Reprinted from *Myocardiology*, Volume 1 of the series entitled *Recent Advances in Studies on Cardiac Structure and Metabolism*, edited by E. Bajusz and G. Rona. University Park Press, Baltimore, London, Tokyo, 1972.

Schering Corporation, Bloomfield, N. J.,
New York Medical College, Pharmacology Department,
Maimonides Medical Center, New York, N. Y., USA

MYOCARDIAL LOSS OF FUNCTIONAL MAGNESIUM

I. EFFECT ON MITOCHONDRIAL INTEGRITY AND POTASSIUM RETENTION

M. S. SEELIG

There is growing interest in the importance of magnesium in myocardial structure and function. Most clinicians in the United States still place greatest emphasis on potassium loss in the etiology or intensification of cardiomyopathy. This paper presents an analysis of the data that indicate that the magnesium loss from the heart, as from other tissues, interferes with the cellular machinery without which cardiac potassium cannot be maintained. Thus, magnesium loss predisposes to potassium loss.

The cardiac loss of potassium seen in magnesium deficiency was first attributed by Vitale and his associates (14, 57) to the necessity of magnesium for the normal functioning and structural integrity of heart mitochondria. They correlated the decreased oxidative activity and/or uncoupling of oxidative phosphorylation by cardiac mitochondria from their magnesium-deficient animals (14, 55, 58) with the biochemically demonstrated importance of oxidative phosphorylation in the active transport of potassium (33, 54). Welt and his associates (59–61) have implicated defective functioning of membrane adenosine triphosphatase (ATPase) as a probable cause of the loss of K by Mg-deficient cells. On the basis of the biochemical work on subcellular membrane particles that showed that Na^+-K^+-ATPase is Mg-dependent and is involved in the transport of Na^+ and K^+, they concluded that magnesium is necessary for the action of "pump" ATPase. Without its optimal activity, the cells apparently lose the capacity to maintain an appropriate concentration gradient across the cell membranes (table 1).

Skou (49–52), first utilizing microsomes from crab nerves and then from mammalian brain and kidney preparations, showed that Mg^{++} is necessary for the activity of ATPase in active transport of Na^+ and K^+ across an electrochemical gradient. Post *et al.* (38) using erythrocyte membranes showed that both Na^+ and K^+-dependent and independent ATPases are activated by Mg^{++}. Dunham and Glynn (15, 19) have also demonstrated that the ATPases require Mg^{++} for full activity, and that Na^+-K^+-ATPase is directly involved in the ionic pump. The presence of Mg-dependent, Na^+-K^+-ATPase has been demonstrated in cardiac microsomes by Auditore and Murray (1, 2) and by Schwartz and Laseter (42, 44).

Table I

Magnesium-dependent Particulate Adenosine Triphosphatases

Investigators	Source of ATPase	Role of Mg^{++}	Inhibitors
Skou (49–52)	Microsomal particle (Crab nerve, guinea pig, rat, rabbit) Brain Kidney	Mg^{++} requirement for Na^+-K^+ transport across electrochemical gradient	Ca^{++} (of Mg-ATPase) Strophanthin (of Na-K-Mg ATPase)
Post *et al.* (38)	Human red blood cell ghosts Guinea pig kidney	Mg^{++} requirement for Na^+-K^+ dependent / independent ATPase	
Dunham and Glynn (15) Glynn (19)	Human red blood cells Microsomes (electric organ)	Mg^{++} requirement for Na^+-K^+ dependent / independent ATPase	Ca^{++} Cardiac glycosides
Auditore (1) Auditore and Murray (2)	Mitochondria; microsomes Rabbit heart	ATPase role in ionic pump Mg^{++} activates: Na^+-K^+ dependent / independent ATPase	G-Strophanthin
Schwartz (41) Schwartz and Laseter (44)	Microsomes Rat heart	Mg^{++}-Na^+-K^+ ATPase Mg^{++}-ATPase	Ouabain

