# Cellular Mechanisms of Genetically Determined Hypertrophic Cardiomyopathy in W/SSM Rats

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Hypertrophic cardiomyopathy, a hereditary pathology of the myocardium, was generates in rats with genetically determined galactose metabolism. It is shown that the increase in the myocardium mass results at first predominantly from an increase in the number of cardiomyocytes and then, after exhaustion of the proliferative potential, from cardiomyocyte hypertrophy.

**Key Words:** *W/SSM rats; hereditary cardiomyopathy, myocardial hypertrophy; cardiomyocyte population; morphometry and stereology*

Genetic factors play an important role in the development of numerous chronic diseases [1], including cardiomyopathies [10]. Therefore, pure-strain animals with genetically determined cardiac pathology are the most suitable models for a detailed study of the mechanisms and patterns of cardiomyopathies [2-5,10,15]. Despite the fact that hypertrophic cardiomyopathy is a common disease, it is one belonging to the noncoronarogenic group, which has not been studied in depth [7,8]. In order to create a model of cardiomyopathy with the corresponding parameters of myocardial hypertrophy, a line of normotensive rats W/SSM was generated at the Institute of Cytology and Genetics (Siberian Division of the Russian Academy of Sciences) [9] by the inbreeding of Wistar rats selected for high sensitivity to the damaging effect of galactose. The morphological manifestations of hereditary hypertrophic cardiomyopathy in W/SSM rats were described previously, and some biochemical mechanisms characterizing the development of a number of pathological signs have also been elucidated [9,12].

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Our objective was to investigate the dynamics of the cardiomyocyte (CM) population and the specific feature of spatial organization of the myocardium on W/SSM rats with hereditary cardiomyopathy.

## MATERIALS AND METHODS

Twenty-five male W/SSM rats aged 3 and 10 months and 8 control male Wistar rats of the same age were used. They were maintained under standard vivarium conditions. The animals were sacrificed by decapitation under chloroform anesthesia immediately after being weighed. The heart and its parts were weighed 1 h after fixation with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 8.0).

General pathological investigation was carried out on paraffin section-histotopograms including the left and right ventricle (LV and RH) myocardium with the interventricular septum. The sections were stained with hematoxylin and eosin (Perls reaction) and colloid iron-PAS-hematoxylin. Some semithin sections were processed for a special histological study. After standard dehydration the tissue specimens were embedded in Epon-Araldite. Longitudinal sections were cut on an LKB III ultratome. The sections (1 μ thick) were stained with azure-II and examined under a Docuval light microscope. Quan-

