

## ***Magnesium Modulation of Calcium Uptake in Cardial Mitochondria: An Ultrastructural Study***

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### INTRODUCTION

A number of factors (inorganic phosphate ( $PO_4$ ), pH, magnesium (Mg)) have been shown to alter or affect mitochondrial calcium (Ca) accumulation (Lehninger *et al.*, 1967). It is generally accepted that Ca accumulation and oxidative phosphorylation are noncompeting processes in mitochondria (Lehninger *et al.*, 1967), that is, mitochondria will preferentially accumulate Ca in lieu of phosphorylation of adenosine diphosphate (ADP). This article describes a modulatory effect of Mg on rapid Ca uptake by heart mitochondria; additional results suggest a "protective" effect of Mg on the phosphorylating capacity of mitochondria during Ca accumulation.

### METHODS

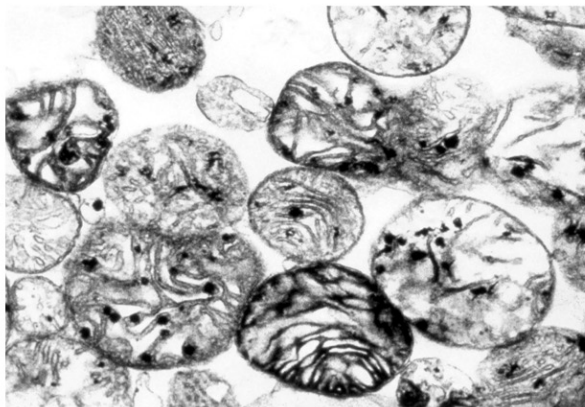
For correlative electron microscopy (EM) of active Ca uptake into the mitochondria, the specimens were rapidly fixed in cold 3% phosphate-buffered glutaraldehyde, postfixed in osmium tetroxide, dehydrated in ethanol, and embedded in Maraglas. The sections were cut on a LKB III Ultratome, placed on 300-mesh copper grids, and stained with uranyl acetate and lead citrate. All specimens were examined in a Philips 300 electron microscope. The micrographs are representative of several hundred samples examined.

Rabbit heart mitochondria were isolated by previously described techniques and mitochondrial respiratory activity was measured by polarographic means (Sordahl *et al.*, 1971). Rapid uptake of Ca was monitored using the chelometric dye murexide (Mela and Chance, 1969) and a DW-2 dual wavelength spectrophotometer (American Instrument Co.) at the wavelength pair 541-507 nm by a modification of the method of Scarpa (1972). The reaction medium for Ca uptake consists of 0.25 mole sucrose, 10 mmoles Tris-HCl (pH 7.2), 70 mmoles NaCl, 8 mmoles phosphate, 50 mmoles murexide, 5  $\mu$ g of rotenone, and 3 mg of mitochondrial protein in a total volume of 3.0 ml. The respiratory substrate succinate is added at a final concentration of 5mM; temperature of the reaction medium 30°C. Changes in the redox state of cytochrome b were also measured by dual wavelength spectrophotometry at the wavelength pair 430-410 nm (Chance, 1963).

## RESULTS

Respiration-supported Ca uptake by rabbit heart mitochondria in the absence of exogenous Mg (Fig. 1) was more than twice that of the initial rate of uptake in the presence of Mg.

MITOCHONDRIA WITH MAGNESIUM IN THE  
INCUBATION MEDIA...NO DESTRUCTIVE  
CRYSTALS SEEN



MITOCHONDRIA WITH NO MAGNESIUM IN THE  
INCUBATION MEDIA...MANY DESTRUCTIVE  
CALCIUM CRYSTALS SEEN

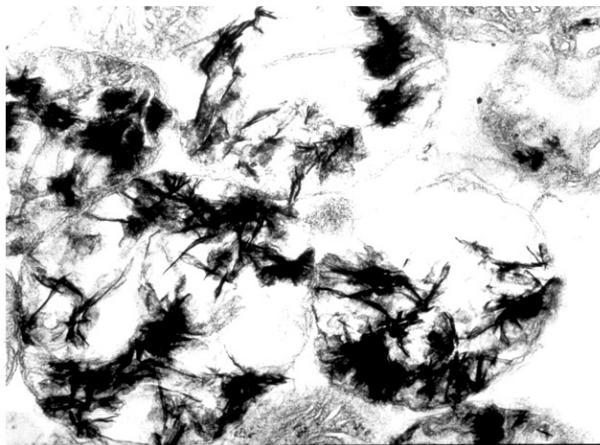


Figure 1. Rabbit heart mitochondria. Respiration supported Ca uptake by rabbit heart mitochondria in the absence of exogenous Mg is double that when Mg was present.