Because under most circumstances magnesium deficiency is associated with hypercalcemia and because agents that cause hypercalcemia have cardiotoxic potential, the demonstration by some investigators (15, 19, 49–52) of inhibition by Ca^{++} of Mg-activated ATPase is especially interesting. The inhibition by cardiotonic agents of the ATPase that is dependent on Na^{+}, K^{+}, and Mg^{++} (1, 2, 15, 19, 42, 44) is also important, because of the loss of myocardial Mg from hearts of animals with digitalis intoxication (25), the increased sensitivity to glycosides of Mg-deficient animals (31, 45, 56, 57), and the efficiency of magnesium in counteracting digitalis toxicity (45, 53, 62).

Review of some of the basic work on the role of magnesium in mitochondrial structure and in the activity of microsomal ATPase reveals a pivotal position of this cation in normal physiology; the present paper cannot encompass the many additional Mg-dependent enzyme systems. The efficacy of Mg in preventing and reversing early damage to mitochondria caused by a variety of challenges provides a clue to the efficacy of magnesium salts against a number of diverse cardiotoxic agents (for reviews of cardioprotective effect of Mg salts, see 3, 4, 46, 47).

Wherever possible, data have been selected from studies with cardiac mitochondria, because of evidence that mitochondria from the heart differ from the parenchymal mitochondria that have been more commonly used in the basic studies. Green and Fleischer (20) have observed that cardiac mitochondria are jam-packed with cristae, whereas liver mitochondria have relatively few cristae, and they are more widely spaced. The greater the oxidative-phosphorylative activity of the cells, the tighter the arrangement of cristae. DiGiorgio *et al.* (14) observed that heart mitochondria are more susceptible than are liver or kidney mitochondria to the impairment of oxidative phosphorylation that is caused by magnesium deficiency and/or thyroxine. Carafoli *et al.* (10) observed that active accumulation of Mg^{++} and Ca^{++} by the liver mitochondria they employed was not great, whereas the heart mitochondria used by Brierley *et al.* (7, 8) had considerable avidity for Mg^{++} and inorganic P ions, coincident with which H^{+} was released. Ernster (16) has considered the evidence that mitochondrial accumulation of divalent cations with inorganic phosphate functions as a buffering system.

Mitochondrial membranes have been described by Gebicki and Hunter (18) as forming a backbone on which the components of the electron transport system and oxidative phosphorylation system are arranged. Green and Fleischer (20) have described the mitochondrion as the structure that provides utilizable energy for the basic cellular functions (table II). Mitochondria are devices for oxidative phosphorylation, or coupling the synthesis of ATP to the oxidative reactions of the citric acid cycle, for fatty acid oxidation, and for utilization of fatty acids in the formation of acetyl coenzyme A. The electron transport system, which participates in oxidation-reduction reactions and active ion transport, is also a

Table II

Mitochondrial Functions

- Provides utilizable energy for basic cellular functions
- Oxidative-phosphorylation (couples synthesis of ATP to the oxidative steps of the citric acid cycle)
- Fatty acid oxidation (in heart mitochondria)
- Fatty acid conversion to acetyl coenzyme A (coupling fatty acid to citric acid oxidation)
- Electron transport system: respiratory chain oxidation-reduction reactions
- Energy for active ion transport

mitochondrial subunit. The arrangement and organization of enzymes and substrates of the mitochondrion implement its functions in cellular metabolism (20). Physiologic swelling and contraction of the mitochondria lead to altered biochemical activity in response to changes in cellular activity; pathologic swelling and disruption of the mitochondria lead to abnormalities in biochemical activity and ultimately to cell death.

