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Left ventricular ultrastructure in pulmonary stenosis and in tetralogy of Fallot

Marcel Toussaint[1, 2], **Claude Planché**[3], **Denis Duboc**[2], **André Pfister**[1], **Christian Da Lage**[1], **François Guérin**[2]

[1] Laboratoire d'Histologie et Embryologie, Faculté de Médecine Necker-Enfants Malades, 156, Rue de Vaugirard, F-75 015 Paris, France
[2] Unité de Cardiologie Infantile, Hôpital Cochin, Paris, France
[3] Service de Chirurgie Cardiaque, Centre Chirurgical Marie Lannelongue, Le Plessis-Robinson, France

Summary. Twelve patients underwent haemodynamic studies and myocardial biopsies: 7 with pulmonary stenosis (PS) and 5 with tetralogy of Fallot (TOF). Their ages ranged between 2 and 43 years. Right ventricular pressure was 128 ± 43 mmHg in PS and 98 ± 8 mmHg in TOF. Aortic blood oxygen saturation was $97.0\% \pm 1.4\%$ in PS, and $88.4\% \pm 6.3\%$ in TOF. Left ventricular (LV) weight was normal in TOF while it was increased in PS: 140.7 ± 74.3 vs 74.0 ± 8.7 g/m^2 ($P < 0.001$). Contractility was altered in both PS and TOF: ejection fractions were $56\% \pm 7\%$ vs $65\% \pm 6\%$ ($P < 0.001$). Light microscopy showed abnormal transverse diameter of left ventricular myocytes in both PS and TOF: $18.6\ \mu m \pm 4.0\ \mu m$ vs $19.4\ \mu m \pm 4.9\ \mu m$. The percentage of interstitium was normal: $29.6\% \pm 3.9\%$ vs $26.2\% \pm 5.1\%$. Transmission electron microscopic examination revealed hypertrophic changes in all patients and degeneration in 7 of them. Hyperfunctional alterations of the myocytes were characterized by the increased number and reduced size of mitochondria, the enlarged Golgi complex, the increased number of ribosomes, the marked folding and convolutions of the nuclear membrane, the dilatation and tortuosity of T tubules. Myofibrillar lysis was the major degenerative change, which was also observed in the right ventricle (RV) of the same patients. No correlation was observed between these alterations and the patient ages, RV pressures, aortic blood oxygen saturations and ejection fractions. These findings led us to conclude that: (1) suprasystemic pressure overload of the RV induces macroscopic LV hypertrophy; (2) mild and suprasystemic pressure overload of the RV induces hyperfunctional changes in the LV; (3) myocardial degeneration is not related to hypertrophy nor to hypoxia, but is part of a more widespread cardiovascular fetopathy.

Key words: Pulmonary stenosis – Tetralogy of Fallot – Myocardial biopsies – Left ventricular hypertrophy – Myofibrillar lysis

Offprint requests to: M. Toussaint at the above address

For a long time, congenital heart diseases have been considered to be isolated problems arising during embryogenesis. Nonetheless, post-mortem investigations have led several authors to report that the myocardium may be the site of histological lesions which are not completely explained by the haemodynamic perturbations resulting from the cardiac malformation. Furthermore, haemodynamic and echocardiographic studies have shown that in the case of malformations that do not involve the left ventricle, such as pulmonary stenosis and tetralogy of Fallot, left ventricular function was altered. These findings led us to study myocardial histology in patients exhibiting these malformations.

Patients and methods

This study was carried out on twelve patients. Seven of them were afflicted with pulmonary stenosis (PS). None presented signs of cardiac failure. Outlines of the heart on chest X-rays were normal or slightly enlarged (the cardiothoracic ratio (CTR) ranged from 0.45 to 0.62). At the time of cardiac catheterisation (Table 1) right ventricular pressure was 128 mmHg $\pm$ 43 mmHg. The average aortic oxygen saturation was $97.0\% \pm 1.4\%$. The left ventricular mass and function were obtained by the area-length method (Yang et al. 1980). These data were compared with those of 20 normal subjects. The left ventricular weight was sharply increased: 140.7 g/m$^2 \pm 74.3$ g/m^2 vs 74.0 g/m$^2 \pm 8.7$ g/m^2 ($P < 0.001$). The ejection fraction (EF) was altered: $56\% \pm 7\%$ vs $65\% \pm 6\%$ ($P < 0.001$). Angiography allowed the localization of the stenosis: it was purely infundibu-

