

# Probing the heart of cardiomyopathies

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Current knowledge of aetiopathogenesis of various forms of human cardiomyopathy is reviewed in this article. There are overwhelming evidences to suggest a pathogenetic link between persistent viral infection, induction of autoimmunity (influenced by immune response determinants in the HLA region) and alteration in the differentiated functions of the myocardium in dilated cardiomyopathy. Genetic studies have identified specific defects in patients with hypertrophic cardiomyopathy but the cellular basis of the disease remains unresolved. Endomyocardial diseases associated with hypereosinophilic syndrome and endomyocardial fibrosis of the tropics appear to be two separate entities. A unifying link among endomyocardial fibrosis of diverse causes could be the uniform response of the cardiac tissue to injuries of heterogenous nature. Application of microtechniques to endomyocardial biopsy promises definitive information on aetiopathogenesis of cardiomyopathies.

CARDIOMYOPATHIES have fascinated cardiologists and basic scientists alike. The reasons are easily found: they offer diagnostic challenge, their pathogenetic mechanisms are elusive, aetiology enigmatic and treatment strategies controversial. A century after the recognition of heart muscle disease as distinct from congenital and acquired valvular, coronary and pericardial diseases, there has been a change in the definition of cardiomyopathy. Myocardial diseases secondary to or part of a systemic disorder are no longer included among cardiomyopathies which are considered as pluricausal or multifactorial and defined as 'heart muscle diseases of unknown cause'<sup>1</sup>.

The standard scheme of classification is based on structural and functional alterations. Though pragmatic for the clinician and the morphologist, the overlapping structural and functional alterations in various forms of cardiomyopathies create difficulties for those who pursue aetiopathogenetic investigations. It is hoped that when precise pathogenetic mechanisms are delineated, the categorization of primary myocardial disease would be more rational. Till then, the WHO classification<sup>1</sup> would remain the reference and provide a basis for the study of disease mechanisms and aetiology (Table 1).

## Dilated cardiomyopathy

Previously known as congestive cardiomyopathy, dilated cardiomyopathy (DCM) is the most common of all

forms of primary myocardial disease and multiple factors are contributory (Table 2).

## Pathologic features

There is dilatation of all cardiac chambers, particularly the left ventricle. Ventricular wall thickness may be normal or moderately increased, but the heart weight is above normal. Most often, patchy thickening of the endocardium and mural thrombi in various stages of organization are present (Figure 1).

Histologic features are nonspecific. Myocyte changes include hypertrophy and nuclear enlargement. Areas of myofibre loss are seen in the myocardium, but myocyte necrosis is not evident. Inflammatory infiltrates are sparse, focal and tend to be interstitial. Fibrosis is nearly always present, and varies from perimyocytic and perivascular to microscopic scars.

Table 1. Classification of primary cardiomyopathies

Dilated cardiomyopathy	Is characterized by ventricular dilatation, modest hypertrophy and severely impaired pump function
Hypertrophic cardiomyopathy	Is recognized by massive hypertrophy often with asymmetrical involvement of the septum, lack of ventricular dilatation and usually with preserved or enhanced pump function, impaired diastolic relaxation, compliance and filling
Restrictive cardiomyopathy	Marked by mild cardiac enlargement with small or normal size ventricular cavity with or without mural endocardial thickening and cavity obliteration, normal systolic function, diminished ventricular compliance and impaired diastolic filling

Table 2. Aetiological classification of dilated cardiomyopathy

Idiopathic
Inflammatory
(i) Infections, e.g. viral (Coxsackie B <sub>2</sub> )
Parasitic ( <i>Trypanosoma cruzi</i> )
(ii) Noninfectious, e.g. Peripartum cardiomyopathy
Toxic
e.g. Alcoholic cardiomyopathy
Cobalt cardiomyopathy
Anthracycline toxicity
Metabolic
e.g. Selenium deficiency
Carnitine deficiency
Familial
Abnormal coronary microvasculature