## Electron Microscopy

Rapid fixation of mitochondria during active Ca uptake revealed dynamic changes in the Ca crystals precipitated intramitochondrially. In the presence of Mg, during active uptake, a spherical, amorphous type of crystal appears in the mitochondrial matrix closely associated with their cristae. After 30 sec of uptake, followed by rapid fixation, all of the heart mitochondria exhibited this spherule-type of Ca accumulation (Fig. 1b). Mitochondria lacking Mg in the incubation medium showed a needle-like Ca accumulation on the same time scale of 1 min (data not shown). After 1 min of active Ca uptake, when Mg was added after absence of Mg, the Ca crystals underwent transformation into an apparently destructive and massive granular type with dendritic crystals which obliterated internal mitochondrial structure (Fig. 1c,d). These crystals had no spheroidal substructure, and appeared to rupture the mitochondrial membrane and extend needle-like processes into the extramitochondrial space. This type of Ca crystal structure also appeared in cardiac mitochondria after 30 sec if no Mg was present (data not shown).

## Polarographic Studies

Measurement of mitochondrial respiratory activity revealed a marked stimulation of succinate-linked respiration by Mg (Fig. 2a). Under conditions identical to those employed for Ca uptake, a distinct stimulation in mitochondrial respiration was observed by the addition of Ca in the absence of exogenous Mg (Fig. 2b). Subsequent addition of ADP failed to produce any significant increase in mitochondrial respiration (Fig. 2b). In the presence of Mg, Ca also produced a distinct stimulation of respiration; the subsequent addition of ADP produced an even greater stimulation of respiratory activity, characterized by an apparent state 3 to state 4 respiratory transition (Fig. 2c).

Changes in the redox state of cytochrome b, concomitant with the Ca uptake and followed by addition of ADP, revealed distinct differences in the presence and absence of exogenous Mg (Fig. 3). In the absence of Mg, cytochrome b did not reach a reduced steady state until all the Ca had been accumulated (Fig. 3a). Subsequent addition of ADP produced a transient oxidation of cytochrome b and a small efflux of Ca from the mitochondria. As cytochrome b returned to the original reduced steady state, the Ca that had been released from the mitochondria was taken back up (Fig. 3a). In the presence of Mg (Fig. 3b), cytochrome b rapidly reached a reduced steady state before all the Ca had been removed from the medium. Subsequent addition of ADP produced a marked transient oxidation of cytochrome b and eventually it returned to a lower, reduced steady state than the original. This is consistent with the observed increase in the rates of oxygen consumption after ADP addition (Fig. 2c). At the point of ADP addition, a substantial amount of Ca was observed to come out of the mitochondria and was re-accumulated as the cytochrome b once again approached a reduced steady state (Fig. 3b).

## DISCUSSION

Electron microscopic studies indicate an effect of Mg on Ca deposition in the intramitochondrial space, in which two forms of Ca crystals were observed. The transformation of amorphous Ca phosphate to crystalline apatite has been studied in aqueous solutions by Eanes et al.

(1973). Their studies emphasize the dependency of the apatite crystallization on the amorphous precursor as natural successors to the spheroidal shape. Shen and Jennings (1972) have shown that ischemic

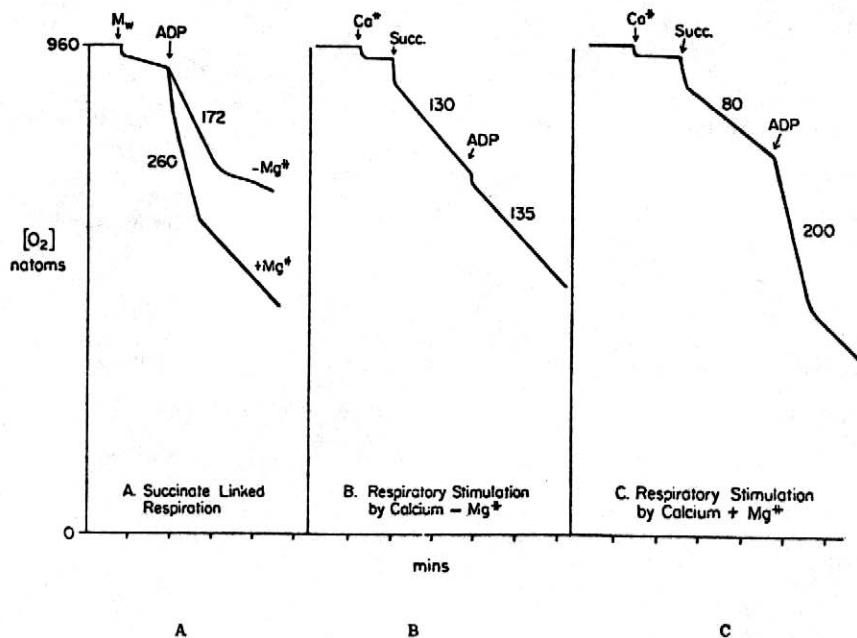


Figure 2. Oxygen electrode traces of mitochondrial succinate-linked respiration during Calcium uptake + Mg. Assay conditions are identical to those in Fig. 1. Magnesium, when present, 3.3mM. The numbers next to the traces indicate the rates of oxygen consumption in natoms/min/mg mitochondrial protein. (A): Coupled respiratory activity  $\pm$  Mg. After addition of mitochondria ( $M_w$ ), ADP (500 nmoles) was added to produce a state 3 respiratory burst. (B): Oxygen consumption during Ca uptake minus Mg. Calcium (300 nmoles) is added, followed by succinate (Succ). Subsequent addition of ADP (500 nmoles) produces no further stimulation in respiration. (C): Oxygen consumption during Ca uptake plus Mg, additions same as B.

