Possible Mechanisms Involved in the Development of the Calcium Paradox

T. J. C. RUIGROK

Department of Cardiology, University Hospital, Catharijnesingel 101, 3511 GV Utrecht, The Netherlands

Abstract. Reperfusion of an isolated heart with calcium-containing solution after a short period of calcium-free perfusion may result in excessive influx of calcium into the cells and irreversible cell damage (calcium paradox). This paper describes the possible routes of calcium entry that occurs during the phase of calcium repletion, and the possible mechanisms involved in the development of the calcium paradox damage. The routes of calcium entry include the glycocalyx, the slow channels, the Na^+—Ca^{2+} exchange mechanism, passive diffusion, and abnormal sites of calcium entry. In addition to an increased influx of calcium, a loss in the ability of the sarcolemma to remove calcium from the cells may contribute to the net gain of tissue calcium. The calcium paradox damage itself, which follows the massive influx of calcium into the myocardial cells, may be a result of calcium-triggered energy dependent reactions and a concomitant acidification of the cytoplasm. Mechanical factors may also be involved in the development of the calcium paradox.

Key words: Calcium paradox — Glycocalyx — Slow channels — Na^+-Ca^{2+} exchange — Na^+/K^+-ATPase

Introduction

Ringer (1883) demonstrated that contraction of the frog heart rapidly ceased during calcium-free perfusion at room temperature, and was restored by the addition of calcium. Similar experiments with isolated rat hearts at a temperature of 37 °C were performed by Zimmerman and Hülsmann (1966). During perfusion with a calcium-free solution contractility was abolished, while electrical activity was maintained. Reintroduction of calcium into the extracellular fluid did not result in recovery of contraction of the heart, but in irreversible loss of electrical and mechanical activity, and myocardial cell death. This phenomenon is known as the calcium paradox (Zimmerman and Hülsmann 1966).

Presented at the 2nd International Symposium on Ionic Channels and Membrane Receptors, 21—26 May 1984, Smolenice, Czechoslovakia

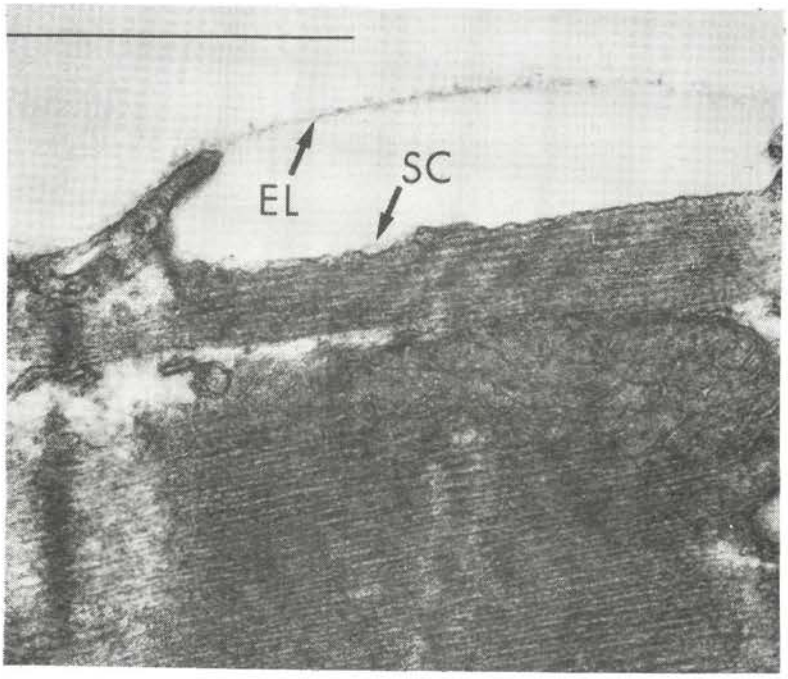

Fig. 1. Rat heart after calcium-free perfusion at 37 °C. This micrograph shows a separation of the external lamina (EL) from the surface coat (SC) of the glycocalyx, resulting in the formation of a cell surface bleb. Bar = 1μ.

