

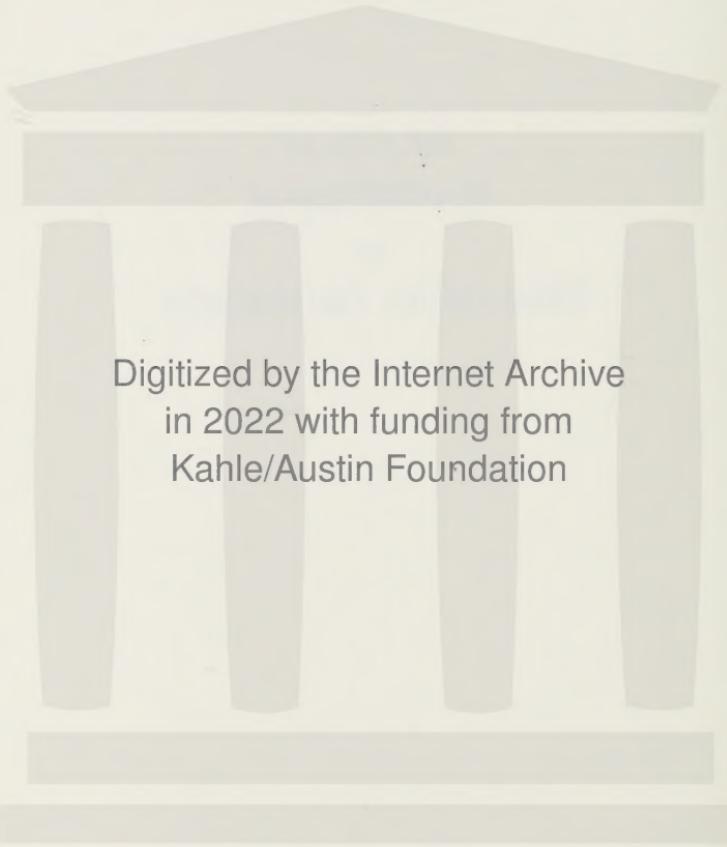








*the role of*  
**MAGNESIUM**  
*in*  
**BIOLOGIC PROCESSES**



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*the role of*  
**MAGNESIUM**  
*in*  
**BIOLOGIC PROCESSES**

*By*

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To

**GORDON MEIKLEJOHN, M.D.,**  
**Gentle Physician**



## PREFACE

WHEN THE RADIOACTIVE isotope of magnesium, Mg<sup>28</sup>, was made available in 1957, it was apparent that this new tool would rapidly extend our knowledge of the role of magnesium in biologic processes. In order to approach the problem of magnesium metabolism in the logical fashion expected of classic experimental medicine, a comprehensive review of the literature was initiated at the University of Colorado. Many of us in the Department of Medicine—and in particular Mrs. Mary Woodruff Snead—spent countless hours poring through references and corraling all pertinent information. Almost two thousand abstracts were copied and indexed. A superficial perusal of this compilation suggested that magnesium was accused, at one time or another, of nearly all medical sins.

The next step was the critical evaluation, selective condensation, and correlation of these reports—and for the crimes of omission and commission in this task I accept responsibility. The present monograph was born of the desire to spare others the tedious and dusty chore of re-reviewing this massive literature.

Having summarized the reported experience with magnesium, we next experimented actively with Mg<sup>28</sup> in the laboratory and on the wards. Some of these studies are summarized in this volume, and a general hypothesis concerning the role of magnesium in biology is suggested. I have attempted to paint a panoramic, perhaps surrealistic, view of the role of magnesium in all life processes, and to suggest a common denominator to explain its seemingly varied functions.

To the U. S. Atomic Energy Commission I owe a considerable debt of gratitude for its continued financial support of our project. The freedom of inquiry encouraged by this organization is in the best tradition of the university. Mrs. Jacqueline Z. Reardon continues to contribute immeasurably to all our efforts. Mrs. Carol

Ouellette typed the many revisions. Dr. Gerald S. Gordon, Miss Eloise L. Rhoades, and many others helped us with the experimental and clinical studies. Mrs. Edward W. Jackson provided the editorial assistance which makes this volume readable.

J. K. A.

## CONTENTS

|  | <i>Page</i> |
|--|-------------|
| <i>Preface</i> .....                               | vii         |
| <i>Chapter</i>                                     |             |
| I. EARLY HISTORY .....                             | 3           |
| The Epsom Spring .....                             | 3           |
| Magnesia .....                                     | 4           |
| Count Rumford (Benjamin Thompson) .....            | 5           |
| Sir Humphry Davy and the Discovery of Magnesium .. | 7           |
| Magnesium in Industry .....                        | 10          |
| Magnesium in Organic Chemistry .....               | 11          |
| Richard Willstatter and Chlorophyll .....          | 12          |
| II. EARLY BIOLOGIC STUDIES .....                   | 15          |
| Mechanism of Purgative Action .....                | 15          |
| First Physiologic Studies .....                    | 16          |
| The Anesthetic Effect of Magnesium .....           | 16          |
| Therapeutic Applications .....                     | 18          |
| III. EXPERIMENTAL MAGNESIUM DEFICIENCY .....       | 21          |
| Clinical and Chemical Changes .....                | 21          |
| Changes in the Bone and Soft Tissues .....         | 23          |
| Cellular Changes .....                             | 25          |
| Pathologic Findings .....                          | 25          |
| Mitochondrial Changes .....                        | 26          |
| IV. MAGNESIUM AND VETERINARY MEDICINE .....        | 29          |
| Grass Staggers .....                               | 29          |
| Clinical Picture and Laboratory Findings .....     | 29          |
| Epidemiology .....                                 | 30          |
| Abnormality in Magnesium Metabolism .....          | 31          |
| Other Syndromes Associated with Hypomagnesemia ..  | 31          |
| Manganese and Its Relationship to Magnesium .....  | 32          |

| <i>Chapter</i>   |  | <i>Page</i> |
|--|--|-------------|
| V. HIBERNATION   |  | 35          |
| Description  |  | 35          |
| Role of Magnesium                                      |  | 36          |
| Artificial Production                                  |  | 37          |
| Metabolic Changes                                      |  | 38          |
| Cold Acclimation                                       |  | 39          |
| VI. THE MEASUREMENT OF MAGNESIUM IN BIOLOGIC MATERIALS |  | 42          |
| Magnesium Ammonium Phosphate                           |  | 42          |
| 8-Hydroxyquinoline                                     |  | 44          |
| Titan Yellow   |  | 44          |
| EDTA-Eriochrome Black T                                |  | 44          |
| Flame Spectrophotometry                                |  | 45          |
| Electrochemical Determination                          |  | 45          |
| Fluorometric Analysis                                  |  | 45          |
| VII. THE ROLE OF MAGNESIUM IN BIOCHEMICAL PROCESSES    |  | 49          |
| Carbohydrate Metabolism                                |  | 49          |
| Glycolysis   |  | 49          |
| Aerobic Metabolism                                     |  | 49          |
| Lipid Metabolism                                       |  | 49          |
| Protein Metabolism                                     |  | 50          |
| The Energy Cycle on Earth                              |  | 51          |
| VIII. THE ROLE OF MAGNESIUM IN HUMAN DISEASE           |  | 53          |
| Changes in Serum Magnesium Concentration               |  | 53          |
| Clinical Symptoms and Signs of Magnesium Deficiency    |  | 54          |
| Response to Magnesium Therapy                          |  | 54          |
| Clinical Syndromes with Hypomagnesemia                 |  | 55          |
| Human Magnesium Deficiency Tetany                      |  | 55          |
| Hyperaldosteronism                                     |  | 55          |
| Tetany Following Parathyroidectomy                     |  | 55          |
| Metabolic Balance Studies                              |  | 55          |
| Magnesium Conservation                                 |  | 56          |
| Experimental Production of Magnesium Deficiency        |  | 56          |
| IX. RADIOMAGNESIUM                                     |  | 60          |
| X. DISTRIBUTION OF MAGNESIUM IN THE HUMAN BODY         |  | 62          |

| <i>Chapter</i>   |   | <i>Page</i> |
|--|---|-------------|
|  | Total Body Content .....                            | 62          |
|  | Bone .....  | 62          |
|  | Muscle .....  | 62          |
|  | Tissue Concentrations .....                         | 63          |
|  | Content of Various Secretions and Fluids .....      | 63          |
| XI. DAILY INTAKE, GASTROINTESTINAL ABSORPTION, AND RENAL EXCRETION ..... |   | 65          |
|  | Daily Intake .....                                  | 65          |
|  | Gastrointestinal Absorption .....                   | 65          |
|  | Renal Excretion .....                               | 66          |
| XII. MAGNESIUM IN THE BLOOD AND CEREBROSPINAL FLUID .....                |   | 68          |
|  | Serum Magnesium Concentration .....                 | 68          |
|  | Total Extracellular Content .....                   | 68          |
|  | Magnesium in the Red Cells .....                    | 69          |
|  | The Nature of Magnesium in Serum .....              | 70          |
|  | Adsorption with Barium Sulfate .....                | 71          |
|  | Ultrafiltrable Magnesium in Thyroid Disorders ..... | 71          |
|  | Ultrafiltrable Magnesium in Other Diseases .....    | 72          |
|  | Magnesium in the Cerebrospinal Fluid .....          | 73          |
|  | Studies with Mg <sup>28</sup> .....                 | 74          |
|  | Lack of In Vitro Binding to Plasma Proteins .....   | 74          |
|  | Lack of In Vivo Binding to Plasma Proteins .....    | 74          |
|  | Dialysis Studies .....                              | 74          |
|  | Ultrafiltration .....                               | 74          |
|  | Electrophoresis .....                               | 75          |
| XIII. THE PLASMA CLEARANCE AND TISSUE UPTAKE OF MAGNESIUM .....          |   | 77          |
|  | Early Studies with a Magnesium Load .....           | 77          |
|  | Tracer Studies with Mg <sup>28</sup> .....          | 78          |
|  | In Rabbits .....                                    | 78          |
|  | In Human Subjects .....                             | 80          |
|  | In Rats .....                                       | 81          |
|  | In Dogs .....                                       | 82          |
|  | In Sheep and Lambs .....                            | 83          |
|  | Magnesium Equilibrium in Muscle .....               | 84          |

| <i>Chapter</i>   |  | <i>Page</i> |
|--|--|-------------|
| Summary .....  |  | 84          |
| <b>XIV. FACTORS INFLUENCING THE TISSUE UPTAKE OF MAGNESIUM .</b>                     |  | <b>87</b>   |
| Alteration of Carbohydrate Metabolism .....  |  | 87          |
| Effect of Insulin and Glucose on the Plasma Clear-<br>ance of Mg <sup>28</sup> ..... |  | 87          |
| Effect of Insulin and Glucose on the Tissue Uptake<br>of Mg <sup>28</sup> .....      |  | 88          |
| In Vitro Studies .....   |  | 89          |
| Alloxan Diabetes .....   |  | 89          |
| Iodoacetate In Vivo .....  |  | 90          |
| Iodoacetate In Vitro .....   |  | 90          |
| Cortisone .....  |  | 90          |
| Pyridoxine and Desoxypyridoxine .....  |  | 91          |
| Digitoxin .....  |  | 91          |
| Uncoupling Agents .....  |  | 92          |
| Thyroxine and Propylthiouracil .....   |  | 92          |
| Sodium Salicylate .....  |  | 93          |
| 2,4-Dinitrophenol .....  |  | 94          |
| Anabolic Agents: Testosterone and Somatotropin .....                                 |  | 94          |
| Catabolic Agents .....   |  | 95          |
| Inhibition of Mitosis by Colchicine .....  |  | 95          |
| X-irradiation .....  |  | 95          |
| Summary .....  |  | 97          |
| <b>XV. THE NATURE OF MAGNESIUM IN BONE .</b>   |  | <b>99</b>   |
| Skeletal Magnesium as a Body Reserve .....   |  | 99          |
| In Vivo Studies on Radiomagnesium Exchange .....                                     |  | 99          |
| Relation of Age to Radiomagnesium Exchange .....                                     |  | 100         |
| In Vitro Studies on Radiomagnesium Exchange .....                                    |  | 100         |
| Methods .....  |  | 101         |
| Comparison of Relative Activity In Vivo and In Vitro                                 |  | 101         |
| Effects of Drugs .....   |  | 102         |
| Effects of Physical Agents and Metabolic Inhibitors                                  |  | 102         |
| Effects of Plasma, Albumin, and Globulin .....                                       |  | 103         |
| Summary .....  |  | 103         |

*Chapter*

|   |       |
|---|-------|
| XVI. THE ATOMIC STRUCTURE OF MAGNESIUM AND ITS ROLE IN<br>THE LIFE PROCESSES, WITH SUGGESTIONS FOR FUTURE STUDIES | 105   |
| Relation of the Atomic Structure of Magnesium to Com-<br>plex Formation .....                                     | 105   |
| Complexing with Water .....   | 106   |
| Areas Where Further Studies are Needed .....  | 106   |
| How the Body Obtains and Uses Magnesium .....   | 107   |
| Gastrointestinal Absorption .....   | 107   |
| Transport .....   | 107   |
| Bone Store .....  | 107   |
| Clinical Problems .....   | 108   |
| Conditions Producing Magnesium Deficiency ..  | 108   |
| Hypermagnesemia .....   | (109) |
| The Role of Magnesium in Biochemistry .....   | 110   |
| Tissue Uptake of Mg <sup>28</sup> as an Index to the Functional<br>Integrity and Activity of Cells .....          | 110   |
| Conclusion .....  | 111   |
| <i>Index</i> .....  | 113   |



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*in*  
**BIOLOGIC PROCESSES**



## *Chapter I*

### EARLY HISTORY

#### THE EPSOM SPRING (1, 2)

EPSOM IS A LARGE village in the County of Surrey, located seventeen miles south of London. During a drought in the summer of 1618, Henry Wicker discovered on the common at Epsom a small hole filled with water, which he enlarged for the purpose of watering cattle. To his surprise, none of his thirsty animals would drink there. Soon thereafter it was found that the bitter-tasting principle in this water healed external ulcers. In 1620, the Lord of the Manor of Epsom enclosed the well with a wall, and erected a shed to shelter sickly visitors who came to take of the water internally. For the first ten years, only the local inhabitants availed themselves of the mineral water.

Lord Dudley North, laboring under a melancholy disposition, drank the mineral water as a medicine and found it to be an effective purgative. Many others obtained similar results. "Epsom water" thus gained fame as an internal remedy and purifier of the blood. It is said that persons of "quality," including Maria de Medici, mother-in-law of King Charles I, were benefited. During the reign of Charles II (1630-1685), Epsom became the fashionable resort of the richer citizens of London. Samuel Pepys recorded in his *Diary* that on a July morning in 1663 he went "up to the Wells" to join "a great store of citizens." By 1690, so many visitors came to Epsom to drink the water that Mr. Parkhurst, Lord of the Manor at that time, enlarged the building at the wells by erecting a ballroom seventy feet long, with other conveniences. Epsom water was sent to those unable to visit the village.

In 1695, Dr. Nehemiah Grew (3) separated the solid Epsom salt in quantity from the natural water and recognized magnesium sulfate as one of the essential constituents. After this, "Epsom salt" or "*sal anglicum*," as it was called on the Continent, sold for 5 shillings an ounce. Dr. Grew conceded that Epsom salt was indeed

unique, differing in its actions, nature, and species from all other salts then known. An old rhyme, purportedly an epitaph, has been quoted as follows (2) :

Here lies I and my three daughters  
Died from drinking the Cheltenham waters;  
If we had stuck to Epsom salts  
We shouldn't be lying in these cold vaults.

At its zenith, Epsom attracted daily as many as 2,000 persons, who came to drink the water or divert themselves (4, 5). Between 1704 and 1715, however, Epsom gradually lost its reputation, apparently because of the knavery of a Mr. John Livingstone. He came to Epsom as an apothecary about 1690, and his practice soon made him wealthy. In 1706 he set himself up as a doctor, purchased some land in Epsom, sank a new well, and erected more buildings near by than were present at the original well. His buildings included an assembly room for dancing and music. He called his establishment the New Wells, and eventually acquired the lease of the old well and had it locked up. Unfortunately, the waters of the new well did not possess medicinal properties. Livingstone died in 1727, but by this time Epsom was doomed as a spa. Though the old well was reopened, it never again became popular.

"Preparations of Magnesia" were advertised as being made from the actual waters of the Epsom well. In the year 1700, George and Francis Moult erected a factory for the production of magnesium sulfate from a spring at Shooter's Hill near London. Then came a large-scale production of "artificial Epsom salt" from the mother liquors left after the preparation of sea salt. At Portsmouth, sodium sulfate (Glauber's salt) was prepared by adding sulfuric acid to the liquors left after the purification of sea salt imported from Portugal and Spain. This too was fraudulently sold as Epsom salt. In 1717, Dr. Friedrich Hoffmann found that the waters of the Seidlitz spring in Germany contained magnesium sulfate. Thus, the terms *Epsom salt* and *Seidlitz powder* were used to designate the same substance long before its composition was known.

## MAGNESIA

During the decline of Epsom, Count di Palma, "a citizen of Rome," had prepared a white powder, "magnesia alba" or Count

di Palma's powder, which he claimed was a panacea for all bodily ailments (4). Di Palma induced a canon of Rome to dispense the material for him. Its composition was kept a secret until 1707, when Michael Bernhard Valentini of Giessen divulged that it was a by-product of the preparation of nitre. Friedrich Hoffmann (1660-1742) then brought a little chemistry into the mystery. By adding potassium carbonate to magnesium chloride (the mother liquor left from the preparation of nitre) he formed a precipitate of magnesium carbonate (magnesia alba). He also distinguished chemically between magnesia alba and "calcareous earth," or calcium carbonate.

It is not clear exactly how the term *magnesia* originated. Magnesia is a district in Thessaly near which deposits of native magnesium carbonate (magnesite) are found. It was also the name of two cities in Asia Minor where magnetic iron ore was first discovered. (The Greeks had previously used the work *magnes* for magnetic iron ore or pyrolucite.) Three minerals were given the name *magnesia*: magnetite, "magnesia nigra" or manganese dioxide, and the white magnesium carbonate itself. It is apparent that the identification of minerals prior to 1700 was in a state of confusion.

The exact nature of magnesia was clarified in 1775 by Dr. Joseph Black of Edinburgh. He demonstrated the presence of carbonate in the compound by expelling from it an "air" which he recognized as the "gas Sylvestre" prepared by Van Helmont, and known now as carbon dioxide. He also distinguished between magnesia and lime, another alkaline earth.

Until the beginning of the nineteenth century considerable confusion existed concerning the chemical nature of the common alkalis. A strange set of circumstances led to the discovery of many alkali and alkaline earth elements. History records that in 1800 a Count Rumford established in London the Royal Institution, at which the elements sodium, potassium, barium, and magnesium were discovered by Sir Humphry Davy.

#### COUNT RUMFORD (BENJAMIN THOMPSON) (6)

Count Rumford (1753-1814) was born Benjamin Thompson at Woburn, Massachusetts, on March 26, 1753. The Thompson family had emigrated from England about the middle of the previ-

ous century, and were moderately wealthy farmers. At the age of 14 Benjamin knew enough about the sciences and higher mathematics to be able to predict a solar eclipse within four seconds. He was apprenticed to a storekeeper in Salem in 1776, and moved shortly thereafter to Boston, where he served as an assistant in another store. At nineteen, he married the widow of Col. Benjamin Rolfe, a woman fourteen years his senior and possessed of considerable property. He became acquainted with Governor Wentworth of New Hampshire, who sent him to England with dispatches. (Thompson apparently had Tory leanings, and was distrusted by friends of the American cause.) While in England he continued his scientific pursuits, and in 1779 was elected a Fellow of the Royal Society of London.

After America had won her independence from England, Benjamin Thompson became acquainted with Prince Maximilian of Bavaria, and entered the civil and military service of that state. He remained at Munich for eleven years as minister of war, minister of police, and grand chamberlain to Prince Maximilian. He contributed considerably to the welfare of Bavaria by reorganizing its army, improving the condition of the industrial classes, and suppressing mendicity. As his reward, the Pope, in 1791, made him a count of the Holy Roman Empire. He chose the title "Rumford" from the original name of the town of Concord, New Hampshire, where Thompson considered his good fortune to have begun.

The new count's wife died in 1792, and in the late seventeenth-nineties he returned to London. Continuing his early interest in science, Rumford was the first to state and demonstrate by experiments that heat is a form of energy; thus he did much to overthrow the phlogistic theory then prevalent. In 1799, with Sir Joseph Banks, he projected the establishment of the Royal Institution, which received its charter from George III in 1800. This institution was to have a profound effect upon the development of science in general, and of chemistry in particular. It was here that Humphry Davy, Michael Faraday, and others made their discoveries.

Count Rumford lived in London until 1802, then moved to Paris, where he married the wealthy widow of Lavoisier, the celebrated chemist.

**SIR HUMPHRY DAVY AND THE DISCOVERY  
OF MAGNESIUM (4, 6)**

Humphry Davy was born on December 17, 1778, at Penzance in Cornwall. His father was a wood carver. As a student at the grammar schools of Penzance and Truro, he showed little taste for scientific pursuits. His best work was done in translating the classics into English verse. After his father's death in 1794, Humphry was apprenticed to Bingham Borlase, a surgeon-apothecary at Penzance; under him he studied metaphysics, ethics, and mathematics. Only in 1797, after reading treatises by Nicholson and Lavoisier, did he become interested in chemistry. He began performing his own experiments with whatever crude apparatus he could secure.

About this time he became acquainted with Davies Giddy (afterwards Gilbert), who was president of the Royal Society from 1827 to 1831. Giddy recommended Davy to Dr. Thomas Beddoes, who in 1798 was establishing his Medical Pneumatic Institution at Bristol to investigate the medicinal properties of various gases. Davy, released from his indenture, was made superintendent of this institution. He was most happy in sharing the delightful home life of Dr. Beddoes and the social contacts with such distinguished literary men as Southey and Coleridge. One of his discoveries at the Pneumatic Institution was the anesthetic property of nitrous oxide, "laughing gas." This discovery, made on April 9, 1799, brought him into prominence.

