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A Unique Non-Invasive Intracellular Magnesium Assay Correlating with Cardiac Tissues, Arrhythmias, and Therapeutic Intervention

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Theophile Theophanides and Jane Anastassopoulou. Magnesium: Current Status and New Developments Theoretical, Biological and Medical Aspects. *Kluwer Academic Publishers*. 1977

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Introduction:

A novel technique using energy dispersive X-ray microanalysis,(EXA_{tm}), for non-invasive intracellular measurement of magnesium has now been accomplished and proven to be a valuable tool in multiple aspects of normal as well as pathological magnesium metabolism.(U.S. patent No. 4-717-826, B.S.) Over the last 15 years data has accumulated using this unique method which is now available to accurately evaluate tissue Mg levels..

Since only 1% of total body Mg^{2+} is found in the intravascular space, serum levels of Mg give little information about a patients overall status with respect to this essential mineral. Using the intracellular analysis it has been determined that Mg levels are significantly reduced in multiple physiological states which may lead to serious pathological conditions.[1] Description of the methodology and examples of data as well as potential applications will focus on intracellular $[Mg^{2+}]_i$ determinations obtained from subjects with cardiovascular disease syndromes related to Mg^{2+} deficiency. .

Examples of the application of EXA_{tm} include examination of intracellular magnesium and other minerals in a wide spectrum of conditions which include : Effects of Microgravity,(NASA) : cardiovascular conditions; arrthymias; heart failure; myocardial infarction; and bypass surgery; standardization of normal controls. (NASA).[2]

Rationale:

Magnesium plays a protean role in intracellular metabolism, second only in potassium as the most common intracellular cation in human physiology. Multiple normal functions as well as pathological syndromes are affected by the cellular levels and availability of the magnesium stores within tissues. Logically, measuring magnesium accurately and easily within tissues is of immense use to the understanding and treatment of a host of diseases in the human condition.

Cytosolic free magnesium acts as a cofactor for every ATPase and regulates calcium and potassium transport into tissues. Because of its many effects, Mg has been called the ”chronic regulator” of cell metabolism. Murphy et al point out , though, that Mg^{2+}i can only modulate intracellular functions if $[Mg^{2+}]$i changes in response to physiological stress.[3]

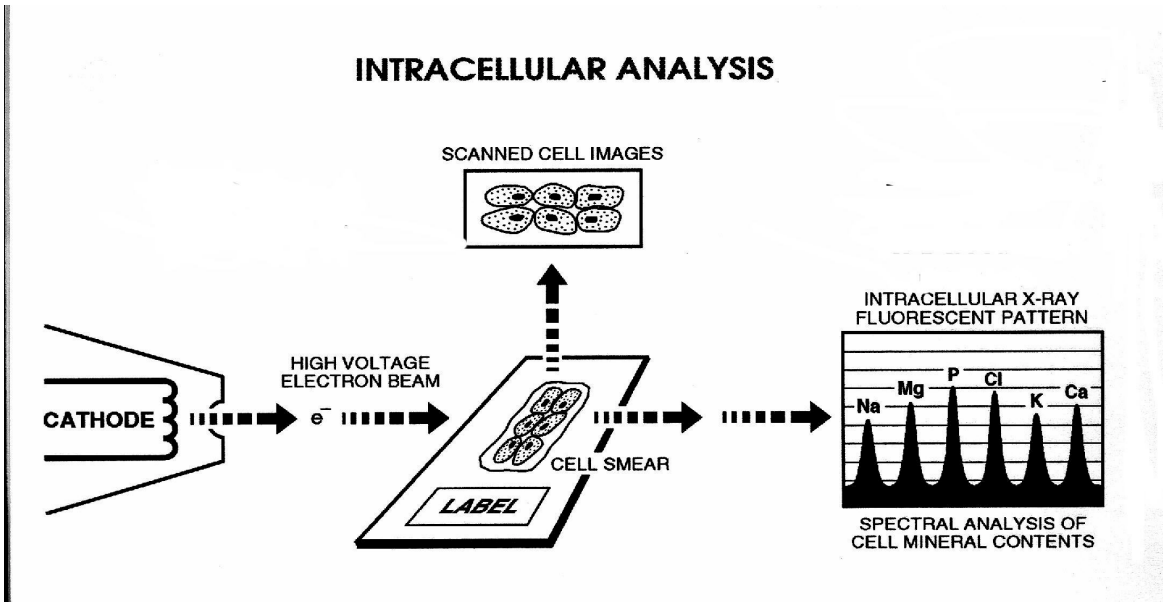
Cellular magnesium activates many enzyme systems including those vital reactions which govern cardiac function. Regulated by Mg levels, sodium-potassium ATPase maintains the normal intracellular concentrations of Na^+ and K^+ vital to normal cardiac function. Given the net effect of a reduction in intracellular Mg on the action potential and the multiple channels and enzymes influenced by the Mg cation, a membrane depolarization would be expected due to cellular K^+ loss, Na^+ gain and CA^{2+} gain.[4]

