A MORPHOMETRICAL STUDY OF MYOCARDIAL DISARRAY ASSOCIATED WITH RIGHT VENTRICULAR OUTFLOW TRACT OBSTRUCTION

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In order to define quantitatively the histological characteristics of the hypertrophied myocardium of the right ventricular outflow tract in patients with congenital heart disease, a light-microscopic study of biopsy specimens obtained from 20 cases with tetralogy of Fallot (TOF) and 14 cases with muscular obstruction of the right ventricular outflow tract (RVOTO) was performed using a semi-automatic sampling counter. As the controls, the crista supraventricularis of 11 autopsied cases without cardiac disease were used. The TOF patients were divided into two groups: those with (9 cases) or without (11) a history of hypoxic spells. The myocyte diameter was calculated according to Chalkley's²⁷ and Arai's method²⁸. The areas of myocardial fibrosis and myocyte disarray were determined quantitatively by the point count method. Myocardial fibrosis was classified into five types: focal, mild perimysial, severe perimysial, perivascular and plexiform. In the control group, the myocyte diameter and area of fibrosis and disarray tended to increase with age. In the obstructive groups, the myocyte diameter and area of disarray were greater than those of the control in any age group. Myocyte diameter tended to increase with age in all groups, except for the TOF cases with a history of hypoxic spells. The latter also had the largest area of myocardial disarray (25.3%) ($p < 0.01$), and a largest area of fibrosis (20.5%) than the TOF cases without spell (14.4%), but severe perimysial fibrosis was observed in only five out of the nine patients with spells. The lack of a relation between the pressure and the presence of an increased area of the myocardial disarray in TOF with spells implies that this myocardial change is brought about by myocardial abnormality accompanied by cardiac malformation.

IT is widely accepted that ischemia¹, hypoxia², coldness and some of endocrine hormones, in addition to pressure or volume loads,³ are experimental or empirical factors in myocardial

hypertrophy. Because of the complexity of the problem, however, the hypertrophy-promoting factors in congenital heart anomalies require further study. Firstly, a clear differentiation between the hypertrophy and the physiological growth of the myocardium throughout the intrauterine and postnatal periods is quite difficult.⁴ Secondly, some peculiar structural changes in the myocardium often coincide with the hypertrophy in progressive infundibular stenosis in cases with subaortic ventricular septal defect⁵

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Fig. 1. Transverse section of the heart in a five-month-old male with TOF. The right ventricular myocardium shows extreme hypertrophy specially in the outflow tract of the right ventricle. RV = right ventricle; VS = ventricular septum; LV = left ventricle; ant = anterior; post = posterior. ($\times 3$).

even after its spontaneous closure.⁶ Hemodynamic stress is generally considered to be the major cause of the hypertrophy,^{7a} but some structural changes may modify the type of hypertrophy. Therefore, both the hypertrophy and structural changes should be estimated by both quantitative and morphological methods for further progress in myocardiology.

As for the structural changes, recent attention has been drawn to disproportionate hypertrophy observed in some cases with aortic stenosis or atresia, pulmonary stenosis or atresia⁸ and TOF.^{9,10} The histopathological feature of this peculiar hypertrophy appears to be disarray or disarrangement of the myocyte lining^{8,11,12} which may be a specific finding of hypertrophic cardiomyopathy (Fig. 1). However, since several papers have described its existence in the hypertrophies with known etiologies,¹³⁻¹⁵ questions have arisen regarding the specificity of the myocardial disarray, that is, whether the morphological resemblance of the muscular infundibular stenosis, including that seen in tetralogy of Fallot (TOF), to hypertrophic obstructive cardiomyopathy can be extended to the histological findings of the myocardial disarray.¹⁶

Furthermore, some patients with TOF experience hypoxic spells,^{17,18} which have been ex-

plained by Brock¹⁹ to be the result of a functional obstruction of the outflow tract. The spells do not usually appear until after the first few months of life, and tend to disappear before six years of age^{7b} with the administration of beta-blockers.²⁰ As this phenomenon is also similar to the syncopal attacks in hypertrophic cardiomyopathy, it is worthwhile to examine whether there is a common pattern in the hypertrophy and the disarray in these two conditions.