Count Rumford at this time was looking for a lecturer in chemistry for the Royal Institution, and in 1801 he engaged Davy as assistant lecturer in chemistry and director of the laboratory. Almost at once he was promoted to lecturer, and subsequently—on May 31, 1802—to professor. It was written of his first lecture: "Sir Joseph Banks, Count Rumford and other distinguished philosophers were present. The audience was highly gratified, and testified their satisfaction by general applause. Mr. Davy, who appears to be very young, acquitted himself admirably well. From the sparkling intelligence of his eye, his animated manner, and the tout ensemble, we have no doubt of his attaining distinguished excellence" (7).

One of Davy's first lectures was on the chemical principles of

tanning, and subsequent lectures dealt with agricultural chemistry. His chief interest at the Royal Institution, however, was with electrochemistry. Some of his early studies showed that pure water, when electrolyzed, does not release acid or alkali. He proposed an electrical theory of chemical compounds which gave an orderly presentation of a host of complex facts.

At that time sodium hydroxide and potassium hydroxide were considered to be elements. In October, 1807, Davy succeeded in decomposing these compounds by electrolysis, thus isolating pure potassium and sodium. This feat created a scientific furore. According to his cousin, Edmund Davy, who was then his laboratory assistant, Humphry Davy was so delighted with his achievement that he danced about the room in ecstasy.

Davy served as secretary of the Royal Society from 1807 until 1812, and during this period conducted his studies on the alkaline earth compounds. After many unsuccessful attempts, and following a suggestion contained in a letter from Professors Berzelius and Pontin of Stockholm, Davy succeeded in producing the amalgams of calcium, barium, strontium, and magnesium. He then isolated the metals by distilling off the mercury. As in the case of the alkali metals, he named these alkaline-earth metals after their oxides (baryta, strontia, chalk, and magnesia), calling them *barium*, *strontium*, *calcium*, and *magnium*. At that time, the word *magnesium* was used to denote manganese. *Magnium* has long been forgotten, however, and the term *magnesium* has been adopted by general usage for the element derived from magnesia.

Davy gave the following description of the characteristics of magnesium: "The metal from magnesia seemed to act upon the glass, even before the whole of the quicksilver had distilled from it. In an experiment, in which I stopped the process before the mercury was entirely given off, it appeared as a solid, having the same whiteness and lustre as the other elements of the earths. It sunk rapidly in water, although surrounded by globules of gas, producing magnesia, and quickly changed in air, becoming covered with a white crust, falling into a fine powder, which proved to be magnesia" (8).

On April 9, 1812, after eleven years at the Royal Institution, Davy gave his farewell lecture as professor of chemistry,\* though

\*He was succeeded by William Thomas Brande, and then by Michael Faraday.

he continued his association with the institution as an honorary professor. In that same month, he was knighted and married to Mrs. Apreese, daughter and heiress of Charles Kerr of Kelso. During a subsequent tour of Europe, Sir Humphry proved that the newly discovered material, iodine, is an element and that diamond is composed of pure carbon.

Upon his return to England in 1815, Davy constructed a miner's safety lamp, which bears his name. Because he took out no patent, the Newcastle coal owners, in September, 1817, presented him with a dinner service of silver plate. Davy's will directed that this service should pass to his brother, Dr. John Davy, on whose decease it was to be melted and sold. The proceeds were to go to the Royal Society "to found a medal to be given annually for the most important discovery in chemistry anywhere made in Europe or Anglo-America." The silver procured 736 pounds, and the interest on the sum is expended on the Davy medal.

In 1818, Davy received a baronetcy for his service to industry. In 1820, he became president of the Royal Society, but his personal qualities did not make for success in that office and he resigned in 1826. During his latter years he directed his attention to various subjects, chiefly electromagnetism; but his efforts were less successful than his earlier experiments. On May 29, 1829, Sir Humphry Davy died at Geneva, where he is buried.

It is said that he was of a sanguine, although somewhat irritable, temperament, and that he displayed characteristic enthusiasm and energy in everything that he pursued. He was a brilliant scientist in every sense of the word, and kept meticulous records. Davy was so thorough and painstaking in his work that he almost invariably rehearsed his lectures before his assistants.

Davy possessed a versatile mind, and continued to write poetry throughout his life. The poet Coleridge declared that if Davy "had not been the first chemist, he would have been the first poet of his age," and Southey said that "he had all the elements of a poet; he only wanted the act." He was extraordinarily popular as a lecturer, and Coleridge went to hear him "to increase his stock of metaphors." Davy once gave the following vivid picture of his beloved rugged coast of Cornwall and lofty St. Michael's Mount, a gigantic rock surmounted by an ancient turreted castle (7) :

The sober eve with purple bright  
Sheds o'er the hills her tranquil light  
    In many a lingering ray:  
The radiance trembles on the deep  
Where rises rough thy rugged steep,  
    Old Michael, from the sea.  
Around the base, in azure pride,  
Flows the silver-crested tide,  
    In gentle winding waves;  
The Zephyr creeps thy cliffs around,—  
Thy cliffs, with whispering ivy crowns,—  
    And murmurs in thy caves.

Occasionally his ambition betrayed him into petty jealousy. Although he trained Michael Faraday, a journeyman bookbinder, to succeed him at the Royal Institution, he became jealous of Faraday's superior performance as a lecturer and tried, without success, to prevent Faraday's election as a Fellow of the Royal Society in 1824. However, to use a phrase which he himself often employed in connection with his invention of the miner's lamp, he was conscious of the "cause of Humanity."

### MAGNESIUM IN INDUSTRY (9, 10, 11)

In 1828, the French chemist, Antoine Alexandre Brutus Bussy, submitted to the Academie Royale a sample of magnesium which he had produced by reducing anhydrous magnesium chloride with potassium. In the same year Wohler synthesized urea from ammonium cyanate, and thus broke down the distinction between organic and inorganic chemistry. Bunsen in 1842 and Matthiessen in 1856 developed other methods for purifying magnesium, but it was not until 1863 that anyone attempted to produce magnesium metal commercially. About the turn of the century the production of magnesium by electrolysis of the anhydrous chloride began in Germany.

During World War I, the demand for magnesium—primarily for use in photography and in incendiary bombs—increased. After the war it declined to such an extent that in 1936 the total world output of magnesium metal was less than 2,230 tons, and in the

United States only one firm—the Dow Chemical Company of Midland, Michigan—was producing it. During and since World War II, magnesium has been in great demand for structural material and for alloys. Because of its lightness and abundance, it has become the glamour metal of the space age. Aluminum, the nearest rival for structural purposes, is one and a half times as heavy. In 1940, magnesium was first processed from sea water at Freeport, Texas, by the Dow Chemical Company.

### MAGNESIUM IN ORGANIC CHEMISTRY (9)

Recognition of the important role of magnesium was as slow to come in the fields of organic chemistry and biology as in the industrial field. Barbier was the first to recognize the importance of magnesium in the field of organic chemistry. In 1900, his student, Victor Grignard, realized the great potentialities of the reaction which later came to bear his name, and continued the work into the Twentieth Century.

In this almost unlimited group of processes, magnesium is bound into compounds of the type,  $\text{RMgX}$ , where R is an organic radical and X is chlorine, bromine, or iodine. Such organomagnesium compounds are primarily important as intermediates in the synthesis of other compounds—for example, in the conversion of aldehydes and ketones to alcohols. In a series of energetically prosecuted studies, Grignard laid the foundation for much of our present knowledge of the reagent and its reactions. Although several other organometallic compounds may be formed, the Grignard reagents are the most important commercially because of the ease with which magnesium reacts with organic halides and other compounds to form organomagnesium compounds, and because of the great versatility of these compounds as intermediates in different reactions. For his meticulous work in providing organic chemists with such a useful tool for synthesis, Grignard was awarded the Nobel Prize in 1912.

While the many reactions in which the Grignard reagents participate have been the subject of much investigation, relatively little effort has been devoted to attempts to understand why magnesium is so effective in this compound formation.

**RICHARD WILLSTATTER AND CHLOROPHYLL**

Also poorly understood is the function of magnesium in chlorophyll, the organic compound which imparts the green color to plants and is essential for photosynthesis. The chemical structure of chlorophyll was unraveled by Richard Willstatter (6, 7), the German organic chemist who was the foremost investigator of the constitution of natural products during the first quarter of the Twentieth Century. Born at Karlsruhe on August 13, 1872, he studied at Munich under Adolph von Baeyer. In 1905, he became a professor at the University of Zurich. Willstatter began his career with an investigation of plant alkaloids, determining, at the age of twenty-six, the structure of tropine, atropine, and cocaine. Three years later he had synthesized tropine, tropilidene, and tropane. There followed a penetrating study of the quinones, culminating in the determination of the structure of aniline black. Next came the work on blood pigments and chlorophyll.

It was characteristic of Willstatter that he attempted only difficult problems, and once remarked that the greatest joy in research came from overcoming obstacles. He also stated that the highest goal of science is the improvement of the lot of humanity; consequently, he was particularly proud of the synthesis of hypnotics and narcotics, which included cocaine and tribromoethanol (Avertin).

During the period 1904 to 1912, Willstatter demonstrated that the structure of chlorophyll consisted of the porphin system, the central magnesium atom with its complex linkage, and the phytol radical. For this work he was awarded the Nobel Prize in 1915. Willstatter later determined the components of the porphin system in his work on the degradation of the chlorophylls.

Willstatter was director of the Kaiser Wilhelm Institute in Berlin from 1912 to 1916, and then succeeded his old teacher, von Baeyer, at the University of Munich. He resigned in 1925 because of anti-Jewish pressure, but continued his work privately—first in Munich and later in Switzerland. He died in forced exile on August 3, 1942.

The discovery that magnesium, bound in the form of a metallic complex, forms an essential component of the molecules of the

natural chlorophylls, caused great surprise at the time. Willstatter showed that magnesium can be split off from chlorophyll by dilute acids, and that this reaction is reversible, since it is possible to re-introduce the magnesium by means of the Grignard reagent.

The exact role of magnesium in chlorophyll, in the assimilation of carbon dioxide by plants, and in photosynthesis remains to be clarified. As a broad generalization (12), it may be stated that organomagnesium compounds appear to play key roles in synthetic reactions, both in organic chemistry and in biologic systems. The exact function and nature of their role remain obscure. Perhaps the most suggestive idea from the biologic viewpoint is that of Willstatter on chlorophyll: that the magnesium linkage in chlorophyll may resemble that present in the Grignard reagents, and hence may be a significant point of reaction with carbon dioxide in photosynthesis. One may then speculate that some of the reductions and condensations necessary for carbohydrate synthesis may also involve Grignard-like reactions. To continue speculatively, the essential role of magnesium in many enzymatic reactions *in vivo* may be as a point of reaction in Grignard-like transformations.

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## *Chapter II*

### **EARLY BIOLOGIC STUDIES**

#### **MECHANISM OF PURGATIVE ACTION**

**A**T THE TIME THAT Richard Willstatter was demonstrating the presence of magnesium in the chlorophyll molecule, magnesium was being used clinically as a purgative on a more or less empiric basis. The discovery of the phenomenon of osmosis in 1828 (1) afforded the first scientific explanation for the action of saline cathartics in general. In 1854, Colin (1) performed the classic experiment of injecting saline solution into an isolated loop of intestines and demonstrating the increase in volume of the aqueous content. By 1883, Hay (2) had shown that a saline purgative always excites more or less secretion from the alimentary canal, the amount varying with the nature of the salt and the strength of its solution. Because of its low diffusibility, salt also impedes the absorption of the secreted fluid. As a result of the stimulated secretion and impeded absorption, fluid accumulates in the intestinal lumen. Purgation occurs when the accumulated fluid, partly because of "ordinary dynamical loss" (2) and partly because of gentle stimulation of the peristaltic movements excited by distention, reaches the rectum.

After the appearance of Hay's article there seems to have been some dispute as to whether the purgative effect of magnesium sulfate results entirely from local action within the lumen or is secondary to its absorption into the blood stream. That such controversy should arise is understandable in view of the inadequacy of the methods then available for chemical analysis of magnesium in biologic material. The development of an accurate method for estimating the amount of magnesium in small quantities of serum made it possible to obtain direct evidence that the purgative action of magnesium salts is independent of the absorption of magnesium into blood. In 1925, Cohen (3) showed that the oral administration of purgative doses of magnesium sulfate is not accompanied

by any rise in the magnesium content of the blood serum. He next demonstrated that the intramuscular injection of magnesium sulfate increases the magnesium content of the blood serum by 50 to 100 per cent, but that this rise is not accompanied by the characteristic purgative effect which follows the oral administration of this salt.

### FIRST PHYSIOLOGIC STUDIES

As far as can be ascertained, the first known physiologic studies with magnesium sulfate were carried out by Jolyet and Cahours (4) in 1869. In dogs, intravenous injections of the drug initially increased the respiratory rate and subsequently produced respiratory paralysis. In dogs under the influence of magnesium, direct stimulation of the sciatic nerve resulted in little muscular response, whereas direct stimulation of the muscle produced a normal response. Magnesium sulfate, therefore, was thought to have only a peripheral paralyzing action similar to that of curare.

### THE ANESTHETIC EFFECT OF MAGNESIUM

More than thirty years later a series of pharmacologic studies by Meltzer and Auer (5) extended our understanding of the metabolism of magnesium. In 1898, Samuel J. Meltzer, head of the department of physiology and pharmacology at the Rockefeller Institute for Medical Research, founder and first president of the Society for Experimental Biology and Medicine, observed that two drops of a 5 per cent 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, in the first of many publications on this subject, Meltzer and Auer (5) reported that the subcutaneous injection of magnesium sulfate can induce general anesthesia in laboratory animals. They stated that the injection of a proper dose of a solution of magnesium sulfate causes the animal to lose all reflexes and signs of sensibility for some time, while the respiration remains intact. They also made

the general statement that the salts of magnesium are capable of inhibiting the entire nervous system, and assumed, in particular, that their inhibitory effects on the central nervous system are capable of producing anesthesia. Meltzer and his co-workers investigated the effects of different modes of administration—intravenous, intraspinal, subcutaneous, and intramuscular—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 subcutaneous injection of magnesium sulfate never has any purgative effect; (3) magnesium is excreted to a great 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 his co-workers (6) labored for many years to establish magnesium sulfate as an anesthetic agent. In the first case in which an operation was performed on a patient under magnesium sulfate anesthesia (7) a 6 per cent solution of the drug was introduced into an exposed cubital vein by means of a burette. Shortly after the infusion was started, the patient became hot and flushed; his forehead was covered with beads of perspiration. He did not complain of nausea, but his pulse and respiratory rate were increased. The patient was fully conscious when the operation was started, but sensation was considerably depressed. During the last part of the operation the patient was under practically complete anesthesia, and evidently had no knowledge of what was happening to him. The observations made in this case proved almost conclusively that the injection of magnesium sulfate produces true anesthesia—that is, temporary loss of sensation as well as of consciousness. The margin between the dose which produces

effective anesthesia and that causing respiratory paralysis is 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 (8) reported that one of three patients in whom magnesium sulfate was used as an adjunct to ether anesthesia died with symptoms of acute poisoning. 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 (9) correlated the narcotic effect of magnesium with the serum concentration. 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. per 100 cc. At a concentration of 14 mg. per 100 cc. 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.

### **THERAPEUTIC APPLICATIONS**

It was not until the twentieth century that magnesium was used therapeutically for purposes other than catharsis. In 1906, Blake (10) reported its use in the treatment of tetanus. In that same year, E. Horn (11) used magnesium sulfate to treat eclamptic convulsions, and reported that it reduced both the systolic and the diastolic blood pressure and increased the urinary volume, thus

relieving convulsions and preventing recurrences. This therapeutic effect was rediscovered in 1925 (12, 13), and the treatment of toxemia of pregnancy by the intravenous injection of magnesium sulfate became fashionable at that time.

Soon thereafter the parenteral use of magnesium sulfate was introduced as a treatment for hemorrhagic nephritis in children. The hypotensive action of magnesium in acute glomerulonephritis was attributed to relaxation of vascular spasm (14). Magnesium sulfate also relieved the convulsions of hemorrhagic nephritis by reducing the cerebral edema associated with this condition (15, 16). Soon after an intravenous infusion of magnesium is begun, muscular twitching ceases. Unlike normal children, children with hemorrhagic nephritis experience no diarrhea following the oral administration of magnesium sulfate.

At present the chief indication for the intramuscular or intravenous administration of magnesium sulfate is the presence of convulsions. An isotonic or slightly hypotonic solution of magnesium sulfate—about 2 per cent of the hydrated sulfate,  $MgSO_4 \cdot 7H_2O$ —is both safe and effective in the control of convulsions. In adults about 500 ml. of this solution is injected intravenously during a period of 30 to 50 minutes. The blood pressure is taken at frequent intervals, and the knee jerks and respiratory rate are also observed closely. Any serious respiratory depression will be preceded by suppression of the deep tendon reflexes; hence the knee jerks are a valuable guide in determining the rate of injection. Since patients with normal renal function will excrete the magnesium within a few hours, repeated injections, when required, can be given with impunity to such patients.

Whenever magnesium sulfate is given parenterally, a solution of calcium chloride suitable for intravenous administration should always be available. If respiratory depression should appear, the calcium chloride should be given by a slow intravenous infusion. If calcium is administered too rapidly, it may induce ventricular fibrillation.

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## *Chapter III*

# **EXPERIMENTAL MAGNESIUM DEFICIENCY**

**T**HE STUDIES of Meltzer and Auer were concerned primarily with the anesthetic and neuromuscular effects of magnesium. It remained for other investigators to demonstrate that magnesium is essential for life. The first attempt to deprive an animal of magnesium appears to have been made by Osborne and Mendel (1) in 1918 during the course of a general study of the inorganic elements in the nutrition of the rat. Their "magnesium-free" diet, however, still contained over 100 parts of magnesium per million, and no untoward effects were apparent. In 1926, Leroy (2) kept five mice on a diet containing only 10 parts of magnesium per million, and found that growth ceased within nine to thirteen days and that three of the mice died within thirty-five days. Finally, McCollum and Orent (3) produced acute magnesium deficiency in young rats and dogs with a diet containing only 1.8 parts of magnesium per million. In the rats this magnesium-deficient diet produced acute hyperemia of the skin and loss of hair, tachycardia, and convulsions. Many of the rats died within eleven days. The term *low magnesium tetany* was applied to this syndrome (4).

### **CLINICAL AND CHEMICAL CHANGES**

In experimental animals, magnesium deficiency produces profound changes in the magnesium and calcium content of the body and the blood (3). The onset of the clinical changes is very rapid, being detectable within a few days after the animals are placed on a low-magnesium diet. The first phase of the deficiency, lasting approximately two weeks, is characterized by vasodilatation, hyperemia, and hyperexcitability. In this phase, the plasma level of magnesium drops sharply and then rises to a peak shortly after the onset of hyperexcitability. The second phase is marked by the development of malnutrition, cachexia, and renal damage. During this phase the blood level of magnesium declines at a relatively slow

rate, finally reaching values on the order of 1 mg. of magnesium per 100 cc. of plasma. The magnesium content of the erythrocytes is reduced to about half the normal amount during the early phase of depletion, and remains fairly constant at this low level. In the heart and muscle the calcium content increases by 50 to 100 per cent, and in the kidney it may be as much as 15 times its normal value.

During prolonged depletion, the body content of magnesium is reduced to about two thirds of the normal value, and the body content of calcium is increased by one and a third times. This retention of calcium in the animal body persists over a period of weeks, until nutritive failure causes loss of calcium.

Because of the abnormal deposition of calcium in the soft tissues (5), any factor which would accelerate or intensify disturbances in calcium metabolism might reasonably be expected to have the same effect on the syndrome of magnesium deficiency.

The interaction between magnesium and calcium suggested by these experiments with a low-magnesium diet has been confirmed by the injection of magnesium and calcium compounds into experimental animals (6). The former increased the excretion of calcium salts, while the latter augmented the output of magnesium salts. When calcium compounds were given orally, however, the effect on magnesium excretion was mitigated if a liberal amount of phosphate was administered simultaneously.

The biochemical changes and nutritive failure produced by diets deficient in both calcium and magnesium are more severe than those occurring in magnesium deficiency alone (7, 8). In rats receiving diets of liberal calcium content and optimum vitamin content, 5 mg. of magnesium per 100 Gm. of food was found to be the minimum amount necessary for good growth (6). 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 two or three weeks after birth. It was concluded that the mother's milk must be deficient in magnesium.

Sexually mature rats maintained on a diet containing less than 1.0 mg. of magnesium per 100 Gm. developed within twenty-three

to twenty-eight days lesions of the skin characterized by erythema, purpural hemorrhages, and eschars (9). The addition of magnesium to the diet resulted in recovery. The fact that symptoms are slower to appear in older animals than in young rats probably reflects greater body stores of magnesium in mature animals.

In dogs on a magnesium-deficient diet, nutritive failure occurs by the fifth week (10), when the blood content of magnesium is low. At this time fat clots often appear in the plasma. A marked increase in blood cholesterol is associated with a concomitant decrease in fatty acids, so that the total fat content of the blood remains constant. This persistent increase in total cholesterol is due principally to elevation of the cholesterol ester fraction. Except for a terminal elevation of nonprotein nitrogen, no other blood constituents are changed. The disturbance in blood lipids was considered to be evidence of failing fat metabolism, discernible outwardly as nutritive failure with loss of weight. The terminal rise in nonprotein nitrogen was explained on the basis of augmented protein catabolism following failure of fat metabolism.

### CHANGES IN THE BONE AND SOFT TISSUES

Orent, Kruse, and McCollum (10) induced convulsions in magnesium-deficient animals. During the few seconds that elapsed between application of the stimulus and the beginning of convulsion, the magnesium content of the blood increased, while that of the bone dropped sharply. This rapid mobilization of magnesium is of particular interest in connection with the composition of bone.

Watchorn and McCance (11), in a study of *subacute* magnesium deficiency in rats, used a diet containing 40 parts of magnesium per million. The blood, bone, and teeth became permanently deficient in magnesium, and calcification of the kidney was found. One of the most unexpected features of the experiment was the finding of normal or only slightly subnormal amounts of magnesium in the organs of the deficient animals. The livers actually showed an increase in magnesium content. The magnesium content of the bone, however, was reduced by a third, and that of the teeth by half. The phosphatase content of blood, bone, and kidney was normal.

