NUMBER AND SIZE OF MYOCYTES, AMOUNT OF INTERSTITIAL SPACE AND EXTENT OF DISARRAY OF THE HEARTS IN PATIENTS WITH SYSTEMIC HYPERTENSION AND ASYMMETRIC SEPTAL HYPERTROPHY

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Wall thickness, the extent of disarray, the number and the size of myocytes and the amount of interstitial space were measured in the ventricular septum (VS) and left ventricular (LV) free wall in hearts of 6 patients with chronic systemic hypertension and asymmetric septal hypertrophy (ASH). Twenty-five subjects (15 with no cardiac disease, and 10 with systemic hypertension) without ASH served as the controls.

In the six patients with ASH, the degree of ASH ranged from 1.3 to 1.6. The extent of disarray in VS was 20% in one heart and within normal limits (mean ± SD = 3 ± 3%) in the other 5. The size of myocytes increased both in the VS and LV free wall and the VS/LV ratio ranged from 0.9 to 1.0. There was no significant difference in the % area of interstitial space between hearts with ASH and controls, and the VS/LV ratio ranged from 0.9 to 1.1. The number of transmural muscle layers (number of myocytes) was 680 ± 90 in the VS and 440 ± 40 in the LV free wall of these with ASH, and 500 ± 60 in the VS and 490 ± 60 in the LV free wall of control subjects. The VS/LV ratio of the number of myocytes ranged from 1.3 to 1.7 and was correlated with the VS/LV ratio of wall thickness. Although the sample is small, our findings suggest that most hearts from patients with chronic systemic hypertension and ASH have no diffuse disarray in the VS and that ASH probably occurs secondary to pressure overload. An increase in number of myocytes in the VS is a pathogenetic factor of ASH, regardless of the extent of disarray or the presence of HCM.

ASYMMETRIC septal hypertrophy (ASH) is highly specific for hypertrophic cardiomyopathy (HCM), but is not a pathognomonic finding for HCM.⁶⁷ ASH is often found in hearts of fetuses and sometimes in hearts of patients with left ventricular or right ventricular overload.⁹ Disarray does not seem to be a direct causative factor of ASH, even in hearts with HCM, because the extent of disarray in the ventricular septum does not correlate with ventricular septal thickness or the degree of ASH in HCM. Therefore, the pathogenesis of ASH is still not clear.

In a previous study⁹ on hearts with HCM, it

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was found that the number of muscle layers increased in the ventricular septum and that the degree of ASH correlated highly with the ventricular septal (VS) to left ventricular (LV) free wall ratio of the number of transmural muscle layers. These hearts with HCM had diffuse disarray in the ventricular septum. Therefore, the pathogenesis of ASH in other cardiac diseases without disarray and in hearts of fetuses remained to be studied.

In the present work, we attempted to define whether the pathogenesis of ASH in hearts with chronic and severe hypertension (LV pressure overload) is due to increase in the member of muscle layers in the ventricular septum.

**MATERIALS AND METHODS**

**Patients studied**

The hearts of thirty-one adult subjects were studied. Included were 6 patients with severe and chronic systemic hypertension and asymmetric septal hypertrophy (ASH), and 25 control subjects. These 6 patients with hypertension and ASH had a clinical history of marked systemic hypertension and the VS/LV (left ventricular free wall) ratio of wall thickness was 1.3 or greater. Marked hypertension of over 180/100 mmHg was present for over five years before death in four patients and over two years in two patients. None of the patients had continuous medication for hypertension. Four patients died following C.V.A., one died of rupture of a dissecting aortic aneurysm, and the other patient died from cancer of the thyroid gland. Family histories for hypertrophic cardiomyopathy (HCM) were negative in two patients examined. The 6 patients included 5 men and one woman, with age ranges from 52–76 years (mean ± SD = 61 ± 9).

The control group included 15 subjects without heart disease and 10 with chronic severe hypertension. In the 10 patients with hypertension, marked hypertension of over 180/100 mmHg had been noted for over 5 years before death in 5 patients and for over 2 years in the other 5 patients. None had continuous medication for hypertension and none of these patients had ASH. The mean VS/LV ratio of wall thickness was 1.0 ± 0.1 (mean ± SD) in patients without heart disease and also in those with hypertension. None of the 31 patients had a coronary arterial narrowing over 75% of luminal diameter or evidence of old or recent myocardial infarction; other types of heart disease, including myocarditis, valvular lesions and congenital anomalies were absent. The age and sex of control patients were similar those of patients with hypertension and ASH.

**Methods for general pathology**

At necropsy, each heart was subjected to a gross examination, then was fixed in 10% formalin. The coronary arteries were transversely and serially cut at 0.2–0.3 cm intervals from the ostium to the periphery and the degree of luminal narrowing was recorded as a percentage.

