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Magnesium: Its Biologic Significance

Jerry K. Aikawa



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MAGNESIUM: ITS BIOLOGIC SIGNIFICANCE

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PREFACE

This volume is the first in a series to be devoted to cations of biologic significance. In this monograph I attempt to synthesize and place in global perspective our current knowledge of magnesium and to develop a hypothesis concerning the role of magnesium in all biologic processes. Its role is traced as far back as possible in the evolution of the planet earth. When viewed in this perspective, the essentiality of magnesium becomes awesomely obvious.

As a scientist probes deeper into the mysteries of life, and as his understanding thereof increases, he hopefully will feel humbler as he glimpses the elegance and intricacies of the mechanisms of life which surpass one's understanding.

To a man who knows nothing
Mountains are mountains
Water is water and
Trees are trees.
When he has studied and
knows a little,
Mountains are no longer
mountains
Water is no longer water and
Trees are no longer trees.
When he has thoroughly
understood,
Mountains are again
mountains
Water is water and
Trees are trees.

Anonymous

THE AUTHOR

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The University of Colorado has been my professional and academic home for the past 26 years. It has been a sanctuary which has given me the opportunity and the privilege to contribute as best I know how to the sum total of human knowledge, welfare, understanding, and hopefully, happiness. For this I am most grateful.

My faithful and efficient secretary, Mrs. Junioretta Cummings, has suffered the many revisions and unreasonable demands; I wish to acknowledge her invaluable assistance.

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Chapter 1

PROLOGUE

I. THE BEGINNING

A. Day 1

In the beginning God created the heaven and the earth.

And the earth was without form, and void; and darkness was upon the face of the deep. And the Spirit of God moved upon the face of the waters.

And God said, Let there be light: and there was light.

And God saw the light, that it was good: and God divided the light from the darkness.

And God called the light Day. And the darkness he called Night. And the evening and the morning were the first day.¹

If one assumes that the sun, the planets, the meteorites, and other debris of the solar system all formed from the same primordial cloud of dust at about the same time, and if one assumes that radioactive-isotopic dating of stony meteorites is accurate, then one is led to conclude that the age of our planet is 4.6 billion years. As the primordial cluster of dust particles coalesced into the new planet earth and as the atmosphere cleared, there was light; and as the new creation rotated on its axis around the sun, there was Day and Night.

B. Day 2

And God said, Let there be a firmament in the midst of the waters, and let it divide the waters from the waters.

And God made the firmament, and divided the waters which were under the firmament from the waters which were above the firmament: and it was so.

And God called the firmament Heaven. And the evening and the morning were the second day.²

For a billion years the atoms on Earth met, parted, and remet until a certain semblance of order evolved, and the elements appeared. And as the elements met, parted, and remet, inorganic chemical compounds grew in complexity, and the firmament appeared 3.5 billion years ago. Two days and nights states the Bible; one billion years reply the scientists.

C. Day 3

And God said, Let the waters under the heaven be gathered together unto one place, and let the dry land appear: and it was so. And God called the dry land Earth; and the gathering together of the waters called he Seas: and God saw that it was good.³

Two hydrogen atoms and one oxygen atom met to form a polar configuration and to become water. As the water rose as vapor, and fell as rain, it was laden with the materials dissolved from the firmament. Thus appeared the ancient sea. The light from the sun shown for millions of years upon the ancient sea. It heated and accelerated the meeting, the parting, and the remeeting of the inorganic chemical compounds, until eventually there appeared organic compounds, the stuff of life.

And God said, Let the Earth bring forth grass, the herb yielding seeds, and the fruit tree yielding fruit after his kind, whose seed is in itself, upon the earth: and it was so.

And the Earth brought forth grass, the herb yielding seed after his kind, and the tree yielding fruit, whose seed was in itself, after his kind: and God saw that it was good.

And the evening and the morning were the third day.⁴

D. Day 4

As organic compounds arranged themselves in myriad ways there arose the most primitive of life in the anaerobic brine — bacteria, we think, but nonetheless life. This ancient form of life captured the energy of the sun to transform the atmosphere from anaerobic to aerobic. A porphyrin ring encircled a magnesium atom and chlorophyll was born. The chlorophyll produced oxygen, adenosine triphosphate, and carbohydrate. Now the Earth was green and the atmosphere laden with oxygen. We call the process photosynthesis. An evening and a morning states the Bible. Another billion years say the scientists.

And God said, Let there be lights in the firmament of the heaven to divide the day from the night; and let them be for signs, and for seasons, and for days, and years:

And let them be for lights in the firmament of the heaven to give light upon the earth; and it was so.

And God made two lights; the greater light to rule the day, and the lesser light to rule the night: he made the stars also.

And God set them in the firmament of the heaven to give light upon the earth.

And to rule over the day and over the night, and to divide the light from the darkness: and God saw that it was good.

And the evening and the morning were the fourth day.⁵

E. Day 5

And so the evolutionary process continued for another billion and a half years. Other organic compounds appeared — nucleotides, nucleic acids, purines, and pyrimidines. A membrane encircled the primitive deoxyribonucleic acid, and thus developed the nucleus, and eukaryotes appeared, initially as algae. More specialization of function occurred as ribonucleic acids synthesized proteins in ribosomes. The energy for this was derived from oxidative phosphorylation in mitochondria. Greater efficiency in energy utilization led to the development of multicellular plants.

And God said, Let the waters bring forth abundantly the moving creature that hath life, and fowl that may fly above the Earth in the open firmament of heaven.

And God created great whales, and every living creature that moveth, which the waters brought forth abundantly after their kind, and every winged fowl after his kind; and God saw that it was good.

And God blessed them, saying, Be fruitful and multiply, and fill the waters in the seas, and let fowl multiply in the air.

And the evening and the morning were the fifth day.⁶

F. Day 6

Multicellular animals appeared about one billion years ago. Vertebrates appeared 400 million years ago, amphibians, 300 million years ago. Mammals first appeared about 225 million years ago. Thus, the fifth biblical day was 600 million years.

And God said, Let the Earth bring forth the living creatures after his kind, cattle and creeping thing, and beast of the Earth after his kind: and it was so.⁷

Homo erectus appeared about 1.8 million years ago; 1.3 million years later his descendant discovered fire and thereafter limited his nomadic ways. Modern man appeared less than 100,000 years ago. And so was completed this sixth biblical day.

G. Day 7

And on the seventh day God rested. Would that God had spent a little more time in perfecting Man.

Table 1
**A COMPARISON OF THE BIBLICAL AND THE SCIENTIFIC SEQUENCE
AND TIME TABLE FOR EVOLUTION**

Biblical			Scientific	
Day	Creation of	Years ($\times 10^6$)	Evolution	Appearance of
1	Light, night, day	4600	Chemical Atomic Inorganic compounds	H, He, Li, Be, B, C, N, O, F1, Na, Mg, Al, Si, P, S, Cl H ₂ , N ₂ , H ₂ O, CH ₄ , NH ₃ , CO, CO ₂ ; No O ₂
2	Firmament	4000	Biological Organic compounds	Aldehydes, carboxylic acids, amino acids
3	Land, water, sea	3500	Anaerobic bacteria	Life Photosynthesis
4	Grass	2500	Anaerobic photosynthetic bacteria	
		2000	Eukaryotic cells	Oxygen atmosphere
		1500	Multicellular plants	Oxidative phosphorylation
5	Creatures in water, fowls, whales	1000	Multicellular animals	Protein synthesis
6	Vertebrates, mammals	400	Modern man	Genetic transcription
	Man	<0.1		

REFERENCES

1. Genesis 1:1-5.
2. Genesis 1:6-8.
3. Genesis 1:9,10.
4. Genesis 1:11-13.
5. Genesis 1:14-19.
6. Genesis 1:20-23.
7. Genesis 1:24.



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Chapter 2

EVOLUTION

This story appropriately commences with Chapter 1 of the Old Testament and with the creation of the planet Earth.

I. GENESIS

The latest scientific evidences suggest that the planet Earth appeared about 4.6 billion years ago following a cosmic event of unimaginable proportions.¹ This planet was initially a blob, in which, in due time, elements predominantly of the lower atomic weights — hydrogen and helium — appeared; the atmosphere was without oxygen. The Earth became stratified into a core, a mantle, and a crust as a result of the heat released as the planet was built up by accretion. The core is made up of iron and nickel and the mantle corresponds roughly in composition to the silicate mineral olivine (FeMgSiO_4).

The original surface of the Earth may have been too hot for water to remain liquid, but as soon as the temperature had fallen below the boiling point, water, released from the interior by outgassing processes, such as volcanism, would have condensed to form the original oceans. Outgassing would have given rise to a secondary atmosphere composed of water vapor (from the water of hydration of minerals), methane, carbon dioxide, carbon monoxide (from the decomposition of metal carbides) ammonia and nitrogen (from nitrides), and hydrogen sulfide (from sulfides). It is from this secondary atmosphere, the character of which was reducing rather than oxidizing, that life presumably arose from nonliving matter some 3.5 billion years ago, most probably preceded by a period of chemical evolution lasting about 500 million years.

II. CHEMICAL EVOLUTION

During this chemical evolution it is generally believed that all of the molecules essential for life were successively synthesized. Starting with pure inorganic compounds, a set of inorganic-organic reactions took place leading to the building stones for proteins and nucleic acids; these are amino acids and nucleotides. Similarly, during this chemical evolution, the mixture of inorganic and organic polyphosphates was synthesized, these being necessary to provide the primary chemical reserve of free energy for further evolution. As a consequence, polymerization and organization occurred; after a further period of development, the first cell evolved.

Since the early 1950s, beginning with the studies of Urey and Miller, evidence has accumulated suggesting that energy sources available on the primitive Earth — perchance, thunder and lightning — could have induced the synthesis of organic compounds from the gases present then.^{2,3} Aldehydes, carboxylic acids, and amino acids have been produced experimentally from inorganic gases under conditions simulating those of the primitive Earth. Biologic monomers, such as amino acids, sugars, phosphates, organic bases, lipids, and other special-purpose molecules, such as flavins, became available. These monomers then polymerized into primitive protein and nucleic acid chains.

A possible mechanism for concentrating prebiologic molecules involves the adsorption of molecules on the surface of common minerals. For example, L-amino acids (L-leucine and L-aspartate) and D-glucose bind in a stereospecific manner to a colloidal

clay, bentonite. The biologically uncommon enantiomers, (D-leucine, D-aspartate, and L-glucose) do not exhibit any selective adsorption on bentonite. It has been suggested that this difference between stereoisomers could account for the evolution of life forms possessing a great preponderance of L-amino acids and D-glucose.⁴ Micas and clay consist of stacked silicate sheets held together by positive ions, with layers of water molecules between the sheets. A cube of kaolinite clay 1 cm on a side provides a total surface area of about 3800 m², or two thirds the area of a football field. It is possible that such a surface could have served as a primitive catalytic center for the polymerization of amino acids, thus being an early step in the evolution of biological protein synthesis.

Living organisms that share an environment with other organisms must be clearly set off from that environment by a boundary surface to avoid being diluted out of existence. There is evidence that an aqueous solution of polymers tends to separate spontaneously into coacervates: polymer-rich colloidal droplets suspended in a water-rich surrounding medium. An important property of coacervates is that substances whose solubility differs in the two phases will be preferentially concentrated in one place or the other.

III. THE BEGINNING OF LIFE

It is possible that over the eons there evolved the type of droplets that contained within themselves the ability to take molecules and energy from their surroundings and incorporate them into substances that would promote the survival not only of the parent droplets but also of the daughter droplets into which the parents were dispersed when they became too big. It is not life, but it is getting close to it. The missing ingredient is an orderly mechanism for ensuring that all the daughter droplets receive the catalysts they need for all the reactions important for their survival. In pragmatic terms, this is the genetic apparatus. Unfortunately, there is no laboratory model for the evolution of the genetic machinery.

A living cell has two central talents: a capacity for metabolism and a capacity for reproduction. The cell survives in the short run by rearranging the atoms of the compounds it ingests into molecules needed for its own maintenance. It survives vicariously over the long run by being able to reproduce itself and give rise to offspring with similar biochemical talents. Both capacities must have developed in parallel. The functioning metabolism is protected against dilution and destruction by its surrounding by some kind of membrane.

IV. MAGNESIUM IN BIO-EVOLUTION

The first primitive cell, in all probability, was not as complicated as the present-day cells. If this very ancient cell functioned in a manner similar to a present cell, a minimum of about 100 different protein molecules would have been required in order to maintain protein synthesis and anaerobic energy production. Incorporated in this ancient cell was a series of metal ions. The main metal ion catalyst at that time, perhaps the only one, must have been the magnesium ion, since almost all of the reactions involved in fundamental cell reactions, such as protein biosynthesis and anaerobic energy production, require Mg²⁺ ions. Also, magnesium ions are known to catalyze several prebiotic condensation reactions. Further assumption that magnesium ions were important early in bio-evolution arises from its presence in seawater and sea sediment, an environment similar, it is assumed, to that from which our cell system once evolved.

A comparison between the extra- and intracellular spaces and between seawater and sediment indicates that there are high concentrations of potassium, magnesium, and phosphate both in the sediment and within the mammalian cell as compared with the concentrations occurring in seawater and the extracellular space, whereas there is a high chloride concentration both in seawater and in extracellular space compared with the amount of chloride present in sediment and cells. These data indicate that a "solid crystalline" phase surrounded by its mother liquor might be regarded as a model for the cell and its extracellular fluid and thus similar to the solid sea sediments surrounded by seawater.

By the biblical third day or 3.5 billion years ago, there was land, water, and sea. When life first appeared on Earth about that time, the atmosphere was reducing rather than oxidizing. The first living cells were presumably small, spheroidal anaerobes. They survived by fermenting organic molecules formed nonbiologically in the anoxic environment. The role of such ready-made nutrients was diminished, however, when the first photosynthetic organisms evolved. This earliest mode of photosynthesis was entirely anaerobic.

V. PHOTOSYNTHESIS

The capacity of the Earth to support life was enormously enhanced by the invention of photosynthesis, which enabled living organisms to capture solar energy for the synthesis of organic molecules. This early photosynthetic mechanism used hydrogen sulfide as the source of hydrogen atoms for reducing carbon dioxide, which is the process conducted today by the green and purple sulfur bacteria.

A little more than two billion years ago aerobic photosynthesis began in the precursors of modern cyanobacteria. The cyanobacteria, a group of prokaryotic organisms, are of particular interest in that they were primarily responsible for the development of the oxygen-rich atmosphere. In their biochemistry these organisms seem to occupy a middle ground between the anaerobes and the eukaryotes. Under anoxic conditions these species not only halt respiration but also adopt an anaerobic mode of photosynthesis, employing hydrogen sulfide (H_2S) instead of water and releasing sulfur instead of oxygen.^{4, 5}

Another activity of some cyanobacteria that seems to reflect an earlier adaptation to anoxic condition is nitrogen fixation. Nitrogen is an essential element of life, but it is biologically useful only in "fixed" form, for example, combined with hydrogen as ammonia (NH_3). Only prokaryotes are capable of fixing nitrogen. The crucial complex of enzymes for fixation, the nitrogenases, is highly sensitive to oxygen. Nitrogen fixation is increased with higher magnesium levels in the soil and higher degrees of saturation of the clay by this nutrient.⁶

VI. OXYGEN

Oxygen was generated by the stromatolite-building microorganisms, but for some 100 million years little of it accumulated in the atmosphere. Instead it reacted with iron dissolved in the oceans, which was then precipitated to create massive banded iron formations. Only when the oceans had been swept free of iron and similar materials did the concentration of free oxygen begin to rise toward modern levels. This biologically induced change in the environment had several effects on biological development. Anaerobic organisms were forced to retreat to anoxic habitats, leaving the

best spaces for photosynthesis to the cyanobacteria. In a similar manner the nitrogen-fixing organisms had to adapt an anaerobic way of life or develop protective heterocyst cells. Atmospheric oxygen also created a layer of ozone (O_3) that filtered out most ultraviolet radiation. Once the oxygen-rich atmosphere was fully established, cells evolved that not only could tolerate oxygen but also could employ it in respiration. The result was a great improvement in metabolic efficiency.⁷

Perhaps the most intriguing mineral evidence for the date of the oxygen transitions comes from an iron-rich deposit: the banded iron formation. These deposits include some tens of billions of tons of iron in the form of oxides embedded in a silica-rich matrix; they are the chief economic reserves of iron in the world. A major fraction of them was deposited within a comparatively brief period of a few hundred million years beginning somewhat earlier than two billion years ago.

A transition in oxygen concentration could explain this major episode of iron sedimentation through the following hypothetical sequence of events. In a primitive, anoxic ocean, iron existed in the ferrous state (that is, with a valence of + 2) and in that form was soluble in seawater. With the development of aerobic photosynthesis small concentrations of oxygen began diffusing into the upper portions of the ocean, where it reacted with the dissolved iron. The iron was thereby converted to the ferric form (with a valence of + 3), and as a result hydrous ferric oxides were precipitated and accumulated with silica to form rusty layers on the ocean floor. As the process continued, virtually all the dissolved iron in the ocean basins was precipitated: in a matter of a few hundred million years the oceans of the world rusted. Only one mechanism is known that could have released oxygen at the required rate for this precipitation: photosynthesis, followed by the sedimentation and burial of the organic matter thereby produced. This process ended about 1.8 billion years ago.

VII. CHLOROPHYLL

By about three billion years ago, the porphyrin ring had been synthesized in the evolutionary process and the inclusion therein of the magnesium atom resulted in the formation of green pigment chlorophyll. Photosynthesis had arrived. The magnesium in this molecule is essential for the process whereby it captures the energy of the sun and converts it to chemical energy in the form of adenosine triphosphate (ATP). ATP is useful for living cells as an energy-storage molecule precisely because the hydrolysis of one bond to produce adenosine diphosphate (ADP) and one unit of organic phosphate releases a large amount of chemical-free energy. The energy in ATP is used to reduce carbon dioxide and water to carbohydrate and oxygen. The photosynthetic process in green plants, over a period of 500 million years, produced sufficient oxygen to provide the present stable oxygen-rich atmosphere, in which one out of every five molecules is oxygen. One consequence was the formation of an ozone layer in the upper atmosphere that sharply reduced the UV radiation on the surface of the earth. Henceforth biological photosynthesis working with the energy of visible wavelengths took over the synthesis of organic matter. The pattern of life driven by solar energy was fixed for all time on the planet, and the stage was set for true biological evolution.

VIII. BIOLOGICAL EVOLUTION

With the stabilization of the oxygen atmosphere, living cells evolved a more efficient mechanism for utilization of energy than was available in anaerobic glycolysis. Oxidative phosphorylation evolved about two billion years ago. Again, ATP is the chem-

ical *sine qua non* for this reaction and magnesium is the essential cation. Since two to three billion years ago, the liberation of the magnesium in the olivene rock by the process of erosion has provided the magnesium essential to the plant for photosynthesis — the synthesis of ATP, carbohydrate, and the liberation of oxygen — and for the efficient utilization of this energy in biologic systems — oxidative phosphorylation. Magnesium is essential for all of these processes.

The original forms of life — the primitive prokaryotes — were unicellular organisms with probably a single loop of deoxyribonucleic acid (DNA) which was loose in the cytoplasm of the cell. Prokaryotes reproduced asexually by binary fission. Most prokaryotes grow best in the absence of oxygen, or in an environment of low oxygen. Concurrent with development of an efficient biochemical energy machine 1.5 billion years ago, cells containing nuclei (eukaryotic cells) appeared in the form of algae. Most eukaryotes have an absolute requirement for oxygen. This observation has lead to the simple hypothesis that the prokaryotes evolved during a period when environmental oxygen concentration was changing, but by the time the eukaryotes arose, the oxygen concentration was stable and rather high.

In eukaryotes, the central metabolic process is respiration, which in overall terms can be described as the burning of the sugar glucose with oxygen to yield carbon dioxide, water, and energy. Part of the released energy is captured in the form of high-energy phosphate bonds, usually in molecules of adenosine triphosphate (ATP). The rest of the energy is lost from the cell as heat.

Through some miracle, the precursor of the elaborate membrane-bound structures that we appreciate today evolved. The genetic material in the new kinds of cells was aggregated in a distinct nucleus and was bounded by a membrane. These nucleated cells were now capable of advanced sexual reproduction, a process whereby the genetic variations of the parent could be passed on to the offspring in new combinations. Because sexual reproduction allows novel adaptations to spread quickly through a population, its development accelerated the pace of evolutionary change.

The majority of the eukaryotes are large, complex, and many celled. Eukaryotic cells also contain other smaller membrane-bound subunits, or organelles, such as mitochondria and chloroplasts. Mitochondria are present in all eukaryotes, wherein they play a central role in the energy economy of the cell. Chloroplasts are present in some protists and in all green plants and are responsible for the photosynthetic activities of these organisms. Magnesium is essential for both functions.

Oxidative phosphorylation, the mechanism for the production of energy for biologic processes, occurs solely in the mitochondria. Respiratory metabolism has two components: a short series of chemical reactions, collectively called glycolysis, and a longer series called the citric acid cycle. In glycolysis, a glucose molecule, with six carbon atoms, is broken down into two molecules of pyruvate, each having three carbon atoms. No oxygen is required for glycolysis, but on the other hand, it releases only a little energy with a net gain of only two molecules of ATP. It seems likely that this was the primitive and original energy-generating process. It also seems likely that the longer series — the citric acid cycle — was simply appended to the existing anaerobic pathway during evolution. The fuel for this citric acid cycle is pyruvate formed by glycolysis. Through a series of enzyme-controlled reactions, the carbon atoms of the pyruvate are oxidized and the oxidations are coupled to other reactions that result in synthesis of ATP. For each 2 molecules of pyruvate (and hence for each molecule of glucose entering the sequence) 34 additional molecules of ATP are formed. The complete respiratory pathway is thus far more effective than glycolysis alone. In respiration the proportion of energy released that can be recovered in useful form (ATP) is higher than it is in fermentation, about 38% instead of only about 30%, and in respiration the net energy yield to the cells is some 18 times greater. By breaking down the glucose

to simple inorganic molecules (carbon dioxide and water) respiration liberates virtually all of the biologic usable energy stored in the chemicals bonds of the sugar.

The construction of biologic molecules demands energy in the form of ATP, and most of the ATP is supplied through the oxygen-dependent citric acid cycle. *Wherever there is ATP, there is an obligatory need for magnesium.*

Further evidence for this proposed evolutionary sequence can be found in the behavior of eukaryotic cells under conditions of O₂ deprivation. For instance, mammalian muscle cells, under prolonged exertion and O₂ deficiency, revert to the glycolytic mechanism. The cells continue to function, albeit at reduced efficiency. Under such condition of oxygen debt, pyruvate is not consumed in the cell, but it can be converted back to glucose in the liver (at a cost in energy of six ATP molecules). Significantly the pyruvate itself is not transported to the liver but instead is converted into lactic acid, which in the liver must then be returned to the form of pyruvate.

Some syntheses have an intrinsic requirement for oxygen, quite apart from metabolic demands. Molecular oxygen is needed, for instance, in the synthesis of biopigments in vertebrates, of chlorophyll a in higher plants, and of the amino acids hydroxyproline and, in animals, tyrosine. The oxygen dependence of two synthetic pathways in particular has been determined in detail — one controls the manufacture of a class of compounds that includes the sterols and the caretenoids and the other is concerned with the synthesis of fatty acids.

IX. SUMMARY

To summarize, the raw material necessary for the evolution of life was available at the time of the creation of the earth 4.6 billion years ago. As the earth cooled, the ocean and the firmament appeared over a period of one billion years. Inorganic compounds in the presence of naturally occurring energy (such as thunder and lightning) joined into organic compounds and, finally, primitive anaerobic bacteria evolved, which were able to reproduce by binary fission. Over a period of another billion years, these bacteria developed the capacity to capture the energy of sunlight and store it in the chemical phosphate bond of adenosine triphosphate (ATP), and to reduce carbon dioxide to carbohydrate, initially using hydrogen sulfide as the source of hydrogen, but eventually using water for this purpose and thereby liberating oxygen. Since, whenever there is ATP, there is an obligatory need for magnesium, it is obvious that magnesium participated in the earliest biochemical steps necessary for the evolution of life; fortunately there was a plentiful supply of this element available in the mantle of the earth.

As the oxygen content of the atmosphere increased and as the efficiency of the biochemical mechanism improved, the molecule chlorophyll evolved, followed by the appearance of various membrane-bound subcellular particles, such as the chloroplast, the mitochondrion, and the nucleus. It is now common knowledge that the magnesium atom is an essential, centrally located component of the chlorophyll molecule. Photosynthesis by green plants produced sufficient oxygen to stabilize its concentration in the atmosphere.

When the sun is not shining, the green plants use the energy stored as carbohydrate for survival. The intricate mechanism of oxidative-phosphorylation which then evolved requires the presence of magnesium. Herbivorous animals eat green plants and thereby indirectly acquire the energy of the sun captured by the photosynthetic process by plants and stored as carbohydrate. The animals rearrange the atoms of the compounds they ingest into molecules needed for their own maintenance — proteins, nucleic acids, lipids, and other special compounds. The source of the energy for these synthetic proc-

esses is the oxidative phosphorylation which takes place in the mitochondrion. In the final analysis, the energy source is the ATP, the product of the most primitive step towards life, which evolved 3.5 billion years ago. Wherever there is ATP, there is an obligatory need for magnesium.

One explanation for the important role of magnesium ions in early life processes (such as prebiotic condensations, protein biosynthesis, anaerobic energy production, and later, photosynthesis) may be attributed to its abundance; at present its concentration (54mM) in the oceans is higher than that of any other divalent metal ion. In primeval seas the Mg^{2+} concentration may have been even higher since NH_4^+ , Fe^{2+} and Mn^{2+} present under the reducing condition could have replaced Mg^{2+} in the silicate phases. Therefore, it is not surprising that Mg^{2+} most likely catalyzed a series of prebiotic condensation reactions, and that it is important for many fundamental steps in life processes, for example, DNA duplication and protein biosynthesis. These subjects will be discussed subsequently in greater detail.

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Chapter 3

SOME FUNDAMENTAL PROPERTIES OF MAGNESIUM

I. OCCURRENCE

It is estimated that magnesium forms about 2.5% of the crust of the Earth. The circumstances of its discovery have been previously reported.¹ Magnesium is widely distributed in nature in a variety of compounds. It occurs as the carbonate in magnesite ($MgCO_3$) and dolomite ($MgCO_3 \cdot CaCO_3$); as the oxide in brucite [$Mg(OH)_2$]; and as the chloride in carnallite ($MgCl_2 \cdot KC\ddagger \cdot 6H_2O$). Magnesium also occurs in silicate minerals, of which the most important are olivine ($(MgFe)_2SiO_4$), serpentine ($3MgO \cdot 2SiO_2 \cdot 2H_2O$), and asbestos [$(CaMg)_2SiO_4$]. The silicate minerals are of worldwide occurrence.

Magnesium is found as chloride in seawater at a concentration of 1.27 g/kg.

II. CHEMICAL PROPERTIES

Chemically, magnesium is a member of Group IIA of the periodic system.

Ordinary magnesium has an atomic weight of 24.312. Its atomic number is 12; its valence, 2. Its isotopic composition is as follows: 24 (77.4%), 25 (11.5%), and 26 (11.1%). The nucleus of the magnesium atom contains 12 neutrons and 12 protons. The configuration of the orbital electrons is as follows: K shell, 2; L shell, 8; and M shell, 2. The tendency to attain to the stable electronic configuration of the inert gases with eight electrons in the outer shell causes magnesium to give up two electrons, thus forming a magnesium ion with two positive excess electrical charges.

Oppositely charged ions form compounds which are held together by electrostatic attraction. This interchange effect does not stop, however, when two equivalent ions with opposite charges apparently neutralize each other. The dominating field effect of an ion can remain and extend to other oppositely charged ions. In the fixation of these excess ions, further considerable amounts of energy can be obtained.

The binding energies of the complex ions are determined above all by the relative sizes of their atoms; the smaller the relative size of the atom, the greater the binding energies and the greater the tendency to form complex compounds. The ionic radii of the alkaline earth elements are as follows: magnesium, 0.78 Å.; calcium, 1.06 Å.; strontium, 1.27 Å.; and barium, 1.43 Å. While barium and strontium show no tendency to complex formation, this tendency is shown increasingly by calcium and magnesium. Among the biologically important cations, magnesium possesses the smallest ionic radius: magnesium, 0.78 Å.; calcium, 1.06 Å.; sodium, 0.98 Å.; and potassium, 1.33 Å.

Because of its small ion volume and its divalence, magnesium forms complex ions to a special degree, complexing not only with other ions but also with many molecules of a dipolar nature. Ligands such as Mg^{2+} contain highly electronegative electron donors; Mg^{2+} is most stably complexed by phosphate and carboxylate anions or by the lone pair of electrons of nitrogen. Because of its greater polarizing ability, Mg^{2+} has a larger hydration energy than Ca^{2+} , and magnesium salts of long-chain organic acids are, for this reason, more soluble than the analogous calcium salts.² The idea of water being bound in a complex may appear strange, but it is supported by the fact that it is impossible to remove water from such complexes as the hydrate, $MgCl_2(H_2O)_6$, by heating; it can be removed only by the destruction of the complex. The capacity to

form a complex with water may be extremely significant in controlling the water content within cells.

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Chapter 4

PHOTOSYNTHESIS

Aerobic photosynthesis evolved about 2.5 billion years ago, but it is only within the twentieth century that man has begun to understand the anatomic and biochemical details of this elegant process. In 1807 Sir Humphrey Davy discovered the element magnesium.¹ A century later in 1903 Willstätter and Stoll reported the then amazing discovery that the magnesium ion occupies the central location in the chlorophyll molecule.² In 1954 Arnon et al. first demonstrated conclusively that carbon dioxide could be reduced by isolated chloroplasts into intermediates of the carbon cycle of photosynthesis.³ In the 1950s, Calvin and Benson, using radioactive carbon, finally proved that atmospheric carbon dioxide is reduced and incorporated into carbohydrate.⁴

I. CHLOROPHYLL

One of the greatest triumphs of early evolution was the invention of a means to harness the energy of the sun which is transmitted as light to drive energy-requiring synthetic processes. This process in higher plants occurs in an especially organized subcellular organelle, the chloroplast. The chloroplast is an organized set of membranes which is crowded with water-insoluble lipid and contains the central pigment chlorophyll. Chlorophyll is the *magnesium chelate* of porphyrin.

It is chlorophyll which produces the oxygen and the foods for all other forms of life on Earth. Wilstätter demonstrated that chlorophyll is comprised of the porphyrin system, the central magnesium atom with its complex linkage, and the phytol radical. There are several different types of chlorophyll molecules in nature, all of them tetrapyrrolic pigments containing up to 0.2% magnesium.⁵ All types exhibit pronounced absorption bands in the blue-green and red regions of the visible spectrum.⁶

The magnesium atom occupies the central position in the chlorophyll molecule, being bound to the four nitrogen atoms of the porphyrin ring. Very little is known about how magnesium is incorporated into protoporphyrin. The magnesium of chlorophyll does not exchange with radioactive magnesium, $^{28}\text{Mg}^{2+}$, in aqueous organic solvents.⁷ Photosynthesis does not proceed when the magnesium atom is removed from the chlorophyll molecule; however, reinsertion of the magnesium atom into the chlorophyll molecule by means of a Grignard reagent causes photosynthesis to begin. Magnesium porphyrins have recently been prepared under anhydrous conditions.⁸

The binding of Mg^{2+} to the nitrogen of the porphyrin ring is thought to be more ionic than covalent, and the metal retains the capacity to bind to electron donors on either side of the plane of the porphyrin ring. Chlorophyll therefore can bind water or polar organic solvents and can form aggregates by binding with magnesium of one ring and the 9-keto group of another. Chlorophyll, as usually prepared in the laboratory, retains a considerable amount of water which probably is bound to the magnesium ion.

When the oxygen concentration in the atmosphere stabilized at about 20%, conditions became thermodynamically favorable for the oxidation of organic compounds. The desired thermodynamically uphill reaction became the photoreduction of the oxidized organic compounds, including carbon dioxide. It is just these photoreactions which are favored by chelating a dipositive closed shell metal ion into the porphyrin ring.