In both rats (5) and calves (12) on a magnesium-deficient diet, bone magnesium was found to be depleted. The soft tissues, however, were usually not depleted of magnesium even in the most severe cases of magnesium deficiency. Apparently bone magnesium represents a store which can be called upon under conditions of deficiency to supply the needs of the soft tissues. By killing rats at different times after instituting a magnesium-deficient diet, it has been shown that their bone magnesium can be rapidly mobilized (5). In calves with varying degrees of hypomagnesemia, there is a direct relationship between bone and plasma magnesium (12). Shortly before death or the appearance of serious clinical symptoms of magnesium deficiency, calves all showed a magnesium content of about 0.2 to 0.3 per cent in the bone ash instead of the normal 0.75 per cent. The appearance of these values coincided approximately with the first decrease in plasma magnesium (to about 0.3 to 0.5 mg. per 100 ml.).

This relationship not only suggests the existence of an equilibrium between plasma and bone magnesium in hypomagnesemia, but also suggests that normal bone, from a physiologic standpoint, is virtually saturated with magnesium. Under normal conditions, when an excess of magnesium is entering the blood, the concentration of plasma magnesium appears to be regulated chiefly by changes in urinary excretion, with the bone playing little or no part.

Recent studies (13) have suggested that about two thirds of bone magnesium is adsorbed on the surface of the mineral structure of bone, with the remaining third replacing calcium in the phosphate molecule. It seems possible that the adsorbed fraction is the part that is available in magnesium depletion. In this case, a plasma magnesium level of 0.3 to 0.5 mg. per 100 ml. would indicate virtually complete removal of this adsorbed magnesium.

Next to the changes in magnesium metabolism, the outstanding metabolic change in animals on a magnesium-deficient diet is the disturbance in *calcium* metabolism (5). In animals on a diet low in magnesium the bone is unusually heavy and overly abundant in ash, calcium, and phosphorus (10). The increased weight is due largely to excessive deposition of calcium during the first five days

on the diet. Thereafter, the rate of calcium deposition in the bone is almost parallel to that in control animals. The weight and intensive calcification imparted to bone by the initial acceleration in calcium deposition is maintained throughout the survival period.

The metastatic calcification of the kidney occurring in magnesium deficiency is directly related to bone metabolism. The histologic findings suggest that magnesium affects bone metabolism in some manner, and that when this element is deficient, bone is not formed, does not grow, or actually atrophies.

### CELLULAR CHANGES

In rats fed a magnesium-deficient diet leukocytosis developed during the hyperemic phase of the magnesium-deficiency syndrome (14). Mononuclear leukocytes and neutrophils contributed to the leukocytosis, but the *eosinophils* were increased to a greater degree, being more than 1,000 per cent above the normal range.

Degranulation of the *dermal mast cells* during the hyperemic phase of magnesium deficiency is followed by regranulation with the disappearance of hyperemia (15). It has been suggested (14) that the hyperemia may be caused by histamine liberated from mast cells as a result of the sudden deprivation of magnesium.

### PATHOLOGIC FINDINGS

Prolonged magnesium deprivation eventually produces degeneration of the kidneys, manifested histologically by degenerative changes in the tubules and progressive calcification, first in the cortico-medullary zone and later in the cortex (16). The renal tissue content of magnesium, however, remains unaltered (17). In calves on a diet deficient in magnesium, the most consistent renal finding at autopsy was a marked proliferation of fibroblasts and fibrosis of the interstitial tissue, with atrophy and necrosis of the parenchyma (18). Magnesium-deficient rabbits (19) also showed damage in the renal corpuscles and tubules, characterized by degeneration of the tubular epithelium and fibrosis of the cortico-medullary region. The glomeruli were often displaced to the periphery by an amorphous, acidophilic-staining mass of material.

Rats fed low-magnesium diets have shown degenerative changes in the liver (20) and heart (21), as well as in the kidney (22). Young rats on a diet containing 1 to 5 mg. of magnesium per 100 Gm. of food showed neuropathologic alterations in the cerebellar tissue, characterized by a degeneration of the cells of Purkinje (19). The cells showed varying degrees of chromatolysis.

In dairy calves a low blood level of magnesium is usually associated with basophilic, hyaline-like necrosis of collagen and of the yellow, elastic connective-tissue elements in the heart, blood vessels, and spleen, and on the peritoneal and pleural surfaces of the diaphragm (20).

### MITOCHONDRIAL CHANGES

The histopathologic changes just described may be explained by the recent observation (23) that magnesium ions are essential in the maintenance of mitochondrial structure and function.

Histochemical methods used for the demonstration of various dehydrogenase and diaphorase activities (24) revealed intracellular alterations in the rat kidney within the first few days on a magnesium-deficient diet. These alterations were confined mainly to distal segments of the proximal convolutions, and consisted of mitochondrial swelling, closely followed by the appearance of lipid droplets. After nine days on a magnesium-deficient diet, a marked decrease of mitochondrial enzyme activity in the damaged areas initiated intracellular calcification and necrosis. Decrease in plasma magnesium was found to be closely related to an increase in plasma calcium and in the mitochondrial alterations observed. A marked rise of kidney calcium content was not accompanied by visible calcification. No change in renal magnesium content was observed throughout the experiment. The study suggested that mitochondria in the various segments of the nephron differ markedly in their response to injury.

Since the mitochondrial changes observed were accompanied by a low plasma magnesium level, it appears that the kidney cells depend on a constant supply of magnesium. *In vitro* studies have also suggested (25) that the integrity of mitochondria and maintenance of oxidative phosphorylation are dependent on the pres-

ence of a magnesium-ATP complex which is dissolved by an increase in calcium ions. This transformation leads to mitochondrial swelling and loss of coenzymes, with subsequent disruption of the energy-producing mechanisms. A similar type of alteration in mitochondria has been induced *in vitro* by suspending mitochondria in a magnesium-free medium (26).

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## *Chapter IV*

# MAGNESIUM AND VETERINARY MEDICINE

## GRASS STAGGERS

**S**JOLLEMA AND SEEKLES (1) were the first to describe a disease of dairy cows—variously known as grass staggers, grass tetany, lactation tetany, and hypomagnesemia—which has a characteristic train of symptoms very similar to those noted by Kruse, Orent, and McCollum (2) in rats deprived of magnesium. Grass staggers has been a disorder of considerable economic importance to dairymen, especially in Australia, New Zealand, England, and Holland. It generally appears within the first two weeks after cattle have been put out to graze in the spring of the year. It is most prevalent during the period of luxuriant growth in spring pastures, although it occasionally occurs in stalled animals in mid-winter.

### Clinical Picture and Laboratory Findings

Uncomplicated grass staggers or grass tetany is sudden in onset (3). Affected cattle often die before any symptoms have been noticed. The disease is characterized by nervousness, anorexia, muscular twitchings, unsteady gait, increased salivation and frothing, and tetany of the muscles of the tail. The development of tonic-clonic convulsions is followed by coma, and frequently by death.

The serum calcium concentration is below normal (6.7 mg. per 100 ml.), and the serum magnesium concentration is markedly reduced, being as low as 0.21 to 1.01 mg. per 100 ml. of serum. The fall in serum magnesium concentration is frequently very rapid; in extreme cases, it may drop to less than 0.7 mg. per 100 ml. of serum within two days after the animal begins grazing in the pasture (4).

In cows sensitized to grass tetany by previous attacks (5), a high potassium intake induced clinical tetany. A low serum concentration of magnesium did not necessarily cause an attack, and no

significant increase in serum phosphorus was observed during an attack.

Some authorities believe that the intravenous injection of a mixture of calcium chloride and magnesium chloride, if given in time, may cure the disease. In Great Britain, rapidly growing nitrogen-stimulated pastures are given heavy top dressings with calcined magnesite in order to prevent grass staggers.

Grass staggers thus appears to be associated in some way with disturbed magnesium and calcium metabolism—although the presence of a causal relationship between hypomagnesemia and grass tetany has not been proved. While it is true that low serum magnesium concentrations have been found in all animals with symptoms of grass tetany, other animals in the same herd may have still lower values in the winter without any signs of tetany (6, 7).

### Epidemiology

Although grass staggers in New Zealand (3), as elsewhere, is a seasonal disease, the most characteristic feature of the incidence in that country is the increase which occurs in unfavorable seasons. Data from New Zealand indicating that underfeeding increases the susceptibility of cows (3) appear at variance with other reports. In cows made susceptible by underfeeding, a number of factors seemed to precipitate the onset of the disease: (1) the act of calving; (2) grazing in short, protein-rich pastures, particularly on highly fertile soils; (3) sudden cessation of feeding out of hay; and (4) short periods of cold, wet weather.

Cunningham (8) attempted to determine whether a dietary deficiency of magnesium actually exists in the areas where the syndrome occurs. He found no shortage of magnesium in the pastures. Because the magnesium content of grass is normally so much above the known mineral requirement, it seems unlikely that a dietary deficiency of magnesium can occur in grazing animals (3). Furthermore, milk from the affected animals contained normal amounts of magnesium, and no magnesium deficit was found in bone or in the soft tissues; the magnesium content of the urine, however, was greatly reduced.

### Abnormality in Magnesium Metabolism

These findings suggest that grass staggers is not caused by a dietary deficiency of magnesium; yet it can apparently be cured or prevented by additional supplies of magnesium. To explain this seemingly contradictory situation, Cunningham (8) suggested the possibility of a temporary impairment in magnesium absorption, or the presence of a toxic principle, elaborated by plants or animals, which has a specific effect on blood magnesium and is inhibited by the administration of additional magnesium.

Rook (4) has suggested that the net uptake of magnesium from the intestine may be reduced with the change from winter ration to grazing. If this is the case, the rapid development of tetany implies that the milking cow has little ability to mobilize the considerable amount of magnesium present in the bone and soft tissues, and that the freely available body store of magnesium must be small in relation to the requirements of a lactating animal. Apparently such animals must depend on a continuous dietary supply for the maintenance of a normal concentration of magnesium in the blood.

Field (9) has investigated the effect of abrupt dietary changes on the magnesium content of body fluids in sheep. A change from typical winter rations to cut spring herbage produced an immediate fall in the urinary excretion of magnesium, even in the presence of an increased magnesium intake. There followed a progressive increase in urinary magnesium excretion, even though the intake remained constant. Such changes in urinary magnesium excretion could be due to alterations in the gastrointestinal absorption or fecal excretion of magnesium.

### OTHER SYNDROMES ASSOCIATED WITH HYPOMAGNESEMIA

In 1929, Montgomerie, Savage, and Dodds (10) described *equine transit tetany*—a disorder first observed in Welsh mountain ponies which had just come off a railway journey under cramped conditions. The disease occurs most often in pasture-reared horses,

generally suckling mares, during and after a long railway journey, or after transfer from free range to confinement. The outstanding physiologic features are muscular fibrillation, clonic spasms, and increased respiration without a marked elevation of body temperature. Biochemical examinations of the blood revealed hypocalcemia and alkalosis.

In 1935, Green, Allcroft, and Montgomerie (11) found that ponies with the unequivocal clinical picture of equine transit tetany showed marked hypomagnesemia in addition to the pronounced hypocalcemia. The average serum magnesium concentration in four tetanic animals was 1.16 mg. per 100 ml; a fortnight later, when the animals were well, the value was 2.12 mg. per 100 ml. This disease is considered similar to grass tetany in cows.

**Tetany of Milk-fed Calves.** In calves reared exclusively on milk, tetany and convulsions, associated with a low concentration of magnesium in the plasma, may develop within a few months after birth (12, 13, 14). The symptoms and signs are relieved by feeding the calves magnesium. In this instance the clinical picture appears to be based solely on a deficiency of magnesium.

In twelve of sixteen calves reared on milk without supplementary vitamin D, all but one, at about five months of age, showed a decrease in plasma calcium concentration which correlated closely with a fall in plasma magnesium concentration (12). The hypomagnesemia was attributed to a magnesium deficiency resulting from a decrease in the ability of calves to absorb dietary magnesium, but the reason for the hypocalcemia was more obscure.

Animals on a diet of wheat straws (14) began to have "fits." The serum magnesium concentration was low during convulsive episodes, and increased immediately afterwards. Adding to the diet 24 mg. of magnesium oxide per pound of body weight eliminated the syndrome and kept the animals in magnesium equilibrium.

#### MANGANESE AND ITS RELATIONSHIP TO MAGNESIUM

Blakemore, Nicholson, and Stewart (15) reported that lactation tetany occurred only in cattle pastured in certain districts. On these farms cows showed a fall in serum magnesium concentration when they were first turned out to pasture in the spring, but re-

gained normal levels in five or six weeks. In the affected districts these changes occurred in animals which remained symptom-free, as well as in animals which later succumbed to the disease, but they were not seen in cows on farms free of lactation tetany.

These investigators carried out detailed chemical and botanical examinations of the affected pastures. They found little or no connection between the type of soil and the distribution of the disease. Lactation tetany was generally associated with pastures growing poor types of grass, although the actual amount of grass available was usually plentiful and the nutritive value of the grass was not abnormal. In some instances the magnesium content of the grass on such farms was actually higher than that of grass in other pastures. The only striking finding was an unusually high content of manganese in the grass from pastures on which lactation tetany commonly occurred: 54 to 132 mg. per 100 Gm. of dry matter, as compared to 1.6 to 10.6 mg. in grass from pastures on which lactation tetany had never been recorded.

This striking difference led the authors to examine the serum manganese levels in cattle grazing upon the two types of pastures. For eight cows grazing in tetany-free areas the average manganese content was 0.0025 mg. per 100 ml. of serum. In six cows which grazed on pasture with a high manganese content the average serum level of manganese was 0.0015 mg. per 100 ml. before they were turned out to pasture, and 0.1599 mg. after they had been in the pasture for twenty days.

In an experimental attempt to link the presence of large amounts of manganese in the grass with the other physiologic changes, manganese chloride was given by stomach tube to rabbits. Lethal doses caused an increase in the blood magnesium level, whereas sublethal doses caused a decrease. Subsequent studies in rabbits, sheep, and cows showed that only the first sublethal dose of manganese consistently depressed the blood magnesium level.

These experimental findings support the thesis that the presence of large amounts of manganese in the grass eaten by cattle can produce a temporary fall in the blood magnesium level. They do not, however, explain the etiology of lactation tetany, since it does not occur in all animals with a low blood level of magnesium. Be-

cause there is no lack of available magnesium in the diet, a magnesium deficiency cannot be held solely responsible for producing either a low blood magnesium level or lactation tetany. The disease must thus be differentiated from the tetany of rats on a low-magnesium diet (2), and from the tetany of calves fed exclusively on whole milk (12).

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## *Chapter V*

# HIBERNATION

**S**URPRISINGLY LITTLE is known concerning the precise mechanisms of sleeping and wakefulness. The phenomenon of hibernation in the animal kingdom has long been recognized, but detailed examinations of the physiologic and biochemical factors inducing this state are meager. As a result of recent renewal of interest in this fascinating dormant field, the First International Symposium on Natural Mammalian Hibernation was held in May, 1959, in Dedham, Massachusetts (1).

### DESCRIPTION

Hibernation 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" (2). 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 support it for the required period.

The fundamental problem in hibernation is that of regulating metabolism and heat production. A study of the biochemical processes involved in hibernation must take into consideration the interdependence of temperature, time, and chemical environment.

Studies of cation concentrations in the serum of hedgehogs have shown that the potassium content is reduced in autumn before hibernation begins, and reaches a minimum during hibernation (3). The sodium content of the serum, on the other hand, is slightly raised. Hibernation does not appear to have any effect on the serum calcium concentration.

The purpose of the present chapter is to marshal all published evidences relating magnesium metabolism to hibernation. After these have been presented, it will become readily apparent that extensive investigations are still needed.

### ROLE OF MAGNESIUM

The magnesium ion seems to be related to temperature regulation and also to cyclic activities of the living organism. It has been reported that a nightly increase in serum magnesium occurs in human beings, and is accentuated by the administration of vitamin B<sub>6</sub> (4). In the summer hedgehogs (3) show typical diurnal variations in the serum 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* (5), and in thirteen-lined ground squirrels, woodchucks, golden hamsters, little brown bats, big brown bats, and hedgehogs. 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 per cent over the control values. In the little brown bat, the elevation of serum magnesium appears to be dependent upon cooling of cells.

Riedesel (6, 7, 8) has presented several theories as to the role of magnesium in the production of hibernation. The sequence of events is pictured as follows: Exposure to cold and reduced activity produce cooling of the peripheral tissues and a release of magnesium from cells to plasma. 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 actually related to 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 (3, 9, 10, 11, 12). In studies on seven hedgehogs (11), this investigator found that the serum magnesium concentration in the autumn, before the onset of hibernation, averaged 3.2 mg. per 100 ml., with a range of 2.9 to 3.6. The serum calcium concentration averaged 10 mg. per 100 ml., with a range of 9.2 to 10.6. In early January, when the hedgehogs were in deep hibernation, the average magnesium content of the serum was 5.43 mg. per 100 ml., with a range of 4.90 to

6.05; the mean calcium value was 10.2, with a range of 9.6 to 10.7. The magnesium values during deep hibernation were 170 per cent of the values before the onset of sleep (11).

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.

### ARTIFICIAL PRODUCTION

In other studies (12, 13) Suomalainen showed that injecting magnesium solutions subcutaneously into hedgehogs and putting the animals into the icebox produced a condition of magnesium anesthesia entirely unlike natural hibernation. In the former, the animal's heat-regulating mechanism appeared to be deranged so that the homoiothermic state was changed into a kind of poikilothermia. Metabolism was diminished; the higher centers of the nervous system were paralyzed; and motility disappeared. Sensibility and muscular tonus, which are preserved in natural hibernation, were greatly diminished, and the animals were very limp. In contrast to the marked hypoglycemia found in hibernating hedgehogs, hyperglycemia was present, and there was an increase in the adrenaline content of the adrenals.

In another series of hedgehogs (13), Suomalainen gave subcutaneous injections of insulin along with the magnesium before transferring them to an icebox. The animals then went into a cold-blooded state which closely resembled natural hibernation. 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. Suomalainen particularly stressed the fact that the blood sugar level adjusted itself to approximate that found in natural hibernation, although the amounts of insulin administered varied widely. Similar amounts of insulin given without magnesium produced fatal hypoglycemia (13).

### 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 one to two hours by hibernating hedgehogs, but for only three to five minutes by nonhibernating animals (14, 15, 16). 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, and it is known that magnesium affects 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 the 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 animals have considerably larger quantities of brown fat than nonhibernating species. The possible relationship between brown fat and anaerobic metabolism, lowered body temperature, and magnesium metabolism remains to be elucidated.

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 (ATP) is known to be the immediate source of energy for metabolic work involving phosphorylation, syntheses, and other chemical processes. Phosphocreatine, glycolysis, and biologic oxidation can effect 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. Anabolic processes of cellular metabolism involving oxidation, carbon dioxide fixation, synthesis, and secretory processes of the cell are related to ATP and the mitochondrial system.

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

### COLD ACCLIMATION

The literature contains many reports 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 (17, 18) that the parenteral administration of magnesium facilitates experimental hypothermia; 2) both in experimental animals and in human beings (19, 20) magnesium has been shown to have an antipyretic action; and 3) an increase in serum magnesium during hypothermia has been reported in both vertebrates and invertebrates (21, 22, 23). In rabbits this increase amounted to 25 per cent.

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 (24); the water content of the muscle increased 2.5 per cent, and that of the skin 3.7 per cent. 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.

Another group of investigators, working with rats, studied changes occurring in the magnesium contents of the serum, heart, liver, and skeletal muscle, in relation to alterations in colonic and footpad temperature, during various stages of the cold acclimation process. During a six-weeks period of exposure to cold, rats were killed at intervals of ten days in order to compare the magnesium contents of the heart, liver, and skeletal muscle with those in con-

trol rats. The serum magnesium was measured at five day intervals. The magnesium contents of the heart and skeletal muscle were significantly elevated after ten days of exposure to cold, the serum magnesium on the first, fifth, tenth, and twentieth days of exposure, and the deep colonic temperature at one, twelve, and twenty-two days. Footpad temperatures decreased throughout the acclimation period. The elevated levels of magnesium in the heart and skeletal muscles would indicate that these tissues did not supply the serum with extra magnesium. The reason for a decrease in the magnesium content of the liver at 40 days of exposure remains obscure. The existence of peripheral hypothermia suggests that hypothermic peripheral tissues such as the skin are the most likely source of the transient increase in serum magnesium which occurs during cold acclimation (25).

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

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## *Chapter VI*

# THE MEASUREMENT OF MAGNESIUM IN BIOLOGIC MATERIALS

**T**HE GREAT VARIETY of methods currently used for determining the amount of magnesium present in biologic materials testifies to the fact that none of them is completely satisfactory. Most are cumbersome, indirect, or inaccurate. The most popular of these methods will be described briefly in this chapter.

### MAGNESIUM AMMONIUM PHOSPHATE

The earliest method for estimating the quantity of magnesium in biologic 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). Kramer and Tisdall (3) first combined calcium and magnesium estimations by the consecutive precipitation of calcium as oxalate and magnesium as magnesium ammonium phosphate ( $MgNH_4PO_4$ ).

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 (6). Unfortunately, this molybdenum blue proved to be unstable (7). Simonsen, Westover, and Wertman (8) next determined the phosphate as the yellow molybdivanadophosphoric acid—a color which is stable over several hours. This method, although cumbersome, has proved to be, in our experience, the most satisfactory one available.

In this method, calcium is first precipitated as calcium oxalate. One of the main difficulties in this step is the separation of magnesium and calcium, since some magnesium is occluded as oxalate (9).

When the radioisotope of magnesium,  $Mg^{28}$ , was added to pooled serum, however, only 2.98 per cent of the amount added was recovered in the calcium oxalate precipitate (10). This residual radioactivity could be removed with a few washings. Precipitation of the calcium oxalate was complete in thirty minutes. Of the  $Mg^{28}$  added to the pooled specimen, 94.9 per cent was recovered in the final  $MgNH_4PO_4$  precipitate, and the phosphate content of the precipitate was constant after thirty minutes. In estimating the magnesium content of normal serum, therefore, the separation of calcium and magnesium does not appear to present any particular problem.