Magnesium is intimately involved in mitochondrial structure and function (fig. 1). It is necessary for formation of the compact Mg-ATP complex (5, 17, 36) at which phase the mitochondrion is "contracted." Calcium, on the other hand, favors the release of ATP from the mitochondria. Ernster and Low (17) have described the mitochondrial arrangement as then being "loose." This is the swollen mitochondrion. As Ca^{++} is taken up by mitochondria (liver), there is an increase in intramitochondrial Na^{+} and a decrease in K^{+}, with reversal of the K : Na ratio (10). It has recently been suggested that, at this phase, there is ATP-induced uptake of Ca^{++} by sarcosomal membranes (11), which is associated with contraction of muscles. (21). Mg^{++}, possibly under the influence of parathyroid hormone, enters the mitochondria in association with phosphate, complexes with ATP in the mitochondria, which then contract. At this phase, K^{+} enters the mitochondria and Na^{+} leaves[1]. Magnesium's complexing with diphosphopyridine nucleotide (DPN) participates as part of the electron-transport respiratory chain in activation of mitochondrial ATPase (37, 48).

Recent findings by Schwartz and his colleagues (28, 29, 43) indicate that K^{+} efflux from mitochondria is an energy-dependent process that is effected by polycationic proteins, including parathyroid hormone and histone. These authors have suggested that histone may enter the mitochondrion with phosphate via an energy-dependent uptake mechanism. Within the mitochondrion, the histone-PO_4 complex would lead to efflux of K^{+}, and possibly of

[1] For more detailed and additional information on the steps involved in the ion transfer reactions, see: Carafoli *et al.* (10), Ernster (16), Rasmussen *et al.* (30, 39), and Sallis *et al.* (41).

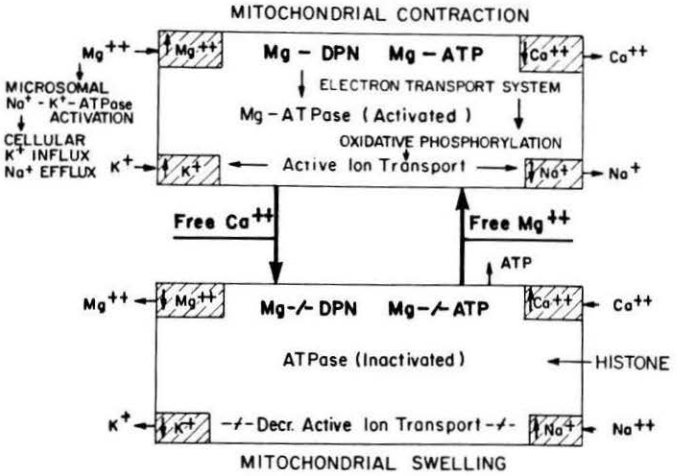

Fig. 1 Physiologic mitochondrial processes.

Mg^{++}, in exchange for the polycationic protein. The mitochondrial swelling and K^+ efflux thus induced is prevented by addition of Mg^{++}.

In their detailed review of the literature on the respiratory chain and oxidative phosphorylation up to 1956, Chance and Williams (12) considered the evidence substantial that Mg acts to preserve the mitochondrial structure. They observed that this function logically explains the prevention by magnesium of the mitochondrial swelling caused either by Ca^{++} or thyroxine. Ernster and his associates (17, 48), Baltscheffsky (5), and Hunter *et al.* (26) have attributed magnesium's stabilization of mitochondria to its complexing with chemical groups on its membrane.

Magnesium deficiency thus causes cellular loss of potassium by several means: a) through decreased microsomal Mg-Na-K-ATPase activity, as a result of which there is decreased ability to maintain an appropriate concentration gradient; b) through basic protein (*i.e.*, histone)-induced K^+ efflux from the mitochondrion; and finally c) through mitochondrial disruption, with destruction of the cellular machinery required for cellular activity (fig. 2).

Lehninger (32) has observed that mitochondrial swelling caused by a wide diversity of chemical agents is preventable or reversible by what appears to be a single, basic mechanism that is ATP-driven. It should be noted, here, that Nanninga (36) has provided evidence that 90% of muscle ATP exists as Mg-ATP, the Mg^{++} probably acting as a bridge, binding substrate to enzyme. In fact, the extent of the mitochondrial swelling has been shown to be inversely proportional to the concentration of Mg^{++} (13).