The calcium paradox is characterized by an excessive influx of calcium into the myocardial cells (Alto and Dhalla 1979), exhaustion of tissue high-energy phosphates (Boink et al. 1976; Bulkley et al. 1978), massive release of enzymes (Zimmerman and Hülsmann 1966; Hearse et al. 1978b), and extensive ultrastructural damage including severe contracture of the myofilaments and swelling of the mitochondria with formation of electron-dense particles (Zimmerman et al. 1967; Muir 1968). When the temperature during the calcium-free period is reduced, the calcium paradox is less evident (Holland and Olson 1975; Boink et al. 1980; Baker et al. 1983). The paradox has been demonstrated in perfused rat, rabbit, guinea-pig, mouse and dog heart (Zimmerman et al. 1967; Hearse et al. 1978a), in superfused strips of the human heart (Lomský et al. 1983), and also in the frog heart although the amphibian heart is more resistant to the calcium paradox than the mammalian heart (Ruigrok et al. 1983).

This paper describes the possible routes of calcium entry that occurs during the phase of calcium repletion, and the possible mechanisms involved in the development of the calcium paradox damage.

Routes of calcium entry

The possible routes of calcium entry that occurs during the phase of calcium repletion have recently been reviewed (Grinwald and Nayler 1981; Nayler et al. 1983a). These routes include the glycocalyx, the slow channels, the Na^+—Ca^{2+} exchange mechanism, passive diffusion, and abnormal sites of calcium entry.

The glycocalyx

The most conspicious ultrastructural alteration brought about by a short calcium-free period is a separation of the external lamina from the surface coat of the sarcolemma (Fig. 1) (Muir 1967; Frank et al. 1977; Crevey et al. 1978; Hearse et al. 1978b; Ashraf 1979; Frank et al. 1982). Under certain conditions that prevent or attenuate the magnitude of the paradox, the normal ultrastructure of the sarcolemma is maintained (Frank 1983). Calcium depletion at 18 °C in the adult rabbit septum prevents separation of the external lamina from the surface coat (Frank et al. 1982) and it is well known that the calcium paradox is less evident at low temperatures (Holland and Olson 1975; Boink et al. 1980; Baker et al. 1983). Addition of 50 μmol/l calcium and other divalent cations during the calcium-free perfusion period has a protective effect on the paradox and prevents glycocalyx separation (Frank et al. 1982; Rich and Langer 1982). The order of effectiveness (calcium > cadmium > manganese > cobalt > magnesium) is related to the ionic radius, with those cations whose radii are closest to that of calcium exerting the greatest protective effect (Rich and Langer 1982). Finally, hearts from neonatal rats do not exhibit significant separation of the glycocalyx even after 20 minutes of calcium depletion (Frank and Rich 1983). These neonatal hearts are insensitive to calcium depletion, as is evident from the return of contractile function and the lack of enzyme release on return to calcium (Chizzonite and Zak 1981).

However, the role for the glycocalyx in regulating calcium permeability is controversial (Isenberg and Klockner 1981; Nayler and Grinwald 1982; Nayler et al. 1983a; Nayler et al. 1983b). When the calcium paradox is evoked in the presence of barium the glycocalyx appears to remain intact but the cells still become overloaded with calcium (Nayler and Grinwald 1982). Moreover, cobalt and manganese do not prevent the glycocalyx separation but do protect against the paradox, as indicated by an absence of calcium overload (Nayler et al. 1983a; Nayler et al. 1983b).