Table 1. Haemodynamic data

Patient number	Dg	Age	Sex	RVP	PAP	LVP	AoP	Sat	LVW	EF
1	PS	19	M	130	20	120	110	98	132	50
2	PS	17	M	90	15	110	110	98	155	53
3	PS	10	F	90	15	130	130	98	95	59
4	PS	2	F	190	16	–	–	–	–	–
5	PS	43	F	180	15	115	115	98	112	48
6	PS	2	F	130	14	105	105	95	280	54
7	PS	8	F	85	17	100	100	95	70	72
m±SD	–	14.4±14.1	–	128±43	16±2	113± 10	111±10	97.0±1.4	140.7±74.3	56±7
Normal values	–	–	–	25	25	90 to 130	90 to 130	98	74.0± 8.7	65±6
P	–	–	–	<0.001	<0.001	NS	NS	NS	<0.001	<0.001
8	TOF	17	M	90	15	90	90	85	81	50
9	TOF	17	F	90	25	90	90	97	65	52
10	TOF	10	M	100	12	100	100	93	106	61
11	TOF	4	M	100	8	100	100	85	65	63
12	TOF	16	M	110	11	110	110	82	99	54
m±SD	–	12.8± 5.7	–	98± 8	14±6	98± 8	98±8	88±6	83.3±19.7	56±5
Normal values	–	–	–	25	25	90 to 130	90 to 130	98	74.0± 8.7	65±6
P	–	–	–	<0.001	<0.005	NS	NS	<0.005	NS	<0.01

Abbreviations: Dg = diagnosis; RVP = peak systolic right ventricular pressure (mmHg); PAP = peak systolic pulmonary arterial pressure (mmHg); LVP = peak systolic left ventricular pressure (mmHg); AoP = peak systolic aortic pressure (mmHg); Sat = aortic oxygen blood saturation (%); LVW = left ventricular weight (g/m^2); EF = ejection fraction (%); PS = pulmonary stenosis; TOF = tetralogy of Fallot

Table 2. Morphometric data

Patient number diagnosis	Right ventricle							Left ventricle						
	D	I	MF	Mito	Golgi	Rib	RER	D	I	MF	Mito	Golgi	Rib	RER
1 PS	21.6	24	1	1	1	1	2	22.6	23	1	3	3	1	1
2 PS	23.0	24	2	1	3	2	1	17.4	31	1	1	1	1	1
3 PS	25.0	29	3	1	2	2	1	16.8	36	3	1	2	3	2
4 PS	17.3	44	2	3	3	2	1	14.9	32	3	3	3	2	1
5 PS	34.3	32	1	3	3	2	2	22.9	32	1	2	3	2	2
6 PS	27.0	28	3	2	3	3	2	10.8	28	2	2	1	1	1
7 PS	23.9	24	1	1	1	1	1	19.2	24	1	1	1	1	1
8 TOF	25.7	35	1	3	3	3	3	16.0	32	1	3	3	3	3
9 TOF	21.9	32	1	2	1	2	1	22.2	30	2	2	3	2	3
10 TOF	25.7	29	1	1	1	1	1	25.2	28	1	3	2	2	1
11 TOF	17.0	33	2	1	3	2	1	18.6	26	3	1	4	3	1
12 TOF	17.1	44	2	3	3	2	1	17.1	33	3	1	3	2	2
m±SD	23.4±4.8	30.6±5.7	1.7±0.8	1.8±0.9	2.3±1.0	1.9±0.7	1.4±0.7	18.6±4.0	29.6±3.9	1.8±0.9	1.9±0.9	2.4±1.0	1.9±0.8	1.6±0.8
Normal values ($n=4$)	19.4±4.9	26.2±5.1	1	1	1	1	1	19.4±4.9	26.2±5.1	1	1	1	1	1
P	<0.05	NS	<0.02	<0.02	<0.001	<0.001	NS	NS	NS	<0.02	>0.01	<0.001	<0.005	<0.05

Abbreviations: D = transverse diameter of the myocytes (μm); I = proportion of interstitial tissue; MF = myofibrillar lysis; Mito = mitochondria; Rib = free ribosomes; RER = rough endoplasmic reticulum. The ultrastructural alterations of MF, Mito, Golgi, Rib and RER are estimated as follows: 0 = normal; 1 = moderate change; 2 = mild change; 3 = important change

lar in one case, and valvular in six, associated with a significant infundibular reaction in two instances.