Brierley (6) has recently suggested that cardiac mitochondrial membrane lesions may favor massive accumulation of magnesium and inorganic phosphate.

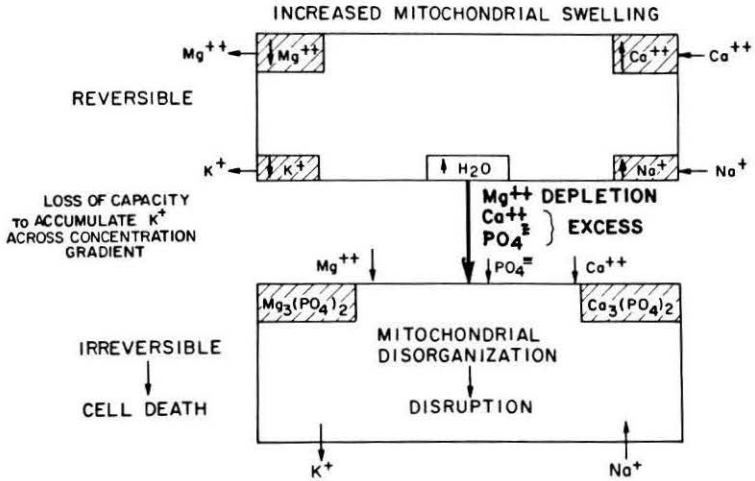

Fig. 2 Mitochondrial changes with magnesium deficiency.

Isolated mitochondria with tightly coupled oxidative phosphorylation accumulated the magnesium more slowly than did those with impaired respiratory control (caused by mechanical damage or by heavy metals). Brierley (6) considers the resultant precipitation of $Mg_3\ (PO_4)_2$ within the damaged mitochondria as a probable pathologic reaction (fig. 2).

This provides the first clear indication that not all of the magnesium in the heart is necessarily functional. Early studies of cardiac mitochondria from magnesium-deficient animals suggested this possibility. For example, Nakamura *et al.* (35) reported that rats on a Mg-deficient diet for 12 days had swollen cardiac mitochondria that contained about the same amount of magnesium (or even slightly more) than did the mitochondria from control rats. That these were not physiologically swollen mitochondria is indicated by the morphologic disorganization of the cristae seen on electron microscopy. Di Giorgio *et al.* (14) proposed that cardiac sarcosomes from Mg-deficient ducks contained Mg which was either insufficient or present in a form unsuitable for coupling of oxidation to phosphorylation.

The particulate electron-dense material seen in the cardiac sarcosomes of Mg-deficient rats was interpreted by Heggtveit *et al.* (22–24) and Mishra and Herman (34) as probable evidence of calcification (fig. 3). That these granules were, indeed, probably calcium is indicated by the observation by Brierley and Slauterback (9) that the fixation procedure for preparation of cardiac mitochondrial pellets, employing osmium tetroxide, removes 80% or more of the inorganic phosphorus associated with magnesium, but only about half that associated with calcium.

Jennings (27) has very recently observed that the development of dense

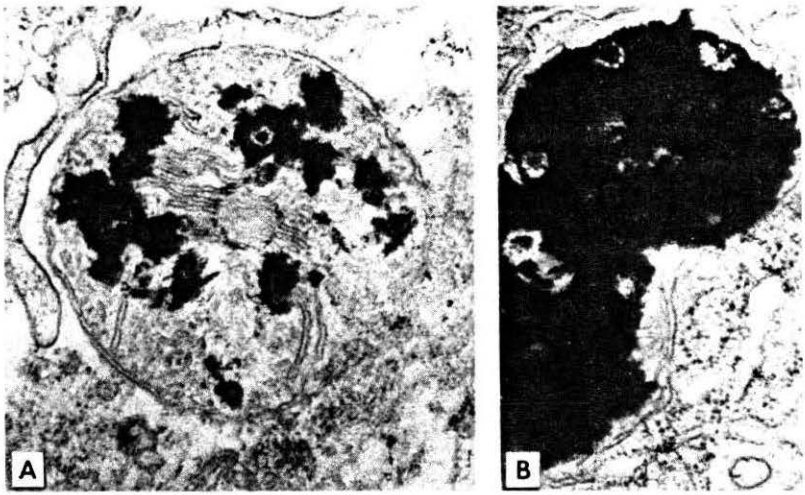

Fig. 3 Degree of filling of mitochondria with electron-dense granules, presumably Ca, in Mg-deficient, stressed rat, day 29. A, Solid electron-dense particles; B, sarcosome filled with tightly packed particles. From Heggtveit *et al.* (24).