The slow channels

The question of whether the enhanced uptake of calcium during the repletion phase is due to an excessive entry of calcium through the slow channels remains controversial. Some investigators have reported that calcium antagonists reduce the loss of proteins and the ultrastructural damage during the calcium paradox (Hearse et al. 1980; Ashraf et al. 1982a, 1982b; Baker and Hearse 1983). Other

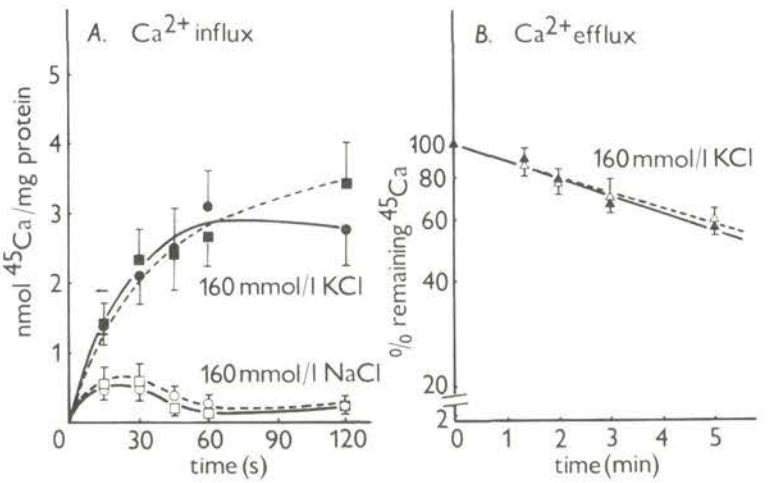

Fig. 2. Time-course of Na^+ dependent Ca^{2+} uptake and passive Ca^{2+} efflux in sarcolemmal vesicles isolated from control perfused and Ca^{2+}-free perfused hearts. (*A*) Vesicles isolated from control perfused (●, ○) and Ca^{2+}-free perfused (■, □) hearts were suspended in 160 mmol/l NaCl, 20 mmol/l MOPS (pH 7.4). Ca^{2+} uptake was assayed by 31-fold dilution in either 160 mmol/l KCl (●, ■) or 160 mmol/l NaCl (○, □) containing 50 μmol/l $^{45}CaCl_2$ and 20 mmol/l MOPS (pH 7.4). (*B*) After 2 min of Ca^{2+} uptake by the Na^+/Ca^{2+} exchange, vesicles isolated from either control perfused (▲) or Ca^{2+}-free perfused (△) hearts were diluted 25-fold in 160 mmol/l KCl containing 0.1 mmol/l EGTA plus 20 mmol/l MOPS (pH 7.4). A first-order plot of the log % of ^{45}Ca remaining in the vesicles is shown. Bars indicate SEM; $n = 7$ for control perfused and $n = 6$ for Ca^{2+}-free perfused hearts. From Lamers and Ruigrok (1983), by permission.

investigators, however, have demonstrated that calcium antagonists do not prevent the massive gain in tissue calcium during the calcium paradox (Alto and Dhalla 1979; Nayler and Grinwald 1981; Ohhara et al. 1982; Meno et al. 1984), although the time course of the events that occur during the development of the paradox may be altered. Verapamil has an energy-sparing effect and reduces the decrease of the mean sarcomere length during the first two minutes of reperfusion (Ruigrok et al. 1980). A reduction of the rate of calcium entry during the early phase of calcium repletion may be responsible for these effects.

It has been argued that the slow channels are not the main route of calcium entry during the calcium paradox because (a) a raised intracellular calcium concentration inactivates these channels; (b) extracellular calcium depletion converts them to sodium channels; (c) concentrations of the calcium antagonists which are sufficient to inactivate the slow channels do not attenuate the gain in calcium that occurs when calcium is readmitted; and (d) calcium antagonists may have other properties apart from their ability to inhibit slow channel transport (Grinwald and Nayler 1981; Nayler et al. 1983a).

Table 1. 5'-Nucleotidase and Na^+/K^+-ATPase activities in control and Ca^{2+}-free perfused rabbit heart

	5'-nucleotidase (nmol/mg per min)		Na^+/K^+-ATPase (μmol/mg per hour)	
	control perfused	Ca^{2+}-free perfused	control perfused	Ca^{2+}-free perfused
n	7	6	7	6
homogenate	1.93 ± 0.18	2.04 ± 0.15	—	—
sarcolemma	12.3 ± 1.9	15.4 ± 2.3	5.5 ± 0.8	1.4 ± 0.5*
purification	6.3 ± 0.7	7.6 ± 1.1	—	—

* Significantly ($P < 0.005$) different from the corresponding values in control perfused hearts. From Lamers and Ruigrok (1983).