Several studies have attempted to resolve this problem on the basis of histopathological methodology.²¹⁻²⁶ However, correlative studies on the clinical and pathological aspects of the myocardial lesions of congenital heart disease have been few. Therefore, we performed a quantitative investigation on the relationship between the hypertrophy and disarray in cases with right ventricular outflow tract obstruction (RVOTO)

MATERIALS AND METHODS

Patient Selection. A histological study was performed on myocardial specimens obtained from 34 patients with right ventricular outflow tract muscular obstruction, whose diagnoses were established by preoperative catheterization,

TABLE I CLINICAL DATA OF PATIENTS WITH TOF AND RVOTO

Case	Age	VSD (mm)	Site of obstruction infundibulum/valve	Other malformation	RV pressure (mmHg) (high pressure chamber)	Gradient (mmHg)	Shunt operation (age)	Appearance of symptoms		Occurrence of murmur (age)
								cyanosis	spell (age)	others
1	2	20 × 18	+	—	90/0	70	—	1M	2M	+
2	2	19 × 16	+	supravalvular	120/0	95	—	—	—	—
3	2	12 × 12	+	—	90/0	78	—	1M	—	2Y
4	2	15 × 15	+	—	80/0	65	2Y	at birth	7M	2M
5	3	17 × 15	+	bicuspid	112/0	98	—	3Y	3Y	—
6	4	23 × 13	+	+	107/—8	89	2Y	5M	2Y	2Y
7	4	23 × 22	+	PFO, PDA	90/0	75	—	10M	4Y	4Y
8	4	20 × 18	+	—	115/0	100	1Y	2M	2M	—
9	4	20 × 20	+	PFO	135/0	—	—	1M	1Y	1M
10	4	25 × 24	+	—	120/0	72	3Y	1Y	2Y	2Y
11	4	23 × 21	+	—	100/6	75	—	—	—	3Y
12	4	15 × 15	+	right aortic arch	110/0	88	—	4M	—	—
13	4	?	+	PFO, PDA	120/0	—	2Y	at birth	—	—
14	5	10 × 8	+	single coronary	108/5	80	—	—	—	—
15	11	?	+	bicuspid	105/0	80	3.5Y	—	—	—
16	16	18 × 16	+	—	110/0	80	—	+	—	+
17	19	30 × 20	+	PLSVC	107/—2	101	—	6Y	—	—
18	22	26 × 24	+	—	124/0	106	—	3M	3Y	3Y
19	23	25 × 20	+	bicuspid	100/10	80	20Y	6Y	—	—
20	23	30 × 20	+	bicuspid	135/0	121	10Y	10Y	—	3M
21	6	5 × 5	+	—	60/—7	33	—	—	—	newborn
22	7	7 × 7	+	—	110/0	85	—	6Y	—	—
23	7	14 × 10	+	—	110/—10	75	—	—	—	—
24	8	5 × 5	+	—	120/—5	100	—	—	—	—
25	10	5 × 5	+	—	66/—6	32	—	—	—	—
26	10	—	+	—	130/—10	120	—	—	—	—
27	11	—	+	—	180/0	160	—	—	—	—
28	11	25 × 20	+	PDA	80/10	30	—	—	—	—
29	12	13 × 10	+	—	150/0	90	—	—	—	—
30	14	—	+	PFO, right aortic arch	150/20	125	—	—	—	—
31	30	—	+	—	200/0	180	—	—	—	—
32	37	—	+	ASD	80/6	65	—	—	—	+
33	39	11 × 8	+	—	70/0	36	—	—	—	—
34	44	15 × 10	+	pulmonary aneurysm	100/0	80	—	—	—	40Y

Cases 1-20 are the patients with TOF, and cases 21-34 are those with RVOTO.

Abbreviations: VSD = ventricular septal defect; RV = right ventricle; PFO = patent foramen ovale; PDA = patent ductus arteriosus; PLSVC = persistent left superior vena cava; ASD = atrial septal defect; Y = year-old; M = month-old.

