Cardiac Hypertrophy

– Scanned Architecture, Ultrastructure and Cytochemistry of Myocardial Cells –

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IN order to define some morphological features which characterize the myocardium in hearts with eccentric, concentric and asymmetrical hypertrophy, a series of studies with scanning

Fig.1. Scanning electron micrographs. A: Ventricular cardiocytes isolated from autopsied human hearts. HCM = hypertrophic cardiomyopathy with asymmetrical hypertrophy. See text. B: En face views of cellular architecture in situ in ventricular myocardium. See text.

Key Words:

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and transmission electron microscopy were performed in our laboratory!¹⁻⁷ The cytochemical activities of some enzymes in hypertrophied myocardial cells were also studied in myocardial

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Fig.2. Dimensions (mean of 100 cells per heart) of ventricular cardiocytes isolated from autopsied human hearts. See text.

biopsies from patients with various types of heart diseases⁸⁻¹⁰ and in spontaneously hypertensive rats (SHR) as an animal model of cardiac hypertrophy.¹¹ This communication reviews our studies on the myocardium in cardiac hypertrophy.

Scanned Architecture of Myocardial Cells in Hypertrophied Myocardium

Scanning electron microscopy revealed the three-dimensional morphology and size of myocardial cells isolated by the EDTA method from autopsied human hearts⁴⁻⁵ and fresh or fixed animal hearts⁶. The connection of the myocardial

Fig.3. Scanning electron micrographs of myocardial cells isolated from left ventricles of 20-week-old SHR and age-matched Wistar-Kyoto rats (WKY). Isolated cells continued beating until fixation with glutaraldehyde.⁶ See text.

cells *in situ* in the myocardium was also elucidated by scanning electron microscope by Evan's method.¹²

Normal Adult Human Hearts. Myocardial cells were isolated from the middle layer of the posterior wall of the left ventricles of 5 normal adult hearts (300–330g in weight). These ventricular cardiocytes were usually cylindrical in shape, with a few short branches (Fig. 1A)⁵ and were, on the average, approximately 62 μ m long and 15.3 μ m wide with a length to width ratio of 4.1 (Fig. 2). These myocardial cells were connected with one another end-to-end at the intercalated discs (Fig. 1) and composed a fascicular network in the ventricular wall or interventricular septum of the heart.

Fig.4. Schematic diagrams of possible sites of sarcomerogenesis in cardiocytes in normal growth (#1) and hypertrophy (#2-4). Myofilaments appear to be synthesized and organized into sarcomeres in the sarco-plasmic matrix adjacent to cell junctions (#1-3), dense material associated with free sarcolemma (#1-4) and already existing sarcomeres, particularly abnormal Z band expansions (#4). In circle #5 is depicted a part of a normal intercalated disc of normal adult or hypertrophied cardiocytes.

Eccentric Hypertrophy. Myocardial cells were isolated from the middle layer of the posterior wall of the left ventricles of 5 human hearts (450–830g in weight) with eccentric hypertrophy caused by chronic combined valvular disease. Many of these cells were markedly elongated and thickened (Figs. 1A and 2). The length to width ratio of these cells was often much larger than that of the cells in normal ventricles or of many hypertrophied cells in ventricles with concentric hypertrophy (Fig. 2). In addition, the branches of the ventricular cells were also thickened and often markedly elongated along the long axis of the cell trunks (Fig. 1A). These elongated and thickened cells were connected with one another end-to-end at the intercalated discs (Fig. 1B) and composed the hypertrophied ventricular wall. Increased volume overload in the ventricular cavity in diastole may widen the branching angles in these cells and

stretch the distal elongated branches away from the trunks so that the layers of these connected myocardial cells readily spread, and the ventricle is dilated eccentrically.

Concentric Hypertrophy. Myocardial cells were isolated from the middle layer of the posterior wall of the left ventricles of 5 human hearts (390–740g in weight) with concentric hypertrophy caused by chronic hypertension. Many of these ventricular cardiocytes were markedly elongated and thickened (Figs. 1A and 2). In contrast to the hypertrophied cells in hearts with eccentric hypertrophy, the length to width ratio of the cells often remained within the normal range (Fig. 2). Compared with the trunks, the branches often did not appear to be very elongated or thickened. Being connected with one another end-to-end at the intercalated discs, these cells composed a concentrically hypertrophied ventricular wall (Fig. 1B).