Methodology:

The measurement of intracellular $[Mg^{2+}]_i$ was performed by x-ray dispersive microanalysis utilizing a specially configured electron microscope which images and irradiates cells with a focused electron beam. Sublingual epithelial cells were chosen as markers since they are easily accessible, are non-cornified, aerobic, have a high cytoplasm to nucleus ratio, turnover in less than 3 days, have long shelf life, exhibit 99% viability, and show significant correlations with cardiac and muscle biopsies taken during bypass surgery. Excitation of cellular atoms displaces inner orbital electrons which are replaced by electrons from higher energy cells releasing fluoresced x-rays which allows quantitation of intracellular elements. EXA_{tm} units equals X-ray intensity (peak divided by background) divided by unit cell volume. Cardiac and muscle tissue biopsies were quick frozen in liquid nitrogen cooled with Freon and freeze dried tissue sections were then carbon coated for x-ray analysis.[5]

EXA_{tm} units are converted to milliequivelants per liter by a conversion constant for each elementderived from reference standard of a highly stable matrix synthetic glass containing known amounts of the elements to be analyzed. The standard was certified by the National Bureau of Standards and Corning Diagnostics. The coefficient of variance of 40 determinations of X-ray emission energies for Mg with the reference standard was 0.82%. Applications of intracellular magnesium analysis will be reported in three investigations incorporating this unique method.