The Na^+—Ca^{2+} exchange mechanism and passive diffusion

It has been proposed that calcium entry through the Na^+—Ca^{2+} exchange mechanism is an important factor in the development of the calcium paradox (Dhalla et al. 1983). However, no difference was found in specific activity of the Na^+—Ca^{2+} exchange mechanism in a crude preparation of sarcolemmal vesicles isolated from calcium-depleted rabbit hearts compared with control perfused hearts (Fig. 2*A*). Likewise, the passive calcium efflux from sarcolemmal vesicles showed rates that were identical with control values, indicating that the calcium permeability of the sarcolemma is not affected by calcium-free perfusion (Fig. 2*B*) (Lamers and Ruigrok 1983; Lamers et al. 1984). It cannot be excluded that the isolation method selected a distinct part of the sarcolemma from the calcium-depleted heart that had no modified permeability barriers to calcium and that another part of the sarcolemma with altered properties was lost during the isolation procedure. Another possibility is that reconstitution processes during the isolation affected membrane permeability properties (Lamers et al. 1984).

Abnormal sites of calcium entry

Excessive influx of calcium during the reperfusion phase may occur through damaged areas of the intercalated discs. It has also been proposed that ion selective channels become distorted in response to calcium depletion and develop an increased carrying capacity for different ions (Grinwald and Nayler 1981).

Loss in ability of the sarcolemma to remove calcium from the cytosol

The previous mechanisms involved an increased influx of calcium into the cells.

Calcium-depleted rabbit hearts have been used to study the biochemical properties of sarcolemma and sarcoplasmic reticulum (Lamers and Ruigrok 1983; Lamers et al. 1984). Na^+/K^+-ATPase activity in sarcolemma isolated from hearts after 10 minutes of calcium-free perfusion is reduced by 75 % compared with the

control activity (Table 1). This reduction is not due to a diminished purity of the sarcolemmal vesicles isolated from these hearts because the relative specific activity of another sarcolemmal marker 5'-nucleotidase was similar in both membrane preparations. This result is consistent with the observation that calcium depletion leads to a loss of tissue potassium and a gain in tissue sodium (Crevey et al. 1978; Alto and Dhalla 1979; Goshima et al. 1980; Cheung et al. 1982; Nayler et al. 1983).

Moreover, calcium depletion causes a decrease in calcium pumping activity of the sarcoplasmic reticulum as estimated in total homogenate (Lamers and Ruigrok 1983), although such a decrease has not been observed in a purified preparation of the sarcoplasmic reticulum (Alto and Dhalla 1981).

These results provide evidence that the net calcium gain of the cells during calcium repletion may be associated, in part, with a loss in the ability of cells to remove calcium from the cytosol.

The calcium paradox damage

Energy dependence

By perfusing isolated rat hearts under anoxic conditions with and without substrate, and by reoxygenating hearts after an anoxic period, the effect of different energy states of the heart on the capacity to develop the calcium paradox has been studied (Ruigrok et al. 1978). Myocardial cell damage was quantitated in terms of release of creatine kinase (CK).

After 35 minutes (30 minutes with calcium and 5 minutes without calcium) of anoxic perfusion in the presence of glucose (Fig. 3*A*) the hearts still contain 80 % of the ATP present at the end of the control perfusion period. No measurable amount of CK is released from the hearts during this period of anoxic perfusion. Reintroduction of calcium to the anoxic glucose-containing perfusate results in a massive CK release from the hearts.

