frequently observed in the intramural arteries. The middle

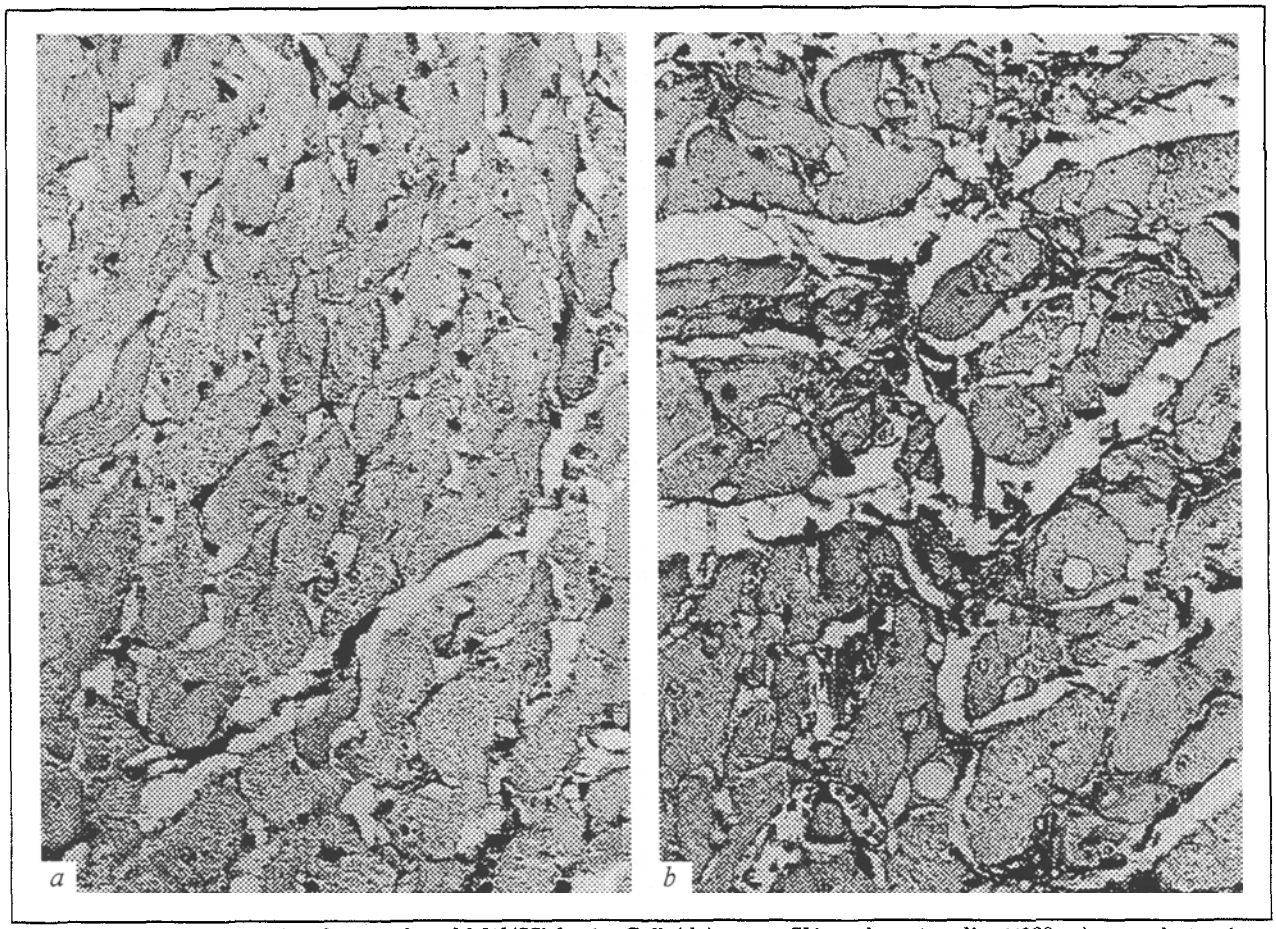

Fig. 2. RV myocardium in 10−month−old W/SSM rats. Colloid iron − SIA − hematoxylin, ×320. *a*) normal structure of the myocardium of a W/SSM−R rat (control). *b*) diffuse roughening of stroma in W/SSM rats; massive deposits of glycosaminoglycans in the intercellular spaces.

arterial layer was thickened, notably in the vessels of the RV. In some animals the vessels (arteries and venous sinuses) were markedly plethoric or filled with plasma; plasmorrhagia was also noted. The arterial adventitia was moderately sclerotized; perivascular infiltrates were found.