granules in the cardiac mitochondria 20 min after coronary occlusion may well be caused by redistribution of Ca^{++} or Mg^{++} within the mitochondria. He has suggested that the crystallization or binding of essential cofactors like inorganic phosphate and Mg^{++} in the granules might contribute to irreversible mitochondrial failure. Demonstration of this shift from functional to nonfunctional Mg may have been provided by Hochrein *et al.* (25) (table III). The progressive drop of myocardial Mg, seen in guinea pigs for the first 4 min of asphyxia, and the

Table III

Myocardial Magnesium in Asphyxia (Guinea Pigs)

Duration of asphyxia (min)	mEq/l	
0	16.6	Control
0.5	14.1	Tachycardia
1.0	11.4	Onset: hypoxic dilatation
1.5	11.4	Complete: hypoxic dilatation
2.0	11.1	AV–block I
2.5	10.9	AV–block II
4.0	10.9	AV–dissociation
8.0	11.7	Cardiac arrest (2 min)
10.0	12.5	Cardiac arrest (4 min)
10.5	15.5	Cardiac arrest (4.5 min)

Translated from Hochrein *et al.* (25).

subsequent rise to almost the control level after 4 1/2 min of cardiac arrest, may have been caused by acute loss of functional Mg, followed by precipitation of the inorganic phosphate salt.

Magnesium normally functions in the high energy phosphate reactions involved in citric acid and fatty acid metabolism, active potassium and sodium transport, in muscle contraction and relaxation, and in maintenance of mitochondrial integrity. When present as an insoluble phosphate salt, magnesium is at best nonfunctional. Whether phosphate precipitates of calcium and magnesium can contribute to cell damage remains to be proved.

SUMMARY

Magnesium is necessary for mitochondrial function and integrity. Cardiac mitochondria are most susceptible to loss of magnesium. Under physiologic conditions, the shift of magnesium out of and of calcium into the mitochondria is associated with impaired mitochondrial structure and function. Inadequate Mg interferes with activation of Na^+-K^+-ATPase, with resultant shifts of K out of and Na into the cell. With pathologically decreased magnesium levels, there is irreversible loss of cellular potassium and, ultimately, mitochondrial disruption and deposition of crystalline phosphates of both calcium and magnesium into mitochondria and microsomes.