injury to intact dog myocardium causes an abnormal uptake of Ca into dense intramitochondrial granules and is an important feature of irreversible cellular injury. The mechanism of this uptake is still not established. The ultrastructural and biochemical data obtained under controlled *in vitro* conditions of actively respiring cardiac mitochondria has indicated that Mg plays a critical role in at least two aspects of pathological Ca accumulation. 1) The presence of Mg appears to modulate the rate of Ca transformation (and uptake) during the dynamic state. 2) Two distinct types of Ca crystals may be present, one spheroidal-amorphous and the other a destructive needle-like crystalloid mass irreversibly destroying structure. The presence of Mg has, in effect, acted as a modulator of both uptake and form of Ca, acting as an

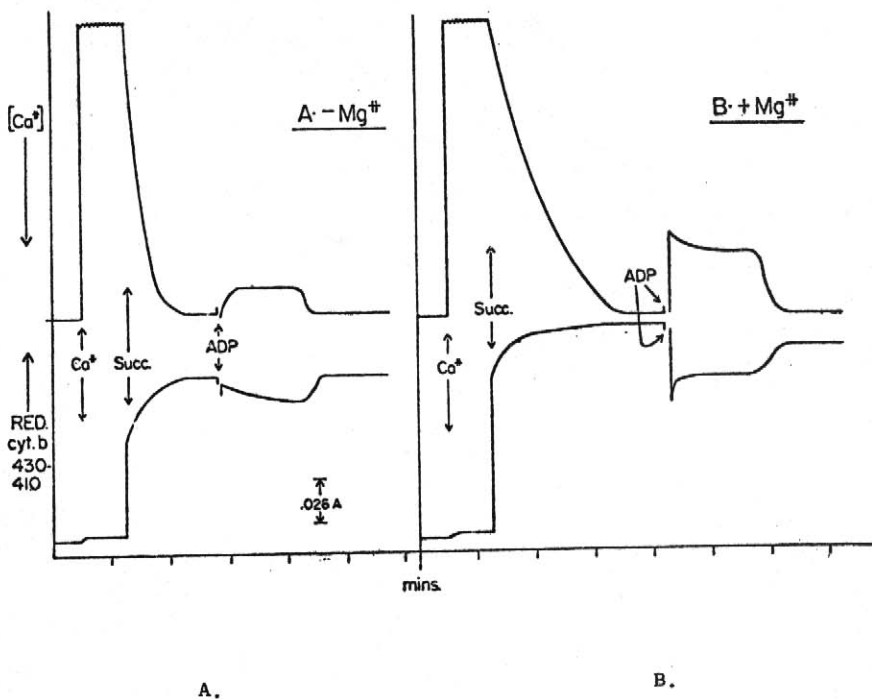


Figure 3. Changes in cytochrome b redox states during Ca uptake and subsequent ADP addition + Mg. The upper traces in each panel represent the independent measurement of Ca uptake + Mg, as in Fig. 1. The lower traces represent cytochrome b reduction (upward deflection) measured at the wavelength pair 430-410 nm by dual-beam spectroscopy. Assay conditions are identical to Fig. 1 except that no murexide is present during assay of cytochrome b. Additions:  $Ca^{++}$  = 300 nmoles, Succ (succinate) = 5mM, and ADP = 500 nmoles. (A): Minus Mg; (B): plus Mg, 3.3mM.

ultrastructural probe which allows visualization of the transitional changes in Ca crystal structure during active metabolism. Modulation of Ca uptake by Mg may play an essential role in protecting the intact myocardial mitochondria during ischemic episodes. This is consistent with the other observed necrotizing effects of Ca on heart muscle cells when intracellular Mg levels are low (Janke *et al.*, 1975; Lehr *et al.*, 1975). The results of these studies suggest that Mg "protects" the high-energy state of mitochondria and modulates Ca-transport and ATP-synthesizing activities of heart mitochondria.

#### SUMMARY

Correlative ultrastructural and biochemical evidence indicates that the presence of Mg alters both the rate and characteristics of Ca uptake in cardiac mitochondria. The ultrastructure of electron-opaque Ca granules appeared to be either amorphous granules or crystal-like deposits within the cristae, depending on the amount of Calcium loading and the type of mitochondria. In the absence of Mg mitochondria accumulate Ca at higher rates than when Mg is present. Calcium loading in heart mitochondria tended to form a second type of crystal structure, apparently destructive to mitochondrial integrity. Stimulation by Ca was also lower in the presence of Mg. A marked stimulation of respiration in the presence of Mg occurred with addition of ADP after Ca uptake. Magnesium appears to protect and modulate the ATP-synthesizing and Ca-transport activity of cardiac mitochondria. Magnesium seems to protect the high-energy state and modulates ATP and Ca transport in heart mitochondria.

#### ACKNOWLEDGMENTS

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