Fig.5. Electron micrograph of unorganized myofilaments immediately adjacent of irregularly interdigitated intercalated disc (ID) of cardiocytes in markedly hypertrophied left ventricle of a 15-week-old SHR. In the vicinity of the intercalated disc, Z bands (Z) are indistinct, fragmented and displaced so that the sarcomeres cannot be identified as complete structural units. Scale, 1 μ m.

Fig.6. Electron micrograph of unorganized myofilaments (f) which are associated with subsarcolemmal dense mats (*) closely attached to cytoplasmic surface of sarcolemmal membrane of hypertrophied cardiocytes in the same specimen as in Fig.5. Scale, 1 μ m.

TABLE 1 HISTOPATHOLOGICAL FINDINGS IN HYPERTROPHIED VARIOUS TYPES OF CHRONIC HEART DISEASES

Diagnosis	Valvular HD															
	AS		AS		ARS		MR					MS				
	T.T.		T.R.		K.A.		T.S.		M.Y.		M.G.		M.M.		S.M.	
	41M		54M		58M		24F		23F		30F		30F		53F	
Biopsy site	LVN	IVS	RVN	LVN	RVN	LVN	LVN	RVN	LVN	LVN	LVN	LVN	RVN	LVN	RVN	LVN
Hypertrophy of myofibers	1	2	2	3	2	3	3	2	3	2	2	2	1	1	2	2
Scarcity of myofibrils	0	1	1	2	2	3	2	0	1	2	1	1	2	1	1	1
Nuclear changes	3	2	2	2	3	3	1	1	2	1	0	2	1	2	3	1
Vacuolization	1	1	0	0	0	0	1	1	0	1	0	0	0	1	1	2
Disarray of myofibers	2	2	1	2	0	2	0	2	2	1	1	2	1	2	2	1
Proliferation of collagen fibers	2	2	0	1	0	0	0	1	2	0	0	1	0	1	1	1
Interstitial edema	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0
Cell infiltration	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fatty Infiltration	0	0	0	0	0	0	0	2	2	1	1	1	1	0	1	1
Endocardial thickening	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

In at least one biopsy from each patient, the myocardial cells were hypertrophied to 20 μ m or more (Score 2 or more) mean transverse diameter. The degree of each change was scored as 0 (no change), 1 (slight), 2 (moderate), 3 (marked) or 4 (extremely marked) according to the criteria proposed by Noda.⁴⁸

Our preliminary study⁶ of the myocardial cells isolated from the left ventricles of 20-week-old SHR revealed that the myocardial cells were markedly hypertrophied and their three-dimensional morphology often resembled that of the hypertrophied myocardial cells isolated from human left ventricles with concentric hypertrophy (cf. Figs. 1A and 3).

Asymmetrical Hypertrophy. In 5 hearts (415–970g in weight) with asymmetrical septal hypertrophy of unknown etiology, the myocardial cells isolated from the middle layer of the interventricular septum were often bizarrely shaped, having several branches of various lengths and oriented in many directions (Fig. 1A).⁵ These cells were occasionally flattened, and large and smooth curvatures were often seen between adjacent branches or a branch and the main body of the cells. Disarray of myofibrils was also seen

beneath the sarcolemma even in the same cell. These bizarrely shaped myocytes appeared to be connected with one another end-to-end, side-to-side or end-to-side at the cell junctions and composed a marked cellular disarray characteristic of hypertrophic cardiomyopathy (Fig. 2).

Ultrastructural Features of Hypertrophied Myocardium

It has been recognized that in cardiac hypertrophy, a variety of ultrastructural changes occur in myocardial cells, interstitial tissue, vessels and nervous elements in the myocardium.