TABLE II SUMMARY OF CLINICAL DATA

Case	Abbreviation	No. of pts.	Age	RV pressure (mmHg) (high pres. chamber)	Outflow valve	obstruction infundibulum
<i>Tetralogy of Fallot</i>	TOF	20	8.1 ± 7.7	108.9 ± 14.7	12	+
TOF with history of spell (Spell (+))		9	5.4 ± 6.2	108.1 ± 18.1	8	+
TOF without history of spell (Spell (-))		11	10.3 ± 8.4	109.5 ± 12.0	4	+
Right ventricular outflow tract obstruction (muscular)	RVOTO	14	17.6 ± 13.5	108.6 ± 38.8	—	+
Control group		11	14.1 ± 11.9	—	—	—

Figures indicate the mean value and the standard deviation

Fig.2. Measurement ("line counting") method of the area of the myocardial disarray.

The screen of the multipurpose test system contains 21 short lines. Non-parallel myocytes on these lines are counted as disarray. The myocytes in the upper part of this photograph are not clearly identified from each other, but the myocyte orientation is easily recognized and one can judge the disarray.

ventriculography and open heart surgery at Juntendo University Hospital School of Medicine. Twenty of the cases had TOF and 14 had subvalvular obstruction of the right ventricle (right ventricular outflow tract obstruction: RVOTO). Cases with a complicated cardiac malformation, such as transposition of great arteries and partial transposition of great arteries (double outlet right ventricle) were excluded, as was TOF with pulmonary atresia. In order to rule out the factors of secondary myocardial hypertrophy

due to valvular stenosis, cases with valvular pulmonary stenosis in RVOTO were also excluded.

Detailed data of the clinical and hemodynamic findings of cases with TOF and RVOTO are shown in Table I. All TOF cases had infundibular obstruction, and 12 of them had an associated pulmonary valvular stenosis or bicuspid pulmonary valves. The average right ventricular systolic pressure and the average pressure gradient between the right ventricle and pulmonary artery in the 18 TOF cases was 110.8 mmHg and 86.3

Fig.3. Measurement of the disarray index.

On the screen at a magnification of $\times 575$ of the sampling counter, seven parallel lines (white lines) are arranged along the main myocyte orientation in a longitudinal section of the myocytes. The intersections C of these lines with the myocyte are counted. Then the screen is rotated 90 degrees. The new intersections C' on these rotated parallel lines (black lines) with the myocytes are counted. The disarray index is defined as C'/C .

mmHg respectively. Shunt operation had been performed previously in eight cases. For a more precise analysis, cases with TOF were divided into two groups on the basis of their history of hypoxic spells (TOF with spells vs. TOF without spells). Because of earlier progression of symptoms, cases with TOF with spells were younger than those without spells (5.4 years vs. 10.3 years). However, there was no difference in right ventricular pressure between the two groups (108.1 mmHg vs. 109.5 mmHg). The average right ventricular systolic pressure and the average pressure gradient between the right ventricle and pulmonary artery in RVOTO cases was 108.6 mmHg and 84.4 mmHg respectively. Clinical and hemodynamic data are summarized in Table II.

Intraoperative Biopsies. In all patients, myocardial specimens from the right ventricular outflow tract were taken during the course of surgical myotomy and myectomy.

Necropsy Specimens. As a control group, myocardial specimens were obtained from 11 age-matched necropsied cases with non-cardiac diseases (mean age, 14.1 years). They included eight cases with malignant tumor and one case each of congenital biliary atresia, ileus and Wiskot-Aldrich syndrome.

The specimens were fixed in 10% neutral formalin and embedded in paraffin. Sections were cut at six microns perpendicular to the endocardium and stained with hematoxylin and eosin, azan, Weigert-van Gieson's, pentachrome

Fig.4. Various types of myocardial fibrosis.

A; Focal fibrosis. Case 28. (H-E stain, $\times 100$), B; Perivascular fibrosis observed surrounding the small arteries and veins. Case 28. (H-E stain, $\times 100$), C; Severe perimysial fibrosis. Case 31. (H-E stain, $\times 200$), D; Plexiform fibrosis and perimysial fibrosis accompanied by myocyte disarray. Case 16. (H-E stain, $\times 400$).

and periodic acid-Schiff methods.