The difficulty is said to arise chiefly when the quantity of magnesium exceeds that of calcium (9), as it sometimes does in the urine. However, when a tracer amount of  $Mg^{28}$  was added to a pooled specimen of urine, a mean of 2.17 per cent of the  $Mg^{28}$  was recovered with the calcium oxalate precipitate and a mean of 99 per cent was found in the washed  $MgNH_4PO_4$  precipitate (10).

For determining the tissue content of magnesium, we have used the following modification of Stutzman's adaptation (11) of Simonsen's method (8). Although tedious, it does result in reproducible values. Tissues are dried to a constant weight in an oven at 98 C. and then ashed in a muffle furnace at 640 C. for three to four hours. After the addition of one drop of methyl red indicator, concentrated ammonium hydroxide and a 5 per cent solution of acetic acid are used to adjust the pH to a range of 5.0 to 5.3. (All reagents used are repurified.) The calcium is then precipitated with 1 ml. of a 4 per cent solution of ammonium oxalate, and the pH is readjusted. From this point on, the procedure is the same as that for determining the calcium and magnesium content of serum (8). In the case of bone, 1 ml. of a 4 per cent solution of ammonium oxalate is added after adjustment of the pH; the precipitate is then allowed to form overnight, and the supernatant fluid is removed by decantation. Another milliliter of the ammonium oxalate solution is added, and this process is repeated until no further precipitate forms. The magnesium is then precipitated as  $MgNH_4PO_4$  from the remaining supernatant fluid.

### 8-HYDROXYQUINOLINE

After calcium is removed from serum, magnesium can be precipitated by 8-hydroxyquinoline (12); 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 (13) 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 (14).

### TITAN YELLOW

In 1927, Kolthoff (15) 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 has been used for the colorimetric determination of small quantities of magnesium (16). Magnesium was at first measured as a specific colored lake (13), which had to be suspended by a colloidal dispersing agent. Soluble starch was initially used, but it tended to give opalescent solutions, and was replaced by Ghatti gum (17), which gave a clear solution. A further modification (18) used hydroxylamine hydrochloride with Titan yellow.

The most recent examination of Titan-yellow methods was made by Orange and Rhein (19), who selected polyvinyl chloride as a colloidal dispersing agent, and claimed that a tenfold increase in sensitivity resulted. Unfortunately, the Titan-yellow method, although simple, is inaccurate and not reproducible.

### EDTA-ERIOCHROME BLACK T

The dye Eriochrome Black T forms a soluble dye complex with magnesium (20-24). Titration with ethylenediamine tetracetate (EDTA) chelates the magnesium, removing it from the ionic form and destroying the dye complex, so that a color change occurs. Since calcium is chelated before magnesium, however, titration of

samples containing both calcium and magnesium yields both these minerals. Usually a second titration is performed, using murexide (ammonium purpurate) as an indicator, in order to determine the concentration of calcium. The difference between the two titrations represents the concentration of magnesium (25). 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 (26).

### **FLAME SPECTROPHOTOMETRY**

Flame photometry, which has so revolutionized studies of alkali metals, would appear to be the method of choice for measuring magnesium in biologic fluids. The inherent emission characteristics of the magnesium atom, however, have led to technical difficulties (27). The relative intensity of the emitted light for magnesium is 0.1 per cent of that for sodium and 0.5 per cent of that for potassium. Because of this low intensity, high photo-detector sensitivity (available only in the photomultiplier type of phototube) is necessary, and a relatively large slit width must be used, thus intensifying interference effects.

In spite of these limitations, it is claimed that satisfactory flame photometers, which are as simple, precise, and accurate as the instrument in use for alkali metals, have been developed for magnesium (28-31). It is hoped that the commercial availability of these instruments in the near future will solve the problem of measuring magnesium in biologic fluids.

### **ELECTROCHEMICAL DETERMINATION**

A unique method untried in the United States is that of Terkildsen (32), which is based on the isolation of magnesium by electrodeposition in the presence of calcium.

### **FLUOROMETRIC ANALYSIS**

Fluorometry is an extremely sensitive method of chemical analysis, and the recent introduction of improved commercial instruments will doubtless accelerate studies of this method (33). Schachter (34) reported that an ethanolic solution of Mg-8-hydro-

xyquinoline exhibited characteristic fluorescence which was maximal at a defined pH. Under appropriate conditions the increment of fluorescence between two different pHs could be used as an index of magnesium concentration. Unfortunately, the results of this extremely simple method are not reproducible in our experience. The greatest difficulty has been with the instability of the fluorescence in the blank solution.

Schachter (35) has subsequently reported the fluometric analysis of an aqueous solution of magnesium-8-hydroxy-5-quinolinesulfonate. We have found this reaction to be extremely unstable and insensitive.

A more sensitive method than the two just mentioned uses the dye reagent, bis(salicylidene ethylenediamine (36). Whether this method is reliable and reproducible enough to replace the molybdivanadate methods (8, 11) remains to be seen.

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## *Chapter VII*

# THE ROLE OF MAGNESIUM IN BIOCHEMICAL PROCESSES

**S**O MANY ENZYMES are known to be activated *in vitro* by magnesium that it is almost impossible to list all of them (1). That these *in vitro* effects of magnesium have a physiologic counterpart *in vivo* is more difficult to demonstrate, but there is evidence that magnesium plays a significant role in intracellular catalysis. In fact, the action of magnesium seems to extend to all the major anabolic and catabolic processes involving the main metabolites.

In this chapter will be summarized very briefly the evidences suggesting the role of magnesium in carbohydrate, lipid, and protein metabolism (2).

### CARBOHYDRATE METABOLISM

#### Glycolysis

Figure 1 summarizes the points at which several sugars enter the glycolytic pathway. Even this superficial analysis shows that lack of magnesium would inhibit at least seven reactions.

#### Aerobic Metabolism

The initial oxidation of pyruvic acid requires magnesium. In the tricarboxylic acid cycle, magnesium is necessary for the conversion of alpha-ketoglutaric acid to succinic acid. In the assimilation of carbon dioxide, the reaction, pyruvate  $\longleftrightarrow$  oxalacetate, requires magnesium. In the pentose monophosphate shunt, the conversion of xylulose-5-phosphate to glyceraldehyde-3-phosphate is magnesium-dependent.

### LIPID METABOLISM

The transformation of pyruvic acid to acetyl-CoA, and that of cholic acid to choly-CoA are both dependent on magnesium.

## MAGNESIUM IN BIOLOGY

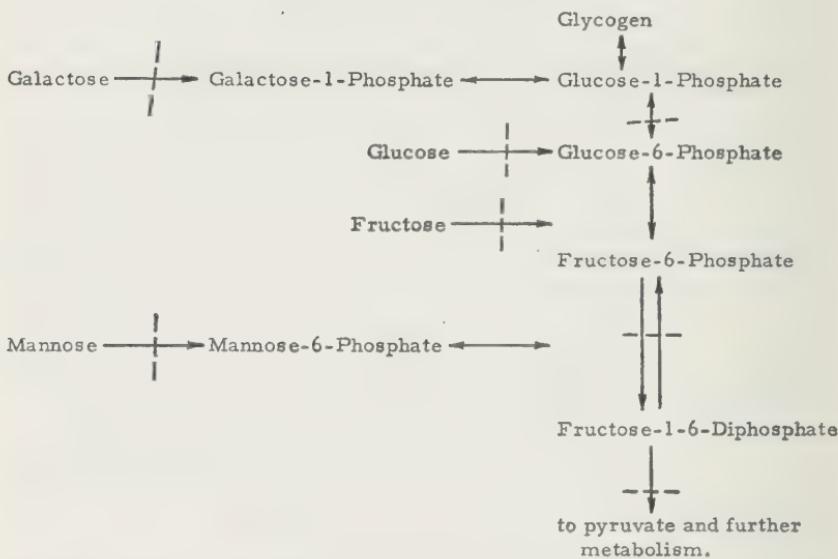


Fig. 1. Summary of Carbohydrate metabolism. The points at which several sugars enter the glycolytic pathway are shown. The dotted lines indicate steps where magnesium is known to be essential.

## PROTEIN METABOLISM

The synthesis of both deoxyribose nucleic acid and ribose nucleic acid would be inhibited by a lack of magnesium.

All the enzymes that catalyze the transfer of phosphate from ATP to a phosphate receptor, or from a phosphorylated compound to ADP (adenosine-diphosphate) are activated by magnesium. In most instances these enzymes may also be activated by manganese. The relationship between this observation and the excessive content of manganese in the feed of animals developing grass staggers remains to be studied.

One of the most intriguing of recent studies concerns the highly purified enzymes that catalyze the transfer of phosphate from ATP to a phosphate receptor, or from a phosphorylated compound to ADP. Extending the previous observation that magnesium increases the activity of ATP (3), Nanninga (4) reported that the

ATP-magnesium complex may be the active enzymatic substrate. This conclusion was suggested by the fact that ATP forms a 1:1 complex with magnesium. Maximal activation occurs when the ratio of magnesium to ATP in the reaction mixture is 1 (5, 6).

In view of the interrelationships known to exist among carbohydrates, lipids, and proteins, it becomes evident even to the neophyte that the enzymatic processes essential to life would cease in the absence of magnesium.

### THE ENERGY CYCLE ON EARTH

The green plants are the major source of food for the other organisms inhabiting this planet. The source of the complex organic compounds produced by plants is photosynthesis—the process whereby radiant energy from the sun, mediated in some yet unknown fashion by chlorophyll (a magnesium-porphyrin compound) transforms simple compounds into complex ones. The over-all reaction leading to the *photosynthesis* of carbohydrate compounds is indicated by the equation:



The equation for *biologic oxidation*, the fundamental process whereby all animals derive energy, is the reverse of the one just given for photosynthesis:



The evolved energy is liberated in a series of gradual steps, in the course of which energy is collected in the form of ATP. This process too is magnesium-dependent.

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## *Chapter VIII*

# THE ROLE OF MAGNESIUM IN HUMAN DISEASE

**T**HE DISCOVERY THAT a neuromuscular disorder in animals might be specifically related to a deficiency of magnesium led to investigations of this ion in various clinical disorders of man. As late as 1931, however, such authorities as Peters and Van Slyke (1) were compelled to introduce their discussion of magnesium metabolism with a comment that no clinical significance had, up to that time, been attached to changes in magnesium metabolism.

### CHANGES IN SERUM MAGNESIUM CONCENTRATION

Because of methodologic limitations, the early studies were confined to changes in the serum magnesium concentration. Such changes were shown to be frequently associated with mental and neuromuscular symptoms (2-5). As early as 1934, Hirschfelder (2) reported the occurrence of muscular twitchings and convulsions in seven patients with low concentrations of magnesium in the serum. There followed innumerable reports of changes in the serum magnesium concentration in various diseases (6). At one time or another, decreases in the serum magnesium have been reported in essential epilepsy, tetany, hyperparathyroidism, bronchial asthma (7), and infantile rickets. No change was demonstrated in cases of thyroid disease, schizophrenia, basophilic adenoma, Addison's disease, cardiac disease, steatorrhea, progressive muscular dystrophy, familial periodic paralysis, myotonia atrophica, and neurocirculatory asthenia. Elevation of the serum magnesium has been reported in tuberculosis, renal insufficiency, toxemia of pregnancy (8), and diabetic acidosis, and during the healing phase of infantile rickets. In the absence of additional knowledge concerning the over-all metabolism of magnesium in human beings, these reports were difficult to interpret.

It is evident now that marked depression of the serum magnesi-

um concentration may occur in the absence of neuromuscular signs. In the following conditions Martin, Mehl, and Wertman (9) found serum magnesium concentrations of less than 1 mEq. per liter, with no evidence of tetany: (1) the postoperative state in patients receiving magnesium-free intravenous fluids, (2) malignancy, (3) diabetic acidosis during treatment, (4) chronic renal disease, (5) the recovery phase of acute renal insufficiency, (6) congestive heart failure, (7) epilepsy, (8) lupus erythematosus, (9) hyperthyroidism, and (10) pancreatitis. Suter and Klingman (10), however, reported that definite symptoms were usually associated with serum magnesium concentrations below 1.5 mEq. per liter; in patients with severe symptoms values of less than 1.25 mEq. per liter were common.

### **CLINICAL SYMPTOMS AND SIGNS OF MAGNESIUM DEFICIENCY**

From these clinical observations there gradually evolved the impression that symptoms of magnesium deficiency include delirium, confusion, muscle tremors, cramps and twitchings, choreiform and athetoid movements, and rarely convulsions. These symptoms and signs, occurring in the absence of hypocalcemia and alkalosis, are consistent with the hypothesis that depletion of magnesium at a site such as the motor end-plate might result in heightened neuromuscular activity, perhaps through the increased production or release of acetylcholine (11, 12). While it is not clear how magnesium depletion produces all of the symptoms, considerable evidence suggests that an intracellular depletion of this ion is of primary importance.

### **RESPONSE TO MAGNESIUM THERAPY**

The mere association of changes in serum magnesium concentration with certain clinical symptoms and signs afforded insufficient evidence of a causal relationship. The evidence was strengthened, however, by the fact that the symptoms were relieved by the parenteral administration of magnesium (13). A group of alcoholic patients with delirium tremens and some nonalcoholic patients with tremors responded to the intramuscular administration of magnesium salts (14, 15). In the tetany of kwashiorkor (16),

magnesium therapy produces a dramatic improvement. Children with neonatal tetany, all of whom had been fed cow's milk, responded to magnesium therapy (17); in these cases, the results duplicated those obtained in naturally occurring magnesium deficiency in animals.

### **CLINICAL SYNDROMES WITH HYPOMAGNESEMIA**

#### **Human Magnesium Deficiency Tetany**

Vallee, Wacker, and Ulmer (18) have recently claimed the discovery of a new specific clinical entity: human magnesium deficiency tetany. This syndrome is virtually identical to that of hypocalcemic tetany, from which it must be differentiated by chemical means. Its manifestations correspond almost exactly to those seen in magnesium-deficient animals. The parenteral administration of magnesium sulfate promptly and completely reverses the syndrome.

The factors which condition the development of the syndrome are said to be: 1) dietary restriction, 2) malnutrition, and 3) malabsorption or increased excretion of magnesium. Thus, magnesium deficiency tetany is most likely to develop in the presence of a severe, debilitating illness, when the patient's intake of magnesium is decreased, and loss of the element is increased.

#### **Hyperaldosteronism**

There have been a few recent reports of hyperaldosteronism with concurrent tetany, in which the serum calcium concentration was normal and the serum magnesium level markedly depressed (19, 20).

#### **Tetany Following Parathyroidectomy**

Tetany following the removal of parathyroid adenomas has been associated with a low serum magnesium concentration (21, 22).

### **METABOLIC BALANCE STUDIES**

Demonstration of a low serum magnesium concentration, even when coupled with response to magnesium therapy, does not afford

conclusive evidence of a prior deficiency state. Metabolic balance studies are helpful in confirming the reality of magnesium deficiency. Montgomery (16) showed that infants with kwashiorkor, during the first few weeks of recovery, may have a positive magnesium balance on the order of 50 mEq. During the period of deficiency, the urinary excretion of magnesium in twenty-four hours was 0.01 mEq. A muscle biopsy during therapy showed that the magnesium content was 9.6 mEq. per kilogram of wet weight—considerably lower than the normal range of 16 to 18 mEq. per kilogram.

Randall, Rossmeisl, and Bleifer (23) found, in twelve adult patients with symptomatic magnesium deficiency, marked retention of the magnesium given therapeutically. Urinary excretion of magnesium initially was greatly suppressed, and in ten of the patients the values for serum magnesium were far below normal.

During the developmental phase of diabetic coma, Butler and his associates (24) found a loss of 0.8 mEq. of magnesium per kilogram of body weight. The total deficit amounted to 50 to 70 mEq. During the recovery phase, a deficit of magnesium amounting to 40 mEq. per 1.73 square meters of body surface was found (25).

### MAGNESIUM CONSERVATION

Barnes, Cope, and Harrison (26) determined the efficiency of the body in conserving magnesium while subjects were on a magnesium-free diet. A liquid diet containing less than 0.12 mEq. of magnesium per liter was given to four volunteers over a ten-day period. Both the urine and the feces showed a striking and prompt decrease in magnesium content. On the average, less than 1 mEq. of magnesium was excreted in the urine daily. After a transitional period, fecal excretion of magnesium was negligible. Like sodium, therefore, magnesium is a component of the body which can be rigidly conserved in the presence of a specific deficiency. The minimal maintenance requirement of magnesium for adults may be on the order of 1 mEq. daily.

### EXPERIMENTAL PRODUCTION OF MAGNESIUM DEFICIENCY

Fitzgerald and Fourman (27), in 1956, reported the first effort to produce magnesium deficiency experimentally in man by the

use of a diet low in magnesium, together with a cation exchange resin to produce a depletion of magnesium in the gastrointestinal tract. For twenty-two and twenty-seven days, two normal men were given, in addition to the cationic resin, a diet containing only 1.1 mEq. of magnesium daily. The total fecal and urinary excretion of magnesium fell to less than 3 mEq. daily, and the total magnesium deficits incurred amounted to 42 and 72 mEq. respectively. The serum magnesium concentration did not fall, however, and the deficiency produced no other outstanding effects. A transient loss of potassium and a gain of sodium and chloride were observed, and presumably there was an increase in extracellular fluid volume.

The experiments were terminated by the intravenous injection of magnesium. Of the 45 and 89 mEq. of magnesium injected, 25 and 45 per cent respectively were retained. In two comparable control experiments in which magnesium was provided throughout, none of the injected magnesium was retained. In the depletion experiments, but not in the control periods, the injections were associated with a loss of sodium and chloride. These observations emphasize the interrelationships among the various ionic constituents of the body.

Recent isotopic studies which extend the above observations will be discussed in the ensuing chapters.

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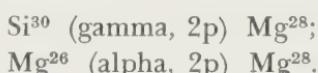
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## *Chapter IX*

### RADIOMAGNESIUM

**T**HE ELEMENT MAGNESIUM is number 12 in the periodic table of elements. The isotopes 24, 25, and 26 occur in nature in the proportions of about 7:1:1, thus, the atomic weight of magnesium is 24.32. The radioactive isotopes 23 and 27, with physical half-lives of 12.3 seconds and 9.58 minutes respectively, are not suitable for biologic studies.

Sheline and Johnson (1), in 1953, reported the artificial production of the isotope of mass number 28, both by a betatron irradiation and by a cyclotron bombardment. The nuclear reactions were as follows:



The half-life of this isotope is 21.3 hours. Jones and Kohman (2) independently produced  $\text{Mg}^{28}$  by the reaction,  $\text{Si}^{30} (\text{p}, 3\text{p}) \text{ Mg}^{28}$ .

Subsequent investigations have shown that  $\text{Mg}^{28}$  decays by emission of 0.42 mev. beta particles and gamma rays of 0.032 mev. (96 per cent), 0.40 mev. (31 per cent), 0.95 mev. (29 per cent), 1.35 mev. (70 per cent).  $\text{Mg}^{28}$  decays to a daughter isotope  $\text{Al}^{28}$ , with which it is in secular equilibrium.  $\text{Al}^{28}$  has a half-life of 2.3 minutes, and decays with emission of a 2.87 mev. beta particle and a 1.78 mev. gamma ray to  $\text{Si}^{28}$ .

$\text{Mg}^{28}$  has been available commercially since early 1957 from the Hot Laboratory Division of the Brookhaven National Laboratory, Upton, Long Island, New York. It is produced in the nuclear reactor by the  $\text{Mg}^{26} (\text{t}, \text{p}) \text{ Mg}^{28}$  reaction. At first the specific activity of this material was on the order of 5 microcuries per milliequivalent of magnesium, but it has steadily improved. In January, 1961,  $\text{Mg}^{28}$  of high specific activity became available. Currently the specific activity exceeds 500 microcuries per milliequivalent.

Because of the availability of  $\text{Mg}^{28}$  and the recent improve-

ments in the chemical methods for assaying magnesium in biologic material, it can be predicted that our understanding of magnesium metabolism will increase rapidly. Our current knowledge of this field may be compared to that of potassium and sodium metabolism in the late nineteen-forties, when flame photometry had just been introduced and the radioisotopes of sodium and potassium had become available.

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## *Chapter X*

# DISTRIBUTION OF MAGNESIUM IN THE HUMAN BODY

### TOTAL BODY CONTENT

**I**NFORMATION ABOUT the chemical composition of the human body is surprisingly scarce—partly because of the difficulty of obtaining bodies for analysis and partly because of the technical problems involved in assaying such large quantities of material. In 1943, Widdowson, McCance, and Spray (1) analyzed four bodies for a variety of chemical components, including magnesium. The magnesium content of the bodies (those of two men, one woman, and a boy aged  $4\frac{1}{2}$  years) ranged between 22.7 and 35.0 mEq. per kilogram of wet weight of tissue—values comparable to those found in rabbits (29.7 and 36.8 mEq. per kilogram of wet tissue [2]). For a man weighing 70 Kg., the body content of magnesium would be on the order of 2400 mEq. (29 Gm.).

### BONE

There is no published report concerning the total content of magnesium in the human skeleton. An adult human skeleton contains about 76,000 mEq. of calcium (3, 4, 5). If one assumes that the skeletal content of magnesium is one fiftieth of this amount, it can be calculated that the bone content of magnesium is about 1,500 mEq., or 60 per cent of the total body content. While this calculation is probably not precise, it does indicate that a large fraction of the total body content of magnesium is in bone.

### MUSCLE

The remaining body content of magnesium (900 mEq., or 40 per cent) must be predominantly intracellular in all the soft tissues, since the total extracellular content of magnesium is less than 30 mEq. Approximately 43 per cent of the body weight of a

70-Kg. man is muscle, and the average muscle content of magnesium is 16 mEq. per kilogram (5). The total muscle content of magnesium, therefore, is calculated to be 482 mEq. All the remaining soft tissues must contain about 418 mEq. of magnesium—less than the total muscle content.