MYOCARDIAL CELLS

In cardiac hypertrophy of any type, a number of myocardial cells undergo hypertrophy in the affected portions of the myocardium. In hypertrophied myocardial cells, a number of

MYOCARDIUM IN VENTRICULAR BIOPSIES FROM 24 PATIENTS WITH

Congenital HD						I C M																Others			
T,F ASD+MR						H C M												C C M						HHD	WPW
M.A.	J.F.	Y.N.				T.N.	K.H.	H.O.	S.M.	H.S.	K.N.				H.M.	Y.M.	Y.I.				M.T.	C.I.			
31M	45F	20M				21M	36M	33M	57M	42M	46F				25M	20M	42M				56M	58F			
RVI	RVN	LVN	RV	LV		RV	LV	RV	LV	RV	LV	RV	LV	RV	LV	RV	LV	RV	LV	RV	LV	RV	RVN		
3	2	0	3	4		2	4	2	2	1	4	1	2	0	3	0	2	2	3	3	3	3	4	2	
1	0	1	2	2		1	2	3	1	1	1	1	1	2	0	2	2	2	2	3	3	1	2	1	1
2	2	1	1	2		2	2	1	1	2	3	3	3	0	1	2	3	2	2	2	2	1	1	2	2
1	0	2	1	1		1	1	1	0	0	1	1	0	2	2	1	1	1	2	4	4	0	1	1	1
1	0	1	2	3		3	3	3	1	3	1	0	3	1	2	2	2	3	3	2	3	1	0	1	1
2	1	1	2	2		1	2	2	1	0	2	0	1	1	2	0	2	2	4	0	2	1	1	1	1
0	1	0	0	0		1	1	0	1	0	1	0	0	0	0	3	0	1	1	0	0	1	1	1	0
0	0	0	0	0		0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
1	2	0	0	0		0	0	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
4	—	—	0	1		0	—	0	1	1	1	0	0	0	0	0	0	3	0	3	0	0	0	—	

ARS, aortic regurgitation and stenosis; AS = aortic stenosis; ASD = atrial septal defect; CCM = congestive cardiomyopathy; F = female; HCM = hypertrophic cardiomyopathy; HHD = hypertensive heart disease; ICM = idiopathic cardiomyopathy; IVS = interventricular septum (needle biopsy); LV = left ventricle (biopsy); LVN = left ventricle (needle biopsy); M = male; MR = mitral regurgitation; MS = mitral stenosis; RV = right ventricle (biopsy); RVI = myectomy of right ventricular infundibulum; RVN = right ventricle (needle biopsy); T/F = tetralogy of Fallot; WPW = WPW syndrome

intracellular organelles appear to show hyperplasia¹³ and, after a long course, a variety of degenerative changes.^{1,14}

Myofibrils

A number of morphometric studies of uncomplicated chronic cardiac hypertrophy have shown a significant increase in the volume fraction of myofibrils and a relative decrease in that of mitochondria accompanied by an appreciable decrease in the mitochondria/myofibril ratio in hypertrophied cardiocytes.¹⁴⁻²² Since the sarcomeres are shorter than in control hearts,^{23,24} they are assumed to multiply in hypertrophied cells. In our comparative observations^{1,2} on the myocardium of SHR and control Wistar-Kyoto rats, both 3 weeks to one year in age, new myofilaments appeared to be formed in multiple sites and were added in parallel and in series in normally growing as well as in rapidly hypertrophying

myocardial cells (Figs. 4-8). In agreement with reports on the hypertrophied myocardium in other animals, new sarcomeres appeared to be synthesized in association with intercalated discs^{1,25,26,29,30} (Figs. 4 (2 and 3) and 5), sarcolemma^{1,28} (Figs. 4 (2-4) and 6), the sarcolemmal matrix adjacent to the nucleus, sarcolemma or myofibrils^{1,17,31} (Figs. 4 (4) and 7) and abnormal Z band expansions^{1,19,25-29} (Figs. 4 (4) and 8) in the hypertrophied cells in SHR. Comparable ultrastructural features of sarcomerogenesis were also seen in hypertrophied myocardial cells in myocardial biopsies obtained from patients with various types of heart diseases (Table I) (Fig. 9).

One of the crucial issues inherent in human myocardial biopsies is that the morphological features may not necessarily represent simple manifestations of hypertrophy process, but also

Fig.7. Electron micrograph of unorganized myofilaments (f) which appear to parallel already existing myofibrils in hypertrophied ventricular cardiocyte in 11-week-old SHR. Rough surfaced endoplasmic reticulum (rer) is well developed. Filamentous structures which transverse myofibrils at Z band (Z) levels may indicate cytoskeletal filaments. Scale, 1 μ m.