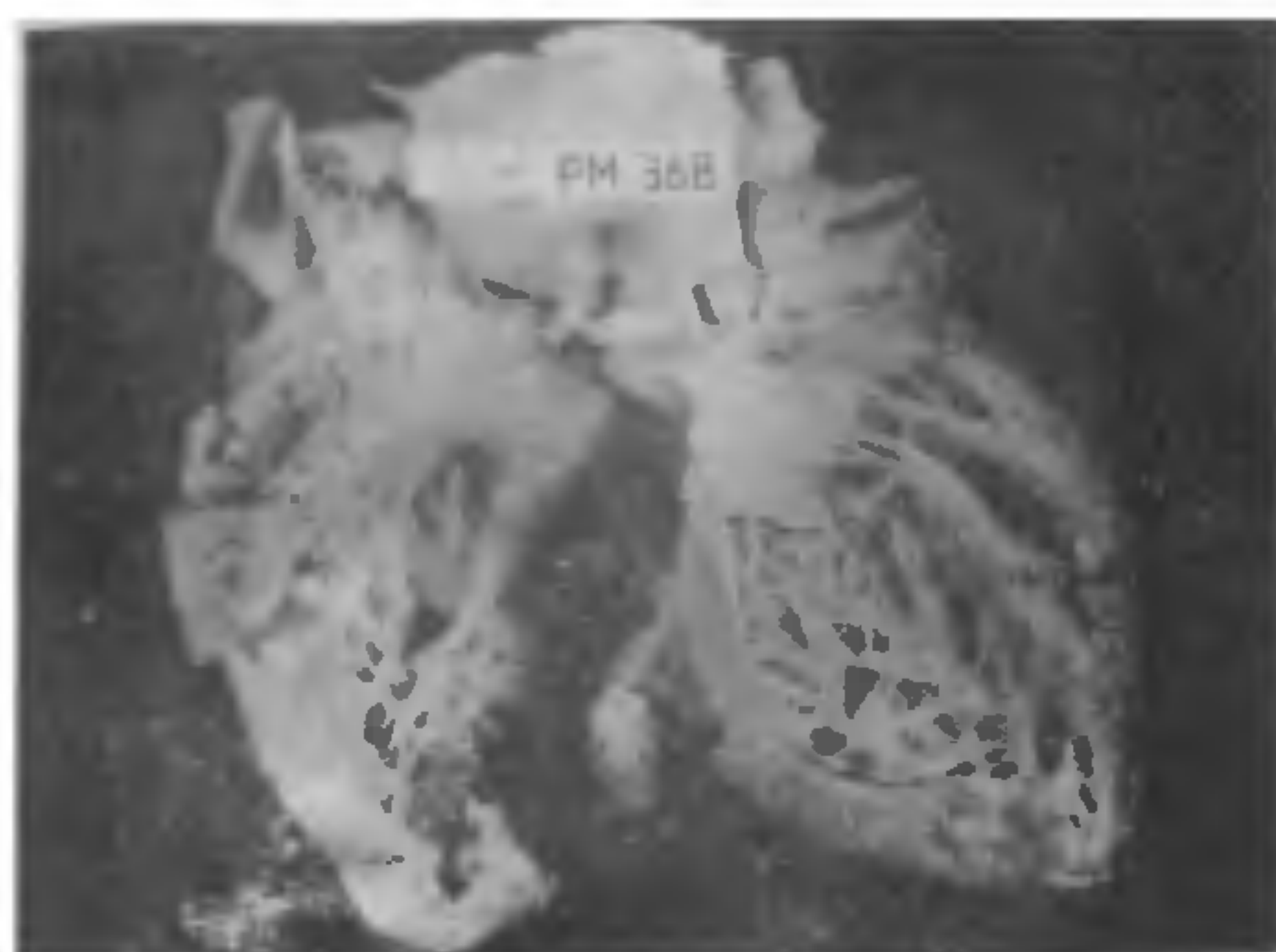


Figure 1. Left ventricular view of an autopsy specimen of dilated cardiomyopathy. Ventricle is dilated, trabeculae are stretched and mural thrombi are seen (arrows).

Electron microscopy findings are those of myocyte hypertrophy as well as myocyte degeneration and hence non-specific.

#### Aetiopathogenesis

A causal relationship between viral myocarditis and idiopathic DCM has been speculated based on circumstantial evidence from clinical, serological, epidemiological and histologic studies<sup>2,3</sup>.

The pathogenetic link between enterovirus myocarditis and DCM has been established by the demonstration, using virus-specific probes of enterovirus RNA sequences in the heart with DCM<sup>4</sup>. After an acute episode of myocarditis, infection can persist through generation of a defective virus, which results from an aberration in the control of viral RNA synthesis<sup>5</sup>. In such instances, neither can the original infectious virus be isolated nor the viral antigens detected in tissues.

