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Editorial Comment

Toward an Understanding of the Molecular Basis of Cardiomyopathies*

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In the early 20th century, hamsters performed acrobatics in streetside circuses in China (1). Scientists found these Chinese hamsters useful as hosts for some infectious diseases, but the strain could not be successfully bred in captivity. One of the hamster's Middle Eastern cousins, the Syrian hamster, was successfully raised in the laboratory and has become a widely used species for study of a variety of human diseases. In 1962, Homburger et al. (2), at the Bio-Research Institute in Cambridge, Massachusetts, described muscular dystrophy (a hereditary muscle disease that is transmitted in an autosomal recessive manner) in a strain of Syrian hamsters (Bio 1.5). Since then, the Bio 14.6 and its descendant lines have become the most intensively studied strain. In addition to skeletal muscle involvement, these myopathic hamsters have progressive cardiac failure. Heart involvement is the most prominent feature of the disease, and premature death occurs in most animals from congestive heart failure. At autopsy, the animals have anasarca, pulmonary congestion and dilated hearts. This Syrian cardiomyopathic hamster has become widely accepted as a model for cardiomyopathy leading to congestive heart failure.

Syrian hamster cardiomyopathy. The cardiac disease can be divided into four phases (3). During the first or prenecrotic phase, the animals appear well and there is no pathologic evidence of disease. The second phase begins when the animals are about 30 days of age, and is characterized by the appearance of focal myocardial necrotic lesions. During this phase the animals still appear well and there is almost no mortality. However, electrocardiographic (ECG) abnormalities can be seen (4). At about 90 to 120 days of age, many of the necrotic lesions have healed, few new lesions appear and hypertrophy of the heart begins. In this phase, clinically evident disease is usually absent, but some animals die suddenly and are found to have intramyocardial white streaks following the direction of the muscle fibers. These streaks represent calcification of the degenerating muscle. The fourth, or terminal, phase is marked by cardiac dilation and overt congestive heart failure. The animals develop anasarca and pulmonary edema and death soon follows. Skeletal muscle lesions occur earlier than the cardiac lesions, and the animals exhibit progressive skeletal muscle weakness, but the cardiac disease is the most prominent feature of the clinical presentation.

The initial muscle lesions consist of focal myocytolysis in which myocyte integrity is lost through slow loss of myofibrils and is replaced by an amorphous material (3). Gradually, all that is left is the shell of the sarcolemma, or cell membrane, which eventually collapses and becomes indistinguishable from the connective tissue of the heart. Another type of lesion appearing during the peak of the second phase consists of larger foci of necrotic cells accompanied by marked cellular infiltration. Contraction band necrosis is often seen with this lesion. Later heart lesions show calcification and fibrosis.

Role of calcium overload in myocardial necrosis. Although calcification is overtly present only in the later lesions, calcium may be active in all three types of lesions. The lesions closely resemble those seen in other pathologic states where cellular calcium overload produces necrosis. For example, reperfusion injury commonly produces contraction bands (5) with later myocytolysis (6), and the same lesions can be seen in catecholamine-induced cardiomyopathy (7). In humans, such lesions are seen 1) after coronary bypass surgery in regions reperfused by the patent bypassed coronary artery (8); 2) in fatal sclerodermatous heart disease in which vascular spasm may lead to ischemia and reperfusion (9); and 3) in cardiomyopathy associated with pheochromocytoma (10,11). In both reperfusion injury and catecholamine cardiomyopathy, calcium overload is thought to play a major role in producing the characteristic histologic appearance. Thus, it is not surprising that several investigators have measured increased levels of calcium uptake in the myocardium of cardiomyopathic hamsters at a time when lesions are first appearing (12,13).

Other lines of evidence point to calcium overload as a major determinant of the pathologic features of this disease. Prolongation of action potential duration has been demonstrated in cardiomyopathic hamsters (14) and could be explained by an enhanced slow inward calcium current through calcium channels. Factor et al. (15) have presented impressive evidence that microvascular spasm is prominent in this disease. Such spasm may play a role in the development

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of necrosis, perhaps as a consequence of ischemia with reperfusion. The vasospasm could be caused by increased calcium ion fluxes across vascular smooth muscle membranes.

Protective effects of verapamil. Persuasive evidence of a calcium-mediated mechanism of injury is the prevention of many of the features of the disease by early administration of the calcium slow channel blocking agent, verapamil. Jasmin and Solymoss (16) reported that administration of verapamil before the appearance of necrotic lesions prevented their appearance. These investigators found that other calcium channel receptor antagonists had similar but less profound protective effects, as did feeding the animals a calcium-deficient diet. Lossnitzer et al. (12) confirmed these protective effects of verapamil against the development of necrosis and correlated it with prevention of myocardial calcium overload as measured by ^{45}Ca uptake. Several investigators have shown that verapamil prevents the occurrence of functional abnormalities in addition to protecting against the necrotic lesions. Factor (15) and Figulla (17) and their colleagues showed that verapamil prevents microvascular circulatory abnormalities seen in the cardiomyopathic hamsters, and Rouleau et al. (18) found preserved myocardial contractility in verapamil-treated myopathic hamsters. Even hamsters in advanced stages of the disease apparently benefit from verapamil treatment. Markiewicz et al. (19) found that short periods of verapamil treatment are sufficient to ameliorate functional and high energy phosphate metabolic abnormalities in animals with early cardiac failure. Educated with these observations, a number of investigators have studied various myocardial cellular elements responsible for electrolyte movement inside or outside of the myocardial cell. Most observed differences were identified after pathologic changes were present and were limited to observations of the heart (20–23).