Fig.8. Electron micrograph of abnormal Z band expansions (Z) along the long axis of hypertrophied cardiocytes in 15-week-old SHR. Scale, 1 μ m.

Fig.9. Electron micrograph of markedly hypertrophied cardiocytes in needle biopsy from left ventricle of 54-year-old man with aortic stenosis. Myofilaments (mf) of the sarcomeres adjacent to irregular intercalated disc (ID) appear to be markedly disorganized. Ultrastructural features of these disorganized myofilaments resemble those seen in Fig.5, which represent a process of sarcomerogenesis in hypertrophying cardiocytes in a 15-week-old SHR. In the late stage of long-standing cardiac hypertrophy in man, however, disorganized sarcomeres may represent, at least in part, degeneration processes. Scale, 1 μ m.

Fig.10. Electron micrograph of markedly hypertrophied cardiocytes in same biopsy as in Fig.9. Some myofibrils appear to have undergone lysis and are scarce in the sarcoplasm. Some mitochondria (Mt) also seem to be damaged. An intercalated disc (ID) follows an irregular course. Scale, 1 μ m.

Fig.11. Electron micrograph of marked disarray of closely packed myofibrils (Mf) in bizarrely shaped cardiocyte in right ventricular endomyocardial biopsy from 29-year-old woman with hypertrophic cardiomyopathy. Several Z bands (Z) are partially expanded, mitochondria (Mt) are small and a T tubule (t) is markedly dilated. Scale, 1 μ m.

Fig.12. Electron micrograph of subsarcolemmal disarray of myofibrils (mf and Z) in cardiocyte in right ventricular endomyocardial biopsy from 47-year-old man with hypertrophic cardiomyopathy. Small myofibrillar bundle terminates in a subsarcolemmal dense mat attached to free lateral sarcolemma (arrow head). is = intercellular space, mt = mitochondria. Scale, 1 μ m.

Fig.13. Electron micrograph of hypertrophied cardiocyte in needle biopsy from left ventricle of 41-year-old man with aortic stenosis. Closely packed myofibrils show marked disarray. Scale, 1 μ m.

various degrees of degeneration.¹⁴ This is because biopsies are usually available only in more or less advanced stages of cardiac disease in man. By the time of biopsy, the myocardium may have undergone pathological hypertrophy beyond the stage of uncomplicated physiological hypertrophy. In fact, in the myocardial biopsies obtained from patients with various types of chronic heart diseases (Table I), a variety of degenerative changes of various degrees were seen in hypertrophied myocardial cells. Among other features, a moderate (Score 2) to marked (Score 3) scarcity of myofibrils in light microscopy (Table I) appeared to correlate well with myofibrillar disorganization or lysis seen in transmission electron microscopy (Fig. 10). These myofibrillar changes included streaming and fragmentation of the Z bands and loss of thick filaments which appeared to exceed that of thin filaments. Some of these morphological aspects of myofibrillar changes did not appear to be distinct from those which were interpreted as representing myofibrillar synthesis in hypertrophying cardiocytes in SHR.¹ These observations suggest that in a chronically overloaded myocardium, degeneration may occur in the myofibrils of otherwise still hypertrophied cardiocytes or *vice versa*.

Expanding the stress theory of cardiac hypertrophy, Grossman et al.³² have proposed a hypothesis that in eccentric hypertrophy, increased diastolic wall stress leads to the addition of new sarcomeres in series, whereas in concentric hypertrophy, increased systolic wall stress leads to the addition of new myofibrils in parallel. As revealed by scanning electron microscopy, the ventricular myocardial cells are markedly elongated and thickened in either eccentric or concentric hypertrophy of the heart. Since myofibrils appear to be abundant in these hypertrophied myocardial cells, the addition of new sarcomeres in series and in parallel must have occurred in both types of cardiac hypertrophy. Between the two types of hypertrophy, however, the general morphology of the hypertrophied cardiocytes was frequently different. In eccentric hypertrophy, the trunks and many branches of the cardiocytes appear to be proportionately elongated as well as thickened, whereas in concentric hypertrophy, the trunks tend to hypertrophy more than the branches. The length to width ratio of hypertrophied cardiocytes is often much larger in hearts with eccentric hypertrophy than in those with concentric hypertrophy. On the assumption that the volume fraction and distri-