The hearts were sliced serially and transversely as 1 cm intervals from the base to the apex. Macrosopic findings, including fibrosis were examined.

**Quantitative measurement of extent of disarray**

A transversely cut slice at the inferior margin of the posterior mitral leaflet was separated into several blocks all of which were embedded in paraffin (Fig. 1). These were sectioned in a plane perpendicular to the long axis of the LV at 25 μ thickness by a microtome, according to the method of Fujiwara et al.5,7 and were stained with hematoxylin and eosin. Hematoxylin and eosin stained tissue sections were directly enlarged at magnification of × 50 (50 × 50 in the area) on a large white paper by a projector (Pradorit color 250, Neitz Company) and areas of disarray were traced on the white paper using a marking pen with a width of 0.2 mm (Figs. 1 & 2). The area of each outlined silhouette in the VS and the LV free wall was then automatically quantitated using an image analyser (Olympus VIP-21, Co.). Disarray defined in previous papers6,7 was counted as abnormal disarray in 25 μ thick section (Fig. 2).

**Measurements of wall thickness, number of transmural muscle layers, mean size of myocytes, and % area of transmural interstitial space**

After a large paraffin block had been sectioned by microtome for measurement of the extent of disarray, two small paraffin tissue blocks were cut transmurally with a sharp knife in a plane parallel to the long axis of the ventricles, from the same large paraffin block (Fig. 3). One block was taken from the middle portion of the VS: the other block was cut from the LV free wall. These paraffin tissue blocks were sectioned transmurally in 2 μ thick slices by a microtome.
Fig.1. Distribution of abnormal disarray (areas encircled by broken lines) in the left ventricular wall of hearts from patients with hypertension and asymmetric septal hypertrophy. Left panel was obtained from a heart with focal distribution of disarray within normal limits. The extent of disarray was 3% in the ventricular septum and 6% in the left ventricular free wall.

The right panel was obtained from a heart with diffuse disarray. The extent of disarray was 20% in the VS and 7% in the LV free wall.

Fig.2. Abnormal disarray in 25 μ thick sections.
left: focal disarray, right: diffuse disarray. The broken line outlines focus of cardiac muscle cell disorganization. (×40, hematoxylin-eosin stain)

with an S-22 blade (Feather Co.,). These sections were stained with hematoxylin-eosin.

The LV free wall and VS thicknesses, and the three elements (the number of transmural muscle layers, the mean size of myocytes and % area of transmural interstitial space) were measured on the same line in each of the stained preparations.

Measurements of the three elements were done with a general-purpose color image processor, model VIP-21 (Olympus Co., Tokyo).

Histologic preparations were imaged in a video imager on which three fine lines (the distance between each line was approximately 100 μ) had been drawn at a magnification of ×1,200

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Fig. 3. Measurements of the numbers and the size of myocytes, and the amount of interstitial space, using VIP 21 (Olympus Co, Tokyo). These were measured using two transmural tissue preparations obtained from the ventricular septum and left ventricular free wall, in a plane parallel to the long axis of the left ventricles (upper panel). The numbers of myocytes crossing each of the three fine dark lines on the color video imagers was counted (middle panel). There are 7 muscle layers in the upper line, 4 in the middle line and 6 in the lower line (V). The myocyte diameters were measured semiautomatically as the shortest diameter at the level of the nucleus (→) in the area between the upper and middle lines of the middle panel. In measurement of the % area of interstitial space, the area of myocytes shown in middle panel was automatically painted in white color (lower panel) on the color video imager. The % area of myocytes was automatically calculated and the % area of interstitial space was obtained by subtracting the % area of myocytes from 100%. MENSEKI RITSU (Japanese) means % area of white color. HEIKIN (Japanese) means mean value.

ANT = anterior; POS = posterior; F = free wall; S = septum; LV = left ventricle
The number of transmural muscle layers which crossed each of the three fine lines was counted by moving the preparation serially and transmurally from the left ventricular endocardium to the epicardium in the free wall and to the right ventricular endocardium in the septum, respectively (Fig. 3). The number of transmural muscle layers in the ventricular septum and in the left ventricular free wall was calculated as an average of the three numbers on the three fine lines.

Myocardial fiber diameter was semi-automatically obtained by measuring the shortest diameters, at the level of the nucleus, with the aid of a light pen, of cells locating between two lines drawn on the video imager, moving the preparation successively and transmurally (Fig. 3). In each preparation, 70 to 150 myocytes were measured.

In measuring the % area of transmural interstitial space, the % area of the myocardial cells in each video imager was calculated by semi-automatic analysis with the aid of a light pen, moving the tissue preparation serially and transmurally (Fig. 3). For each tissue preparation, the number of measurements was about 40 to 80, and the % area of transmural interstitial space was calculated by subtracting the % area of transmural myocardial cells from 100%.