Recently, the dihydropyridine binding site related to the voltage-dependent slow channel for calcium entry has been identified and studied by radioligand binding techniques (24). One unifying hypothesis for both the myocardial and vascular lesions would be an increase in the number of calcium channels (and thereby increased calcium entry) indexed by dihydropyridine binding sites. In studies reported in 1986 (25), our group found an increased number of binding sites for the dihydropyridine [^{3}H]nitrendipine in the heart and brain of 30 day old Syrian hamsters. We also found an increase in [^{3}H]desmethoxyverapamil binding. This compound labels a second type of calcium antagonist receptor allosterically linked to the dihydropyridine receptor (26). However, other receptors not related to the calcium channel studied by radioligand binding including muscarinic cholinergic receptors, alpha$_1$ adrenoceptors, dopamine D_2 receptors, adenosine A_1 receptors and beta-adrenoceptors showed no increase. Finkel et al. (27) found similar results nearly simultaneously. Kobayashi et al. (28) in this issue of the Journal report an increase in the number of dihydropyridine binding sites in the hearts of myopathic animals and a normal number of adrenoceptors in the early stages of the disease. Recently, calcium uptake mediated by Na^+–Ca^{2+} exchange was found to be enhanced in cardiac sarcolemmal and brain nerve terminal membrane (synaptosome) preparations from myopathic hamsters (29). However, direct physiologic evidence that cellular calcium overload occurs because of the increase in calcium channels or through Na^+–Ca^{2+} exchange has not been obtained, and the pathologic consequences of the hypothesized calcium overload in muscle have not been explicitly defined.

Microvascular spasm followed by reperfusion could also contribute to cardiac myocyte calcium overload. Perfusion abnormalities of the microcirculation have been observed in the cardiomyopathic hamster (15,17) and their vascular smooth muscle exhibits increased contractility in response to pharmacologic stimulation (30). These phenomena could be caused by increased calcium uptake through the calcium channel of vascular smooth muscle. The salutary effects of verapamil could be explained by the potent antispasm action on vascular smooth muscle as well as an effect on myocardial cells. At this point, it is not possible to distinguish between a direct effect on the myocardial cell and a secondary effect mediated by vascular smooth muscle spasm. Because smooth muscle from the esophagus of these animals has increased dihydropyridine binding (25), it is possible that smooth muscle from small vessels might share the same abnormality.

Other studies of the pathogenesis of Syrian hamster cardiomyopathy have emphasized biochemical alterations, including abnormalities in high energy phosphate metabolism (31–34) and contractile protein abnormalities (35,36). However, these findings have largely been in older animals with more advanced stages of the disease. These abnormalities could thus be secondary phenomena of failing cardiac muscle rather than underlying mechanisms of the disease.

The study by Kobayashi et al. (28) This study not only confirms the observation that calcium antagonist receptors are increased in cardiac tissue in the early stage of the disease but also substantiates that early administration of verapamil ameliorates the myopathy. Although some investigators (37,37a) have not found this increase in dihydropyridine binding sites, we are now aware of a third confirmatory study (38).

Kobayashi et al. also suggest a role of free radical production in the pathogenesis of the disease. Recently, linkage between calcium overload and free radical injury related to myocardial reperfusion has been substantially strengthened. Reperfusion after ischemia results in a burst of oxygen radicals (39). Free radicals will inactivate the calcium adenosinetriphosphatase responsible for calcium removal from the myocardial cell (40) and may possibly lead to enzyme changes that increase calcium entry (41,42). Pathologic findings after

myocardial ischemia induced by coronary occlusion and reperfusion are similar to those found in the early stages of the hamster cardiomyopathy, namely, myocytolysis and contraction band necrosis. Thus, at least part of the myopathy of the Syrian hamster might be explained by a process proposed by Sonnenblick et al. (43): coronary spasm produces recurrent ischemia and reperfusion of myocardial cells prone to calcium overload. This would explain the histologic picture of calcium overload and reperfusion-like injury in the early stages of the disease, but it is not clear that this would explain the later hypertrophic phase and dilated myopathy. Ongoing ischemia produced by coronary spasm with secondary necrosis and reactive hypertrophy is a possible cause. An enhanced inotropic state caused by increased cell calcium with increased wall stress may also provide the stimulus for hypertrophy and later cardiac failure.

Clinical implication. A human paradigm may exist in that human hypertrophic cardiomyopathy is a disease in which abnormalities of myocyte calcium handling appear to play a role. In many patients with hypertrophic cardiomyopathy, abnormal diastolic function improves after administration of verapamil. The possibility that these patients might have an increase in trans-sarcolemmal calcium flux similar to that in the hamsters is worth further investigation.

Thus, the Syrian hamster cardiomyopathy appears to provide one vehicle for understanding human cardiac diseases. There is a need to further understand the interplay of the calcium channel, calcium overload and free radical generation in creating the myopathy of these hamsters. New molecular biology approaches applied to the Syrian hamster should be able to identify a specific gene defect in channel regulation if the increased number of dihydropyridine binding sites in cardiac and smooth muscle is the primary mechanism. The possible relevance of abnormal cellular calcium handling to heritable hypertrophic myopathy has already been mentioned, but this model may also provide insights into human disorders of ischemia and reperfusion, including scleroderma, in which spasm of small arteries is suspected. These hamsters may provide more than entertainment in the Chinese circus.

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