A number of studies implicate immune regulatory defects in DCM. Immunologic findings include the presence of a wide array of autoantibodies directed against cardiac tissues, myocyte-specific cytotoxic T cell responses, decreased natural killer cell activity and functional deficiency in the activity of suppressor cells<sup>6-15</sup>. Since such abnormalities relate to patterns of major histocompatibility complex expression, a series of investigations have evaluated HLA A, B, DR and DQ antigen expression in patients with DCM. A positive association with increase in the frequency of HLA DR<sub>4</sub> and HLA DQW<sub>4</sub> was specifically confirmed in a prospective analysis. HLA-DR<sub>4</sub>-DQW<sub>4</sub> haplotype is only seen in patients with idiopathic DCM. The significant increase in HLA DR<sub>4</sub> frequency has also been indicated in a metaanalysis of combined studies<sup>16</sup>. These results raise the possibility that immune response

determinants in the HLA regions may contribute to disease susceptibility or resistance in patients with viral myocarditis.

Most of the autoantibodies in patients with DCM reflect a non-specific response to myocardial injury. However, some of them are directed against proteins critical for pump function of the heart, as for example, intracellular transport proteins, gap junctional proteins and ion channel proteins.

An antibody showing cross reactivity between two-cell surface proteins (the calcium channel and connexin) and a mitochondrial protein, the adenine nucleotide translocator (ADP-ATP carrier protein), has been identified<sup>17</sup>. Functionally, these organ-specific antibodies inhibit nucleotide transport from heart mitochondria by specific binding to the substrate/ligand binding site of the carrier protein<sup>18</sup>. These antibodies can penetrate myocardial cells and disturb energy metabolism<sup>19,20</sup>. Interestingly, the adenine nucleotide translocator, the main autoantigen in viral heart disease, and *Coxsackie B<sub>3</sub>* virus, a causative agent in human viral myocarditis, have homology in their antigenic determinant molecular structure<sup>21</sup>. The demonstration that this homology is translated into immunologic cross reactivity suggests that the sharing of cross reacting antigenic determinants may play a role in the pathogenesis of viral heart disease.

The precise cellular basis of contractile dysfunction in patients with DCM is unknown, but a metabolic cause has been attributed. Abnormalities in calcium handling in the cardiomyopathic tissue may be involved in the pathogenesis of the disease<sup>22,23</sup>. The cause for these abnormalities is not yet established. While a lower rate of calcium uptake by sarcoplasmic reticulum has been suspected by some workers<sup>22,24</sup>, another suggestion is that an increased calcium entry through voltage-dependent sarcolemmal channels and a diminished capacity to restore resting calcium levels during diastole may be responsible<sup>23</sup>. The existence of antibodies against ADP/ATP carrier that cross-react with cardiac calcium channel in patients with DCM suggests that these antibodies which enhance calcium channel activity may contribute to the pathogenesis of DCM by inducing chronic calcium overload<sup>25,26</sup>. The pathophysiological significance of the antibodies has been demonstrated through the use of isolated and perfused working heart preparations, in which the antibodies cause cardiac dysfunction<sup>20</sup>. Immunologic mechanism could also play an important role in modifying the function of sarcoplasmic reticulum, causing a decline in calcium transport in patients with DCM<sup>27</sup>.

Another observation of possible pathophysiological significance in patients with DCM is the presence of autoantibodies that react with cardiac beta adreno-receptors<sup>28</sup>. The development of antireceptor antibodies

has been linked to the presence of DW<sub>4</sub> subtype of HLA DR<sub>4</sub>-phenotype<sup>29</sup>. Possibly as a consequence of antibeta adrenoreceptor antibodies, there is decrease in density of membrane-associated beta-adrenoreceptors in patients with DCM<sup>30</sup>. Additionally, there is marked down-regulation of beta-1 receptors, mild uncoupling of beta-2 receptors from the stimulatory G protein G<sub>s</sub> and mild upregulation of the G<sub>i</sub> protein<sup>31</sup>. An increase of alpha subunits of the inhibitory guanine nucleotide-binding protein might contribute to the reduced effects of endogenous catecholamines in DCM<sup>32</sup>. Alterations in signal transduction pathway through which the receptors stimulate the contractile apparatus could also be the cellular basis of contractile dysfunction<sup>33</sup>.

Recently, autoantigens against alpha and beta cardiac myosin heavy chain isoforms have also been shown to be present in DCM<sup>34</sup>.