Tetralogy of Fallot was diagnosed in five patients. Two of them had previously undergone a Blalock's operation. The degree of cyanosis was variable: the average aortic oxygen saturation was 88.4% ± 6.3%. The X-ray outlines of the heart were of normal size in three cases and enlarged in two (the CTR ranged from 0.42 to 0.70). Catheterisation (Table 1) revealed equal pressure in both ventricles (98 ± 8 mmHg). Left ventricular weight was normal: 83.0 g/m^2 ± 19.3 g/m^2 vs 74.0 g/m^2 ± 8.7 g/m^2. The EF was altered: 56.0% ± 5.7% vs 65.0% ± 6.0% ($P<0.01$). The angiocardiograms showed that the site of the pulmonary stenosis was infundibular in all cases and in three patients it was associated with valvular stenosis.

Right and left ventricular biopsies were performed with a Tru-Cut* needle (Travenol Laboratories, USA), during surgery, prior to cardiopulmonary bypass. The tissue fragments were immediately immersed in 1% procaine hydrochloride and fixed for 24 h in 2% cold glutaraldehyde in 0.1 M Sörensen's buffer, pH 7.3. After washing, the tissue was post-fixed in 1% osmium tetroxide in phosphate buffer, dehydrated and embedded in Epon. Semithin sections (1 μm thick) were stained with alkaline toluidine blue.

Photographs of cross-sectional areas were taken at a magnification of 250×. The average myocyte diameter was obtained by measuring the shortest diameter of at least 15 cells (Fuster et al. 1977). The quantitation of interstitial tissue was obtained by analysing the micrographs with a semi-automatic digital planimeter (Digiplan*, Kontron). Ultrathin cross-sectional and longitudinal sections were stained with uranyl acetate and Reynold's lead citrate, and examined with a Philips EM 300 electron microscope. A semi-quantitative study of the lesion was performed (Table 2). Data were compared statistically by Student's t test.

Results

The transverse diameters of the myocytes were normal in the right ventricle (23.4 μm ± 4.8 μm vs 19.4 μm ± 4.9 μm). Only the 43 year old patient had an increased myocyte diameter: 34.3 μm. The proportion of interstitial tissue when compared with myocytes was normal in both the right ventricle (30.4% ± 5.9%) and the left ventricle (29.6% ± 3.9%) (Table 2). On semithin sections, the myocytes had a regular, uniform orientation and no areas of disarray were observed. In half of the patients, the myocytes observed by electron microscopy presented no lesions. In the other half, consisting of three tetralogies of Fallot and three pulmonary stenoses, the major alteration was a marked loss of contractile material (Figs. 1–3). The myofibrillae were replaced by cytoplasmic zones particularly rich in glycogen particles with rare mitochondria dispersed among the other organelles. Within the same cell, major modifications coexisted with areas that were completely normal. At times, the myofibrillae themselves were affected: the Z bands were normal, but myofilaments had disappeared to such an extent that empty spaces were observed even inside the sarcomere. All these lesions varied from one cell to another and were distributed randomly. Thus it is necessary to observe many samples in order to quantify the importance of these changes precisely. The number of intercalated discs was not increased, but the defective insertion of myofibrillae was sometimes seen at this level. Myofibrillar lysis was also observed in the right ventricular myocardium. The mitochondria were either normal or small and numerous (Fig. 2). They, nonetheless, did not present structural anomalies. In all cases studied the Golgi apparatus was extremely well developed. In general, a Golgi complex was located at each pole of the nucleus and its saccules and vesicles were more numerous than normal. Sometimes, numerous free ribosomes were observed, dispersed throughout the cytoplasm (Fig. 4). The nuclei presented multiple convolutions in their membranes. Residual bodies were sometimes found. Those, which were plentiful, were small, circular and had only a few concentric lamellae. Others had larger diameters, their form was irregular, they had many lamellae and they were uni or multicentric (Fig. 4). Small residual bodies could be associated with the mitochondria.