REFERENCES

1. AUDITORE, J. V.: Sodium-potassium activated G-strophanthin sensitive ATPase in cardiac muscle. Proc. Soc. Exp. Biol. Med. *110:* 595–597 (1962).
2. AUDITORE, J. V. and MURRAY, L.: The localization of the Mg+Na+K activated G-strophanthin sensitive ATPase in cardiac muscle. Arch. Int. Pharmacodyn. *145:* 137–146 (1963).
3. BAJUSZ, E.: Electrolytes and Cardiovascular Diseases (Karger, Basel 1965).
4. BAJUSZ, E.: Nutritional Aspects of Cardiovascular Diseases. (Lippincott, Philadelphia 1965).
5. BALTSCHEFFSKY, H.: Mitochondrial respiratory control and phosphorylative activities in a Mg-free medium. Biochim. Biophys. Acta. *25:* 382–388 (1957).
6. BRIERLEY, G. P.: Ion transport by heart mitochondria. J. Biol. Chem. *242:* 1115–1122 (1967).
7. BRIERLEY, G. P.; BACHMANN, E. and GREEN, D. E.: Active transport of inorganic phosphate and Mg ions by beef heart mitochondria. Proc. Nat. Acad. Sci. *48:* 1928–1935 (1962).
8. BRIERLEY, G.; MURER, E.; BACHMANN, E. and GREEN, D. E.: Studies on ion transport. II. The accumulation of inorganic phosphate and Mg ions by heart mitochondria. J. Biol. Chem. *238:* 3482–3489 (1963).
9. BRIERLEY, G. P. and SLAUTTERBACK, D. B.: Studies on ion transport. IV. An electron microscope study of the accumulation of Ca^{2+} and inorganic phosphate by heart mitochondria. Biochim. Biophys. Acta. *82:* 183–186 (1964).
10. CARAFOLI, E.; ROSSI, C. S. and LEHNINGER, A. L.: Cation and anion balance during active accumulation of Ca^{++} and Mg^{++} by isolated mitochondria. J. Biol. Chem. *239:* 3055–3061 (1964).
11. CARVALHO, A. P. and LEO, B.: Effects of ATP on the interaction of Ca^{++}, Mg^{++} and K^+ with fragmented sarcoplasmic reticulum isolated from rabbit skeletal muscle. J. Gen. Physiol. *50:* 1327–1352 (1967).

12. CHANCE, B. and WILLIAMS, G. R.: The respiratory chain and oxidative phosphorylation. Adv. Enzymology *17:* 65–134 (1956).
13. CONNELLY, J. L. and LARDY, H. A.: The effect of adenosine triphosphate and substrate on orthophosphate-induced mitochondrial swelling at acid pH. J. Biol. Chem. *239:* 3065–3070 (1964).
14. DI GIORGIO, J.; VITALE, J. J. and HELLERSTEIN, E. E.: Sarcosomes and magnesium deficiency in ducks. Biochem. J. 82: 184–187 (1962).
15. DUNHAM, E. T. and GLYNN, I. M.: Adenosinetriphosphatase activity and the active movements of alkali metal ions. J. Physiol. *156:* 274–293 (1961).
16. ERNSTER, L.: Control of cell metabolism at the mitochondrial level. Fed. Proc. *24:* 1222–1236 (1965).
17. ERNSTER, L. and LOW, H.: Reconstruction of oxidative phosphorylation in aged mitochondrial systems. Exp. Cell Res. (Suppl. *3:*) 133–153 (1955).
18. GEBICKI, J. M. and HUNTER, F. E.: Determination of swelling and disintegration of mitochondria with an electronic particle counter. J. Biol. Chem. *239:* 631–639 (1964).
19. GLYNN, I. M.: "Transport adenosinetriphosphatase" in electric organ. The relation between ion transport and oxidative phosphorylation. J. Physiol. *169:* 452–465 (1963).
20. GREEN, D. E. and FLEISCHER, S.: The Mitochondrial System of Enzymes in Metabolic Pathways. (Academic Press, New York 1960).
21. HAUGAARD, N.; HAUGAARD, E. S.; NAM HEA LEE and HORN, R. S.: Possible role of mitochondria in regulation of cardiac contractility. Fed. Proc. *28:* 1657–1662 (1969).
22. HEGGTVEIT, H. A.: The cardiomyopathy of magnesium deficiency in: Electrolytes and Cardiovascular Diseases pp. 204–220 (Karger, Basel 1965).
23. HEGGTVEIT, H. A.: Myopathy in experimental magnesium deficiency. Ann. N.Y. Acad. Sci. *162:* 758–765 (1969).
24. HEGGTVEIT, H. A.; HERMAN, L. and MISHRA, R. K.: Cardiac necrosis and calcification in experimental magnesium deficiency. Amer. J. Path. *45:* 757–782 (1964).
25. HOCHREIN, H.; KUSCHKE, H. J.; ZAQQA, Q. und FAHL, E.: Das Verhalten der intracellularen Magnesium-Konzentration in Myokard bei Insuffizienz, Hypoxie und Kammerflimmern. Klin. Wschr. *45:* 1093–1096 (1967).
26. HUNTER, F. E.; LEVY, J. F.; FINK, J.; SCHUTZ, B.; GUERRA, F. and HURWITZ, A.: Studies on the mechanism by which anaerobiosis prevents swelling of mitochondria *in vitro:* Effect of electron transport chain inhibitors. J. Biol. Chem. *234:* 2176–2186 (1950).
27. JENNINGS, R. B.: Symposium on the pre-hospital phase of acute myocardial infarction. Part II – Early phase of myocardial ischemic injury and infarction. Amer. J. Card. *24:* 753–765 (1969).
28. JOHNSON, C. J.; MAURITSEN, C. M.; STARBUCK, W. C. and SCHWARTZ, A.: Histones and mitochondrial ion transport. Biochem. *6:* 1121–1127 (1967).
29. JOHNSON, C. L.; ORO, J. and SCHWARTZ, A.: Basic protein induction of low amplitude energy-linked mitochondrial swelling. Arch. Biochem. Biophys. *131:* 310–315 (1969).
30. KIMMICH, G. A. and RASMUSSEN, H.: The effect of parathyroid hormone on mitochondrial ion transport in the terminal portion of the cytochrome chain. Biochim. Biophys. Acta. *113:* 457–466 (1966).
31. KLEIGER, R. E.; SETA, K.; VITALE, J. J. and LOWN, B.: Effects of chronic depletion of potassium and magnesium upon the action of acetylstrophanthidin on the heart. Amer. J. Card. *17:* 520–527 (1966).