Myocyte Diameters. The myocyte diameters were calculated morphometrically in most of the biopsied materials according to the method of Chalkley et al²⁷ and Arai et al²⁸. In the control cases and in certain other cases, the diameters were measured at the longitudinal section by an eye-piece micrometer at the level of the nucleus, when the number of the transversely sectioned myocytes was too few to apply Chalkley's and Arai's methods.

Myocardial Disarray. In order to estimate quantitatively the degree of myocardial disarray, its definition was established as the non-parallel arrangement of myocytes. According to this definition, myocardial disarray can be observed not only in cardiomyopathy, but even in the normal heart.

The area of myocardial disarray was counted by a semi-automatic sampling counter (Wild-Heerbrugg, M501) at a magnification of $\times 142$,

using 21 short lines 130 microns in length on the projected screen at the low power view, on the multipurpose test system (Wild-Heerbrugg Instrument Inc., CH-9435)²⁹. When non-parallel myocyte or fascicle arrangement and Y-shaped branching portion were across the short lines, the positive disarray point was counted. All areas of each specimen were scanned except the edges and surgically crushed portion (Fig. 2). A few specimens containing myocytes insufficient to decide myocyte orientation were excluded from this study.

In order to quantify the myocyte disarray in each case, a disarray index was established: it expressed the degree of bias from parallel myocyte orientation. On the screen at a magnification of $\times 575$ of the sampling counter, there is a seven parallel line arrangement in 28-micron intervals in a 192×192 micron-square unit. These parallel lines were set along the longitudinal section of the main myocyte orientation. The number of intersections, C of these parallel lines with the myocytes were counted, then the screen

TABLE III PATHOLOGICAL DATA OF TOF, RVOTO AND CONTROL

Case	Age	Diameter of myocardial cell (μ)	% area of disarray (%)	%area of fibrosis (%)	Types of fibrosis					Disarray index
					focal	perimysial		perivascular	plexiform	
						mild	severe			
1	2	15.4	19.4	20.6	—	+	—	+	—	2.0
2	2	12.4	13.7	16.6	—	—	+	+	—	2.7
3	2	14.7	12.5	12.1	—	+	—	+	—	1.7
4	2	19.2	26.7	13.5	—	—	+	+	+	2.0
5	3	14.1	20.9	29.4	—	—	+	+	+	1.5
6	4	17.7	38.0	20.0	—	—	+	+	—	2.3
7	4	13.1	37.5	19.3	—	—	+	+	+	2.0
8	4	12.7	5.5	10.6	—	+	—	+	—	1.9
9	4	22.6	30.7	31.0	—	+	—	+	—	2.1
10	4	15.0	22.0	24.2	+	—	+	+	—	2.3
11	4	16.0	9.7	10.9	+	—	+	+	—	1.8
12	4	14.5	5.0	12.3	+	+	—	+	—	1.5
13	4	19.2	11.9	8.3	—	—	+	+	—	1.9
14	5	15.5	19.5	19.7	—	—	+	+	+	1.6
15	11	17.8	11.0	15.2	—	—	+	+	+	1.9
16	16	23.1	6.7	8.7	—	—	+	+	—	1.6
17	19	19.4	8.6	14.1	+	—	+++	+	—	1.6
18	22	16.2	26.7	15.6	+	+	—	+	+	1.7
19	23	21.2	9.4	22.8	—	—	+++	+	—	1.6
20	23	21.3	4.3	17.8	—	—	+	+	—	2.1
21	6	11.2	17.0	10.5	—	++	—	+	—	1.8
22	7	13.5	6.3	14.5	+	++	+	+	—	1.6
23	7	14.5	18.9	9.8	—	+	—	+	—	1.5
24	8	15.8	21.7	17.9	+	+	—	+	—	1.9
25	10	12.9	20.0	20.3	—	++	+	+	—	1.7
26	10	16.7	12.5	11.7	—	++	+	+	—	1.4
27	11	17.2	18.7	21.4	—	+	+	+	—	1.5
28	11	16.7	16.5	13.8	—	+	++	++	+	2.0
29	12	16.7	13.9	19.2	—	++	+	++	—	2.5
30	14	15.5	10.8	14.3	—	+	++	++	—	1.7
31	30	14.7	11.1	23.6	—	+	+++	+	—	1.5
32	37	23.8	8.0	11.0	—	++	—	+	—	1.6
33	39	15.2	10.5	13.7	—	+	—	+	—	1.7
34	44	24.4	5.8	20.1	—	+	+++	+	—	1.8
4714	1M	7.8	3.0	3.6	—	—	—	+	—	1.6
4397	7M	7.1	8.8	4.2	—	+	—	+	—	1.8
4891	2	7.7	4.0	3.6	—	—	—	+	—	1.8
4765	3	7.9	3.9	8.6	—	+	—	+	—	2.7
4595	11	9.6	7.0	4.3	+	—	—	+	—	2.4
4840	13	13.7	8.9	6.7	—	+	—	+	—	1.6
4568	14	10.8	1.1	8.7	—	+	—	+	—	2.2
4039	22	11.3	6.0	11.1	—	+	—	++	—	1.5
4877	25	11.3	11.4	18.2	+	++	—	+	—	1.4
4536	28	8.2	1.4	7.6	—	+	—	+	—	1.6
4873	35	10.3	11.4	8.0	—	+	—	+	—	1.8