Mauzerall hypothesizes that the purpose of the ionically bound magnesium in the vacant hole in the porphyrin ring is to stabilize the structure so that it would undergo

perfectly reversible one-electron oxidations.⁹ The redox potential of chlorophyll correlates very well with the electronegativity of the central magnesium ion. With photoactivation, the chelated magnesium makes the excited state a powerful reductant and stabilizes the resting cation. Why magnesium rather than some other metal in the photosynthetic pigments? Fuhrhop and Mauzerall suggest that if the aim of the biological system is a minimal redox potential combined with maximum stability in a protic solvent, then magnesium is a good minimax solution to the requirements.¹⁰ That is to say, the excited magnesium porphyrin complex is capable of undergoing reversible photochemical reduction or oxidation, that is, of accepting or donating an electron to the partner molecule.¹¹ There was an abundant supply of magnesium available at the time when the evolutionary process required an ion for such a function 2.5 billion years ago. The reason why magnesium plays the role that it does in photosynthesis may be that it was simply available in the quantity needed for this purpose and that it was ideally suited for this function. Magnesium is because it is.

II. ANATOMY

Photosynthesis takes place in the chloroplasts, where chlorophyll is located. Considering the size of the granum (0.1μ in diameter) in which chlorophyll is concentrated, the chlorophyll concentration could be as high as $0.1 M$. Such a high chlorophyll concentration in the photosynthetic apparatus naturally leads to the question of the manner of chlorophyll distribution. The chemical structure of chlorophyll suggests that these molecules could be located at some sort of interface formed between hydrophilic and hydrophobic phases. The existence of such an interface is suggested by the presence of lamellar structures in the chlorophyll-bearing grana in chloroplasts, as revealed by electron microscopy. It has been estimated from electron micrographs and chlorophyll content that the total available lamellar area is just enough to accommodate all the chlorophyll molecules in a closely packed monomolecular layer.¹²

In higher plants, the chloroplast from top view is seen to consist of a green field filled with small (0.2 to 1.0μ) totally absorbing bodies called grana. Side views of the chloroplast show that the grana regions are interconnected by material indistinguishable from the grana themselves.

Each chloroplast is surrounded by a double membrane system. In all the chloroplasts the internal membrane system is embedded in a matrix called the stroma. The internal membranes are actually closed, flattened sacs (thylakoids). In any higher plant chloroplast, small thylakoids are stacked to make grana structures. Electron microscopy has shown that the chloroplasts consist of two phases, a thylakoid phase which contains the chlorophyll, and a stromal phase which is the site of carbon cycle enzymes and other synthetic capacities of the chloroplast.

The light reactions of photosynthesis and the associated electron transport reactions leading from the oxidation of water to the reduction of ferredoxin occur within the internal membrane system of chloroplast, while the CO_2 fixation reactions of the carbon cycle occur within the stromal region of the chloroplast. Enzymatic systems other than CO_2 fixation systems are present in the stroma, the most notable being specific chloroplast ribosomes and an apparent ability to synthesize proteins.

A. Micromorphology

Membranes are the site of the oxidation of water to produce oxygen gas and electron transport with accompanying phosphorylation to the level of a reducing agent which will reduce ferredoxin.

A considerable amount is known about the chemical composition and the morphol-

ogy of the internal membrane systems of the chloroplast. The greatest gap in knowledge falls in the area which lies between solution chemistry and present electron microscopic techniques. It is the micromorphology of the association of discrete molecules within the membrane which will finally help us to explain not only the *in vivo* environment of chlorophyll molecules, but also the entire photosynthetic, quantum conversion, and electron transport process.

III. THE BIOCHEMISTRY OF PHOTOSYNTHESIS

The simple equation that summarizes the process of photosynthesis has been known since the nineteenth century: water (H_2O) + carbon dioxide (CO_2) yields some form of carbohydrate (represented by CH_2O) and oxygen (O_2). The reaction is driven by light energy. Photons are first absorbed by chlorophyll and other photosynthetic pigments.¹³

A. Absorption of Light Energy

Once the light energy is absorbed, it is used for two purposes. First, it is used to generate "reducing power". Reduction involves the addition of electrons or the removal of protons, or both. Molecules that are rich in reducing power can transfer electrons to more oxidized molecules. The reducing agent produced by photosynthesis is NADPH, the reduced form of nicotinamide adenine dinucleotide diphosphate (NADP). Second, the light energy becomes converted into the energy-rich phosphate compound adenosine triphosphate (ATP). Both NADPH and ATP are needed to reduce CO_2 , a relatively oxidized molecule, into carbohydrate. The overall balance sheet for photosynthesis shows that three molecules of ATP and two molecules of NADPH are required for each molecule of CO_2 reduced.

Photosynthesis in higher plants occurs in the intricate membrane-filled structure, the chloroplast. Light energy is trapped within the chloroplast and therein occur the rapid photophysical and photochemical reactions that generate the ATP and NADPH. The subsequent reduction of CO_2 to carbohydrate occurs at a more leisurely pace. Once ATP and NADPH have been formed in the membrane, they are released into the soluble phase of the chloroplast. The fixation of carbon dioxide proceeds in the stroma in the absence of light, assisted by a number of soluble enzymes.

When chlorophyll absorbs photons, the pigment passes to a higher energy, or excited, state. Two structurally different chlorophyll molecules, *a* and *b*, have been identified, which have their characteristic absorption spectra. Chlorophyll *b* does not participate directly in photosynthesis but assists in gathering light energy, which is transferred to chlorophyll *a* in a photosynthetic reaction center. Only the latter is involved in the subsequent reactions of photosynthesis.

B. The First Chemical Step

Once energy is captured in the reaction center, the chemistry begins. The excitation energy is used to form an oxidant and a reductant. The former is capable of oxidizing water, that is, splitting the water molecule into free oxygen, protons, and electrons. The reductant accepts the reducing equivalents (electron and protons) that arise from the oxidation of water. The electrons are transported through a series of reactions, ultimately to yield NADPH and ATP. Ultimately these equivalents are used in the reduction of carbon dioxide. The oxidants and the reductants must be formed within the very short lifetime of the excited state of chlorophyll *a*.

C. The Biochemical Phase

It appears that the biochemical phase of photosynthesis actually involves two light reactions (instead of one as tacitly assumed so far). The Hill-Bendall hypothesis assumes that electrons could be transported along a biochemical chain in which two separate light reactions are triggered by light in two different photochemical systems. Each system has a reaction center in which an oxidant and a reductant are formed. Photochemical system II (PS II) sensitizes a reaction that results in the oxidation of water and in the formation of a weak reductant. The size of this active pigment array is not constant but can be increased by the addition of Mg^{2+} , indicating that Mg^{2+} affects chloroplast membrane structure.¹⁴ Photochemical system I (PS I) sensitizes a reaction that yields a weak oxidant and a strong reductant. Mg^{2+} can induce conformational changes in PS I particles, which is suggested as being a fine-tuning mechanism for the regulation of excitation energy transfer in chloroplasts.^{15, 16} The two photochemical systems are linked in series by electron carriers, so that the weak reductant produced in PS II is oxidized by the weak oxidant produced in PS I. Subsequently the reducing power is utilized to reduce CO_2 to the level of carbohydrate.¹⁷

IV. REGULATION OF PHOTOSYNTHESIS BY MAGNESIUM

Lin and Nobel observed an increase in the concentration of chloroplast Mg^{2+} *in vivo* caused by illuminating the plant, the first direct evidence indicating that changes in magnesium level actually occur in the plant cell.¹⁸ Krause reported that light-dependent proton pumping leads to an increase in Mg^{2+} concentration in chloroplast stroma of about 2 mM at pH 7.6. This increase in concentration is sufficient for a significant role of Mg^{2+} in the light activation of the photosynthetic carbon reduction cycle.¹⁹ Thus, the change in magnesium in chloroplasts upon illumination may be a regulatory mechanism whereby light controls photosynthetic activity. In the daytime when the plant is illuminated, the rise in the magnesium concentration in the chloroplast would enhance photosynthetic activity since many of the enzymes involved are magnesium-dependent.

At night the energy source for photosynthesis is gone and so photophosphorylation and CO_2 fixation would cease. Magnesium may then flow out of the chloroplast into the cytoplasm where it could be involved in certain Mg^{2+} -dependent biosynthetic reactions. Since chloroplasts account for approximately 40% of the cytoplasmic volume, such light-induced Mg^{2+} movements might influence cytoplasmic enzymes in addition to playing a major role in regulating photosynthetic activity. During the past decade much evidence has accumulated which indicated that Mg^{2+} -induced regulation of the excitation energy distribution between the photosystems is important in regulating the quantum efficiency of photosynthesis.^{20, 21}

V. A VIEW OF PHOTOSYNTHESIS

From the characteristic lamellarity of the granum which incorporates the chlorophyll molecule evolves the concept of a unit membrane held together with the assistance of magnesium. Magnesium could stiffen lipoprotein membranes by bridging neighboring carboxylate groups.²² Chlorophyll functions in photosynthesis by virtue of its ability to produce and to maintain a charge separation in the highly ordered lamellar structure of the chloroplast. An inward proton transport across the chloroplast thylakoid membrane establishes a sizable proton concentration gradient which serves as a driving force for ATP formation. In the steady state, Mg^{2+} appears to be the major counterion to proton uptake, with important regulatory functions in the photosynthetic appara-

tus.²³ The entire photosynthetic process can be viewed as the capture of the energy of photons in the form of high-energy electrons, followed by a stepwise passage of energy down an energy gradient in a structured membrane held together by the coordinating properties of the magnesium atom. This model is essentially the unifying concept first proposed by Szent-Györgyi; it is now modified to explain the role of magnesium.¹⁴

The subsequent synthetic process may be summarized as follows: $6\text{CO}_2 + 6\text{H}_2\text{O} + 18\text{ATP} + 12\text{NADPH} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 18\text{ADP} + 18\text{Pi} + 12\text{NADP} + 6\text{O}_2$.²⁵ ATP and chemical reducing power operate to produce carbohydrate via common intermediates which require 12 separate enzymes. All enzyme reactions that are known to be catalyzed by ATP show an absolute requirement for magnesium. It is obvious that magnesium is essential for practically every aspect of photosynthesis and plays a role in regulating the entire process.

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Chapter 5

THE ROLE OF MAGNESIUM IN CELLULAR PROCESSES

I. OXIDATIVE PHOSPHORYLATION

With the stabilization of the oxygen concentration in the atmosphere of the earth about 2.5 billion years ago, there evolved the photosynthetic mechanism for the transduction of solar energy to chemical energy. What was needed next was a mechanism to utilize stored chemical energy for the maintenance of life and for various synthetic processes. This problem was solved by the evolution over a period of about 500 million years of the mechanism for oxidative phosphorylation.

In the absence of sunlight, plants rely on stored chemical energy to maintain life. This stored energy is released by oxidative phosphorylation, a process which occurs in the mitochondrial membrane of both plant and animal cells. The primary function of all mitochondria is to couple phosphorylation to oxidation. Adenosine triphosphate, ATP, the main fuel of life, is produced in oxidative phosphorylation. All enzyme reactions that are known to be catalyzed by ATP show an absolute requirement for magnesium. These reactions encompass a very wide spectrum of synthetic processes.¹

So fundamental and widespread are the reactions involving ATP that it must influence practically all processes of life. Many enzymes are activated by the magnesium cation; this group includes all those utilizing ATP or catalyzing the transfer of phosphate. ATP is known to form a magnesium complex, with Mg²⁺ binding usually to the phosphate moiety. Magnesium has a single divalent state and does not form highly stable chelates with organic complexes, as do the transitional metals. It is perhaps this quality which allows it to act as a bridge in the large number of chemical reactions not requiring redox reactions, but resulting in transfer of organic groups from one molecule to another. When organic phosphate takes part in a reaction, magnesium is usually its inorganic cofactor. All partners in reactions known to be dependent on ATP are capable of chelating with magnesium. The effect of magnesium chelation in such reactions is to lower the free energy of activation of the rate-determining step.

The ATP molecule is usually depicted as existing in a linear configuration, with the purine and phosphate ends separated by the pentose. Szent-Györgyi has suggested that the spacial configuration of the ATP molecule is such that it could function as a transformer as well as a storage battery.² The phosphate chain can touch the purine ring; magnesium can form a very stable quadridentate chelate connecting the two ends of the ATP molecule, and energy in the form of electrons can now pass from the phosphate to the purine. The magnesium may not only actually connect the two ends of the molecule, but it may also make one single, unique electronic system of the phosphate chain and the purine with common nonlocalized electrons which could transport energy.

The roles of sunlight, chlorophyll, and magnesium in the primary synthetic process on earth have already been discussed. In photosynthesis, carbon dioxide and water are synthesized into carbohydrate, and oxygen is released. In the absence of sunlight, plants rely on stored chemical energy to maintain life. This stored energy is released by oxidative phosphorylation, a process which occurs in the mitochondrion of both plant and animal cells. The biosynthesis of ATP coupled to the oxidation of substrate is known as oxidative phosphorylation and takes place in the mitochondrial membrane. The primary function of all mitochondria is to couple phosphorylation to oxidation. The transduction is the conversion of chemical energy from bond energies of certain metabolite to the bond energies of ATP. Whereas phosphorylation in the chlo-

roplast is light-dependent, phosphorylation in the mitochondrion is dependent not on light but on oxygen. Whereas photosynthesis combines carbon dioxide and water and evolves oxygen, oxidative phosphorylation does just the reverse. It requires oxygen and evolves carbon dioxide and water, thus completing the carbon cycle on earth and returning electrons to ground state.

ATP, the main fuel of life, is produced in both photosynthesis and oxidative phosphorylation. In both cases, ATP is produced by an electric current, i.e., the energy released by "dropping" electrons.

The mitochondrion represents, much as the chloroplast, a general blueprint that is characteristic of all membrane systems; in fact, it is characteristic of all energy-transforming systems of the cell.³ The basic design of the mitochondrion is copied in all other systems in the cell that have to do with the transformation or use of energy. Under the electron microscope, the mitochondrion, just like the chloroplast, is seen to consist of a lamellar membrane.

Magnesium is distributed in rat liver mitochondria as follows: outer membranes, 4%; intermembrane compartment, 50%; inner membranes, 5%; and matrix, 41%. The highest Mg/protein ratio was found in the intermembrane compartment.⁴ The total content of magnesium was found to be 20 to 25 nmol/mg protein.

The inner membrane forms invaginations (cristae). The intermembrane and intercristal spaces are thought to be continuous and to form a central compartment. The intermembrane compartment contains high molecular weight compounds, most likely proteins which form complexes with magnesium ions.⁵ The matrix, which is surrounded by the folded inner membrane, comprises the second compartment, and the entire mitochondrion is thought to be a two-compartment system. The cristae contain a strictly regulated respiratory chain along which electrons are transferred by the difference in redox potential. Along this respiratory chain, the oxidation-reduction energy is converted into phosphate bond energy in the form of ATP. The optimum concentration of magnesium for the process appears to be 10^{-4} to $10^{-5} M$. The respiratory enzymes — cytochromes and flavoproteins — which sequentially release the energy of electrons, may be embedded in the mitochondrial membranes that are structurally organized into respiratory units. The oxidizing enzymes in the inner mitochondrial membrane are assembled asymmetrically in a way that gives rise to a vectorial movement of protons. Racker feels that this proton current is the driving force in the production of biologically useful energy in the form of ATP.⁶

The data available at the present time are in conformity with Mitchell's chemiosmotic hypothesis, which states that translocation of protons takes place across the mitochondrial membrane via the respiratory chain, going from the inside of the membrane to the outside, thereby establishing a proton concentration gradient as well as a membrane potential; this is the oxidative component.⁷ Secondly, there is a proton pump that reverses the proton flux — from outside to inside — which leads to the generation of ATP from ADP and Pi; this is the phosphorylation step. A Mg^{2+} -induced conformational change in the membrane may facilitate this final step.⁶

Magnesium is present inside the mitochondrial membrane at a concentration of about 1 nmol/mg protein. The intramitochondrial free magnesium concentration may be a potential regulator of enzyme activity.⁸ Enzyme/ATP/ Mg^{2+} ternary complexes with intramolecular stacking have been demonstrated by nuclear magnetic resonance measurements; this stacking may explain the enhanced stability of the complex.⁹ Magnesium plays an essential regulatory role in the maintenance of membrane integrity; the presence of magnesium on a number of membrane sites appears to be necessary to maintain the impermeability of the mitochondrial inner membrane.¹⁰ Specific pathways for electrophoretic penetration of monovalent cations are present in the inner membrane of the mitochondrion; Mg^{2+} bound by a limited number of high-affinity sites in

or near these pathways can control monovalent cation permeability.¹¹ The mitochondrion can be made to swell and to contract experimentally. Although swelling can be caused by a large variety of different chemical agents, it is significant that only ATP together with magnesium can cause contraction. ATP is always split during contraction of swollen mitochondria.

A rapid swelling of heart and liver mitochondria can be produced in rats fed a magnesium-deficient diet for 10 days, whereas no significant decrease in the magnesium content of the mitochondrion results. ATP reverses the swelling of mitochondria from heart and liver of magnesium-deficient rats. The addition of 1 mM MgCl₂ to partially swollen rat liver mitochondria initiates contraction, inhibits respiration, and alters the ultrastructural configuration of the inner membrane-cristae-matrix continuum.¹²

Not only is magnesium essential for the process of oxidative phosphorylation, but recent studies suggest that magnesium regulates the process.

II. MAGNESIUM AND THE RIBOSOME

Ribosomes probably evolved about 1.5 billion years ago. Palade in 1955 first reported the identification of a "small particulate component of the cytoplasm" which is now recognized as the ribosome.¹³

Ribosomes are of universal occurrence in microorganisms, higher plants, and animals. Regardless of the source, ribosomes exhibit a remarkable similarity in structure and composition. They are approximately spherical particles, generally 150 to 350 Å in diameter, and are composed of approximately equal parts of protein and ribonucleic acid (RNA). Within the cells, the ribosome may be free or may be attached to strands of endoplasmic reticulum. Ribosomal sedimentation constants are similar (70 to 80 S) and their molecular weights are about three or four million. They are highly hydrated.

Information from DNA in the cell nucleus is brought via messenger RNA (mRNA) to the ribosomes. mRNA interact with another molecule called transfer RNA (tRNA), which continually brings amino acids to the ribosome as raw materials that are subsequently assembled into proteins.

The principal and probably the only function of the ribosome is the biosynthesis of protein. Ribosomes are found either bound to membranes or free in the cytoplasm and consist of two parts: a small subunit with 21 different proteins and a larger subunit with about 34 proteins. The same subunits also contain RNA molecules — transcripts of the DNA molecules in the cell nucleus. Together the ribosomal proteins and the RNA act to receive and carry out genetic instructions for making cellular protein molecules. The rate of protein synthesis is proportional to the number of ribosomes present.

The only cell devoid of ribosomes is the adult erythrocyte. The immature cell, the reticulocyte, in which hemoglobin is being synthesized, is rich in ribosomes.¹⁴ Its magnesium content is higher than that of the mature erythrocyte.¹⁵ Increasing the extracellular magnesium concentration retards the rate of reticulocyte maturation in vivo and in vitro. Magnesium thus retards reticulocyte maturation rather than stimulating reticulocyte production.

The mature reticulocyte demonstrates a clear example of a correlation between protein synthesis and cellular magnesium content. As the reticulocyte matures, the concentration of magnesium ions in the soluble fractions of the cell decreases and protein synthesis declines.¹⁴ The ribosomal fraction is largely responsible for this decline, since ribosomes require magnesium ions for their stability and dissociate when the magnesium concentration becomes very low.

Ribosomes require magnesium ions in order to maintain their physical stability; they

dissociate into smaller particles when the magnesium concentration becomes low.^{16,17} Mg²⁺ stabilizes the tertiary structure of the ribosomes primarily by reducing the repulsion between the negative phosphate groups in the RNA backbone. There appears to be a subtle balance of forces which permit the ribosomes to be stable but flexible enough to undergo functionally significant but as yet undetected conformational changes.¹⁸ An optimal intracellular concentration of magnesium is required for the integrity of the macromolecules necessary for RNA synthesis.¹⁹ The process of RNA synthesis consists of three major steps, initiation, elongation, and termination. Manganese preferentially activates the initiation step in vitro, whereas either manganese or magnesium is necessary for the elongation step of RNA synthesis.²⁰

A. The Role of Magnesium

The concentration of magnesium ion has a profound influence on ribosomal structure. Chao in 1957 first suggested that the function of the divalent cation is to bind smaller ribonucleoproteins into a macromolecule.²¹ Ribosomes require the presence of low concentrations of magnesium (approximately 10⁻³ M) for the maintenance of structural integrity. If Mg²⁺ concentration is increased about ten fold, two ribosomes "dimerize" to form a new particle with a molecular weight twice that of the original ribosome. If the Mg²⁺ concentration is lowered approximately tenfold, each ribosome (70 S particle) separates into two smaller particles, comprising respectively about two thirds and one third of the original ribosome. Thus a 70 S ribosome splits into two particles with sedimentation constants of 50 S and 30 S. These subunits and "dimers" are incapable of catalyzing the synthesis of amino acids into proteins. Until recently, it was felt that only the 70 S ribosomes were able to synthesize protein. Recent studies suggest, however, that in vivo protein synthesis begins with attachment of the native 30 S ribosome subunit to the mRNA, followed by the attachment of the 50 S subunit.

Repeated association and dissociation of the ribosomal particles is essential in the living cell. The initiation of protein synthesis in *Escherichia coli* requires dissociation of resting 70 S ribosomes into 30 and 50 S subunits that, after binding of several factors to the 30 S particle, reassociates to yield activated ribosomes. The magnesium dependence of the association reaction obeys a true equilibrium.²² Both the association and the dissociation reactions can involve conformational changes within quite a number of ribosomal proteins rather than simple joining of rigid association-specific interfaces.²³

The physical size of the RNA aggregates is controlled by the concentration of magnesium, and polypeptide formation cannot proceed unless magnesium concentration is optimal.²⁴ Mg²⁺ probably acts to stabilize a favorable protein conformation.²⁵ Presumably the role played by Mg²⁺ in the ribosome is to maintain essential secondary structure of rRNA and to stabilize certain RNA-protein electrostatic interactions.²⁶

tRNAs have about five strong binding sites for magnesium not found in either tRNA fragments or double helical or single-stranded RNA. These strong binding sites are intimately associated with tRNA tertiary structure.²⁷ The binding of magnesium by tRNA may be via phosphate groups, all of which have the same intrinsic binding affinity.²⁸ Polyamines also may be necessary to maintain the conformation of tRNA for performing its ordained biologic function.²⁹

B. Summary

To summarize, a ribosome appears to be a porous sphere with a high content of water. The particle consists of a large number of basic protein subunits of low molecular weight and of two or three RNA molecules of high molecular weight. These subunits are bound together principally by a relatively large number of divalent cations, in particular Mg²⁺. In addition, a template and the tRNA may be held on the ribosome

by salt linkages through magnesium and probably also by hydrogen bonds between the RNA bases. Magnesium may also serve as a coenzyme. The only well-established biologic function of the ribosome is its role in the synthesis of polypeptides by specific mRNA. Since the Mg²⁺ concentration controls the aggregation of ribosomes and the maintenance of their secondary structure, the role of magnesium appears to be that of a chelating agent.³⁰

Cells respond to external effectors as an integrated unit, coordinately increasing its metabolism, differentiated function and growth rate in response to stimulants, and decreasing them in response to inhibitors. The intracellular availability of free magnesium (Mg²⁺) is proposed as the central coordinating factor in metabolism. The coordination is effected by lowering the availability of Mg²⁺ for Mg²⁺-dependent metabolic reactions, particularly those involving transphosphorylations. This is brought about by increasing the binding of Mg²⁺ at the internal surface of the plasma membrane, or by decreasing the membrane permeability to Mg²⁺. Conversely, the entire range of reactions which constitute the coordinate response is stimulated by increasing the availability of Mg²⁺. Since the estimated concentrations of free Mg²⁺ in animal tissues are lower than those necessary for maximum activity of several key regulatory enzymes, small changes in free Mg²⁺ can exert considerable metabolic control.³¹ In normal cells, the regulatory effect of Ca²⁺ is primarily mediated through the Mg²⁺-dependent processes.³²

III. MAGNESIUM AND THE CELL NUCLEUS

Life could have been no more than an experiment of nature until protoorganisms developed dependable machinery to reproduce themselves about one billion years ago. It is of considerable theoretical interest that all forms of life on Earth have basically the same system for this purpose — it is summed up in the familiar initials, DNA (deoxyribonucleic acid). This substance is almost exclusively confined to the cell nucleus, 45% of which (by dry weight) may itself be composed of DNA. DNA is the carrier of genetic information.

Our understanding of the biosynthesis of DNA dates back to 1956, when Kornberg and associates isolated and purified a polymerase from *E. coli*.³³ In the presence of a DNA primer and Mg²⁺, the material catalyzes the polymerization of the 4-deoxyribonucleoside triphosphates to form a new polynucleotide, the composition of which is determined by the primer. It is reasonable to visualize Mg²⁺ as an intermediate complexing agent responsible for the unwinding of the parent DNA chain, a step that must take place before the DNA content is doubled and cell duplication occurs, or to theorize that magnesium plays a role in stabilizing polynucleotide structure formation of RNA on a double-stranded DNA template.³⁴ Binding with Mg²⁺ increases the stability of the DNA helical structure, the effect being observed mainly between 10⁻⁶ to 10⁻⁴ M.³⁵

During the past 20 years, scientists have obtained substantial understanding of how information is stored and replicated in DNA molecules, how it is passed on to RNA molecules and finally to proteins, and how the three-dimensional structure of protein depends upon the linear arrangement of the constituent amino acids. This information storage in molecular structure and its subsequent readout is dependent upon the presence of magnesium in optimal concentration.³⁶ The rather complicated three-dimensional structures assumed by some polymers are a consequence of their primary sequence. The interactions of these to form even more complicated multicomponent complexes are also determined by their chemistry.

It has long been recognized that divalent cations, including Mg^{2+} , Ca^{2+} , and Mn^{2+} are strongly bonded to the naturally occurring nucleic acids. Since divalent cations are known to form a complex with organic phosphates, the most likely sites of binding are the negatively charged phosphate groups of the pentose-phosphate backbone. It is highly probable that the phosphates are the *only* important binding sites and that the base groups of DNA are not directly involved.³⁷ The DNA molecule has a wide spacing of neighboring phosphate groups, and the rigid configuration of the molecule serves to prevent close contact of Mg^{2+} with more than one phosphate group.

Much of the magnesium in the cell nucleus is combined with those phosphoric acid groups of DNA that are not occupied by histone. The chemical factors that control the variable activity at the sites along a chromosome are largely unknown. There is a suggestion that the sites along the DNA chain at which the phosphoric acid groups are combined with histone are inactive and, conversely, that those at which they are combined with magnesium are active.³⁸

Both magnesium and ATP are involved in the synthesis of nucleic acids. Since sections of the chromosomes in the nucleus are held together by calcium and magnesium, it seems likely that changes in the concentration of magnesium in the medium might determine the degree of chromosomal aberration.³⁹ Variations in the concentration of magnesium *in vivo* must exert a control on DNA synthesis.⁴⁰ The incorporation of ¹⁴C-labeled cytidine 5'-monophosphate into DNA by soluble enzyme extracts from 5-day-old chick embryos was markedly affected by the concentration of magnesium. The progression of rat thymocytes *in vitro* into mitosis is profoundly affected by the level of magnesium in the medium. This mitogenic action appears to be due to the ability of magnesium to stimulate the initiation of DNA synthesis.⁴¹

All known DNA polymers require either Mg^{2+} or Mn^{2+} as an added divalent cation for activity. The fidelity of DNA synthesis may be influenced by the metal activators during catalysis.⁴²

It is now well known that there are macromolecules which undergo conformational changes in the course of their function and that allosteric effects may be important in the regulation of cellular activities.⁴³ Evidence is accumulating which suggest that tRNA may function in a similar fashion. Mg^{2+} can induce conformation changes in tRNA.

IV. THE ROLE OF MAGNESIUM IN BIOCHEMICAL REACTIONS

A. Chelation

There is very little doubt that magnesium is essential for life on Earth. The exact function of magnesium in the chlorophyll molecule, as well as in maintaining the structural integrity of the granum, is conjectural; however, it seems possible that magnesium, because of its inherent atomic composition is, in this particular situation, able to capture and transmit energy more efficiently than any other element. Moreover, the magnesium atom is able to hold reacting groups together to thus maintain the physical configurations that are optimal either for the transfer of energy in the form of excited electrons or for the transmutation of energy into ATP. The one fundamental property of magnesium upon which all of these photosynthetic processes depends is *chelation*. It seems that the capture, conversion, storage, and utilization of solar energy are all dependent upon a chelating function which is unique to, and specific for, the magnesium atom.

Two of the basic functions of solar energy in living cells are *genetic transcription* and *protein synthesis*. Recent studies in molecular biology have established that inter-

relations exist among the three major biologic macromolecules: DNA, RNA, and proteins. Genetic information stored in DNA is transcribed into messenger RNA, which in turn translates that information into amino acid sequences in the newly synthesized protein. At literally every turn in these processes, magnesium plays a vital role. The physical integrity of the DNA helix appears to be dependent upon magnesium. The physical size of the RNA aggregates is controlled by the concentration of magnesium, and polypeptide formation cannot proceed unless magnesium concentration is optimal.

B. Magnesium in Enzymatic Reactions

Magnesium serves as an activator for many enzyme systems of intracellular metabolism. Most important among these enzymes are those that hydrolyze and transfer phosphate groups, including the enzymes concerned with reactions involving ATP. As ATP is required for glucose utilization, fat, protein, nucleic acid, coenzyme synthesis, muscle contraction, methyl group transfer, and other reactions, interference with magnesium metabolism can affect all of these functions.

Among other enzymes activated by magnesium is alkaline phosphatase which is necessary in the formation and maintenance of bone. Hexokinase, fructokinase, diphosphopyridine-nucleotide phosphorylase, and some protein-splitting peptidases are also dependent on the magnesium ion for their activity.

Magnesium has a complex set of interactions with other cations present in the body. As it is necessary for the activation of ATP and as ATP, in turn, is the prime constituent of the active transport mechanism of sodium and potassium across cell membranes, it has been postulated that hypokalemia may partially be the result of hypomagnesemia.

V. MAGNESIUM IN COORDINATE CONTROL

Rubin et al. have recently proposed a model for the coordinate control of metabolism, differential function, and growth through the activity of the magnesium ion.⁴⁴⁻⁴⁶ In this model, the compartmentalization of Mg²⁺ within the cell serves as the key element in this coordinate control by regulating those metabolic pathways in which the rate-limiting steps are transphosphorylation reactions. Much of the Mg²⁺ in cells is bound to membranes and only a fraction is free, or bound to adenine nucleotides. Since Mg²⁺ is the only co-factor common to the three regulatory steps of glycolysis, and indeed to all transphosphorylation reactions, Rubin proposes that the rates of control reactions are determined by the concentration of complexes of adenine nucleotide and magnesium which have been shown to be the appropriate substrates in transphosphorylation reactions.

VI. MAGNESIUM AS A SECOND MESSENGER

A "second messenger" is defined as a substance that transmits the instructions of hormones or other small molecules at the cell membrane to produce physiological changes within the cell.⁴⁷ Current concepts of insulin action hold that this protein hormone negotiates its action without ever gaining access to the interior of the cell.⁴⁸ Interaction of insulin with a specific receptor in the plasma membrane of responsive cells generates a signal that culminates in synthetic activity and cell growth. Such a mechanism was first suggested for hormones in general by Hechter,⁴⁹ and for insulin in particular by Krah⁵⁰

Ion translocation, an early event in insulin action, may constitute an effective second messenger system. It is proposed that insulin stimulation of Mg²⁺ translocation from extracellular to intracellular loci may constitute an important component of the insulin effect on translation.⁵⁰ Insulin enhances the accumulation of Mg²⁺ in the intracellular phase of uterine muscles in which there is concurrent stimulation of protein synthesis. This stimulus by insulin depends upon the functioning of plasma membrane (Na⁺ + K⁺)–ATPase (EC3.6.1.3). The Mg²⁺ so accumulated intracellularly may then serve as a second messenger to mediate diverse effects ascribed to insulin.