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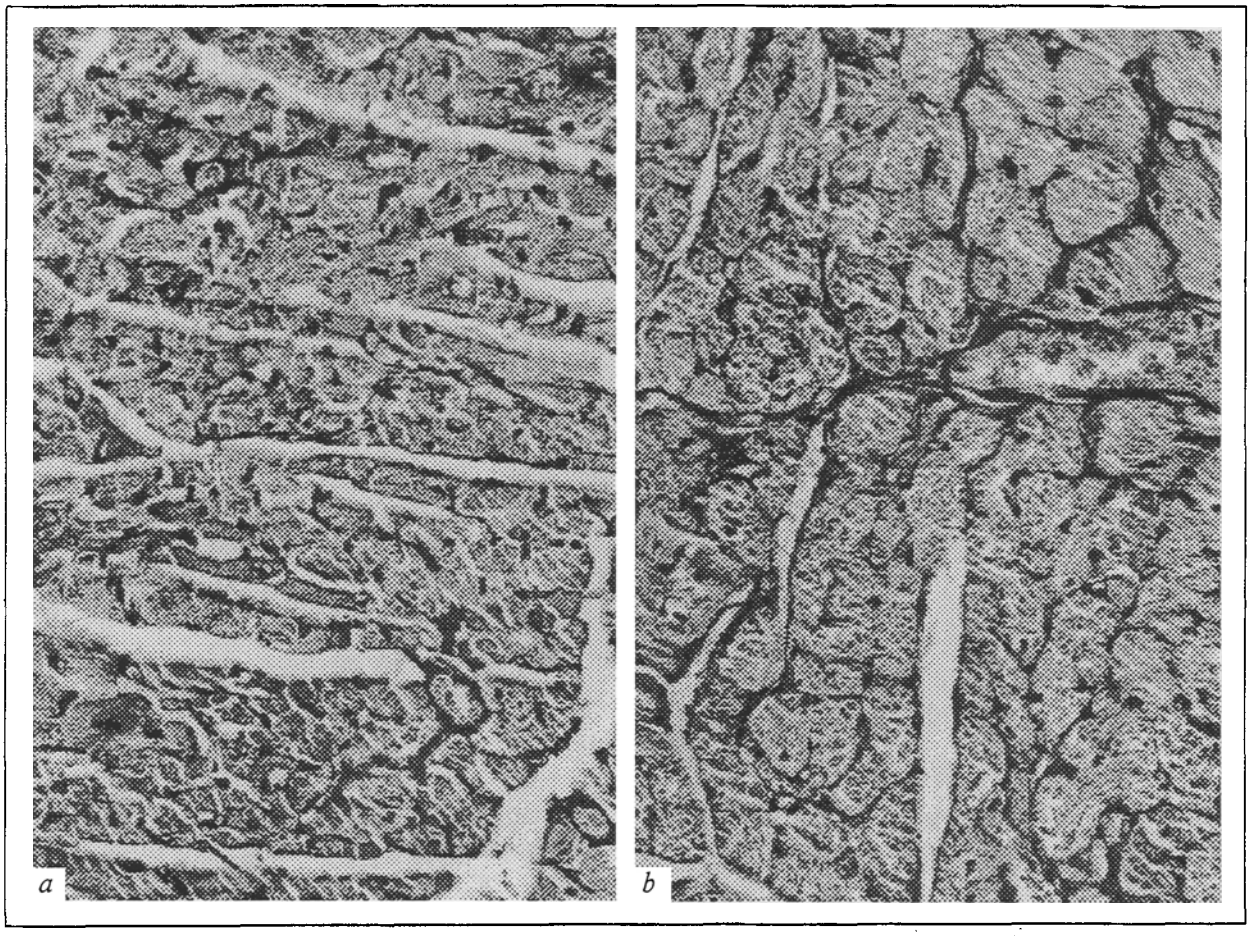


Fig. 1. LV myocardium of 10-month-old rat. Colloid iron-PAS-hematoxylin. ×320. *a*) normal structure of myocardium in Wistar rat; *b*) myocardial hypertrophy in W/SSM rat with hereditary cardiomyopathy. Massive deposits of acid glycosaminoglycans in intercellular spaces; diffuse roughening of stroma.

titative analysis of the CM population was performed after alkaline dissociation [10,11]. The myocardium was fixed for 14 days, and then washed with 0.1 M phosphate buffer (pH 8.0) for 24 h; tissue specimens (20-25 mg) were incubated in 50% KOH for 18 h at 8°C. The CM suspension thus obtained was stained with 0.1% etidium bromide. The suspensions were analyzed in a Fuchs-Rosenthal chamber under a Dialux microscope.

Quantitative morphological study was carried out using the morphometric and stereological methods [5,6]. The mean diameter of CM was determined in each group of rats at a magnification of 1000, and tissue stereological analysis was performed. We determined the relative volume (volume density) of CM, their nuclei, capillary lumens, endothelial cells, the interstitial cells, ground substance, and fibers of the myocardium stroma and the relative surface area (surface density) of CM, their nuclei, inner surface of the capillaries, and connective tissue cells. On the basis of these data the surface-volume ratio of tissue structure and the ratio of volume and surface densities of stromal structures to the volume density of heart parenchyma were calculated. The results were processes using mathematical and statistical methods described elsewhere [5,11].