was rotated 90-degrees. The number of intersections, C' of these rotated parallel lines with the myocytes which were perpendicular to the main myocyte orientation, were counted consecutively. The disarray index is defined as the ratio of

C' to C .

Five areas with severe disarray were counted in each of the 34 cases and the controls, and the average count was calculated.

Fig. 5. Histopathological findings of TOF and RVOTO.

A; Y-shaped-branching of myocyte. Case 21. (H-E stain, $\times 400$), B; Myocyte disarray in a case of TOF. Case 1. (H-E stain, $\times 200$), C; Severe myocyte disarray in a case of RVOTO. Case 44. (H-E stain, $\times 400$), D; Severe interstitial edema and small round cell infiltration. Slight degenerative changes in myocytes. Case 25. (H-E stain, $\times 200$).

Myocardial Fibrosis. The histological features of myocardial fibrosis were classified into five types: focal, mild perimysial, severe perimysial, perivascular and plexiform (Fig. 4 a-d). Focal myocardial fibrosis was defined as scattered small replacement fibrosis of less than 1500 micron.³⁰ Perimysial fibrosis was a collagen network surrounding each myocyte, and mild or severe indicates a loose or tight network. Perivascular fibrosis was collagenosis around intramyocardial small arteries and plexiform fibrosis was a combination or advanced form of various types which show a spider or caput medusa form extension of collagen mass.³¹ Each type of myocardial fibrosis was graded in three ranks from (1+) to (3+). The area of fibrosis was also measured by the point count method using the 42 points on the medium power magnified screen ($\times 142$) of the semi-automatic sampling counter. Points that fell on the intima and the media of the vessels and the endocardium, and obvious edema in the interstitium were omitted from the counting.

Statistical Methods. Data were compared and analyzed using the student t test, chi-square test or analysis of variance. A *p* value was considered as statistically significant when it was less than 0.05.

RESULTS

Myocyte diameters, areas of myocardial disarray, areas of myocardial fibrosis, types and grade of myocardial fibrosis and the disarray index in TOF, RVOTO and the control groups are listed in Table III.