Fig.14. Electron micrograph of hypertrophied cardiocyte in left ventricular lateral wall of one-year-old SHR. Myofibrils show disarray and a small myofibrillar bundle (Mf) appears to terminate in a subsarcolemmal dense mat (*) attached to the free lateral sarcolemma (SL). Scale, 1 μ m.

bution of myofibrils are even in the sarcoplasm, the addition of new sarcomeres in series and/or in parallel may vary topographically depending upon the type of cellular hypertrophy. For example, in hypertrophied cardiocytes with increased length to width ratios, the addition of new sarcomeres in series might have been preponderant over their addition in parallel; in thickened and elongated branches of cells in hearts with eccentric hypertrophy, the addition of new sarcomeres both in series and in parallel must have been more prominent than in the short and thin branches of hypertrophied cardiocytes which are common in hearts with concentric

hypertrophy. In this context, the concept of the addition of new sarcomeres in series or in parallel with reference to eccentric or concentric hypertrophy proposed by Grossman et al.³² may explain the topographical preponderance of sarcomere synthesis depending upon the type of hypertrophy.

In the bizarrely shaped cardiocytes which are characteristic of hearts with asymmetrical hypertrophy, the myofibrils varied in amount and arrangement. They were occasionally closely packed and markedly disoriented from each other (Fig. 11). The myofibrillar disarray occasionally extended from the subsarcolemmal

area to the perinuclear area. Abnormal Z band expansions were also seen in these myofibrils (Fig. 11). Although most myofibrillar ends terminated at the fasciae adherentes of the cell junctions, some appeared to be connected with the Z band-like material associated with the plasma membrane of the free lateral wall of the cardiocytes (Fig. 12). It is likely that the disarray of bizarrely shaped cardiocytes and the myofibrillar disorganization in these cells interfere somehow with the normal contraction and relaxation of the affected cell groups or muscle segments in ventricles with asymmetric hypertrophy.

It should be noted that disarray of myofibrils *per se* is not a hallmark of the bizarrely shaped cardiocytes in hypertrophic cardiomyopathy. Some myofibrils showed marked disarray in hypertrophied ventricular cardiocytes in human patients with valvular heart disease (Fig. 13), congenital anomalies or congestive cardiomyopathy and in SHR (Fig. 14).^{1,3} In these other types of heart disease, however, myofibrillar disarray tends to be localized near the periphery of the myocardial cells.

Mitochondria

As previously mentioned, the mitochondrial fraction was often significantly decreased in myocardial cells in chronic cardiac hypertrophy. Our study of SHR showed that the mitochondrial fraction decreased in hypertrophied cardiocytes as blood pressure increased markedly after 11 weeks of age.² In younger rats, no difference was noted in shape, size or mitochondrial fraction in the cardiocytes of SHR and Wistar-Kyoto rats. At 15 weeks of age and thereafter, maximum hypertension was sustained and the mitochondria were often much smaller than normal; they were often increased in number (mitochondriosis) and clustered in some places, particularly in the perinuclear areas.^{1,2} In some other places, however, mitochondria were rare. Some mitochondria were swollen, cristae were scarce and the matrix became electron lucent. Other mitochondria contained glycogen deposits or moderately electron dense inclusions. These various changes of mitochondria appeared to represent an advanced stage of cardiac hypertrophy and, together with myofibrillar lysis, proliferation of sarcoplasmic reticulum and lipofuscin granules, hypertrophy of Golgi complexes and rough surfaced elements of endoplasmic reticulum, nuclear changes, etc., they may represent hypertrophied myocardial cells undergoing degeneration.^{1,2}

Essentially similar changes of mitochondria and other organelles were frequently seen in hypertrophied myocardial cells in biopsies obtained from patients with various types of chronic heart diseases, as listed in Table I.

In our attempts to correlate enzymatic activities and morphological changes in hypertrophied cardiocytes in SHR, β -hydroxybutyrate dehydrogenase (HBD), monoamine oxidase (MAO) and cytochrome oxidase (Cyt Ox) activities were studied with light and electron microscopic cytochemistry (Fig. 15). Although all these enzymes were located in mitochondria, the activity of each enzyme appeared to vary among many mitochondria (functional heterogeneity).