### TISSUE CONCENTRATIONS

Data on the amounts of magnesium contained in human tissues are limited (6). Table 1 summarizes the available information (5, 8, 9). Bone cortex has the highest concentration of magnesium. Most of the soft tissues contain between 10 and 20 mEq. per kilogram.

TABLE I  
TISSUE MAGNESIUM CONCENTRATIONS IN MAN AND RABBIT  
(MEQ./KG. WET WEIGHT OF TISSUE)

|                  | <i>Man</i>        |                          | <i>Rabbit (8)</i> |
|------------------|-------------------|--------------------------|-------------------|
|                  | <i>Shohol (5)</i> | <i>Aikawa et al. (9)</i> |                   |
| Adrenal          | 6.8               |                          | $16.40 \pm 0.90$  |
| Blood cells      | 4.2               |                          |                   |
| serum            | 2.5               |                          |                   |
| Bone marrow      |                   | 39.4                     | $10.30 \pm 1.15$  |
| Brain            | 11.0              |                          | $11.40 \pm 0.31$  |
| Heart            | 11.9              |                          | $12.96 \pm 0.40$  |
| Intestine        | 5.1               |                          | $18.60 \pm 0.84$  |
| Kidney           | 14.4              |                          | $12.23 \pm 0.66$  |
| Liver            | 15.3              |                          | $12.35 \pm 0.50$  |
| Lung             | 5.1               |                          | $11.75 \pm 0.76$  |
| Lymph node       |                   |                          | $14.80 \pm 1.22$  |
| Muscle           | 16.1              | 11.5                     | $21.49 \pm 0.15$  |
| Pancreas         | 13.6              |                          |                   |
| Skeleton         | 74.6              | 269.7a<br>322.4b         | $298.1 \pm 10.2$  |
| Skin             | 9.3               | 5.3                      | $6.35 \pm 0.27$   |
| Spleen           | 10.2              |                          | $16.20 \pm 0.43$  |
| Subcutaneous fat |                   | 1.97                     |                   |
| Testicle         | 6.8               |                          | $10.40 \pm 0.46$  |
| Thyroid          | 6.8               |                          |                   |
| Uterus           | 11.9              |                          |                   |

a-fibula

b-tibia

### CONTENT OF VARIOUS SECRETIONS AND FLUIDS

The limited information on this subject is summarized in table 2 (7, 10).

TABLE 2  
MAGNESIUM CONTENT OF VARIOUS SECRETIONS AND FLUIDS OF MAN (7)

|                             | <i>mEq/l</i>           |                   | <i>mEq/l</i>           |
|-----------------------------|------------------------|-------------------|------------------------|
| Amniotic fluid              | 1.8                    | Pericardial fluid | †                      |
| Cerebral spinal fluid       | $2.40 \pm 0.14^{(10)}$ | Peritoneal fluid  | 0.5                    |
| Gastrointestinal secretions |                        | Plasma            |                        |
| Gastric juice               | (1.8-7.7)              | Adult             | $1.95 \pm 0.21^{(10)}$ |
| Duodenal secretion          | †                      | Fetal             | †                      |
| Bile                        | 1.5                    | Pleural fluid     | 1.71 (0.72-2.41)       |
| Pancreatic secretion        | 0.3                    | Prostatic fluid   | 1.0                    |
| Jejunal secretion           | †                      | Saliva            | 0.58 (0.16-1.06)       |
| Ileal secretion             | 18.8 (15.1-22.9)       | Semen             | 12                     |
| Lymph                       | †                      | Sweat             | (0.003-0.24)           |
| Milk                        |                        | Synovial fluid    | †                      |
| Colostrum                   | 3.3 (2.5-6.6)          | Tears             | †                      |
| Transitional                | 3.3 (1.6-4.1)          | Transudates       | 2.0 (1.6-2.4)          |
| Mature                      | 3.3 (1.6-4.9)          |                   |                        |

† No data available.

Unless otherwise designated, all values are from reference 7.

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## *Chapter XI*

# DAILY INTAKE, GASTROINTESTINAL ABSORPTION, AND RENAL EXCRETION

### DAILY INTAKE

MAGNESIUM IS READILY available to the organism in ingested food, since the magnesium content of meat is on the order of 15 mEq. per kilogram, and any plant contains chlorophyll. Although quite resistant to alkali, chlorophyll readily loses its magnesium in a weakly acid solution. The dietary intake of magnesium must vary considerably, but the usual American diet is said to contain between 20 and 40 mEq. of magnesium daily (1).

### GASTROINTESTINAL ABSORPTION

Tibbetts and Aub (1) reported that two thirds of the ingested magnesium is excreted in the feces. When a tracer dose of  $Mg^{28}$  was administered orally to twenty-six subjects (2), fecal excretion within 120 hours accounted for 60 to 88 per cent of the administered dose. The concentration of radioactivity in the plasma was maximal at four hours, but the actual increase in serum magnesium was negligible. The fecal magnesium appears to be primarily magnesium from food which is not absorbed by the body, rather than magnesium secreted by the intestine (3, 4). When  $Mg^{28}$  was injected intravenously into a normal human subject, only 1.79 per cent of the radioactivity was recovered in the stool in seventy-two hours.

In rabbits, less than one third of the ingested magnesium appears to be absorbed from the upper portion of the small intestine, and absorption from the large intestine is negligible (5). Absorption is not affected by variations in the pH induced by feeding either sodium carbonate or hydrochloric acid (6).

Until recently, it was stated (7) that no known factor controlled the gastrointestinal absorption of magnesium in the way that vitamin D controls the absorption of calcium. Meintzer and Steen-

bock (8), however, recently reported that vitamin D slightly increases the gastrointestinal absorption of magnesium in the rat.

There is now some evidence of an interrelationship between the absorption of calcium and that of magnesium. Alcock and MacIntyre (9) reported that calcium absorption from the gastrointestinal tract is increased in the absence of magnesium, and magnesium absorption is increased in the absence of calcium. In magnesium deficiency, the urinary excretion of calcium is decreased—possibly because of increased tubular reabsorption of calcium. Alcock and MacIntyre advanced the hypothesis that magnesium and calcium are absorbed by a common transport mechanism from both the intestine and the renal tubule.

### RENAL EXCRETION

The major excretory pathway for absorbed magnesium is the kidney (10, 11). In subjects on a normal diet, one third or less of the ingested magnesium (5 to 17 mEq.) is excreted by the kidney. The mean twenty-four hour urinary content of magnesium in 12 normal men on an unrestricted diet was  $13.3 \pm 3.5$  mEq. (7). Normal women on a controlled diet containing 21 mEq. of magnesium per day excreted an average of 7.9 mEq. in the urine (12). Following the intravenous injection of a tracer dose of  $Mg^{28}$  in 12 to 16 mEq. of stable magnesium (13), the daily urinary excretion of magnesium in eight normal subjects ranged between 6 and 36 mEq. (13). Urinary excretion of magnesium increased as the parenteral dose was increased.

The diffusible magnesium in plasma is filtered in the glomeruli and absorbed by the renal tubule. There is some evidence that magnesium may also be secreted by the renal tubule (7). The high content of magnesium in the urine of the aglomerular goosefish affords evidence that—in this species, at least—magnesium is excreted by a secretory process (14). Whether such a process exists in the glomerular kidney of mammals remains to be established.

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## *Chapter XII*

### **MAGNESIUM IN THE BLOOD AND CEREBROSPINAL FLUID**

**I**T WAS ESTABLISHED in the previous chapter that only a small proportion of the magnesium ingested in food is absorbed through the intestinal mucosa. What next happens to this small amount of magnesium so absorbed? Some of it is stored extracellularly—in the blood and the interstitial fluid. The nature of this extracellular magnesium and that in the red cells will be discussed in this chapter.

#### **SERUM MAGNESIUM CONCENTRATION**

The results of the various methods for determining serum magnesium have previously been summarized (1). In our study in human beings (2), in which the molybdivanadate method (3) was used, the mean value for 220 determinations of the serum magnesium concentration in hospitalized subjects was  $1.59 \pm 0.32$  mEq. per liter. Values lower than 1.1 mEq. per liter were obtained in patients with congestive heart failure, cirrhosis, or renal failure after dialysis. All values higher than 2.10 mEq. per liter were found in patients with renal failure prior to therapy (2). The various other diseases associated with changes in the total serum magnesium concentration were discussed in Chapter VIII.

#### **TOTAL EXTRACELLULAR CONTENT**

If the mean concentration of magnesium in the extracellular fluid space is 1.6 mEq. per liter and if 20 per cent of the total weight of a 70-Kg. man is extracellular fluid, then  $14 \times 1.6$ , or 22.4 mEq., of the total body content of magnesium is extracellular. Since the body of a man weighing 70 Kg. is assumed to contain approximately 2,400 mEq. of magnesium, less than 1 per cent of the total body content of magnesium is extracellular. Like potassium, magnesium is located predominantly within cells. Although serum magnesium

represents only a small proportion of the body content of magnesium, it is this proportion that is most accessible for chemical analysis, and hence of greatest importance clinically.

### MAGNESIUM IN THE RED CELLS

The magnesium concentration in the red cells of healthy hospital personnel is  $4.67 \pm 0.92$  mEq. per liter (4). An increase in magnesium was observed clinically in the red cells of patients with reticulocytosis, and experimentally in rabbits with reticulocytosis produced by phenylhydrazine. There is apparently no exchange of magnesium between the plasma and the red cells in the peripheral circulation; *in vitro* experiments with mature and immature human and rabbit erythrocytes showed no uptake of  $Mg^{2+}$  from the suspending medium. The increase in the magnesium content of red cells observed in reticulocytosis appears to occur in the bone marrow prior to their release; in animals made anemic with phenylhydrazine and exhibiting marked reticulocytosis, the relative tissue uptake of  $Mg^{2+}$  in the bone marrow was significantly increased (4).

A recent study (5) suggests that significant abnormalities of magnesium concentration in the red cells are common in human disease. Both the erythrocyte magnesium concentration and the serum magnesium concentration were low in patients with hyperthyroidism, malnutrition, or chronic liver disease. In advanced renal disease, the magnesium concentration of both plasma and erythrocytes was elevated. In viral hepatitis and in hypoparathyroidism the concentration of magnesium was altered in the red cells, but not in the plasma; in the former condition the erythrocyte magnesium concentration was decreased, and in the latter it was increased. Under certain conditions causing an extracellular depletion or excess of magnesium, the concentration of magnesium in the red cells may be altered in a similar direction. The authors concluded that the red cell membrane permits only slow diffusion of magnesium, and that alterations in the extracellular magnesium must persist for long periods of time before they affect the intra-erythrocytic concentration. There was some evidence that in such conditions as hypoparathyroidism active transport of magnesium from plasma into erythrocytes may occur.

Assuming that the red cell volume is 30 ml. per kilogram, a 70-Kg. man would have 2,100 ml. of red cells with a mean magnesium concentration of 4.67 mEq. per liter. Thus, his total red cell magnesium content would be 9.8 mEq. It is evident that the magnesium in the red cells is not a labile or quantitatively important store of this element.

### THE NATURE OF MAGNESIUM IN SERUM

Watchorn and McCance (6) were probably the first investigators to show that the magnesium in serum can be divided by ultrafiltration through collodion membranes into two parts, one diffusible and the other nondiffusible. In their series an average of 25 per cent of the total serum magnesium was nondiffusible. The average ratio of the ultrafiltrable (or diffusible) portion to the total serum magnesium was 0.750. The lowest individual figure was 0.628 and the highest 0.915, while the average deviation for the whole series was 0.058. The proportion of ultrafiltrable magnesium appeared to remain constant, regardless of the total magnesium content of the serum. In cases of uremia and nephritis associated with marked nitrogen retention, the total serum magnesium concentration ranged from 1.29 to 8.5 mEq. per liter, but the ratio of ultrafiltrable to total magnesium remained within normal limits.

Watchorn and McCance showed that the amount of magnesium found in the ultrafiltrate is practically unaffected by the level of protein in the serum; they suggested that some other factor affects the ultrafiltrable magnesium, and may often be the chief agent affecting the nondiffusible fraction. They postulated that part of the magnesium may be combined with the organic lipoid phosphoric acids of the serum—that is, that magnesium may form colloidal phosphate complexes. There was no relation between the levels of calcium and magnesium in the serum.

During pregnancy the serum magnesium concentration is lowered, and the ultrafiltrable fraction of the total serum magnesium is increased. In the pregnant women studied by Watchorn and McCance, the average ratio for ultrafiltrable to total magnesium was 0.796, as compared with 0.750 for the whole series. This observation could not be satisfactorily explained on the basis of the

diminished quantities of protein in the serum during pregnancy. The underlying mechanism still remains obscure, although Watchorn and McCance have pointed out that the higher ultrafiltrable fraction would facilitate transfer of magnesium through the placenta, since everything that passes the placental barrier must presumably be in an ultrafiltrable form. The physicochemical changes that take place in the blood during pregnancy, therefore, favor the passage of magnesium from mother to fetus. Other recent studies on the placental transfer of magnesium will be discussed in Chapter XIII.

### **Adsorption With Barium Sulfate**

Studies with barium sulfate (7) have shown that a portion of the diffusible magnesium in serum can be adsorbed with this material, while another portion cannot. The significance of these findings remains obscure.

### **Ultrafiltrable Magnesium in Thyroid Disorders**

In thirty-one patients with Graves' disease studied in 1937, Soffer and his co-workers (8) found the total serum magnesium concentration to be in the normal range of 1.53 to 2.43 mEq. per liter, while the percentage of bound (nondiffusible) magnesium varied between 21.5 and 61.6 per cent, with an average of 36 per cent. In most of the normal subjects studied, the bound magnesium made up less than 20 per cent of the total serum magnesium. After administration of Lugol's solution to the hyperthyroid patients, the bound fraction varied between 6 and 33 per cent. No explanation has been found for this marked increase in the proportion of bound magnesium found in the serum of patients with untreated hyperthyroidism.

Two patients with myxedema who were studied (9) had no circulating bound magnesium, although the total serum magnesium concentration was within the normal range. In subsequent experimental studies, total thyroidectomies were performed in four dogs. Serum magnesium partition studies were conducted before the operation and at frequent intervals after it. In each instance, a profound drop in the percentage of bound magnesium occurred

within seventeen days to five weeks after operation, thus confirming the clinical observations in myxedematous patients.

These results were confirmed by Dine and Lavietes (10), who suggested that magnesium may be an integral part of the circulating thyroid hormone complex. Cope and Wolff (11), however, were unable to demonstrate that the sera of hyperthyroid and normal persons contained appreciably different percentages of bound magnesium.

In a subsequent study in dogs, Soffer and his associates (12) investigated various factors which might conceivably influence the blood magnesium partition. The intravenous administration of sodium iodide and magnesium sulfate and the subcutaneous injection of thyroxine produced no appreciable change. The subcutaneous injection of thyrotropic hormone, however, produced an increase in the percentage of bound magnesium comparable to that seen in patients with Graves' disease, followed by a sharp drop to levels as low as those found in myxedema. After injections of this drug were discontinued, the percentage of bound magnesium again increased beyond the control levels.

Cosgrove and Perry (13) reported that the proportion of non-diffusible magnesium in the plasma was relatively constant (about 50 per cent) in a wide range of conditions, including severe thyroid dysfunction. They suggested that the variations in results obtained by different investigators must indicate differences in techniques or methods.

Data obtained by Silverman and Gardner (14) indicated that the total and ultrafiltrable magnesium in the serum of normal infants and children is maintained at concentrations no different from those found in adults. This study also failed to support the observations of Soffer *et al.* (8, 9) and of Dine and Lavietes (10) that the concentration of ultrafiltrable magnesium in the serum rises in hypothyroidism.

#### **Ultrafiltrable Magnesium in Other Diseases**

Increases in the proportion of ultrafiltrable serum magnesium have recently been reported (15) in the following diseases: multi-

ple myeloma, uremia, hypothyroidism, disseminated lupus erythematosus, hyperparathyroidism, generalized peritonitis, and myasthenia gravis. An increase in the percentage of ultrafiltrable magnesium was always associated with increased total magnesium. The diffusible serum magnesium was found to be decreased in the nephrotic syndrome, and in hyperthyroidism, alcoholic cirrhosis, delirium tremens, disseminated lupus erythematosus, and rickets resistant to vitamin D. Among the factors thought to be responsible for maintaining normal levels of total and ultrafiltrable magnesium are the serum proteins, the thyroid, and the kidneys. Some other unknown factor or factors, however, also appear to play a part (15).

### **MAGNESIUM IN THE CEREBROSPINAL FLUID**

The concentration of magnesium in the spinal fluid was reported by Barrio (16) to vary between 1.15 and 5.02 mEq. per liter, the average concentration being 125 per cent of that in the blood serum. In other reports, the ranges have been 0.83 to 2.90 mEq. per liter, with an average of 2.49 (17), and 2.49 to 2.99 mEq. per liter (18). When the magnesium concentrations in the serum and spinal fluid of normal, nonfasting adult males were determined simultaneously (19), the mean serum value was  $1.95 \pm 0.21$  mEq. per liter, the mean spinal fluid value  $2.40 \pm 0.14$  mEq. per liter. While the spinal fluid has a higher concentration of magnesium than the serum, it contains less of the ultrafiltrable fraction (20, 6).

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 cerebrospinal fluid. Supporting this hypothesis are the observations of Cohen (21), who compared the range of magnesium concentrations in the cerebrospinal fluid of patients with and without meningitis. In the latter, the magnesium concentrations in the spinal fluid exceeded the values for plasma by 0.39 to 1.40 mEq. per liter. In patients with meningitis, the spinal fluid magnesium never exceeded the plasma magnesium by more than 0.09 mEq. per liter,

and in some instances the plasma contained higher magnesium concentrations—in one case, 0.51 mEq. per liter more than that in the cerebrospinal fluid.

### **STUDIES WITH Mg<sup>28</sup> (22)**

#### **Lack of In Vitro Binding to Plasma Proteins**

Three pools of sera were obtained: one from hyperthyroid patients, one from hypothyroid patients, and one from normal individuals. A tracer amount of Mg<sup>28</sup> was added to each pool, and the mixture was incubated for thirty minutes in a water bath at 37 C. After the protein was precipitated with sodium hydroxide and zinc sulfate, the radioactivity content of the three precipitates averaged 30 per cent. Three washings of the precipitates with water decreased the radioactivity content to 0.6 per cent.

#### **Lack of In Vivo Binding to Plasma Proteins**

Blood was obtained from a rabbit two hours after the intraperitoneal injection of 2 mEq. of magnesium tagged with Mg<sup>28</sup>. The unwashed protein precipitate contained 30 to 34 per cent of the radioactivity in the untreated serum. Washing of the precipitate resulted in a progressive decrease in radioactivity.

#### **Dialysis Studies**

Tracer amounts of Mg<sup>28</sup> were added to three pools of sera, obtained from hyper- and hypo-thyroid patients and from normal individuals. After being incubated for thirty minutes at 37 C., each pool was dialyzed for twenty-four hours against physiologic saline solution at room temperature. Eighteen to twenty per cent of the radioactivity in all three types of sera remained in the cellophane bags.

When specimens of serum obtained from two rabbits two hours after the intraperitoneal injection of Mg<sup>28</sup> were dialyzed in a similar manner, 14 per cent of the radioactivity was recovered in the bags.

#### **Ultrafiltration**

Pools of hypo-, hyper-, and eu-thyroid sera were studied in the Toribara ultrafiltration apparatus. In all three types of sera the

proportions of bound and ultrafiltrable magnesium were 60 and 36 per cent respectively.

### Electrophoresis

Pooled human serum to which Mg<sup>28</sup> had been added was subjected to paper electrophoresis overnight. There was no binding of Mg<sup>28</sup> to any portion of the paper strip, and all of the radioactivity was recovered in the buffer solution.

The sum of these observations suggests that the binding of serum magnesium to plasma proteins is nonspecific, and is probably a physicochemical adsorption phenomenon.

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## *Chapter XIII*

### **THE PLASMA CLEARANCE AND TISSUE UPTAKE OF MAGNESIUM**

**T**HE NATURE of magnesium in the extracellular fluid was reviewed in the previous chapter. The next logical question is: What happens to this extracellular magnesium? An answer to that query will be attempted in this chapter.

#### **EARLY STUDIES WITH A MAGNESIUM LOAD**

Smith, Winkler, and Schwartz (1) were among the first to study the fate of parenterally administered magnesium. They based their interpretations on changes in magnesium concentrations occurring in the blood, urine, and feces of human subjects within three to four hours after the intravenous infusion of isotonic magnesium sulfate. In this period of time none of the injected magnesium was excreted in the feces. On the assumption that the injected magnesium was distributed in a volume of fluid at the concentration of magnesium found in the plasma, they calculated the "space" in which the injected magnesium was distributed. This "magnesium space" was equal to the "sulfate space" and amounted to 20 or 25 per cent of the body weight. Since the "sulfate space" is thought to be an index of the extracellular fluid volume, it was concluded that magnesium is initially distributed throughout the extracellular fluid.

Mendel and Benedict (2) reported that between four and twenty-four hours after the infusion, a variable proportion of the magnesium injected, over and above that excreted in the urine and stools, leaves the extracellular fluid and is stored somewhere in the body. This storage in the body is known to be temporary, and ultimately all the injected magnesium is excreted almost entirely in the urine; the amount of magnesium in the feces is not increased, and the injection of magnesium produces no purgative effect. The

urinary excretion of magnesium is quite rapid immediately following an injection, but is not complete for several days (3).

### TRACER STUDIES WITH Mg<sup>28</sup>

#### In Rabbits

The availability of radiomagnesium has made it possible to observe the behavior of magnesium under more physiologic conditions. In rabbits given by vein 1 mEq. of magnesium tagged with Mg<sup>28</sup>, serial blood specimens showed that Mg<sup>28</sup> disappeared from the blood stream rapidly during the first six hours, and more slowly thereafter throughout the twenty-four hour period of observation (4). The apparent volume of distribution of Mg<sup>28</sup>, as calculated from the plasma concentrations, exceeded the thiocyanate space of rabbits in less than an hour, and between the third and fourth hour exceeded the total body water (70 per cent of the body weight).