32. LEHNINGER, A. L.: Reversal of various types of mitochondrial swelling by adenosine triphosphate. J. Biol. Chem. *234:* 2465–2471 (1959).
33. LEHNINGER, A. L.; WADKINS, C. L.; COOPER, C.; DEVLIN, T. M. and GAMBLE, J. L: Oxidative phosphorylation. Experiments with fragments of mitochondria offer new information about respiratory energy conversion. Science *128:* 450–456 (1958).
34. MISHRA, R. K. and HERMAN, L.: Preliminary observation on the ultrastructure of rat cardiac muscle following a Mg-deficient regime and cold stress in: Proc. European Reg. Conf. on Electron Microscopy, pp. 907–911 (1960).
35. NAKAMURA, M.; NAKATINI, M.; KOIKE, M.; TORII, S. and HIRAMATSU, M.: Swelling of heart and liver mitochondria from magnesium deficient rats and its reversal. Proc. Soc. Exp. Biol. Med. *108:* 315–319 (1961).
36. NANNINGA, L. B.: Calculation of free magnesium, calcium, and potassium in muscle. Biochim. Biophys. Acta. *54:* 338–344 (1961).
37. PACKER, L.: Metabolic and structural states of mitochondria. II Regulation by phosphate. J. Biol. Chem. *236:* 214–220 (1961).
38. POST, R. L.; MERRITT, C. R.; KINSOLVING, C. R. and ALBRIGHT, C. D.: Membrane adenosine triphosphatase as a participant in the active transport of sodium and potassium in the human erythrocyte. J. Biol. Chem. *235:* 1796–1802 (1960).
39. RASMUSSEN, H.; FISCHER, J. and ARNAUD, C.: Parathyroid hormone, ion exchange and mitochondrial swelling. Proc. Nat. Acad. Sci. *52:* 1198–1203 (1964).
40. SALLIS, J. D. and DELUCA, H. F.: Action of parathyroid hormone on mitochondria. Magnesium- and phosphate-independent respiration. J. Biol. Chem. *241:* 1122–1127 (1966).
41. SALLIS, J. D.; DELUCA, H. F. and RASMUSSEN, H.: Parathyroid hormone-dependent uptake of inorganic phosphate by mitochondria. J. Biol. Chem. *238:* 4098–4102 (1963).
42. SCHWARTZ, A.: A sodium and potassium stimulated adenosine triphosphatase from cardiac tissues–I Preparation and properties. Biochem. Biophys. Res. Comm. *9:* 301–306 (1962).
43. SCHWARTZ, A.: The effect of histones and other polycations on cellular energetics I Mitochondrial oxidative phosphorylations. J. Biol. Chem. *240:* 939–943 (1965).
44. SCHWARTZ, A. and LASETER, A. H.: A sodium- and potassium-stimulated adenosine triphosphatase from cardiac tissues–II The effects of ouabain and other agents that modify enzyme activity. Biochem. Pharmacol. *13:* 337–348 (1964).
45. SELLER, R. H.; NEFF, M. H.; MENDELSSOHN, S.; KIM, K. E. and SWARTZ, C. D.: Magnesium sulfate in digitalis toxicity. Amer. J. Card. *25:* 127 (1970).
46. SELYE, H.: The Chemical Prevention of Cardiac Necrosis. (Ronald Press Co., New York, 1958).
47. SELYE, H.: The Pluricausal Cardiopathies. (Charles C Thomas, Springfield, Ill. 1961).
48. SIEKEVITZ, P.; LOW, H.; ERNSTER, L. and LINDBERG, O.: On a possible mechanism of the adenosinetriphosphatase of liver mitochondria. Biochim. Biophys. Acta. *29:* 378–391 (1958).
49. SKOU, J. C.: Enzymatic basis for active transport of Na^+ and K^+ across cell membrane. Physiol. Rev. *45:* 596–617 (1965).
50. SKOU, J. C.: Further investigations on a Mg^{++} + Na^+-activated adenosinetriphosphatase, possibly related to the active, linked transport of Na^+ and K^+ across the nerve membrane. Biochim. Biophys. Acta. *42:* 6–23 (1960).
51. SKOU, J. C.: The influence of some cations on an adenosine triphosphatase from peripheral nerves. Biochim. Biophys. Acta. *23:* 394–401 (1957).
52. SKOU, J. C.: Preparation from mammalian brain and kidney of the enzyme system involved in active transport of Na^+ and K^+. Biochim. Biophys. Acta. *58:* 314–325 (1962).