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Chapter 6

THE ROLE OF MAGNESIUM IN SOME FUNDAMENTAL PHYSIOLOGIC PROCESSES

I. HIBERNATION

A. Description

From the evolutionary point of view we may consider the phenomenon of hibernation as an adaptive mechanism for the conservation of energy during the winter season when food is difficult to obtain. Survival is a fundamental attribute in nature. Some species survive during the winter months by reducing metabolic activity and heat production to a minimum by the process of hibernation, which has been defined as "a periodic phenomenon in which body temperature falls to a low level, approximating ambient, and heart rate, metabolic rate, and other physiologic functions fall to correspondingly minimal levels."¹ The purpose of hibernation seems to be the conservation of energy, so that the animal's food supply whether in the form of depot fat or physically stored food—can be made to suffice for the required period.

B. Role of Magnesium

Elevation of the serum magnesium appears to be a characteristic of hibernation. From several different laboratories have come reports of elevated serum magnesium during hibernation in the snail *Helix pomatia*, and in thirteen-lined ground squirrels, woodchucks, golden hamsters, little brown bats, big brown bats, and hedgehogs.² The extent of the increase in serum magnesium appears to be dependent upon the species of the hibernator; in the hedgehog there is an increase of 92% over the control values.

Riedesel has presented the following hypothesis for the role of magnesium in the production of hibernation: exposure to cold and reduced activity produce cooling of the peripheral tissues and a release of magnesium from cells to plasma.³ This process may start when the animal is asleep. The action of the elevated serum magnesium on the heat loss center in the hypothalamus causes the body temperature and metabolism of the animal to drop to hibernation levels. The unanswered question is whether the elevated serum magnesium is causally related to initiation of the hibernation or is purely a consequence of cold.

The most extensive studies of the influence of magnesium on natural hibernation are those reported by Suomalainen.⁴ In studies on hedgehogs, this investigator found that the serum magnesium concentration in the autumn, before the onset of hibernation, averaged 3.2 mg/100 ml. In early January, when the hedgehogs were in deep hibernation, the average magnesium concentration of the serum was 5.43 mg/100 ml, an average increase of 170% of the values before the onset of sleep.⁵ According to Suomalainen, the typical features of hibernation are as follows: the transformation of a warm-blooded animal to a cold-blooded one, an increase in serum magnesium concentration, hypoglycemia, and a decrease in the adrenaline content of the adrenals.

In the ground squirrel, the concentration of plasma magnesium during hibernation was significantly higher in females than in males, while there was no difference between the sexes in active animals.⁶ These findings may help to explain the clinical observation that females hibernate better than males. During the period of hibernation, the concentration of plasma magnesium declines gradually, eventually reaching the level main-

tained during the animal's active period. It seems likely that this decline is responsible for terminal arousals and that the hibernating behavior of animals is directly related to the changes in plasma magnesium.

C. Artificial Production

Suomalainen subsequently demonstrated that injecting magnesium solutions subcutaneously into hedgehogs and placing the animals in an icebox produced a condition of magnesium anesthesia entirely unlike natural hibernation.⁷ When subcutaneous injections of insulin were given along with the magnesium, however, before transferring them to an icebox, the animals then went into a cold-blooded state which closely resembled natural hibernation.⁸ Sensibility and muscle tone were preserved. The animals were rolled up in a natural manner, and one of them continued sleeping for three days. The blood sugar concentration and the adrenaline content of the adrenals were approximately the same as those in natural hibernation. Animals given insulin without magnesium developed fatal hypoglycemia.

Magnesium, insulin, glucose, the regulation of body temperature, and hibernation thus appear to be interrelated.

D. Metabolic Changes

Studies of hibernating and nonhibernating hedgehogs exposed to pure nitrogen and to varying mixtures of carbon dioxide and oxygen have shown that anoxia is tolerated for 1 to 2 hr by hibernating hedgehogs, but only for 3 to 5 min by nonhibernating animals.⁹ It is known that rapid cooling prolongs survival under conditions of hypoxia.

The depression of temperature and metabolism occurring in hypoxia are thought to be regulated by the central nervous system. These facts suggest that anaerobic metabolism may be the predominant mechanism during hibernation, and that the elevation of serum magnesium may be related to a mechanism for forcing this anaerobism. There is a possibility that the animal may use anaerobic pathways more extensively during hibernation than in nonhibernating state. On the other hand, data on hamsters, woodchucks, and hedgehogs indicate that hibernating animals maintain the pH, carbon dioxide tension, and oxygen content of the blood near the values found in active animals.

Data on the respiratory quotients and blood glucose levels indicate that fat is the principal source of energy during hibernation. Hibernating mammals have considerably larger quantities of brown fat than nonhibernating species. The possible relationship among brown fat and anaerobic metabolism, lowered body temperature, and magnesium metabolism remains to be clarified.

The degree of hypoglycemia occurring during hibernation varies from species to species. The cardiac glycogen reserve is maintained at the expense of glycogen stores in the liver and skeletal muscle. Adenosine triphosphate is the immediate source of energy for metabolic work involving phosphorylation, synthesis, and other chemical processes. Phosphocreatine, glycolysis, and biologic oxidation can affect a steady resynthesis of ATP. During hibernation, gluconeogenesis, primarily from fat, feeds metabolites slowly into the glycolytic cycle. The role of the endocrine glands in hibernation is questionable.

The fact that oxidative phosphorylation is localized in the mitochondria of muscle stimulates interest in the possible structural changes occurring in the cardiac and skeletal muscles during hibernation. ATP is necessary to maintain the functional integrity of the contractile elements of the muscle: actin and myosin.

It is of interest that death from irradiation is postponed by hibernation. Death occurs after hibernation if the animals are kept in a warm environment.

E. Reports of a Blood-Borne Trigger

Dawe and Spurrier reported the existence of a blood-borne substance which, when transferred to summer-active ground squirrels, induced hibernation among the recipients.¹⁰ This material in serum was dialyzable.¹¹

Extracts of partially purified polypeptide from the brain of hibernating ground squirrels have been shown to cause a significant and long-lasting reduction in body temperature and oxygen consumption when administered intravenously to normothermic white rats.¹²

F. Role of Thyroid Hormone

The ground squirrel *Spermophilus tridecemlineatus* completely ceases release of hormone from its thyroid gland just before the hibernating season.¹³ It is suggested that this cessation of thyroid hormone secretion is necessary for changes to occur in membrane lipid composition so that, at the low body temperatures of hibernation, this animal's membranes would remain in a fluid state. Thyroid hormones do in fact influence the composition, structure, and function of mitochondrial membranes in a non-hibernator.¹⁴ Hulbert proposes that the primary locus of thyroid hormone action is cellular membranes and more specifically on the fatty acids of the membranes, altering the level of their unsaturation.¹⁵ Many of the effects of the thyroid hormones can then be explained as secondary results of a change in membrane structure and function. Such an action also explains the difference in membrane composition and membrane function between homeotherms and poikilotherms. These hormones are also involved in the tolerance of low temperature during hibernation.

Hillier demonstrated that thyroid hormones bind to phospholipid membranes.¹⁶ There is a strong correlation between *in vivo* tissue thyroxine concentration and the phospholipid content of various tissues from the laboratory rat. This binding of the thyroid hormone is very rapid and appears to be related to the hydrophobic nature of the aromatic portions of both L-thyroxine and 3,5,3'-triiodothyronine. Within the cells, thyroxine is bound at multiple sites. It appears that in the process of exerting their actions, the thyroid hormones are deiodinated at cellular membranes.

In the early 1950s, the mechanism of thyroid thermogenesis was proposed to be via the uncoupling of oxidative phosphorylation which would result in an elevated mitochondrial oxygen consumption without a corresponding increase in ATP synthesis. This theory has been largely discarded because the measurement of uncoupling required high doses of thyroid hormones. An action of the thyroid hormones on the mitochondrial membranes would seem most appropriate especially since it now appears that the coupling of electron transport and phosphorylation occurs via a hydrogen ion gradient that is created across the inner mitochondrial membrane.¹⁷ A large decrease in unsaturation of mitochondrial membrane fatty acids uncouples oxidative phosphorylation.¹⁸

A large part (but not all) of the calorigenic effect of the thyroid hormone can be accounted for by stimulation of the sodium pump.¹⁹ Thyroid hormone stimulation of the sodium pump in heart tissue appears to be due, not to changes in the surrounding membrane lipid, but due to increased synthesis of the Na^+/K^+ -ATPase system.²⁰ Thyroid hormones can also stimulate the transport of both calcium and magnesium in liver tissue.²¹

Thyroid hormone has a direct stimulatory action on the cellular transport of magnesium. A probable but not invariable result of hormonal stimulation of magnesium transport appears to be a preservation of normal cellular concentrations of magnesium despite a tendency to depletion of extracellular magnesium in hyperthyroidism and to an excess of extracellular magnesium in hypothyroidism.²²

There are many indications that the endocrine system is in some way involved in

hibernation. However, there is not at the present a really satisfactory description of specific roles for particular hormones in hibernation.²³

II. COLD ACCLIMATION

There are many reports in the literature concerning the biochemical and physiologic changes induced by exposure to cold. The relation of the magnesium ion to heat loss has been demonstrated by studies outside the field of hibernation: (1) it has been shown that the parenteral administration of magnesium facilitates experimental hypothermia,²⁴ (2) both in experimental animals and in human beings magnesium has been shown to have an antipyretic action,²⁵ and (3) an increase in serum magnesium during hypothermia has been reported in both vertebrates and invertebrates.²⁶ In rabbits this increase amounted to 25%.

The source of this additional magnesium is not definitely known. In turtles exposed to cold, magnesium seemed to be drawn largely from skin and skeletal muscle rather than from the liver; the water content of the muscle increased 2.5%, and that of the skin 3.7%.²⁷ The percentage of dialyzable magnesium in the serum of the turtles was not changed appreciably when the serum magnesium was increased by exposure to cold. The skin appears to be the most likely source of the transient increase in serum magnesium which occurs during cold acclimation.²⁷

The relationship of magnesium metabolism to the control of body temperature, sleep, hibernation, and wakefulness remains to be clarified.

III. CONTROL OF BODY TEMPERATURE

The mechanism for control of body temperature among hibernators was a mystery until recently although brown adipose tissue was identified with hibernation as early as 1551.²⁸ Carlier noted in 1895 that the "hibernating gland" of the hedgehog was largest at the beginning of hibernation and that it decreased gradually during the winter. Recent studies suggest that the brown fat provides an internal heating jacket which overlies parts of the systemic vasculature; on signal, it becomes an active metabolic heater which is applied directly to the flowing bloodstream as it passes to and from the cooler periphery. The exact biochemical function that this tissue plays in the arousal from dormancy is still poorly understood.

The cells of brown adipose tissue contain an extraordinary potential for metabolic power, both absolutely and relatively to other cell types. Structurally, the central nucleus is surrounded by cytoplasm carrying large numbers of mitochondria, which are themselves frequently in juxtaposition with the numerous fat vacuoles. Electron micrographs show the mitochondria internally to be heavily lamellated with cristae. In vitro studies suggest that brown fat mitochondria possess an energy-conservation mechanism which is coupled to electron transport. Brown fat contains the enzymes involved in the synthesis of glycogen from glucose. In addition, it contains both the enzymes necessary for glycolysis via the hexose-monophosphate shunt as well as the Embden-Meyerhof pathway, and the enzymes associated with the tricarboxylic acid cycle. Brown fat has a higher respiratory capacity. Further, it also contains several steroid compounds — cortisone, corticosterone, and deoxycorticosterone. The biochemical mechanism for thermogenesis in brown fat may be the uncoupling of oxidative phosphorylation.²⁹

The physiological mediator by which brown fat metabolism is stimulated is most probably norepinephrine, which is released through the sympathetic innervation to the

tissue. Not yet completely understood is the biochemical mechanism by which norepinephrine can induce a sustained elevation of the rate of oxygen consumption in brown fat.

IV. CELLULAR ADHESION

The ability of individual animal cells to adhere to one another or to glass requires the presence of some divalent cation in the external milieu.³⁰ At the turn of the century, in a study that is now classic, Herbst showed that the cells of the sea urchin larva separate from one another when the larva is placed in seawater lacking calcium and magnesium ions.³¹

Recent studies of the significant physical forces in cellular adhesion suggest that they are identical to those forces which are of greatest importance in determining the properties of lyophobic colloids.³² From the purely physical standpoint, animal cells in suspension may be thought of as colloidal particles. If this assumption is valid, then the stability of cells in suspension would depend upon the interaction of two sets of opposing forces: the zeta potential (which tends to disperse the particles) and the London-van der Waals forces (which tend to agglomerate the particles). The zeta potential of cells would be a reflection both of the net surface charge on the cell and the dielectric constant and the ionic composition of the suspending solution.³³

The surface charge of cells is apparently due mainly to ionogenic groups which are part of the surface structure. Under physiologic conditions, the net surface charge is negative for most cells investigated so far. Curtis has suggested that the primary role of the positively charged divalent cations in promoting cell adhesion is to reduce the electrostatic repellent forces between individual cells by lowering the net negative surface charge of the cells.³⁴ When these repellent forces are reduced sufficiently, adhesion can occur. Riddick has demonstrated progressive clumping of particles as zeta potential is lowered from -30 mV to zero.³⁵ The zeta potential of rat Walker sarcoma cells and a standard silica particle was considerably more negative in distilled water or balanced salt solution than when albumin or calcium was added; in the former two solutions, zeta potential was -9 to -13 mV, a level close to the critical value for particle agglomeration.³⁵

Different cations, however, have been found to differ in their ability to produce reaggregations of dissociated tissue cells, even though all cause the same degree of reduction in the surface charge.³² This finding suggests that divalent cations have more than one role to play in promoting cell adhesion. In a preparation of chick limb-bud cells, cells were most adhesive in the presence of magnesium ions, then calcium and strontium, then barium. When magnesium and calcium ions were present together in similar concentrations the calcium ions modified the action of the magnesium ions. Thus, the effect of Mg²⁺ on cellular adhesion appears to be unique and specific.³⁶

V. NERVE EXCITATION

Tasaki has attempted to describe and to interpret the process of nerve excitation on the basis of physicochemical concepts and principles.³⁷ In his model, the excitable membrane of the squid giant axon is visualized as a macromolecular complex of proteins and phospholipids. A relative excess of fixed negative charges at the external layer of the membrane confers cation-exchange properties on the system. The macromolecules in the critical layer of the membrane are assumed to be bound together by

the divalent cations from the external medium and to form complex coacervates. Such complexes can be disrupted (reversibly) by the addition of univalent cations which have a strong affinity for the negatively charged sites contributing to the complex formation.

The process of excitation involves a rapid, reversible cation-exchange mechanism involving transitions between "two stable states" of the membrane macromolecules. In the resting stable state, the anionic sites in the membrane are occupied primarily by divalent cations derived from the external medium; in the excited state, these sites are occupied predominantly by univalent cations. During nerve excitation, it is highly probable that the membrane macromolecules undergo a drastic conformational change which leads to an increase in the membrane water content. It is postulated that the membrane macromolecules undergo some kind of phase transition when the chemical composition of the external medium is varied.³⁸ The conformation of the membrane macromolecules and the properties of the cation exchange are determined primarily by the univalent/divalent ratio within the membrane.

Excitation is terminated when divalent cations derived from the external medium once again form stable complexes with the anionic sites. Termination is associated with reduction in the univalent/divalent cation ratio in the membrane macromolecules and the return of macromolecular conformation to that of the resting state. This process of transition from the excited to the resting state is endothermic. The mechanism for the regulation of intracellular calcium and magnesium in the squid axon is still unclear.³⁹

VI. FERTILIZATION

When sea urchin eggs were inseminated in seawater free of Mg²⁺, little fertilization took place. Spermatozoa that had been treated with egg jelly to induce the acrosome reaction also failed to fertilize eggs in seawater free of magnesium.⁴⁰ Mg²⁺ is required at least in some process(es) between acrosome reaction and fertilization membrane elevation, such as sperm penetration or membrane fusion.⁴¹

The total amount of magnesium contained in two species of sea urchin eggs was 0.128 µg/µgPO₄ in the unfertilized egg; this content did not change upon fertilization. However, the amount of bound magnesium decreased remarkably in 15 min after fertilization and recovered the original value in a further 15 min. The investigators attributed the observation to participation of magnesium in "the rearrangement of the protein."⁴²

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Chapter 7

MAGNESIUM IN GEOCHEMISTRY

I. GEOCHEMISTRY

Rocks and minerals, which have been in existence at least four billion years, usually contain a higher percentage of magnesium than do soils; this reflects the loss of magnesium during weathering.¹ Bowen reports the following magnesium compositions: igneous rocks, 2.33%; shales, 1.5%; sandstones, 1.1%; limestones, 0.3%; and dolomites, 20%. This contrasts with 0.3% for soils. Seawater contains approximately 1350 ppm, compared to an average of 4.1 ppm for fresh waters.

A. Soil Solubility Relations

During weathering, magnesium may be precipitated in clay minerals, a process which plays a significant role in governing the solubility of magnesium in soil. The Mg²⁺ ion often replaces Al³⁺ and leaves a net negative charge of -1 for each substitution in the mineral lattice. Magnesium montmorillonites reflect this type of substitution. Also, the substitution of Mg²⁺ for Fe²⁺ is common, but here there is no alteration in charge associated with the exchange. A major portion of soil magnesium, therefore, consists of weathered primary minerals and secondary alumino-silicates in which Mg²⁺ is substituted for Al³⁺.

In soil solution, magnesium ion reacts with water, forming MgOH⁺, which does not significantly affect the pH range of soils. The level of soluble magnesium in soils is generally in the range of 10⁻³ to 10⁻⁴ M, slightly less than that of Ca²⁺. These levels may result in a significant removal of both Mg²⁺ and Ca²⁺ under heavy rainfall conditions.

Mg²⁺ is held as an exchangeable cation by negatively charged silicate minerals and generally comprise from 5 to 30% of the total exchange capacity. Thus, exchangeable magnesium constitutes the major reserve of magnesium readily available to plants. Magnesium deficiencies usually occur when exchangeable magnesium drops below 5% of the total cation-exchange capacity.² This happens mainly in coarse-textured soils in areas of high rainfall. Excessive leaching favors the removal of magnesium from the exchange site and the development of acid soils. A familiar agricultural practice is to add dolomite (CaMg[CO₃]₂) where magnesium comprises less than 5 to 10% of the total exchangeable cation. A guideline often used is that the ratio of magnesium to potassium should be greater than two.³

B. Soil Science

That magnesium is the key metallic substance in chlorophyll has been discussed previously in Chapter 2. Magnesium is more abundant in the parts of a plant that are concerned with vital processes, such as seed and foliage, than in roots and stems.⁴

The lack of magnesium in early spring grass may result in so-called grass tetany in animals grazing on young grass in soils low in available magnesium or where the soil solution contains a high concentration of the stronger cations which may limit absorption of the weaker magnesium ion by certain plants. The accumulation of magnesium and phosphorus in the seed and other storage organs of the plant indicates that there is a very definite relation between magnesium and phosphorus in reproduction and growth.

Deficiencies of magnesium in plants may be due to a number of conditions such as

insufficient supply in the virgin soil, insufficient rate of solubility, enhanced leaching through additions of anions forming soluble magnesium compounds, and insufficient rate of absorption due to selective absorption of the stronger nutrient cations such as K^+ , Na^+ , Ca^{2+} , or NH_4^+ .

Soil areas in which the supply of magnesium is inadequate are usually associated with regions of relatively heavy precipitation and leaching. Light sandy loams in the humid areas are most likely to be deficient in magnesium.

Crops containing a relatively large quantity of magnesium, such as potatoes, cotton, tobacco, tomatoes, and other vegetables, often give a marked response, as indicated by yields, to applications of magnesium in mixed fertilizers. The addition of large quantities of sulfates, chlorides, or nitrates will increase the solubility of magnesium in the soil and result in a heavy loss of magnesium through leaching in regions of high rainfall.

A broadcast application of dolomite limestone may supply the magnesium requirement for a number of years. In the production of high-yielding crops with a short growing season such as tobacco, cotton, potatoes, and certain vegetables, the addition of the equivalent of 20 to 30 lb/acre of magnesium oxide in soluble magnesium salts will usually satisfy the annual magnesium requirement in most soils.

Magnesium is a factor in nitrogen fixation by nodule bacteria of legumes. In its absence a rather characteristic chlorosis develops in green plants. This results in poor growth and low vigor of the plant.⁵ Crops differ to a marked degree in the response to a deficiency of magnesium in the soil. For instance, buckwheat and spinach are most affected, and turnips, mangel, corn, and tobacco considerably so. The small greens, grasses, clovers, and potatoes are only slightly affected, and other plants not at all.

Plants sensitive to magnesium deficiency develop characteristic physiologic symptoms which have value in diagnosis. In corn, chlorosis appears in the intervascular tissue, although incipient chlorosis sometimes appears first on the margin and tip of the leaves; the typical striped chlorosis progresses to necrosis.⁶ The pattern of chlorosis developed by other plants follows rather closely the type of venation of the leaves. Leaves having parallel veins, such as certain small grains and grasses, develop the same type of chlorosis as corn, but of a mild degree. Susceptible plants with netted venation as a rule develop a mottled pattern similar to the well-known appearance of tobacco leaves affected by "sand-drown".⁷ Buckwheat and turnips (and tobacco) are the outstanding plants with this type of chlorosis. In advanced stages, cupping and curling of the leaf margin is common; the tissue turns yellow or brown, and the leaves drop from the plant.⁸ A necrosis of intervascular tissue similar to sunscalds often occurs, particularly in leaves exposed to full action of the rays of the sun.

Recent ultrastructural studies have shown a reduction in the grana-fret system and the conspicuous starch grains in magnesium-deficient plants. The weakly developed frets were ascribed to incomplete development of the chloroplasts because of magnesium deficiency. The presence of starch grains in the magnesium-deficient chloroplasts was attributed to the need for magnesium for phosphorylation of starch.⁹

It appears that a soil should contain from 30 to 40 ppm of easily replaceable magnesium, or 60 to 80 lb/acre, to avoid magnesium deficiency.

II. GEOCHEMICAL STUDIES

A. Drinking Water: A Possible Geochemical Factor in Human Health

The use of glacial milk, essentially an aqueous extract (solution and suspension) of

rocks, by the natives of Hunza, West Pakistan, has been cited as a major factor contributing to purported excellent health and unusual longevity of these people. A suspension of rock flour can not only supply immediately assimilable substances in solution, but also continuous delayed-action, mineral-nutrient reserves after ingestion.¹⁰

B. U.S. Geological Survey Studies

From 1963 to 1965, the U.S. Geological Survey coordinated a cooperative study with the Heart Disease and Stroke Control Program, U.S. Public Health Service, of two groups of contiguous or nearly contiguous counties known to have greatly different cardiovascular mortality rates.¹¹ Nine contiguous counties of northern Georgia have a low mortality rate for cardiovascular diseases (including coronary heart disease, stroke, hypertensive diseases, and other diseases of the heart and blood vessels) for white males age 35 to 74, which range from 560 to 682 per 100,000 population. The principal geochemical characteristic of the soil from this area is its content of weatherable minerals which serve as a constant source of element enrichment in the process of soil development. Nine counties of central and south-central Georgia, in contrast, have a high cardiovascular mortality rate ranging between 1151 to 1446 per 100,000. The soil in these counties is largely composed of materials that generally are deficient in weatherable minerals. The concentrations of the elements that were studied in both garden soils and uncultivated soils from the two areas tended to be significantly different and were generally higher in the low-death-rate area. Garden soil from the low-rate area contained almost nine times as much magnesium as soil from the high-rate area.

The greater abundance of many chemical elements in soils from the low-death-rate area (northern Georgia) compared with their abundance in soils from the high-death-rate area (south-central Georgia) is believed to reflect the differences in the character of the bed rock which provided the materials for soil formation. The sediment in the high-rate area probably had been extensively weathered and leached during its original deposition. Soils formed in these sediments, consequently, are relatively deficient in many elemental constituents. The present soils in the low-death-rate area, on the other hand, are being continuously supplied with elements from the weathering of fresh igneous and metamorphic rocks.

The analysis of tree samples and some vegetables tend to reveal the geochemical differences in soils from the two areas only when examined on a broad scale. The difference between the two areas in the levels of various chemical elements present in vegetables appeared to be so slight as to be unlikely to contribute appreciably to the observed differences in death rate in middle-aged humans. The data showed that garden vegetables do not clearly reflect the chemical composition of the soils in which the vegetables grew. The chemicals in soils, however, may enter the human food chain in water, in food plants not sampled in this study, and in meat and milk from animals that have fed on cultivated forage plants and native vegetation. Of these alternatives, *entrance into the food chain in the water* appears to be the most attractive hypothesis. The authors concluded that if geochemical differences between the two areas do, in fact, have a causal relationship to death from cardiovascular diseases, the cause would appear to be a deficiency, rather than an excess, of the elements studied.

Shacklette et al. extended their studies of geochemical factors to the entire U.S.¹² Samples of soils or other regoliths, taken at a depth of approximately 8 in. from locations about 50 mi apart throughout the conterminous U.S., were analyzed for their content of elements. Surficial materials of the western U.S. generally contained more calcium, magnesium, strontium, potassium, sodium, aluminum, and barium, but contained less titanium and zirconium than did those of the eastern half. The magnesium

content of surficial material from the western U.S. (west of the 97th meridian) averaged 7800 ppm, whereas that in the eastern U.S. averaged 2300 ppm. Surficial materials in the Atlantic Coastal Plain tended to have much lower concentrations of most metals than are common in other regions, whereas these materials in the Basin and Range province, in parts of the Rocky Mountains and in Maine and adjacent states generally have high concentrations.

A further discussion of the relationship of geochemistry to human disease is pursued in Chapter 10.

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Chapter 8

PHYSIOLOGY OF MAGNESIUM IN MAN

I. NORMAL DISTRIBUTION AND TURNOVER

A. Body Content

The limited data available from analysis of human carcasses indicate that the magnesium content of the human body ranges between 22.7 and 35.0 meq/kg (272 to 420 mg/kg) wet weight of tissue.¹ Extrapolations from tissue analyses performed on victims of accidental death indicate that the body content of magnesium for a man weighing 70 kg would be on the order of 2000 meq (24 g).² 89% of all the magnesium in the body resides in bone and muscle. Bone contains about 60% of the total body content of magnesium at a concentration of about 90 meq/kg wet weight. Most of the remaining magnesium is distributed equally between muscle and nonmuscular soft tissues. The magnesium contents obtained in various soft tissues in a study in Bombay, India, performed on 20 cases of instantaneous accidental death of normal adults, ranged from a maximum of 28.3 meq/kg wet weight in breast tissue to a minimum of 11.7 meq/kg in uterine tissue (Table 1).³

Approximately 1% of the total body content of magnesium is extracellular. The levels of magnesium in serum of healthy people are remarkably constant, remaining on the average of 1.7 meq/l and varying less than 15% from this mean value.⁴ The distribution of normal values for serum magnesium is identical in men and women and remains constant with advancing age.⁵ Approximately one third of the extracellular magnesium is bound nonspecifically to plasma proteins. The remaining 65%, which is diffusible or ionized, appears to be the biologically active component. The ratio of bound to unbound magnesium, as well as the total serum levels, is remarkably constant. The magnesium content of erythrocytes varies from 4.4 to 6.0 meq/l (Figure 1).^{6,7}

B. Intake

The average American ingests daily between 20 and 40 meq (240 to 480 mg) of magnesium; magnesium intakes of from 0.03 to 0.35 meq/kg/day (3.6 to 4.2 mg/kg/day) are thought to be adequate to maintain magnesium balance in normal adults.⁸ A daily intake of 17 meq (0.25 meq/kg) may meet nutritive requirements provided that the individual remains in positive magnesium balance. The U.S. National Research Council recommends a magnesium allowance of 25 to 42 meq/day (300 to 500 mg/day) for adults.⁹ Using the data from 941 magnesium balance experiments collected from the literature, Seelig claimed that, after allowing for sweat losses, the minimum magnesium requirement was 0.5 meq (6 mg)/kg of body weight per day or about 33 meq (400 mg)/day.¹⁰ Marshall, Nordin, and Speed performed 208 magnesium balances and found a linear relation between magnesium intake and output with a slope of 0.83 and an intercept at theoretical zero intake of 37 mg. Regression of output vs. intake yielded a mean equilibrium value of 18.6 meq (223 mg).¹¹

Schroeder et al. first called attention to the theoretic relationship of dietary magnesium deficiency to serious chronic diseases, including atherosclerosis.² The possible relationships among geochemistry, dietary magnesium intake, and human diseases will be discussed in Chapter 10. The estimated daily requirement for a child is 12.5 meq (150 mg).¹² The greater importance of magnesium in childhood is suggested by the relative ease with which deficiency states are produced experimentally in young animals as compared with adult animals.¹²

Table 1
MAGNESIUM CONTENT IN HUMAN
TISSUES

Tissue	% Dry/Wet Wt.		Magnesium Content (meq/kg of Wet Wt.)	
	(Mean)	± (SD)	(Mean)	± (SD)
Adrenal (18)*	37.5	11.8	15.0	6.7
Aorta (20)	30.69	7.51	21.7	16.7
Breast (4)	29.41	20.64	28.3	10.0
Cerebrum (14)	19.94	3.23	16.7	7.5
Cerebellum (14)	19.77	4.19	15.0	6.7
Esophagus (9)	22.83	6.53	17.5	7.5
Heart (19)	21.20	5.60	23.3	12.5
Ileum (16)	21.46	9.54	20.0	10.8
Kidney (22)	19.49	3.95	18.3	7.5
Liver (21)	26.48	4.96	20.0	6.7
Lung (21)	16.76	4.41	15.0	8.3
Muscle (21)	23.07	5.60	22.5	10.8
Pancreas (19)	26.84	12.33	21.7	10.8
Prostate (4)	21.38	2.33	23.3	7.5
Skin (17)	42.81	18.66	24.2	30.0
Spleen (19)	23.32	10.25	18.3	6.7
Stomach (17)	21.55	5.80	16.7	8.3
Testis/ ovary (13)	21.78	14.64	15.0	7.5
Thymus (4)	40.02	22.75	25.8	12.5
Thyroid (14)	21.42	5.77	17.5	8.3
Tongue (19)	25.66	10.75	20.0	9.2
Trachea (21)	30.0	10.79	21.7	15.8
Urinary bladder (18)	32.13	11.65	17.5	20.8
Uterus (3)	36.95	18.94	11.7	2.5

* Number in parenthesis indicates number of samples analyzed.

Modified from Soman, S. D., Joseph K. T., Raut, S. J., Mulay, C. D., Parameshwaran, M., and Panday, V. K., *Health Phys.*, 19, 641, 1970.