In the control group, the myocyte lining showed a normal parallel arrangement, and the area of disarray was only 6.1%. The disarray was composed of the fascicular type, not the myocyte type. The fibrosis in the interstitium was also minimal (1.85% of the sample). Interstitial fibrosis tended to increase with age ($r = 0.58$, $p < 0.1$). The diameters of the myocyte and the areas of disarray tended to correlate with age,

TABLE IV SUMMARY OF HISTOPATHOLOGICAL DATA

	No. of pts	Diameter of myocardial cell (μ)	% area of disarray (%)	Disarray index	% area of fibrosis (%)	Incidence of severe perimysial fibrosis
TOF	20	17.06 $\pm$ 3.29**	17.0 $\pm$ 10.5**	1.89 $\pm$ 0.31	17.1 $\pm$ 6.3**	14/20**
Spell (+)	9	16.22 $\pm$ 3.17**	25.3 $\pm$ 10.0**	1.98 $\pm$ 0.26	20.5 $\pm$ 6.9**	5/9*
Spell (-)	11	17.73 $\pm$ 3.38**	10.2 $\pm$ 4.3*	1.82 $\pm$ 0.34	14.4 $\pm$ 4.6**	9/11**
RVOTO	14	16.34 $\pm$ 13.57**	13.7 $\pm$ 5.2**	1.73 $\pm$ 0.28	15.8 $\pm$ 4.5**	9/14**
Control	11	9.60 $\pm$ 2.06	6.1 $\pm$ 3.7	1.85 $\pm$ 0.41	7.7 $\pm$ 4.3	0/11

** and * indicate significant correlation from the control and the two age-matched groups, respectively.

** = $p < 0.01$; * = $p < 0.05$

but there was no statistical significance.

In TOF and RVOTO, myocytes showed considerable hypertrophy and disarray (Fig. 5 a–c). The interstitium exhibited various types of fibrosis, edema and small round cell infiltration (Fig. 5d). Degenerative changes, such as vacuolar degeneration of the myocytes, were also frequently observed.

In TOF, the diameter of the myocytes correlated with age ($p < 0.05$), and the areas of disarray had a significant relation to the areas of interstitial fibrosis ($p < 0.05$). In TOF without spells, the diameters of the myocytes correlated with age ($p < 0.01$). However, in TOF with spells, they had no relation with age, intracardiac pressure, diameters of the myocytes or areas of disarray and fibrosis.

In RVOTO, the diameters of the myocytes correlated with age ($p < 0.01$).

The mean myocyte diameters, the percent area of disarray, the disarray index, the percent area of fibrosis of each group are summarized in Table IV. There were significant differences in all mean values ($p < 0.01$) except for the disarray index in each group. TOF with spells, TOF without spells and RVOTO showed significant increases in the myocyte diameter, the percent area of disarray, the percent area of fibrosis and the incidence of severe perimysial fibrosis, when compared to the control.

In TOF with spells, there was a significant increase in the area of disarray compared to TOF without spells and RVOTO. The disarray index of TOF with spells was higher than that of RVOTO ($p < 0.05$), but there was no significant difference among the groups ($p < 0.01$, analysis of variance). TOF with spells also showed a higher area of fibrosis than TOF without spells

($p < 0.05$). The severity and distribution of perimysial fibrosis tended to be less than TOF without spells and RVOTO.

Perivascular fibrosis was observed in various degrees in all groups. Plexiform fibrosis was seen in 4 out of the 9 cases of TOF with spells and in 2 among the 11 cases of TOF without spells.

DISCUSSION

Quantitative analysis of myocardial disarray can be performed by the scanning method using a high magnification microscope,³² the point count method, or by enclosing the area of myocardial disarray in the magnified images of the photographs of tissue sections.^{15,33} However, these methods have their drawbacks. Myocardial disarray is a three-dimensional structure, and the disarray is composed of at least two myocardial fibers. Consequently, it is theoretically impossible to determine the presence of myocyte disarray at any given point and it is also impossible to draw the exact line enclosing the myocardial disarray, especially in fascicular type disarray. Though the scanning method is a simple and common technique, it is difficult to determine the difference between the normal myocyte arrangement and the fascicular type disarray at the high power view.

In our study, we attempted to determine myocardial disarray using the pattern of the myocardial fibers between two points that are on the line shown in the screen (Fig. 2). Disarray is defined as non-parallel oriented myocardial fibers on the short line. This "line counting" method can be applied not only to myocyte disarray, but also to fascicular disarray. The method based on using the pattern of the myocardial fibers corre-

sponds, a physiological point of view, with the assumption of myocardial fiber contraction and relaxation. Determination of the presence of disarray is an important histological finding, because the disarray is thought to reduce the efficacy of contraction and relaxation. It is difficult to demonstrate disarray in longitudinally oriented myocytes, and this is a drawback of this line counting method.