Discussion

The aim of this study was to attempt to answer to the following questions: 1. What is the nature of the histological modifications observed in the myocardium in these two cardiac malformations? 2. Above all, what causes the histological modifications of the left and right ventricles?

The ultrastructural modifications that we observed are not specific. They have previously been described by Ferrans (1972) and by Hatt et al. (1979) in their studies of valvular diseases and myocardiopathies. They can be classified into two distinct groups with different consequences. The first group manifest intense metabolic activity: in these cases we observed highly developed Golgi apparatus, rough endoplasmic reticulum, numerous ribosomes, and very active nuclei, which are the ultrastructural signs of hypertrophy. In fact, we noted that the left ventricular myocardial weight is augmented without a modification in the proportion of interstitial tissue. The cell diameter is normal, excepted in the adult patient. It may be that the mechanism of cellular hypertrophy is different in children and that it occurs by increasing the length of the myocytes, but we have not been able to demonstrate this possibility: biopsies represent a tissue mass too limited to allow the isolation of cells and to perform precise morpho-

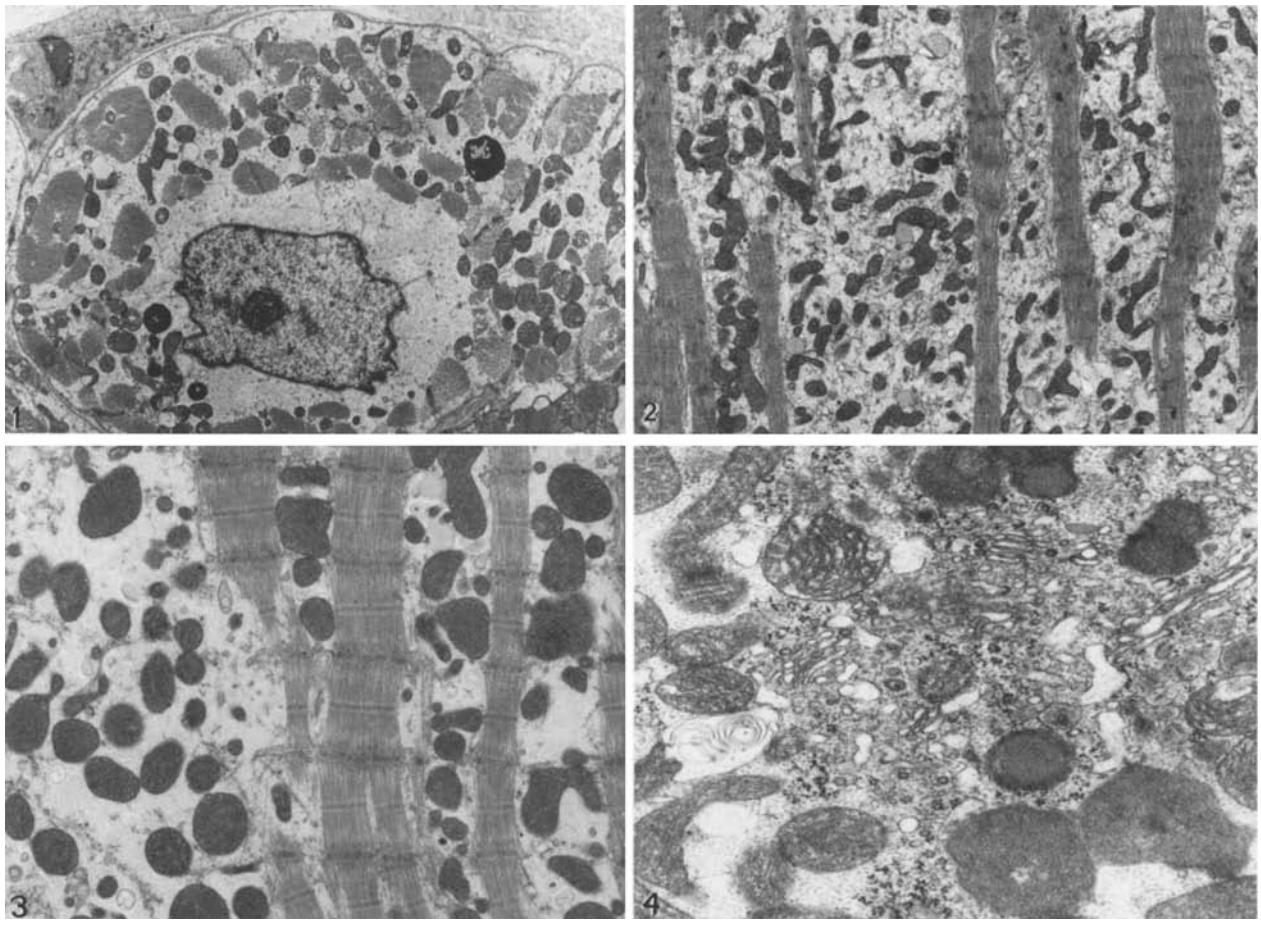

Fig. 1. Myofibrillar lysis. Pulmonary stenosis (obs. n°6). Transverse section of a left ventricular myocyte (× 70000)

Fig. 2. Myofibrillar lysis. Pulmonary stenosis (obs. n°6). Longitudinal section (× 3500)

Fig. 3. Myofibrillar lysis. Tetralogy of Fallot (obs. n°9). Longitudinal section (× 6300)