Figure I



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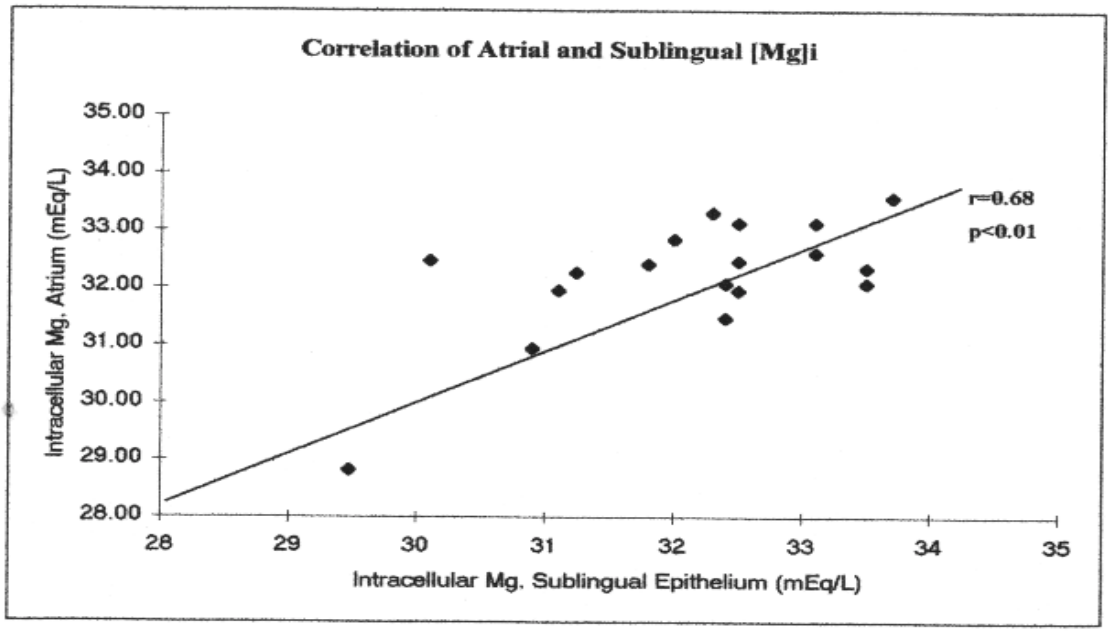
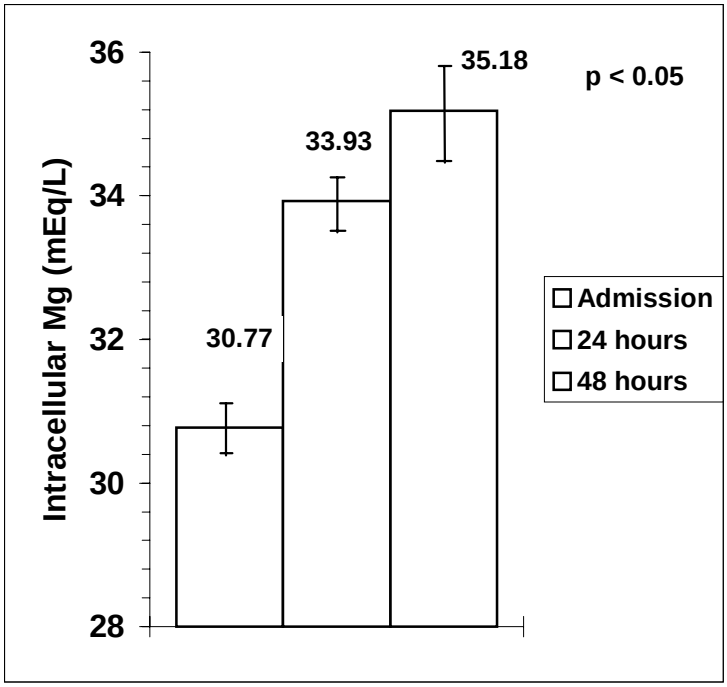
I. Correlation of Mg levels between Sublingual cells and Atrial Tissues Taken at Surgical Bypass and the Effect of IV Mg in Patients with ST elevation and Acute Myocardial Infarction.

Results: We have found that energy dispersive x-ray analysis (EXA_{tm}) of sublingual epithelial cells correlate well with atrial specimens obtained at cardiopulmonary bypass. Seventeen subjects, average age 65.7 years, had sublingual smears made prior to atrial biopsies at the time of bypass surgery. Their sublingual levels were compared to healthy volunteers. Atrial biopsy tissue frozen as described and elemental x-ray analysis was performed. Serum vs. Intracellular $[Mg^{2+}]_i$ in Surgical Patients**:** Mean serum Mg levels for the cardiac surgery patients was within normal range, 1.87±0.06 mEq/L. Despite normal serum Mg, intracellular $[Mg^{2+}]_i$ levels in the sublingual epithelium of bypass patients were reduced when compared to healthy subjects despite normal serum levels. Linear regression comparing the individual values is shown in figure 2. A strong correlation exits between the sublingual and cardiac cells. ($R=0.68$, $p<0.002$)(Figure #2)

Correlative Study of Intracellular Magnesium
from Atrial Tissues and Sublingual Cells
Atrial biopsy tissues frozen at surgery
Sublingual Tissues Taken within 8 hours of surgery or at surgery