Two major problems can occur when measuring the three elements. First, if the stained preparation has myocytes with disarray, it is difficult to measure precisely the size of myocyte because of the bizarre shape with branching. Maron and Roberts reported that disarray was present more frequently and to a greater extent in transverse than in longitudinal plane sections in the ventricular septum in cases of hypertrophic cardiomyopathy. Therefore, in the present study, longitudinal plane sections were used for measurement of the three elements. The second problem is the overlapping of myocytes which makes the boundary of fibers unclear. Two μ sections in this study solved this problem, to some extent. As a result, in the 2 μ preparations sectioned in a plane parallel to the long axis of the ventricle, most of the myocytes in the left ventricular wall were cut transversely, disarray and cells of a bizarre shape were rare, and the boundary of myocytes was clear.

Data analysis

The differences between groups were analysed statistically by one way analysis of variance with Duncan’s multiple comparison. All data are given as the mean value ± S.D. Statistical significance and rejection of the null hypothesis was achieved at p < 0.05.

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RESULTS

In six patients with severe and chronic hypertension and asymmetric septal hypertrophy (ASH) there was a moderate-to-marked concentric hypertrophy of the left ventricle (LV). The hearts ranged in weight from 450 to 700g (average 540g). In the 15 normal hearts the weight was 190 to 330g (mean 280g), and in the 10 hypertensive hearts without ASH the weight was 350 to 650g (mean 460g). The wall thickness was 24 ± 4 mm in ventricular septum (VS) and 16 ± 2 mm in LV free wall (Fig. 4). The VS/LV (ventricular septum to left ventricular free wall) ratio was from 1.3 to 1.6 (mean 1.4) (Fig. 4).

There was no evidence of massive fibrosis, myocardial infarction, marked coronary arterial stenosis, acute myocarditis, or congenital and valvular cardiac disease. Thrombi in the cavities and significant thickening of the endocardium were absent.

Extent of disarray in 25 μ thick sections

One of the 6 hearts from patients with hypertension and ASH had diffuse disarray of 20% in the VS (Fig. 1). However, the extent of disarray in the other 5 hearts in cases of hypertension and ASH ranged from 1 to 5% (mean 3%) in the VS and from 1 to 6% (mean 3%) in the LV free wall (Fig. 1). There were no significant differences between the group with ASH, the group with hypertension and no ASH, and the group without cardiac disease (3 ± 2% in the VS of

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control and 4 ± 2% in the LV free wall of control.

**Number of transmural muscle layers, mean size of myocytes and % area of interstitial space**

The number of transmural muscle layers was 680 ± 90 in the VS and 440 ± 40 in the LV free wall. The number of transmural muscle layers increased significantly in the VS and showed no significant difference in the LV free wall, compared to findings in the control groups (Fig. 5). The VS/LV ratio ranged from 1.3 to 1.7 (mean 1.4) (Fig. 5). The mean size of myocytes was 21 ± 3 μ in the VS and 22 ± 4 μ in the LV free wall (Fig. 6). The VS/LV ratio ranged from 0.9 to 1.0 (mean 1.0) (Fig. 6). The mean size of myocytes in patients with hypertension and ASH increased significantly when compared to that of normal hearts and was similar as that in hearts of patients with hypertension and no ASH.

The % area of the interstitial space was 44 ± 5% in the VS and 44 ± 3% in the LV free wall (Fig. 7). The VS/LV ratio ranged from 0.9 to 1.1 (mean 1.0) (Fig. 7). Significant differences of the % area of the interstitial space were not apparent in the group with ASH and the two control groups.

In a study made of the correlations of VS/LV ratios between wall thickness and the number of transmural muscle layers, the mean size of myocytes, the % area of transmural interstitial space and the extent of disarray, the correlation was positive only between VS/LV ratios of the number of transmural muscle layers and of wall thickness (Fig. 8).

**DISCUSSION**

The present study revealed that size of myocytes and % area of interstitial space were similar between the ventricular septum (VS) and left ventricular (LV) free wall, of the same hearts. An increase in the number of transmural muscle layers in the VS was detected in hypertensive hearts with asymmetric septal hypertrophy (ASH) and the degree of ASH correlated well with the VS to LV free wall ratio of the muscle layers.

**Relationship between hypertrophic cardiomyopathy and asymmetric septal hypertrophy seen in hypertension**

Maron et al.3,8 reported two patients with ASH and systemic hypertension. These two patients had no family history of HCM or diffuse abnormal disarray in the VS. In the present study, diffuse distribution of disarray in the VS, which is considered to be highly specific for HCM and to be primary, was found only in one of 6 patients with chronic and severe systemic hypertension and ASH. The family history of HCM was negative in the two patients examined. Therefore, ASH in the 5 patients without diffuse disarray in the VS is probably unrelated to HCM and occurs secondarily to pressure overload. ASH in one patient with diffuse disarray may be a manifestation of the underlying HCM.