## Hypertrophic cardiomyopathy

### Pathologic features

The characteristic morphologic marker of hypertrophic cardiomyopathy (HCM) is a nondilated left ventricle with asymmetrical hypertrophy of its wall, in the absence of any other cardiac or systemic disease capable of producing left ventricular hypertrophy (Figure 2).

Bizarre and disorganized arrangement of the muscle bands, particularly in the ventricular septum, is the histologic hallmark in HCM. There is also abnormality of myocardial cell-to-cell arrangement (disarray), and intramural coronary artery lesions which consist of intimal proliferation and smooth muscle hyperplasia.

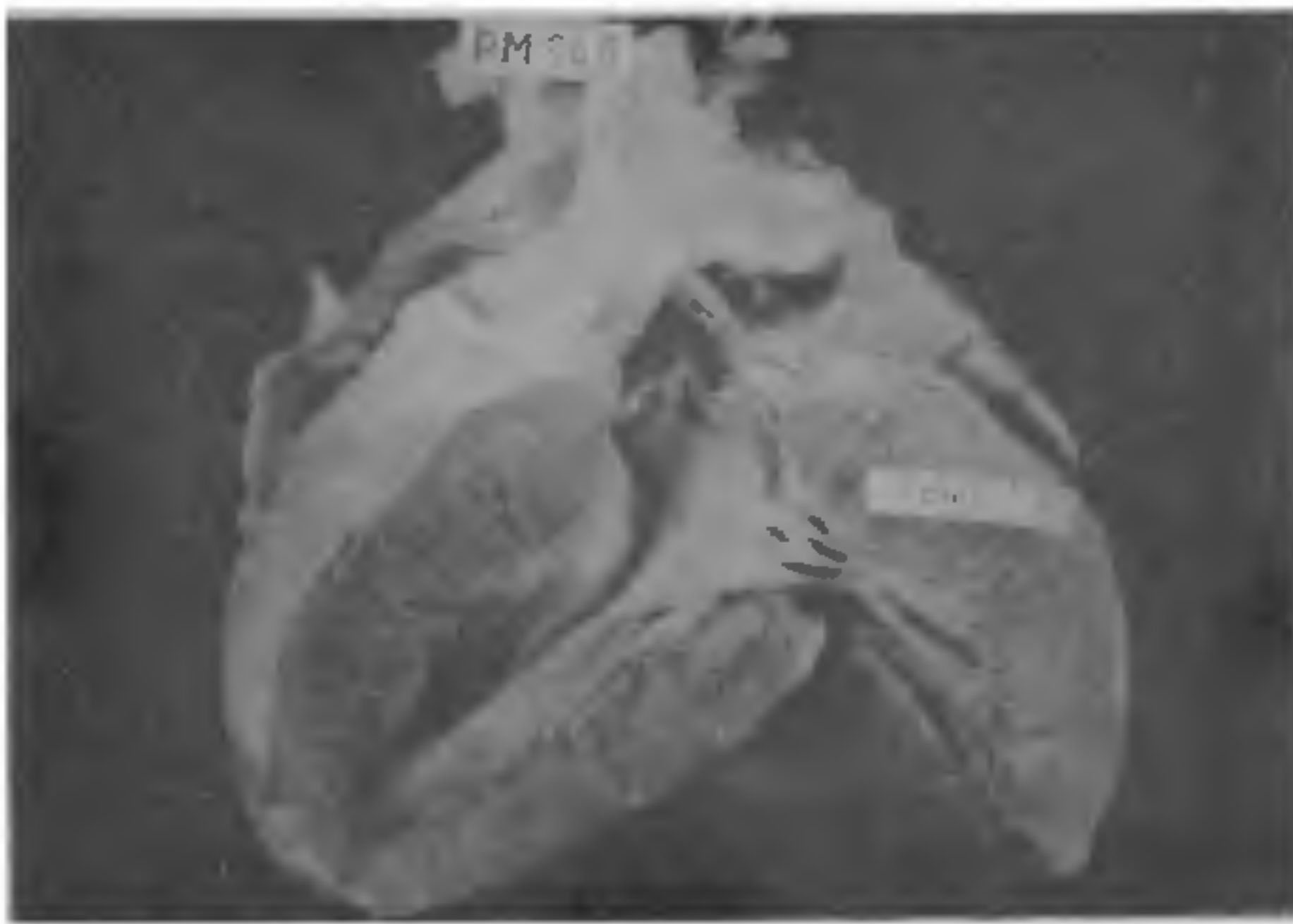


Figure 2. Left ventricular view of an autopsy specimen of hypertrophic cardiomyopathy. Asymmetric hypertrophy with bulging interventricular septum in subaortic region is seen.