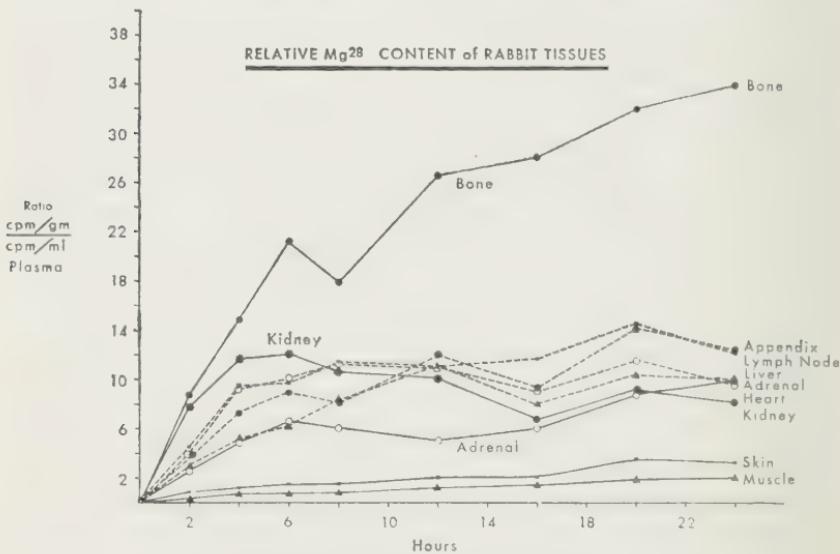


Fig. 2.

The tissue distribution of Mg<sup>28</sup>, expressed as the ratio of the radioactivity content of the tissues to that of plasma obtained at the time of death, is summarized in Figure 2. The relative radio-

activity was considerably higher in bone than in any of the other tissues studied. The radioactivity ratio of bone to plasma was 8.7 at two hours, and increased steeply to a value of 26.5 at twelve hours. Between the fourth and sixth hour, the relative activities of the other tissues were in the following order of decreasing ratios: kidney, 12; heart, 10; appendix, 9; liver, 6; skin, 1.6; and muscle, 1. The ratios in the heart, appendix, liver, and kidney had become stable within twelve hours after injection, and showed little variation during the next twelve hours.

The data obtained in studying the tissue distribution of Mg<sup>28</sup> suggested that equilibration of the injected radioactive atoms with most of the body content of magnesium may have occurred within sixteen to twenty-four hours. The exchangeable magnesium content of the intact rabbit was measured by the isotope dilution principle. When the dose of magnesium injected intravenously was less than 0.5 mEq. per kilogram of body weight, the mean exchangeable magnesium content in fifteen determinations was 36.5 ± 7.7 mEq. per kilogram. This mean value closely approximates the amounts of magnesium found by direct analysis of two whole carcasses: 36.8 and 29.7 mEq. per kilogram. Further studies of the tissue specific activities of magnesium are indicated before it can be concluded that the injected Mg<sup>28</sup> indeed reaches equilibrium within twenty-four hours with all the native magnesium atoms in the body of a rabbit.

When Mg<sup>28</sup> was injected intravenously into pregnant rabbits (5), its clearance from the blood stream was similar to that previously observed in normal young adult rabbits. Its distribution in the tissues of the mother was also similar to that in nonpregnant animals, except that uptake was slower in the bone and muscle of pregnant rabbits. It is known that magnesium is mobilized from maternal tissues during pregnancy.

The observation that the placental concentration of Mg<sup>28</sup> at two hours was higher than the concentration in maternal plasma suggests that the placenta actively concentrates magnesium. The specific activity of magnesium in all the fetal tissues studied reached a fairly constant value by twenty-six hours. Magnesium turnover

in the tissues of the fetus *in utero*, especially in bone and muscle, is considerably more rapid than that in the respective tissues of the mother. Previous studies (5) support the interpretation that rabbit fetuses late in gestation are rapidly increasing in bone and muscle mass. Apparently the rate of uptake of magnesium by various tissues is related to anabolic activities of the cells involved.

### In Human Subjects

When nine normal human subjects were given intravenous infusions of 12 to 30 mEq. of magnesium tagged with Mg<sup>28</sup>, the material was very rapidly cleared from the extracellular fluid (6). Within a few hours the volume of fluid available for the dilution of this ion, as calculated from the plasma concentrations of Mg<sup>28</sup>, exceeded the volume of total body water. The amount of Mg<sup>28</sup> excreted in the urine during this period was only a small fraction of the injected dose; hence renal excretion alone could not account for this rapid clearance of Mg<sup>28</sup> from the blood. Data obtained from biopsies of tissue removed from hospitalized patients showed concentrations of Mg<sup>28</sup> in liver, appendix, fat, skin, and subcutaneous connective tissue which could not be attributed solely to the extracellular components of these tissues. All these observations suggest that Mg<sup>28</sup> rapidly enters cells, and that 70 per cent or more of the infused magnesium is retained in the body for at least twenty-four hours.

Of interest is the observation that the twenty-four hour urinary excretion of *stable* magnesium following the infusion of Mg<sup>28</sup> approximated the amount of radioactive magnesium infused. This increased urinary excretion of magnesium led previous investigators to the erroneous interpretation that most of the infused magnesium was rapidly excreted by the kidneys. Our 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 which were administered.

After about eighteen hours 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 of magnesium, 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. per kilogram of body weight). It appeared, therefore, that less than 16 per cent of the total body content of magnesium was being measured by the Mg<sup>28</sup> exchange technique, in contrast to the apparent 100 per cent exchange in the rabbit.

The results of the external survey and of the tissue analyses suggest 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 is exchanged very slowly.

The application of this tracer technique to the study of the pathophysiology of various clinical disorders is eagerly awaited. The recent availability of Mg<sup>28</sup> of considerably higher specific activity and the more sensitive detection of radioactivity made possible by the whole-body counter should enable us to prolong the period of experimental observation. Silver, Robertson, and Dahl (7) were able to follow the magnesium turnover for periods up to ninety hours after the intravenous injection of Mg<sup>28</sup> into human subjects. Equilibration of the isotope with the magnesium in the body was slow, and at forty to sixty hours amounted to about 10 to 25 per cent of the body's total; at ninety hours perhaps a third of the body's magnesium had reached equilibrium with the isotope. Graphic analysis of urinary Mg<sup>28</sup> curves in terms of exponential components revealed a slow component, with a half-time of fourteen to thirty-five hours, accounting for 10 to 15 per cent of the injected dose; and two more rapid components, with half-times of one and three hours each, accounting for 15 to 25 per cent of the injected dose. The large fraction remaining—about 25 to 50 per cent of the body's total—had a turnover rate of less than 2 per cent per day. Presumably this latter fraction is made up largely of the magnesium in bone.

### In Rats

The previous two studies (6, 7) with Mg<sup>28</sup> were performed in human adults. Breibart, Lee, McCoord, and Forbes (8) have re-

ported a study in rats which suggests that the exchange of magnesium by the bone cortex is related to the age of the animal. They found that the exchange of  $Mg^{28}$  in cortical bone was five to ten times greater in rats twenty to thirty days old than in rats 60 to 180 days old. The bone also showed a progressive decline in water content and a progressive increase in stable magnesium content as the animals became older. This greater degree of magnesium exchange by the bone of young animals may be related to the smaller size of the bone crystals or to the greater water content of the bone, which might give it greater accessibility to circulating body fluids.

Rogers and Mahan (9) concluded that magnesium is present in rats in at least two physiologic states: one with a turnover time of 1.2 hours, and the other with an exchange time of about twenty-five hours. In the exchange of plasma magnesium with that in the cells, tissues seemed to fall into two classes. In the liver, heart, and kidney the exchange was rapid, and complete equilibrium was reached in about three hours. In the brain, testes, red cells, and skeletal muscle the exchange was not complete after seven hours. A stimulated skeletal muscle showed a more rapid exchange than an unstimulated muscle. The specific activity of the plasma decreased at a slightly faster rate in immature rats than in older rats. Unfortunately, the bone cortex was not included in this study.

### In Dogs

Brandt, Glaser, and Jones (10) studied the tissue distribution and plasma disappearance of  $Mg^{28}$  administered intravenously to dogs. The rapid disappearance of  $Mg^{28}$  from plasma could not be accounted for solely by urinary excretion and distribution in the extracellular fluid; it apparently resulted from rapid entry of the radioactive magnesium into the intracellular space. At twenty-four hours, the highest concentrations of  $Mg^{28}$  per gram of dry weight were found in the heart, kidney, liver, and pancreas. During the forty-eight hours of observation the concentration of  $Mg^{28}$  was found to be higher in heart muscle than in any other tissues, including the bone cortex. The uptake of isotopic magnesium by bone was extremely variable.

Lazzara and his co-workers (11) gave rapid intravenous injections of an isotope with high specific activity to thirteen dogs. Of seventy-eight tissues sampled at death, thirty-six reached the maximal concentration of radioactivity within sixty-eight hours. Times (hours) of maximal concentration for a few tissues were as follows: heart, 9 to 24; pancreas, 22 to 68; adrenal gland, 1.5 to 24; liver, 2.5 to 24; lung, 9 to 36. Calculated values for "exchanging" magnesium (mEq. per kilogram) were: ventricular myocardium, 23; pancreas, 23; adrenal gland, 14; liver, 14; atrium, 14; lung, 11. These values correspond closely to available chemical analyses. Important tissues not reaching equilibrium within sixty-eight hours were the brain and spinal cord, cortical bone, and skeletal muscle. Body "exchanging" magnesium mass during the interval from twenty-four to sixty-eight hours in plasma equivalents ranged from 4.9 to 5.7 mEq. per kilogram.

$Mg^{28}$  excretion was primarily urinary, and was maximal during the first twenty-four hours (6 to 20 per cent of the dose). This rate is substantially less than that reported by others, probably because smaller amounts of carrier magnesium were used.

### In Sheep and Lambs

$Mg^{28}$  administered intravenously to normal lambs disappeared rapidly from the plasma during the first two hours, and more slowly thereafter; within approximately eight to ten hours most of the radioactivity was gone from the blood (12). Uptake of  $Mg^{28}$  by red blood cells was very small. Over a thirty-five hour period 3.6 to 5 per cent of the intravenous dose was excreted in the feces, and 5.9 to 7.2 per cent in the urine. The liver contained the largest amount of  $Mg^{28}$ , while the highest concentration was in the heart, followed in order by the liver, spleen, and kidney.

After an oral dose of  $Mg^{28}$ , radioactivity in the plasma reached a maximum at twelve to fourteen hours. Although the heart, liver, spleen, lungs, and kidneys were the sites of greatest  $Mg^{28}$  activity when the isotope was given orally, the ubiquitous character of this ion in the animal body was shown by its widespread distribution in other tissues. The long bones, particularly the shank portion,

contained the highest isotope activity. It was concluded that a dietary deficiency of magnesium results in mobilization of the magnesium reserve in bone.

In the first five days after ruminal administration of 30 to 40 microcuries of cyclotron-produced "carrier-free" Mg<sup>28</sup> to sheep (13), 79.2 per cent of the dose was excreted in the feces and only 0.07 per cent in the urine. Following intravenous injection, 11.9 per cent of the dose was recovered in the urine in five days and 19.4 per cent in the feces in four days and eight hours (13).

### MAGNESIUM EQUILIBRIUM IN MUSCLE

Gilbert (14) used Mg<sup>28</sup> to study the permeability of isolated frog sartorius membrane to magnesium. Muscle was immersed in Ringer's solution containing 2 millimoles of magnesium tagged with Mg<sup>28</sup>, and the uptake of the radioactive magnesium was measured under controlled conditions. The uptake proceeded in three stages lasting respectively about 0.5, 30, and 300 minutes, and accounting respectively for about 0.21, 0.71, and 0.67 millimole of magnesium per kilogram of muscle. It was assumed that the first stage represented surface adsorption, the second stage represented uptake by extracellular water and connective tissue, and the third stage, entry inside the cell. It was estimated that the maximum intracellular magnesium concentration is about 1.1 millimole, and that only about 0.6 millimole of magnesium per liter of intracellular water is exchanged per hour. The maximum energy required per hour to pump the magnesium out of the cell against the electrochemical gradient was calculated to be only 1.5 calories per kilogram of muscle. About 75 to 80 per cent of the magnesium in muscle is nonexchangeable and difficult to remove by diffusion.

### SUMMARY

The sum of these data indicates that intravenously injected magnesium rapidly reaches a state of equilibrium with the stable magnesium in the extracellular fluid, and that various tissues subsequently take up this ion at different rates. Uptake is rapid in the soft tissues, including the heart, liver, kidneys, adrenals, lymph

nodes, and appendix. It is slow in the skin and muscle. In adult animals, uptake is slow in the bone. Fetuses and growing animals concentrate magnesium rapidly in bone and muscle.

In dogs and human beings, equilibration of the Mg<sup>28</sup> with the body magnesium content was not complete sixty hours after intravenous administration of the isotope. For this reason, the isotopic dilution method, used so successfully with potassium, cannot be employed to quantitate the total body content of magnesium in these species.

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## *Chapter XIV*

# FACTORS INFLUENCING THE TISSUE UPTAKE OF MAGNESIUM

## ALTERATION OF CARBOHYDRATE METABOLISM

EARLY STUDIES SUGGESTING a causal relationship between magnesium and carbohydrate metabolism have been summarized by Stutzman (1). Among other observations, it has been reported that the parenteral administration of magnesium may cause a temporary hyperglycemia or hypoglycemia, depending on the dose given. The intravenous injection of small amounts of magnesium decreases the rate of removal of glucose from the blood stream—probably because of impaired peripheral utilization, accelerated glycolysis, or both. The intravenous administration of insulin may result in a temporary increase in the serum magnesium concentration, followed by a decrease. Glucose infusions have been reported to decrease serum magnesium concentrations. Insulin and magnesium are said to have a synergistic effect.

### **Effect of Insulin and Glucose on the Plasma Clearance of Mg<sup>28</sup>**

Twelve normal adult male rabbits were each given an intravenous injection of a tracer dose of Mg<sup>28</sup> (2). Two hours later, intravenous injections containing 2 units of crystalline zinc insulin per kilogram of body weight and 10 ml. of a 5 per cent solution of dextrose were given to six of the rabbits. For six hours after the administration of Mg<sup>28</sup>, blood specimens were obtained hourly for determinations of the concentration of radioactivity (Table 3).

During the first hour after the injection of insulin and glucose, the plasma Mg<sup>28</sup> concentration decreased at a significantly faster rate in the animals given insulin and glucose than in the control group. Since the two groups showed no difference in the urinary excretion of Mg<sup>28</sup> during the period of observation, the more rapid decrease in Mg<sup>28</sup> concentration in the plasma of the test animals is

interpreted as indicating an increased rate of uptake of Mg<sup>28</sup> by the tissues. Magnesium metabolism thus appears intimately related to that of glucose and insulin.

TABLE 3

| Time Interval<br>After Injection<br>of Mg <sup>28</sup> (Hours) | THE EFFECT OF INSULIN AND DEXTROSE ON THE PLASMA CLEARANCE OF Mg <sup>28</sup>         |             |            |            |           |
|---|--|-------------|------------|------------|-----------|
|   | 1-2  | 2-3         | 3-4        | 4-5        | 5-6       |
|   | Decrease in the Plasma Concentration of Mg <sup>28</sup> During<br>the Hour (Per Cent) |             |            |            |           |
| Control <sup>a</sup>  | 44.8 ± 1.1 <sup>c</sup>  | 23.5 ± 1.7  | 18.9 ± 1.7 | 11.6 ± 5.7 | 9.3 ± 4.0 |
| Test <sup>b</sup>   | 44.1 ± 17.9  | 35.5 ± 2.9* | 19.5 ± 8.8 | 15.3 ± 6.5 | 7.2 ± 4.2 |

\*Statistically significant difference when compared with control mean ( $P = <0.01$ ).

<sup>a</sup>Six rabbits given only a tracer dose of Mg<sup>28</sup>.

<sup>b</sup>Six rabbits given Mg<sup>28</sup>, followed after two hours by an intravenous injection of insulin, 2 units per kilogram, and dextrose, 10 ml. of a 5 per cent solution.

<sup>c</sup>Mean ± standard error.

### Effect of Insulin and Glucose on the Tissue Uptake of Mg<sup>28</sup>

The simultaneous injection of magnesium, insulin, and glucose did not alter significantly the serum magnesium concentration or the blood glucose level. It did increase the uptake of Mg<sup>28</sup> in all tissues studied: bone cortex, kidney, heart, liver, appendix, skin, and skeletal muscle (3). Since the magnesium content of bone, liver, and skin did not increase significantly, the accelerated Mg<sup>28</sup> uptake by these tissues was attributed to more rapid exchange of Mg<sup>28</sup> between their intracellular and extracellular compartments. In skeletal muscle and heart, on the other hand, there was a significant increase in magnesium content as well as in the Mg<sup>28</sup> relative activity. It was concluded, therefore, that in these two locations actual deposition of increased amounts of magnesium had occurred. Less striking changes were noted when insulin or dextrose alone was given with the injection of magnesium.

The biochemical mechanisms whereby insulin and dextrose accelerate Mg<sup>28</sup> turnover in the tissue are obscure, but the results suggest that the metabolism of magnesium is intimately related to that of carbohydrate, and that two of the factors regulating the turnover of magnesium in tissues are insulin and dextrose.

### In Vitro Studies

In a subsequent study (2), pieces of fresh rabbit diaphragm were incubated at 4 C. and 37 C. for as long as seven hours in various bath solutions (such as physiologic saline solution, balanced salt solution, and plasma) which contained tracer amounts of Mg<sup>28</sup>. In these *in vitro* systems, the addition of insulin and glucose did not change the rate at which Mg<sup>28</sup> was taken up by the diaphragm. The same result was obtained in a few studies performed with heart, kidney, and bone cortex under similar *in vitro* conditions. In the absence of an intact circulatory system, insulin and glucose have no effect on the *in vitro* uptake of Mg<sup>28</sup> from the incubating medium.

A rabbit liver homogenate in sucrose containing a tracer amount of Mg<sup>28</sup> was divided into two identical portions. Insulin and glucose were added to one, and both portions were then subjected to differential ultra-centrifugation and assayed for radioactivity content in Fractions I, II, and III and the supernatant fluid. The test and control specimens contained respectively the following percentages of the total radioactivity: Fraction I, 18.1 and 19.2; Fraction II, 7.0 and 5.5; Fraction III, 13.6 and 14.5; and supernatant fluid, 56.2 and 56.5. In the absence of the cell membrane, insulin and glucose had no effect on the distribution of Mg<sup>28</sup>.

### Alloxan Diabetes

Since the administration of exogenous insulin and glucose accelerated the tissue uptake of Mg<sup>28</sup>, it was of interest to determine whether the experimental production of diabetes would have the opposite effect. Alloxan readily produced diabetes in rabbits; hyperglycemia, polyuria, glycosuria, and weight loss became evident within a few days after its administration (4).

Although the mean exchangeable magnesium content was decreased significantly in the diabetic rabbits, there was no change in the external balance of magnesium, and the relative radioactivity of muscle, skin, appendix, lung, liver, heart, kidney, and bone did not differ significantly in the test and control groups. There was, however, a significant reduction in the magnesium content of

the skin and a significant increase in the magnesium content of the bone. These results indicate that the dynamics of magnesium metabolism were altered by diabetes; but the exact significance of the changes remains to be unraveled.

### Iodoacetate In Vivo

Since the administration of supplementary insulin and glucose accelerated tissue uptake of Mg<sup>28</sup>, it was thought that inhibition of the glycolytic cycle might decrease tissue uptake of Mg<sup>28</sup>. Iodoacetate specifically blocks the conversion of the triosephosphate enzyme 2 (1,3-phosphoglyceraldehyde) to mutase (2 [2-phosphoglycerate]).

Eleven rabbits were given intravenous injections containing 30 to 40 mg. of sodium iodoacetate per kilogram of body weight. Thirty minutes later a tracer dose of Mg<sup>28</sup> was injected intravenously. The eight animals which survived were killed four hours after the injection of Mg<sup>28</sup>, and the relative tissue uptake of Mg<sup>28</sup> was determined (2). Statistically significant reductions in the relative tissue uptake of brain, bone cortex, heart, and kidney were found.

### Iodoacetate In Vitro

Bone cortex, liver, kidney, heart, diaphragm, and intestine were incubated for three hours at 37 C. in baths containing 0.05 molar sodium iodoacetate at pH 8.12 (2). The Mg<sup>28</sup> uptake of these tissues did not differ from that of similar tissues incubated without iodoacetate.

## CORTISONE

The pharmacologic actions of cortisone are manifold. Since it is known to produce diabetes under certain circumstances, and since previous studies have linked magnesium and carbohydrate metabolism, the effect of cortisone on magnesium metabolism was investigated (5).

External balance studies for magnesium and serial isotopic determinations of the exchangeable magnesium content were made in seven adult male rabbits before and during a fourteen-day series

of daily subcutaneous injections of cortisone acetate (10 mg. per kilogram of body weight). Significant reductions in serum magnesium concentration were found at eight and fourteen days. The exchangeable magnesium content was increased on the eighth day. In another group of rabbits, relative tissue activity studies showed that the uptake of Mg<sup>28</sup> by the heart, appendix, and muscle was increased on the eighth day of cortisone administration.

These results suggest that cortisone produces subtle changes in the distribution of magnesium in the body, which cannot be attributed to its diabetogenic or anti-inflammatory effect. Further studies may show that cortisone stimulates anabolic activities in certain tissues such as the heart and the connective tissue.

### PYRIDOXINE AND DESOXYPYRIDOXINE

In a search for experimental conditions suitable for studying the relationship between protein metabolism and magnesium metabolism, these two drugs were used in experiments with rabbits (6). It was found that pyridoxine deficiency alters both protein and magnesium metabolism, and produces changes in enzymatic reactions.

In eight rabbits given two intravenous injections of pyridoxine (100 mg. each) twenty-four hours apart, the relative tissue activity of Mg<sup>28</sup> was increased in the appendix and the heart—the same tissues affected by cortisone. In fifteen rabbits given seven daily intravenous injections of desoxypyridoxine, 40 mg. per kilogram, a significant *decrease* occurred in the relative activity of the kidney, lung, and bone cortex, and a significant increase in the serum magnesium concentration. These experiments showed that the rate of uptake of Mg<sup>28</sup> by various tissues is altered by the administration of pyridoxine or desoxypyridoxine.