After 35 minutes (30 minutes with calcium and 5 minutes without calcium) of anoxic perfusion in the absence of glucose (Fig. 3*B*) the myocardial ATP content is only 2 % of that at the end of the control perfusion period. The absence of glucose in the perfusate causes a slow release of CK. After reintroducing calcium to the anoxic glucose-free perfusate enzyme release increases slightly, indicating that the calcium paradox does not occur in hearts subjected to anoxic substrate free perfusion. However, reoxygenation results in a prompt and massive release of CK. This oxygen-induced calcium paradox is completely inhibited when KCN is present during the last 15 minutes of anoxic perfusion and during reoxygenation. This indicates that reactivation of the electron transport system is responsible for the sudden cell damage. CK release on reoxygenation after a merely anoxic sub-

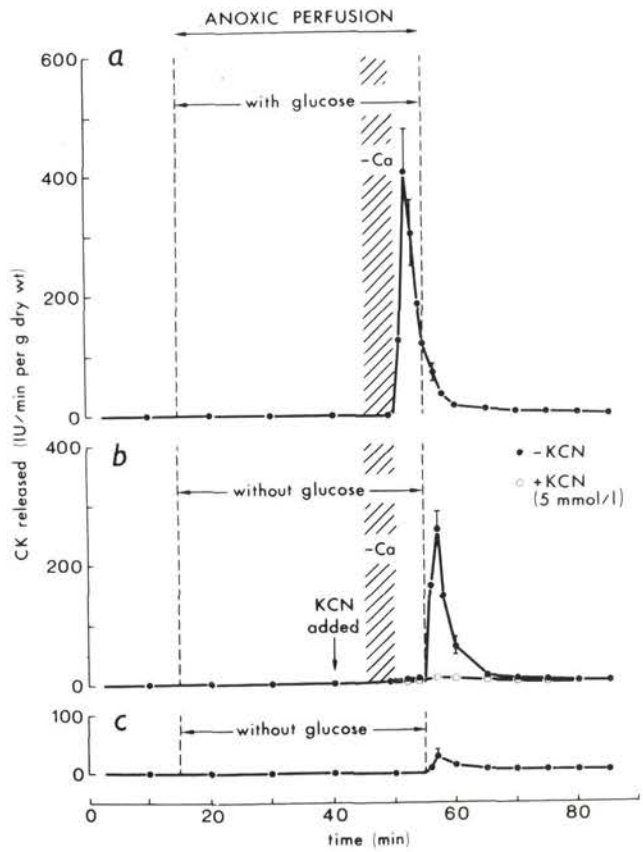

Fig. 3. Effect of reperfusion with Ca^{2+} after a Ca^{2+}-free period (shaded bar), in the anoxic rat heart [(*A*) in the presence of glucose; (*B*) in the absence of glucose], on the release of CK. (*A*) Reperfusion with Ca^{2+} results in an immediate and massive release of CK. (*B*) Massive release of CK does not occur on reperfusion with Ca^{2+}, but only on reoxygenation of the heart. In the presence of KCN oxygen-induced CK release is completely inhibited. (*C*) Effect of reoxygenation after anoxic glucose-free perfusion on the release of CK from isolated rat heart. Reoxygenation results in a relatively slight release of CK. Values are given as mean ± SEM ($n = 8$). From Ruigrok et al. (1978), by permission.

strate-free perfusion is much less pronounced than with a preceding calcium-free period (Fig. 3*C*).

These data show that the calcium paradox fails to occur in the absence of energy but occurs as soon as the electron transport system is reactivated (Fig. 3*B*). Electron transport is not a condition for the calcium paradox; in the absence of electron transport the paradox occurs, provided that ATP is present (Fig. 3*A*).