53. SZEKELY, P. and WYNNE, N. A.: The effects of magnesium on cardiac arrhythmias caused by digitalis. Clin. Sci. *10:* 241–247 (1951).

54. ULRICH, F.: Active transport of potassium by heart mitochondria. Amer. J. Physiol. *198:* 847–854 (1960).

55. VITALE, J. J.; HEGSTED, D. M.; NAKAMURA, M. and CONNORS, P.: The effect of thyroxine on magnesium requirement. J. Biol. Chem. *226:* 597–601 (1957).

56. VITALE, J. J.; HELLERSTEIN, E. E.; NAKAMURA, M. and LOWN, B.: Effects of magnesium-deficient diet upon puppies. Circulat. Res. *9:* 387–394 (1961).

57. VITALE, J. J.; NAKAMURA, M. and HEGSTED, D. M.: The effect of magnesium deficiency on oxidative phosphorylation. J. Biol. Chem. *228:* 573–576 (1957).

58. VITALE, J. J.; VELEZ, H.; GUZMAN, C. and CORREA, P.: Magnesium deficiency in the cebus monkey. Circulat. Res. *12:* 642–650 (1963).

59. WELT, L. G.: Experimental magnesium depletion. Yale J. Biol. Med. *36:* 325–349 (1964).

60. WELT, L. G. and TOSTESON, D. C.: The mode of activation of membrane ATPase by Mg^{++}. South. Soc. Clin. Res. *12:* 73 (1964).

61. WHANG, R. and WELT, L. G.: Observations in experimental magnesium depletion. J. Clin. Invest. *42:* 305–313 (1963).

62. ZWILLINGER, L.: Uber die Magnesiumwirkung auf das Herz. Klin. Wschr. *14:* 1429–1433 (1935).

Mailing address: Mildred S. Seelig, M.D., Department of Medical Statistics, Schering Corporation, Bloomfield, New Jersey 07003 (USA).