In 3-month-old rats microscopic examination did not show pronounced signs of focal lesions or of muscle fiber hypertrophy in the myocardium of either ventricle. A quantitative analysis of the CMC population in such animals demonstrated that the myocyte counts per mg myocardial tissue in either the LV or the RV were the same in rats of the compared groups (Table 2). This suggests that an increase in the myocardial weight was due to the increase in the total CMC count owing to stepped-up cell division. In other words, the increase in the ventricular myocardial weight resulted from CMC hyperplasia.

Although in the W/SSM and control rats the total CMC counts per mg myocardial tissue were equal, the ratio between the number of mononuclear CMC in the LV and RV and the number of binuclear cells was higher in 3-month-old W/SSM rats than in the same tissues of the control animals (Table 2). This may also be attributed to enhanced cell division. In 10-month-old W/SSM rats macroscopic investigation of the heart showed enlarged ventricular cavities, notably in the RV, where parietal clots were frequently observed.

Histological study of the myocardium of these rats demonstrated that the density of the muscle segments in the LV and RV myocardium varied, decreasing from the epicardial toward the subepicardial zone. The most evident fraying of the muscle tissue was observed in the middle, deeper myocardial layers. Mosaic staining of the muscle segments was pronounced. Slight contractures of CMC were observed in polarized light.

Glycogen in the form of powdery clusters was found predominantly in the outer and inner layers of the ventricular myocardium and not in all muscle cells. The venous sinuses in the subepicardial layers were plethoric. Arteries were in a state of spasm, their walls thickened. The vessels of the microcirculatory bed in some areas were dilated and contained erythrocyte sludges.