Acidification of the cytoplasm

The capacity of mitochondria to accumulate large amounts of calcium is considered to be an important defence mechanism by which cells try to control an increased influx of calcium from extracellular spaces. Since readmission of calcium after a calcium-free period leads to an increased tissue level of calcium (Alto and Dhalla 1979) and the formation of intramitochondrial electron-dense particles (Zimmerman et al. 1967; Holland and Olson 1975; Yates and Dhalla 1975), the mitochondria most likely play an important role in the origin of the calcium paradox. The sudden uptake of calcium by mitochondria is accompanied by an excessive release of protons (Boink et al. 1976). However, mitochondria are not the only site of energy-dependent reactions which are triggered by calcium. Other reactions are the activation of myosin ATPase and the uptake of calcium by the sarcoplasmic reticulum. Each of these reactions is activated by an increased intracellular calcium concentration and each requires ATP (Grinwald and Nayler 1981). Since dephosphorylation of ATP results in release of protons (Gevers 1977) these reactions will also contribute to acidification of the cytoplasm. A sudden and large release of protons upon readmission of calcium could precipitate cell damage since the phospholipases and proteases in the cytoplasm and lysosomes are both calcium- and proton-dependent (Grinwald and Nayler 1981).

Contracture-mediated enzyme release

It has been argued that contracture may be a mediator of sarcolemmal injury and enzyme release in the calcium paradox (Ganote 1983; Ganote et al. 1983; Ganote et al. 1984; Ganote and Sims 1984). Myocardial cells are coupled mechanically by fascia adherens junctions of intercalated discs. During calcium-free perfusion separation of fascia adherens junctions develops so that the cells are no longer mechanically coupled. Under these conditions contracture would cause cells to pull apart and mechanical forces would be transmitted to still intact nexus junctions. These junctions are capable of withstanding mechanical tension but the membranes adjacent to the junctions would tear, thus giving rise to enzyme release (Ganote et al.1983). Although the conditions of the above experiments were not identical to those of the classical calcium paradox experiments, it is nevertheless conceivable that mechanical factors may contribute to membrane damage and to the release of intracellular constituents.

Acknowledgement. I am grateful to Prof. Dr. F. L. Meijler and Dr. J. M. J. Lamers for their helpful comments.

References

Alto L. E., Dhalla N. S. (1979): Myocardial cation contents during induction of calcium paradox. Am. J. Physiol. **237**, H713—H719

Alto L. E., Dhalla N. S. (1981): Role of changes in microsomal calcium uptake in the effects of reperfusion of Ca^{2+}-deprived rat hearts. Circ. Res. **48**, 17—24

Ashraf M. (1979): Correlative studies on sarcolemmal ultrastructure, permeability, and loss of intracellular enzymes in the isolated heart perfused with calcium-free medium. Am. J. Pathol. **97**, 411—432

Ashraf M., Onda M., Benedict J. B., Millard R. W. (1982): Prevention of calcium paradox-related myocardial cell injury with diltiazem, a calcium channel blocking agent. Am. J. Cardiol. **49**, 1675—1681

Ashraf M., Onda M., Hirohata Y., Schwartz A. (1982): Therapeutic effect of diltiazem on myocardial cell injury during the calcium paradox. J. Mol. Cell. Cardiol. **14**, 323—327

Baker J. E., Bullock G. R., Hearse D. J. (1983): The temperature dependence of the calcium paradox: enzymatic, functional and morphological correlates of cellular injury. J. Mol. Cell. Cardiol. **15**, 393—411

Baker J. E., Hearse D. J. (1983): Slow calcium channel blockers and the calcium paradox: comparative studies in the rat with seven drugs. J. Mol. Cell. Cardiol. **15**, 475—485

Boink A. B. T. J., Ruigrok T. J. C., de Moes D., Maas A. H. J., Zimmerman A. N. E. (1980): The effect of hypothermia on the occurrence of the calcium paradox. Pflügers Arch. **385**, 105—109

Boink A. B. T. J., Ruigrok T. J. C., Maas A. H. J., Zimmerman A. N. E. (1976): Changes in high-energy phosphate compounds of isolated rat hearts during Ca^{2+}-free perfusion and reperfusion with Ca^{2+}. J. Mol. Cell. Cardiol. **8**, 973—979