Fig. 4. Golgi apparatus and numerous free ribosomes. Pulmonary stenosis (obs. n°4). Longitudinal section (× 28000)

metric measurements. In addition, it is possible that during childhood myocytes retain a certain capacity to divide, but nothing permits us to confirm this hypothesis at this time. Astorri et al. (1971); Astorri et al. (1977) has nevertheless demonstrated that this mechanism exists in adults with very severe hypertrophies. In tetralogy of Fallot, the left ventricular mass is normal and we observed the same ultrastructural modifications. We believe that the method for the determination of ventricular mass by means of angiography is too crude to allow the demonstration of small variations.

The second group of ultrastructural modification is represented by myofibrillar lysis which was observed in the right or left ventricle of seven of the patients studied. This is not a phenomenon of adaptation, but is indeed an alteration of the cell. In these cases it cannot be attributed to a hypertrophy which has surpassed the compensatory stage, since it is present in three patients with tetralogies of Fallot in whom the left ventricular masses were normal. No relationship could be established between this observation and either age or aortic oxygen saturation.

We feel that the left ventricular manifestation has a double origin: a physiological reactional hypertrophy, as a consequence of right ventricular hypertrophy; and a congenital myocardial disease, expressed by lesions such as those of myofibrillar lysis or by an authentic cardiomyopathy.

The left ventricular mass was normal in patients with tetralogy of Fallot regardless of their ages and it was significantly increased in those with pulmonary stenosis. Thus it seems that only a ma-