In a recent study of the nutritional requirements for magnesium during pregnancy, ten healthy, pregnant, white women were observed on a self-selected diet. The mean magnesium intake was found to be only 60% of the recently established recommended dietary allowance.¹³

Some common foods can be ranked in order of decreasing mean concentrations of magnesium as follows: nuts, 162 meq/kg; cereals, 66; seafoods, 29; meats, 22; legumes, 20; vegetables, 14; dairy products, 15; fruits, 6; refined sugars, 5; and fats, 0.6. This order differs when the concentrations are ranked on the basis of caloric values of the foods, as follows: vegetables, legumes, seafoods, nuts, cereals, dairy products, fruit, meat, refined sugars, and fats. Noteworthy is the very small contribution of fats and refined sugars to the total intake of magnesium. These two, the major sources of caloric energy, are virtually devoid of magnesium.²

C. Intestinal Absorption

When a tracer dose of ²⁸Mg was administered orally to 26 subjects, fecal excretion

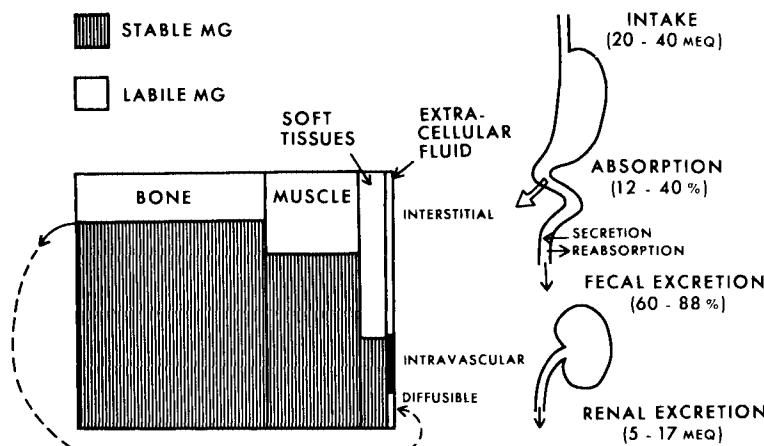


FIGURE 1. Distribution of magnesium in man.

within 120 hr accounted for 60 to 80% of the administered dose.¹⁴ The concentration of radioactivity in the plasma was maximal at 4 hr, but the actual increase in serum magnesium concentration was negligible. When ²⁸Mg was injected i.v. into a normal human subject, only 1.8% of the radioactivity was recovered in the stool within 72 hr.¹⁵ The fractional absorption of ²⁸Mg (the proportion of orally administered ²⁸Mg as compared with ²⁸Mg administered i.v.) in 16 normal individuals was $41 \pm 8\%$ (mean \pm SD), with a range from 34 to 62%.¹⁶

Magnesium absorption was studied in the normal human jejunum and ileum by in vivo intestinal perfusion, using 0 to 20mM Mg. As luminal magnesium concentration was increased, the rate of absorption in the jejunum rose progressively with a tendency towards saturation at the higher concentrations. The kinetics and rates of magnesium absorption in the ileum were comparable to those in the jejunum. Calcium had little or no influence on magnesium absorption. Magnesium absorption in the human appeared to be mediated by a transport process different from that which facilitates calcium absorption. Normal magnesium absorption may be dependent on vitamin D. It is still not established whether the normal intestine can absorb magnesium against an electrochemical gradient.¹⁷

D. Intestinal Secretion

There undoubtedly is considerable secretion of magnesium into the intestinal tract from bile and pancreatic and intestinal juices. This secretion is followed by almost complete reabsorption. Parotid saliva contains about 0.3 meq/l and pancreatic juice about 0.1 meq/l of magnesium.¹⁸ The concentration of magnesium in other secretions varies considerably. The observation that hypomagnesemia can occur in patients suffering from large losses of intestinal fluids suggests that intestinal juices contain enough magnesium to deplete the serum when the magnesium is not reabsorbed by the colon.

Studies are just beginning on the role played in the transport of divalent cations by biochemical changes in the cells of intestinal mucosa.¹⁹ Further investigations may show that the cells of the intestinal mucosa, like those in the kidney and elsewhere in the body may depend in part upon metabolic activity for the uptake and release of calcium and magnesium.

E. Excretion

Most of that portion of the magnesium which is absorbed into the body is excreted

by the kidney.^{20, 21} Fecal magnesium represents largely the unabsorbed fraction. In subjects on a normal diet, one third or less of the *ingested* magnesium (5 to 17 meq) is excreted by the kidney. After the i.v. injection of a tracer dose of ²⁸Mg in 12 to 16 meq of stable magnesium, the daily urinary excretion of magnesium in eight normal subjects ranged between 6 and 36 meq as the parenteral dose was increased.¹⁵ The maximal renal capacity for excretion is not known, but it probably is quite high, perhaps greater than 164 meq/day.²

The diffusible magnesium in plasma is filtered by the glomeruli and is reabsorbed by the renal tubule, probably by an active process, although the control mechanisms are not known. There is some evidence that magnesium may be secreted by the renal tubule.²² Both the mercurial and the thiazide diuretics increase excretion of magnesium, calcium, potassium, and sodium.

Magnesium excretion also occurs in sweat.²³ When men are exposed to high temperature for several days, from 10 to 15% of the total output of magnesium is recovered in sweat. Acclimatization does not occur, as in the case for sodium and potassium. Under extreme conditions, sweat can account for 25% of the magnesium lost daily; this factor would be important when the intake of magnesium is low.²⁴

F. Magnesium Conservation on a Low-Magnesium Diet

It is primarily the ionic fraction of the magnesium in plasma which appears in the glomerular filtrate. Any protein-bound magnesium which is filtered is probably returned to the circulation via lymph. The excretion of magnesium may be greater than normal in renal diseases associated with heavy proteinuria.

Magnesium clearance, corrected for protein binding, increases as a linear function of serum magnesium concentration and approaches the inulin clearance at high plasma levels of magnesium. There normally appears to be almost maximal reabsorption of magnesium.²⁵

In spite of the probability of diets being low in magnesium under certain circumstances, clinical magnesium deficiency does not occur in human beings with healthy kidneys. The explanation for this clinical observation appears to be that renal mechanisms are efficient enough to conserve all but about 1 meq of magnesium per day. Fecal losses are minimal.²⁶

G. Abnormal Magnesium Levels in the Blood

Values lower than 1.1 meq/l have been obtained in patients with congestive heart failure, cirrhosis, or renal failure after hemodialysis. All values higher than 2.0 meq/l were found in patients with renal failure before therapy.¹⁴

II. THE PLASMA CLEARANCE AND TISSUE UPTAKE OF MAGNESIUM

A. Early Studies

Mendel and Benedict reviewed much of the early literature on the absorption and excretion of magnesium.²⁷ These investigators showed quite clearly that rapid renal excretion of magnesium followed the s.c. injection of various magnesium salts, whereas intestinal excretion was minimal. Hirschfelder and Haury, however, reported that in seven normal adults, 40 to 44% of an injected dose of magnesium appeared in the urine within 24 hr.²⁸ Tibbetts and Aub, by means of classic balance techniques, studied the excretion of magnesium in normal subjects; they found that individuals on an oral intake of 40 to 74 meq/day excreted 41 to 66 meq, of which slightly over one

TISSUE	TOTAL CONTENT = 2,000 MEQ (IN A 70 KG MAN)			EXTRA- CELLULAR FLUID
	BONE	MUSCLE	SOFT TISSUES	
MAGNESIUM CONCENTRATION (MEQ/KG WET WT)	90	15	10-15	1.7
CONTENT (MEQ)	1,200	580	200	20
% OF TOTAL	60	89	99	100

FIGURE 2. Turnover of magnesium in man.

half was in the stools.²⁹ Smith et al. studied the excretion of magnesium in dogs after the i.v. administration of MgSO₄ and concluded that the magnesium distributed itself throughout the extracellular fluid during the first 3 to 4 hr; during subsequent hours, some of the ion appears to be segregated from the extracellular fluid and not excreted (Figure 2).³⁰

B. Tracer Studies in Human Beings

The introduction of the radioactive isotope of magnesium, ²⁸Mg, for clinical studies in 1957 made possible determination of the "exchangeable" pool in human subjects. When nine normal subjects were given i.v. infusions of 12 to 30 meq of magnesium tagged with ²⁸Mg, the material was very rapidly cleared from the extracellular fluid.¹⁴ The concentration of radioactivity in plasma and urine was too low to follow beyond 36 hr. Within a few hours, the volume of fluid available for the dilution of this ion, as calculated from the plasma concentration of Mg²⁸, exceeded the volume of total body water.

The clearance curves in general showed a rapid phase during the first 4 hr, a subsequent more gradual decline up to about 14 hr, and a slow exponential slope thereafter. Biopsies of tissues contained concentrations of ²⁸Mg in liver, appendix, fat, skin, and s.c. connective tissue which could not be attributed solely to the extracellular components of these tissues. All of these observations suggested that ²⁸Mg rapidly entered cells of the soft tissues and that 70% or more of the infused magnesium was retained in the body for at least 24 hr.

Of interest is the observation that the 24-hr urinary excretion of stable magnesium following the infusion of ²⁸Mg approximated the amount of nonradioactive magnesium infused, whereas only 20% of the ²⁸Mg infused was recovered. Previous investigators without the benefit of the radioisotopic data have assumed that most of the infused magnesium was rapidly excreted by the kidney. The additional isotopic data indicate that the infusion of fairly large amounts of magnesium results in a compensatory renal excretion of the body store of magnesium and that the material excreted is probably not the ions that were administered.

Serial external survey of radioactivity over the entire body revealed the maximal distribution of radioactivity at the end of infusion over the right upper quadrant of the abdomen. This finding suggests initial concentration of magnesium in the liver. At 18 hr, the specific activity in bile was equal to that of serum. This equilibration of the infused ²⁸Mg had occurred earlier in bile than in any other tissue or fluid available for study.¹⁵

After about 18 hr, the specific activities in plasma and urine showed only a slight gradual increase, suggesting that the infused material had equilibrated with the stable magnesium in a rather labile pool and that further exchange was occurring very slowly in a less labile pool. The size of this labile pool in normal subjects ranged between 135 and 397 meq (2.6 to 5.3 meq /kg of body weight). Since the body content of magnesium is estimated to be 30 meq/kg, it appears that less than 16% of the total body content of magnesium is measured in the ^{28}Mg exchange technique.

The results of the external survey and the tissue analyses suggests that the labile pool of magnesium is contained primarily in connective tissue, skin, and the soft tissues of the abdominal cavity (such as the liver and intestine) and that the magnesium in bone, muscle, and red cells exchanges very slowly.

In another study, Silver et al. followed the turnover of magnesium for periods up to 90 hr after ^{28}Mg was injected i.v. into human subjects.³¹ Even at 90 hr, only one third of the body's magnesium had reached equilibrium with the isotope. The results confirmed the impression that the GI absorption of magnesium is very limited. Graphic analysis of urinary ^{28}Mg curves in terms of exponential components yielded a slow component with a half-time of 13 to 35 hr, which accounted for 10-15% of the injected dose, and two more rapid components with half-times of 1 and 3 hr each, accounting for 15 to 25% of the injected dose. The large fraction remaining — about 25 to 50% of the body's total — had a turnover rate of less than 2% per day. Because approximately 25 to 50% of the total body content exchanges at a turnover rate of less than 2% per day, this isotopic dilution method, used so successfully with sodium and potassium, cannot be employed to quantitate the total body content of magnesium in man. In rabbits, however, the exchangeable magnesium value at 24 hr agrees well with the total carcass content of magnesium.³² During starvation, the renal excretion of magnesium amounts to 61.7 meq/kg of wet weight.³³

C. Magnesium Equilibration in Bone

The reactivity of the skeleton, as measured by isotopic exchange, declines with age.³⁴ The exchange of ^{28}Mg , expressed as bone/serum-specific activity, is much more rapid in younger animals than in older ones. ^{28}Mg accumulates in the bones of young rats about twice as fast as in the bones of adult rats.³⁵

The exchange of ^{28}Mg in cortical bone occurs much more rapidly in young rats than in old ones. The stable magnesium content of bone increases with age and varies inversely with the water content of bone. ^{28}Mg studies in lambs indicate that the magnesium reserve in bone is mobilized during dietary magnesium deficiency.³⁶

D. ^{28}Mg Compartmental Analysis in Man

Avioli and Berman used a combination of metabolic balance and ^{28}Mg turnover techniques in order to develop a mathematical model for magnesium metabolism in man.³⁷ The data thus derived were subjected to compartmental analysis using digital computer techniques.

After the i.v. administration of ^{28}Mg , the decline in the specific activity of plasmas or urine can be expressed as the sum of several exponential terms by the method of graphic analysis. On the basis of such analyses, Silver et al. defined in man three exchangeable magnesium compartments with half-times of 38, 3, and 1 hr.³⁸ MacIntyre et al. described three exchangeable magnesium compartments containing 7.3, 24.4, and 98.7 meq of magnesium.³⁹ Zumoff et al. obtained similar data.⁴⁰

Multicompartmental analysis indicates that in man there are at least three exchangeable magnesium pools with varied rates of turnover: compartments one and two, exemplifying pools with a relatively fast turnover, together approximating extracellular

fluid in distribution; compartment three, an intracellular pool containing over 80% of the exchanging magnesium with a turnover rate of one half that of the most rapid pool; and compartment four, which probably accounts for most of the whole-body magnesium. Only 15% of whole-body magnesium, averaging 3.54 meq/kg body weight, is accounted for by relatively rapid exchange processes.^{37, 41}

III. GASTROINTESTINAL ABSORPTION

A. Daily Absorption in Man

In normal individuals on regular diets, the average daily absorption of magnesium from the GI tract is 0.14 meq/kg, an amount approximately 40% of the size of the extracellular pool. The rate of entry of magnesium into the intracellular pool would be approximately 0.0058 meq/kg/hr if one assumes that absorption occurs continuously throughout the day. This rate of entry is approximately 1% of the rate of removal of magnesium from the extracellular pool by all routes.⁴²

The stable isotope ²⁶Mg has recently been used to study magnesium absorption in man.⁴³

B. Factors Affecting Absorption

No single factor appears to play a dominant role in the absorption of magnesium as does vitamin D in the absorption of calcium. Several studies using ²⁸Mg suggest that the absorption of magnesium in man is influenced by the load presented to the intestinal mucosa.^{44, 45} On an ordinary diet containing 20 meq of magnesium, 44% of the ingested radioactivity was absorbed per day. On a low magnesium diet (1.9 meq/day), 76% was absorbed. On a high magnesium diet (47 meq/day), absorption was decreased to 24%.

Absorption begins within 1 hr of ingestion and continues at a steady rate for 2 to 8 hr; it is minimal after 12 hr. In man, absorption throughout the small intestine is fairly uniform, but little or no magnesium is absorbed from the large bowel.⁴⁵

C. Site of Absorption

Evidence from a variety of animals suggests that the small intestine is the main site of magnesium absorption, but that the pattern of absorption varies with the species studied.^{45, 46} Absorption from the large intestine is negligible in the rabbit.³² In male albino rats, more than 79% of the total absorption of ²⁸Mg takes place in the colon, and excretion of endogenous magnesium occurs predominantly in the proximal gut.⁴⁷ Both magnesium and calcium are bound to phosphate and to nonphosphate binding material of an unknown nature in the ileal content of ruminating calves.⁴⁸

There appears to be an interrelationship between the absorption of magnesium and calcium in the proximal part of the small intestine in the rat.^{49, 50} The suggestion has been made that there is a common mechanism for transporting calcium and magnesium across the intestinal wall,^{51, 52} but another recent study contradicts this impression.¹⁷

D. The Role of Ionic Magnesium

At the present time, there is no unequivocal evidence that magnesium is actively transported across the gut wall.⁵³ It seems reasonable to assume that the net amount of dietary magnesium absorbed is directly related to the intake and to the time available for absorption of the magnesium from the small intestine. Therefore, apart from a small effect from the difference in potential across the wall of the small intestine, the concentration of *ionic* magnesium in the digest at the absorption site must be the main factor controlling the amount absorbed in a given time.⁴⁸

IV. RENAL EXCRETION

A. Control of Body Contents

The kidney is the major excretory pathway for magnesium once it is absorbed into the body.²⁷ In subjects on a normal diet, this renal excretion amounts to one third or less of the 5 to 17 meq of magnesium which is ingested every day. The mean daily excretion of magnesium in the urine of 12 normal men on an unrestricted diet was 13.3 ± 3.5 meq.⁴ Following the i.v. injection of a tracer dose of ^{28}Mg in 12 to 16 meq of stable magnesium, the daily urinary excretion of magnesium in eight normal subjects ranged between 6 and 36 meq.⁴⁴ Urinary excretion of magnesium increased as the parenteral dose was increased.

Metabolic balance studies in 27 subjects on a self-selected diet of normal composition showed a close positive correlation between the level of dietary intake and the magnesium excretion in both the urine and the feces.⁵⁴ These results suggest that the absorption of magnesium from the intestinal tract is a poorly controlled process which is determined largely by the dietary intake of the element. The kidney must, therefore, be the organ principally responsible for regulating the total body content of magnesium. When dietary intake of magnesium is increased or decreased, urinary excretion of magnesium is increased or decreased respectively without any significant change in the plasma level of magnesium.

B. Effect of Dietary Restriction of Magnesium

Retention of magnesium by the kidney occurs rapidly in response to the dietary intake.^{26, 55} This is why it is so difficult to produce magnesium depletion in the adult without some source of abnormal loss from the body.

Diurnal variations in the urinary excretion of calcium and magnesium have been demonstrated in patients in a metabolic ward.⁵⁶ A reduction in the excretion of calcium, magnesium, sodium, and creatinine occurs at night. There are slight but constant diurnal variations in the serum concentration of calcium and magnesium with the values being lower in the morning than in the evening. Diet and physical activity appear to play the dominant roles in the diurnal fluctuation, but there also might be an associated rhythmicity in the function of the parathyroid gland.

C. Mechanism of Renal Excretion

The mechanism of excretion of magnesium by the mammalian kidney is still unclear.⁵⁷ It could involve glomerular filtration and partial reabsorption of the filtered material by the renal tubules, or the filtered material could be completely reabsorbed and the excreted magnesium appear by tubular secretion as is believed to occur with potassium. A recent micropuncture study in the young rat revealed that the ascending limb of the loop of Henle was the major site for magnesium reabsorption.⁵⁸ Tubular secretion of magnesium undoubtedly occurs in the aglomerular fish,⁵⁹ but stop-flow studies with radioactive magnesium in dogs have produced conflicting evidence about secretion of magnesium by the tubules.^{60, 61} In the rabbit, the renal excretion of magnesium appears to be essentially glomerular; the tubular wall appears to be impermeable to magnesium throughout its length.⁶²

D. A Possible Renal Threshold

The amount of magnesium that is filtered at the glomerulus in an adult human is about 9.6 meq/hr, assuming a glomerular filtration rate of 130 ml/min, total plasma magnesium concentration of 1.6 meq/l and an ultrafiltrable fraction comprising 75% of the total. The mean rate of magnesium excretion in the urine (about 0.33 meq/hr)

therefore represents only 3.5% of the filtered load. Moreover, the whole range of excretion observed under physiologic conditions in man can be explained if the tubular reabsorption of magnesium varies between 91 and 99% of the amount filtered at the glomerulus. In the rat,⁶³ sheep,⁶⁴ and cattle,⁶⁵ there is evidence for the existence of a renal threshold for excretion of magnesium at a value close to the lower limit of the normal blood level. There is reduction in tubular reabsorption of magnesium above a total serum magnesium concentration of 1.2 to 1.4 meq/l; this could be due to either a decrease in the maximum capacity for tubular reabsorption or an increase in tubular secretion of magnesium.

E. Tubular Secretion

The possibility of secretion of magnesium by the renal tubules has been investigated under conditions of magnesium loading.⁵⁴ At serum concentrations of above 6.2 meq/l, the amount excreted exceeded twice the filtered load, thus demonstrating tubular excretion of magnesium beyond any likely experimental error. The response to the administration of 2,4-dinitrophenol suggested that magnesium is also secreted by the tubules under physiologic conditions.

All the available evidence in the rat until recently has been consistent with a mechanism for renal excretion which involves reabsorption of the filtered material, with the excreted magnesium derived chiefly by tubular secretion. This secretion only appears to commence when the magnesium concentration in serum exceeds a critical value which is close to the lower limit of the normal range. However, studies with the stop-flow techniques did not find magnesium secretion in acutely magnesium-loaded rats undergoing mannitol or sulfate diuresis.⁶⁶

In the dog, magnesium excretion, like sodium and calcium excretion, is determined by filtration and reabsorption alone without evidence for tubular secretion.⁶⁷ There is a maximal tubular reabsorptive capacity (Tm) for magnesium of approximately 11.5 $\mu\text{eq}/\text{min}/\text{kg}$ body weight. The parathyroid hormone may directly enhance tubular reabsorption of magnesium (Figure 2).

V. HOMEOSTASIS

We do not understand yet the physiologic mechanisms which are responsible for maintaining plasma magnesium concentration at a constant level.⁶⁸ Both calcitonin⁶⁹ and parathormone may be involved. Nevertheless, animals and human beings on an adequate intake of magnesium do remain in magnesium balance and the two chief regulatory sites appear to be the gastrointestinal tract and the kidney.

A. Effects of Parathyroid Hormone

There is considerable evidence for the hypothesis that the parathyroid hormone may help to control the concentration of plasma magnesium through a negative feedback mechanism.⁷⁰⁻⁷²

Magnesium deficiency in the intact rat is accompanied by hypercalcemia and hypophosphatemia, provided the parathyroid glands are intact. The concentration of ionic calcium in plasma is elevated. In the absence of the parathyroid gland, magnesium deficient rats do not develop hypercalcemia or hypophosphatemia. Moreover, parathyroidectomized animals with magnesium deficiency develop a concentration of ionized calcium in plasma that is lower than that observed in parathyroidectomized rats on a normal diet.^{73, 74}

These observations help to establish a relationship between an apparent increased function of the parathyroid gland and magnesium deficiency.⁷⁴ Recent studies suggest that magnesium depletion may result in either impaired synthesis or release of parathyroid hormone in man, or both.⁷⁵⁻⁷⁷ All hypomagnesemic patients were observed to have an immediate rise in serum immunoreactive parathyroid hormone (IPTH) concentration after administration of magnesium and regardless of the basal IPTH concentration.⁷⁸ Although it seems clear that impairment of PTH secretion is an important factor in the pathogenesis of hypocalcemia in magnesium deficiency, the presence of normal or increased serum IPTH concentration in some patients indicates that a state of peripheral resistance to PTH also exists. Thus, PTH responsiveness in hypomagnesemic patients may, at least in part, be dependent upon the adequacy of intracellular magnesium stores.⁷⁹

B. Effects of Hypomagnesemia

If parathyroid regulation is influenced by the concentration of magnesium in plasma, hypermagnesemia should diminish parathyroid gland activity.⁸⁰ This hypothesis was tested in intact and chronically parathyroidectomized rats which were nephrectomized to eliminate the urinary excretion of calcium as a variable in this study. Isotonic magnesium chloride was administered s.c. to the experimental animals and normal saline was administered to the controls. A significant decrease in the concentration of ionic calcium was observed in the magnesium-treated animals with the intact parathyroid gland. In contrast, magnesium-treated parathyroidectomized animals failed to develop a significant change in the concentration of ionic calcium in comparison to saline-treated parathyroidectomized controls. These observations suggest that hypermagnesemia may inhibit parathyroid gland activity. The results are consistent with the hypothesis that the parathyroid regulatory mechanism which is involved in calcium homeostasis is modified by alteration in the concentration of plasma magnesium.⁸¹

C. Perfusion Studies

The influence of the plasma magnesium concentration on parathyroid gland function was evaluated in goats and in a sheep by perfusion of the isolated parathyroid gland with whole blood with varying magnesium concentration.⁸² The concentration of PTH in venous plasma from the gland was estimated by a specific radioimmunoassay. In each animal, the concentration of parathyroid hormone in the effluent plasma diminished when the concentration of magnesium was raised; the concentration of hormone increased when the concentration of magnesium was lowered. The response of the parathyroid hormone concentration to changes in plasma magnesium concentration occurred rapidly within minutes. Magnesium appeared to have a specific influence on the rate of release of PTH.

D. Studies in Organ Culture

Sherwood et al. recently developed an organ culture system utilizing normal bovine parathyroid tissue.⁸³ Studies with this system provide direct evidence that the release of PTH is inversely proportional to both the calcium and the magnesium ion concentrations. These two cations are equipotent in blocking hormone release.

E. Relationship Between Bone and Extracellular Magnesium

Magnesium deficiency in the rat has been shown repeatedly to cause lowering of the magnesium concentration in bone.⁸⁴ The observation of a close direct relationship between the magnesium concentration in the plasma and the femur of magnesium-defi-

cient rats, calves, and man⁸⁵ supports the view that the skeleton provides the magnesium reserve in the body and suggests that there exists an equilibrium between the magnesium of the plasma and the bone. Recent clinical studies indicate that bone and extracellular fluid magnesium and the major magnesium pools in man increased during magnesium excess and decreased during magnesium depletion.⁶ This equilibrium is apparently independent of enzymatic activity and must, therefore, be physicochemical in nature. The fact that the equilibrium is dependent upon the concentration of magnesium in both the medium and the bone suggests that the relationship between bone and extracellular fluid magnesium is analogous to the ionization of a poorly dissociated salt, with the magnesium in bone corresponding to the undissociated salt.

F. Effects of Parathyroid Extract In Vitro

Parathyroid extract increases the rate of magnesium loss from either fresh or boiled bone in vitro in a magnesium-low medium containing 50% bovine serum; however, the extract has no effect in a protein-free medium. These observations are consistent with the hypothesis that the physicochemical action of parathyroid preparations may involve the binding of divalent cations by a parathyroid-albumin complex.^{84,86} This phenomenon in dead tissue, which may partially explain an important biologic function, certainly is not in accord with current concepts of the mechanism of hormonal action.

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Chapter 9

MAGNESIUM DEFICIENCY IN MAN

I. THE CLINICAL SYNDROME

For many years, there was doubt about the existence of a pure magnesium deficiency state in man. Now it is established that there is such a condition.^{1,2} It is characterized by the following features: (1) spasmophilia,³ gross muscular tremor, choreiform movements, ataxia, tetany, and, in some instances, predisposition to epileptiform convulsions;⁴ (2) hallucinations, agitation, confusion, tremulousness, delirium, depression, vertigo, muscular weakness, and organic brain syndrome;⁵ (3) a low serum magnesium concentration associated with a normal serum calcium concentration and a normal blood pH; (4) a low-voltage T wave in the electrocardiogram,⁶ low-voltage PQRS complexes, and a short fixed P-R interval;⁷ (5) positive Chvostek and Trousseau signs; and (6) prompt relief of the tetany when the serum magnesium concentration is restored to normal.² Durlach recognizes the presence of other manifestations of clinical magnesium deficiency, such as phlebothrombosis, constitutional thrombasthenia, and hemolytic anemia, an allergic or osseous form of the deficiency, and oxalate lithiasis.^{8,9} Dysphagia and vertical nystagmus have recently been reported to be an unusual symptom and a sign of magnesium deficiency.^{10,11}

II. EXPERIMENTAL PRODUCTION OF A PURE MAGNESIUM DEFICIENCY

It is difficult to achieve a significant magnesium depletion in normal individuals by simple dietary restriction because of the exceedingly efficient renal and GI mechanisms for conservation. The urinary magnesium in normal individuals falls to trivial amounts within 4 to 6 days of magnesium restriction.^{12, 13} In spite of these conservatory mechanisms, Dunn and Walser did induce in two normal subjects deficits approaching 10% of the total body content of magnesium by infusing sodium sulfate and adding calcium supplements to the magnesium-deficient diet.¹⁴ The concentration of magnesium in plasma and erythrocytes fell moderately. Because the muscle magnesium content remained normal, the presumption was that bone was the source of the loss. No untoward clinical effects were noted.

Randall et al reported data suggesting that total body depletion of magnesium may result in psychiatric and neuromuscular symptoms.¹⁵ Administration of magnesium by the parenteral route or in the diet was associated with clinical improvement which occasionally was dramatic.

The best study to date of magnesium deficiency in man is that recently reported by Shils.¹⁶⁻¹⁸ Seven subjects were placed on a magnesium-deficient diet containing 0.7 meq of magnesium per day. The concentration of magnesium in plasma declined perceptibly in all subjects within 7 to 10 days. Urinary and fecal magnesium decreased markedly, as did urinary calcium. At the height of the deficiency, the plasma magnesium concentration fell to a range of 10 to 30% of the control values, while the red cell magnesium declined more slowly and to a smaller degree. All male subjects developed hypocalcemia; the one female patient did not. Marked and persistent symptoms developed only in the presence of hypocalcemia. The serum potassium concentration decreased, and, in four of the five subjects in whom the measurement was made, the ⁴²K

space was decreased. The serum sodium concentration was not altered significantly. Three of the four subjects with the severe symptoms also had metabolic alkalosis.

A positive Troussseau sign which occurred in five of the seven subjects was the most common neurologic sign observed. Electromyographic changes, which were characterized by the development of myopathic potentials, occurred in all five of the patients tested. Anorexia, nausea, and vomiting were frequently experienced. When magnesium was added to the experimental diet, all clinical and biochemical abnormalities were corrected.

III. CLINICAL CONDITIONS ASSOCIATED WITH DEPLETION OF MAGNESIUM IN ADULTS

Magnesium deficiency can occur in congestive heart failure, after diuresis, with furosemide, ethacrynic acid and mercurials, and with digitalis intoxication, diabetic acidosis, acute and chronic alcoholism, delirium tremens, cirrhosis, malabsorption syndromes, protracted postoperative cases, open heart surgery, the diuretic phase of acute tubular necrosis, and with primary hypoparathyroidism, primary aldosteronism, juxtaglomerular hyperplasia, and pancreatitis.¹⁹

A. Fasting

Prolonged fasting is associated with a continued renal excretion of magnesium.²⁰ After 2 months of fasting, the deficit in some subjects may amount to 20% of the total body content of magnesium. Despite evidence for depletion of magnesium in muscle, the concentration of magnesium in plasma remains unchanged. The excess acid load presented for excretion to the kidney and the absence of intake of carbohydrate might be factors contributing to the persistent loss of magnesium. The magnitude of the excretion of magnesium parallels the severity of the acidosis. The ingestion of glucose decreases the urinary loss of magnesium.

B. Excess Loss from the GI Tract

Persistent vomiting or prolonged removal of intestinal secretions by mechanical suction coupled with the administration of magnesium-free intravenous infusions can induce clinical magnesium deficiency.^{21, 22}

C. Surgical Patients

There are postoperative changes in magnesium metabolism in patients undergoing a variety of operations involving a moderate degree of trauma.^{23, 24} A lowered serum magnesium concentration is observed from the day after operation in 56% of the patients, but it is usually corrected by the second or third postoperative day. Surgery is followed by a negative magnesium balance days in duration and similar changes are observed after dietary restriction in normal subjects. However, the magnitude of the magnesium loss following surgery is minimal and usually does not result in symptomatic magnesium deficiency.²⁵⁻²⁷

D. GI Disorders

The intestinal tract plays a major role in magnesium homeostasis. The rate of transport of magnesium across the intestine appears to be slower than that of calcium and directly proportional to intestinal transit time.²⁸ Malabsorption of magnesium, therefore, occurs in conditions in which intestinal transit is abnormally rapid or in which the major absorbing site, the distal small intestine, has been resected.

Malabsorption — Hypomagnesemia is associated frequently with malabsorption due to a variety of causes. In general, there appears to be a correlation between the degree of hypomagnesemia and the severity of the underlying disease. The increased fecal loss of magnesium that has been demonstrated in this disorder may be due to steatorrhea.²⁹

Idiopathic steatorrhea — A case of idiopathic steatorrhea with hypomagnesemia was reported by MacIntyre et al., whose careful studies utilized balance techniques, bone and muscle biopsies, and measurements of the exchangeable magnesium content.³⁰ The amount of magnesium in muscle was reduced to two thirds of the normal value, but the quantity in bone was affected little. The presence of magnesium depletion was further confirmed by the finding that the 24 hr exchangeable magnesium content was markedly reduced. Balance studies showed that fecal loss of magnesium was the cause of the deficiency and that this loss was intensified by a high intake of calcium. The results of the study appear to refute the concept that the bone magnesium always acts as a reservoir. The suggestion that bone magnesium in the adult man may be largely unavailable in disease states appears to correlate with the evidence that very little intracellular magnesium is mobilized during magnesium deficiency.

Malabsorption syndrome — The magnesium deficiency of severe malabsorption syndrome is associated with metabolic abnormalities which affect the renal and intestinal transport of several other electrolytes.³¹ Negative balances of magnesium, calcium, phosphorus, and potassium have been reported. Total exchangeable potassium content is reduced by one third during magnesium deficiency. The total 24 hr exchangeable magnesium content is reduced by more than 50%, due mainly to a decrease in the size of the slow pool which is believed to include skeletal muscle magnesium. In patients with steatorrhea and tetany associated with hypomagnesemia, hypocalcemia, and absence of demonstrable bone disease, the replenishment of magnesium results in a very rapid restoration of serum calcium towards normal. This rapid change suggests that secretion of parathyroid hormone had been insufficient during magnesium deficiency and that replenishment of magnesium in some way stimulates the parathyroid glands.

Regional enteritis — Patients with inflammatory bowel disease may develop symptomatic hypomagnesemia with symptoms and signs comparable to those induced in experimental animals placed on a diet deficient in magnesium. Although not a common complication, patients with this disease are uniquely disposed to the development of magnesium deficiency, either as a result of the underlying disease or as a consequence of therapy. The symptoms of hypomagnesemia are readily reversible. Unrecognized magnesium depletion may interfere with the complete restoration of deficits of other ions such as calcium and potassium.