Bulkley B. H., Nunnally R. L., Hollis D. P. (1978): "Calcium paradox" and the effect of varied temperature on its development. A phosphorus nuclear magnetic resonance and morphologic study. Lab. Invest. **39**, 133—140

Cheung J. Y., Thompson I. G., Bonventre J. V. (1982): Effects of extracellular calcium removal and anoxia on isolated rat myocytes. Am. J. Physiol. **243**, C184—C190

Chizzonite R. A., Zak R. (1981): Calcium-induced cell death: susceptibility of cardiac myocyte is age-dependent. Science **213**, 1508—1511

Crevey B. J., Langer G. A., Frank J. S. (1978): Role of Ca^{2+} in maintenance of rabbit myocardial cell membrane structural and functional integrity. J. Mol. Cell. Cardiol. **10**, 1081—1100

Dhalla N. S., Alto L. E., Singal P. K. (1983): Role of Na^{+}—Ca^{2+} exchange in the development of cardiac abnormalities due to calcium paradox. Eur. Heart J. **4** (Suppl. H), 51—56

Frank J. S. (1983): Ca depletion of the sarcolemma — ultrastructural changes. Eur. Heart J. **4** (Suppl. H) 23—27

Frank J. S., Langer G. A., Nudd L. M., Seraydarian K. (1977): The myocardial cell surface, its histochemistry, and the effect of sialic acid and calcium removal on its structure and cellular ionic exchange. Circ. Res. **41**, 702—714

Frank J. S., Rich T. L. (1983): Ca depletion and repletion in rat heart: age-dependent changes in the sarcolemma. Am. J. Physiol. **245**, H343—H353

Frank J. S., Rich T. L., Beydler S., Kreman M. (1982): Calcium depletion in rabbit myocardium. Ultrastructure of the sarcolemma and correlation with the calcium paradox. Circ. Res. **51**, 117—130

Ganote C. E. (1983): Contraction band necrosis and irreversible myocardial injury. J. Mol. Cell.Cardiol. **15**, 67—73

Ganote C. E., Grinwald P. M., Nayler W. G. (1984): 2,4-Dinitrophenol (DNP)-induced injury in calcium-free hearts. J. Mol. Cell. Cardiol. **16**, 547—557

Ganote C. E., Sims M. A. (1984): Parallel temprerature dependence of contracture-associated enzyme release due to anoxia, 2,4-dinitrophenol (DNP), or caffeine and the calcium paradox. Am. J. Pathol. **116**, 94—106

Ganote C. E., Sims M. A., VanderHeide R. S. (1983): Mechanism of enzyme release in the calcium paradox. Eur. Heart J. **4** (Suppl. H), 63—71

Gevers W. (1977): Generation of protons by metabolic processes in heart cells. J. Mol. Cell. Cardiol. **9**, 867—874

Goshima K., Wakabayashi S., Masuda A. (1980): Ionic mechanism of morphological changes of cultured myocardial cells on successive incubation in media without and with Ca^{2+}. J. Mol. Cell. Cardiol. **12**, 1135—1157

Grinwald P. M., Nayler W. G. (1981): Calcium entry in the calcium paradox. J. Mol. Cell. Cardiol. **13**, 867—880

Hearse D. J., Baker J. E., Humphrey S. M. (1980): Verapamil and the calcium paradox. J. Mol. Cell. Cardiol. **12**, 733—739

Hearse D. J., Humphrey S. M., Boink A. B. T. J., Ruigrok T. J. C. (1978): The calcium paradox: metabolic, electrophysiological, contractile and ultrastructural characteristics in four species. Eur. J. Cardiol. **7**, 241—256

Hearse D. J., Humphrey S. M., Bullock G. R. (1978): The oxygen paradox and the calcium paradox: two facets of the same problem? J. Mol. Cell. Cardiol. **10**, 641—668

Holland C. E., Olson R. E. (1975): Prevention by hypothermia of paradoxical calcium necrosis in cardiac muscle. J. Mol. Cell. Cardiol. **7**, 917—928