**Number of transmural muscle layers and disarray**

The wall of the heart contains cardiac muscle cells and interstitial components. Generally, left ventricular myocardial fibers from endocardium to epicardium run in a tangent direction to the surface of the LV cavity through the VS or the LV free wall, although the successive layers of myocardium from endocardium to epicardium displayed a progressive change in orientation, like an open Japanese fan. Therefore, in the LV free wall with a minimum extent of disarray or no disarray, quantification of transmural muscle layers does not depend on myocardial fiber orientation, and the wall thickness is dependent on three elements: number of transmural layers, size of myocytes, and extent of transmural interstitial space. In the present study, 5 of 6 hearts with ASH and all of controls were such cases.

In the tissue areas with disarray, myocardial fibers run in various directions in a cell-to-cell or fascicle-to-fascicle fashion and we observed myocardial fibers running vectorially parallel to a line placed from the endocardium to the epicardium. Therefore, in the wall with diffuse abnormal disarray, the wall thickness may depend on the fiber orientation, in addition to the above 3 elements. Theoretically, the number of transmural muscle layers decreases, in comparison with that in the wall without disarray. In the present work, 1 of 6 hearts with ASH had a diffuse disarray occupying 20% of the ventricular septum. However, on a line of the VS where the wall thickness, and number of transmural muscle layers were measured, the wall thickness was 28 mm and the extent of disarray occupied only 3 mm, as shown in Fig. 1. The ventricular septal to left ventricular free wall ratio was
28 mm/18 mm (= 1.6) in wall thickness and 865/505 (= 1.7) in numbers of transmural muscle layers. Thus, the effect of disarray on the numbers of transmural muscle layers was minimum in this case.

Linzbach\(^{13}\) reported that the number of transmural muscle layers in the LV free wall was the same in concentric hypertrophy without ASH as in normal hearts and averaged 520, although there were considerable individual differences. Our data on the muscle layers in the LV free wall in normal and hypertensive hearts without ASH confirm the findings of Linzbach.

**Transmural interstitial space and size of myocytes**

The present study revealed that size of myocytes and % area of interstitial space were similar between the VS and the LV free wall in each of the hypertensive hearts with ASH and two control groups without ASH. The data on the size of myocytes in the two control groups are compatible with data in our previous papers\(^{9,14}\) and with the data of other investigators.\(^{15}\) Fuster et al.\(^{15}\) reported that, in normal and hypertensive hearts without ASH, the amounts of interstitial tissue were similar in the basal and apical regions of the LV free wall and that there was no significant difference in interstitial space in the LV free walls of normal and hypertensive hearts without ASH. Our data confirm these findings. However, % area of the interstitial space was 32% in normal hearts and 36% in hypertensive hearts in the data of Fuster et al.\(^{15}\) and was 41% in normal hearts and 44% in hypertensive hearts in the present study. Interstitial space includes intermyocytes, vessels and the perivascular area, and perimysum (interfascicular space).\(^{16}\) Fuster et al.\(^{15}\) measured only several selected areas obtained from the subendocardial and subepicardial tissue, and the outline of myocytes was manually traced at a magnification of ×1,200. Therefore, the areas with small vessels and perimysum were not included. In the present study, interstitial space was semiautomatically traced and calculated serially on a line placed from endocardium to epicardium, where small arteries and perimysum were included, at a magnification of ×1,200. In the areas with small vessels and/or perimysum, % area of the interstitial space was frequently near 100%. This may explain the difference in the % areas of interstitial spaces.

**Pathogenesis of asymmetric septal hypertrophy**

It was previously found that, in hearts with HCM and ASH, the size of myocytes and % area of interstitial tissue were similar between the VS and LV free wall, of the same hearts. The number of transmural muscle layers increased in the VS and decreased in the LV free wall, compared to control hearts without ASH. Although these hearts with HCM had a diffuse extent of disarray in the VS, the degree of ASH did not correlate with the extent of disarray. As a result, the VS/LV ratio of the number of transmural muscle layers correlated with the degree of ASH.

The present study revealed an increase in the number of transmural muscle layers in the VS and a high correlation between the degree of ASH and the VS to LV free wall ratio in hypertensive hearts with ASH and without diffuse disarray. The number of transmural muscle layers in the LV free wall did not increase in hearts with concentric hypertrophy without ASH,\(^{9,13}\) although a secondary reduction in the number of transmural muscle layers was seen in the LV free wall of hearts with eccentric hypertrophy, due to rearrangement of the muscle fibers.\(^{13}\) Therefore, the pathogenesis of ASH is probably hyperplasia in the ventricular septum, regardless of the presence or absence of HCM.

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