Celiac disease — Hypomagnesemia may occur in this disease in which the fecal loss of magnesium is increased, renal excretion of magnesium is decreased, and sodium and potassium are retained in the body. All mineral balances are restored to normal when gluten is eliminated from the diet.

Ulcerative colitis — Patients with severe ulcerative colitis may develop hypomagnesemia along with subnormal levels of serum sodium, potassium, and chloride.

Acute pancreatitis — Magnesium apparently forms fatty soaps in the necrotic omental fat and causes hypomagnesemia.³²

E. Acute Alcoholism

The mean serum magnesium value in patients with delirium tremens in one study was 1.53 ± 0.27 meq/l. In alcoholics without delirium tremens, it was 1.89 ± 0.22 meq/l. In the control group of 157 nonalcoholics, the mean serum magnesium value was 1.84 ± 0.18 meq/l. There was a tendency for the lowest serum magnesium levels to coincide with the highest values for serum glutamic oxalacetic transaminase.³³ Hy-

pomagnesemia occurs frequently in patients with chronic alcoholism with and without delirium tremens. Patients exhibiting alcohol withdrawal signs and symptoms³⁴ have low serum and cerebrospinal fluid levels of magnesium, low exchangeable magnesium levels,^{35, 36} a lowered muscle content of magnesium,^{37, 38} and conservation of magnesium following i.v. loading.³⁹ A transient decrease in serum magnesium may occur during the withdrawal state even though prewithdrawal levels are normal. An ethanol-induced increase of magnesium in the urine occurs only when the blood alcohol level is rising. It does not persist once the subject has established high blood alcohol levels. However, in the presence of hypomagnesemia and delirium tremens, sudden death can occur as a result of cardiovascular collapse, infection, and hyperthermia.⁴⁰ The red cell concentration of magnesium is abnormally low in all patients with delirium tremens, whereas the plasma concentration is abnormally low in only 58% of them.⁴¹ Intracellular fluid levels of magnesium as reflected in the erythrocyte correlate better with clinical symptoms and signs than do extracellular fluid levels. The predominant factor accounting for magnesium depletion in acute alcoholism is most likely an inadequate intake of magnesium, but another factor may be increased excretion of magnesium in the urine and feces.^{42, 43}

Independent of the phenomena described above, an abrupt and significant fall in serum magnesium levels may occur following cessation of drinking. This acute fall of serum magnesium level is associated with a transient decrease in concentration of other serum electrolytes and with respiratory alkalosis⁴⁴ and coincides with the onset of neuromuscular hyperexcitability that characterizes the withdrawal state.³⁴ Hypomagnesemia appears to be directly related to the syndrome of alcoholic encephalopathy; adequate treatment with magnesium reverses the syndrome.⁴⁵ A kinetic analysis of radiomagnesium turnover was performed in a group of partially repleted alcoholic subjects. Despite the continued presence of hypomagnesemia and of decreased urinary excretion of magnesium, there was little evidence of continued depletion of magnesium in the extracellular space and in the tissue pools.⁴⁶

F. Cirrhosis

The magnesium content of the liver tissue per unit weight is decreased in cirrhosis.⁴⁷ This decrease appears to be due mainly to the substitution of parenchymal tissue of high magnesium content with connective tissue of low magnesium content. There is a good relationship between histological changes (extent of fibrosis and degree of infiltration of inflammatory cells) and decrease of the magnesium concentration per number of cells. The actual changes in the concentration of magnesium in the parenchymal cells of the cirrhotic liver appear to be negligible.

Patients with cirrhosis may have clinical features consistent with magnesium deficiency in the presence of a normal serum magnesium value but with a low skeletal muscle magnesium content and a normal bone and erythrocyte magnesium content.⁴⁸

G. Mastocytosis

A patient with the telangiectatic type of urticaria pigmentosa had clinical evidence of intestinal malabsorption and increased amounts of mast cells and histamine in the skin, stomach, and gut.⁴⁹ This patient had daily attacks of tetany; the serum magnesium concentration was 1.1 meq/l. There was an excessive fecal loss of calcium and magnesium.

Another patient with systemic mastocytosis had attacks of facial flushing, abdominal pain, tachycardia, and explosive diarrhea.⁵⁰ These clinical symptoms and signs occurred coincidentally with a decrease in serum magnesium concentration.

H. Diseases of the Parathyroid Gland

There have been references for years to abnormalities of magnesium metabolism in hyperparathyroidism. In clinical hypoparathyroidism, serum magnesium concentration tends to be lower than the average for normal individuals. Patients with primary hyperparathyroidism prior to treatment tend to develop a negative magnesium balance; this tendency appears to be related to the degree of hypercalcemia, the presence of advanced secondary renal disease, and inadequate intake of magnesium in the diet.⁵¹ These patients retain an abnormally large portion of an injected dose of magnesium. Individuals reverting from hyperparathyroidism to normal or to the hypoparathyroid state store magnesium. The probable causes of the hypomagnesemia are an increased excretion of magnesium due to impaired renal conservation and an impaired absorption of magnesium from the gut. Severe hypomagnesemia may result in an end-organ unresponsiveness to the physiological effects of parathyroid hormone.⁵² Parathyroid hormone responsiveness in hypomagnesemic patients may, at least in part, be dependent upon the adequacy of intracellular magnesium stores.

The effect of parathyroid hormone on magnesium metabolism is qualitatively similar to its influence on calcium metabolism but clearly less prominent. Significant and sustained hypomagnesemia after parathyroidectomy occurs only in patients with generalized bone disease. Its development appears to be dependent upon the inadequacy of the ordinary diet to meet the combined requirements for magnesium for formation of new soft tissue and for deposition of mineralizing bone.

There are reports of patients with an acute parathyroid crisis treated by surgical removal of a parathyroid adenoma who postoperatively developed evidence of magnesium deficiency; this cleared dramatically following the parenteral administration of magnesium.^{53, 54}

I. Burns

Patients with extensive burns may develop the magnesium-deficiency syndrome.⁵⁵ Serum magnesium may decrease significantly and clinical symptoms of magnesium deficiency may develop. Some of the psychiatric symptoms exhibited by many patients with burns may be due to or may be aggravated by magnesium deficiency.

J. Renal Disease

Hypomagnesemia has been observed in patients with glomerulonephritis, hydronephrosis, pyelonephritis, and renal tubular acidosis. Although this reduction is presumably due to a defective tubular reabsorptive mechanism, its occurrence is unpredictable. Frequent hemodialysis may result in severe muscle cramps due to acute hypomagnesemia if the dialysis fluid contains an inadequate amount of magnesium.⁵⁶

Hypomagnesemia due to renal disease of unknown etiology may be associated with tetanic convulsions, arthritic pain, hypermagnesuria, hypokalemia, hypercalciuria, progressive nephrocalcinosis, and chondrocalcinosis.⁵⁷ Children may have hypomagnesemia with carpopedal spasm and fail to thrive due to a renal tubular defect in the reabsorption of magnesium.⁵⁸

K. Cardiovascular Disorders

It was recognized but not widely appreciated as early as 1952 that magnesium deficiency occurs in congestive heart failure,⁵⁹ and that hypomagnesemia follows the administration of mercurhydrin.³²

Some patients with hypertension and others with primary aldosteronism have subnormal serum magnesium levels. The exchangeable magnesium content per kilogram of body weight is decreased in hypertensive men as compared to controls. These changes have not been found in hypertensive women.⁶⁰

The administration of hydrochlorothiazide to patients with hypertension results in a decrease in serum magnesium concentration; despite this, the erythrocyte magnesium concentration increases significantly. The change in diastolic pressure induced by hydrochlorothiazide correlates with the ratio of the difference of intracellular potassium to intracellular magnesium. These results suggest that changes in magnesium metabolism influence vascular tone and play an integral role in the control of blood pressure.⁶¹

Congestive heart failure — Although the serum magnesium levels in patients with congestive heart failure are similar to those in healthy individuals, the magnesium content of erythrocytes may be decreased in patients with longstanding failure. Also reduced are the magnesium concentrations in heart and skeletal muscles. There is a correlation between the intracellular deficiency of magnesium and that of potassium, yet the deficiency of potassium is greater.⁶²

Digitalis toxicity — Many patients who are receiving digitalis have a significantly lowered magnesium level which predisposes them to digitalis toxicity.⁶³ This toxicity can be terminated promptly by the administration of magnesium sulfate. Cardiac glycosides may induce magnesium deficiency associated with arrhythmias such as ventricular tachycardia and atrial or ventricular fibrillation.¹⁹ It is recommended that levels of serum magnesium as well as those of serum potassium be determined routinely in patients suspected of digitalis toxicity.

Paroxysmal ventricular fibrillation — Paroxysmal ventricular fibrillation, which is not associated with heart block, may occur in patients with hypomagnesemia.¹⁹

Liquid protein diet and sudden death — Sudden death in adults without serious underlying heart disease has emerged as a major complication of the liquid protein diet. Postmortem examination of the heart in a patient dying of such a condition revealed diffuse reduction in size of myofibers, fragmentation of fibers and lipofuscin deposition.⁶⁴ The presence of a prolonged QT_c interval and death from ventricular fibrillation, and the presence of hypokalemia and a borderline low serum magnesium concentration is superficially suspicious of a magnesium deficiency. High protein diets can be used in rats to produce severe magnesium deficiency.⁶⁵ Severe magnesium deficiency produces an obligate loss of potassium and potassium deficiency that cannot be repleted by potassium alone.⁶⁶ Also produced is cardiac myopathy characterized by myocardial cellular necrosis and focal acute and chronic inflammation.⁶⁷

L. Hypomagnesemia Associated with Drug Therapy

Prolonged treatment with certain drugs, such as gentamycin, can result in hypomagnesemia associated with hypocalcemia and hypokalemia.^{68, 69} In the case of gentamycin, the primary abnormality appears to be renal magnesium wasting, although its pathogenesis is unclear.

IV. MAGNESIUM DEFICIENCY IN CHILDHOOD

A. Primary Hypomagnesemia in Infancy

Attention has recently been drawn to a disease entity in infancy characterized by primary hypomagnesemia with secondary hypocalcemia, and apparently due to an isolated malabsorption of magnesium from the gastrointestinal tract.⁷⁰ In such an infant, an interruption of magnesium substitution leads to a drastic fall in magnesium in blood and to a rapid but less extensive hypocalcemia. The Ca to Mg ratio increases threefold. The intracellular concentrations of Mg and Ca are more stable and the Ca to Mg ratio remains unchanged. Magnesium depletion is followed by an excess of sodium in intracellular water, possibly due to Ca-induced inhibition of enzymatic steps in the "sodium

pump". The mechanism behind the secondary hypocalcemia, which is considered likely, is PTH unresponsiveness of bone cells due to the imbalance between Ca and Mg at the outer cell membrane. Magnesium depletion and a relative excess of calcium ions leads to a decreased stimulation of the membrane-bound adenylyl cyclase system.

A low urinary elimination of magnesium always corresponded to the hypomagnesemia. In such patients, intestinal absorption of both stable magnesium and radioactive ²⁸Mg was also reduced. ²⁸Mg exchangeable pool was decreased. Magnesium concentration in sweat was increased.⁷¹ An alteration of the apical part of the cytoplasm of epithelial cells of the intestinal mucosa was observed on electron microscopy. The endoplasmic reticulum was dilated and mitochondrial swelling was observed. The brush border, however, was normal.

B. Neonatal Magnesium Deficiency

Tsang has recently reviewed this subject.⁷² Alterations in the physiology of the gastrointestinal tract by surgical procedures, such as resection or creation of short circuits and fistulae, can result in neonatal magnesium deficiency.

Convulsions and tremors have been reported recently in children with hypomagnesemia but without associated hypocalcemia. Upon recovery, either spontaneously or after administration of magnesium, the serum magnesium concentration returns to normal levels. Hypomagnesemic convulsions have been reported in well-nourished infants in association with gastroenteritis, in a newborn infant from a hypophosphatemic mother, and in association with maternal hyperparathyroidism.

C. Sudden Infant Death Syndrome (Crib Death)

A very prevalent and puzzling tragedy is the discovery of a presumably previously healthy infant dead in its crib. Caddell has advanced the hypothesis that sudden unexpected death in infancy is a preventable condition which results from the magnesium-deprivation syndrome of growth.⁷³ She tested this hypothesis in a magnesium-deficient weanling rat model.⁷⁴

D. Kwashiorkor and Protein-Calorie Malnutrition (PCM)

Protein-calorie malnutrition is the major nutritional deficiency syndrome of the world. Magnesium supplementation improves the rate and extent of recovery from severe PCM⁷⁵.

A characteristic feature of diets which give rise to kwashiorkor is often the complete lack of milk. A pint of milk contains 6 to 7 meq of magnesium; a satisfactory curative diet for kwashiorkor contains 13 meq, of which 11 are derived from milk. A kwashiorkor-producing diet contains about one half of the magnesium in a curative diet.

The first controlled clinical studies of the relationship between magnesium deficiency and PCM were reported from Nigeria by Caddell.⁷⁵ Children with PCM have several clinical signs or symptoms compatible with magnesium deficiency. These include weakness, emaciation, anorexia, sleeplessness, trophic changes, and hyperirritability.

It is now well documented that magnesium deficiency occurs in PCM. This is evidenced by the low magnesium content of muscle, by a very low level of urinary magnesium, and by a very prolonged positive balance of magnesium during recovery. This positive balance is much greater than could be accounted for by the corresponding retention of nitrogen. The magnesium content of the serum and red blood cells could be within normal range. There is impaired GI absorption of magnesium in kwashiorkor, as well as a loss of tissue potassium, a retention of sodium, and osteoporosis. Children with kwashiorkor had an increased concentration of sodium and calcium and a decreased concentration of magnesium in the nails when compared with a control group.

Although the replacement of the magnesium deficit was not essential for recovery from PCM in Guatemalan children, the rate of recovery was accelerated by approximately 2 weeks in those children who received a supplement of magnesium sulfate.⁷⁶

E. Familial Disorder of Magnesium Metabolism

Since 1966 there have appeared reports in the literature of chronic congenital hypomagnesemia which seem to indicate it is a familial disorder with autosomal recessive transmission. Such patients may have repeated episodes of hypomagnesemia, symptoms of intermittent weakness and abnormal personality behavior, and a history of dermatographism and bronchial asthma.

There have been several other recent reports of a familial hypomagnesemia. In a Scandinavian report, two brothers at the ages of 15 and 23 days developed repeated tetanic convulsions.⁷⁷ The tetany, hypomagnesemia, hypocalcemia, and hypophosphatemia all subsided when magnesium was given as the only form of therapy. Histologic examination by electron microscopy revealed hepatic cellular necrosis, dilatation of the endoplasmic reticulum, and mitochondrial swelling. Net intestinal absorption of magnesium as measured by ²⁸Mg was abnormally low, whereas its renal handling appeared normal; no definite signs of generalized malabsorption were found. Therefore, the defect appeared to be one related specifically to the intestinal absorption of magnesium.

V. MAGNESIUM DEFICIENCY IN PREGNANCY

There may exist a subclinical deficiency of magnesium even in normal pregnancy. In 87 normal subjects, the average value for serum magnesium was 1.6 ± 0.14 meq/l. The value for erythrocytes in 56 specimens was 5.2 ± 0.81 meq/l. For normal pregnant women in their third trimester, the mean value for 105 serum specimens was 1.41 ± 0.04 meq/l. The mean value for 104 erythrocyte specimens was 4.35 ± 0.41 meq/l. These respective differences between the two groups are statistically significant.⁷⁸

Magnesium deficiency may be the cause of the spasmophilic gravid uterus. Administration of magnesium reduced uterine hyperexcitability, insomnia, anxiety, and asthenia. In 24 of 80 observations, the serum magnesium level was less than 1.48 meq/l.⁷⁹

Excessive lactation may result in tetany attributable to hypomagnesemia. In one case, the i.v. administration of 12.5 meq of magnesium resulted in the retention of some 78% of the administered dose, indicating the prior existence of a depletion of this ion.⁸⁰ Studies are in progress to determine by means of a parenteral magnesium load in postpartum women the nutritional status for this element.⁸¹

VI. DIAGNOSIS

In many patients, the clinical symptoms and signs, although nonspecific, accompanied by a low serum magnesium concentration, confirm the diagnosis. However, a normal serum magnesium level does not exclude magnesium deficiency.

Since serum magnesium is regulated largely by renal control of urinary magnesium excretion, urinary output of the element has been used as an index of magnesium deficiency.⁸² Caddell has described a magnesium load test in infants up to 6 months of age.⁸³ A 56-hr test measured cation and creatinine excretion before and after an intramuscular load of 0.49 meq Mg/kg of body weight. This approach has been ex-

tended to postpartum American women.⁸¹ An intravenous test dose of 0.4 to 0.5 meq Mg/kg of estimated lean body weight was administered and the net retention calculated. Thorèn has used an i.v. dose of 0.25 mmol of Mg/kg of body weight; more than 80% of this dose should be excreted in the urine within 24 hr if tissue reserves are adequate.²³

VII. THERAPY

In patients with the clinical symptoms and signs of magnesium deficiency, the deficit of magnesium is on the order of 1 to 2 meq/kg of body weight. Since less than one half of the administered magnesium is usually retained in the body, the required therapeutic dose is 2 to 4 meq/kg which can be administered parenterally over a 4-day period. In order to administer the dose safely, one should first determine the adequacy of renal function, then monitor the plasma level of magnesium during therapy.⁸⁴

Flink recommends an initial loading dose of 49 meq (6.0 g of MgSO₄ in 1000 ml of solution containing 5% glucose) given i.v. over a period of 3 hr, followed by additional doses of 49 meq every 12 hr.⁸⁴

Another suggested regimen is the i.v. administration of 98 meq on the first day (2.0 g [16.3 meq] every 2 hr for three doses and then every 4 hr for four doses), followed by 33 to 49 meq/day in divided doses for 4 days.

For the treatment of arrhythmia, Iseri et al. administered 10 to 15 ml of a 20% magnesium sulfate solution, given i.v. over 1 min, followed by a slow 4- to 6-hr infusion of 500 ml of a 2% magnesium sulfate in 5% dextrose water.¹⁹ A second infusion of magnesium sulfate may be necessary should the arrhythmia recur.

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Chapter 10

THE RELATIONSHIP OF MAGNESIUM TO HUMAN DISEASES

I. THE RELATIONSHIP OF GEOCHEMISTRY TO HUMAN DISEASE

Various geochemical factors which might influence the availability of magnesium for human consumption and thereby influence health have been discussed in Chapter 7. In this section evidences will be reviewed suggesting an inverse relationship of magnesium in drinking water to cerebrovascular and cardiovascular diseases. Cardiovascular diseases, including hypertension and coronary artery disease (leading to sudden death), are major causes world-wide of both morbidity and mortality and are of unknown etiology. Such being the case, it is intriguing to hypothesize that magnesium might be causally related.

A. The “Drinking Water” Story

Investigations into the potential effects on health of the geochemical environment appear to have begun in the late 1950s and in the early 1960s independently in several areas of the world. At about the time that the studies by Shacklette et al.¹ were being conducted in Georgia, Takahashi hypothesized that deficiencies or excesses in the content or availability of trace elements in rocks and soils, or in water flowing through them, may be a possible cause of certain chronic diseases — cerebrovascular and cardiovascular diseases.² Kobayashi was apparently the first investigator to suggest an inverse relationship between the incidence of strokes and the hardness of drinking water.³ This observation more generally in relation to cardiovascular diseases was confirmed by Schroeder and others in the U.S. and Canada⁴⁻⁸ and in England.⁹ Magnesium ions are one of the two main components of what has commonly been called “hardness” in water. “Hardness” refers to the number of polyvalent ions in water; quantitatively, the two chief ions are calcium and magnesium.

With several exceptions, studies in eight countries have shown that death rates from cardiovascular diseases are usually higher in areas served by soft water than in areas served by hard water.^{10,11} In Europe, the mean national death rate increases with increasing age of surface rocks and underlying strata in each country or groups of countries.

The hardness of water is due to magnesium and calcium salts. The factors in hard water which are responsible for the protective effect are not known, although there is evidence that the crucial one is the magnesium content of the drinking water.¹²

In 1969, the World Health Organization (WHO) in collaboration with the International Atomic Energy Agency (IAEA) began a multidisciplinary research program on trace elements in relation to cardiovascular diseases. Of the many studies concerning the water to cardiovascular disease relation, only three, coordinated by WHO, continue in depth: one in England,^{13, 14} one in Finland,¹⁵ and one in Canada.¹¹ WHO is also carrying out some other more recent studies in New Guinea and in five European cities.¹⁶

B. The English Study

Glasgow has an extremely soft water supply and the highest death rate for cardiovascular disease in Britain, whereas greater London has extremely hard drinking water and a much lower mortality rate from such disease. In a study of postmortem specimens from these two areas, more myocardial scars were found from the Glasgow area

than from the London area, and more cases of atheroma and of luminal stenosis in those aged 30 to 44 years. In cases of accidental death, there were more men from Glasgow less than 40 years old with extremely low concentrations of magnesium and calcium in the coronary arteries than from the London area.¹⁷

Sixty-one towns of comparable size were ranked according to the hardness of their water supplies. Death rates from cardiovascular diseases in the six towns with the hardest water proved to be lower than those in the six towns with the softest water. Moreover, the populations of the hard-water towns also generally were found to have relatively lower blood pressures, lower heart rates, and lower blood-cholesterol levels. Socioeconomic, dietary, and environmental factors appeared to have no influence on these associations.

Chipperfield and Chipperfield found that myocardial magnesium concentrations were lower in subjects dying of coronary thrombosis or myocardial degeneration than in subjects dying from other noncardiac causes: $173 \pm 36 \mu\text{g/g}$ and $205 \pm 33 \mu\text{g/g}$ wet weight, respectively.¹⁸ There was no difference in the myocardial concentration of zinc or of potassium. Myocardial enzyme activation requires magnesium and a deficiency of this element is associated with an increased liability to arrhythmia. Treatment with magnesium has been shown to improve survival after myocardial infarction.

Both Tolley¹⁹ and Dean²⁰ have suggested that there is one group of individuals who might merit close study for an answer to the "water story" — those who have been taking Epsom salt in one form or another regularly over a period of years, and particularly, those people in soft-water areas.

C. The Finnish Study

Behr and Burton²¹ reported a confirmation of the results reported by Chipperfield and Chipperfield.¹⁸ Heart muscle magnesium was depleted in 18 cases of acute coronary thrombosis causing sudden death in the first attack, as compared with the myocardial magnesium concentration in those who had had long-standing coronary heart disease or who died of other causes. There was no significant difference in magnesium concentrations in skeletal muscle among the three groups.

Karppanen and Neuvonen reported that soil concentrations of magnesium are three times higher in the southwestern part of Finland than in the eastern part, where coronary heart disease mortality is twice as high, and that in rural areas the magnesium intake would broadly reflect the magnesium content of the local soil.²² These investigators, too, stressed that magnesium deficiency could be involved in the development of arrhythmias and sudden death.

D. The Canadian Study

In Canada, general mortality, as well as cardiovascular mortality, was found to be higher in the soft-water areas.

Anderson and colleagues in Canada compared coronary deaths during a 12-month period in three areas of Ontario differing in water hardness.²³ Whereas death rates from other causes showed little variation, the standardized coronary death rate declined with water hardness. Anderson and le Riche subsequently showed that sudden coronary deaths were 20 to 30% higher in a soft-water city than in a hard-water city.²⁴ This observation has been confirmed by Peterson et al. in a study in the state of Washington.²⁵ Anderson and co-workers subsequently studied muscle specimens from persons dying from acute coronary heart disease, accidents, or other causes, some from a hard-water group of cities and some from a soft-water group.²⁶ They found that: (1) magnesium was the only element with a significantly lower myocardial concentration in the accident (healthy) cases from the soft-water groups (93% of that in the hard-

water accident cases); (2) myocardial, diaphragmatic, and skeletal muscle concentrations of magnesium were lower in soft-water coronary cases than in hard-water coronary cases; (3) comparing *all* the coronary cases and *all* the accident cases, there were significantly lower concentrations of magnesium in the myocardium than in diaphragmatic muscle among the former. Alcohol abuse and diuretic usage as possible causes of the differences in myocardial magnesium concentration were ruled out among the accident cases in the soft- and hard-water areas.

Shaper pointed out two interesting facts: first, hypertension is common in soft-water areas, and secondly, so is sudden death.²⁷ El Shahawy emphasized that chronic magnesium deficiency may be as important as potassium depletion in abnormal myocardial function and that such a deficiency may result in cardiovascular lesions, and lead to disorders of rhythm.²⁸

E. Experimental Studies in Rabbits

The Neals have shown that the magnesium present in "hard" drinking water can have a significant effect in the prevention of atherosclerotic lesions in rabbits, whose blood serum contained 2% cholesterol; corroborative studies revealed a marked protective effect of magnesium, but only a slight effect of calcium.²⁹

F. Prevention of Cardiovascular Diseases

The important role of magnesium in the prevention of cardiac disease has been emphasized by Durlach.³⁰

The WHO study in five European cities includes a standardized assessment of the rates of occurrence of, and extent of damage from, atherosclerosis, hypertension, and myocardial infarction in autopsy cases from Prague, Czechoslovakia, Malmö, Sweden, and Ryazan, Fallin, and Yalta, U.S.S.R.¹⁶ Preliminary results indicate an inverse relation between water hardness and extent of cardiovascular damage.

The WHO study in New Guinea confirms the findings in England and Finland that higher blood pressure is associated with areas having softer water than in those with harder water.¹⁰

II. THE RELEVANCE OF DIETARY MAGNESIUM TO GEOCHEMISTRY AND HUMAN DISEASE

Although, as discussed in Chapter 8, the U.S. National Research Council recommends a daily magnesium allowance of 25 to 42 meq (300 to 500 mg), in fact, the usual food intake may not satisfy this requirement in the U.S. and Europe. The reason is the loss of magnesium during the processing of food staples.³¹ For instance, 80% of the magnesium is lost in refining flour from wheat; 83% in polishing rice; 99% in extracting white sugar from molasses; and 97% from producing starch from corn.

A dietary intake of 17 meq (200 mg) of magnesium daily should be considered minimal.³² In a recent study of magnesium intakes of university students, magnesium content was analyzed in the three main meals served in a university cafeteria. The average magnesium content of the meal was 200 to 270 mg/day, a quantity lower than the recommended dietary allowance of Canada and the U.S.³³ In a survey of the serum magnesium status of 32 random subjects, aged 40 to 60, over 50% of the magnesium values were below the defined normal range, while none was above. Highly significant (inverse) correlations were discovered between magnesium and cholesterol, i.e., $r = -0.87$.³⁴ Thus, magnesium deficiency may exist among populations in which cardiovascular disease is endemic, and may also be associated with low potable water magnesium.

Hard waters can contribute from 3.3 to 8.0 meq (39 to 96 mg) or more of magnesium daily. Therefore, water-borne magnesium can make the difference between inadequacy and sufficiency.³⁵ Marier³⁶ has recommended daily magnesium supplementation of 300 mg and Simpson³⁷ has discussed the possibility of magnesium supplementation in drinking water.

Aleksandrowicz has pointed out that it is not accidental that the two diseases — infarcts and lymphatic leukemia — in civilized countries correspond with magnesium deficiency in such people and in the soil, water, and vegetation. He states that traditionally utilized fertilizers contain NPK ions, which are responsible for diminishing magnesium, iron, and copper horizons. It seems that this is the essence of disruptions in the equilibrium in biocoenoses as a result of man's activities.³⁸

III. MAGNESIUM AND THE CARDIOVASCULAR SYSTEM

Heart disease is the most common cause of death today in the U.S. and in Northern Europe.³⁹ Nearly one half of these deaths occur suddenly and are considered to be associated with cardiac arrhythmias.⁴⁰ A greater proportion of sudden deaths due to ischemic heart disease has been noted in cities with soft water, relatively lacking in magnesium and calcium.^{23, 41} A deficiency of potassium and/or magnesium may increase myocardial irritability and result in sudden death.

Magnesium deficiency may also be a factor in some deaths associated with mechanical failure of the heart, since magnesium is essential for oxidative phosphorylation, and necessary for the replenishment of adenosine triphosphate to maintain strength of muscular contraction. Seelig⁴² and Seelig and Heggtveit⁴³ have recently reviewed the evidences for involvement of magnesium in heart disease in animals; Burch and Giles, its role in human cardiovascular disease.⁴⁴

Although the effect of magnesium on the cardiovascular system has been studied since 1900, these studies were hampered until recently by the lack of a simple and precise method for measuring magnesium.⁴⁵ The primary cardiovascular effects of magnesium involve myocardial conduction and contraction as well as regulation of blood pressure. Magnesium sulfate administered i.v. causes a prolongation of the P-R interval as the serum magnesium concentration increases. With continued administration, intraventricular conduction is prolonged. In general, the electrocardiographic manifestations of hypermagnesemia are similar to those of hyperkalemia, while the changes of hypomagnesemia are similar to those of hypokalemia.

Clinically, magnesium has been found to have a vasodilator effect in hypertensive patients. It also increases cardiac output in all patients.^{39, 46} It has been used in the stabilization of cardiac rhythmicity.⁴⁷

A. Hypomagnesemia in Cardiac Disorders

The antiarrhythmic effect of magnesium sulfate in digitalis-induced arrhythmias has been known for many years.⁴⁸ Hypomagnesemia facilitates the development of digitalis toxicity.⁴⁹ Intravenous magnesium sulfate has been used successfully in the treatment of several arrhythmias including paroxysmal atrial tachycardia, ventricular tachycardia, and digitalis toxicity. The precise mechanism of the digitalis-induced arrhythmia remains to be determined, although there is some evidence that the digitalis-induced loss of myocardial potassium may be a contributing factor.

Iseri and co-workers stressed the influence of the magnesium ion upon the activity of the heart.⁵⁰ Although the exact mechanism is still unclear, one theory concerns the action of magnesium upon the Na-K-ATPase. According to this theory, a magnesium

deficiency would lead to a cellular loss of potassium and eventually to a lowering of the intracellular concentration of potassium in the face of a normal serum potassium level. Since the resting membrane potential is largely dependent upon the ratio between the intracellular and extracellular potassium, a lowering of only one of these parameters would change the resting membrane potential. A lowering of the intracellular potassium would lead to a less negative resting membrane potential, which would make the cell more easily excitable.

In a recent study by Dyckner and Wester in patients on diuretic therapy, after magnesium infusion there was a significant decrease in the frequency of ventricular ectopic beats.⁵¹ This decrease paralleled the increase in muscle potassium content which was measured after magnesium infusion. On the other hand, the infusion of potassium did not result in any increase in cellular potassium content, neither did it influence the frequency of the ventricular ectopic beats.

B. ^{28}Mg Studies in Patients with Coronary Heart Disease

Boddy et al. recently studied the oral absorption of magnesium, its myocardial uptake, and exchangeable magnesium in patients with coronary heart disease and controls using the radioactive isotopic ^{28}Mg .⁵² This study was performed in the west of Scotland, a soft-water area with a high incidence of coronary heart disease. No significant difference was found in oral absorption or myocardial uptake between patients and controls. However, the exchangeable magnesium per kilogram body weight was significantly lower by about 20% in the patients than in the controls, with mean values at 24 hr of 2.34 ± 0.10 (SEM) in the patients and 2.91 ± 0.18 in controls.

It is important to determine both serum magnesium and potassium in all cases of digitalis toxicity, since diuretic therapy can result in hypomagnesemia as well as hypokalemia. If hypomagnesemia is present, Seller recommends that 7 to 15 ml of 25% magnesium sulfate be administered slowly i.v. under electrocardiographic monitoring.⁴⁹

C. Magnesium and the Electrocardiogram

Early electrocardiographic signs of magnesium deficiency showed slightly widened QRS complexes, peaked T waves, and ST segment depression. As the magnesium deficiency becomes more severe the QRS complex widens and conduction disturbances and arrhythmias occur.^{53, 54}

1. Magnesium and the QT_c Interval

QT_c is the QT complex, which is the interval measured from the beginning of the QRS complex to the end of the T wave. It represents the total duration of ventricular systole, i.e., the combined phases of depolarization and repolarization. QT intervals are clinically important. The QT_c interval usually does not exceed 0.42 sec in men and 0.43 sec in women. A delayed QT_c interval results from delayed repolarization of the ventricular myocardium. It is during this period that the patient is highly vulnerable to cardiac arrhythmias with myocardial infarction and death. During this period, endogenous or exogenous stimulation can provoke ventricular flutter or fibrillation. Sudden death with a long QT interval is common.