In previous biochemical and histochemical studies,³³ HBD activity showed an appreciable increase at 9 weeks of age and a tendency to decrease after 11 weeks of age in the ventricular myocardium of SHR. In the electron microscopic study of HBD activity, the copper ferrocyanide method was employed.³⁴ Electron dense enzymatic reaction products were present in the cristate membranes, intracristate space and outer compartment of mitochondria. In 9- and 15-week-old Wistar-Kyoto rats, approximately one-half of the mitochondria showed a markedly positive reaction, whereas in most of the others, the reaction was decreased in varying degrees. In 9-week-old SHR, most mitochondria showed a markedly positive reaction, suggesting increased enzymatic activity in cardiocytes without any change in the intracellular volume fraction of mitochondria (Fig. 15 *a* and *b*). In 15-week-old SHR, markedly positive HBD reactions occurred in clustered mitochondria, while much milder or no reactions were noted in other mitochondria, particularly those which underwent marked swelling. A significant decrease in the mitochondrial volume fraction suggests a possible decrease in enzymatic activity in hypertrophied cardiocytes at this age. All these results appeared to be very compatible with the biochemical and histochemical findings reported previously.³³ It is well known that HBD is involved in fatty acid metabolism. Increased activity of this enzyme in 9-week-old SHR strongly suggests an increase in fatty acid metabolism in cardiocytes in the initial stage of cardiac hypertrophy. So far no evidence is available of increased fatty acid metabolism in hypertrophied myocardial cells in advanced stage of cardiac hypertrophy in SHR.

Previous biochemical and histochemical studies

Fig.15. Comparative enzyme-cytochemistry in cardiocytes in left ventricles of SHR and control Wistar-Kyoto rats (WKY). *a* and *b*: Electron micrographs of β -hydroxybutyrate dehydrogenase (HBD) reaction¹¹. In 9-week-old SHR (*a*), most mitochondria showed a markedly positive enzymatic reaction, whereas in 9-week-old WKY (*b*), only about one-half of mitochondria showed a markedly positive reaction and the remainder a much weaker reaction. With no significant difference in the intracellular volume fraction of mitochondria between SHR and WKY at 9 weeks of age^{1,2}, the above findings suggest a significant increase in intracellular HBD activity in SHR prior to the development of marked hypertension and ventricular hypertrophy. See text. *c* and *d*: Histochemistry of monoamine oxidase (MAO) reaction³⁶. Even at 8 weeks of age, MAO reaction was apparently more prominent in SHR (*c*) than in WKY (*d*). *e*: Electron microscopy³⁷ showed the MAO reaction to be occasionally located only in the outer compartment of a mitochondrion. See text. *f* and *g*: Histochemistry of cytochrome oxidase (Cyt Ox) reaction in 3 μ m thick epon sections⁹. In one-year-old SHR (*f*) the reaction was decreased. *h* and *i*: Electron micrographs of Cyt Ox reaction. In one-year-old SHR (*h*), many mitochondria showed a decreased reaction (see Fig.16), whereas in age-matched WKY (*i*), most mitochondria showed a marked homogeneous reaction. See text.

CYTOCHROME OXIDASE ACTIVITY IN CARDIOCYTES

Fig. 16. Schematic diagrams of electron microscopic patterns of decreased Cyt Ox activity in cardiocytes. Decreased color density and decreased number of bars indicate decreased enzymatic activity in mitochondria. See text.

Fig. 17. Semiquantitative histochemical activity (1-4) of Cyt Ox⁹ and case incidence in relation to mean transverse diameters of myofibers (cells) in ventricular biopsies. For this study, 55 biopsies were obtained from 39 patients with various types of heart diseases⁹. Irrespective of the diagnosis, histochemical activity (reaction) of Cyt Ox decreased as cellular hypertrophy developed. See text.

revealed significant increases in MAO activity in the ventricular myocardium in SHR.^{33,35} This was confirmed in our histochemical study with the tetrazolium method³⁶ even in 8-week-old SHR (Fig. 15 *c* and *d*). In electron microscopic cytochemistry with the BSPT method,³⁷ electron dense enzymatic reaction products were located in the outer compartment in some mitochondria in cardiocytes (Fig. 15 *e*). These findings suggest that in SHR, monoamine metabolism has been enhanced in cardiocytes even before significant hypertension and cardiac hypertrophy develop.