Ultrastructural features are those of myofibre hypertrophy and are non-specific.

### Aetiopathogenesis

There are only speculations on the aetiology of HCM.

**Genetics.** Several studies indicate a genetic origin of HCM. The pattern of inheritance is consistent with an autosomal-dominant trait with a high degree of penetrance or of reduced penetrance<sup>35</sup>. Family studies have shown that the phenotype of HCM may vary between affected members of the same family. Age, gender and factors which cause secondary hypertrophy in their own right as well as neurohumoral mechanisms are possibly important factors which influence the degree of gene penetrance in HCM.

An important advance in our understanding of HCM is the discovery that two families with HCM have mutations in the cardiac myosin heavy chain genes. In one, an abnormal alpha-beta chain hybrid gene was present while in the other, a point mutation was identified<sup>36,37</sup>. The mutation would cause arginine to be replaced by a glutamine residue in the functional region of the protein that binds to actin. It has been suggested that the disorganized myofilament structure in HCM is the result of the mutation which may produce a disruptive protein.

Genetic linkage studies report a strong linkage to an area of chromosome 14 in several families<sup>38</sup>. However, there is also evidence, from linkage studies, that familial HCM is a genetically heterogenous disease and that it can be caused by genetic defects in at least two loci<sup>39</sup>. In a recent study based on eight French families, a genetic linkage of hypertrophic cardiac disease allele to alpha-beta myosin heavy chain and to cardiac actin gene was not found. The study suggests that the expression of familial HCM is compounded by genetic heterogeneity<sup>40</sup>.

Several cellular abnormalities are hypothesized for the pathogenesis of HCM. Among them three potential mechanisms have received considerable attention.

(i) *Catecholamine hypothesis.* According to Perloff, familial HCM is caused by a defective response of developing cardiac cells to sympathetic stimulation<sup>41</sup>. Support for this hypothesis is drawn from the demonstration by Pearse in 1964 that norepinephrine content and sympathetic innervation are increased in the operatively excised myocardium from patients with HCM. The hyperdynamic state of the left ventricle and the therapeutic benefit of beta adrenergic blockers in controlling symptoms in most patients as well as the fact that norepinephrine can induce myocardial hypertrophy in cell cultures and animals are compatible with

the speculation. However, there are reports that the histochemical techniques used by Pearse and co-workers were not specific for norepinephrine<sup>42</sup>.

Recent studies have rekindled interest in the concept. Increased rate of production of norepinephrine in the heart and impaired neuronal uptake of cardiac norepinephrine have been demonstrated in patients with HCM<sup>43</sup>. The elevated norepinephrine levels at the neuroeffector junctions of myocardial and vascular smooth muscle cell adrenoreceptors could explain myocyte hypertrophy and smooth muscle hyperplasia in small coronary arteries. As adrenergic stimulation can cause coronary vasoconstriction, secondary myocardial ischaemia could contribute to the extensive scarring in the myocardium.

(ii) *Defective cytosolic calcium regulation.* Impaired ventricular relaxation and the symptomatic relief offered by calcium antagonists prompted the view that increased cytosolic calcium levels may account for the pathogenesis of HCM<sup>44,45</sup>. An interesting evidence for the hypothesis has recently been reported. The density of dihydropyridine-binding sites (dihydropyridine is a prototype of a major class of calcium antagonists which include nifedipine) is elevated in patients with HCM. This increase probably represents an increase in the density of voltage-sensitive calcium channels<sup>46</sup>.

(iii) *Proliferation disease concept.* The concept that HCM is a primary proliferative disorder involving several cardiac cell types originates from the morphologic findings of myofibre hypertrophy, smooth muscle hyperplasia in small coronary arteries, neovascularization and excess myocardial fibrous tissue. Supportive evidences have been obtained by demonstrating potent mitogens, presumably acidic and basic fibroblast growth factors, in the hearts of patients with HCM<sup>47</sup>.

### Restrictive cardiomyopathy

These disorders can result either from myocardial or endomyocardial disease (Table 3)<sup>48</sup>. Endomyocardial diseases are typified by endomyocardial fibrosis with or without eosinophilia.