Davis and Ziady gave 4 to 6 tablets, each containing 0.5 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, at night to 25 randomly selected patients for periods varying from 6 weeks to 2 years.⁵⁵ Each patient served as his own control. A statistically significant shortening of both the QT_c and the QU_c occurred after administration of Mg^{2+} . With continuing therapy, both QT_c and QU_c intervals shortened progressively.

D. Electrophysiological Studies

Electrophysiological interactions of magnesium and potassium ions were studied in isolated rabbit heart preparations. A threefold increase in extracellular magnesium concentration, in the presence of either high (7.5 mM), normal (4.5 mM), or low (1.5 mM) potassium concentration, tended to increase the amplitude of the action potential, the resting membrane potential, the maximal rate of depolarization, and the duration of the action potential of the ventricular muscle fibers, and prolong the A-V conduction time. A reduction of magnesium concentration to one third produced opposite results. In the presence of high magnesium concentration, the effective refractory time was prolonged more than the duration of the action potential. This resulted in a better propagation of the earliest premature response. Low magnesium concentration shortened the effective refractory time, but tended to prolong the relative refractory period, and, thus increased ventricular vulnerability.⁵⁶

E. The Influence of Thiazide Diuretics on Cation Balance

The benzothiadiazide diuretics cause diuresis and magnesium and potassium wasting in normal and hypertensive human subjects.⁵⁷ Decreased serum magnesium levels are associated with chronic diuretic therapy.^{58,59} The addition of oral magnesium replacement during diuretic therapy had no effect on any measured values beyond those seen in subjects who took diuretics without magnesium replacement. Thus, magnesium replacement did not reduce urinary potassium loss. However, urinary calcium losses increased when magnesium was given to subjects who were not receiving diuretics. Small doses of oral magnesium enhanced calcium excretion in hypertensive subjects.

F. Magnesium in Heart Muscle

There is a paucity of information concerning this subject.⁶⁰ However, additional knowledge is needed, since recent experiments on skeletal muscle indicate that Mg²⁺ may be a critical modulator of the tension with which the contractile apparatus of striated muscle responds to the prevailing ionized calcium concentration.⁶¹

Content and intracellular concentration of magnesium — Watchorn and McCance many years ago noted the high magnesium content of mammalian ventricular muscle.⁶² The cellular magnesium content of rat ventricular muscle is 43.4 ± 0.4 mmol/kg dry weight.⁶³ The calculated intracellular magnesium concentration is 17.3 ± 0.2 mmol/kg cell water, a value roughly comparable to the 16.1 mM for rat skeletal muscle.⁶⁴ Human subjects dying suddenly with ischemic heart disease had significantly lower levels of myocardial tissue magnesium and potassium than controls (men dying of acute trauma). The four lowest potassium values were obtained for the only men in the group with a history of angina. Three of the men with angina also had the lowest tissue magnesium levels.⁶⁵ This finding supports the hypothesis that an inapparent deficiency of one or both of these elements might contribute to sudden cardiac death in a susceptible person.

Intracellular distribution — Surprisingly little is known about the intracellular distribution of magnesium among the various cellular subcompartments and organelles. An analysis of subcellular fractions isolated by fractionation after homogenization of rat ventricular muscle indicates that less than 15% of the cellular magnesium is associated with the mitochondria or myofibrils.⁶⁶ The efflux of Mg²⁺ ions from heart mitochondria appears to be respiration-dependent and may require phosphate.⁶⁷ Of the remaining 85% a major fraction is presumably present as Mg-complexes of ATP, ADP, and AMP. On a priori grounds it seems reasonable that most of the adenine nucleotides (and hence most of the magnesium) in myocardial cells should be associated with the myofibrils, whose total energy consumption greatly exceeds that of other cellular components.

Intracellular functions of magnesium — It is probably significant for the cellular physiology of heart muscle that many Mg-dependent processes and reactions in heart muscle (such as force development by and relaxation of myofibrils, release of calcium from sarcotubules, and mitochondrial ATPase related to oxidative phosphorylation) proceed rapidly when Mg^{2+} is in the range of 1 mM or less. Polimeni and Page stressed the dependence of myofibrillar contraction and relaxation on (Mg ATP) and (Mg^{2+}) and the dependence of the uptake and the release of calcium from the sarcotubules on (Mg^{2+}).⁶⁰

Magnesium transport in heart muscle — The plasma membrane of rat left ventricle myocardial cells is the locus of a transport mechanism by which about 98% of cellular magnesium exchanges at a very slow single rate both *in vivo* and *in vitro*.⁶⁸ At 37°C and at the near-physiological extracellular magnesium concentration of 0.56 mM, the rate was 0.15 ± 0.02 mmol Mg exchanged per min/kg dry left ventricle or about 0.21 ± 0.02 pmol exchanged per sec per cm^2 of plasma membrane. Since the electrochemical gradient would favor the diffusion of magnesium into the cell, a carrier-mediated transport of magnesium out of the cell is postulated to prevent a physiologically inappropriate rise in (Mg^{2+}) within the cell.

Accumulation or loss of magnesium by myocardial cells — *In vivo*, myocardial cells accumulate magnesium in response to stimuli which cause the cells to grow or hypertrophy.⁶⁹ A net loss of magnesium by myocardial cells can occur under many pathologic conditions. In evaluating a reduced total magnesium content in a sample of heart muscle, it is important to distinguish between a reduction in the total number of myocardial cells because of focal processes as contrasted with a diffuse loss of magnesium because of a process affecting all cells. One such diffuse process is the net loss of magnesium associated with oxygen deficiency.⁷⁰

G. Energy Source for the Myocardium

Myocardial cells are provided with energy largely due to the processes of aerobic oxidation of fatty acids and glucose and the synthesis of ATP in mitochondria.^{71, 72} The mechanism of oxidative phosphorylation and the transport of adenine nucleotides across the inner mitochondrial membranes have been extensively studied.⁷³ Further steps in the transport of energy from mitochondria to the active sites of its utilization on myofibrils are not well understood.

Heart mitochondria contain the specific mitochondrial isoenzyme of creatine phosphokinase which accounts for 30% of total cellular creatine phosphokinase activity.⁷⁴ It has been suggested that this isoenzyme plays an important role in intracellular energy transfer from ATP of the intermembrane space of mitochondria to cytoplasmic creatine with the formation of creatine phosphate. Energy transport via creatine phosphate can only occur if ATP synthesized by oxidative phosphorylation under conditions characteristic for the intracellular medium (the presence of ATP, ADP, and relatively high concentrations of creatine phosphate and creatine) is effectively transformed into creatine phosphate in mitochondria.

The regulatory role of magnesium — Mg^{2+} ions play a regulatory role in the creatine phosphokinase reaction. In the presence of all substrates and products of the reaction the ratio of the rates of forward and reverse reactions can be effectively regulated by the concentrations of Mg^{2+} ions. At limited Mg^{2+} concentrations creatine phosphate is preferentially synthesized while at high Mg^{2+} concentrations ATP synthesis takes place.⁷⁵

H. Alcoholic Cardiomyopathy

Chronic ingestion of alcoholic beverages is associated with hypomagnesemia and

decreased skeletal muscle potassium.⁷⁶ Since about 20% of the body magnesium resides in skeletal muscles, a deficiency of magnesium in this tissue indicates a significant intracellular deficit throughout the body, and including the heart.⁷⁷ There is a linear relationship between retention of infused magnesium and the increment of increase in skeletal muscle magnesium.

Alcohol may increase the renal excretion of magnesium by a direct effect on tubular reabsorption or by increasing production of some metabolic intermediates which could bind magnesium ions as they are excreted.⁷⁸ It is of interest that a high rate of urinary excretion of magnesium continues in alcoholics during alcohol withdrawal despite low serum magnesium levels.⁷⁹

Arrhythmia — Serious arrhythmias occur fairly often in the alcoholic withdrawal syndrome.⁸⁰ The average positive magnesium balance during recovery from the alcohol withdrawal syndrome was 1.0 meq/kg; there was a significant decrease in muscle magnesium, potassium, and phosphate concentrations.⁸¹

I. Myocardial Disease in Experimentally Induced Magnesium Deficiency

Greenberg et al.⁸² in 1936 reported myocardial degeneration with fibrosis and polyblastic infiltration in rats fed a low magnesium diet from birth. The same group subsequently reported in detail the basic histologic lesions of magnesium deficiency in the rat heart.⁸³ There were distinct inflammatory and necrotic foci around small blood vessels. In the acute stage, the lesions were characterized by a collection of inflammatory cells, associated with some areas progressing to necrosis and subsequent scar formation.

Heggelviet, Herman, and Mishra reported both light and electron microscopic findings.⁸⁴ After 14 days of magnesium depletion, half of the experimental rats showed gross myocardial lesions ranging from small, pale yellowish-gray patches to large zones of necrosis and calcification. Light microscopy revealed focal areas of necrosis and exudative inflammation in most magnesium-deficient rats after 10 days, particularly in subendocardial regions. Muscle fibers adjacent to areas of necrosis showed increased sarcoplasmic eosinophilia, patchy loss of cross-striations, vacuolization, and accumulation of periodic acid-Schiff (PAS)-positive material. Calcification in the granulomatous lesions was seen in approximately one half of the animals. There was progression of the lesions to scarring.

The low-magnesium diet did not affect the activity of ATPases and cytochrome oxidase. The areas of myocardial degeneration and necrosis were free of the activity of alkaline phosphatase and succinic dehydrogenase.⁸⁵

Electron microscopic evidence of myocardial damage was seen as early as 5 days after starting on a magnesium-deficient diet. The mitochondria exhibited the earliest changes, consisting of swelling and vacuolization, compression and distortion of the cristae, and accumulation of an unidentified material on or between the cristae. The myofibrillae were deranged and fragmented and were separated by accumulation of intracellular fluid. Nuclear changes were noted, consisting of marginal clumping of chromatin, loss of nucleoli, and vesiculations. In addition edema of the vascular endothelium was noted.

The sequence of structural abnormalities suggested that interference with magnesium-dependent enzymes involved in oxidative phosphorylation played an important role in the pathogenesis of the lesions observed.

There are reports of similar studies in puppies with similar results.^{86, 87} Moore et al. reported focal cardiac necrosis and calcification in calves fed a magnesium-deficient diet.⁸⁸ Swelling of myofibrils and degeneration of Purkinje fibers was noted.

J. Regulation of Vascular Tone and Reactivity

The role of magnesium in the physiology and biochemistry of the heart and the blood vessels is discussed in this section

1. Early Studies

It has been recognized for many years that the concentration of extracellular magnesium can affect blood flow, blood pressure, and vascular reactivity in intact mammals. Hazard and Wurmser in 1932 first demonstrated that elevations in serum magnesium can produce profound and rapid vasodilatation and hypotension.⁸⁹ Conversely, acute hypomagnesemia in man and animals is often associated with elevations in blood pressure and peripheral vascular resistance.⁹⁰ Interest in magnesium increased during World War II because of the observation that individuals with severe trauma had elevated serum magnesium levels. Beecher et al. in 1947 reported that increased serum magnesium levels appeared to correlate with the severity and the duration of circulatory shock and trauma.⁹¹

2. Recent Studies

Acute hypomagnesemia in animals and man is often associated with rises in blood pressure and elevations in peripheral vascular resistance in several regional beds. Altura and Altura studied the relationship between the extracellular magnesium and calcium concentrations and vascular tone and responsiveness, using a variety of rat and rabbit blood vessels which were observed directly with a closed circuit television-microscope recording system.⁹⁰ Extracellular magnesium concentration was found to differentially affect a variety of hormone- and drug-induced contractions in isolated rat and rabbit arteries as well as isolated perfused rat mesenteric arterioles. Progressive elevations in extracellular magnesium concentration above physiologic levels dose-dependently produce progressive, increased inhibition of most contractile substances, such as acetylcholine and angiotensin; these results were not due to changes in osmolarity. The inhibitory effects of elevated extracellular magnesium were attributed to a block in calcium influx at the membrane and to membrane stabilization. Withdrawal of extracellular magnesium induced contractions of rat arteries as well as of isolated perfused rat mesenteric arterioles.

In isolated rat portal veins, withdrawal of extracellular magnesium resulted in rapid enhancement of the spontaneously evoked mechanical responses and increases in rhythmic contractility. The spontaneously evoked mechanical responses were abolished in hypermagnesemic solutions.

An elevation of the extracellular magnesium to as little as 2.4 mM inhibited the spontaneous mechanical activity seen in isolated rat aorta; raising the extracellular magnesium concentration to 6 mM not only completely inhibited all prior spontaneous mechanical events but, in addition, lowered baseline tension.⁹² The contractile responses observed upon withdrawal of extracellular magnesium were dependent upon the extracellular calcium concentration and the polarity of the membrane; they, however, were not related to inhibition of Na⁺, K⁺-ATPase.⁹³ With rat aortic strips, addition of CaEDTA (which has affinity for Mg²⁺) potentiated contractions induced by withdrawal of Mg²⁺, while EGTA (which selectively chelates Ca²⁺) promoted rapid relaxation.

The data obtained suggested that extracellular magnesium played an important role in regulating membrane permeability to extracellular calcium, that extracellular magnesium probably occupies sites which are exchangeable with membrane-bound calcium in arterial, venous, and arteriolar smooth muscle, and that it acts intracellularly as well to compete with calcium for certain divalent cation sites.^{94, 95} The fact that the ionic radius of magnesium (0.78 Å) is smaller than that of calcium (1.06 Å) may facil-

itate this competition. Altura and Altura suggested that magnesium might play an important role in regulating arteriolar tone and blood pressure.⁹²

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Chapter 11

MAGNESIUM EXCESS

I. HYPERMAGNESEMIA

A. Introduction

The most common cause of hypermagnesemia is impaired renal function.¹ When the inulin clearance is less than 10 ml/min, the increase in magnesium excretion per nephron is insufficient to maintain a normal serum magnesium level.² The other commonly associated finding is the use of magnesium-containing medications. Quite often it is iatrogenic, associated with purgation, antacid therapy, replacement in deficiency states, and with treatment of neonatal tetany, hyperuricemia and hyperlipidemia, lithium toxicity, hyperthyroidism, pancreatitis, hepatitis, phlebitis, coronary artery disease, arrhythmia, and digitalis intoxication. An unusual iatrogenic cause of hypermagnesemia is due to the use of magnesium-containing urologic irrigation solutions for dissolution of urinary calculi.³ In pediatrics, it is well documented that symptomatic hypermagnesemia is encountered in neonates delivered from eclamptic mothers treated with excessive doses of magnesium sulfate.^{4,5} Because of the severe dehydration, one may encounter hypermagnesemia in patients with cholera in the presence of magnesium depletion.⁶ Since the subject has recently been reviewed in depth,⁷ only a brief summary is presented here.

B. Clinical Recognition

Elevations in serum magnesium levels above 4 meq/l are usually associated with symptoms. Hypotension occurs at concentrations of 3 to 5 meq/l. Above 5 meq/l, the individual feels drowsy and hot and appears flushed. At levels of 7 to 10 meq/l, there is depression of deep tendon reflexes, weakness, atonia, and slurred speech. There is nausea, followed by bradycardia and nonspecific alterations in the electrocardiogram, with lengthening of the P-R interval. Oliguria appears. With higher serum magnesium concentrations, the hyporeflexia progresses to areflexia which precedes respiratory paralysis. This is why great attention must be paid to testing the deep tendon reflexes during magnesium therapy. If the ventilatory insufficiency is combated with artificial respiration, at ten times the normal serum level, coma occurs, followed by anoxia and death with auriculo-ventricular dissociation.^{8,9}

II. EFFECTS OF EXCESS MAGNESIUM

A. Effects on the Nervous System

Excess magnesium affects the peripheral nervous system; it produces paralysis by suppressing the release of acetylcholine and blocking transmission at the neuromuscular junction.¹⁰

In the autonomic nervous system, magnesium excess also diminishes acetylcholine release and blocks transmission in sympathetic ganglia and in vagal terminals of the sino-atrial node. Magnesium appears to diminish the amount of transmitted substance released and it also diminishes the sensitivity of the postsynaptic membrane to a given amount of the transmitter.

In the central nervous system, magnesium is not an anesthetic nor even a major depressant, unless given intrathecally or intraventricularly or applied directly to nerv-

ous tissue. A secondary nervous system depression develops in part due to hypotension and hypoxia.

B. Effects on the Heart and the Cardiovascular System

The electrocardiographic changes induced by excess magnesium include an increase in P-R interval at concentrations of 5 to 10 meq/l which then may progress to complete heart block at levels greater than 15 meq/l; at a higher concentration, the heart arrests in asystole. Intraventricular conduction defects, consisting of an increased QRS duration and an increased QT interval, appear at 10 to 15 meq/l of magnesium. The P-wave voltage may be decreased as well as there being a variable degree of T-wave peaking. However, no classic hypermagnesemic EKG changes have been described.

Transient tachycardia followed by sinus bradycardia has been reported in experimental animals.¹¹ The use of magnesium to treat digitalis-induced arrhythmia appears to be effective.¹²

Hypermagnesemia is associated with hypotension.¹³

III. TREATMENT

Magnesium toxicity can be promptly but only transiently reversed by the administration of calcium. Dialysis is the treatment of choice, either peritoneal or hemodialysis. Magnesium losses are dependent on the dialysis gradient. A dialysate concentration of about 1.0 meq/l appears to normalize the serum magnesium concentration.¹⁵

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Chapter 12

THE INTERACTION OF MAGNESIUM WITH OTHER CATIONS

I. EXPERIMENTAL BIOENERGETICS

The steady state of a cell must be dependent upon the ATPase enzyme concentration, the ion concentrations which it helps to maintain, and the availability of ATPase.¹ New work in bacteria indicates that there is a stoichiometric relationship between the internal concentrations of potassium, magnesium, and phosphorus.² However, the amounts of these elements which are associated with the outer membrane can be considerable. The ratios of in-cell concentrations of Mg/K/P remain close to 1:2:10 when these external amounts of the elements are taken into account.

In bacteria, cells moving out of stationary phase pick up K, Mg, and P, before RNA formation, and, in turn, protein synthesis.³ This is in keeping with the observation that in vitro K and Mg are required to stabilize RNA. The inorganic content of the cell is a reflection of its ability to use energy in growth. It could then be that limitation of ion uptake controls growth.

There has been a recent rekindling of interest in phosphorus metabolism. The possible relationship of hypomagnesemia to phosphate depletion has been reviewed.⁴ Experimental phosphate depletion in the rat is associated with: (1) magnesuria due to a decrease in the net renal tubular reabsorption of magnesium with the main source of the urinary loss being bone magnesium; (2) hypomagnesemia secondary to the renal leak of magnesium; (3) negative magnesium balance; and (4) increase in the intestinal fractional absorption of magnesium.⁵

II. CLINICAL STUDIES

Recent clinical studies have provided evidence supporting the theory that magnesium is essential for keeping the intracellular potassium constant.⁶ In malnourished Jamaican children, Montgomery demonstrated both lowered muscle potassium and magnesium levels.⁷ MacIntyre and co-workers reported a slight decrease in skeletal muscle potassium in three magnesium-deficient patients.⁸ When Shils induced magnesium deficiency in seven patients, he found that the total exchangeable potassium fell during the period of deficiency, and that it could not be restored by potassium supplementation.⁹ Magnesium repletion, however, was associated with increased total exchangeable potassium, even though the potassium intake was unaltered. In malnourished Thai children, Caddell and Olsen showed a concurrent depletion of both magnesium and potassium.¹⁰

A sustained increase in magnesium excretion may lead to a cellular magnesium deficiency, which by an insufficient activation of Na-K-ATPase may result in an inability of the cell to maintain the high intracellular potassium concentration.¹¹ Thus, the cell fails to attract potassium despite an abundant supply. As the resting membrane potential is mainly a function of the logarithmic ratio between the intracellular and extracellular potassium, a change in only one of these factors results in a change in membrane potential; thus, a decrease in intracellular potassium will lead to a less negative potential. In this way, the resting membrane potential approaches the threshold potential and the cell becomes more excitable.

Preliminary results suggest that giving increased potassium supplements to patients taking diuretics over a long period does not necessarily augment the cellular uptake of

potassium. This may be explained by a concomitant cellular magnesium deficiency with an insufficient activation of the Na-K-ATPase. This theory is further supported by the increase in cellular potassium concentration obtained after magnesium infusion.¹¹

Percutaneous muscle biopsy studies on patients with hypokalemia and/or treatment with diuretics revealed no relation between the extra- and intracellular concentrations of sodium or magnesium. The intracellular potassium concentration correlated strongly with the intracellular magnesium concentration. These results imply that magnesium deficiency could alter the intra- and extracellular potassium balance.¹²

Low plasma magnesium concentration may be associated with a reduced lymphocyte content of magnesium and potassium. Intramuscular injection of MgSO₄ may, within a 24-hr period, result in increased plasma and lymphocyte magnesium. Lymphocyte potassium increased in parallel with the lymphocyte magnesium, suggesting that magnesium can play an important role in the restoration and maintenance of tissue potassium.¹³

III. ANIMAL STUDIES

A. Studies in Magnesium-Depleted Rats

Whang and his associates have shown in several animal studies that a dietary deprivation of magnesium is accompanied by muscle potassium deficit despite an abundant supply of potassium.^{14, 15} Thus, a deficit of magnesium exerts an important influence during potassium depletion as it does during potassium repletion.¹⁶

B. Studies in Potassium-Depleted Rats

In rats, a diet depleted of potassium caused a significant hypokalemia and hypermagnesemia, a diuresis and natriuresis, a magnesiuria, and a decrease in the fecal excretion of magnesium. Balance studies revealed that magnesium metabolism was positive and higher than in control rats. Potassium and magnesium contents in muscles were reduced, whereas the sodium level was increased and plasma aldosterone was significantly lower. The elevation in plasma magnesium level appeared to be the result of a more positive metabolic balance of magnesium and the shifting of magnesium from the tissue into the plasma compartment. The hypermagnesemia may have been mediated through the depression in mineralocorticoid activity induced by the depletion of potassium.¹³

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Chapter 13

MAGNESIUM AND THE CENTRAL NERVOUS SYSTEM

I. EARLY STUDIES OF THE CEREBROSPINAL FLUID

In 1923 Barrio reported that the concentration of magnesium in the cerebrospinal fluid (CSF) varied between 1.15 and 5.02 meq/l, the average concentration being 125% of that in the blood serum.¹ In other reports, the ranges have been 0.83 to 2.90 meq/l, with an average of 2.49,² and 2.49 to 2.99 meq/l.³ When the magnesium concentrations in the serum and spinal fluid of normal, nonfasting adult males were determined simultaneously, the mean serum value was 1.95 ± 0.21 meq/l, the mean spinal fluid value 2.40 ± 0.14 meq/l.⁴ While the spinal fluid has a higher concentration of magnesium than the serum, it contains less of the ultrafiltrable fraction.⁵

II. CONSTANCY OF THE CSF VALUE

On the average, 64 to 75% of the magnesium in the serum is diffusible.⁶ There exists a concentration gradient for the magnesium in the ultrafiltrable plasma and in the CSF. There does not appear to be a simple relationship between the diffusible serum magnesium and the CSF magnesium.⁷ The CSF magnesium tends to be constant, completely ultrafiltrable, and independent of the serum value. When the concentration of magnesium in the plasma of dogs was raised from 6 to 24 meq/l by infusion of magnesium salts, the concentration of magnesium in the CSF did not change.⁸ In early studies, induction of severe hypomagnesemia in adult rabbits⁹ and in young and adult rats¹⁰ by feeding a low magnesium or a magnesium-free diet produced no changes in the concentration of magnesium in the CSF. In man also, the level of magnesium in CSF is maintained in the presence of low levels of plasma magnesium.¹¹ Thus, the concentration of magnesium in the CSF cannot be explained by ultrafiltration alone; it appears to be fixed at a definite level by an active cellular process.¹² The central nervous system appears to be protected either by a barrier or by a regulating mechanism which keeps the physiologically active concentration of Mg²⁺ within certain limits.^{13,14}

III. SECRETION

The hypothesis which seems most compatible with these findings is that magnesium is secreted into the spinal fluid by cells of the choroid plexus, and that the blood-brain barrier must be intact in order to maintain the higher concentration of magnesium in the CSF. Supporting this hypothesis are the observations of Cohen who compared the range of magnesium concentrations in the CSF of patients with and without meningitis.¹⁵ In the latter, the magnesium concentrations in the spinal fluid exceeded the values for plasma by 0.39 to 1.40 meq/l. In patients with meningitis, the spinal fluid never exceeded the plasma magnesium by more than 0.09 meq/l, and in some instances the plasma contained higher magnesium concentrations — in one case, 0.51 meq/l more than that in the CSF. A significant increase in magnesium concentration has recently been reported in epileptics, presumably by release of magnesium ions from CNS into the CSF.¹⁶ In patients with cerebral thrombosis or cerebral hemorrhage, the mean

serum and CSF magnesium values were significantly lower than those obtained from control subjects.¹⁷

IV. ^{28}Mg STUDIES

^{28}Mg has been used to study the exchange of magnesium between plasma and CSF.¹⁸ The normal ratio in the dog of CSF to plasma magnesium is 1.34. When the plasma magnesium concentration was raised to 300 to 400% of normal, the CSF magnesium rose only to a maximum of 21% above the control value at the end of 5.5 hr. $^{28}\text{MgCl}_2$ administered i.v. entered the CSF rapidly and reached equilibrium with the nonisotopic magnesium within 2 to 3 hr. The exit rate of magnesium from the CSF had a half-life of about 70 min, regardless of whether the CSF magnesium levels were close to normal or greatly elevated. This rate was independent of an elevated plasma magnesium concentration.

In the present state of our knowledge, there is evidence for the existence of blood-CSF and blood-brain barriers regulating magnesium homeostasis within the central nervous system. In mature animals, systemic interstitial fluid magnesium appears to be actively transported across the choroid plexus and the vasculo-glial (blood-brain) complex into the extracellular fluid of the CNS against electrochemical gradients.¹² The choroid plexus is involved in maintaining the constancy of the CSF-Mg concentration by sensing changes in the normal CSF-Mg concentration and altering appropriately its rate of active secretion of magnesium.¹⁹ Passive efflux of magnesium from the CNS takes place via the arachnoid villi and presumably across the cerebrospinal capillaries.

V. EXPERIMENTAL MAGNESIUM DEFICIENCY AND THE CENTRAL NERVOUS SYSTEM

In recent studies young rats became hypersensitive to pure tone stimuli 48 to 72 hr after being placed on a low magnesium diet.²⁰ The CSF-Mg remained normal for the first 24 hr of magnesium restriction despite marked hypomagnesemia. It then decreased and continued to fall thereafter for at least 8 to 13 days. Somewhat over 98% of the brain magnesium was "intracellular". A portion of the magnesium in brain appeared to be associated with mitochondria, synaptosomes, myelin-rich subfractions, and acidic lipids. Magnesium in the brain is found in those areas composed predominantly of gray matter. The mean concentrations of magnesium in cerebral cortex ranged from 29 to 33 $\mu\text{mol/g}$ dry weight compared to 17 to 22 $\mu\text{mol/g}$ for white matter.

Intracellular magnesium decreased exponentially after the first day of magnesium restriction²¹ and the central neurologic hyperexcitability correlated best with a decrease in brain magnesium content. This decrease was not accompanied by any significant changes in the monoamines — norepinephrine, dopamine, and 5-hydroxytryptamine.²² All the major regions of the brain appeared to be involved in this decrease in brain magnesium. Thus, marked changes in plasma, CSF, and brain magnesium developed extremely early in the course of a particularly severe form of magnesium deficiency in the young rat.²³ These studies provide evidence that the mechanism whereby magnesium deficiency acts to induce hyperexcitability lies in the CNS rather than peripherally.

When magnesium was administered parenterally to the deficient rats, CSF and skeletal muscle magnesium promptly returned to normal, but the correction of cellular

deficits in the brain required several hours. There was complete restoration of magnesium losses within 2 hr in the cerebral cortex and the cerebellum and within 4 to 6 hr in the diencephalon-midbrain and pons-medulla. The loss of magnesium was greater from predominantly white matter than from predominantly gray matter.^{24,25} Supracortical injections containing 22 µg Mg as sulfate reduced the seizure activity of magnesium-deficient mice, even when the serum magnesium levels remained low. Magnesium-deficient weanling rats exhibited an increase in nonspecific excitability level (NEL) and in audiogenic seizure susceptibility.²⁶ When deficient rats were injected i.p. with MgCl₂, raising the serum magnesium concentration to 6.6 meq/l, NEL decreased to normal while audiogenic seizure susceptibility remained. Both the NEL and audiogenic seizure susceptibility in rats appeared to reflect CNS magnesium concentration, except when serum magnesium concentration was very high. Very high serum magnesium concentration lowered NEL but did not lower audiogenic seizure susceptibility if the CSF magnesium was low.

Buck et al. made cerebral intraventricular injections of magnesium in artificial CSF in magnesium-deficient rats and found progressive depression of excitability.²⁷ A low magnesium diet resulted in an increase in brain serotonin. Serum magnesium concentration remained unchanged, confirming the hypothesis that the primary effect of magnesium status on excitability is due to its action on the central nervous system and not upon the musculature.

VI. VENTRICULOLUMBAR PERfusion STUDIES

Allsop and Pauli²⁸ developed a technique for ventriculolumbar perfusion of the CSF space in unanesthetized sheep. Perfusion with synthetic CSF solution containing less than 0.6 mg magnesium per 100 ml produced episodes of tetany which were abolished by perfusion with a solution of normal magnesium concentration. Perfusion with a solution containing no magnesium at all resulted in mild episodic signs lasting up to 10 sec, involving fine muscle tremors of the head and neck, tetanic extension of the neck and forelegs, and paddling, mainly of the forelegs. The effect was apparently not mediated through any large changes in blood magnesium concentration. Removal of calcium from the CSF also caused characteristic signs, which were distinct from those induced by lack of CSF magnesium.

VII. STUDIES ON THE ANESTHETIC EFFECTS OF MAGNESIUM

In 1898, Meltzer observed that two drops of a 5% solution of magnesium sulfate injected intracerebrally into dogs produced complete anesthesia and relaxation for several hours. Hypothesizing that magnesium is an inhibitory factor in the life phenomena, Meltzer followed these observations by a prolonged study of the effects of magnesium on the animal's body. In 1905, Meltzer and Auer reported that the s.c. injection of magnesium sulfate can induce general anesthesia in laboratory animals.²⁹ The animals lost all reflexes and signs of sensibility for some time, while the respiration remained intact. Meltzer and co-workers investigated the effects of different modes of administration — i.v., intraspinal, s.c., and i.m. — in animals and also, to a considerable degree, in human beings. Magnesium given by any route was found to have an unmistakably depressant effect.

Among the more important findings reported by Meltzer and his co-workers were the following: (1) the hypodermic injection of a concentrated solution of magnesium

sulfate has a profound depressing effect upon the nervous system without a preceding excitatory phase; (2) the s.c. injection of magnesium sulfate never has any purgative effect; (3) magnesium is excreted to a large extent through the kidneys; (4) the action of calcium in the body is antagonistic to that of magnesium, potassium, and sodium, and the effects of magnesium given parenterally can be rapidly reversed by the intravenous administration of a solution of calcium chloride.

Meltzer and Auer³⁰ labored for many years to establish magnesium sulfate as an anesthetic agent. As a matter of fact, they reported an operation performed on a patient under magnesium anesthesia, which was induced by the i.v. administration of a 6% solution of the drug.³¹ The margin between the dose which produces effective anesthesia and that causing respiratory paralysis was so slight, however, that the use of this drug as an anesthetic agent was soon abandoned.

These early studies on the use of magnesium sulfate for anesthesia demonstrated that magnesium has two sites of action on the nervous system: a peripheral blocking effect on the neuromuscular junction and a central nervous system effect. The anesthetic effect may or may not be accompanied by paralysis of the endings of the motor nerves to the skeletal muscles.