### DIGITOXIN

Since digitoxin has been found to alter potassium metabolism (7), its effect on magnesium metabolism was also studied (8).

In a test group of eight rabbits the administration of digitoxin in doses of 0.1 mg. per kilogram, given by intravenous injection daily for eight days, produced no significant changes in the external

balance of magnesium. On the day following the last injection, however, the exchangeable magnesium content was significantly increased. At the same time, all the tissues studied showed an increase—though not a significant one—in the mean values for relative radioactivity. A significant decrease, which could not be adequately explained, was observed in the magnesium content of the skin. The results suggest that the experimental conditions produced an internal redistribution of magnesium, which was not reflected in the external balance data.

### **UNCOUPLING AGENTS**

By *coupled phosphorylation* or *oxidative phosphorylation* is meant the series of biochemical events in the respiratory sequence whereby energy is generated from oxidation of the hydrogen carriers by the enzymes of the mitochondria, and transferred from exergonic to endergonic reactions by the conversion of adenosine diphosphate (ADP) to adenosine triphosphate (ATP). ATP then serves as the "common currency" to supply energy for other reactions. Various drugs are known to "uncouple" this chain of events so that the free energy is not accumulated as ATP, but is dissipated as heat. Such an uncoupling process may not necessarily affect the accompanying respiratory processes. The effects of the uncoupling agents on magnesium metabolism were studied in the hope that they might furnish insight into a possible interrelationship.

### **Thyroxine and Propylthiouracil**

The previous contradictory evidences for and against an association between alterations in magnesium partition in the serum and disorders of the thyroid gland have been reviewed in Chapter XII.

In experiments employing rabbits, external balance studies for magnesium and isotopic studies with  $Mg^{28}$  were performed following the administration of thyroxine or propylthiouracil (9). In eight rabbits given two intravenous injections of thyroxine sodium (0.3 mg. per kilogram of body weight) no significant changes were noted in the body weight, magnesium balance, or exchangeable

magnesium content, although the serum magnesium concentration was decreased. In another group of eight rabbits the relative tissue activity of Mg<sup>28</sup> was determined within four days following the intravenous injection of 1 mg. of thyroxine per kilogram of body weight. The relative activity was increased in liver, skin, appendix, and heart.

Eight rabbits given propylthiouracil orally showed a decrease in the exchangeable magnesium content at the end of the third week. At the same time a significant decrease was found in the relative radioactivity of the liver and bone. The value for exchangeable magnesium content appeared to be related to the rate of turnover of Mg<sup>28</sup> in bone and muscle—the two largest body stores of magnesium.

These studies indicate that stimulation of cellular activity by thyroxine accelerates the uptake of Mg<sup>28</sup>, while suppression of cellular activity with propylthiouracil decreases the uptake of Mg<sup>28</sup>.

### Sodium Salicylate

Rabbits given sodium salicylate in a single intravenous dose of 200 mg. per kilogram of body weight died within a few minutes.

Six rabbits were given a smaller intravenous injection of sodium salicylate (150 mg. per kilogram), followed immediately by a tracer dose of Mg<sup>28</sup> and two hours later by another injection of the same amount of sodium salicylate. When the animals were killed two hours after the last injection, tissue assays revealed that the relative radioactivity of the bone marrow, spleen, and adrenal gland was significantly increased, and that of the appendix significantly decreased.

Eight rabbits received sodium salicylate for five days in daily doses of 40 mg. per kilogram (20 mg. per kilogram given by interscapular injection at 9 A.M. and again at 4 P.M.). On the sixth day an injection of 20 mg. per kilogram was followed immediately by the intravenous administration of a tracer dose of Mg<sup>28</sup>. When the animals were killed four hours later, significant increases in relative radioactivity were found in the following tissues: kidney, lung, heart, bone marrow, and spleen.

## 2,4-Dinitrophenol

2,4-Dinitrophenol uncouples oxidative phosphorylation so rapidly and so effectively that fever develops within thirty to sixty minutes after injection of the drug into rabbits.

Eight rabbits were given 2,4-dinitrophenol interscapularly in daily doses of 10 mg. per kilogram (5 mg. per kilogram at 9 A.M. and again at 4 P.M.) for five days (2). Thirty minutes after the final injection of the drug, Mg<sup>28</sup> was injected intravenously, and the animals were killed four hours later for determinations of relative tissue activity. A significant increase in relative radioactivity was found in the following tissues: liver, kidney, lung, appendix, heart, bone marrow, spleen, brain, and stomach.

Comparison of the effects of the three uncoupling agents which we have studied indicates a positive relationship between the known intensity of pharmacologic effect and the number of tissues and the degree of increase in the relative radioactivity of Mg<sup>28</sup>.

## ANABOLIC AGENTS: TESTOSTERONE AND SOMATOTROPIN

If magnesium metabolism is related to cellular hypertrophy and hyperplasia, as is suggested by previous studies on fetal tissues, protein-anabolic agents such as testosterone and somatotropin would be expected to increase the Mg<sup>28</sup> uptake of various tissues. To test this hypothesis, the following experiments were performed (2) :

Four rabbits were given, by subcutaneous injection, pork pituitary growth hormone in a single dose of 4 mg. per kilogram, followed by a tracer dose of Mg<sup>28</sup>. When the uptake of radioactivity by various tissues was compared with that in the plasma, the relative radioactivity was found to be significantly increased in the spleen and bone marrow.

In rabbits given testosterone propionate intramuscularly in a single dose of 10 mg. per kilogram, there were significant increases in the relative radioactivity of the heart and stomach, as well as the spleen and bone marrow.

These experiments suggest that an increase in the activity, size, or number of cells is associated with an increased uptake of Mg<sup>28</sup>.

## CATABOLIC AGENTS

### Inhibition of Mitosis by Colchicine

In the experiments reported thus far, tissue uptake of Mg<sup>28</sup> was increased by those substances which stimulate metabolic activity of cells, and decreased by those agents which suppress this activity. In view of these findings, it appeared reasonable to suspect that inhibition of cellular mitosis with a poison such as colchicine would suppress the metabolism of magnesium.

Colchicine in sublethal doses was injected subcutaneously into rabbits, and its effects were studied by both the external balance technique and the use of Mg<sup>28</sup> (2). A total dose of 6 to 7 mg. per kilogram decreased the relative radioactivity in the heart, appendix, lung, kidney and bone cortex, and increased the serum magnesium concentration.

In another group of rabbits given subcutaneous injections of colchicine, 0.15 mg. per kilogram daily for four days, there was no change in the external balance studies or in the exchangeable magnesium content. A single dose of 2 mg. per kilogram, however, produced a significant decrease in the exchangeable magnesium content.

Apparently the suppression of mitosis, the associated functional metabolic alterations, or the combination of both factors caused a decrease in the rate of uptake of Mg<sup>28</sup> by various tissues. The decrease in the exchangeable magnesium content was thought to be due primarily to the suppression of Mg<sup>28</sup> uptake in bone.

### X-irradiation

Total body X-irradiation is the best experimental method available for producing generalized cellular catabolism and death. Such wholesale injury to cells would be expected to suppress magnesium metabolism. To test this hypothesis, external balance studies for magnesium and serial isotopic determinations of the exchangeable magnesium content and relative tissue uptake of Mg<sup>28</sup> were made in male rabbits subjected to 600 r of whole-body X-irradiation (2).

Within three hours Mg<sup>28</sup> uptake by the adrenal gland was in-

TABLE 4  
EFFECT OF VARIOUS AGENTS ON THE RELATIVE TISSUE UPTAKE OF Mg<sup>65</sup> IN THE RABBIT

| Tissue      | <i>Insulin Iodo-</i><br><i>Control and ac-</i><br><i>glucose tate</i> | <i>Corti-</i><br><i>Desoxy-</i><br><i>pyri-</i><br><i>doxine</i> | <i>Thy-</i><br><i>nitro-</i><br><i>uracil</i> | <i>Propyl- 2,4-Di-</i><br><i>thio-</i><br><i>phenol</i> | <i>Salicylate (2)</i><br><i>300 mg/Kg daily for</i><br><i>6 days</i> | <i>Tes-</i><br><i>cine (2)</i> | <i>Colchi-</i><br><i>cine (2)</i> | <i>600 R X-irra-</i><br><i>diation (after</i><br><i>8 hrs.)</i> |
|-------------|---|--|---|---|--|--------------------------------|-----------------------------------|---|
| Adrenal     | 5.10  | 9.90   | 11.64*  | 5.57*   | 7.15   | 6.36                           | 7.81*                             | 6.54  |
| Appendix    | 7.66  | 22.10*   | 9.14*   | 12.67   | 9.29*  | 6.24*                          | 10.90*                            | 6.94  |
| Bone cortex | 12.38   |  |   |   | 12.89  | 15.13                          | 10.59                             | 9.64  |
| Bone marrow | 2.12  |  |   |   |  | 5.27*                          | 5.43*                             | 3.09*   |
| Brain       | 0.65  |  | 0.46*   |   |  | 1.01*                          | 0.69                              | 4.01*   |
| Heart       | 8.44  | 12.60*   | 5.68*   | 13.29*  | 6.85*  | 7.89                           | 13.13*                            | 0.98  |
| Kidney      | 10.46   | 14.60*   | 6.60*   | 13.76*  | 8.65   | 11.20                          | 10.24                             | 13.06*  |
| Liver       | 5.10  | 9.80*  | 5.86  | 5.66  | 4.65   | 9.92*                          | 3.47*                             | 11.07*  |
| Lung        | 3.77  |  | 3.02  | 4.83  | 3.19   | 3.98                           | 3.58                              | 14.07*  |
| Muscle      | 0.76  |  | 1.24  | 0.50  | 1.23   | 0.58                           | 0.41                              | 10.69   |
| Skin        | 1.36  |  | 2.84*   | 1.26  | 1.63   | 1.43                           | 1.39                              | 6.02*   |
| Spleen      | 5.01  |  |   |   |  |                                | 16.86*                            | 5.65*   |
| Stomach     | 7.05  |  |   |   |  |                                | 12.31*                            | 4.23  |
| Testis      | 5.10  |  |   |   |  |                                | 6.17                              | 4.55  |
|             |   |  |   |   |  |                                | 3.49                              | 5.30  |
|             |   |  |   |   |  |                                |                                   | 6.28  |
|             |   |  |   |   |  |                                |                                   | 2.51  |

\*Statistically significant difference when compared with the control ( $P = <0.01$ ).

creased. At eight hours, relative radioactivity was decreased in the appendix, heart, kidney, stomach, and bone cortex. At twelve hours, decreases in relative radioactivity were observed in the appendix, kidney, stomach, bone cortex, and testis. At twenty-two hours, only the heart and kidney showed decreased relative radioactivity. A decrease in the exchangeable magnesium content was not observed until the sixth day after exposure. External balance studies carried out for six days after exposure showed a significantly decreased turnover of stable magnesium.

If the suppression of  $Mg^{28}$  uptake in tissues is an index of functional cellular damage, this experiment seems to indicate that the bone cortex, kidney, and heart—tissues previously considered to be radioresistant—are as radiosensitive as the appendix, stomach, and testis.

### SUMMARY

The studies reported in this chapter suggest that insulin is necessary to transfer magnesium across the cell membrane. This role of insulin in the metabolism of magnesium appears to be similar to its role in the metabolism of glucose and potassium. Magnesium within the cell is essential for proper cellular functioning—certainly for the oxidation of glucose. Since magnesium appears to be involved in oxidative phosphorylation and the production of ATP, it seems to play a secondary role in protein and fat metabolism as well.

Studies of the various factors affecting the tissue uptake of magnesium suggest that magnesium metabolism is influenced by any condition which alters 1) cellular integrity or volume, 2) the exercise of specialized cellular functions, 3) cell growth or division, or 4) the synthesis of special metabolites and storage products. These changes may be so subtle that they cannot be detected by the classic external balance method, but can be demonstrated only by the radioisotopic technique. Further studies must be made before the significance of these latter changes is fully understood.

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## *Chapter XV*

### **THE NATURE OF MAGNESIUM IN BONE**

**T**HE SKELETAL SYSTEM contains up to 60 per cent of the total body content of magnesium. The exact nature and function of this magnesium in bone, however, are still poorly understood.

#### **SKELETAL MAGNESIUM AS A BODY RESERVE**

In the rat the skeletal magnesium serves as a reserve which can supply magnesium to the soft tissues during periods of acute deficiency of the element in the diet (1). In rats with subacute magnesium deficiency, the magnesium content of the bones was reduced to approximately two thirds of the normal level (2). In growing rats placed on a deficient diet, the quantity of magnesium released from the skeleton and its apparent rate of mobilization were related to the rate of bone growth. The absence of an initial lag suggests that there are no other reserves of significant magnitude and lability. Apparently a falling level of plasma magnesium is responsible for the outflow of magnesium ions from the bone salt in rats (3).

In calves bone-ash magnesium appears to play little or no part in the control of plasma magnesium when the diet contains a plentiful supply; when the diet is deficient in magnesium, both the concentration of plasma magnesium and the bone content of magnesium decrease (4).

#### **IN VIVO STUDIES ON RADIOMAGNESIUM EXCHANGE**

When rabbits were fed a magnesium-deficient diet containing 6.6 mEq. of magnesium per kilogram, the urinary excretion of magnesium dropped to less than 0.8 mEq. daily (5). By the end of the first week the body weight had decreased significantly and the serum magnesium concentration had dropped to 1.2 mEq. per liter, where it remained through the thirtieth day. The exchange-

able magnesium content was one third of the baseline value by the end of the first week, and continued to decrease thereafter.

After the animals had received the diet for one month, the magnesium content of most soft tissues was unchanged; only lung and bone showed a significant reduction. At the same time, study of the relative radioactivity content of the tissues showed that most soft tissues continued to accumulate magnesium at the usual rate. Relative radioactivity was significantly decreased only in skin and bone.

This study confirms previous observations that bone serves as the reservoir from which magnesium is drawn during the ingestion of a magnesium-deficient diet (5).

#### **RELATION OF AGE TO RADIOMAGNESIUM EXCHANGE**

The exchange of Mg<sup>28</sup> is five to ten times greater in rats twenty to thirty days old than in rats 60 to 180 days old. The stable magnesium content of bone increases with age, and varies inversely with the water content (6). The magnesium ion is adsorbed onto the surface of bone crystals (7). The greater degree of exchange exhibited by the bone of the young animal may be related to the smaller size of the mineral crystals (8) or to the greater water content of young bone, which might make it more accessible to circulating body fluid (9).

In puppies relatively greater amounts of radioactive magnesium were found in the epiphyseal line than in the epiphysis or diaphysis (10). The epiphyseal line is the growing, actively metabolizing portion of bone in immature animals.

#### **IN VITRO STUDIES ON RADIOMAGNESIUM EXCHANGE**

Histologically, bone consists of apatite (the microcrystalline mineral structure), bone cells, and the matrix. *In vivo* studies in rabbits (described in the preceding chapter) revealed that insulin and glucose increased the relative uptake of Mg<sup>28</sup> by bone cortex at three hours from a mean control value of 12.38 to 22.10 (11). Iodoacetate, propylthiouracil, desoxypyridoxine, colchicine, and X-irradiation all *decreased* the ratio significantly. All of these agents would be expected to affect living, active cells, and would

not be expected to influence the uptake of Mg<sup>28</sup> by the crystalline structure of bone. In an effort to understand further the mechanism of absorption of magnesium by bone, *in vitro* studies were performed under various conditions (12).

## Methods

The basic incubating medium contained sodium chloride, 120 mEq. per liter, and sodium bicarbonate, 30 mEq. per liter. The pH of this solution, unadjusted, was 8.0. To this solution was added a sufficient amount of the stock solution of Mg<sup>28</sup> to make the concentration of radioactivity high enough for ready assay. The final concentration of magnesium in the bath was usually less than 0.01 mEq. per liter. The drug to be tested was added to obtain the desired final concentrations.

Rabbits were killed by exsanguination. The shaft of the tibia was isolated, and cylindrical sections 0.7 to 1.0 cm. in length were cut. A maximum of six sections was usually obtained from each shaft. After the marrow had been removed with a cotton-tipped applicator stick, the samples were ready for use.

Each sample of tibia was placed in an individually stoppered, 50-ml. Erlenmeyer flask usually containing 25 or 50 ml. of the incubation mixture. After the flasks had been incubated for three hours in a water bath at 37 C., the bone was removed, wiped dry with tissue paper, weighed, and assayed for radioactivity content. The counting rate per gram (wet weight) of bone was compared with that per milliliter of bath mixture, and the results expressed as the ratio,

$$\frac{\text{cpm/Gm.bone}}{\text{cpm/ml.bath.}}$$

## Comparison of Relative Activity In Vivo and In Vitro

After three hours in the basic incubating medium, bone samples had a relative activity of 6.22. By comparison, the relative activity in bone cortex three hours after the intravenous injection of Mg<sup>28</sup> into rabbits was 12.38. This finding suggests that cellular activity is involved in the absorption of Mg<sup>28</sup> by bone *in vivo*.

### Effects of Drugs

None of the following drugs altered the *in vitro* uptake of Mg<sup>28</sup> by bone: digitoxin, cortisone, 2,4-dinitrophenol, propylthiouracil, colchicine, insulin and glucose, and phenylhydrazine.

The finding that drugs which influence Mg<sup>28</sup> uptake *in vivo* do not alter the ratio *in vitro* suggests that the absorption of Mg<sup>28</sup> by bone cells *in vitro* is not due to the presence of active cells. Whether these negative results indicate that bone cells are dead or that the drugs, because of the loss of circulation in bone, do not reach the cells by simple diffusion cannot be determined from these observations.

### Effects of Physical Agents and Metabolic Inhibitors

When the bone was boiled for five minutes before incubation, the relative activity increased. Fluoride and cyanide also increased the uptake of Mg<sup>28</sup>, but exposure to one normal hydrochloric acid for several hours decreased the uptake. The increase in Mg<sup>28</sup> uptake produced by boiling and by incubation with fluoride and cyanide was the same in dried bone as in fresh bone. Increasing the pH of the incubation mixture from 6.0 to 10.0 resulted in a progressive increase in the relative activity of Mg<sup>28</sup> up to a pH of 9.0.

Although fluoride and cyanide are metabolic inhibitors *in vivo*, they are known to be adsorbed very avidly to bone apatite. In the absence of metabolic activity of bone *in vitro*, the effect of these agents in the incubating medium is to increase the physicochemical adsorption of magnesium to bone apatite. The similarity in the behavior of dried bone and fresh bone appears to be conclusive evidence that living cells are not necessary to explain the observed results—and that they must, therefore, be due to adsorption of Mg<sup>28</sup> to the bone matrix, the crystalline structure, or both. Since decalcification of bone resulted in loss of all the radioactivity, it seems most probable that the major portion of the Mg<sup>28</sup> is adsorbed to the crystalline apatite structure. The increased uptake resulting from boiling of bone could be explained by the removal of some of the matrix or cells, or by greater exposure of the crystalline structure.

### Effects of Plasma, Albumin, and Globulin

The *in vitro* uptake of Mg<sup>28</sup> by bone cortex was suppressed to a marked degree by the addition of rabbit plasma to the incubating medium, and to a lesser degree by the addition of human serum albumin or gamma globulin. While the degree of suppression was proportionate to the concentration of albumin in the incubation mixture, there appeared to be little correlation between the concentration of human gamma globulin and the degree of suppression. This finding suggests that bone competes with the plasma proteins—particularly albumin—for the adsorption of Mg<sup>28</sup>.

### SUMMARY

Taken as a whole, these observations suggest that living bone cells play a role in the concentration of magnesium by bone cortex *in vivo*. Comparison of the *in vivo* and *in vitro* values for relative activity at three hours suggests that roughly half the concentrating ability of the bone cortex is a function of living cells, and half is due to physicochemical adsorption of magnesium to the mineral structure of bone.

The cellular activities related to the metabolism of glucose and insulin accelerate the uptake of Mg<sup>28</sup> *in vivo*, and agents and drugs which suppress the metabolic activities of bone cells decrease this uptake. The physicochemical adsorption of magnesium to bone apatite *in vitro* is conditioned by the pH of the surrounding medium and by the presence of fluoride and cyanide. Agents and treatments which alter the bone matrix do not inhibit adsorption of magnesium onto the apatite. Adsorption of magnesium by bone *in vitro* is suppressed, however, by incubating the bone in serum or serum albumin.

It may well be that the relative distribution of magnesium in bone and in the extracellular fluid is governed by the differential adsorption of this ion to bone and serum albumin.

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## *Chapter XVI*

# **THE ATOMIC STRUCTURE OF MAGNESIUM AND ITS ROLE IN THE LIFE PROCESSES, WITH SUGGESTIONS FOR FUTURE STUDIES**

**T**HIS MONOGRAPH WILL have served its purpose if the reader has been made aware of the essential role of magnesium, not only in human biology, but in all life processes. Without chlorophyll there would be no life on earth; when this single fact is realized, it is humbling to have to admit that science still does not understand fully the role of the magnesium atom at the core of the chlorophyll molecule. Is the chlorophyll molecule a chance product of evolution? Could it be that the key role of magnesium in so many vital processes can be explained simply by the fact that the magnesium ion tends to form hydrates and double salts, and hence has the capacity for complex formation?

### **RELATION OF THE ATOMIC STRUCTURE OF MAGNESIUM TO COMPLEX FORMATION**

If the answer to this question is affirmative, then the biologic role of magnesium can be related to its atomic structure. Ordinary magnesium has an atomic weight of 24.32. Its nucleus contains twelve neutrons and twelve 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 (1). The ionic radii of the alkaline earth elements are as follows: magnesium, 0.78 A.; calcium, 1.06 A.; strontium, 1.27 A.; and barium, 1.43 A. 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 A.; calcium, 1.06 A.; sodium, 0.98 A.; and potassium, 1.33 A.