### Primary restrictive cardiomyopathy

Some patients may manifest features of a restrictive cardiomyopathy in the form of restrictive physiology and yet not exhibit either fibrosis or infiltration in the heart. Pathologic findings include biatrial dilatation, often with thrombi in the atrial appendages, normal left ventricular cavity size and myofibre hypertrophy. Rarely, the disease is familiarly linked. This entity has

Table 3. Classification of restrictive cardiomyopathy

Myocardial diseases
Non-infiltrative
Idiopathic restrictive cardiomyopathy
Infiltrative
e.g. Amyloidosis, Gaucher's disease
Storage diseases
e.g. Haemochromatosis.
Endomyocardial diseases
Endomyocardial fibrosis of the tropics
Endomyocardial disease associated with hypereosinophilia syndrome.
Carcinoid
Radiation induced
Anthracycline toxicity
Methysergide toxicity

been classified as idiopathic primary restrictive cardiomyopathy<sup>49-51</sup>.

### Endomyocardial fibrosis

The commonest cause of restrictive cardiomyopathy in the tropics is endomyocardial fibrosis (EMF), the recognition of which dates back to 1948, when Davies described the disease in Kampala, Uganda<sup>52</sup>.

### Pathologic features

The macroscopic appearances of EMF are distinctive and include monstrous dilatation of the atria which often contain massive organizing thrombi, a characteristic notch on the right border of the heart at the apex, contracted ventricles and increased amounts of epicardial fat<sup>52,53</sup>. There may be associated massive pericardial effusion. Severe endocardial thickening, occasionally calcified, is seen in the ventricles and is either diffuse or focal in distribution (Figure 3).

The thickened endocardium consists of a superficial compact layer of collagen with hyalinization and occasional calcification and a deeper spongy layer of fibrous tissue with numerous capillary channels and scanty lymphocytic infiltrates<sup>54</sup>.

Electron microscopic findings comprise accumulation of cellular and extracellular elements of connective tissue in the endocardial layer, myocyte changes of atrophy and hypertrophy, interstitial fibrosis and presence of myocardial microvascular alterations<sup>55</sup>.

### Aetiopathogenesis

Various factors, viz. nutritional deficiency, serotonin consumption through bananas and plantains, vitamin E deficiency, obstruction of cardiac lymphatics, tropical immunology syndrome, hypersensitivity response to