Using the EXA$_{tm}$ sublingual cell evaluation, magnesium intravenous intervention studies were done on 22 myocardial infarct patients who were compared to healthy controls and non-cardiac patients. Mean Mg in infarct patients was 30.7±0.4 compared to 15 control subjects whose cellular Mg levels were 35.0±0.5, p<.0001. Infarct patients received a mean dose of 36±6 mmol /24 hrs of intravenous Mg_2S0_4. No Mg was given after the second 24 hours of the study. Intracellular $[Mg^{2+}]_i$ rose significantly in the infarct patients over the first 24 hours and the magnitude of the increase was greater in those who received higher doses of intravenous magnesium sulfate. Despite the fact that Mg_2SO_4 was not given after the first 24 hours, mean sublingual magnesium continued to rise for 48 hours after the first dose of magnesium sulfate (from 30.7±0.4 to 35.2±0.6 mEq/L) suggesting that magnesium may be moving from the vascular space to the tissues over 48 hours.(Figure #3)

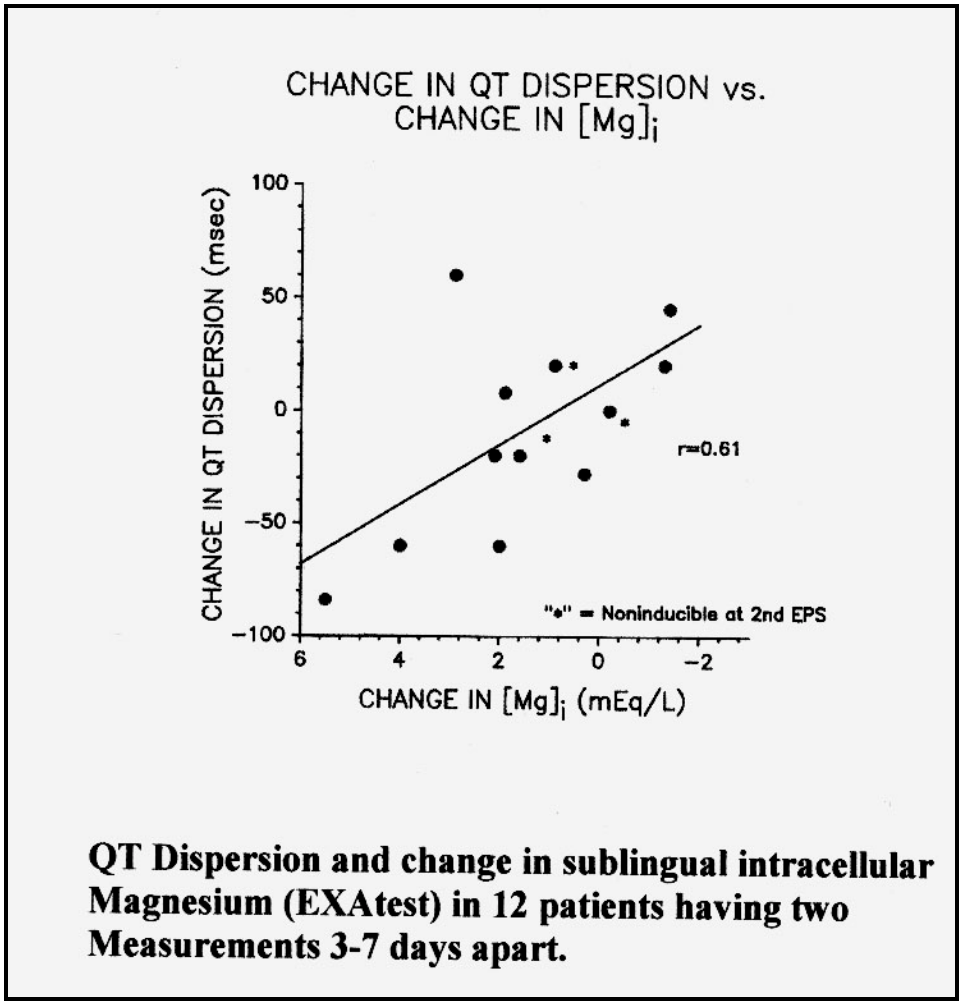


INCREASE OF INTRACELLULAR Mg IN MI PATIENTS FOLLOWING Mg INFUSION FROM ADMISSION THROUGH 48 HOURS POST -TREATMENT

II. Abnormal QT dispersion Correlates with Tissue Magnesium