Light and electron microscopic cytochemistry of Cyt Ox activity was demonstrated by the DAB method³⁸ in the ventricular myocardium in SHR. Light microscopy showed decreased enzymatic activity in hypertrophied cardiocytes in one-year-old SHR (Fig. 15 *f* and *g*). Electron microscopy demonstrated electron dense reaction products in cristate membranes, intracristate spaces and the outer compartment in most mitochondria in control rats (Fig. 15 *i*). In one-year-old SHR, electron microscopy (Fig. 15 *h*) revealed that decreased histochemical activity of the enzyme indicates: 1) decreased mass of mitochondria (Fig. 16 (1)), 2) enzymatic hypoactivity in apparently well-preserved mitochondria (intrinsic hypoactivity) (Fig. 16 (2)), 3) destruction of cristate membranes in mitochondria (Fig. 16 (3))

Fig.18. Electron micrograph of extensively spread side-to-side junction (arrows) between adjacent cardiocytes in right ventricular endomyocardial biopsy from 36-year-old man with hypertrophic cardiomyopathy. In one cardiocyte, myofibrils (Mf) show disarray, and a double membrane-bound vacuole (V) is seen near the periphery. Scale, 1 μ m.

Fig.19. Electron micrograph of multiple intercalated discs of hypertrophied cardiocytes in right ventricular endomyocardial biopsy from 56-year-old man with hypertensive heart disease. Scale, 1 μ m.

Fig. 20. Schematic diagrams of tunnel capillary traveling through sarcolemmal cleavage and intracellular tunnel in hypertrophied cardiocyte in SHR^{1,2}.

and/or 4) increased mass of defective mitochondria as mentioned above [2) and 3)] (Fig. 16 (4)) in hypertrophied cardiocytes.

In our cytochemical study⁹ of Cyt Ox, 53 ventricular biopsies were obtained from 39 patients with various heart diseases including athlete's heart, cardiomyopathies, valvular heart diseases, congenital anomalies, etc. Irrespective of the diagnosis, the histochemical activity of Cyt Ox appeared to decrease as the mean transverse diameter of myocardial cells (myofibers) increased (Fig. 17). Electron microscopy revealed that decreased histochemical activity of the enzyme indicated one or any combination of the four patterns of mitochondrial response mentioned above for SHR (Fig. 16). These findings suggest a potential or actual disturbance in the electron transport system in hypertrophied cardiocytes in both SHR and human patients with advanced cardiac hypertrophy.

Other Intracellular Organelles

In hypertrophied myocardial cells, T tubules were occasionally dilated, tubular structures of sarcoplasmic reticulum were dilated and/or multiplied in several places, rough surfaced elements of sarcoplasmic reticulum were often well-developed, Golgi complexes were also often enlarged, and lipofuscin granules were occasionally increased in number and clustered in the perinuclear region. The nuclei were often

markedly hypertrophied and irregular in shape.³⁹ Although multinucleation has been described,⁴⁰ no mitotic figures have been identified in hypertrophied myocardial cells in adult hearts.⁴¹ Glycogen granules, mostly β particles and less commonly α particles, were often increased in amount, occupying the sarcoplasmic areas. Although not specific for cellular hypertrophy, vacuoles measuring 2 to 15 μ m in diameter were occasionally present in the sarcoplasm (Fig. 18).^{10,42} They were usually bounded by a double membrane structure. Some vacuoles contained pale amorphous substance; in others there were mitochondria, lipofuscin granules, myelin figures, vesicular structures and/or abundant glycogen granules. Although the origin of these vacuoles has been discussed in connection with mitochondria⁴² or intercalated discs,⁴³ many may have been *de novo* in origin representing the autophagic function inherent in myocardial cells. In fact, these vacuoles occasionally appeared to have been transferred to the periphery of the cell, and their contents enveloped by the inner membrane of the double membrane boundary appeared to be extruded and expelled into the extracellular space.