## RESULTS

Over the entire observation period there were no statistically significant differences in the mean body weight of control and experimental animals of the same age (Table 1). The mean heart weight of 3-month-old W/SSM rats increased 21.8% compared with that of the control Wistar rats. The LV myocardium weight increase by 17.6% and that of the RV myocardium by 40.1% ($p<0.05$). The cardiac index increased correspondingly. Morphological study revealed no focal metabolic necrobiotic alterations in the myocardium of either ventricle. It should be noted that in the RV myocardium the muscle fibers were packed more densely while the myofilaments were packed more loosely.

Quantitative analysis of the CM population revealed no differences in the concentrations of the

TABLE 1. Morphometric Parameters or Cardiac Hypertrophy in Rats with Hereditary Cardiomyopathy ($M \pm m$)

| Parameter | Age of rats, months | | | |
|---|---|---|---|---|
| | Wistar | | W/SSM | |
| | 3 | 10 | 3 | 10 |
| Body weight, g | 213.4±6.1 | 387.5±15.5 | 223.7±4.7 | 386.0±11.7 |
| Absolute weight, mg | | | | |
| heart | 613.4±7.6 | 1136.3±40.3 | 747.3±28.9* | 1202.8±19.3 |
| RV | 103.0±4.2 | 211.5±10.1 | 144.3±9.0* | 246.8±6.3* |
| LV | 512.8±9.1 | 924.8±37.3 | 603.0±23.7* | 956.0±19.0 |
| Cardiac index | 2.80±0.06 | 2.94±0.09 | 3.34±0.07* | 3.12±0.06 |
| CM diamater, μ | 13.9±0.2 | 15.1±0.5 | 16.1±0.4** | 23.1±0.4*** |

Note. Here and in Tables 2 and 3: one asterisk indicates $p<0.05$, two asterisks $p<0.01$, and three asterisks $p<0.001$ when the parameters of Wistar rats (control) are compared with those of age-matched W/SSM rats (hereditary cardiomyopathy).

nuclei and cells in the LV and RV myocardium (Table 2). Nevertheless, the total number of CM in the LV in 3-month-old W/SSM rats increased by 13% and in the RV by 46%. The diameter of the myofibrils in W/SSM rats increased 15.8% compared with Wistar rats. From these observations it followed that the augmentation of the weight of the ventricular myocardium in W/SSM rats during the first 3 months of life is due predominantly to an increase in the number of CM, but not in their volume and weight. This is indirectly confirmed by a certain prevalence of mononuclear CM in W/SSM rats.

By the 10th month of life, the heart and LV weights in W/SSM rats did not differ significantly from those in the control animals, while the RV weight had increased 16.7% ($p<0.05$). Macroscopically, the ventricles, particularly the right one, were widened, and mural thrombi were often seen in the RV. Microscopically, the myocardium of control animals was normal. In experimental animals CM were hypertrophied, the stroma had become rough, and the myofilament in CM were very loosely packed (Fig. 1). All these signs are indirect evidence of a chronic contractile cardiac insufficiency. This is confirmed by phenomena of stagnation in the lungs, such as thickening of the interalveolar septa, dystelectases, and accumulation of siderophages in the alveoli.

Quantitative analysis of the CM population revealed leveling of CM counts in the LV of experimental and control animals (compared with 3-month-old rats) as a result of a decreased rate of cell population growth. In control animals the CM count in the RV had risen 96.9% and in experimental rats 42.6% compared with the counts in 3-month-old rats. However, in experimental rats the increase in the RV growth rate was 16.7% greater than in the control ($p<0.05$) due to CM hypertrophy. If the cardiosclerotic alterations which were revealed by histological study are taken into account, the weight of an individual CM in W/SSM rats had increased by 10%.