Abnormal QT dispersion(QTd) on the 12 lead EKG , and magnesium deficiency have both been shown to predict a higher risk for sudden cardiac death, in patients with CHF, alcoholic liver disease, or requiring antiarrhythmic drugs. We tested the hypothesis that Mg deficiency predisposes to abnormal repolarization in the (QTd). In 37 subjects with malignant arrhythmias, Mg in serum and sublingual epithelial cells, were measured in a blinded fashion. Results were correlated with the EKG , ($r=0.56$, $p=0.0001$).Linear regression showed QTd correlated with depressed tissue Mg measured using energy dispersive x-ray analysis, (EXA_{tm}). Mean serum levels showed no such correlations. Repeat Mg levels obtained in 12 treated patients 3-7 days later showed increase in tissue Mg (1.5± /-0.6 mEq/l ($p<0.0001$) and a decrease in QTd by 10 ±12 msecs ($p<0.0001$). Delta QTd [MG] correlated at $r=0.61$, $p<0.05$. QT dispersion was shown to correlate with

tissue Mg levels suggesting Mg deficiency may be a risk factor for sudden cardiac by its effect on cardiac repolarization as indexed by QTd.(Figure #4).



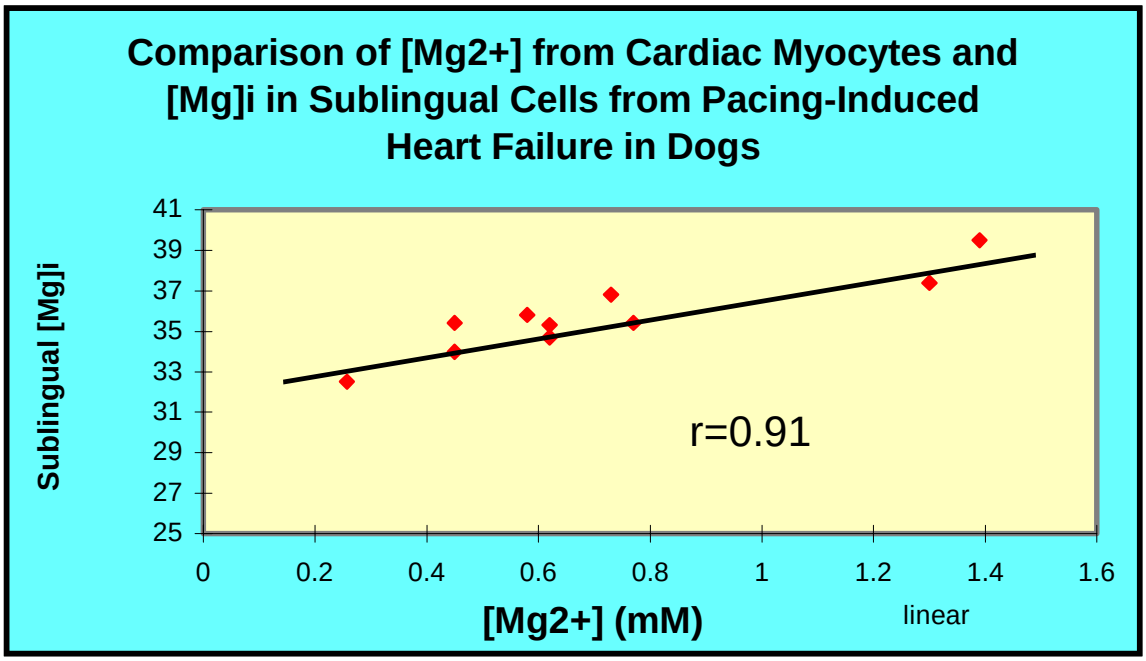
QT Dispersion and change in sublingual intracellular Magnesium (EXAtest) in 12 patients having two Measurements 3-7 days apart.