Sarcolemma and Cell Junctions

In normal adult ventricular myocytes, the intercalated disc is subdivided into transverse and longitudinal portions.⁴⁴ Although no quantitative estimation was made, either portion of the cell junctions often appeared to be increased in size in hypertrophied ventricular myocytes. In some transverse portions, a number of interdigitating processes appeared to be markedly elongated and attenuated (Fig. 5).^{1,25} Some other intercalated discs had lost their normal steplike appearance and showed an irregular and oblique course with respect to the long axis of the cell (Figs. 9 and 10). These altered portions of intercalated discs often seemed to be associated with unorganized sarcomeres (Figs. 5 and 9). In addition to the intercalated discs, side-to-side connections also were occasionally increased in size between hypertrophied cardiocytes (Fig. 18). In any type of cardiac hypertrophy, special cell junctions termed multiple intercalated discs^{14,15} were occasionally formed *de novo* between adjacent myocardial cells. The multiple intercalated discs were characterized by lateral projections of sarcoplasm which protruded from the side of one cell into that of the adjacent cell (Fig. 19).^{2,14} In the hypertrophied myocardium of ventricles with hypertrophic cardiomyopathy,

bizarrely shaped cardiocytes were often polyhedrally connected with one another at irregularly oriented cell junctions.

In the advanced stage of cardiac hypertrophy, some cell junctions showed dissociation of various degrees between apposed plasma membranes of adjacent myocardial cells; total dehiscence of the cell junctions could occur in degenerated myocardial cells.⁴⁴ In hypertrophied and degenerated cardiocytes, intracytoplasmic junctions and a number of spherical microparticles were also seen in association with the sarcolemma or dissociated cell junctions.¹⁴

CAPILLARIES IN HYPERTROPHIED MYOCARDIUM

In both concentric and eccentric hypertrophy of the heart, the general consensus appears to favor hyperplasia of the capillaries in the hypertrophied myocardium. The heart in SHR is not an exception^{1,2}; in this animal, sarcolemmal cleavages and intracellular tunnels develop in some cardiocytes as they hypertrophy. One or more capillaries are usually seen in each cleavage or lumen of the tunnels (Fig. 20). These capillaries may represent a form of adaptive cardiac hypertrophy and appear to be of great benefit in limiting the disturbance of oxygen diffusion between the capillaries and the centers of hypertrophied cells. It should be noted that more or less similar profiles of capillaries have been described in hypertrophied human hearts in which occasional capillaries were almost completely surrounded by thickened cardiocytes so that a capillary was seen deep in the cleavage of the cell.⁴⁶ Similar features have also been documented in the capillaries of hypertrophied skeletal muscle in the rat.⁴⁷

Concluding Remarks

Scanning electron microscopy revealed that some crucial differences exist in the three-dimensional morphology of isolated ventricular cardiocytes and the cellular architecture *in situ* in the ventricular wall in hearts with eccentric, concentric and asymmetrical hypertrophy.

A review of the literature, including our own electron microscopic studies on chronological changes of the myocardium in SHR, shows that the sites of increased sarcomerogenesis in hypertrophied cardiocytes may be related to the addition, in parallel and in series, of new myofilaments to previously existing myofibrils.

In light and transmission electron microscopy,

hypertrophied cardiocytes in biopsies obtained from 24 patients with various types of heart diseases often showed a wide spectrum of morphological changes including myofibrillar lysis, mitochondrial damage, localized hyperplasia of dilated sarcotubules, rough surfaced elements of sarcoplasmic reticulum, Golgi complexes and lipofuscin granules, metamorphosis of cell junctions, autophagic vacuoles and a number of spherical microparticles, hypertrophy of nuclei, and increased amounts of glycogen granules in the sarcoplasm. Considering the clinical setting under which the biopsies were obtained, some of these changes, particularly myofibrillar lysis and mitochondrial damage, appear to represent degenerative processes in hypertrophied cardiocytes. Our comparative study with the advanced stage of cardiac hypertrophy in old SHR seems to support this conclusion.

In the cardiocytes of SHR, intramitochondrial activities of β -hydroxybutyrate dehydrogenase, monoamine oxidase and cytochrome oxidase were studied with light and electron microscopic cytochemistry. Although the morphological and cytochemical findings are semiquantitative, they may have some significance in terms of the biochemical changes in the mitochondria in hypertrophied cardiocytes. The decreased cytochrome oxidase activity in hypertrophied cardiocytes in human myocardial biopsies suggests a potential or actual disturbance in the electron transport system in the mitochondria.

The possible functional significance of tunnel capillaries in hypertrophied cardiocytes in SHR was discussed in reference to the similar capillaries seen in hypertrophied cardiocytes in human hearts.

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