### **Complexing with Water**

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 character. It is commonly forgotten that water is one of the dipolar molecules which can form complex ions with magnesium. The idea of water's being bound in a complex may appear strange, but is supported by the fact that it is impossible to remove water from such complexes as the hydrate,  $MgCl_2 \cdot (H_2O)_6$ , by heating; it can be removed only by destruction of the complex. This capacity to form a complex with water may be extremely significant in controlling the water content within cells.

Other compounds which are capable of forming complex ions with magnesium include ammonia, amino acids, heterocyclic nitrogen compounds, and organic oxygen-containing compounds. The key role of magnesium in biochemistry may be related to this ability to bring reacting substances physically close to one another and to water.

### **AREAS WHERE FURTHER STUDIES ARE NEEDED**

The availability of a radioactive isotope of magnesium and of simpler and more precise methods for measuring magnesium in biologic materials has already aroused greater interest in the problems related to magnesium metabolism. It is probably true

that our current understanding of magnesium metabolism is comparable to our knowledge of potassium in the early nineteen-forties, when flame photometry and the nuclear reactor had just been introduced to medicine. Greater understanding of magnesium metabolism will almost certainly add immeasurably to our comprehension of basic biochemical and physiologic mechanisms; yet, paradoxically, may be of little immediate value in the clinical management of patients. Cases of pure magnesium deficiency, like cases of pure potassium deficiency, probably occur rarely. Certainly, however, our understanding of the role of potassium in metabolic alkalosis and diabetic acidosis has helped considerably in the management of these conditions.

### **How the Body Obtains and Uses Magnesium**

**Gastrointestinal Absorption:** Although the precise mechanism by which magnesium is absorbed from the gastrointestinal tract is still obscure, it appears that the ordinary diet contains an excess of magnesium, and that the gastrointestinal mucosa absorbs this ion selectively and variably. The factors controlling its absorption are still obscure. Only a small proportion of the magnesium exposed to the surface of the mucosa is absorbed; if it were otherwise, the pharmacologic purgative effect of magnesium sulfate, to cite one obvious example, could not take place.

**Transport:** Less than a third of the ingested magnesium is absorbed by the upper portion of the small intestine. The magnesium which enters the extracellular fluid compartment from the intestinal mucosa may be bound to the water which is fixed to elements of the connective tissue. Within the vascular compartment, a portion of the extracellular magnesium is adsorbed to plasma proteins, especially the serum albumin. This union appears to be a loose electrostatic bond which can be readily broken. Although the fraction adsorbed to protein is small in comparison to the total body content of magnesium, it nevertheless may serve as the most readily available and labile pool of magnesium for supplying the needs of the soft tissues. The dialyzable magnesium in the plasma and the interstitial fluid is probably the direct source of magnesium for soft tissues.

**Bone Store:** The experiments described in the preceding chap-

ters leave very little doubt that the bone store of magnesium is labile and functions to maintain the usual concentration of magnesium in soft tissues and extracellular fluid. It appears that the concentration of magnesium in the extracellular fluid is maintained within its usual narrow range by a balance between the magnesium adsorbed to plasma protein and that adsorbed to bone. No hormonal factor has yet been uncovered to explain this regulatory mechanism, and the most plausible explanation available at present is one based on the atomic structure of the element magnesium.

The younger the animal, the more rapid is the uptake of magnesium by bone; in the adult most of the bone store of magnesium exchanges so slowly that it is not possible to measure the total body pool of magnesium by the isotopic dilution technique. More than half the total bone store of magnesium is released before the serum magnesium concentration decreases. For an ideal man weighing 70 Kg., this amounts to about 750 mEq.—a relatively large deficit.

### Clinical Problems

The greatest clinical problem relating to magnesium metabolism appears to be that of acquiring a thorough understanding of the physiology and the biochemistry of magnesium metabolism, to the end that the alert clinician may be able to anticipate those conditions which produce magnesium deficiency, and take appropriate prophylactic steps. The detection of magnesium deficiency by determination of the red cell magnesium content or the plasma concentration of magnesium is already possible; additional data are needed, however, to evaluate the relative merits of these two methods.

**Conditions Producing Magnesium Deficiency:** Because of the ubiquitous nature of magnesium, creation of a magnesium deficiency is extremely difficult. Unless the subject remains on a pure milk diet or a synthetic diet low in magnesium for prolonged periods, deficiency due to *inadequate intake of magnesium* is not likely to occur. Starvation decreases the body content of magnesium, but does not create a magnesium deficiency; the tissue, plasma, and bone concentration of magnesium remains unchanged.

The increased neuromuscular irritability observed in domestic animals with grass staggers and transit tetany may be related to *competitive inhibition of enzymes* by trace elements such as manganese. It seems that the confusion originally encountered in separating magnesium and manganese chemically persists still at the biochemical level. In the absence of such competitive enzymatic inhibition, magnesium deficiency may develop as part of a *general nutritional deficiency*—as, for instance, in kwashiorkor or severe alcoholic cirrhosis. Milk tetany may be an exceptional instance of insufficient dietary intake of magnesium aggravated by *excessive ingestion of an antagonist of magnesium, calcium*.

While as yet there is no convincing evidence of any *hormonal factor* regulating magnesium metabolism, the precise role of the parathyroid gland, the thyroid gland, and the adrenal cortex should be explored by further studies. The observation that hypomagnesemia is characteristic of primary hyperaldosteronism should be followed up by studies with  $Mg^{28}$  and measurement of the tissue content and turnover of magnesium.

Since a deficit in intake is highly improbable and the role of hormonal factors uncertain, the only known major cause of magnesium deficiency is excessive loss of magnesium from the body by *renal excretion*.

The normal kidney has the capacity to compensate for tremendous variations in the organism's magnesium load. For instance, the rapid intravenous infusion of 97 mEq. of magnesium resulted in the renal excretion of 79 mEq. within fourteen hours (2). On the other hand, the renal excretion of magnesium may decrease to less than 1 mEq. per day if it becomes necessary to conserve body magnesium.

The renal excretion of magnesium can be increased by the administration of mercurial and nonmercurial diuretic agents. The possibility of inducing magnesium deficiency by vigorous treatment of the various fluid-retention states should be studied further.

**Hypermagnesemia:** The problem of hypermagnesemia usually occurs only in association with renal insufficiency, where the elimination of all waste products from the body is reduced.

## The Role of Magnesium in Biochemistry

Biochemists have long accepted, perhaps too tacitly, the necessity for magnesium in many enzymatic reactions. The essential role of magnesium in the various steps of the glycolytic cycle has been emphasized. Because of the proven interrelationship of carbohydrate, protein, and fat metabolism, and because the energy from ATP is necessary for the proper functioning of many subsequent enzymatic reactions, it is not surprising that experimental magnesium deficiency produces a host of seemingly unrelated disorders and pathologic pictures. To date, however, very little effort has been made to correlate enzymatic phenomena with the physiology of magnesium metabolism.

Among the questions which remain to be answered concerning the biochemical role of magnesium are the following: Is all the magnesium in the various tissues a necessary part of various enzymatic mechanisms? What is the exact state of the magnesium—amounting to approximately 15 to 20 mEq. per kilogram—in most soft tissues? The limited studies performed to date on the sub-cellular distribution of magnesium tagged with Mg<sup>28</sup> suggest that magnesium is specifically concentrated by certain subcellular fractions, and does not distribute itself passively in the water content of the various fractions.

## Tissue Uptake of Mg<sup>28</sup> as an Index to the Functional Integrity and Activity of Cells

Studies of the factors affecting the *in vivo* uptake of magnesium by tissues have shown that insulin and glucose facilitate the transfer of magnesium across the intact cell membrane. In general, any condition, drug, or agent which suppresses cellular activity or damages cells will cause a decrease in magnesium uptake. Any condition, factor, or agent which stimulates cellular activity—be it hyperactivity, hypertrophy, or hyperplasia—will increase the uptake of magnesium.

These findings suggest that the rate of uptake of Mg<sup>28</sup> may serve as an index to the functional integrity of the cells in a particular tissue. If this hypothesis is true—and it is supported by all the

studies performed with Mg<sup>28</sup>—, then the finding that X-irradiation suppresses the uptake of Mg<sup>28</sup> in the bone cortex, heart, and kidney, as well as in the stomach and appendix, suggests that the former three tissues are more radiosensitive than was previously suspected. In certain species and conditions the heart takes up Mg<sup>28</sup> faster than bone. If cardiac tissue is as active as this finding indicates, the heart would be unusually susceptible to various conditions upsetting homeostasis.

It has previously been assumed that bone cells are relatively inert. Although insulin and glucose are the only agents which have been found to increase the uptake of Mg<sup>28</sup> by bone, many agents—iodoacetate, propylthiouracil, desoxypyridoxine, colchicine, and X-irradiation—suppress its uptake of Mg<sup>28</sup>. Thus the absorption of magnesium to bone appears to depend partially on the metabolic activity of bone cells. *In vitro* studies indicate that the rest of the adsorptive capacity is based on physicochemical factors.

### CONCLUSION

If the supply of magnesium were not plentiful in most parts of the earth, organisms dependent on magnesium for such fundamental biologic processes as the maintenance of the conscious state, neuromuscular transmission, and hibernation could not have evolved. Much further study is needed for a clear understanding of the fundamental nature of these phenomena. Certainly the nature and function of magnesium in the cerebrospinal fluid should be further investigated.

It would be superfluous to reiterate that further studies on the role of magnesium in all biologic processes are indicated.

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# INDEX

## A

- Aerobic metabolism, 49  
Alcock, N., 66  
Alkaline earth compounds, 8  
    physical characteristics, 106  
Alloxan diabetes, 89  
Aluminum, 11  
Anabolic agents, 94  
Anoxia in hibernating hedgehogs, 38  
Atomic structure of magnesium, 105  
ATP, 50  
Auer, J., 16

## B

- Balance studies in kwashiorkor, 56  
Banks, Sir Joseph, 7  
Barium, 8  
Barnes, B. A., 56  
Barrio, N. G., 73  
Bavaria, 6  
Beddoes, Thomas, 7  
Biochemistry,  
    role of magnesium, 110  
Biologic oxidation, 51  
Biopsies, 80  
Blakemore, F., 32  
Body content of magnesium, 62  
Bone content of magnesium, 23, 62  
Bone  
    exchange of magnesium in rat, 81  
    in-vitro studies of Mg<sup>28</sup> exchange, 101  
        effect of drugs, 102  
        effect of metabolic inhibitors, 102  
        effect of physical agents, 102  
        effect of plasma albumin, globulin, 103  
    nature of magnesium in, 99  
    skeletal magnesium as body reserve, 99  
Brandt, J. L., 82  
Breitbart, S., 81  
Bussy, Antoine Alexandre Brutus, 10  
Butler, A. M., 56

## C

- Calcium, 8  
    carbonate, 5  
    chloride, 19  
Carbohydrate metabolism, 49, 87  
Carbon dioxide, 5  
Catabolic agents, 95  
Cattle, grass staggers in, 29  
Cerebral edema, 19  
Cerebrospinal fluid magnesium, 73  
Chlorophyll, 12, 15  
Clinical problems, 108  
Cohen, H., 73  
Colchicine, 95  
Cold acclimation, 39  
Complex formation, 105, 106  
Connective tissue changes, 26  
Conservation of magnesium, 56  
Convulsions  
    in patients with hypomagnesemia, 53  
    therapy of, 54  
Cope, C. L., 72  
Cortisone, 90  
Cosgrove, J. B. R., 72  
Cunningham, I. J., 30

## D

- Davy, Edmund, 8  
Davy, Sir Humphry, 7  
Davy Medal, 9  
Deficiency of magnesium  
    clinical symptoms and signs, 54  
    experimental production, 21  
    intracellular depletion, 54  
Desoxypyridoxine, 91  
Diabetic coma, 56  
Diffusible magnesium in plasma, 66  
Digitoxin, 91  
Dine, R. F., 72  
2,4-dinitrophenol, 94  
Dow Chemical Company, 11

**E**

- Electrolysis, 8  
 Electrophoresis of serum, 75  
 Epsom, 3  
 Epsom salt, 3  
 Epsom Spring, 3  
 Epsom water, 3  
 Equine transit tetany, 31  
 Exchangeable magnesium content, 79  
     in dogs, 83  
 External survey, 81

**F**

- Faraday, Michael, 6, 10  
 Feces, excretion of magnesium, 65  
 Field, A. C., 31  
 Fitzgerald, M. G., 56  
 Food, magnesium content of, 65

**G**

- Gardner, L. I., 72  
 Gastrointestinal absorption, 65, 107  
 Gilbert, D. L., 84  
 Glauber's salt, 4  
 Glucose  
     effect on plasma clearance of Mg<sup>2+</sup>, 87  
     effect on tissue uptake of Mg<sup>2+</sup>, 88  
 Glycolysis, 49  
 Grass staggers  
     abnormality in magnesium metabolism, 31  
     clinical picture, 29  
     epidemiology, 30  
     laboratory findings, 29  
 Graves' disease, 71  
 Green, H. H., 32  
 Grew, Nehemiah, 3  
 Grignard reagents, 11, 13

**H**

- Hedgehog hibernation in, 35  
 Hibernation  
     artificial production, 37  
     description, 35  
     hypermagnesemia, 36  
     hypoglycemia, 38  
     metabolic changes, 38  
     role of magnesium, 36

Hirschfelder, A. D., 53

Hoffmann, Friedrich, 4

Hyperaldosteronism, 55

Hypermagnesemia, 109

    in hibernation, 36, 37

    in renal failure, 68

Hypocalcemia, in equine transit tetany, 32

Hypoglycemia

    from parenteral magnesium, 87

    in hibernation, 38

Hypomagnesemia, 29

    clinical syndromes with, 55

    in equine transit tetany, 31

    in experimental deficiency, 21

    in grass staggers, 29

    in human diseases, 68

Hypothermia, 39

**I**

Incendiary bombs, 10

Insulin

    effect on plasma Mg<sup>2+</sup> clearance, 87, 88

    effect on tissue Mg<sup>2+</sup> uptake, 88

Intake, daily, of magnesium, 65

Intravenous infusion of Mg<sup>2+</sup>, 80

Iodoacetate effects

    in-vitro studies, 90

    in-vivo studies, 90

Ionic radii, alkaline earth elements, 106

**K**

- Kidney degeneration with magnesium deficiency, 25  
 Kolthoff, J. M., 44  
 Kramer, B., 42  
 Kruse, H. D., 23, 29  
 Kwashiorkor, 54  
     tetany, 55  
     magnesium balance in children, 56

**L**

Labile pool in normal subjects, 81

Lactation tetany, 29

Laughing gas, 7

Lazzara, P., 83

Leroy, J., 21

Lipid metabolism, 49

Livingstone, John, 4

**M**

Magnes, 5  
Magnesia, 4, 5  
Magnesia alba, 5  
Magnesia nigra, 5  
Magnesium  
anesthetic effect, 16  
atomic structure, 105  
bone content, 23, 24  
complex formation, 105  
complexing with water, 106  
deficiency  
    acute phase, 21  
    conditions producing, 108  
    second phase, 21  
discovery of, 7, 8  
distribution in human body, 62  
early studies with magnesium load, 77  
effects on nervous system, 18  
equilibrium in muscle, 84  
erythrocyte content, 22  
experimental deficiency, 21, 56  
    body content, 22  
    calcium retention, 22, 24  
    cellular changes, 25  
    chemical changes, 21  
    clinical changes, 21  
    convulsions, 23  
    eosinophilia, 25  
    in dogs, 23  
    in rats, 21  
    kidney, calcification, 25  
    leukocytosis, 25  
    mitochondrial changes, 26  
    nutritive failure, 22  
    pathologic findings, 25  
    skin lesions, 22  
    in blood and cerebrospinal fluid, 68  
    in bone, 99  
    in cerebrospinal fluid, 73  
    in chlorophyll, 12  
    in industry, 10  
    in organic chemistry, 11  
    in red cells, 69  
    isotopes, 60  
    key role in biochemistry, 106  
    measurements in biologic material, 42  
        EDTA-Eriochrome black T, 44

electrochemical determination, 45  
flame spectrophotometry, 45  
fluorometric analysis, 45  
8-hydroxyquinoline, 44  
magnesium ammonium phosphate method, 42  
    Titan yellow, 44  
physiologic studies, 16  
purgative action, 15  
relation to grass staggers, 30  
relation to veterinary medicine, 29  
role in biochemical processes, 49  
role in hibernation, 36  
role in human disease, 53  
serum, 53, 68  
subacute deficiency, 23  
therapeutic applications, 18  
tissue distribution, 78  
Magnesium ammonium phosphate, 42  
Magnesium carbonate, 5  
"Magnesium space," 77  
Magnesium sulfate, 16, 19  
    as an anesthetic agent, 17  
    acute poisoning, 18  
        post mortem examination, 18  
Magnetite, 5  
Magnium, 8  
Manganese, 5, 32  
    relationship to magnesium, 32  
Martin, H. E., 54  
Maximilian, Prince, of Bavaria, 6  
McCance, R. A., 23, 62, 70  
McCollum, E. V., 21, 23, 29  
Medical Pneumatic Institution, 7  
Meltzer, Samuel J., 16, 21  
Mendel, L. B., 21, 77  
Meningitis, 73  
 $Mg^{88}$   
    decay scheme, 60  
    dialysis studies, 74  
    equilibration in the body, 81  
    external survey in rabbits, 81  
    equilibrium in muscle, 84  
    exchange in bone, 99  
        relation to age, 100  
    physical properties, 60  
    plasma clearance, 87  
        effect of insulin and glucose, 88  
studies in dogs, 82

- studies in human subjects, 80  
 studies in pregnant rabbits, 79  
 studies in rats, 81  
 studies in sheep and lambs, 83  
 studies of binding to plasma proteins, 74  
 studies of magnesium ammonium phosphate method, 42  
 tissue uptake, 110  
     effect of insulin and glucose, 87  
 tracer studies in rabbits, 78  
 Mitosis, inhibition of, 95  
 Montgomerie, R. F., 32  
 Montgomery, R. D., 56  
 Muscle  
     magnesium equilibrium, 84  
     content of magnesium, 63
- N**
- Nanninga, L., 50  
 Nephritis, treatment of, 19  
 Nitre, 5  
 Nobel Prize, 11, 12  
 Nuclear reactor, 60
- O**
- Orange, M., 44  
 Orent, E. R., 21, 23, 29  
 Organomagnesium compounds, 13  
 Osborne, T. B., 21  
 Osmosis, 15  
 Oxidative phosphorylation, 92
- P**
- di Palma, Count, 4  
 Pepys, Samuel, 3  
 Peters, J. P., 53  
 Phenylhydrazine, 69  
 Phlogistic theory, 6  
 Photography, 10  
 Photosynthesis, 12, 13, 51  
 Placenta, concentration of magnesium, 79  
 Plasma clearance of magnesium, 77  
 Potassium, 8  
     content in hibernation, 35  
     isolation, 8  
 Propylthiouracil, 92
- Protein metabolism, 50  
 Pyridoxine, 91
- R**
- Radiomagnesium, 60, 74  
 Randall, R. E., Jr., 56  
 Red cells  
     abnormalities in magnesium, 69  
     concentration of magnesium, 69  
 Relative radioactivity, 79  
     in tissues of rabbits, 79  
     studies with pyridoxine, 91  
 Renal excretion, 66, 80  
 Reticulocytosis, 69  
 Riedesel, M. L., 36  
 Rogers, T. A., 82  
 Rook, J. A. F., 31  
 Royal Institution, 6, 7, 8, 10  
 Royal Society, 7, 8, 9  
 Rumford, Count, 5, 7
- S**
- Sal anglicum, 3  
 Schachter, D., 45  
 Seekles, L., 29  
 Seidlitz powder, 4  
 Serum magnesium  
     adsorption with barium sulfate, 71  
     changes in human disease, 53  
     concentration, 68  
     during pregnancy, 70  
     electrophoresis, 75  
     in grass staggers, 29  
     nature of, 70  
     ultrafiltration studies, 74  
 Sheline, R. K., 60  
 Silver, L., 81  
 Silverman, S. H., 72  
 Simonsen, D. G., 42  
 Sjollema, B., 29  
 Skeletal content of magnesium, 62  
 Smith, P. K., 77  
 Sodium  
     content in hibernation, 35  
     isolation, 8  
     salicylate, 93  
 Soffer, L. J., 71, 72  
 Soft tissue content of magnesium, 63

- Somatotropin, 94  
Specific activity of magnesium, 79  
  in fetal tissues, 79  
  in plasma and urine of rabbit, 80  
  in plasma of rat, 82  
St. Michael's Mount, 10  
Strontium, 8  
Stutzman, F. L., 43, 87  
Subcutaneous injection of magnesium, 16  
"Sulfate space," 77  
Suomalainen, P., 36, 37  
Suter, C., 54
- T**  
Testosterone, 94  
Tetanus, treatment of, 18  
Tetany  
  equine transit, 31  
  following parathyroidectomy, 55  
  in grass staggers, 29  
  low magnesium, 21  
  of human magnesium deficiency, 55  
  of milk-fed calves, 32  
Therapy, magnesium, response to, 54  
Thompson, Benjamin, 5, 6  
Thyroid disorders and ultrafiltrable magnesium, 71  
Thyroxine, 92  
Tibbets, D. M., 65  
Tisdall, F. F., 42
- Tissue concentration of magnesium, 63  
Tissue uptake of magnesium, 87  
  factors affecting, 97  
  in-vitro studies, 89  
Total extracellular content of magnesium, 68  
Toxemia of pregnancy, 19  
Transport, 107
- U**  
Ultrafiltrable magnesium  
  in diseases, 72  
  in thyroid disorders, 71  
Ultrafiltration of serum, 74  
Uncoupling agents, 92  
Urinary excretion of magnesium, 80, 81
- V**  
Valenti, Michael Bernhard, 5  
Vallee, B. L., 55  
Van Slyke, D. D., 58
- W**  
Wacker, W. E. C., 55  
Watchorn, E., 23, 70  
Water, 106  
Widdowson, E. M., 62  
Willstatter, Richard, 12, 15
- X**  
X-irradiation, 95



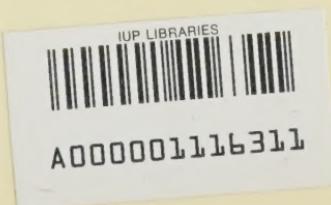




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