Subsequent studies by Meltzer and Auer in 1913 and 1914 showed that magnesium sulfate and ether have a synergistic action. In 1921, however, Curtis reported that one of three patients in whom magnesium sulfate was used as an adjunct to ether anesthesia died with symptoms of acute poisoning.³² Postmortem examination demonstrated marked jaundice, acute fatty changes in the liver, cloudy swelling of the parenchyma, and remarkable petechial hemorrhages in the pleura, pericardium, and endocardium. A high concentration of magnesium was found in the liver, and death was attributed to magnesium sulfate.

In 1932, Neuwirth and Wallace correlated the narcotic effect of magnesium with the serum concentration.³³ Evidence of depression of central nervous system activity — a state of quiet, relaxation, and lessened responsiveness — first appears with a serum magnesium concentration of 5 to 6 mg/100 ml. At a concentration of 14 mg/100ml ataxia appears, and a concentration of 20 mg produces complete unconsciousness, absence of muscular movements, and insensibility to pain — in other words, full surgical anesthesia. The margin between this concentration and the lethal concentration is extremely small.

There have recently been some doubts raised concerning the anesthetic effects of magnesium. Somjen and co-workers failed to produce analgesia or electronencephalographic changes in two human volunteers whose serum magnesium levels were as high as 15 meq/l.³⁴ Other studies have shown that the i.v. injection of magnesium produced changes in the cardiovascular system, including alterations in arterial blood pressure,³⁵ pulse rate,³⁶ peripheral resistance,³⁷ and electrocardiogram.³⁸ The results of a study in dogs³⁹ suggest that magnesium does not have direct general anesthetic properties, but that the "sleeplike" state is due to depression of cardiac function and probably also due to hypoxia secondary to respiratory muscle paralysis. EEG changes were observed which could be explained on the basis of significant arterial hypotension and elevation of the central venous pressure. The narcotic properties of magnesium were questioned.

VIII. PHARMACOLOGIC STUDIES

Mice rendered physically dependent on phenobarbital exhibited significantly lower whole-brain and serum-magnesium concentrations than did control mice. The symptoms of phenobarbital withdrawal are remarkably similar to those seen in magnesium-

deficient mice exposed to a low-magnesium diet without drug exposure. Belknap et al. suggest that brain magnesium deficits produced by chronic phenobarbital withdrawal could contribute to the observed phenobarbital withdrawal syndrome.⁴⁰ Administration of MgSO₄ during withdrawal significantly reduced the incidence of tonic-clonic and lethal tonic seizures.

Effect of magnesium on epileptic foci — Magnesium sulfate infused i.v. can suppress neuronal burst firing and interictal EEG spike generation in neuronal populations rendered epileptic by topical penicillin. The degree of suppression corresponds closely to the increasing serum magnesium concentration and is reversible as the serum level declines. These results corroborate the clinical observations in patients that magnesium can produce an anticonvulsant effect apart from neuromuscular blockade.⁴¹

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Chapter 14

THE RELATIONSHIP OF MAGNESIUM TO NEPHROLITHIASIS

I. INTRODUCTION

Patients with recurrent formation of urinary calculi composed of calcium oxalate and calcium phosphate present a stubborn and curious puzzle to the urologist. The typical subject is free of demonstrable metabolic disease such as hyperparathyroidism or tumor metastases in bone, has a normal serum calcium, uninfected urine, and normal renal function. In North America, there appears to be an uneven distribution of stone incidence which has led to the designation of a so-called "stone belt" across the mid-Atlantic and southeastern U.S., but the condition is by no means exclusive to that area. In a Czechoslovakian study, in a group of 57 children with urolithiasis, hypomagnesemia was found in 26.3%.¹

II. PHYSICOCHEMICAL ASPECTS

Most stones formed in the kidneys are composed of calcium oxalate, calcium phosphate, or a mixture of the two. There are two main mechanisms which are of possible significance in the formation of renal calcium stones² (1) the concentration of precipitating substances, i.e., mainly calcium, oxalate, and phosphate, and the solubility of the mineral phase formed, and (2) the inhibitory activity protecting against the precipitation and aggregation of these calcium stones. The urine from most stone-formers is supersaturated with respect to calcium oxalate or calcium phosphate. Subjects without renal stone disease excrete either no crystals or only individual crystals in the urine, whereas stone-formers excrete greater amounts of crystals and, in addition, tend to excrete large aggregations of crystals.³ The main inhibitors of the precipitation of calcium oxalate and calcium phosphate crystals are citrate, pyrophosphate, and magnesium, whereas glucosaminoglycans, besides citrate and pyrophosphate, inhibit the aggregation of formed crystals in the urine.²

III. THE POSSIBLE ROLE OF MAGNESIUM IN THE PATHOGENESIS OF RENAL STONES

In 1929 Hammarsten reported that magnesium can increase the solubility of calcium oxalate in vitro.⁴ Others have shown experimentally that magnesium decreases the incidence of calcium oxalate stone formation.^{5,6} Desmars and Tawashi emphasized the fine kinetic control which magnesium exerts on calcium oxalate formation, because of the greater solubility product for magnesium oxalate than for calcium oxalate.⁷

Young rats fed on a magnesium-deficient diet rapidly develop renal calcification.⁸ The calcium is present as apatite crystals in the proximal tubular cells.^{9,10} This phenomenon can also be produced with a vitamin B₆-deficient diet in rats, whereas a diet containing abundant magnesium protects against stone formation.¹¹

The renal calcium accumulation characteristic of magnesium deficiency is an intranephronic calculosis.¹² The calculi appear to be derived from the accretion of calcium phosphate upon laminated periodic acid Schiff (PAS) positive bodies in the tubular lumen. The origin and identity of the stone nidus have not been established.

The PAS positive matrix is a conglomerate containing significant quantities of cholesterol, fatty acids, phosphate, glucose, and one or more proteins.⁸ These substances account for about 60% of the dry matrix mass. The data point towards the brush border of the renal proximal tubular cells as the primary source of the calcifiable matrix. This region is strongly PAS positive, rich in neutral polysaccharides, and poor in the mucosubstances which contain sulfated hexuronic acids. The mechanism of release of the matrix from the brush border is currently unknown.

IV. CLINICAL OBSERVATIONS

Since a low urinary excretion of magnesium may, from a physicochemical point of view, be expected to be associated with a reduced inhibitory activity and, hence, an increase in stone formation, the output of magnesium in the urine has been determined in a number of clinical studies. The consensus of these studies¹³⁻¹⁵ indicates that stone-formers excrete magnesium in the urine in the same manner as healthy, non-stone-forming control subjects. In other studies, however, stone formers are reported to have a low urinary excretion of magnesium.^{16, 17}

The urinary excretions of magnesium and calcium are positively correlated; that is, for increasing values of urinary calcium, the urinary magnesium rises. Most stone formers have high urinary calcium excretion associated with a disproportionately low magnesium output.¹⁴ The ratio of urinary magnesium to urinary calcium has been used as an index of the stone-forming propensity, and a low value has been reported to be associated with an increased frequency of stones.^{14, 17-19} Further, in epidemiologic studies, it has been shown that, irrespective of the level of urinary calcium, a high ratio of urinary magnesium to urinary calcium is associated with a low prevalence of stones.¹⁴ Thus, although the total urinary magnesium in stone formers often is not significantly different from that observed in control subjects, there is evidence that magnesium plays a protective role in renal stone disease. Determination of the serum and urinary magnesium concentrations have not suggested that magnesium deficiency is a feature of renal stone disease.

Magnesium has been used in clinical trials as a prophylactic treatment against recurrent renal stone formation.²⁰⁻²² The available data indicate that the recurrence rate is significantly reduced during the treatment, but unfortunately only one study reports on the natural history in untreated control subjects.²³

In a recent study, Johansson determined that the fractional intestinal absorption of magnesium was no different in healthy subjects and in renal stone formers.²⁴ Renal stone formers, however, showed an increased intestinal absorption of calcium, indicating that this is a common cause of the hypercalciuria often seen in renal stone disease.²⁵ The stone former have a mean value of urinary magnesium similar to that observed in the control subjects. However, in comparison with the healthy subjects, the stone-forming subjects had a tendency to lower magnesium output for any given level of urinary calcium. Thus, the stone-forming subjects had a lower ratio of urinary magnesium to urinary calcium, mostly as a consequence of their greater urinary calcium output. When the stone formers were given prophylactic magnesium treatment, their urinary magnesium excretion increased, whereas the calcium output remained unchanged and, consequently, the ratio of urinary magnesium to urinary calcium increased on treatment and approached the value found in healthy subjects.²⁴ When a magnesium-loading test was performed, magnesium retention was found to be within the normal range in the renal stone formers.

V. THERAPEUTIC TRIAL

Prophylactic treatment with magnesium hydroxide (400 to 500 mg daily) was instituted in 56 of the 70 patients with renal stone disease who had previously been investigated with regard to their magnesium metabolism. The urinary excretion of magnesium increased approximately 1.5 mmol/24 hr during the first month, irrespective of the pretreatment excretion, and this larger excretion persisted during the follow-up period. No changes occurred in the serum and urinary calcium concentrations. Therefore, the urinary magnesium to calcium ratio increased and approached the value found in healthy subjects.

At the time of the report, most of the patients had been under treatment for at least 2 years, and only six of them had experienced recurrence or formation of new stone.²⁴ The mean stone-episode rate during treatment was 0.03 stones/year, compared with a pretreatment rate of 0.8 stones/year. Thirty-four stone-forming patients in the same clinic who were not on medical treatment had a total recurrence rate during 2 years of 44%. Thus, treatment with magnesium hydroxide appeared to reduce the recurrence rate and was indeed beneficial. Besides minor GI discomfort, no adverse effects were noted during therapy.

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Chapter 15

MAGNESIUM DEFICIENCY IN RUMINANTS

I. EARLY HISTORY

Dairymen in the Netherlands have known for many years that cows may develop tetany soon after being turned out from the stall to grass.¹ This condition is known as grass staggers, grass tetany, or lactation tetany. It generally appears within the first 2 weeks after cattle have been put out in the spring of the year to graze in lush, rapidly growing pasture, although occasionally it occurs in stalled animals in mid-winter. The symptoms, which include hyperexcitability, muscular twitching, opisthotonus, and convulsions, may be very acute in onset or of long duration. When the onset of clinical signs is acute, death can occur within 30 min.

Grass staggers may be fatal to as many as 30% of the animals with clinical signs; therefore, it has been a disorder of considerable economic importance to dairymen, especially in Australia, New Zealand, England, and Holland. The disease is most prevalent in countries that are in temperate climate zones with wet weather and cool temperatures in the range of 40 to 60°F. Its incidence is increased with improvement of pasture. Grass tetany is now occurring in the U.S.^{2,3}

II. ASSOCIATION WITH HYPOMAGNESEMIA

Sjollema and Seekles in 1932 first associated hypomagnesemia with this disease.⁴ Blood magnesium level falls rapidly within 2 days to 2 weeks after the animals are turned out to grass; the incidence of clinical signs, both with and without hypocalcemia, is high and not necessarily associated with calving.⁵ In Scotland, the overall incidence of asymptomatic, low levels of serum magnesium in dairy cows is 8.7%; one half to 2% of dairy cows may develop clinical hypomagnesemia. However, in individual herds of dairy cattle, the incidence of hypomagnesemia during the spring grazing period may be greater than 50%.⁶

The usual range of plasma magnesium concentration in dairy cows is 1.5 to 3.2 meq/l. Moderate hypomagnesemia usually does not result in clinical disease, but the danger of tetany increases as the plasma level falls below 0.8 meq/l. Not all animals with severe hypomagnesemia develop clinical signs; some may maintain critically low levels for a long time and remain clinically normal. The reason for this difference was unknown until recently.⁷ Pauli and Allsop in 1974 reported that in dairy cows with clinical signs of grass tetany the onset of the hypomagnesemic tetany was more closely associated with low cerebrospinal fluid magnesium levels than with hypomagnesemia.⁸ Hypocalcemia did not appear to be essential for the nervous signs.

III. DIAGNOSIS

Diagnosis of most of the cases of hypomagnesemia must depend upon a clinical or postmortem finding because only rarely is it possible to obtain blood samples before death. Magnesium levels in the heart muscles of dead cattle and sheep suspected of hypomagnesemia are significantly lower than those in normal animals.⁹

IV. ETIOLOGIC FACTORS

Hypomagnesemic tetany occurs almost entirely in ruminants; the only comparable condition in non-ruminant species is transit tetany in horses or tetany in lactating mares or those approaching parturition.¹⁰ Cases in nonpregnant female or male animals are rare. The condition occurs in calves confined to an all-milk diet. Therefore, the stresses of lactation or rapid fetal development must be important etiologic factors.¹¹

The hypomagnesemic tetany of milk-fed calves and of nonlactating adult bovine animals on poor upland pasture could be attributed to a simple dietary deficiency of magnesium. It could not be so for the classic grass tetany which affects cows on lush spring grass or for the more recent outbreaks on intensely managed pasture, since most grasslands contain more magnesium than the theoretic minimum requirements. In North Ireland there is evidence of marked differences in incidence of hypomagnesemic tetany in adjacent areas where soils are derived from different geologic formations.¹¹

The magnesium content in pastures in northern Europe is lowest in early spring. In spite of this, the daily intake of magnesium by dairy cows grazing on spring grass usually would be 15 to 20 g, which is well above the minimum daily requirement of 9 to 11 g. Therefore, under these conditions, hypomagnesemia may be regarded as a conditioned magnesium deficiency.

V. CONDITIONING FACTORS

Many studies have been made of possible conditioning factors. Heavy fertilizer treatment appears to decrease the apparent availability of magnesium; the tricarboxylic acid of pasture may act as a chelating agent and interfere with absorption of magnesium. Variations in dietary components such as water, phosphorus, sodium, potassium,¹² calcium, sulfate, nitrate, citrate,¹³ and phytate,¹⁴ all appear to influence the degree of absorption of magnesium from the gut.^{15,16} Simesen suggests a binding of calcium and magnesium to suspended materials of the digesta as the most acceptable explanation for the etiology of hypomagnesemia.¹⁷ The general conclusion to date appears to be that hypomagnesemia rarely occurs when the magnesium content of the pasture exceeds 0.20% of the pasture dry matter. Present methods for control of prevention of hypomagnesemic tetany depend almost entirely on increasing the magnesium intake of susceptible animals during critical periods. A novel method for prevention of hypomagnesemia recently proposed is the oral administration of magnesium alloy bullets;¹⁸ unfortunately, its efficacy is questionable.¹⁹

VI. MAGNESIUM HOMEOSTASIS

The net amount of magnesium required for maintenance of an adult cow would be about 1.8 g/day; and for production of milk, 0.5 to 0.6 g/10 lb of milk secreted. Assuming that 33% of the magnesium in the diet is available, the minimum daily requirement of magnesium for a cow producing 20 to 30 lb of milk would be 9 to 11 g.¹¹

There are very few estimates of the true availability of magnesium in foodstuffs; this is because of the difficulty in estimating endogenous excretion. Recent tracer studies with ²⁸Mg indicate that the endogenous fecal loss ranges from 3 to 5 mg/kg/day for both cattle and sheep.¹¹

Of the magnesium of the animal body, 60 to 70% is in the skeleton and only about

1% is in the extracellular fluid. The total magnesium in the extracellular fluid of a 1000 lb (450 kg) cow is approximately 2 g (165 meq). The serum magnesium concentration can fall rapidly in lactating animals if they are subjected to sudden change in diet — such as a change from winter ration to spring pasture — or to a reduction of magnesium intake. In some cows serum magnesium values as low as 0.3 to 0.4 meq/l were obtained within 2 to 4 days after the change.¹¹

It appears that only the extracellular magnesium is markedly affected in hypomagnesemic disorders of adult ruminants. This represents only about 1% of the total body magnesium. The relatively large amount of magnesium in bone and soft tissues does not appear to be readily available to meet a critical situation.

Ruminants have a complex 3- or 4-chambered stomach. The rumen is the large first compartment of the stomach of a ruminant from which food is regurgitated for rumination and in which cellulose is broken down by the action of bacterial and protozoan symbionts. From the rumen the food passes *seriatim* to the reticulum, the omasum, and finally the abomasum.

VII. ABSORPTION AND EXCRETION

Early studies with ²⁸Mg in sheep indicated that absorption takes place mainly from the middle third of the small intestine. Other areas of the digestive tract of the ruminant animal were not thought to be important absorptive areas under normal dietary conditions. Ordinarily, urinary magnesium represents the excess of absorption over requirements. Hypomagnesemic animals have a very low output of magnesium in the urine.²⁰ Recent reinvestigation of this problem indicates that the rumen — the large first compartment of the stomach of a ruminant — is an important site of net magnesium absorption. This absorption is mediated by an active transport process.²¹ In sheep, Tomas and Potter reported significant absorption of magnesium from either the omasum or abomasum; absorption of magnesium postrumenally was thought to be insufficient to maintain normal magnesium status in the animals.²²

The main excretory pathway is via the feces, which contain the unabsorbed portion of dietary magnesium and magnesium that has re-entered the gut in digestive secretions. The daily secretion of magnesium in the digestive fluids of the sheep is approximately equivalent to the total magnesium content of the extracellular fluid.²³ Since only a small fraction of the total body magnesium of adult animals is rapidly exchangeable, secretion into the digestive tract may play an important part in the rapid lowering of serum magnesium levels that results from a reduction of magnesium intake or absorption. The rapidity with which hypomagnesemia can develop in lactating ruminants suggests that there is no hormonal feedback mechanism to control the concentration or content of magnesium in the extracellular fluid.²⁴

In summary, the sudden decrease in the magnesium content of the diet can cause a rapid decrease in plasma magnesium concentration, especially in lactating animals. For cows at pasture, any factor which restricts grazing time or food intake, such as estrus or inclement weather, also contributes toward the development of hypomagnesemia. Intake and/or availability of magnesium would appear to be the two dominant factors.

VIII. EXPERIMENTALLY INDUCED MAGNESIUM DEFICIENCY IN RUMINANT FARM ANIMALS

Low-magnesium diets given to calves, lactating cattle, or sheep cause a progressive fall in serum magnesium concentration to less than 1.5 meq/l.²⁵ The renal excretion

of magnesium quickly falls virtually to zero. Fecal excretion of magnesium shows a slower fall to a very low level, and a negative magnesium balance results. Hyperirritability and other early signs of magnesium deficiency are noted at serum magnesium concentrations of 0.6 meq/l. A more marked fall in serum magnesium concentration to about 0.4 meq/l is required before the convulsive phase is observed. Convulsion may occur when the serum calcium concentration is normal.

Two of nine lambs fed a magnesium-deficient ration developed severe signs of hypomagnesemia.²⁶ In these two lambs, the serum values of lactic dehydrogenase, alpha-hydroxybutyrate dehydrogenase, aldolase, isocitric dehydrogenase, and creatine phosphokinase were increased. There was a decrease in the serum alkaline phosphatase activity. At necropsy, the liver from both animals displayed a yellow tinge and microscopically there was very mild fatty degeneration of the parenchyma. In another study, mature sheep fed a magnesium-deficient diet developed a definite decrease in serum albumin and definite increase in alpha-1 and gamma globulins, but the total serum protein remained constant.²⁷ Several sheep developed icterus; microscopic examination of the livers revealed proliferation and distention of the bile capillaries and a slight increase in connective tissue around the portal triads.

In lactating animals that develop hypomagnesemia, there is no reduction in the magnesium content of the milk secreted. Provision of magnesium for milk (0.5 g/gal) appears to have a high priority. An inadequate intake of magnesium from the gut must be supplemented from body reserves. The magnesium immediately available is that of the serum and other extracellular fluid. Consequently, the serum concentration falls, with a progressive reduction in renal excretion to zero as the serum concentration approaches the threshold value. To maintain serum magnesium concentration would require a mobilization of the considerable reserves of magnesium present in the cells of the soft tissues and of the skeleton, but, unfortunately, only a very small part of these reserves is labile.^{28, 29} Therefore, when the nutritional deficit is large and the requirement for milk secretion is high, the limited reserves of the serum and extracellular fluids are rapidly depleted and there is a sharp fall in serum magnesium concentration.

Studies of electrical stimulation of muscles in calves made hypomagnesemic with a low-magnesium milk diet suggest that the primary lesion causing tetany is not likely to be in the muscle fibers nor in the central nervous system. The lesion may be described as facilitation of neuromuscular transmission occurring at, or just prior to, tetany.³⁰

IX. RELATIONSHIP TO FAT METABOLISM

Rayssiguier infused adrenaline i.v. into ewes and found hypomagnesemia and an increase in nonesterified fatty acids in the blood.³¹ He interpreted the findings as follows: β -adrenergic stimulation by adrenaline resulted in an increase in the level of intracellular cyclic AMP, which resulted in hypomagnesemia, which appears to be correlated with stimulation of lipolysis. By inhibiting the phosphodiesterase, theophylline has an effect similar to adrenaline. These observations were thought to have important implications in the etiology of grass tetany.

X. MAGNESIUM EXCESS

There is a paucity of studies dealing with the feeding of excess magnesium to ruminants. Gentry et al. fed Holstein bull calves 1, 2, and 4% supplemental magnesium as

magnesium oxide.³² Diarrhea resulted, the extent and intensity of which was closely related to the dietary magnesium content. Supplements of 2 and 4% resulted in reduced feed consumption and weight. In calves receiving the 4% added magnesium, the plasma magnesium values tripled above those of the controls. Urinary magnesium excretion was increased. Within 1 week after the calves were returned to control diet, magnesium in the urine and plasma declined to control values.

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Chapter 16

MAGNESIUM DEFICIENCY IN EXPERIMENTAL ANIMALS

The first attempt to deprive an animal of magnesium was made in 1918.¹ It was unsuccessful because the "magnesium-free" diet still contained over 100 ppm of magnesium (8 meq/kg), and no untoward effects were apparent. Several years later, a diet containing only 10 ppm of magnesium (0.8 meq/kg), when fed to mice, resulted in cessation of growth within 9 to 13 days and death within 35 days. Finally, McCollum and Orent produced acute magnesium deficiency in young rats and dogs with a diet containing only 1.8 ppm of magnesium (0.15 meq/kg).² In the rat this magnesium-deficient diet produced acute hyperemia of the skin and loss of hair, tachycardia, and convulsion.³ Many of the rats died within 11 days.

The recent availability of a magnesium-deficient diet commercially has relieved investigators of the tedious chore of preparing it themselves, and the introduction of atomic absorption spectrophotometry for the quantitation of magnesium has simplified the analytic process.

I. EFFECTS OF MAGNESIUM DEFICIENCY DURING PREGNANCY

Magnesium plays a role in the maturation of the ovum, the viability of the sperm and in the process of fertilization.⁴

Hurley and co-workers have been studying for several years the effects of magnesium deficiency in the pregnant rat.⁵ A diet severely deficient in magnesium given from the 6th to the 14th day of pregnancy produced fetal death and resorption or gross congenital malformations. A diet which was less severely deficient in magnesium and fed throughout pregnancy also produced congenital malformations and some resorptions.⁶

Mated female Sprague-Dawley rats fed a magnesium-deficient casein diet containing 2.5 mg Mg per 100 g of feed gained less weight than the control animals fed a stock diet. The maternal plasma magnesium levels at day 21 of pregnancy were 0.74 ± 0.02 mg Mg per 100 ml in the deficient animals and 2.43 ± 0.09 mg Mg per 100 ml in the control animals. There was an increased incidence of intrauterine deaths. The fetuses surviving to term in the magnesium-deficient mothers weighed significantly less than those from control mothers. Of the fetuses from magnesium-deficient mothers 44% exhibited malformations, anemia, and edema.⁷

In the magnesium-deficient fetuses, erythropoiesis was significantly greater in liver, adrenal glands, and spleen than in the controls; maturation was normoblastic. Peripheral blood smears showed an obvious macrocytosis, poikilocytosis, and erythroblastosis. There was no evidence of alteration in the chemical structure of the hemoglobin molecule. The anemia was attributed to the direct result of red cell malformation. The morphologic features were consistent with the hypothesis that reduction of hemoglobin synthesis and abnormalities in the red cell membrane are the most important factors in the production of the anemia.⁷ The total plasma protein from magnesium-deficient fetuses was significantly lower than in the control fetuses and was a reasonable explanation for the edema. A level of 40 mg Mg per 100 mg diet was considered suboptimal for pregnancy in rats.⁷

In another study, Whang et al. fed Sprague-Dawley rats a 0.008% Mg diet during pregnancy.⁸ This resulted in a high incidence of stillbirths. Over 90% of live offspring died during the first week postpartum. Litter size was not affected, but the body weight

and the magnesium content of the offspring were significantly reduced. When fed during lactation, the deficient diet caused death in five of the nine dams. At the time of death or when killed on day 21 of lactation, all dams had lost body weight and showed decreased levels of blood and bone magnesium. Thus, maternal depletion during pregnancy affected the condition of the offspring most severely, but depletion during lactation had more serious effects on the mother.

Magnesium deficiency for a period of more than 3 months resulted in sterility of both males and females.⁹

II. GROWTH REQUIREMENTS

In rats receiving diets of liberal calcium content and optimum vitamin content, 5 mg Mg per 100 g of food (4 meq/kg) was found to be the minimum amount necessary for good growth.¹⁰ Female rats on this diet gave birth to young of normal weight and normal magnesium content. While suckling, however, the young rats developed all the symptoms of magnesium deficiency 2 or 3 weeks after birth. The mother's milk appeared to be deficient in magnesium.

III. GENERAL CLINICAL COURSE^{11,12}

A. First Phase

When experimental animals are placed on a synthetic diet containing less than 1 meq of magnesium per kilogram of feed, the onset of clinical changes is very rapid, being detectable within a few days. During this first phase, which lasts approximately 2 weeks, peripheral vasodilatation and hyperemia regularly appear within 3 to 7 days after the animals are placed on a low-magnesium diet. Edema of the ears and paws appears, and hyperexcitability develops. Audiogenic seizures can be provoked in most animals. Up to 60% of the animals may die in convulsions. The plasma level of magnesium drops sharply and then rises slightly to a peak shortly after the onset of hyperexcitability. Hypomagnesemia persists for at least 28 hr after the animals are placed on a control diet which contains magnesium. In magnesium-deficient rats, body temperature and blood pressure decrease.¹³

Magnesium deficiency in dogs with clinical tetany is associated with the following electrocardiographic findings: shortened P-Q and QRS intervals and a normal duration of the Q-T interval. Negative T waves with deep inversion occur frequently.

B. Second Phase

This phase is marked by nutritive failure. Both food consumption and body weight decline. Malnutrition, cachexia, and renal damage develop. The hair becomes coarse and ulcerative lesions develop about the head and neck. Some animals develop diarrhea and appear jaundiced. During this phase, the level of magnesium in plasma declines at a relatively slow rate, finally reaching a value on the order of 0.8 meq/l. The magnesium content of the erythrocytes drops to about one half the normal amount during the early phase of depletion and then remains fairly constant.

C. Age Dependency

Acute deficiency in the young is characterized by growth retardation, convulsions, and a high mortality rate; in the mature, by growth retardation and skin necrosis; and in the old, by the absence of these manifestations. Hypomagnesemia occurs in all de-

ficient rats irrespective of age, but in direct proportion to dietary magnesium intake. The concentration of bone magnesium is decreased in proportion to dietary intake, but the degree of response is strongly age dependent, being greatest in the young rats. No significant decrease in skeletal muscle magnesium occurs in any deficient animals at any age, but subacute deficiency produces a significant fall in heart muscle magnesium in rats of all ages. The manifestations of magnesium deficiency appear to be age dependent, and bone magnesium concentration in experimental magnesium deficiency is also a function of growth rate. Skeletal muscle magnesium remains normal in the face of severe magnesium depletion and is an unsatisfactory guide to the magnesium stores of the body.¹⁴

IV. SPECIES DIFFERENCES

A dietary deficiency of magnesium has been shown repeatedly to cause hypercalcemia in the adult rat, whereas cattle, sheep, pigs, and dogs develop hypocalcemia under comparable conditions. This hypercalcemia in the rat is accompanied by hypomagnesemia, hypophosphatemia, and hyperphosphaturia, findings usually associated with hyperparathyroidism. However, the intestine does not have an increased ability to transport calcium, so that the hypercalcemia may be mediated by a mechanism independent of an adaptive increase in intestinal calcium absorption.¹⁵

The hypocalcemia observed in hypomagnesemia is said to be the consequence of a decrease in calcium mobilization from bone, secondary to a deficit in the release of parathyroid hormone, a skeletal resistance to the action of PTH, and a decrease in heteroionic exchange between the skeleton and the extracellular fluid.¹⁶

V. BIOCHEMICAL CHANGES

A. In the Rat

Magnesium depletion in the adult rat is associated with the following findings: hypomagnesemia; a diminution in the content of magnesium in the erythrocyte, muscle, and total carcass; a consistent hypercalcemia; a consistent depletion of muscle potassium; an increased renal secretion of potassium; an increased renal excretion of phosphorus with no alteration in the serum level of phosphorus; no increase in excretion of nitrogen, with augmented renal excretion of either potassium or phosphorus; and azotemia associated with a renal lesion characterized by intraluminal deposits of calcium. Serum and duodenal alkaline phosphatase are reduced.¹⁷

Protein synthesis — Magnesium-deficient rats have low levels of the serum protein components usually synthesized in the liver. The associated findings of a decreased concentration of hepatic free lysine and normal liver protein content suggest an abnormality in the ability of the liver to deliver protein to the circulation, either by defective synthesis or impaired release of the formed proteins.¹⁸ Previous studies have shown that high levels of dietary protein enhance susceptibility to magnesium deficiency.¹⁹ Conversely, magnesium deficiency reduces overall protein utilization; amino acid incorporation into liver albumin was reduced in the absence of measurable changes in incorporation into total liver protein.²⁰ Male albino rats on a magnesium-deficient diet show a significant decrease in hepatic serine and threonine dehydrase activity with a significant increase in arginase activity and blood urea levels, while tryptophane pyrolase was unaffected. The total protein level showed a significant decrease, the hepatic RNA level an appreciable rise, and there was no change in the DNA content in the deficient animals.²¹

B. In the Rabbit

In domestic rabbits placed on a magnesium-deficient synthetic diet containing 6.6 meq of magnesium per kilogram of diet and followed for 6 weeks, the body content of magnesium was reduced to about two thirds of the normal value, and the body content of calcium was increased by a factor of one and a third. The concentration of magnesium in serum fell from a mean baseline value of 2.0 meq/l to 1.2 meq/l at 8 days and remained at this level for 22 days. The mean baseline value for exchangeable magnesium of 58.0 meq/kg fell progressively to 13.2, 8.2, 6.6, and 6.0 meq/kg at 8, 14, 22, and 30 days, respectively. In the skin and bone, ^{28}Mg uptake was decreased. The magnesium content was significantly decreased in the lung and the bone.

Animals which survived for 4 to 6 weeks stopped eating, lost weight, and revealed biochemical evidences of hepatic and renal failure. Serum concentrations of the following constituents were elevated: total and conjugated bilirubin, cholesterol, lactic dehydrogenase, glutamic oxalacetic transaminase, alpha and beta globulins, creatinine, and urea nitrogen. The following serum concentrations were decreased: calcium, magnesium, phosphate, potassium, sodium, and carbon dioxide content. The mean content of magnesium in most tissues of the experimental group was lower than in tissues of the control group, being significantly lower in the appendix, bone cortex, and large intestine. Histologic evidence of biliary cirrhosis was present.²²

VI. PATHOLOGIC CHANGES

Young Long Evans rats showed a uniform histopathologic lesion in all tissues during magnesium deficiency.²³ This was an inflammatory reaction in loose mesenchymal tissues around blood vessels of precapillary and capillary size which developed within 2 weeks on a deficient diet.

Rats severely depleted of magnesium by dietary means have significant necrobiotic changes in the skeletal muscle characterized by multifocal hyaline, vacuolar, granular, and floccular necrosis frequently associated with calcification of the myofibers. Ultrastructural studies showed early mitochondrial damage which appeared to be fundamental to the pathogenesis of the lesion.