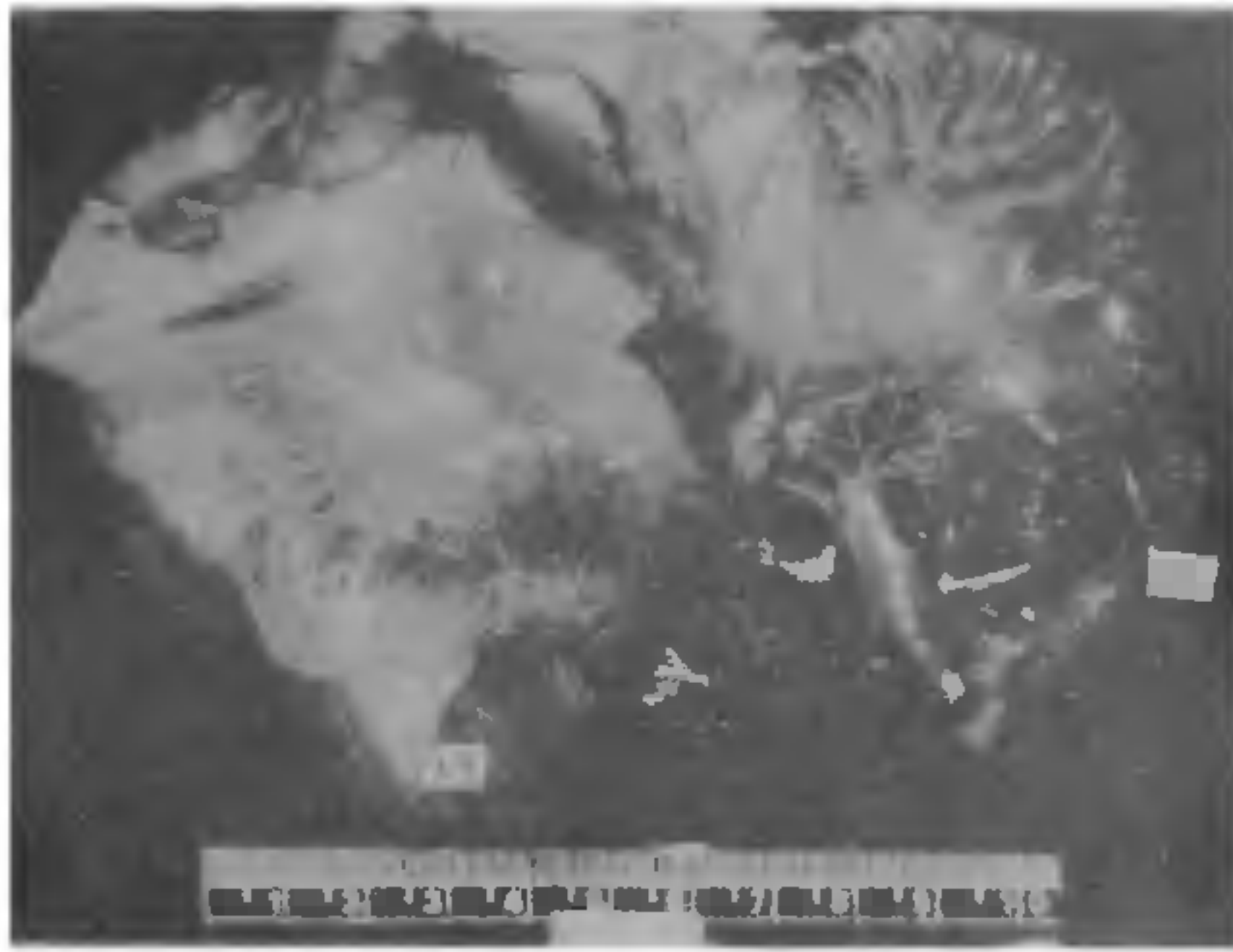


Figure 3 Gross photograph of an autopsy specimen of biventricular endomyocardial fibrosis. There is massive endocardial thickening of the left ventricle and focal involvement in the right ventricle.

malaria and filariasis, viral infections, toxoplasma infection and eosinophilia have all been considered and investigated in the search for the aetiology of EMF in the tropics<sup>56,57</sup>

Among the divergent views on its causation, the view that EMF and Loeffler's endomyocardial disease are cardiac manifestations of eosinophilia and that EMF is the final stage where hypereosinophilia is muted has been aggressively advanced by Olsen and coworkers<sup>58</sup>. But any role for eosinophilia as a determinant of tropical EMF is still unproven. Several workers have recently suggested that the concept should be abandoned<sup>59</sup>.

The geographic distribution of EMF in Kerala and its spatial coincidence with laterite and monazite soils prompted analysis of cardiac tissue for elemental constituents of laterite and monazite<sup>60</sup>. The observation of magnesium deficiency and elevated levels of cerium in cardiac tissues of patients with EMF led to the geochemical hypothesis<sup>61</sup>. The enhancement of cerium level in tissues by magnesium deficiency has been demonstrated in an animal model<sup>62</sup>. Studies on the molecular basis of cerium-induced myocardial injury demonstrate that cerium can functionally mimic magnesium in biological reactions, that cerium cytotoxicity is enhanced by magnesium deficiency and that cerium at nanomolar levels can stimulate collagen synthesis in cardiac fibroblasts<sup>63-65</sup>

New insight into the pathogenesis of EMF is offered by a recent study of macroscopically uninvolved portions of the heart with EMF<sup>66</sup>. An increase in interstitial cellularity and interstitial matrix components along with predominant perivascular and interfibre fibrosis in the absence of significant myofibre lesions and inflammation suggests that EMF is an interstitial heart disease and the fibrosis, reactive in nature.

Progress of lesions in endomyocardial disease

associated with hypereosinophilic syndrome and the role of the eosinophil are better delineated. An acute necrotic phase characterized by striking eosinophilic infiltrates is followed by a thrombotic phase and a late fibrotic stage which is indistinguishable from EMF seen in the tropics<sup>55</sup>. Initial studies confirmed that most of the patients with cardiac damage have hypogranulated eosinophils in the circulation and raised serum levels of eosinophil-derived cationic and major basic proteins<sup>67</sup>. Moreover, toxic effects of eosinophils on myocardial cells have been demonstrated *in vitro*<sup>68</sup>. There is also immunocytochemical evidence for the presence of eosinophil granule proteins in cardiac tissues of patients with eosinophilic endomyocardial disease<sup>69</sup>.