Stereological analysis of the tissue organization of the myocardium of W/SSM rats aged 3 and 10 months showed that the statistically significant decrease in volume and surface densities of the capillaries and the increase in the volume density of the ground substance and the connective tissue fibers (compared with Wistar rats of the same age)

TABLE 2. Changes in the CM Population in RV and LV in Rats for Modeled Hereditary Cardiomyopathy ($M \pm m$)

| Parameter | Age of rats, months | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Wistar | | | | W/SSM | | | |
| | 3 | | 10 | | 3 | | 10 | |
| | LV | RV | LV | RV | LV | RV | LV | RV |
| Concentration of CM nuclei, $10^6$/mg | 38.1±1.9 | 41.6±3.3 | 29.4±0.8 | 37.1±1.3 | 35.2±1.4 | 42.6±1.8 | 29.1±0.8 | 34.3±0.6 |
| Number of CM nuclei, $10^6$/ventricle | 19.5±1.1 | 4.2±0.2 | 27.1±0.6 | 7.8±0.3 | 21.2±1.1 | 5.9±0.3 | 27.8±0.9 | 8.6±1.5 |
| Number of CM, $10^6$/ventricle | 10.3±0.6 | 2.3±0.2 | 14.4±0.3 | 4.6±0.3 | 11.6±0.5 | 3.4±0.2* | 14.8±0.5 | 4.8±0.1 |
| Number of nuclei in 1000 CM | 1906±8 | 1802±10 | 1878±13 | 1726±26 | 1823±23 | 1746±15 | 1880±12 | 1786±14 |

TABLE 3. Stereological Parameters of the Myocardium of W/SSM Rats with Hereditary Hypertrophic Cardiomyopathy ($M \pm m$)

| Parameter | Age of rats, months | | | |
|---|---|---|---|---|
| | Wistar | | W/SSM | |
| | 3 | 10 | 3 | 10 |
| Relative volume, $mm^3/cm^3$ | | | | |
| CM | 836.8±6.5 | 838.2±10.3 | 841.5±5.3 | 814.1±6.3 |
| CM nuclei | 10.8±0.2 | 10.6±1.1 | 11.7±0.9 | 6.9±0.7* |
| capillaries | 48.9±4.0 | 45.8±2.2 | 35.6±1.1* | 38.4±1.3* |
| endothelial cells | 18.4±1.1 | 17.3±2.1 | 10.1±0.9** | 12.8±1.1 |
| connective tissue cells | 12.3±1.4 | 11.6±2.6 | 13.2±1.2 | 13.9±1.1 |
| ground substance and the fibers of connective tissue | 72.8±1.3 | 76.5±6.4 | 87.9±2.8 | 113.9±6.8** |
| Relative surface area, $m^2/cm^3$: | | | | |
| CM | 0.1103±0.0032 | 0.1041±0.0022 | 0.1316±0.0059* | 0.0924±0.0048 |
| CM nuclei | 0.0070±0.0008 | 0.0069±0.0007 | 0.0078±0.0010 | 0.0065±0.0008 |
| capillaries | 0.0362±0.0016 | 0.0350±0.0011 | 0.0305±0.0012* | 0.0302±0.0014* |
| connective tissue cells | 0.0130±0.0014 | 0.0116±0.0016 | 0.0138±0.0011 | 0.0140±0.0010 |
| Surface–volume ratio, $m^2/cm^3$: | | | | |
| CM | 0.132±0.007 | 0.124±0.004 | 0.153±0.006 | 0.113±0.006 |
| CM nuclei | 0.648±0.021 | 0.651±0.036 | 0.667±0.033 | 0.942±0.037 |
| capillaries | 0.740±0.043 | 0.764±0.045 | 0.856±0.051 | 0.786±0.046 |
| connective tissue cells | 1.057±0.051 | 1.000±0.073 | 1.045±0.029 | 1.007±0.032 |
| capillaries to CM | 0.043±0.002 | 0.041±0.004 | 0.036±0.003 | 0.037±0.004 |
| Volume ratio, number: | | | | |
| stroma to parenchyma | 0.180±0.009 | 0.178±0.011 | 0.172±0.006 | 0.218±0.012* |
| capillaries to CM | 0.058±0.007 | 0.054±0.006 | 0.042±0.005 | 0.046±0.006 |

were the most important events (Table 3). In 3-month-old animals the decrease was the greatest in the case of capillary parameters 27.2 and 15.7% *vs.* 16.2 and 13.7% in 10-month-old rats. This determined the more pronounced decrease in the volume and surface-volume ratio of the capillaries to CM in 3-month-old W/SSM rats: 27.6 and 16.3 *vs.* 14.8 and 9.8% in 10-month-old rats. By contrast, the volume density of the connective tissue acellular components increased to a greater extent in 10-month-old W/SSM rats (by 48.9%), which resulted in a statistically significant increase in the volume ratio of stroma to parenchyma (by 22.4%) at this period of ontogenesis.