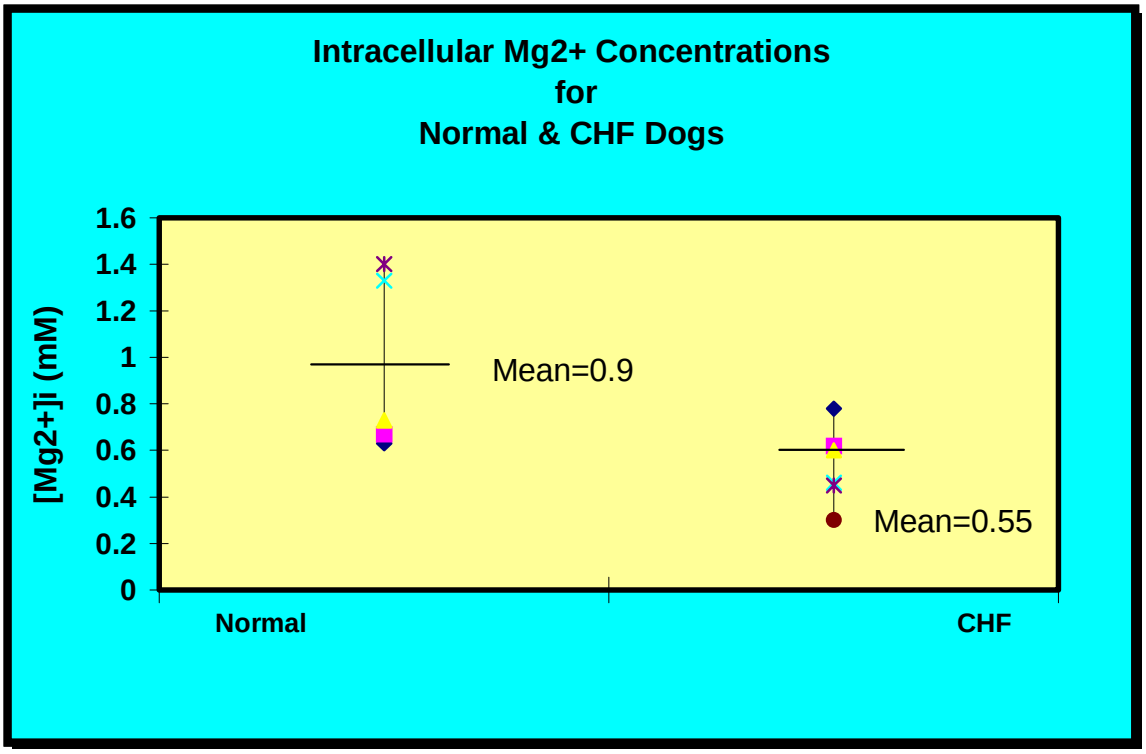
III. Effect of Experimental Heart Failure on Sublingual Tissue MG and Cardiac Ionized Mg.