jor pressure overload can induce controlateral ventricular hypertrophy. In fact in the tetralogy of Fallot, the ventricular septal defect plays the role of a haemodynamic regulator; thus the systolic pressure in the right ventricle cannot surpass that of the left ventricle, whereas in the case of isolated pulmonary stenosis, it can reach suprasystemic values. Several experimental studies have attempted to specify the response of the left ventricle to right ventricular hypertrophy. The results vary depending on the authors. Spann et al. (1967) performed banding of the pulmonary artery in 30 cats, and followed up with haemodynamic and anatomic controls, 1 day to 90 days after the initial intervention. Right ventricular hypertrophy appeared after one day, but there was no increase in the left ventricular mass. Archie et al. (1974) also used the technique of pulmonary artery banding in 21 h to 28 h lambs, which he evaluated 5 weeks to 12 weeks later. He did not observe a significant difference between the left ventricular mass of treated animals and those of controls, however, right ventricular hypertrophy was quite important. The protocol used by Laks et al. (1969) is similar in that he studied the ventricular mass in a serie of 8 dogs, 2 weeks to 40 weeks after they had undergone banding. The right ventricular mass increased very early and reached its maximum at 18 weeks and remained stable thereafter. The left ventricular mass increased only after four months and henceforth increased consistantly. A correlation exists between the duration of the banding and the myocardial weight ($r=0.94$, $P<0.001$). The absence of left ventricular hypertrophy in the animals studied by Spann et al. (1967) and Archie et al. (1974) could be explained by the insufficient duration of the observation periods. We have observed no correlation between the ages of our patients and their left ventricular weights. Several authors have demonstrated the presence of left ventricular hypertrophy in pulmonary stenosis. Becu et al. (1976), in a clinical series of 53 patients, found two cases, and Harinck et al. (1977) reported an augmentation of the wall thickness, in 10 out of 16 patients, that was correlated with patient ages. Harinck et al. (1977) suggested that the changes imposed on the haemodynamics of the right heart cause modification of the geometry of the left ventricle. He bases this hypothesis on the experimental studies of the mechanical interactions between the two ventricles. Elzinga et al. (1974) uses a model involving an isolated heart mounted in such a manner that the circulations are independent. He shows that an increase in the right heart preload leads to an elevation of the end diastoc pressure of the left ventricle and an acute decrease of the cardiac output. Using a slightly different protocol, Maruyama et al. (1983) observed a diminution of left ventricular complicance while the right ventricular end-diastolic pressure increase, and a decreased systolic function, evidenced by a lowered pressure peak. Bemis et al. (1974) studied the geometric modifications of the left ventricular cavity as function of the right ventricle filling pressure, by means of an experimental model almost identical to that of Elzinga et al. (1974). When he raised the right ventricular end diastolic pressure, the left ventricular end diastolic pressure increased, the transverse diameter decreased by 4.5% and the anterior-posterior diameter increased 4.4%, but the left ventricular systolic pressure did not vary. These authors stressed that these effects were more striking if the pericardium was kept intact. Kelly et al. (1971) performed pulmonary artery banding and induced tricuspid regurgitation in dogs and studied the haemodynamic effects three weeks later. He noted a decrease in the indices of left ventricle contractility (dP/dt max, VCE max).

These experimental models all differ from pulmonary stenosis and tetralogy of Fallot. They create acute volumetric overload whereas our patients have chronic pressure overloads. Nonetheless, they are most interesting, since they show that the compression of the left ventricle by the right ventricle provokes a decrease in contractility and compliance, thus corresponding to an inversed Bernheim's (1910) syndrome. Severe pulmonary stenoses induce marked concentric hypertrophy of the right ventricle, leading to the outward bulging of the septum into the left ventricular cavity. In fact, Herbert and Yelline (1969), in a series of pulmonary stenosis patients, found that the left ventricular end diastolic pressure was elevated when the hypertrophy involved the septum. The increased left ventricular mass could be a consequence of the flattening of the cavity by the bulging septum, as suggested by Harinck et al. (1977). Indeed, the augmented anterior-posterior diameter demonstrated by Bemis et al. (1974), leads to a rise in the intraparietal pressure (whose value is determined by the following formula: $T=PD/4e$, where P is the systolic pressure, D is the diameter, and e is the wall thickness) and thus to an increase in the work performed by the heart and oxygen consumption. The increased wall thickness and thus the left ventricular weight could be an attempt by the organ to normalize the intraparietal pressure, by acting upon the denominator of the equation.

The cause of myofibrillar lysis that we observed

in 7 patients is an interesting problem. We demonstrated that it is not related to age, to aortic blood desaturation or to ventricular hypertrophy. We propose that the cytological lesions of the right and left ventricular myocardium observed in pulmonary stenosis and in tetralogy of Fallot are a part of a more widespread cardiovascular fetopathy. Cardiac malformations are often associated with a disease of the myocardium or with malformations of other organs. Becker and Anderson (1981), Somerville (1981) and Schneeweiss (1983) report cases of congenital malformation of the heart associated with hypertrophic cardiomyopathy. Pernot et al. (1977) reported on a series of 23 cases of atypical pulmonary stenosis in conjunction with syndromes presenting multiple malformations (Leopard's syndrome, Watson's syndrome); he cites the important proportion of associated cardiomyopathies. We believe that the malformation of the heart (pulmonary stenosis, tetralogy of Fallot) and the myofibrillar lysis which is observed in some patients are two components of a same congenital disease.

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