The reorganization of the myocardium of W/SSM rats is similar to that observed in spontaneously hypertensive rats (SHR) during the development of cardiac hypertrophy [3]. It is likely that such a dynamics of quantitative changes in parenchymatous and stromal structures reflects the genetically determined peculiarities of their regeneration at different periods of ontogenesis and represents a species-specific development of hypertrophic cardiomyopathy independently of the background against which it occurs: normotensive or hypertensive.

In the myocardium of W/SSM rats with genetically determined disorders of carbohydrate metabolism and elevated activity of lysosomal enzymes [9] cardiomyopathy leads to disturbances in muscle contractility , which induces an increase in the heat weight. Our results show that the compensatory growth of the ventricular myocardium obeys certain regularities: at first, cell proliferation is triggered, and when the proliferate potential is exhausted, the diameter of muscle fibers starts increasing - in other words, hypertrophy culminating in cardiac decompensation [12,14].

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# The Postradiation Demyelination of the Optic Nerve

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Neutron and x-radiations induce focal dose-dependent demyelination of the optic nerve. Myelin sheaths are more radiosensitive than axis cylinders. It is shown that phagocytic activity of fibrillary astroglia and endocytosis of myelin by altered axons play a key role in postradiation demyelination.

**Key words:** *neutrons; x-rays; optic nerve*

Damage to myelin sheaths under the impact of ionizing radiation has been reported in various regions of the brain and spinal cord [5,7]. However, the mechanisms of this phenomenon are not completely understood and, as a rule, are attributed to radiosensitivity of myelin-producing oligodendrocytes [1,6].

In view of the high probability of radiation injury of the optic nerve occurring in oncoradiology [4], the aim of the present investigation was to identify the role of glial and neuronal elements in demyelination of the optic nerve after exposure to sparse and dense ionizing radiation.

## MATERIALS AND METHODS

The study was performed on 45 guinea pigs of both sexes with an initial weight of 400-450 g, 25 of which were exposed to a single whole-body x-raying with a dose of 4.5 Gy ($LD_{50/30}$) using an RUM-17 apparatus (40 cm focal distance, 0.5 mm Cu filter, dose rate: 0.64 Gy/min) and 20 animals were the control. The right eye of 45 male chinchilla rabbits (2-2.5 kg weight) was exposed to fractionated neutron radiation with a dose of 1.5 Gy per fraction twice a week (Monday and Friday) according to the treatment protocol for cancer patients used at the Oncological Institute of the Tomsk Science Center, Russian Academy of Medical Sciences. Fast neutrons (6.2 MeV) were produced by bombardment of a beryllium target in a U-120 cyclotron (proportion of gamma-quanta 8-10%, dose rate 0.15 Gy/min, 4×6 cm field). Forty-four control rabbits were exposed to sham irradiation and kept under the same conditions as the treated animals with the usual 24-h light regimen in the vivarium. Decapitation of the animals and collection of the material (the optic nerves) were performed 1, 5, 10, 25, and 60 days after whole-body x-raying and after local neutron exposure 1 day and 6 months later if the total doses attained 3 and 15 Gy, 1, 10, and 30 days and 6 months later at 7.5 Gy, and after 24 h at 40.5 Gy. Bioptates from 16 cancer patients with paraorbital tumors that had not spread to the eyeball and optic nerve were studied, 12 of these patients having received a course of radiotherapy with a total focal dose of 9-10.5 Gy prior to surgery. With

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