### Arrhythmogenic right ventricular cardiomyopathy

Arrhythmogenic right ventricular cardiomyopathy is a recently described entity characterized by an electrical instability of the right ventricular myocardium due to fibrous and/or fatty replacement<sup>70</sup>.

#### *Pathologic features*

The striking feature is the isolated right ventricular involvement and the localized or diffuse atrophy of its free wall. Two distinct forms are identified on histology. Myocardium is replaced either by lipomatous or fibrolipomatous tissue in which residual myocytes are seen scattered. Occasionally, myocyte vacuolization, necrosis and infiltration by macrophages are seen. Nuclear abnormalities are absent.

#### *Aetiology*

The cause of the disease is unknown. It is considered to be an acquired progressive atrophic condition. Reports of familial occurrence suggest a genetic disorder with autosomal dominance and variable expression and penetrance<sup>71</sup>.

### Infantile cardiomyopathy

#### *Pathologic features*

Evident abnormalities include increased heart weight and nodular areas of yellowish white discoloration of the myocardium<sup>72</sup>. Endocardial thickening, cardiac dilatation and mural thrombi may be present.

The characteristic histologic finding is the presence of large round or oval cells (20-40  $\mu$ m in diameter) which resemble foamy histiocytes and lack myofibrils, seen singly or in clusters in the interstitium.

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Ultrastructurally, the cells lack myofibrils, sarcoplasmic reticulum and T-tubules, but have marked increase in the volume fraction of mitochondria and accumulation of glycogen and lipids. Aetiopathogenesis is unknown.

### Predictions for the future

Technologic advances and the availability of powerful techniques have enhanced our ability to study the causative factors, pathogenesis and natural history of various forms of cardiomyopathies. Endomyocardial biopsy would offer a distinct advantage to study the pre-heart failure stages of heart muscle disease. Recent trends suggest that biopsy would be used to define cellular and molecular abnormalities and the biochemical pathology of cardiomyopathies. From phenomenological data, the next decade would bring in aetiologically definitive information. Biochemical analysis to study energy metabolism, molecular biology techniques for identification of viral genomes and radioactive ligand assays for hormone receptors are being applied to endomyocardial biopsy specimens<sup>73-76</sup>. Viable myocytes have been isolated from biopsy specimens for cell culture studies<sup>77</sup>.

Future research would address the following questions<sup>2, 78</sup>.

- (1) How do viral antigens or autoantigens interact with HLA peptides and T-cell receptor complex?
- (2) What is the mechanism by which altered immunity produces myocyte injury in dilated cardiomyopathy?
- (3) What is the link between the genetic defect and the many cellular dysfunctions in hypertrophic cardiomyopathy?
- (4) Could restrictive cardiomyopathy be an interstitial heart disease?
- (5) Can specific genetic and biochemical diagnostic markers be found for various types of cardiomyopathy?

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## Polyamine biosynthesis inhibitors: New protectants against fungal plant diseases

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Polyamines (PAs) have been shown to be involved in a variety of growth and developmental processes in a wide range of organisms, including fungal systems. Because of their vital role in cell proliferation and differentiation, the specific inhibition of PA biosynthesis has provided a novel approach for new therapies. The PA biosynthesis inhibitors like  $\alpha$ -difluoromethylornithine (DFMO), an inhibitor of ornithine decarboxylase have been shown to be very effective protectants against various kinds of fungal plant diseases, especially rust infections and

powdery mildews. DFMO is highly potent, persistent, fast acting and translocatable. Interestingly, DFMO is non-phytotoxic and has no effect on the growth and endogenous PA pools of the host plants. It thus affords a possible new means of controlling plant diseases of fungal origin in the future, and may generate a new class of target-specific pesticides for use in plant disease control. The current status of this newly emerging field is reviewed here.

POLYAMINES (PAs) are one of the most important and interesting groups of naturally occurring polycationic low molecular aliphatic nitrogenous compounds that are present in all cells<sup>1</sup>. The most common PAs are the diamines putrescine (PUT) and cadaverine (CAD),

triamine spermidine (SPD) and tetraamine spermine (SPM) (Figure 1). In general, prokaryotic cells contain fairly large amounts of PUT, small quantities of SPD and no SPM, while eukaryotes have little PUT, more SPD and considerable SPM<sup>2</sup>. In addition to the above