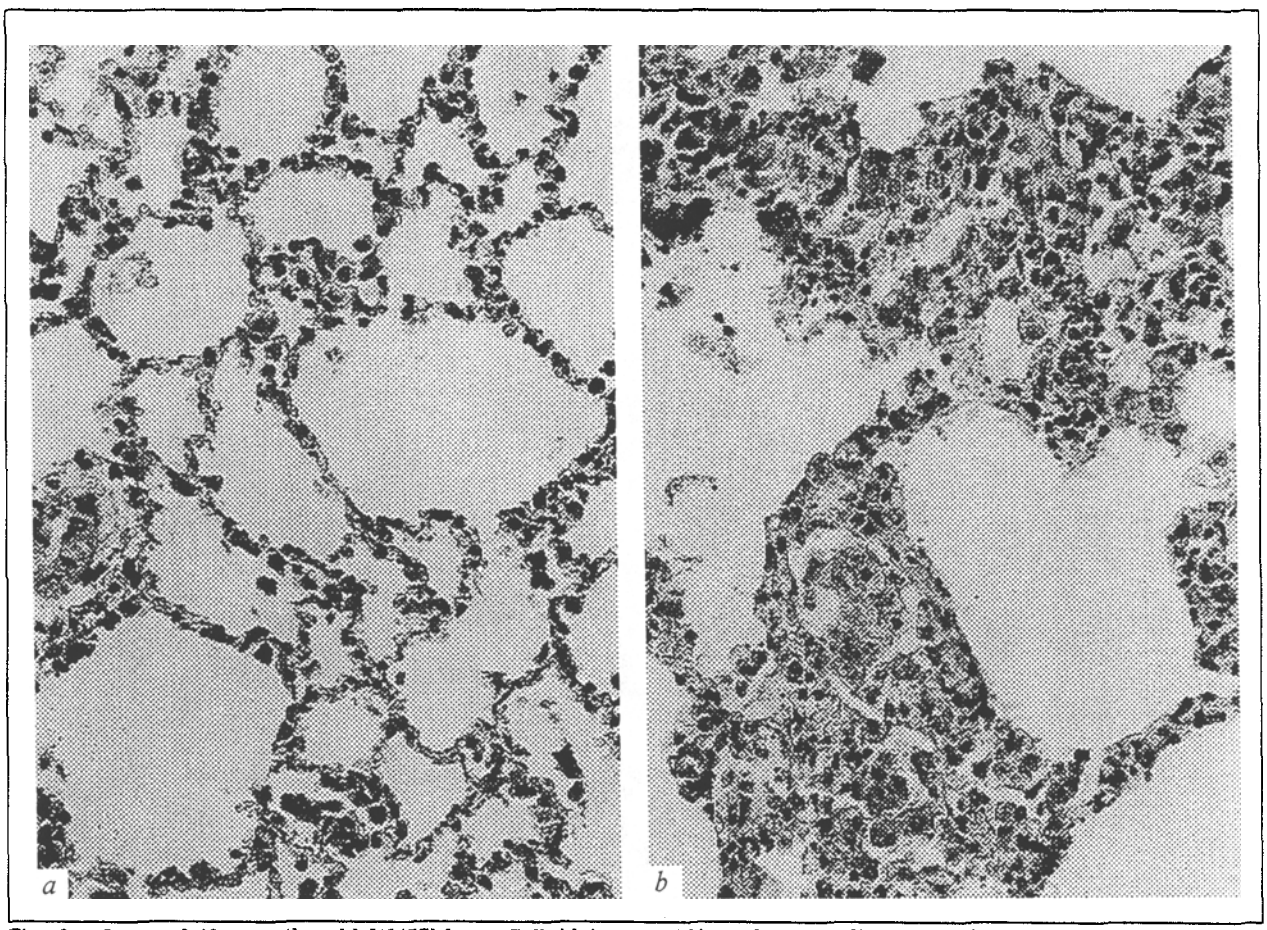

Fig. 3. Lung of 10-month-old W/SSM rat. Colloid iron – SIA – hematoxylin, ×320. *a*) normal structure of lungs of W/SSM-R rat (control); *b*) congestion and marked thickening of interalveolar septa; effusion and cell elements in the lumen of dilated alveoli.

TABLE 2. Number of CMC and of Mononuclear and Binuclear Cells in the LV and RV of W/SSM and W/SSM−R Rats (per mg Tissue, Mean of 4 Experiments)

Strain	CMC		Mononuclear cells		Binuclear cells	
	LV	RV	LV	RV	LV	RV
W/SSM	19.25±0.75	24.48±0.77	18.4±1.50	26.5±2.30	80.5±1.40	77.5±2.2
W/SSM−R	19.80±1.34	23.07±1.87	11.4±0.60	21.0±1.47	87.2±0.40	78.0±1.47
p	>0.001	>0.001	<0.001	<0.001	<0.001	>0.001

In many animals of this age the stroma was considerably collagenized. Myoelastofibrosis of the intramural arteries was noted. In some cases perivascular connective tissue was infiltrated with mononuclear cells; bundles of collagen fibers spreading into intermuscular connective tissue were also found there. Mast cells and siderophages were observed perivascularly against the background of moderate edema.

In 10-month-old W/SSM rats microscopic examination showed muscle fiber hypertrophy in the LV myocardium, which was attended by diffuse stroma collagenization and focal sclerotic changes (Fig. 2), and by pulmonary congestion (Fig. 3) in the form of thickening of interalveolar septa, dystelectases, and siderophage clusters in the lumen of the alveoli [10]; i.e., signs of chronic heart failure were observed.

Thus, determination of the myocardial weight and histological and morphological investigation of both ventricles of W/SSM rats demonstrated changes in the myocardium which were similar to those found in humans with HCM in the absence of anatomical and physiological reasons for CMC hypertrophy [4,5,7].

Previous studies had suggested that in W/SSM rats multiple structural and functional disturbances in organs and cells are caused by an enhanced intercellular accumulation of hexoses resulting from their abnormally intensive transport via the plasma membranes, which is due to a high specific activity of the glucose transporter [1,2,9].

An increase of the cell sugar level may boost the formation of superoxide and hydroxyl radicals [11-13], which are capable of initiating lipid peroxidation and thus damaging the cell membranes (plasma membranes, lysosomal and mitochondrial membranes, etc.) [3]. Damage to the cell membranes may be the major cause of the development of a multiple pathology, including damage to the myocardium in W/SSM animals which is similar to HCM in humans.

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