Isenberg G., Klockner U. (1981): Glycocalyx is not required for slow inward calcium current in isolated rat heart myocytes. Nature **284**, 358—360

Lamers J. M. J., Ruigrok T. J. C. (1983): Diminished Na^+/K^+ and Ca^{2+} pump activities in the Ca^{2+} depleted heart: possible role in the development of Ca^{2+} overload during the Ca^{2+} paradox. Eur. Heart J. **4** (Suppl. H), 73—79

Lamers J. M. J., Stinis J. T., Ruigrok T. J. C. (1984): Biochemical properties of membranes isolated from calcium-depleted rabbit hearts. Circ. Res. **54**, 217—226

Lomský M., Ekroth R., Poupa O. (1983): The calcium paradox and its protection by hypothermia in human myocardium. Eur. Heart J. **4** (Suppl. H), 139—142

Meno H., Kanaide H., Nakamura M. (1984): Effects of diltiazem on the calcium paradox in isolated rat hearts. J. Pharmacol. Exp. Ther. **228**, 220—224

Muir A. R. (1967): The effects of divalent cations on the ultrastructure of the perfused rat heart. J. Anat. **101**, 239—261

Muir A. R. (1968): A calcium-induced contracture of cardiac muscle cells. J. Anat. **102**, 148—149

Nayler W. G., Elz J. S., Perry S. E., Daly M.J. (1983): The biochemistry of uncontrolled calcium entry. Eur. Heart J. **4** (Suppl. H), 29—41

Nayler W. G., Grinwald P. M. (1981): The effect of verapamil on calcium accumulation during the calcium paradox. J. Mol. Cell. Cardiol. **13**, 435—441

Nayler W. G., Grinwald P. M. (1982): Dissociation of Ca^{2+} accumulation from protein release in calcium paradox: effect of barium. Am. J. Physiol. **242**, H203—H210

Nayler W. G., Perry S., Daly M. J. (1983): Cobalt, manganese and the calcium paradox. J. Mol. Cell. Cardiol. **15**, 735—747

Ohhara H., Kanaide H., Nakamura M. (1982): A protective effect of verapamil on the calcium paradox in the isolated perfused rat heart. J. Mol. Cell. Cardiol. **14**, 13—20

Rich T. L., Langer G. A. (1982): Calcium depletion in rabbit myocardium. Calcium paradox protection by hypothermia and cation substitution. Circ. Res. **51**, 131—141

Ringer S. (1883): A further contribution regarding the influence of the different constituents of the blood on the contraction of the heart. J. Physiol. **4**, 29—42

Ruigrok T. J. C., Boink A. B. T. J., Slade A., Zimmerman A. N. E., Meijler F. L., Nayler W. G. (1980): The effect of verapamil on the calcium paradox. Am. J. Pathol. **98**, 769—790

Ruigrok T. J. C., Boink A. B. T. J., Spies F., Blok F. J., Maas A. H. J., Zimmerman A. N. E. (1978): Energy dependence of the calcium paradox. J. Mol. Cell. Cardiol. **10**, 991—1002

Ruigrok T. J. C., Slade A. M., Poole-Wilson P. A. (1983): The calcium paradox in isolated frog heart: Ringer revisited. Eur. Heart J. **4** (Suppl H), 89—96

Yates J. C., Dhalla N. S. (1975): Structural and functional changes associated with failure and recovery of hearts after perfusion with Ca^{2+}-free medium. J. Mol. Cell. Cardiol. **7**, 91—103

Zimmerman A. N. E., Daems W., Hülsmann W. C., Snijder J., Wisse E., Durrer D. (1967): Morphological changes of heart muscle caused by successive perfusion with calcium-free and calcium-containing solutions (calcium paradox). Cardiovasc. Res. **1**, 201—209

Zimmerman A. N. E., Hülsmann W. C. (1966): Paradoxical influence of calcium ions on the permeability of the cell membranes of the isolated rat heart. Nature **211**, 646—647

Received September 11, 1984/Accepted October 18, 1984