The myocardium showed occasional focal areas of inflammation and necrosis. Electron microscopic studies revealed fine structural abnormalities, such as loss of density in the matrix in the mitochondrion, disruption of cristae, and occasionally, total disorganization.

Experimental production of magnesium deficiency in the dog resulted in degeneration of skeletal muscle and widespread calcification of the kidney, heart, blood vessels, larynx, trachea, lungs, and smooth muscle of the stomach and uterus. Magnesium may affect the integrity of the mucopolysaccharides in the body. Since bone is a specialized form of connective tissue, this suggestion of a relationship between magnesium and metabolism of connective tissue may be extremely important.

In animals given a synthetic diet containing 0.4 to 0.6 meq/kg, the earliest histologic alteration in the kidney was swelling of the tubular epithelium, which was most severe in the pars recta of the proximal convoluted tubules. Coincidental with this swelling, there was an increased amount of intracellular calcium deposits which were needle-like and possess the electron diffraction pattern of apatite. There soon appear in the thin limb of Henle's loop spherical microliths which are composed of a matrix of the para-amino salicylic acid positive substance in calcium phosphate. These microliths grow by accretion to form intranephronic calculi. This development of nephrocalcinosis in magnesium deficiency appears to be dependent to some extent upon the relative

amounts of calcium, phosphorus, and magnesium in the diet. Magnesium-deficient kidney calcification is closely associated with lowered kidney ATP levels.²⁴

Renal calculi have been reported in tubules of trout fed a magnesium-deficient diet. Electron probe microanalysis showed that these calculi contained or comprised tricalcium phosphate. The skeletal muscle contained significantly more sodium than that of normal trout, indicative of an increase in extracellular fluid in the muscle of magnesium-deficient trout.²⁵

Thyroxine injected i.p. at the rate of 10 µg daily per 100 g body weight completely prevented renal calcification of magnesium-deficient rats; but, if thyroxine injections were not begun within less than 48 hr after initiation of magnesium deficiency, the anticalcifying effect could not be demonstrated. Magnesium deficiency was found to increase ⁴⁵Ca uptake in vitro by isolated kidney mitochondria, and thyroxine inhibited ⁴⁵Ca uptake regardless of the magnesium status of the animals.²⁶

VII. SUBCELLULAR CHANGES

There is considerable evidence that a fundamental metabolic disturbance occurs within the mitochondria of magnesium-deficient animals. Histologic evidences for early mitochondrial lesion have been reported in the kidney²⁷ and the heart^{28,29} of magnesium-deficient rats. Cellular fractionation studies indicate that the magnesium loss and calcium deposition in liver occur predominantly in the mitochondrial fraction of the cells.³⁰ Mitochondria from magnesium-deficient rats are swollen and have a lowered P/O ratio, whereas microsomal preparations function normally when provided with a source of energy. These observations suggest that the inhibited protein synthesis and changes in cellular potassium and sodium concentrations are all secondary to a primary disturbance in energy metabolism.³¹

Heaton and George recently undertook to determine in liver mitochondria the locus of the alteration in magnesium distribution during magnesium deficiency — whether there was selective involvement of any of the four main regions: the outer membrane, the inner membrane, the intermembranal space, or the matrix.³² In normal mitochondria, magnesium was present mainly in the intermembranal compartment and the matrix. During magnesium depletion, magnesium was lost primarily from the inner membrane and additional calcium was deposited in the inner membrane and the matrix; these findings suggest that metabolic processes which occur in this region of the mitochondria, such as electron transport and oxidative phosphorylation, will be more susceptible to disruption during magnesium deficiency.

VIII. CHANGES IN BONE

The renal excretion of hydroxyproline is decreased in magnesium deficiency; repletion of magnesium results in a rapid increase in the rate of its excretion. Some think that the hypocalcemia of magnesium deficiency in animals other than the rat is the result of a reduced rate of bone metabolism due to a direct action of magnesium deficiency on bone. There is a significant decrease in the concentration of magnesium in the mandible and femur of both the young and adult rats given the magnesium-deficient diet; the decrease is significantly greater in the young than in the adult rats.

Various histochemical reactions have been used to identify those components of bone and dentin that are affected by magnesium deficiency.³³ Animals fed a magnesium-deficient diet for 19 days show a decrease in the rate of growth. The epiphyseal

cartilages of the posterior head of the tibia are characterized by a widened hypertrophied zone and a narrower proliferating zone. The diaphyseal trabeculae and the incisal dentin stain deep red with the periodic acid-Schiff stain and exhibit an abundance of Alcian blue-positive material, which also stains a very intense violet with toluidine blue. These staining reactions would indicate that there is an interference with calcification of cartilage, bone, and dentin.

The exchangeable bone magnesium is the surface-limited bone magnesium. During magnesium depletion, magnesium concentration in both the exchangeable and nonexchangeable pool decreases. The surface-limited magnesium pool is available during magnesium depletion.³⁴

Smith and Nisbet reported marked osteoporosis and complete cessation of growth in the proximal end of the tibias of both young and adult magnesium-deficient rats.³⁵ Nielsen found that the magnesium concentration in the extracellular fluid regulated calcification of young bone and the transformation of amorphous salt to hydroxyapatite.³⁶ The magnesium content of femoral ash and of the dry bones paralleled the magnesium intake per body weight in animals of similar ages, but the sensitivity of bone to magnesium depletion appeared to be age dependent.^{14,37} Magnesium deficiency in the rat specifically affects the cellular activity in the proximal ends of the long bones; abnormal bone shape was associated with defective regulation of calcification. Cell-mediated mineralization may be partly an intracellular process in which the mitochondria may be involved in sequestering calcium and possibly phosphate. The intimate relationship between magnesium and the mitochondria in energy production may be a basis for intracellular regulation of mineralization by magnesium.³⁸

Incisors of rats maintained on a magnesium-deficient diet revealed a marked reduction in eruption rate. Histologically, these teeth showed atrophy of the formative basal tissues and degenerative changes in the odontoblasts and enamel epithelium with disturbances in calcification. The apical tissues of incisors showed a decrease in mitosis.³⁹

In magnesium-deficient young rats, the acid and alkaline phosphatase activities were decreased in the distal femoral metaphysis. The activity of bone alkaline phosphatase was increased in vitro upon the addition of magnesium to bone from magnesium-deficient rats; this observation suggests that magnesium is essential for the stabilization of the tertiary structure of the alkaline phosphatase molecules in vitro.⁴⁰ The mean ash content of the dry fat-free bone was significantly higher in the magnesium-deficient rats than in pair-fed controls, suggesting that the matrix contents of the bones of deficient rats was less than that of the controls. An impairment of synthesis of both proteinpolysaccharides and collagen may partially explain the disturbance in skeletal growth of magnesium-deficient rats. The magnesium-deficient bones were rather brittle and hypermineralized.⁴⁰

A diet containing 0.8 to 2 mg Mg per 100 g fed to young or adult rats for 18 to 21 days produced generalized medullary bone growth (osteomyelosclerosis) and also periosteal tumors of the desmoid type, at the femoral linea aspera. These changes were accompanied by a decrease in bone magnesium concentrations of 52 to 71%. These masses probably represent an accumulation of cells which are unable to differentiate properly. The lack of magnesium could then be envisaged as responsible for enzyme malfunction.⁴¹ In the presence of periosteal tumors, parathyroidectomy favored cartilage formation. The administration of parathyroid extract inhibited cartilage formation in favor of fibrous tissue and bone production. The addition of magnesium to the diet quickly restored the normal appearance of the tissues.⁴¹

Apatite crystals were synthesized under approximately physiological conditions and in the presence of magnesium.⁴² After aging from 4 to 21 days, the crystals were analyzed and their exchange of incorporated magnesium studied with the use of ²⁸Mg.

Both the kinetics and the extent of the exchange showed that the magnesium ions were strongly rejected by the apatite lattice. In aged crystals, nearly 90% of the magnesium was located in readily exchangeable (surface) positions. These findings support the concept that apatite magnesium is essentially a surface-limited ion and help to explain the ready availability of skeletal magnesium in animals on a magnesium-deficient diet.

The role of magnesium in bone metabolism was studied in chick embryo tibiae in organ culture.⁴³ The study revealed that: (1) magnesium had very little effect on the mineral mass in bones incubated with viable cells; (2) there was an inverse relationship between the magnesium levels and the net mineral accumulation seen in nonvital systems when the bone cells were killed; and that (3) in embryonic chick tibiae, magnesium deficiency did not result in loss of responsiveness to parathyroid hormone. The results suggested that cellular control of magnesium may be an important factor in controlling the rate of net increase of bone mineral. It appeared that a large portion of magnesium in bone is free to move passively in and out of bone.

IX. PHYSIOLOGIC CHANGES

The induction of magnesium deficiency in rats by dietary means produces peripheral vasodilatation as well as other manifestations which are typical of histamine release — degranulation of mast cells, elevation of plasma and urinary levels of histamine, and the appearance of eosinophilia.⁴⁴ Magnesium appears to be essential for the maintenance of the mast cell. Rats on a magnesium-deficient diet show a decrease in the population of mast cells in the s.c. tissue. The lack of magnesium might decrease the binding capacity of tissues for histamine, thereby causing a passive release of the amine. The total number of mast cells in the tongue of magnesium-deficient rats decreased progressively with the duration of the deficiency. Mast cells in the bone marrow, however, increased five- to sixfold.⁴⁵ Histamine levels in plasma and tissue increased rapidly and reached a maximum after 10 days of magnesium deficiency. In view of the fact that magnesium deprivation causes growth retardation, decrease in the synthesis of sulfated mucopolysaccharides, and defective skin regeneration of mast cells, one can explain the increase in marrow mast cell population on the basis of retardation in the release of these cells.⁴⁵ The administration of testosterone to magnesium-deficient males and estradiol to magnesium-deficient females prevented the increase in marrow mast cells.

The blood and tissue eosinophilia of magnesium deficiency is especially prominent in the acute phase of the syndrome. During this phase, there is marked dermal hyperemia and degranulation of mast cells. Such magnesium-deficient animals develop splenomegaly. The eosinophilia may be due to the endogenous release of histamine and possibly also to an interference with the histamine-inactivating system.

Magnesium deficiency in the rat results within several days in a cutaneous hyperemic reaction accompanied by intense pruritus. There is diminution in the number of mast cells in the skin, a parallel decrease of tissue histamine content, and an inversely elevated level of plasma and urinary histamine. The hyperemic reaction is accompanied by an intense leukocytosis with marked eosinophilia.

The coupling of pharmacologically active agents and magnesium may result in an inactive form which is stored within cells; magnesium deficiency may liberate the active agents for release into the circulation. Coordination complexes may be of major importance in understanding the biologic phenomena of binding of the catecholamines. The metals magnesium, copper, iron, and zinc are present in synaptosomes from the brain in sufficient quantities for chelation to be of biologic significance. An ATP-

metal-norepinephrine ternary complex might represent a mechanism for storage or binding of the catecholamine molecules. It seems likely that magnesium chelates with the alpha-and beta-phosphate groups.

Divalent cations might form coordination complexes with membrane constituents and, hence, be importantly related to membrane structure and function. One could envision a ternary metal-ATP-phospholipid complex in which the metal and ATP might play a role as modifiers of the configuration assumed by phospholipid in aqueous solutions. Such a complex can be seen as forming lamellar sheets in agreement with the most generally accepted model depicting the cellular membrane.

X. HEMATOLOGIC EFFECTS

A. Granulocytic Leukemia and Thymoma

Rats fed a synthetic magnesium-deficient diet containing about 4 meq of magnesium per kilogram of diet have been observed for as long as 12 months. Four strains of rats on this regime developed a three- to fourfold persistent granulocytic and lymphocytic leukocytosis. Depending upon the strain, a chronic lymphocytic meningoencephalitis, a chronic granulocytic leukemia, or a malignant thymoma was observed. The granulocytic leukemia and the thymoma were transmissible by injection of viable neoplastic cells.⁴⁶ The leukocytosis of the magnesium-deficient animals was reversed either by the administration of parenteral supplements of MgSO₄, or by dietary supplementation. Both the total white blood cell count and the percentage of granulocytes decreased to their control levels. However, once the animal had developed leukemia, dietary supplementation had no effect on either the leukocytosis or the granulocytosis. No evidence of infection was present in the postmortem examination of the deficient animals. It has been suggested that the chronic lack of magnesium at the cellular level might increase the movement of magnesium out of the nucleus into the cytoplasm, thereby causing chromosomal aberration and cell mutation.⁴⁴

B. Chromosomal Aberrations

Previous studies have suggested that Mg²⁺ binds to sites of fundamental importance to chromosome structure.⁴⁶ Magnesium is a constituent of nucleohistone;⁴⁷ it has been implicated in chromosome metabolism. Magnesium is an obligate cofactor for DNA synthesis; in its absence DNA synthesis is arrested and cellular division is stopped.⁴⁷ The magnesium effects could also be on the macrostructure of the chromosomes by affecting tertiary formations. In any event, in a study of the effect of dietary deficiency during pregnancy of zinc or magnesium on maternal and fetal chromosomes, both magnesium- and zinc-deficient maternal bone marrow and fetal liver cells showed significantly more chromosomal aberrations than did those of controls.⁴⁸ The chromosomal aberrations occurring in highest incidence in magnesium-deficient animals were terminal deletions and fragments. A higher than normal incidence of "stickiness" was also observed in cells from magnesium-deficient animals.

C. Anemia

It has been known since 1933 that dogs on a magnesium-deficient diet become anemic.⁴⁹ This observation has subsequently been confirmed in humans⁵⁰ and in other experimental animals.⁵¹ The anemia of magnesium deficiency is characterized by a shortened erythrocyte survival, reticulocytosis, spherocytosis, microcytosis, and erythroid hyperplasia of the bone marrow.⁵² Erythrocytes during magnesium deficiency were characterized by decreased: intracellular magnesium content, glucose utilization,

lactate production, and ATP and 2,3-DPG concentrations. A progressive decrease in red cell deformability as measured by cell elastometry occurred. Defective membrane construction in a magnesium-deficient environment was suspected.⁵³ Of the three factors affecting erythrocyte mean survival — dietary magnesium intake, magnesium content of the erythrocyte, and splenectomy — the most important appears to be dietary magnesium intake. This factor apparently exerted its effect by lowering plasma, and consequently red cell, magnesium. The mechanism of the anemia of magnesium deficiency is currently still unclear. The spleen does no play a major role in shortening survival of red cells in magnesium-deficient rats. A recent study showed a change in red cell membrane structure in rats on a magnesium-deficient diet, which may condition these erythrocytes to hemolysis.⁵⁴ It therefore appears that the lowering of plasma magnesium concentration leads to an increased rate of intravascular hemolysis of erythrocytes.

In glycolyzing human red cells maintained at 37°C in an atmosphere of 5% CO₂, 84 ± 4% and 78 ± 4% of the total ATP were complexed to Mg²⁺ in the aerobic and anaerobic states of the cells, respectively. The intracellular concentration of free Mg²⁺ was calculated to be 0.25 ± 0.07 mM in the aerobic and 0.67 ± 0.15 mM in the anaerobic state in a sample of normal red blood cells. Since the Mg²⁺ in the red cells is largely complexed, the threefold increase in free Mg²⁺ under fully anaerobic conditions would significantly affect the rates of enzymatic reactions.⁵⁵

XI. IMMUNE RESPONSE

The synthesis of antibodies, major components of the total resistance system, is regulated by the supply and utilization of factors involved in protein synthesis. Magnesium has been linked with protein metabolism in several ways. It activates enzymes critical to the ATP needed for peptide bond synthesis.⁵⁶ It is required for activation and transfer of amino acids to soluble RNA,⁵⁷ and for stabilization of DNA, RNA, and ribosomes.^{58,59} In addition, magnesium affects tissue growth in the thymus,⁶⁰ which is involved with the establishment of immunologically competent cells.

The relation of magnesium to protein synthesis as a part of the resistance mechanism has received little attention. Alcock and Shils reported decreased levels of immunoglobulin G (IgG or 7S antibody) in serum or plasma of magnesium-depleted rats.⁶¹ When these animals were repleted, marked increase in serum IgG occurred immediately. Because it is a major component of gamma globulin, IgG concentration serves as an index to total antibody formation.

Alcock et al. reported that, in rats fed a magnesium-deficient diet for 65 days, the thymus was enlarged in 18 to 52% of deficient animals surviving more than 6 to 7 weeks in various experiments. The enlarged thymus showed marked cellular changes with the normal structure being replaced by cells that resembled transformed lymphocytes. The changes were interpreted as hyperplastic rather than neoplastic. Marked leukocytosis was present during the early stages of the deficiency. Splenomegaly was consistently found.⁶²

Synthesis of antibodies from free amino acids⁶³ has been shown to follow the general pattern of mammalian protein synthesis.⁶⁴ The role of magnesium in the synthesis of protein, therefore, assumes increased significance. Participation by magnesium in the control of cell mutation and division has also been suggested. Bois postulated that a chronic lack of cellular magnesium could increase the movement of magnesium from the nucleus into the cytoplasm, thereby causing chromosomal aberrations and cell mutations.⁶⁵ Battifora et al. advanced a related hypothesis to explain the leukemia occur-

ring in magnesium-deficient rats and indicated that intracellular magnesium deficiency may possibly interfere with DNA replication during cell division.⁶⁶

The influence of magnesium on ribosomal aggregation with consequent effects on protein biosynthesis has been well documented in vitro.⁶⁷ Studies provide evidence for decreased protein synthesis in the spleen at the lowest intake of magnesium (10 ppm). Assessment of serum antibody actively provided a sensitive index to the participation of magnesium in maintaining functional proteins.

The concentration of four serum immunoglobulins decreased in magnesium-deficient mice. The ability of the humoral immune system within the spleen to respond to an antigenic stimulus was progressively impaired by dietary magnesium-deficiency in mice. The impairment in protein biosynthesis is systemic in nature and not confined to the immune system.⁶⁸

Magnesium deficiency produced a drastic fall in the primary and secondary immune responses, as measured by the number of spleen antibody-forming cells.⁶⁹ The number of rosette-forming cells was also much lower in the spleen of deficient animals. Consequently, the spleen immune system is deeply affected by this deficiency.

Spleen cells and thymocytes from magnesium-deficient rats, fed a magnesium-deficient diet for 10 to 13 weeks, incorporated [³H]-thymidine at one half the rate in normal cells.^{61,68} These cells are less stimulated by lectins than those from normal animals.⁷⁰ These effects correspond to the reduced control of gamma globulin in serum in magnesium deficiency.

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Chapter 17

EPILOGUE

The scientific evidences currently available suggest that this earth first appeared as an entity 4.6 billion years ago. From hydrogen and helium evolved the other elements, then simple inorganic chemical compounds, progressing to relatively insoluble oxides, sulfides, and silicates. Magnesium participated early on in evolution in the development of the crust of the earth, primarily as silicate compounds. The magnesium which was released from this deposit by the leaching action of water was available as a catalyst in pre-biologic chemical condensation reactions almost 4 billion years ago. The primitive precursor of the biologic membrane may have been the silicates.

Life probably first appeared as anaerobic bacteria about 3.5 billion years ago. In the succeeding 500 million years of chemical evolution, all the molecules essential for life were synthesized. In the subsequent evolutionary processes, magnesium has been found to play a key role in practically every important step, starting with photosynthesis and the development of the oxygen-rich atmosphere, the evolution of chlorophyll, the synthesis of ATP in the early glycolytic mechanism, and the subsequent appearance of oxidative phosphorylation. The reproductive mechanism which requires DNA and the cell nuclear apparatus also has an absolute need for magnesium; the mechanism for the synthesis of protein needs magnesium too. Thus, magnesium is essential for the capture of solar energy, its conversion to chemical bond energy, its release in oxidative phosphorylation, and its utilization in synthetic processes.

What is it about magnesium that makes this ion so well suited for its various functions? Since it was available in abundance when needed early on in the evolutionary process, biologic evolution must have been molded around the unique capabilities of the ions and the chemical compounds available during the evolutionary process. When I was searching for a general hypothesis concerning the biochemical and cellular functions of magnesium 9 years ago, the common denominator appeared to be its ability to act as a chelating agent or as a biologic cement.¹ Although this hypothesis may have been useful then, it seems now to be a terribly simplistic explanation, considering the immense complexities of the mechanisms under consideration. A more attractive hypothesis is that proposed by Rubin,² namely, that the magnesium ion coordinates the control of metabolism, differential function, and growth. To be sure, the chelating properties of magnesium are necessary for the repeated association and dissociation of molecules and for the maintenance of certain tertiary conformations, but, more importantly, Mg²⁺ in physiologic concentrations coordinates the complex intracellular regulatory processes essential for the maintenance of life. Its responsibilities then are mind boggling and awesome beyond comprehension.

Until recently there has not been much consideration of the influence of geochemical factors on the health of all forms of life. There are now sufficient data to indicate that suboptimal amounts of exchangeable magnesium in the soil affects plant life and hence agricultural productivity. For instance, farmers recognize the magnesium deficiency in tobacco leaves as "sand-drown" and correct for the deficiency by adding dolomite to the soil.

There is some evidence that domestic animals grazing on grass grown in magnesium-deficient soil develop clinical manifestations of magnesium deficiency. No doubt there are many conditioning factors which trigger the clinical picture, but we do not yet understand why these manifestations occur so suddenly. The explanation may be related to the "availability" of the magnesium in the feed and/or the body stores and its gastrointestinal absorption. The pathogenesis of the central nervous system manifestations is still poorly understood.

Magnesium deficiency can now be produced consistently in experimental animals. Depending upon the degree and the duration of the deficiency, pregnant animals will abort or produce offspring with congenital abnormalities and disturbances of protein metabolism and synthetic processes. The clinical manifestations of acute magnesium deficiency are explicable on the basis of physiologic disturbances due to an "ungluing" phenomenon.¹ The most unexpected but fascinating finding is that of chronic granulocytic leukemia and thymoma in rats fed a synthetic magnesium-deficient diet. Chromosomal aberrations have been demonstrated in magnesium-deficient animals. Can these findings be explained as due to a discombobulation of the homeostatic mechanism due to magnesium deficiency and leading to an unbridled proliferation of abnormal cells?

It is very troubling that in spite of extensive recent investigations, we have not been able to identify the regulatory mechanisms which maintain constancy of magnesium in the internal environment. Although GI absorption and renal excretion are involved and parathyroid gland participates, a precise delineation of the regulatory mechanism still eludes us. Perhaps the system is so primitive and so predates the creation of life that it operates on the basic assumption that magnesium will be available in adequate amounts.

Recent human epidemiologic studies suggest a relationship between a subclinical deficiency of magnesium and disorders of pregnancy. Of greater clinical significance is the suggestion that large populations of people are dependent on an adequate intake of magnesium in drinking water to maintain health. Where the soil, and therefore the drinking water, is deficient in magnesium, there is a higher incidence of cardiovascular disease, including hypertension and coronary atherosclerosis, with a higher incidence of sudden death due to cardiac arrhythmia. The dietary deficiency of magnesium may be culturally and technologically conditioned in the developed nations.

In the less fortunate parts of the earth, malnutrition still remains the most urgent health problem. Many subjects with kwashiorkor or protein-calorie malnutrition also have magnesium deficiency. Malnutrition has a regulatory influence by controlling population growth. I would like to suggest that the ultimate role of magnesium may be that of the coordination of life on earth.

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APPENDIX 1

THE MEASUREMENT OF MAGNESIUM IN BIOLOGIC MATERIALS

The great variety of methods used in the past for determining the amount of magnesium present in biologic materials testifies to the fact that none of them was completely satisfactory. Most were cumbersome, indirect, or inaccurate. The most popular of these methods will be summarized prior to a description of the current method of choice — atomic absorption spectrophotometry.

I. MAGNESIUM AMMONIUM PHOSPHATE

The earliest method for estimating the quantity of magnesium in biological fluids was to weigh the precipitate of magnesium ammonium phosphate.¹ There followed many micromethods applicable to serum which were based on estimation of the phosphate in the precipitate by such procedures as the decolorization of ferric thiocyanate.² Then followed a method for magnesium measurement by the consecutive precipitation of calcium as oxalate and magnesium as magnesium ammonium phosphate ($MgNH_4PO_4$).³

The colorimetric estimation of phosphate, introduced in 1920,⁴ was soon applied to the $MgNH_4PO_4$ precipitate.⁵ In this reaction magnesium is determined as phosphate by the reduction of phosphomolybdic acid to a blue substance, the amount of which is determined colorimetrically. Unfortunately, this molybdenum blue proved to be unstable. Simonsen, Westover, and Wertman next determined the phosphate as the yellow molybdivanadophosphoric acid — a color which is stable over several hours.⁶ This method, although cumbersome, has proven to be, in our experience, the most satisfactory chemical method available.

II. TITAN YELLOW

The Titan-yellow method, although simple, is inaccurate and not reproducible. It is mentioned here because many of the early clinical studies employed this method.

In 1927, Kolthoff⁷ discovered that magnesium imparts a pink or red color to alkaline solutions of two acridine sulfo dyes: Titan yellow and Clayton yellow. The Titan-yellow reaction was used for the colorimetric determination of small quantities of magnesium. Magnesium was at first measured as a specific colored lake, which had to be suspended by a colloidal dispersing agent. The most recent modification of this method was introduced by Orange and Rhein,⁸ who selected polyvinyl chloride as a colloidal dispersing agent.

III. 8-HYDROXYQUINOLINE

After calcium is removed from serum, magnesium can be precipitated by 8-hydroxyquinoline;⁹ this precipitate is so light that difficulties are encountered in further handling. Magnesium hydroxyquinoline has been brominated; loss of bromine through evaporation is the chief source of error in this method. Titration of hydroxyquinoline with ceric sulfate has the advantage that the amount of ceric sulfate consumed is about eight times as great as the bromine in the bromimetric titration. Another method is to

decompose the 8-hydroxyquinoline by heating it to 450°C, and then measure the magnesium as magnesium chloride.

IV. EDTA-ERIOCHROME BLACK T

The dye Eriochrome Black T forms a soluble dye complex with magnesium.¹⁰ Titration with ethylenediamine tetraacetate (EDTA) chelates the magnesium, removing it from ionic form and destroying the dye complex, so that a color change occurs. Another method is to separate magnesium from calcium by differential elution from a Dowex 50® chromatographic column, before titration with EDTA and Eriochrome Black T in a buffer at pH 10.5.¹¹

V. ATOMIC ABSORPTION SPECTROPHOTOMETRY

Walsh¹² in 1955 reported the first application of atomic absorption spectrophotometry to the determination of magnesium. He devised a light source — a hollow cathode lamp with the cathode made of magnesium metal — which emitted a light beam with the wavelengths characteristic for magnesium. This light beam is focused through a flame which contains the unknown concentration of magnesium atoms at ground state. Absorption of a portion of the light beam occurs in the flame in proportion to the concentration of magnesium in solution. The energy of the emerging beam is measured with a galvanometer.¹³

Since the mid-1960s, the commercial availability of atomic absorption spectrophotometers for magnesium analysis has considerably improved the precision and accuracy and simplified this determination.

Atomic absorption spectrophotometry is currently the method of choice for determining magnesium because:

1. It is highly specific, using the 285.2 nm line of a Mg hollow cathode lamp. There is also no interference with other elements.
2. Sample preparation is minimal and for most routine analysis involves only dilution of body fluids.
3. Instrumentation is simple to operate.
4. The method is sensitive enough to insure precision in small samples.
5. The determination can be performed rapidly.^{14,15}

Magnesium is aspirated at a suitable dilution into a three-slotted air-acetylene or air-propane burner, and the absorbance of the Mg hollow cathode lamp output at 285.2 nm is recorded. Lanthanum is used as the internal standard.

The results of atomic absorption spectrophotometry are in good agreement with a standard ammonium phosphate precipitation method, and recovery of Mg added to serum is quantitative.¹⁶ Between-run precision (95% limits) of the method is about \pm 5% for serum values in the normal range.

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APPENDIX 2

OTHER RECENT LEADS CONCERNING THE ROLE OF MAGNESIUM IN BIOLOGIC PROCESSES

The following are several intriguing recent studies suggesting additional functions of magnesium in biologic processes, leads which might warrant further studies.

I. VIRUS ADSORPTION TO CELLS

Eylar, O. R. and Wiseman, C. L., An usually high divalent cation requirement for attachment of West Nile virus to primary chick embryo cells, *Proc. Soc. Exp. Biol. Med.*, 157, 322, 1978.

The concentration of magnesium sulfate plays an important role in the productive adsorption of West Nile virus to chick embryo cells. Mg^{2+} serves as a mechanism by which the negatively charged virus and host cell can come into intimate contact to initiate the adsorption and penetration process.

II. PROLIFERATION OF VIRUS-INFECTED FIBROBLASTS

Balk, S. D., Polimeni, P. I., Hoon, B. S., Lestourgeon, D. W., and Mitchell, R. S., Proliferation of *Rous sarcoma* virus-infected, but not of normal, chicken fibroblasts in a medium of reduced calcium and magnesium concentration, *Proc. Natl. Acad. Sci. U.S.A.* 76, 3913, 1979.

Both normal and *Rous sarcoma* virus-infected chicken fibroblasts proliferate actively in a culture medium containing physiological concentrations of calcium (1.2 mM) and magnesium (0.7 mM). Reduction of either calcium or magnesium concentration resulted in a significant decrease in the proliferation of the normal, but not of the neoplastic fibroblasts. It has been hypothesized that fibroblast replication is initiated when cytosolic concentrations of calcium, magnesium, or both rise above a critical level, and that the autonomous initiation of replication of neoplastic fibroblast is a result of failure of cytoplasmic divalent cation homeostasis.

III. MG EMISSION BY SNAILS: EFFECT ON SCHISTOSOMES

Stibbs, H. H., Chernin, E., Ward, S., and Karnovsky, M. L., Magnesium emitted by snails alters swimming behaviour of *Schistosoma mansoni* miracidia, *Nature (London)*, 260, 702, 1976.

Sponholtz, G. M. and Short, R. B., *Schistosoma mansoni* miracidia: stimulation by calcium and magnesium, *J. Parasitol.*, 62, 155, 1976.

Schistosoma mansoni is a parasitic flatworm transmitted by snails which infects millions of people in tropical countries with poor sanitation. Larval worms, emerging from fresh water snails, penetrate human skin and mature in intestinal veins where they lay eggs. Many eggs remain and damage tissues, while others pass out in excreta and hatch in water, releasing embryos called miracidia. These short-lived, multicellular, ciliated, free-swimming miracidia must find, penetrate, and reproduce within certain snails; larvae infective of man thence emerge. Snails emit substances (miraxones)

which alter the swimming behavior of miracidia and may help them to locate and attack the snails. The biologically active miraxones appear to be the magnesium ion. It is not known how the snails release magnesium.

IV. MG AND THE SKIN

Dimond, R. L., Erickson, K. L., and Wuepper, K. D., The role of divalent cations in epidermolysis, *Br. J. Dermatol.*, 95, 25, 1976.

Very little is known about the factors that maintain epidermal integrity. Divalent cations play a critical role in maintaining skin structure, not only between dermis and epidermis, but also with the epidermis itself. Magnesium rather than calcium is needed to maintain cellular adhesion and to prevent epidermolysis.

V. HYPOMAGNESEMIA AND RADIATION SICKNESS

Tansy, M. F., Nichini, F. M., Baker, H. W., and Chrzyanowski, J., Association of low serum magnesium concentration with severity of gastrointestinal symptomatology in the irradiated patient, *J. Surg. Res.*, 11, 213, 1971.

The blood serum magnesium concentration in patients receiving radiation therapy for ovarian carcinoma were, in all cases, low and associated with the onset of gastrointestinal symptomatology comparable to that observed in animals.

VI. GEL FORMATION BY MAGNESIUM AND AN ANTIASTHMATIC DRUG

Woodward, G. D., McDonald, R., and Cox, J. S. G., Interaction of cromolyn sodium in aqueous solution with magnesium ions, *J. Pharm. Sci.*, 67, 1403, 1978.

The antiasthmatic drug cromolyn sodium is thought to act on the biochemical events in the mast cell that follow the antigen-antibody interaction on the membrane. Thixotropic gels were formed between 4×10^{-2} M cromolyn sodium and 4×10^{-4} M Mg²⁺. The relationship of the changes in viscosity and actual gel formation to the pharmacologic action of this drug is intriguing but poorly understood.

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