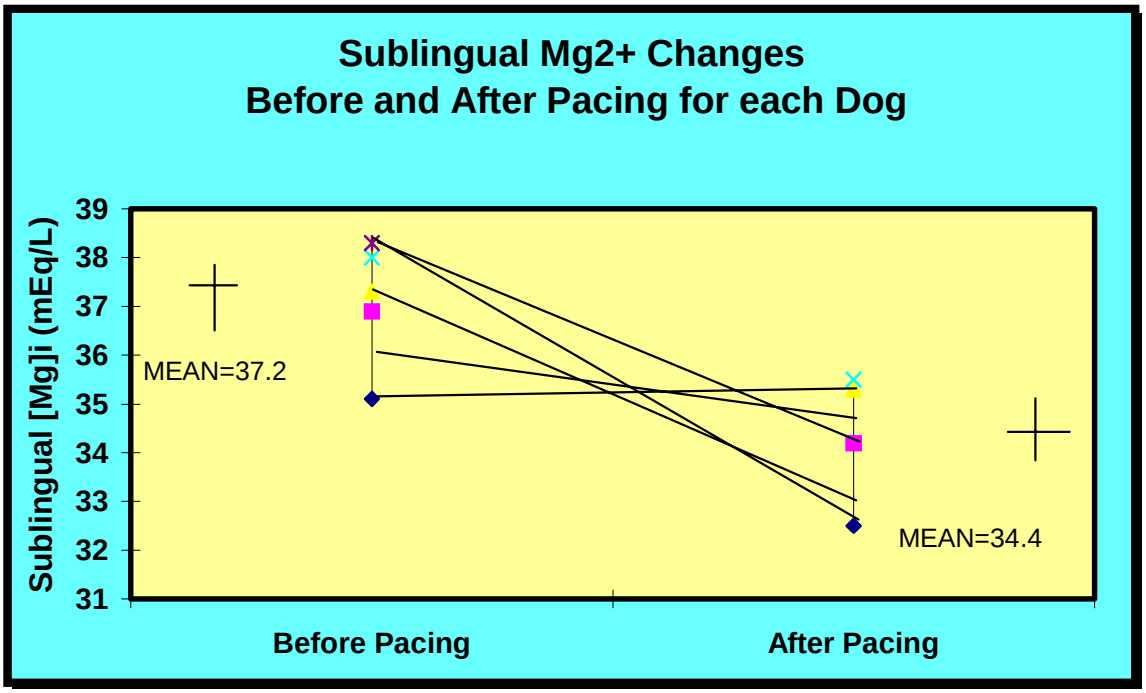
To test the hypothesis that CHF results in a rapid loss of tissue Mg in the absence of confounding drugs or diseases, sublingual smears were obtained from normal dogs (n=4) which were exposed to 4 weeks of ventricular pacing at 250bpm. . All animals manifested bi-ventricular failure. Sublingual cell Mg was measured by energy dispersive x-ray analysis (EXA_{tn}) . Cardiac myocytes were measured with the fluorescent probe Mg-Indo to measure $[Mg^{2+}]_{i.}$

Sublingual cells demonstrated significantly lower $[Mg^{2+}]_i$ in paced dogs, (34.8mEq/L±0.8 vs. 38.7mEq/l±0.5 $2p<0.05$.)The fluorescence ratios indexing $[Mg^{2+}]_i$ was also significantly reduced in the paced animals compared to the normal dogs. $(0.573\pm0.007, n=49)_{paced}$ vs. normals $(0.749\pm0.029)_{normal}$,(n=18 cells, $2p<0.001$) . The

reduction in total cellular Mg detected in the sublingual cells was (8%) yet was associated with a ~40% reduction in $[Mg^{2+}]_i$ compared to controls. It seems that free Mg is not well buffered but fluctuates with changes in changes in total Mg. A small reduction in total cellular Mg would cause a much large decrement in free Mg which constitutes <5% of total stores. The data suggests that a reduction in tissue Mg results in a relatively large fall in free cardiac Mg. Measures of sublingual Mg (EXA_{tn}) my be useful in future clinical investigation regarding the significance of Mg in heart failure. More importantly, EXA_{tm} testing will allow accurate serial measurement of Mg to guide Mg repletion therapy in a wide variety of syndromes in which Mg deficiency is a factor. (Figures 5, 6)




Intracellular Mg2+ Concentrations
for
Normal & CHF Dogs
[Mg2+]i (mM)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Mean=0.9
Mean=0.55
Normal
CHF


Sublingual Mg2+ Changes
Before and After Pacing for each Dog
Sublingual [Mg]i (mEq/L)
39
38
37
36
35
34
33
32
31
MEAN=37.2
MEAN=34.4
Before Pacing
After Pacing

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4. Agus ZS, Morad M. Modulation of Cardiac Ion Channels by Magnesium: Annu Rev. Physiol. 1991;299-307
5. Haigney, MCP, Silver B, Tanglao E, Silverman HS, Hill JD, Shapiro E, Gerstenblith G, Schulman SP: Non-invasive Measurement of Tissue Magnesium and Correlation with Cardiac Levels: Circ, 1995;92:2190-2197

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