The disarray index of myocyte disarray in each case was ascertained in order to evaluate the degree of the myocyte disarray from the parallel arrangement. This index theoretically becomes 1.0 where disarray is the highest. But the mean disarray index in the non-disarray segment of the control group was 2.83. One of the weak point of the disarray index is the difficulty in defining the orientation of myocytes and the center of the screen. When the myocytes are slanted, the disarray index becomes lower. Disarray index must be measured in sections with horizontally oriented myocytes. Using this disarray index, quantification of myocyte disarray in each case can be made.

Jones et al.¹⁰ resected the crista supraventricularis in 59 patients with congenital heart disease, including 27 patients with TOF and 8 with infundibular pulmonary stenosis who had not an atrial septal defect. They stated that myocardial hypertrophy correlated with the systolic outflow gradient and a high right ventricular end-systolic pressure (6 mmHg or greater), but not with the patient's age. They also reported that very severe hypertrophy was present in 14 of the 54 cases with obstruction of the outflow tract. Kato et al.²⁵ observed that right ventricular hypertrophy in 104 patients with TOF was found to have its onset immediately after birth, that the myocyte diameter increased with age and that there was no relation between myocyte diameter and oxygen saturation of the arterial blood. Jones et al. and Maron et al.⁵ also noted that the resected right ventricular outflow tract muscle in patients with right ventricular outflow obstruction showed focal and minimal myocardial disarray.

We demonstrated a relatively large area of myocardial disarray in patients with TOF and RVOTO, compared to the results of Jones et al.¹⁰ This may be due to the marked difference in the definition of myocardial disarray between our study and that of Jones et al.. They only examined five to 15 tissue blocks ranging from one to two mm in size. On the contrary, we scanned

the entire area of the tissue specimens with a light microscope.

The mean disarray indices in each group were not significantly different. This indicates that there are no qualitative differences in each myocyte disarray from a physiological point of view. We postulate that extensive myocyte disarray can cause greater impairment of cardiac function than the rather small amount of advanced disarray seen in the hypertrophic cardiomyopathy.

Cases of TOF with spells showed an increase in the area of myocardial disarray and no difference in the diameters of the myocytes as compared to RVOTO and TOF without spells, despite the fact that the mean age of TOF patients with spells was 5.4 years as opposed to 17.6 years in RVOTO patients and 10.3 years in TOF patients without spells. This myocardial hypertrophy and increased area of disarray in TOF cases with spells appears to be characteristic of the histological findings in TOF with spells, suggesting a similar contraction abnormality of the right ventricular outflow tract muscle in TOF, to that of the hypertrophic obstructive cardiomyopathy. Furthermore, the relatively low incidence of severe perimysial fibrosis in TOF cases with spells suggests that hypoxic spells are induced by a functional obstruction rather than a fixed obstruction in the outflow tract. The disarray of myocytes is thought to require more energy for contraction and may act as a generator of cardiac hypertrophy. Focal hypertrophy of the myocardium, in turn, will act as another uneven distributor of the myocardial contraction. Thus, myocardial hypertrophy will progress and result in the narrowing of the outflow tract in same congenital heart disease which is similar to that seen in hypertrophic obstructive cardiomyopathy. The contraction of the hypertrophied myocardium in TOF patients with spells will cause stenosis of the outflow tract and reduce pulmonary blood flow which causes anoxic spells.

In our study, the myocardium of patients with TOF and RVOTO showed myocardial hypertrophy and increases in the area of myocyte disarray and interstitial fibrosis. Furthermore, the diameters of the myocytes correlated with the patient's age, but not with the hemodynamic variables. The lack of a relation between the pressure and the presence of the increased area of the myocyte disarray in TOF with spells implies that this myocardial

changes is brought about by myocardial abnormality accompanied by cardiac malformation in the prenatal period, rather than ordinary hypertrophy that preserves the normal structure in the myocardium.

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